Urban bees: reproductive success, colony health and foraging in an anthropogenic environment

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Author declaration

I declare that all work is entirely my own, except for in the following cases. In Chapter Three and Four, the "creating a land cover map" part of the land classification process for 30 out of 38 sites was performed by a research assistant (Alexis Gkantiragas). In Chapter Five, the *Nosema* screens were carried out jointly by myself and a technician (Kel Lui). In Chapter Four, the ground surveys of agricultural sites were performed by a research assistant (Maggie Fitzherbert). In Chapter Six, 31% of waggle dances were decoded by nine research assistants and the R code for land-use preference analysis was written collaboratively by myself and Dr Roger Schürch (Virginia Tech). For all chapters, manuscripts were commented on by all named authors.

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Abstract

Bees are extremely important pollinators but are under threat from reduction in forage availability, parasites and disease and pesticide exposure. Urbanisation is a rapidly expanding driver of land-use change that is likely to interact with these threats, but it is unclear whether urban areas support or impair bee populations. While there is evidence that the abundance and diversity of bee species may be higher in urban areas, it is not known whether this is driven by effects of land-use on reproductive success or migration, and which mechanisms are behind these effects. This thesis employs a colony-level approach to investigating the effects of urbanisation on honeybees and bumblebees. I first developed a land classification protocol to analyse land-use attributes at a resolution relevant to pollinator use of the landscape. Using this protocol, I investigated bumblebee colony success in city, village and agricultural sites by placing lab-reared colonies of *Bombus terrestris* into the field and monitoring their development. I found reduced reproductive output, colony size, longevity and queen survival in colonies in agricultural areas. These colonies were also less likely to contain pollen and nectar stores. Building on these findings in wild bees, I investigated the effect of urbanisation on honeybee pollen foraging and colony health by sampling 51 beehives located across a gradient of urbanisation in South-East England in the spring and autumn. Here I found increased pollen species richness, larger colony sizes and lower *Nosema* infection in colonies located in urban areas. These results in honeybees and bumblebees suggested forage availability may play an important role in mediating the relationship between urbanisation and colony success. To investigate differences in forage availability between urban and rural areas I decoded waggle dances performed by honeybees in ten urban and ten rural sites across an entire foraging season. Urban bees showed consistently lower foraging trip distances, suggesting higher forage availability. This was not compensated for by differences in nectar sugar content, with urban bees collecting nectar with a higher average sugar content than rural bees. Analysis of land-use preferences highlighted the role of residential areas containing gardens in the city and the reliance on mass-flowering crops in the countryside. The results of this thesis suggest that bees are able to thrive in urban areas, and serve to highlight the poor suitability of agricultural land to provide habitat resources for bees and other pollinators.

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Chapter One: Introduction

Bees: ecology and conservation

Bees are a diverse and ecologically important group of organisms, comprising over 16,000 species (Danforth 2007) distributed across all continents but Antarctica (Michener 2000). They play a crucial role in providing a pollination service to both wild and agricultural plants (Corbet et al. 1991; Klein et al. 2007): 87.5% of angiosperms are pollinated by animals (Ollerton et al. 2011), with bees representing the dominant group of animal pollinators (Brown and Paxton 2009). In 2009, the economic value of this service was estimated to total €153bn or 9.5% of global food production value (Gallai et al. 2009); in the US, honeybees alone contribute $14bn per year to the economy with native bees providing an additional $3bn (Danforth 2007).

There is cause for concern, therefore, that growing evidence suggests bees may be in decline. Wild bees in particular appear to be at risk, with evidence from the UK and the Netherlands showing large declines in non-*Apis* bee diversity since 1980 (Biesmeijer et al. 2006). Distributions of many bumblebee species are contracting in the UK (Goulson et al. 2008) and worldwide (Arbetman et al. 2017), and syntheses of land-use gradient studies as proxies for temporal change suggest widespread losses of pollinator diversity and abundance due to habitat loss (Potts et al. 2010a). However, not all species are under threat: populations of some generalist species appear to be stable or even on the rise (Ghazoul 2005). In addition, it appears that reductions in NW-European wild bee species richness have begun to slow down and in some cases reverse in recent decades (Carvalheiro et al. 2013).

Honeybees (*Apis mellifera*) provide the majority of the world's pollination service (Hung et al. 2018), particularly in large-scale agricultural systems where wild bee populations are insufficient (Klein et al. 2007; Grünewald 2010). Despite considerable media attention surrounding the plight of honeybees, the evidence for a honeybee decline is much more equivocal than for wild bees, confounded partly by the fact that the vast majority of colonies are managed by humans (Jaffé et al. 2010). In some areas of the world a significant reduction in the number of managed hives is evident; in the UK this number has decreased from 300,000 to 135,000 in the last 60 years (Alton and Ratnieks 2013) and hives in Europe as a whole declined by 16% between 1985-2005 (Potts et al. 2010b). Similarly, the US experienced a 60% reduction in colonies since 1940 (Aizen and Harder 2009a). However, these regional trends are counterbalanced by increases elsewhere, resulting in a 45% overall global rise in honeybee stocks over five decades (Aizen and Harder 2009b). Although anthropogenic factors including the spread of invasive parasites such as *Varroa destructor* are likely to play a role in regional declines (Boecking and Genersch 2008), this geographic variation in honeybee trends has been largely attributed to the economic viability of beekeeping (Aizen and Harder 2009b; Potts et al. 2010b; Grünewald 2010).

Some evidence remains of another process at play, with unusually high winter colony losses reported in some regions (Gross 2009; Neumann and Carreck 2010). One manifestation of this that is particularly apparent in the US is described as Colony Collapse Disorder (CCD). This is characterised by the disappearance of large numbers of adult bees from the hive and rapid colony failure (Oldroyd 2007) for which various explanations have been proposed including novel pathogens, pesticides or interactions between multiple factors (Ratnieks and Carreck 2010). However, it remains unclear whether the recent high incidence of colony losses is an indication of a wider problem or consistent with the normal peaks in colony losses seen in the historical record (Neumann and Carreck 2010). Less controversial is the decline in feral (i.e. unmanaged) honeybee colonies, which remain at very low densities in most regions due to the ubiquity of *Varroa*, which causes colonies to rapidly die out in the absence of treatment (Kraus and Page 1995; Wenner and Bushing 1996). However, recent reports suggest some feral populations are able to coexist with *Varroa* and other parasites (Seeley 2007; Le Conte et al. 2007) and feral honeybees have been shown to harbour fewer pathogens and display stronger immune gene expression than domesticated bees (Youngsteadt et al. 2015a), suggesting that these populations may be a source of ecological resilience.

Several potential drivers and emerging threats to wild and managed bees have been identified. Habitat loss (including agricultural intensification) leading to loss of forage and nesting resources has been a major driver of bee declines in the 20th century (Le Féon et al. 2010; Potts et al. 2010a; Marshall et al. 2017; Papanikolaou et al. 2017a). Environmental contaminants such as pesticides pose significant threats (Goulson 2013), while invasive species, including plants (Stout and Morales 2009) and other bees (Thomson 2004), may also impact bee populations. Both novel pathogens (Paxton et al. 2015) and climate change (Potts et al. 2015; Marshall et al. 2017) have been identified as significant recent and emerging drivers, and these and other threats are predicted to interact (Vanbergen et al. 2013). These drivers will be discussed in more detail below in the context of urbanisation.

Effects of urbanisation on bees

In recent years, increasing attention has been devoted to urbanisation in ecological research (Hahs and Evans 2015), reflecting the growing impact of land use on biodiversity (Grimm et al. 2008). Over half the world's human population currently reside in cities and by 2050 this is predicted to reach 68% (United Nations 2018). The physical extents of urban areas are expanding at an even faster rate, with an estimated three-fold increase in global urban land cover predicted between 2000 and 2030 (Seto et al. 2012). Currently, 4% of the terrestrial land surface is comprised of urban land, with the ecological footprint of each developed area extending well beyond its city boundaries (Goddard et al. 2010). Urbanisation affects the local biota via multiple routes, including fragmenting natural habitat, modifying microclimate and hydrology, facilitating invasion and altering species composition and diversity (Alberti 2005; Grimm et al. 2008; Elmqvist et al. 2013). Whether this has a negative or positive impact varies widely depending on the taxon or ecosystem involved; while some bird species such as sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*) show higher abundance in cities (Evans et al. 2009) and plant species richness is positively associated with moderate urbanisation (Mckinney 2008), many taxa such as carabid beetles show decreasing diversity with increasing urbanisation (Niemelä and Kotze 2009).

Bees are distributed over a wide range of habitats and geographical regions, making contact with urban areas a regular occurrence for many species and populations. Despite this, it is not fully clear how urbanisation affects bees. The following review of the literature refers to studies employing a range of definitions of urban land, including descriptive classification (e.g. Goulson et al. 2002) and quantitative definitions based on GIS land classification (e.g. Lecocq et al. 2015), and carried out across a range of geographical regions. As such the urban-rural comparisons may differ between studies, although the broad patterns are likely to remain informative. To preserve the original classifications I use the terms stated in the study in question (e.g. urban, suburban, rural).

Several studies have investigated bee diversity and abundance within and outside urban areas. A review of 59 studies by Hernandez *et al.* (2009) found that species richness was generally negatively correlated with urbanisation and/or anthropogenic modifications, while a meta-analysis by Winfree *et al.* (2009) similarly found a negative effect of habitat loss or fragmentation on bee abundance and species richness, although the magnitude of effects was small. However, results from individual studies show a varied and often contradictory picture. For example, negative correlations with urbanisation were found for bumblebee diversity in Stockholm, Sweden (Ahrné et al. 2009), wild bee species richness in Germany (Dauber et al. 2003) and Pennsylvania, US (Choate et al. 2018), bee diversity and abundance in Birmingham, UK (Bates et al. 2011), and stingless bees and eusocial wasps in Belo Horizonte, Brazil (Zanette et al. 2005). A temporal comparison of bee communities pre- and post-urbanisation in Brazil also indicated negative impacts of land-use change (Cardoso and Gonçalves 2018). In contrast, other studies found positive associations with urbanisation, including for bumblebee abundance in San Francisco, US (McFrederick and LeBuhn 2005); bee abundance in New Jersey, US (Winfree et al. 2007a); species richness (but not abundance) in multiple cities across the UK (Baldock et al. 2015) and colony growth in bumblebees in South-East England (Goulson et al. 2002). In addition, some experiments have found no clear effect of urbanisation on bees: for example, Hausmann *et al.* (2015) found no difference in bee diversity between built-up and green areas in Berlin, but a higher abundance of honeybees in the built-up areas and higher wild bee abundance in green areas. Similarly, Banaszak-Cibicka and Żmihorski (2012) found that wild bee species richness and diversity was stable across an urbanisation gradient in Poznan, Poland (although species composition differed), and Osborne *et al.* (2008) demonstrated similar nest densities of bumblebees in gardens and linear rural habitat.

The variation in reported responses to urbanisation is likely to be due in part to the differing magnitude of land-use change in the various sites studied; for example, bees exhibit more negative responses when human land-use is highly intense (<5% natural habitat remaining; Winfree et al. 2011). The major driver of response variation, however, can be attributed to the wide range of life history traits often displayed by sympatric bee species. Cane *et al.* (2006a) showed that bees' responses to urban habitat fragmentation are predicted by ecological traits including dietary breadth and nesting ecology; for example, social bees are more strongly affected by isolation from natural habitat than solitary bees (Williams et al. 2010), and generalists are often less affected by urbanisation than specialists (Zanette et al. 2005; Hernandez et al. 2009). Banaszak-Cibicka and Żmihorski (2012) suggest that urbanisation acts as a filter for specific ecological traits that facilitate colonisation of urban habitat. This provides a challenge for conservation, in that efforts to conserve one species or guild may harm others.

The effects of urbanisation on managed honeybees must be considered separately to wild bees because of their vastly different life histories and domestication. A major current influence on the interaction between honeybees and urban land-use is the recent surge in popularity of urban beekeeping, bringing many more honeybee colonies into contact with cities. In London, for example, the number of beekeepers rose from 464 to 1,237 between 2008 and 2013 and the number of hives doubled to over 3,500 (Alton and Ratnieks 2013), while in Berlin the number of beekeepers increased by 53% and hives by 44% between 2006 and 2012 (Lorenz and Stark 2015). This has been driven by an increased awareness of threats to honeybees from media coverage of the neonicotinoid debate and CCD, a change in motive from economic to ecological, improved access to training and resources and the advent of new hive types suitable for urban beekeeping (Lorenz and Stark 2015). The belief that cities constitute a better habitat for honeybees than rural areas seems widely held (e.g. McCallum and Benjamin 2012; Peters 2012), but the empirical evidence is less clear. For example Clermont *et al.* (2015) found that urban land-use (particularly transport, industry and leisure) was associated with high honeybee colony losses in Luxembourg, while Winfree *et al.* (2009) found no effect of anthropogenic disturbance on either managed or feral honeybees. Further research on the effects of urbanisation on honeybees in the context of specific drivers is discussed below.

Any factor that affects pollinator populations is likely to impact pollination services, and evidence suggests that this is the case for urbanisation. Hennig and Ghazoul (2012) found that bee visitation rate to red clover was positively correlated with the extent of vegetated area in the surrounding urban matrix, driven primarily by the dominant pollinator *Bombus pascuorum*. In contrast, Lowenstein *et al.* (2015)

found increasing bee abundance and richness with increasing human population density, which led to higher visitation of potted Echinacea. In sites with higher population density, bee communities shifted to species that carry more pollen and are more active pollinators including honeybees and some native species. The positive relationship between human population density seems to have been mediated by the effect of people on floral resources, as more densely populated areas had higher floral diversity. Similarly, Pereira-Peixoto *et al.* (2014) found a positive effect of proximity to urban areas on flowering crop visitation due to spillover of the urban pollinator population into adjacent agricultural fields and Theodorou *et al.* (2016) demonstrated enhanced pollination in urban areas linked to higher pollinator density. However, higher visitation rates may not always lead to increased pollination. Baldock *et al.* (2015) found that flower visiting insects were less specialised in cities than in farmland due to the higher floral diversity and hypothesised that this would lead to less effective pollination. In support of this theory, a recent study found lower seed set in experimental plants placed in urban and agricultural areas than in natural, despite higher visitation rates (Leong et al. 2014).

Urban drivers of bee ecology

Habitat loss

Alteration of nesting resources represents a major driver of urban effects on bees (Harrison and Winfree 2015a). For wild bees, urbanisation can either create or remove nesting opportunities. Several studies have found that the magnitude of the effect of urbanisation on bee abundance is predicted by the nesting ecology of the species in question, with cavity-nesting species consistently more abundant in urban areas than ground-nesters (Zanette et al. 2005; Cane et al. 2006b; Hinners 2008; Matteson et al. 2008; Neame et al. 2013), due to the availability of artificial cavities such as eaves and masonry and the reduction in ground-nesting opportunities with an increase in impervious surface. In the UK, the recently colonising bumblebee *Bombus hypnorum* has been shown to be strongly associated with urbanisation, attributed to its cavity-nesting behaviour (Crowther et al. 2014). However, the density of ground-nesting bumblebee nests was found to be higher in gardens than in non-linear rural habitat (Osborne et al. 2008), which may be driven by other factors such as the availability of forage.

Forage availability has a strong effect on whether urban areas support healthy bee populations; Wojcik and McBride (2012) found that floral resource availability was a stronger predictor of bee species occurrence than landscape, suggesting that urban areas can be good habitats for bees providing sufficient forage is available. Similarly, Bates *et al.* (2011) found a positive correlation between bee diversity and abundance and floral abundance; in this case, however, bee populations were negatively correlated with urbanisation, implying insufficient forage availability in urban areas. Competition may also affect forage availability: honeybee hives are often present at much higher densities in urban than rural areas (Alton and Ratnieks 2013), potentially increasing competitive interactions with wild bees (Goulson and Sparrow 2009). Despite this, bumblebee colony productivity (weight gain) has been shown to be higher in suburban (Goulson et al. 2002) and urban (Parmentier et al. 2014) areas than in the countryside. Pollinator community phenologies may also be affected by changes in forage availability associated with urbanisation (Leong et al. 2016).

Cities and towns often offer high floral abundance and diversity in the form of gardens and parks. 87% of UK homes have a garden, with a total area of 432,924 ha (Davies et al. 2009; Theodorou et al. 2017). Gardens are recognised as extremely important in the protection of urban biodiversity, providing heterogeneous habitat, high connectivity and fostering a wider interest in nature (Goddard et al. 2010). Bees regularly show positive associations with gardens: Goulson et al. (2010) found that the land use class with the most consistent effect on bumblebee nest density and survival was gardens, and several surveys of garden pollinator assemblages have shown that high diversity (e.g. Fetridge et al. 2008; Foster et al. 2017) and resource provision (Kaluza et al. 2016) is supported. Similarly, Baldock et al. (2019) identified gardens and allotments as hotspots for pollinators in urban areas. This has been demonstrated to have positive knock-on effects for pollination. Samnegård et al. (2011) found higher bee abundance and species richness nearer to gardens, which led to increased seed set in experimental plants, while Cussans et al. (2010) found greater seed set in two bee-pollinated wildflower species in gardens compared to arable land.

Gardens are often associated with higher proportions of non-native plants: 70% of the UK garden flora is exotic in origin (Loram et al. 2008). The implications of this for bees is contested (Vanbergen et al.

2018). Matteson and Langellotto (2011) found that adding native plants to urban gardens did not increase beneficial insect richness with some bees preferentially utilising exotic species; in contrast, a study in north-western Ohio found that gardens with more native plants supported higher wild bee abundance and altered species composition (Pardee and Philpott 2014). The presence of exotic plants can extend the seasonal availability of forage for bees; in London, UK, bumblebees can achieve high foraging rates throughout the winter months despite few native plants being in bloom (Stelzer et al. 2010), and gardens may act as buffers between mass-flowering crop blooms in agricultural areas (Requier et al. 2015; Langellotto et al. 2018). However, while there are benefits for bees, native plants may be negatively impacted by pollinator visitation of exotics. A meta-analysis found that visitation to native plants was significantly reduced by proximity to non-native plants compared to native plants, an effect that was magnified when plants were phenotypically similar (Morales and Traveset 2009). In addition, the benefits of exotic plants may be experienced by only a subset of bees: several studies show that floral specialists are underrepresented in urban areas (McFrederick and LeBuhn 2005; Cane et al. 2006a; Fetridge et al. 2008; Hernandez et al. 2009; Harrison and Winfree 2015a). Even for generalist bee species, not all flowers are attractive, with some artificially selected ornamental varieties displaying inaccessible nectaries or exotic varieties possessing adaptations for vertebrate pollination (Garbuzov and Ratnieks 2014a). A survey of flower-visiting insects in a public garden in Lewes, UK found that only 4% of the varieties in bloom were highly attractive to insects (Garbuzov et al. 2014) and a study of flowering garden plants in an experimental plot found a 100-fold variation in attractiveness to insects (Garbuzov and Ratnieks 2014a).

Honeybees are affected differently to wild bees by the habitat alteration associated with urbanisation. As predominantly domesticated animals they are not limited by nesting resources, but are highly dependent on abundant high quality forage to support colonies that are many times larger than the largest wild bee colonies (Naug 2009). One method of investigating foraging behaviour unique to honeybees is waggle dance decoding: honeybee foragers communicate locations of profitable resources by performing a figure-of-eight ‘dance’ on the comb in which is encoded the angle from the sun’s azimuth and the distance to the resource (Couvillon 2012). Garbuzov *et al.* (2015b) studied this in the context of urbanisation, finding that distances communicated by bees in a city were typically lower than those communicated by rural bees in a comparable study in the same region (Couvillon et al. 2014a) and showed less seasonal variation (i.e. average distances were similar during different months). As honeybees are sensitive to foraging economics, distance acts as a proxy for forage availability (Couvillon et al. 2014a) so the lower distances travelled by urban bees suggest it was easier to find high-quality forage near to the hives. The low inter-month variation implies abundant forage throughout the season, consistent with the diversity of floral varieties and bloom periods seen in gardens and parks (Loram et al. 2008). Waggle dance communicated distances in suburban Florida and California were also found to be shorter than those in a comparable experiment in temperate deciduous forest (Visscher and Seeley 1982; Waddington et al. 1994) again suggesting that urban areas provide abundant forage. In contrast, a study analysing dance frequency for different land-use types while correcting for distance found no evidence for a preference for urban areas; indeed, there was a non-significant trend towards avoidance of urban land (Couvillon et al. 2014b). Similarly, a "choice test" for hives placed on an urban-rural boundary suggested a preference for rural land (Sponsler et al. 2017). However, no study to date has compared waggle dances from urban and rural hives in the same experiment.

Honeybee foraging success can also be monitored by analysis of food collection, the results of which have been largely inconsistent. Lecocq *et al*. (2015) showed that hives in urban areas gained more weight than hives in agricultural or mixed landscapes, driven by strong differences in weight gain in July and August (periods of low forage availability in some agricultural areas, Couvillon et al. 2014a). In contrast, Sponsler and Johnson (2015) found that food storage was negatively correlated with the ratio of urban to cropland, and Naug (2009) found a positive correlation between open (non-urbanised) land area in US states and state-wide honey yields and a corresponding negative correlation between colony losses and the ratio of open to developed land.

Parasites and disease

Pathogens represent a key threat to pollinators (Grünewald 2010); in honeybees, parasites such as *Nosema* and *Varroa* are responsible for considerable colony losses (Boecking and Genersch 2008; Paxton 2010). Recent evidence has emerged for an interaction between urbanisation and parasite prevalence: Youngsteadt *et al*. (2015a) found higher pathogen loads (*Nosema ceranae* and Black Queen Cell Virus) in honeybee colonies in more urbanised areas. This correlation was not mediated by immune response, implying a role for environmental factors. In addition, the probability of survival of bees in laboratory conditions declined three-fold across the increasing urbanisation gradient. Studies in bumblebees have found a similar pattern, with prevalence of three protozoan parasites increasing with urbanisation in *Bombus terrestris* (Goulson et al. 2012), and prevalence of *Crithidia bombi* higher in *Bombus* populations in urban areas (Theodorou et al. 2016). This could be mediated by several factors. The high densities at which both bumblebees (Osborne et al. 2008; Goulson et al. 2010; Theodorou et al. 2016) and honeybees (Alton and Ratnieks 2013) occur in urban areas could increase direct and indirect contact between bees (Mallinger et al. 2017), resulting in higher transmission rates, both within (Youngsteadt et al. 2015a) and between (Fürst et al. 2014; Pirk et al. 2017) species. Patchy resources in cities may concentrate pollinators in small fragments, increasing opportunities for horizontal disease transmission (Youngsteadt et al. 2015a). Temperature-sensitive parasites such as *Nosema ceranae*, which displays inhibited germination following cold exposure (<4°C, Gisder et al., 2010), may be favoured by the warmer microclimate found in cities (Gago et al. 2013), and in honeybees, disease transmission may be increased by the higher proportion of novice beekeepers in cities (Alton and Ratnieks 2013). Conversely, urban areas may provide protection against parasites in the form of greater pollen diversity (Goulson et al. 2002), which has been shown to increase survival in *N. ceranae* infected honeybees (Di Pasquale et al. 2013).

Environmental contaminants

Exposure to environmental contaminants interacts with land-use, with different pressures on urban compared to rural bees. Pesticides, particularly insecticides, represent the major contaminant threat to bees. Although the vast majority of research in this field concerns agricultural areas (Goulson 2013), bees may also come into contact with pesticides in urban areas (Bonmatin et al. 2015). In the UK, for example, 208 insecticide products are authorised for use on ornamental plants and 701 tonnes of pesticides were applied for amenity use in 2012 (Goulds 2012). Although total agricultural pesticide use may be higher than in urban areas (Goulds 2012; Botías et al. 2017), bees may be exposed to higher localised application rates in a garden context (Hopwood et al. 2012). Some home pesticide products recommend application of 12 to 16 times the amount of imidacloprid (a neonicotinoid insecticide) permitted in agriculture, and unlike in crops, residues of neonicotinoids in ornamental plant flowers at label application rates may exceed the LD50 for honeybees (Hopwood et al. 2012). Indeed, a survey by Johnson and Pettis (2014) found the highest imidacloprid concentrations in water sources near honeybee hives in urban areas compared to suburban or rural, including a sample of 131ppb near a garden nursery, and 70% of a sample of ornamental plants sold for horticultural use were found to be contaminated with neonicotinoids (Lentola et al. 2017). Use of neonicotinoids on lawns with flowering plants has also been shown to be harmful to bees: bumblebee colonies allowed to forage on lawns treated with clothianidin showed delayed weight gain, reduced reproductive output and higher worker mortality (Larson et al. 2013).

Neonicotinoids have been shown to negatively impact bee cognition (Samuelson et al. 2016; Siviter et al. 2018b), reproductive success (Whitehorn et al. 2012) and foraging behaviour (Gill et al. 2012), and consequently became subject first to an EU moratorium restricting their use on flowering crops (European Commission 2013) and subsequently to a complete ban on outdoor agricultural use of imidacloprid, thiamethoxam and clothianidin (European Commission 2018a, b, c). However, horticultural use is unaffected, raising the possibility of increased exposure to neonicotinoids in urban areas compared to rural. In support of this Nicholls et al. (2018) found neonicotinoid residues in bumblebee nests decreased following implementation of the moratorium in rural but not urban areas.

Other contaminants may be particularly prevalent in urban areas. Porrini *et al.* (2003) found heavy metals were more likely to accumulate inside bees in cities; nickel is known to reduce bumblebee visit duration (Meindl and Ashman 2014) and cadmium, lead and zinc all increase mortality in solitary bees (Moroń et al. 2012). Air pollution from transport may also modify pollination interactions, as diesel exhaust has been shown to degrade floral volatiles, making them undetectable to honeybees (Lusebrink et al. 2015). Nitrogen deposition from industry and transport results in altered competitive dynamics in urban flora (e.g. from forbs to grasses, Burkle and Irwin 2010) and modification of reward provision, with knock-on effects for pollination interactions (Harrison and Winfree 2015a).

Urban warming

Temperatures in cities can be up to 12°C higher than the surrounding countryside due to heat trapped by buildings and roads and high urban energy expenditure (Rizwan et al. 2008; Gago et al. 2013). As such, cities provide valuable natural experiments for the effects of climate change on ecosystems (Youngsteadt et al. 2015b), and any current impacts of urban warming on pollinators might be expected to be magnified in the future (Papanikolaou et al. 2017b). Although research in this area is still in its infancy, Hamblin, et al. (2018) found temperature was an important predictor of urban bee community structure. Theoretically, urban heat islands may filter urban bee communities, either directly or indirectly through altering of plant community composition (Harrison and Winfree 2015a; Hamblin et al. 2017). Pollinator phenologies may also be changed by urban warming: in London, for example, *Bombus terrestris* queens and workers have been observed throughout the winter, suggesting colonies with a winter-active generation (Stelzer et al. 2010). Although a temporal mismatch between these bees and their native food plants would have been possible, this is offset by the abundance of winter-blooming exotic plants available in urban areas (Stelzer et al. 2010). Warm winter temperatures cause earlier emergence and increase weight loss in hibernating adult bees, possibly due to higher metabolic activity (Fründ et al. 2013). As described above, some diseases may be favoured by urban warming (Gisder et al. 2010), and a warmer microclimate may also affect thermoregulatory costs for bees (Fahrenholz et al. 1989).

Conclusions and research gaps

The research to date investigating the effects of urbanisation on bees has produced mixed results: while there is evidence that urbanisation can have negative impacts, many studies have found positive associations between bees and urban areas. Much of the research effort has focussed on abundance/diversity studies, with recent evidence demonstrating higher abundance and diversity of bees in urban areas (e.g. Baldock et al. 2015; Theodorou et al. 2017; Martins et al. 2017). However, it is unclear whether these population differences are driven by migration between small-scale land-use patches, forager concentration at resources or by effects of urbanisation on true population size and fitness (Crone and Williams 2016). In bumblebees, a small number of studies have approached this question at the colony level, but the use of commercial colonies has precluded investigation of total reproductive output throughout the colony life-cycle (Goulson et al. 2002; Parmentier et al. 2014). In honeybees, colony-level studies have typically examined a single driver of urban effects in isolation (e.g. Youngsteadt et al. 2015a). As such, holistic colony-level investigation of the effects of urbanisation on honeybees and bumblebees represents a significant research gap.

Forage availability and habitat loss has been identified as a major driver of bee declines (Senapathi et al. 2015), but there is a scarcity of research investigating the relationship between forage availability and urbanisation. A number of studies have measured food store accumulation in honeybees across urbanisation gradients (Naug 2009; Sponsler and Johnson 2015; Lecocq et al. 2015), but investigation into what bees are foraging on and where they are foraging in urban areas remains largely unexplored. The tools of pollen analysis and waggle dance decoding are well suited to answering these questions, but to date palynology has been under-utilised in an urban context and waggle dance studies have been restricted to single sites (Waddington et al. 1994; Garbuzov et al. 2015b; Sponsler et al. 2017), necessitating comparison between studies with different methodologies.

Research to date has identified several key drivers of the effects of urbanisation on bee populations. However, significant gaps remain in linking drivers to fitness and elucidating effects at the colony-level for social bees. Exploitation of the characteristics of social bees that facilitate experimentation such as the ability to rear colonies in the lab and decoding of waggle dances represents a useful tool for understanding effects of urbanisation on central-place foraging social bees, and to some extent provide an insight into pressures that may also affect other pollinators. This is particularly relevant if generalist foraging species are used as such much of their response to land-use may be extrapolated to other pollinator taxa with overlapping forage requirements, although no single species will reflect responses of all pollinator taxa as population drivers including nest site availability, forage specialism and larval food plant distribution vary widely across taxa (Cariveau and Winfree 2015).

Summary of research chapters

The research chapters in this thesis (Chapters Three to Six) are presented in the format in which they were submitted as manuscripts, except for the referencing styles which have been standardised for consistency. Electronic Supplementary Material is supplied as appendices to each chapter. Chapter Two ("Methodology") presents a critical evaluation of the methods used in the experiments.

In Chapter Three I developed a land classification protocol relevant to pollinator ecology and applied it to a case study of 38 sites across an urbanisation gradient in South-East England. This method was developed for the study in Chapter Four and I subsequently applied variations of the method to Chapters Five and Six. Chapter Four investigated the effect of urbanisation on bumblebee colony fitness alongside parasite load and foraging success, by monitoring lab-reared *Bombus terrestris* colonies placed at 38 sites in city, village and agricultural land throughout the colony life-cycle. In Chapter Five I built on the results from wild bees and examined the effect of urbanisation on pollen foraging and colony health in honeybees. I analysed samples of stored pollen, *Nosema* spores, *Varroa* mites and colony strength from May and September from 51 honeybee colonies located in urban, suburban, rural open and rural wooded sites across South-East England.

The results from Chapters Four and Five highlighted forage availability as an important driver of the relationship between urbanisation and bee success. To investigate this in more detail, in Chapter Six I examined the effect of urbanisation on forage availability using the honeybee waggle dance. I decoded waggle dances from ten urban and ten rural sites over an entire foraging season to investigate differences in foraging distance as a proxy for forage availability. I also analysed nectar sugar content to test whether differences in foraging distance are compensated for by differences in forage quality, and investigated forage locations to identify preference for land-use types within the urban and rural landscapes.

Appendix One contains a review of honeybee foraging behaviour published as a book chapter in the *Encyclopaedia of Animal Cognition and Behaviour.* Appendices Two and Three contain Supplementary Tables from Chapters Four and Five respectively.

Chapter Two: Methodology

In this chapter I critically evaluate methods used in the four research chapters of this thesis. This is not an exhaustive description of the methods used, which are described in more detail in the Methods sections and Supplementary Material for each research chapter. I refer to the experiments as follows:

Chapter Three: Development of a land classification protocol for pollinator ecology research

Chapter Four: Effects of urbanisation on bumblebee reproductive success

Chapter Five: Effects of urbanisation on honeybee pollen foraging and colony health

Chapter Six: Using the waggle dance to investigate pollinator forage availability in urban and rural land

General approach

The overall approach to the experiments in this thesis was to combine manipulative and observational techniques. Land-use research is often correlative due to the necessity of collecting data from existing sites representing the land-use type(s) in question; it is not possible to recreate a land-use type in a controlled laboratory setting. As such, many studies into the effects of urbanisation on bees focus on surveys of bee abundance and diversity across varying degrees of urbanisation (Hall et al. 2016). For the experiments presented here, I aimed to introduce more experimental control by measuring not only bee populations already present at sites, but also by randomly placing colonies of honeybees or bumblebees reared in the lab or elsewhere into the field as much as possible. Where this was not possible (for example in the study in Chapter Five) the aim was to select colonies to minimise collinearity of covariates with land-use, such as beekeeper experience, so that causal links between land-use and response variables could be more reliably interpreted than from a purely correlative study.

This aim in part drove the focus on two study species, *Apis mellifera* and *Bombus terrestris*. Both are social bee species that can be reliably reared in captivity. *A. mellifera,* the European honeybee, lives in colonies of up to 60,000 workers and a single queen (Seeley 1995) and has been domesticated by humans since c. 4,000BC (Tatli 2017), with widely available techniques and equipment for rearing colonies in man-made beehives. *B. terrestris* has been domesticated less recently, but due to the increased demand for intensive agricultural pollination, protocols for rearing colonies in captivity have been developed (Velthuis and van Doorn 2006). As such these species are well-suited to manipulative land-use experiments. As important pollinator species (Goulson 2010; Hung et al. 2018), there is an economic and ecological argument for studying the effects of urbanisation on *A. mellifera* and *B. terrestris*. I decided to work on both species to investigate whether the effects of urbanisation are consistent across species with different colony sizes and foraging ranges, to understand how representative the results obtained may be for other similar pollinators. In addition, characteristics of the two species allow for different questions to be studied: in bumblebees it is possible to directly measure reproductive output (Crone and Williams 2016), allowing examination of land-use effects on fitness not possible in other taxa, while in honeybees it is possible to obtain detailed information on foraging behaviour using the waggle dance (Couvillon 2012).

While no single species can encompass characteristics of all pollinator taxa or even all bee species, both *A. mellifera* and *B. terrestris* are generalist foragers (Seeley 1995; Goulson 2010), meaning that their responses to forage availability may reflect those of some other generalist insect pollinators. However, even amongst generalist taxa differences exist in preferences for forage sources (Petanidou et al. 2008), foraging behaviour and range (McArt et al. 2017), communication ability (Stout and Goulson 2001; Couvillon 2012) and colony/individual nutritional requirements (Vaudo et al. 2015). Specialist foragers are likely to respond very differently to land-use than generalists (Goulson et al. 2005; Cariveau and Winfree 2015) . Furthermore, pollinator populations are additionally driven by non-forage factors such as parasite pressure (Potts et al. 2010a), nesting requirements (Williams et al. 2010) and larval food plants (Biesmeijer et al. 2006). Therefore, while experiments with these species provide an indication of how other pollinators may respond to urbanisation, the results should be interpreted alongside other studies of pollinator responses to urbanisation to gain a holistic view of species responses to this type of land-use change.

Colony-level approach

Research into abundance and diversity of pollinator species gives important insights into the suitability of different habitats for pollinators. However it is not possible to elucidate the reasons behind population differences using this method; for example it is unclear whether habitats supporting high pollinator abundance and diversity is due to positive effects on reproductive success or due to migration into the habitat from surrounding areas or forager concentration at resources (Crone and Williams 2016; Iles et al. 2018). While colony densities can be extrapolated from bumblebee worker counts (Glaum et al. 2017) or molecular analysis (Darvill et al. 2004), experimental placement of bumblebee colonies in different habitats, as in Chapter Four, allows a direct assessment of reproductive fitness by counting the number of queens and males produced. Similarly, colony strength measures and colony-level parasite counts in honeybees provide information more directly relevant to their unit of reproduction (the colony) than data derived from population-level surveys in the environment.

Participant recruitment and selection

The development of a wide selection of sites to place colonies was achieved by recruiting beekeepers and garden owners to a participant network. For honeybee studies (Chapters Five & Six) I recruited sites managed by existing beekeepers so that the beekeepers could help with hive management, while for the bumblebee study (Chapter Four) I recruited sites managed by non-beekeepers to avoid an effect of presence of honeybee hives. Beekeeper recruitment primarily used Beekeeper Association (BKA) avenues, including contacting BKA chairs (as listed on bbka.org.uk), placing adverts in BKA newsletters and *BBKA News*, and writing an article in *Beecraft* magazine. I used more generic routes to recruit non-beekeeper garden-owners, including social media posts, internal university emails and a project website. Over the course of the project the network of participants to draw from numbered approximately 130 beekeepers and 115 garden owners, which I maintained by giving regular talks at BKA meetings and disseminating study results to participants.

Collaborating with participants had both advantages and disadvantages. Using hives managed by beekeepers allowed me to have much larger sample sizes across a larger spatial range than if I had managed hives entirely myself, and for all studies the site owners checked colonies regularly and contacted me if there were problems, allowing more frequent monitoring than could be achieved alone. Frequent contact with beekeeper communities also helped me to gather anecdotal evidence about pressures facing beekeepers in urban and rural areas and gave me access to a knowledge base that improved my own beekeeping skills. Using beekeeper equipment also kept costs down and reduced the risk of disease transfer between sites. Disadvantages of participant collaboration included the time required to maintain the network and reduced control over management techniques and equipment; for example in Chapter Six observation hives were not all the same size and in Chapter Five beekeepers used different varroa treatments, although this was accounted for statistically. In addition, data collection by non-scientists (citizen science) is generally more suited to larger scale data collection and can be unreliable (Dickinson et al. 2010; Ratnieks et al. 2016), so I decided not to ask participants to collect data themselves.

A rigorous selection protocol helped to minimise the noise generated by varying management techniques. Before selecting sites/participants from the larger pool for each experiment I carried out data exploration to identify variables collinear with urbanisation. For example for honeybee sites the main collinear variables were beekeeping experience and apiary size (more experienced and larger apiaries in rural areas), so I selected sites to balance those two variables (see individual chapters for study-specific selection procedures). In addition, spatial autocorrelation, where nearer sites are more closely correlated with each other in variables of interest, can be a problem in spatial analyses (Dormann et al. 2007). I minimised this by prioritising distance between sites in my selection process and formally tested for spatial autocorrelation in all analyses both graphically (correlogram and spatial distribution of residuals) and statistically (Moran's I Test).

Land classification

Land-use ecology is a complicated field, with a wide array of different approaches to land classification available. Urban ecology suffers from additional complexity due to the intersection of human geography and wildlife ecology (Hahs and Mcdonnell 2006). Extracting characteristics of the urban environment that are relevant to how the taxon in question interacts with the habitat is an important part of developing an urban land classification protocol, with human-focussed definitions of urban land such as demographic variables often ill-suited to pollinators (Brunbjerg et al. 2018; Gren and Andersson 2018). Equally, land-cover measures such as impervious surface do not always capture differences in resource provision for pollinators. For this reason I developed a land-classification protocol from the bottom up that classifies urban land at a resolution relevant to pollinators using categorisation based on pollinator use of the landscape (see Chapter Three for a full description and critical evaluation). I then applied this protocol in the subsequent Chapters (Four-Six), demonstrating its flexibility for different pollinators and different research questions.

Land classification driven by attributes of the land itself rather than broad categorisation into city and countryside helps to disentangle the urban-rural divide that is often not directly relevant to pollinators (Gren and Andersson 2018). Instead, it allows identification of urban attributes such as gardens and urban green spaces that provide traditionally rural or natural habitat resources, deconstructing human-defined land-use types into measures of resource provision for the taxa in question (Matteson et al. 2013). Another consideration for all land-use studies is the scale at which land-use is measured. This largely depends on the study organism's use of the landscape; in the case of bees, their foraging range (Steffan-Dewenter et al. 2002). For honeybee studies I used waggle dance data to select an ecologically relevant land-use radius, while for the bumblebee experiment I used model selection to select from a range of possible radii (see Chapter Three and Four) as foraging range for bumblebees is less accurately known (Osborne et al. 1999; Knight et al. 2005).

The land classification protocol used in this thesis generates categorical land-use variables through clustering sites based on the constitution of the surrounding land. Investigation of the principle component (PC) scores (see Chapter Three, Fig. 5) allows interpretation of the underlying features of a particular cluster and selection of a descriptive name. For example, a cluster with high scores on the urban-agricultural PC and high scores on the open-covered PC may be designated "suburban" as a descriptive name. However, these names are a convenient shortcut and the primary interpretation is carried out from the underlying features of a land-use category based on the PC scores.

Comparison between urban and agricultural habitat

Agriculture is the most common primary land-use in Europe (Eurostat 2016), with semi-natural habitat constituting only a small fraction of the landscape (Levers et al. 2018). Urban areas typically occur as land-use fragments within the wider agricultural landscape (Antrop 2000), and as such a comparison between urban and agricultural land represents a realistic picture of the habitats available to pollinator populations in Western Europe. Consequently I chose not to include semi-natural habitat in the gradient of land-use types studied, as although this may include habitats close to the environments in which bees evolved (Danforth 2007), it does not reflect the availability of habitats to pollinators and to mass-action conservation efforts in modern European landscapes.

Animal husbandry

Ethics

There are no regulations for the ethical treatment of insects in scientific research in the UK. However, I selected non-lethal sampling techniques wherever possible (see *e.g.* varroa sampling and nectar sampling) and care was taken to avoid spreading disease between site visits (equipment was washed and stored in a soda crystal solution between visits). Ecological impact was minimised by using local wild-caught bumblebee queens rather than imported commercial colonies.

Honeybees

Where I managed colonies, I followed standard beekeeping approaches as advised by the BBKA. Where beekeepers were primarily responsible for managing hives I instructed them to continue their usual beekeeping practice and recorded their techniques (*e.g.* varroa treatment, swarm control, feeding, honey harvesting). I excluded from the studies beekeepers that used uncommon techniques, including unusual hive types (such as OSB, Warré and Smith) and commercial beekeepers. Observation hives (glass-fronted beehives used in Chapter Six) were managed differently to standard beehives; see Chapter Six for full details. A common difficulty encountered during observation hive management was avoiding overcrowding to prevent swarming; this was achieved by removing a frame of brood and bees (to another hive in the apiary) whenever overcrowding was detected. Another problem was blockage of the entrance pipe by dead bees; this was particularly common in observation hives with entrances at the base and was rectified by creating side entrances. For both these problems frequent monitoring by beekeepers additional to my fortnightly visits was useful.

Bumblebees

For the experiment in Chapter Four I reared *B. terrestris* colonies from wild-caught queens in the lab prior to placement in the field (see Chapter Four Supplementary Methods). I chose this method over using commercial colonies because this allays ethical concerns of releasing non-native *B. terrestris* workers and reproductives into the environment (Ings et al. 2005, 2006; Colla et al. 2006; Velthuis and van Doorn 2006) and allows colonies to remain in the field until the end of the colony life cycle. Locally caught queens are also more representative of local populations' response to land-use than imported commercially-reared colonies (Ings et al. 2006), and commercial colonies are often delivered at a late stage of colony development meaning the response to differing land-use at the critical early-development stage cannot be monitored. It was also possible to screen the lab-reared colonies for parasites several times during the rearing process to ensure all colonies went out into the field parasite-free, and colony sizes could be standardised at the start of the experiment.

Field techniques

Visit randomisation

All three experiments involved visiting sites multiple times to collect data. This required a visit sequence that was a) randomised and balanced across land-use types and b) consistent. Initial randomisation depended on the study. For Chapter Five sites were grouped into land-use types and a visit sequence constructed that equally distributed land-use types across the four weeks of the experiment with timings of visit within week depending on beekeeper availability; for Chapter Four six transects connecting six-seven sites across the gradient of urbanisation were designed based on minimising travel distances, so that each night an even spread of land-use types were visited; for Chapter Six nearby sites were paired and visited on the same day, with urban and rural sites being visited on alternate days in a randomised order. The aim of these procedures was to achieve a balance between complete randomisation and minimising cost and time involved in travelling between sites. For all experiments, the ideal visit sequence was kept consistent across the weeks of the study. This was often disrupted by weather; when this was the case specific site visits were switched with the aim to maintain the order of land-use group visits and the amount of time between visits to the same site. Because the ideal visit sequence was reverted to the following visit set, despite frequent changes to the sequence there was no overall drift away from the ideal sequence. In each experiment there was redundancy built into the sequence so that if weather meant a day had to be skipped, the schedule of visits in the following visit set would not be affected (e.g. Chapter Four had six data collection days every week and Chapter Six had ten data collection days every fortnight).

Weather and temperature recording

Initially I planned to use temperature data loggers (RC-5, Elitech, UK) for field experiments. These proved to be unreliable when tested with several loggers at the same site. Recording temperature at visit times was also not suited to the experiments, as for colony success measures I was interested in broad temperature patterns over the period colonies were in the field rather than point recordings, and for the waggle dance study temperatures recorded at the hive sites were not representative of outside temperatures as hives were often housed in buildings. For Chapters Four and Six I therefore used local weather station data obtained from www.wunderground.com. For Chapter Four, I located the nearest weather station with records for wind speed, rainfall and temperature and extracted weekly averages for these variables. For Chapter Six I needed only temperature data, so I used data from the weather station at London Heathrow for all sites as analysis of weather station data from Chapter Four showed that there was no relationship between land-use and temperature.

Vegetation surveys

A limitation of GIS land classification using satellite imagery, which is not updated annually, is the inability to record transient land-use types such as crops, which change between and within years (see Chapter Three). Where information about crop types is required land classification should be supplemented by real-time surveys. I achieved this either by ground vegetation surveys (Chapter Four) or aerial surveys (Chapter Six). For Chapter Four, I needed to differentiate between grassland types (e.g. flower-rich meadow or improved pasture) and locate flowering crop fields to ascertain the area of flower-rich habitat in the areas surrounding bumblebee colonies. I initially created GIS land-use maps according to the standard protocol (Chapter Three) and identified polygons containing fields. A research assistant then visited the sites and visually assessed the habitat type of each field to a number of pre-defined categories. This information was then incorporated into the GIS data, and each habitat was scored as “flower-rich” (e.g. wildflower strip, oilseed rape field) or “flower-poor” (e.g. improved pasture, cereal crop). For Chapter Six, ground surveys were not feasible due to the large foraging range of honeybees. For this experiment I was specifically interested in the location of oilseed rape (OSR) fields as a source of spring forage for honeybees, so I conducted aerial surveys using a drone (DJI Phantom 4) in May at all rural sites. The drone was flown to a height of 120m directly above the hive and a 360° video of the landscape recorded, with a visual field radius of up to 4000m. OSR fields could be identified as bright yellow patches in the video and this information was incorporated into the GIS maps. This proved to be a time- and cost-efficient method of surveying OSR, although the technique did not allow discrimination of other land-use types such as grassland types.

Bumblebee field techniques

Colony boxes

In the field, bumblebees were housed in an inner wooden nest box contained within an outer plastic field box. The wooden nest box (W 280 x L 320 x D 160mm) was large enough to hold colonies at their peak (up to 200 workers). Two circular apertures (diameter: 75mm) in the Perspex lid were sufficient to insert forceps to remove bees at any location within the nest without allowing non-target bees to escape. Bees were unable to walk over the underside of the Perspex lid, preventing bees crawling through the apertures when they were open. Colony waste presented a problem in larger colonies where faecal matter and dead bees filled the bottom of the wooden box. In wild nests this liquid would be absorbed into the ground, so future iterations of this design should include absorbant material (e.g. cat litter) to solve this problem. Access to the outside was via a plastic tube (length: 100mm, diameter: 32mm) which was sufficient to allow entry and exit of workers, males and queens.

The primary design aims for the outer box (W 440 x L 710 x D 310mm; Really Useful Box, Kingston, UK) were protection from predators (e.g. badgers), insulation, and waterproofing. The first aim was achieved by securing the box to the ground with screw or groundhog pegs at each corner and attaching a ratchet strap around the middle of the box. This was successful: none of the colonies were destroyed by predators despite evidence of badger attack (tooth marks on the entrance tube) at one of the sites. Insulation was achieved by lining the boxes with insulation wrap (Thermawrap, Creswell, UK); temperature monitoring of a pilot colony showed that this protected the colony from the extremes of daily temperature fluctuations. Waterproofing was achieved by making the box completely sealed; however this resulted in high humidity (see below). In the wild, *Bombus terrestris* nests underground, typically in existing cavities such as abandoned mouse nests (Goulson 2010). In this study, the size of the internal nest box, light intensity and entrance size reflect approximately natural conditions. In contrast, the external box was more visible to predators and brood parasites than a natural nest, which may have influenced brood parasite invasion and (unsuccessful) badger predation.

Mass monitoring

The initial experimental design for Chapter Four involved continuous mass monitoring via scales attached to a data logger in each colony box. These were designed in collaboration with A&D Instruments, Abingdon, UK to consist of three parts: an EM-30KAM head unit, which contained the computer and displayed the mass; a scale unit which consisted of a 300 x 350mm pan on which the wooden nest box could permanently rest and a data logger (AD-1688) plugged into the head unit programmed to record a mass reading every 15 minutes. The head unit battery lasted c. one week and could be replaced with a charged unit each weekly visit. The data loggers stored data in csv format which I uploaded to a tablet on each visit. The data loggers and head units were calibrated at the start of the experiment to ensure readings were the same. The scales were accurate to a resolution of 2g, approximately the mass of four workers.

Pilot tests in the lab with bumblebee colonies set up on scales and allowed to forage freely in a foraging arena were promising, with data loggers recording typical daily fluctuations in colony mass, with a gain over the day from collection of nectar followed by a gradual decrease as stores were used up in the night (Fig. 1). It was also possible to identify peaks in foragers leaving and returning to the nest, so it was hoped that the mass data could provide information not only on long-term mass changes over the course of the experiment but also on differences in foraging activity between the colonies. Unfortunately at the end of the experiment it became clear that the mass data were unreliable, probably due to high humidity levels in the plastic nest boxes which were not captured during piloting of the scales. Throughout the experiment I checked the scales with a 1kg bag of sugar, which showed extremely high variation in recorded mass. Continuous mass data from the data loggers also contained high levels of variation and unexpected mass changes. Variation was higher in larger colonies, consistent with an effect of humidity. Consequently I decided not to use the mass data and instead use worker counts as a measure of colony size.

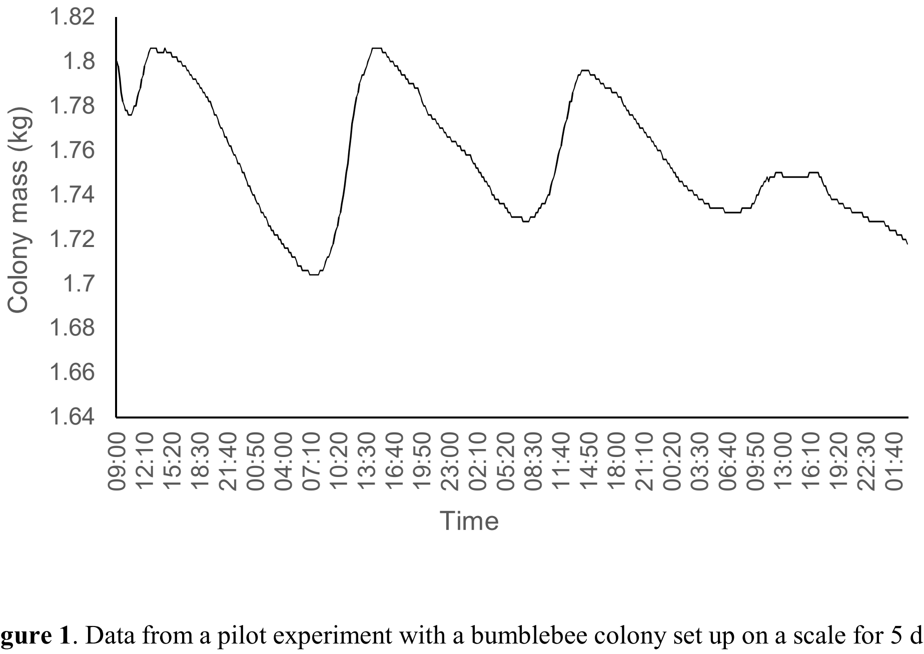

Figure 1. Data from a pilot experiment with a bumblebee colony set up on a scale for 5 days, showing changes in colony mass over time.

Night monitoring

I conducted colony visits at night for several reasons. First, all bees are inside the nest, giving accurate worker and reproductive counts. Second, bumblebees do not fly in the dark so colony inspections could be carried out without a beesuit and without risking bees escaping through the lid apertures. All inspections were carried out with a red light headtorch. Night checks also ensured that store level data were more standardised between visits, as fluctuation is higher over the course of a day than a night. Driving between sites was also unimpeded by traffic at night. Disadvantages included access to gardens, which needed to be arranged with owners in advance, and the difficulty of fitting all visits into the hours of darkness around midsummer, when it is dark for approximately six hours in the study region.

Sample collection and data recording

The primary objective of the field methodology used was to build up a picture of colony development over time, in contrast to traditional methods of a snapshot of colony status at a particular point in the colony life cycle, usually before reproductive production or at the end of the life cycle (Goulson et al. 2002; Whitehorn et al. 2012; Williams et al. 2012). To achieve this I collected samples of workers, males and gynes at each weekly time point (see Chapter Four for full methods). I recorded gynes and males as raw counts, rather than applying a correction based on fitness value (e.g. recording reproductive success as males + 3*gynes (Pelletier and McNeil 2003)). This decision was made because fitness values cannot be accurately calculated, with varied methods appearing in the literature (e.g. Imhoof and Schmid-Hempel 1999; Baer and Schmid-Hempel 2003; Pelletier and McNeil 2003; Whitehorn et al. 2012); raw counts represent a conservative estimate of fitness due to asymmetrical reproductive investment in gynes and males.

Stores were monitored by scanning storage pots in the nests for the presence of nectar and pollen and recording a binary response (presence/absence) for each food type. This method was chosen for its rapidity and the fact that it highlights colonies experiencing food shortages (i.e. colonies with no stored food). More quantitative measures would be difficult to implement due to different colony sizes and the fact that a proportion of storage pots are obscured by the nest architecture in larger colonies. However, for future experiments development of an accurate quantitative measure of food storage would be preferable as land-use was found to have a strong effect on this variable.

Brood parasites

Field studies with bumblebee colonies employ various methods for dealing with cuckoo bumblebee (brood parasites of "true" bumblebees; Goulson 2010) invasion, including protecting against invasion (Siviter et al. 2018a), removing invaders (Pelletier and McNeil 2003) andleaving invaders in the colony (Carvell et al. 2008). A pilot of queen excluders (10mm washers) suggested I could not be confident that all workers could exit the nest and that the entrance tube would not get blocked, so I decided against using these in the field. Instead I chose to record presence of cuckoo bees (invariably *B. vestalis)* in the nest (alive, injured or dead) and remove them immediately. This allowed investigation of *B. vestalis* invasion as a response variable, while minimising harm to the founding queen. Invading *B. vestalis* queens usually remain in the colony for several days before killing the founding queen (van Honk et al. 1981), so weekly visits were sufficient to remove most invaders before the founding queen was killed.

Honeybees

Colony strength

Colony strength was included both as a predictor and a response variable in analyses and was recorded following methods in Delaplane *et al.* (2013a) which involve estimating bee-covered surface. In Chapter Five the number of sides of frames >50% covered by bees were counted alongside a record of the frame size (deep or shallow) and type (national, commercial etc). The known surface areas of frame types were multiplied by the number of frames covered with bees to achieve an estimate of bee-covered surface. As the observation hives are smaller, higher resolution was recorded in Chapter Six (number of frames covered with bees to the nearest 0.5).

Sample collection

Where possible, I used non-lethal sampling methods. This included *Varroa* screening in Chapter Five, which was carried out using the icing sugar shake method (Macedo et al. 2002). This has advantages over other *Varroa* screening methods such as alcohol wash that kill the sampled bees or debris examination on *Varroa* boards that are non-instantaneous in the field (Dietemann et al. 2013). To collect crop contents from foragers in Chapter Six, I collected returning foragers and stimulated regurgitation in cold-anaesthetised bees by manipulation of the abdomen with forceps (Couvillon et al. 2014a). This similarly represents an instantaneous, non-lethal method that can be carried out in the field. For non-*Varroa* parasite analyses, lab analysis was required, necessitating the collection of bee samples. These were killed immediately by freezing in dry ice, and stored in dry ice until transfer to storage at -80°.

Lab techniques

Waggle dance decoding

I followed methods outlined in Couvillon *et al.* (2012) to manually decode recorded waggle dances using video analysis. This is an extremely time-consuming method (dance decoding for 2017 data took approximately 8 months in total) but is highly accurate as waggle dances are analysed via frame-by-frame playback. It is a well-established protocol allowing confidence in the results obtained (Beekman et al. 2004; Couvillon et al. 2012; Schürch et al. 2013a). It is also robust to different observers, meaning that I could outsource a proportion of the decoding (31%) to research assistants. From personal experience, speed in using the method increases considerably with practice, but accuracy is high early on in learning to dance decode. I formally tested this in two ways: 1) including decoder ID as a predictor in the analysis of waggle run duration and 2) decoding a subset of dances decoded by research assistants myself. Both methods showed that there was no significant difference between dances decoded by myself (7 years decoding experience) and trained research assistants (up to 6 months decoding experience).

While using this established method for my data, I was keen to explore other avenues to reduce the time-consuming aspect of the method while retaining the accuracy. One route that has received some attention in recent years is decoding automation (Wario et al. 2015). Some progress has been made, but current methods typically require a strictly controlled filming set-up with a non-standard observation hive (Wario et al. 2015, 2017). This was not possible with my large-scale experiment involving several hives in public places. I therefore explored the possibility of developing a method allowing more flexible filming conditions. For this I collaborated with a computer scientist, Alex Pearce-Kelly, an animator, Matthew Robertson and an invertertabrates curator, Paul Pearce-Kelly, based at ZSL London Zoo. The aim was to develop a neural network that could recognise and measure waggle dances which is taught by information from manual dance decoding data and the associated videos. This method is still in development.

Pollen analysis

Pollen analysis is a powerful tool to identify forage sources, as pollen grains are extremely varied in morphological attributes including size, shape, number of colpi (grooves) and surface structure (Sawyer and Pickard 1981). As such, many pollen types can be reliably identified to species- or genus- level (Moore et al. 1991). To achieve the most accurate levels of identification, a reference collection of pollen from plants in the study region is necessary (Balfour and Ratnieks 2017). This is impossible in an urban area, as a large diversity of exotic and horticultural plant varieties are present in gardens and parks. I therefore decided to focus primarily on differentiating pollen types and classifying them to morphospecies to achieve an accurate measure of pollen diversity, species composition and richness. I then identified all possible morphospecies to species, genus, or family level with 50% of pollen types (n=24) in spring and 51% (n=44) in autumn confidently identified, using a combination of published (Sawyer and Pickard 1981; Moore et al. 1991) and online (Pollen-Wiki 2016; AutPal 2017) sources. To create pollen slides, I followed methods in Balfour and Ratnieks (2017). Because each sample came from a single storage cell, samples were almost always homogenous so one morphospecies was recorded per sample. In rare cases where more than one type was present I recorded the most abundant type.

Parasite screening

In all experiments where parasite screening was carried out I used microscopic methods rather than molecular methods. In some cases, such as parasite screening during bumblebee colony rearing in Chapter Four, this was because immediate information about queens' parasitisation status was required. In other cases (*Nosema* and *Varroa* screens in Chapter Five and *Locustacaris,* Conpoidae, Braconidae, *Crithidia, Apicystis,* and *Nosema* screens in Chapter Four) I decided to use microscopic methods as similar resolution data could be achieved to molecular methods at a considerably lower financial and time cost. For all measured parasites except *Nosema* in honeybees, species (or taxon level of interest) can be reliably identified under a dissection or compound light microscope. The two *Nosema* species present in honeybees (*N. apis* and *N. ceranae*) cannot be differentiated under the microscope, but as *N. ceranae* is uncommon in the UK (Klee et al. 2007) I decided that identification to genus level was sufficient to investigate the research question. Following the results of strong differentiation in *Nosema* levels between urban and rural sites in Chapter Five, however, it would be interesting to follow this up with molecular techniques to investigate the contribution of the two *Nosema* species.

Body fat analysis and ovary dissection

Dissection of sampled bees from colonies in Chapter Four provided an efficient method of gathering large amounts of different data simultaneously. As well as parasite screens from three organs (hindgut, malphigean tubules and fat body), dissection allowed screening for macroscopic parasites (conopid and braconid larvae), ovary development, body fat analysis and dry mass measurements. To investigate the effect of urbanisation on nutrition in Chapter Four I analysed the body fat content of 393 workers, 46 gynes and 418 males following methods in Brown *et al.* (2000; see Chapter Four supplementary material for full methods). Alongside this, I recorded dry mass of whole bees and abdomens only, and bee size. I also measured ovary development in all collected workers to investigate whether colonies in which the queen had died had switched to worker laying, or whether other stressors related to land-use had induced worker laying.

Statistical techniques

In all chapters I employed an Information Theoretic approach to statistical analysis (Johnson and Omland 2004; Burnham et al. 2011). I carried out an initial data exploration procedure to identify outliers, remove collinear variables and identify biologically relevant interactions (Zuur et al. 2010). I then followed an “all-subset” approach to model selection, building models containing all combinations of covariates, including only biologically relevant interactions. I used AICc (Akaike’s Information Criterion corrected for small sample sizes) to compare models, selecting models within 2 ΔAICc from the model with the lowest AICc as the optimal model set (Symonds and Moussalli 2011). I model averaged this subset of models to obtain model-averaged parameter estimates and confidence intervals

(Grueber et al. 2011). Models were validated graphically to assess fit and adherence to assumptions (Zuur and Ieno 2011).

Concluding remarks

The methods used in this thesis span a range of field and lab techniques. The primary aims in designing protocols were to collect colony-level data, minimise environmental noise, generate manipulative rather than correlative data where possible, maximise sample size and streamline sampling to focus on hypothesis-driven data. Through this, I have developed a toolkit of methods for analysing the effects of land-use on pollinators at the colony-level, which may also be applied in future research.

Chapter Three

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A land classification protocol for pollinator ecology research: an urbanisation case study

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Running title: Land classification in pollinator ecology

Abstract

1. Land-use change is one of the most important drivers of widespread declines in pollinator populations. Comprehensive quantitative methods for land classification are critical to understanding these effects, but co-option of existing human-focussed land classifications is often inappropriate for pollinator research.
2. Here we present a flexible GIS-based land classification protocol for pollinator research using a bottom-up approach driven by reference to pollinator ecology, with urbanisation as a case study. Our multi-step method involves manually generating land cover maps at multiple biologically relevant radii surrounding study sites using GIS, with a focus on identifying land cover types that have a specific relevance to pollinators. This is followed by a three-step refinement process using statistical tools: 1) definition of land-use categories, 2) Principal Components Analysis (PCA) on the categories and 3) cluster analysis to generate a categorical

land-use variable for use in subsequent analysis. Model selection is then used to determine the appropriate spatial scale for analysis.

3. We demonstrate an application of our protocol using a case study of 38 sites across a gradient of urbanisation in South-East England. In our case study, the land classification generated a categorical land-use variable at each of four radii based on the clustering of sites with different degrees of urbanisation, open land and flower-rich habitat.
4. Studies of land-use effects on pollinators have historically employed a wide array of land classification techniques from descriptive and qualitative to complex and quantitative. We suggest that land-use studies in pollinator ecology should broadly adopt GIS-based multi-step land classification techniques to enable robust analysis and aid comparative research. Our protocol offers a customizable approach that combines specific relevance to pollinator research with the potential for application to a wide range of ecological questions, including agroecological studies of pest control.

Keywords: anthropogenic stressors, bees, GIS, land classification, land-use change, pollinator, urbanisation, agricultural pest control

Introduction

A large body of evidence suggests that insect pollinators, including bees, are under threat (Biesmeijer et al. 2006; Potts et al. 2010a). Multiple anthropogenic drivers have been identified (Goulson et al. 2015), with land-use change and the associated loss of habitat proposed as one of the most critical threats (Potts et al. 2015). Strong negative effects of landscape alteration on bee and wasp species richness and composition have been documented (Senapathi et al. 2015), with habitat- and food-specialist pollinator taxa particularly vulnerable (González-Varo et al. 2013). However, the impacts of land-use on different aspects of pollinator ecology and on different pollinator taxa can be complex, with effects varying depending on pollinators' dietary and dispersal strategies (Steffan-Dewenter et al. 2002; Winfree et al. 2009) and the type and magnitude of the land-use change in question (Cariveau and

Winfree 2015; Senapathi et al. 2017). As a result, the impact of land-use change on pollinator populations remains a considerable knowledge gap.

Comprehensive quantitative methods for classifying the land surrounding study sites are critical to producing a robust analysis of the effects of land-use (Owen et al. 2006). The more rigorous the land classification, the greater the flexibility of the questions that can be asked about its effects, and the less subjective the interpretation of land-use types. In the pollinator literature, methods used vary widely, and there has historically been no single commonly adopted land classification approach. Broadly, the approaches used can be grouped into three categories: 1) simple visual classification; 2) Geographical Information System (GIS)-based single-step classification and 3) GIS-based refined classification. The former typically involves locating study sites in extreme and/or representative examples of land-use types (e.g. nature reserve, agricultural land, city) and using these qualitatively defined types as a categorical land-use variable, often associated with qualitative descriptions of features of the land-use types but with no further analysis (e.g. Goulson et al. 2002; Banaszak-Cibicka et al. 2018). GIS-based single-step classification typically employs a more quantitative approach, using unmanipulated variables directly extracted from existing data layers or remote-sensing data such as "proportion impervious surface" or "proportion agricultural land" as defined by the classification system of the data layer in question (e.g. Williams et al. 2012; Youngsteadt et al. 2015a), or a combination of a number of these variables (e.g. Donkersley et al. 2014; Baldock et al. 2015; Senapathi et al. 2015). These variables may be categorised by non-statistically defining criteria e.g. "Agricultural = More than 50% of the surrounding designated landscape composed of agricultural areas" (Lecocq et al. 2015). Finally, GIS-based refined classification involves an additional step or steps to manipulate combinations of relevant land variables into a smaller number of variables containing the same information using statistical tools (e.g. Verboven et al. 2014; Sponsler and Johnson 2015). This type of approach typically affords more capability to generate a land classification tailored to the study question, as we will argue below.

As land classification methods have advanced, there has been a slow shift within the field of pollinator ecology towards adopting the latter approach. However, uptake has been far from universal and land classification protocols are typically less powerful than those currently in common use in geographical disciplines. A reasonable criticism of land classification in pollinator studies is that using land-use variables that have been developed from a human perspective, such as proportion urban land as defined by a topographic mapping data layer, can be an ill fit for the aspects of the landscape that are relevant to pollinators (Senapathi et al. 2017). For example, urban land consisting of residential houses and gardens may represent a considerably richer habitat for bees than an industrial estate or central business district (Foster et al. 2017), or agricultural areas growing flowering crops may be richer than those growing cereals (Riedinger et al. 2015). This information may be lost in extracting data from existing classifications, particularly if demographic variables such as human population density are used (Matteson et al. 2013). In essence, it can be argued that adopting human-focussed land classification for pollinator research is at best a proxy for land classification from the pollinator's perspective.

Techniques for generating a land classification from raw data as a bottom-up approach can draw on existing methods used in geographical disciplines (Hahs and Mcdonnell 2006; Owen et al. 2006), and allow flexibility in adapting the land classification to the specific research question. For example, in studies where transient land cover information is required, such as crops grown and bloom stage, data from ground surveys may be incorporated into the land classification. The resolution of the land classification can also be tailored to the space-use of the taxon in question; available land cover data layers are often at resolutions too low to be appropriate for the resolution at which pollinators interact with the land (Büttner et al. 2004). A bottom-up approach also allows extraction of multiple land-use variables at different levels of categorisation. For example the question "how does agricultural land-use affect pollinator abundance?" may be followed up by investigating whether any effect found is driven by the extent of wildflower strips in the surrounding area. The spatial scale at which a pollinator responds to the surrounding land depends on its space use (e.g. foraging range) and the response variable in question (e.g. relating to nesting, foraging, or mating behaviour; Steffan-Dewenter et al. 2002; Westphal et al. 2006); a pollinator-focussed land classification protocol can include data-driven methods for assessing this.

In this paper, we develop a flexible approach to land classification that is appropriate for research into the effects of land-use on pollinators, using urbanisation as an example. The advantages of a bottom-up approach are particularly apparent for urban land classification, as its high level of heterogeneity at a fine resolution is often missed with coarser classification methods, and its typically intransient land cover patches are well-suited to visual classification from satellite imagery. Urban ecology is a growing field (Adams 2005), and in recent years attention has begun to focus on the effects of urbanisation on pollinators (Baldock et al. 2015; Harrison and Winfree 2015b). The wide array of land classification techniques that have been employed in this growing body of literature can make comparisons between studies difficult, generating a call for wider adoption of geographical approaches (Winfree et al. 2011).

The protocol that we present combines primary land cover classification using GIS with a focus on identifying land cover types that have a specific relevance to bees and other pollinators, followed by information refinement using statistical tools (Fig 1). Refinement consists of a three-step process: 1) definition of land-use categories, 2) Principal Components Analysis (PCA) on the categories and 3) cluster analysis to generate a categorical land-use variable for use in subsequent analysis. We present a case study for land classification of 38 sites in South-East England across a gradient of urbanisation, within which bumblebee colonies were placed for a study investigating the effects of urban land-use on colony success.

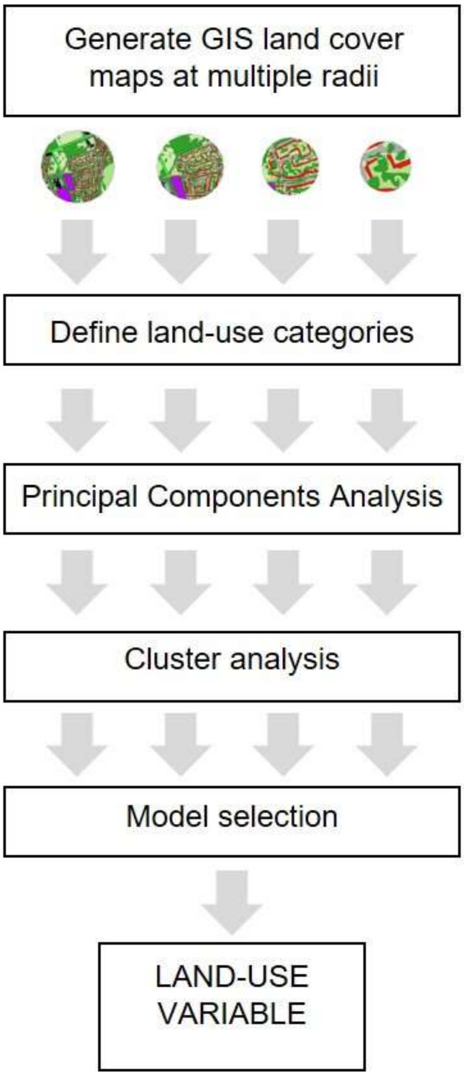

Figure 1. Overview of the multi-step protocol presented for land classification in pollinator ecology research

Methods

Study area

38 sites were located across a c.5000km2 area in SE England (Fig. 2) spanning an urbanisation gradient from dense continuous urban development in central London (most easterly site: 51° 32' 59.5644" N, 0° 2' 25.3284" W) to agricultural land in the counties of Hampshire, Surrey and Berkshire (most westerly site: 51° 20' 17.1096" N, 1° 12' 24.9469" W). This represents a typical urbanisation gradient in Western Europe, with dense urban land transitioning into a wide suburban belt before giving way to agriculture.

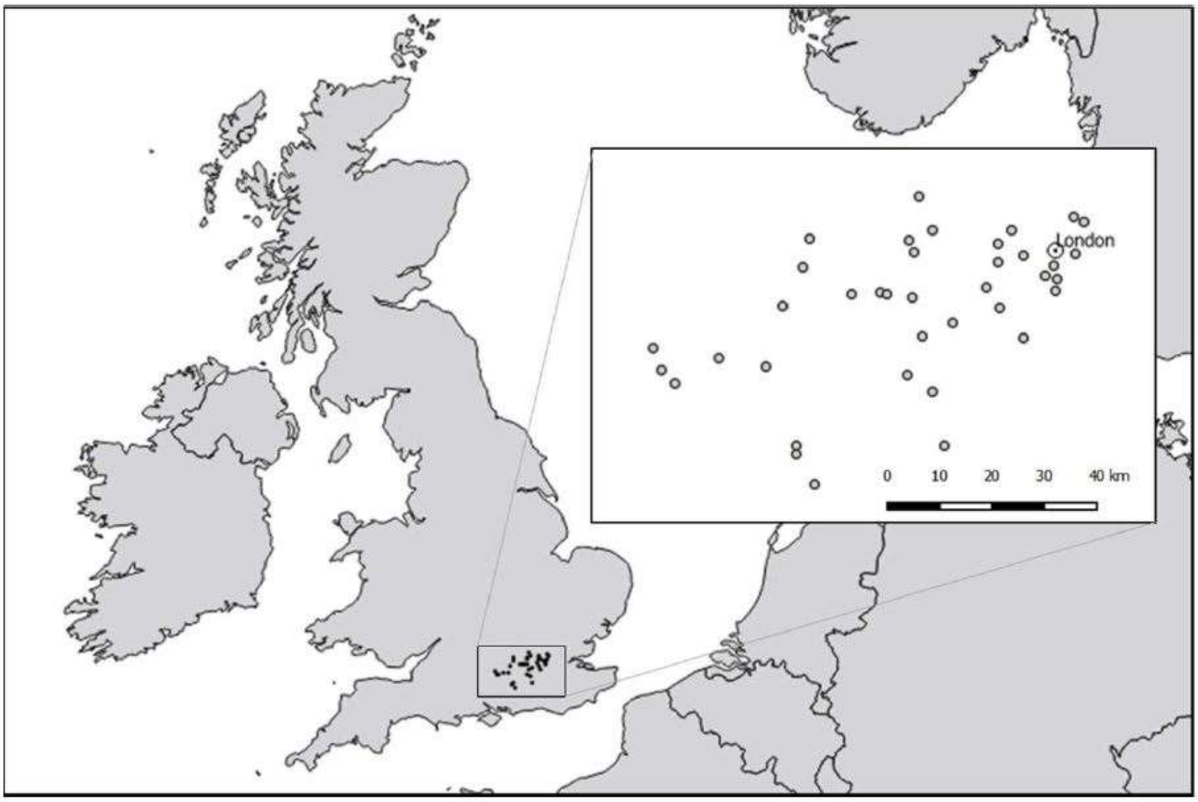

Figure 2. Location of 38 sites in SE England for which land classification was carried out using the protocol presented here.

Creating a land cover map

For the purposes of this paper, we use the term land cover to refer to surface cover and land-use to refer to data generated from land classification containing information about various aspects of the land. Our protocol involves manual generation of a land cover map based on visual inspection rather than using existing data layers, to increase flexibility in selecting resolution, allow later combination with ground survey data and increase relevance to pollinator-specific use of landscape through discrimination of relevant habitats (e.g. gardens or wildflower strips). Sites were located using Google Earth (version 7.1.5.1557) by navigating to the nearest postcode and visually adding a Placemark at the exact location of colony placement at an "eye altitude" of 500m. The site locations were imported as a .kml file into QGIS version 2.16 and saved as a .shp file for manipulation as a data layer in QGIS. The sites data layer was overlaid onto the web-based satellite imagery layer Bing Aerial from the OpenLayers plugin (http://www.openlayers.org). A 750m circular buffer (the largest spatial scale of four selected for the land classification (see below), based on B. terrestris typical foraging range; Osborne et al. 1999; Darvill et al. 2004; Knight et al. 2005) was generated around each site with a separate data layer for each site.

Land cover patches were classified within the buffers surrounding each site. At a scale of 1:5000m in agricultural areas, or 1:2500m in built-up areas, polygons were drawn around each land cover patch using the QGIS "Split Features" and "Fill Ring" tools, separating the buffer layer into a series of features representing individual patches of a single land cover type, at a resolution separating individual buildings (or joined sets of buildings), fields and gardens (Fig 3a.). The resolution at which patches are separated may be adapted to the focus of the study; for example it may be more appropriate to group areas of similar density of urban development rather than separating individual buildings for a honeybee study, due to the greater foraging range of honeybees. Each polygon was visually assigned to one of 34 initial land cover classes (e.g. house, residential garden, arable field, hedgerow; for full list see Appendix S1 in Supporting Information) by entering a two-letter code in the "Description" field of the attribute table. For visualisation purposes a layer style was generated with a colour assigned to each land cover class (Fig 3b).

Maps at multiple radii

The spatial scale at which pollinators respond to the surrounding landscape varies depending on aspects of behaviour and ecology such as foraging range and the response variable in question (Steffan-Dewenter et al. 2002; Westphal et al. 2006). Land cover maps at multiple biologically-relevant radii may therefore be generated for later comparison using model selection techniques (see below). In addition to the 750m buffer, buffers of 500m, 250m and 100m (representing steps of spatial scales at which bees may interact with the surrounding land (Moreira et al. 2015; Carvell et al. 2017) were added by clipping the initial buffer layer to generate new data layers at the specified radii. Each site thus had four associated land cover map layers (Fig 3c).

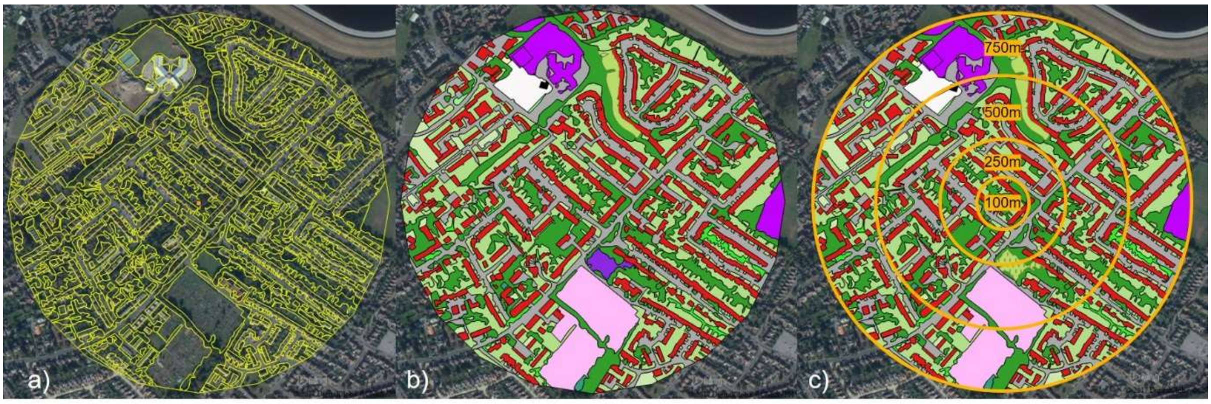

Figure 3. Illustration of the steps involved in manually generating a land cover map for a 750m radius around a study site in QGIS, using an example site in the suburban region to the South-West of London, UK. **a)** The first step involves drawing polygons around each land cover patch at a set scale (1:5000m in agricultural areas or 1:2500m in built-up areas) to split the data layer into a series of features representing each patch. **b)** Each patch is visually classified to one of 80 land cover classes; for colour legend see Appendix S1 (Supporting Information). **c)** The buffer is clipped to multiple radii representing different spatial scales at which the study taxon may interact with the surrounding land based on ecology of the organism.

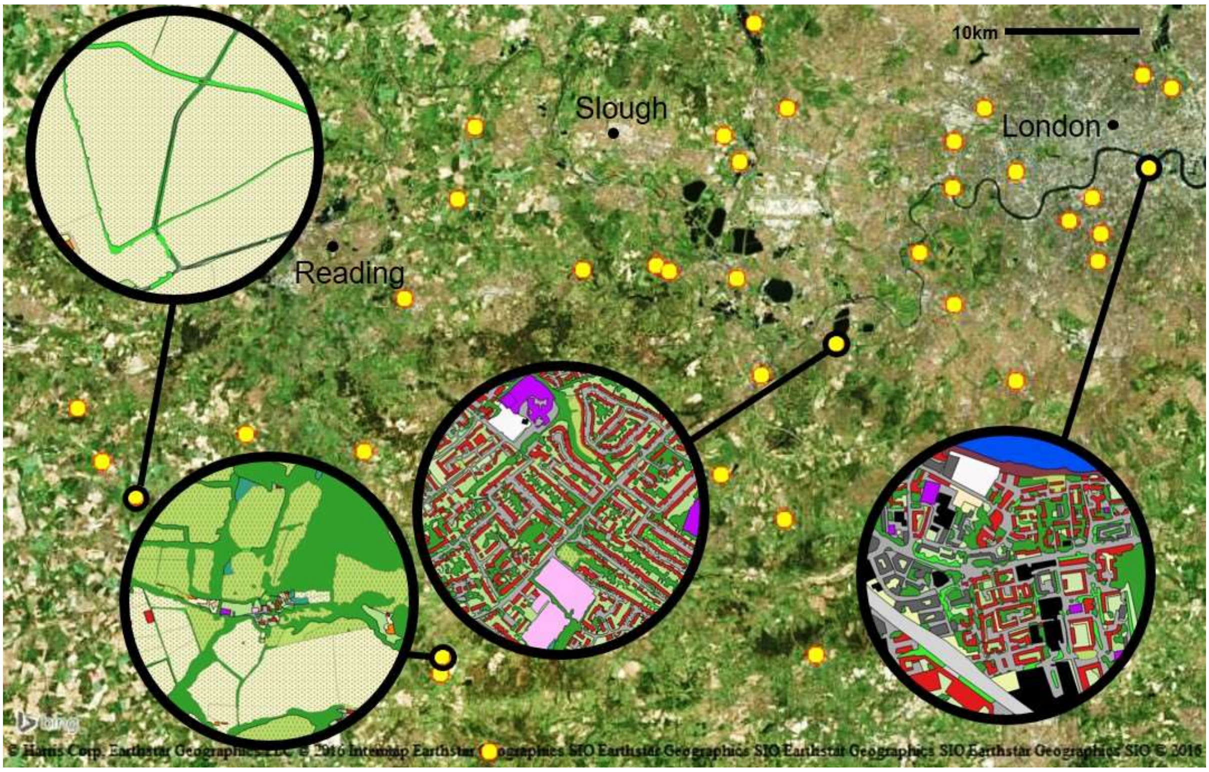

Figure 4. Land cover maps at a 750m radius (inset circles) were generated for 38 sites in South-East England; four representative sites across a gradient of urbanisation are shown (large inset circles). Yellow circles indicate locations of sites.

Ground surveys

Visually classifying land cover using satellite imagery is suitable for intransient land cover types such as urban or water body land classes, but not for transient land cover classes such as crops because readily available satellite imagery is typically not updated annually. In addition, crops may not be imaged during their flowering period, making them unidentifiable from satellite imagery. It is therefore recommended to supplement GIS classification with ground surveys to produce an up-to-date picture of the land-use at the time of the study. This is particularly important for bee research, as bees may forage on floral resources such as oilseed rape which are highly transient between seasons (Riedinger et al. 2015).

Ground surveys were carried out in May 2016, while bumblebee colonies were in the field, at all sites which contained agricultural land within a 750m radius (n=19). For each site, agricultural fields were visited by car or on foot and the crop grown, bloom stage and presence of wildflower strips and other floral resources recorded. This information was incorporated into the existing GIS, splitting polygons where necessary to add wildflower strips. This resulted in a total of 80 land cover classes.

Defining land-use variables

Eight land-use categories were defined which group land cover classes that share expected drivers of bee populations based on reference to the literature (Table 1): impervious surface (including buildings), flower-rich habitat, domestic infrastructure (including parks), gardens, tree cover, agricultural land, open land, and road (excluding vegetated verges). This categorisation aims to integrate multiple existing measures of urbanisation and known land-use factors that affect bees to generate a holistic classification that captures a wide array of ways in which land-use interacts with bees. Each of the 80 land cover classes was coded according to whether it belonged to each category (see Appendix S2 in Supporting Information); for example, flower-rich habitat contained gardens, flowering crops and urban parks and tree cover contained woodland, hedgerow and free-standing trees. The proportion of each of the eight categories at each radius was calculated by summing the total area of all land cover classes contained within a category and dividing by the total area of the circle.

Land-use category	Description and justification	References
Impervious surface	Paved land including buildings, roads and car parks. This is a common quantitative measure of urbanisation in the literature.	(Williams et al. 2012; Youngsteadt et al. 2015a)
Flower-rich habitat	Includes land-classes scored as flower-rich based on reference to the literature (e.g. gardens, parks, cemeteries, allotments) and ground surveys (e.g. wildflower strips, flowering crops, flower-rich grassland). This is a proxy for nutritional quality of the land as it does not take into account attractiveness or reward of different flowering plants.	(Baldock et al. 2019)
Domestic infrastructure	Includes urban land-classes such as parks from a human perspective, as these are subject to different levels of human disturbance than non-urban classes.	(Winfree et al. 2007b; Markovchick-Nicholls et al. 2008)
Gardens	Private gardens, which have been shown to be positively associated with bee abundance and diversity .	(Osborne et al. 2008; Baldock et al. 2015, 2019)
Tree cover	Freestanding trees, woodland and hedgerows. Many tree species provide a source of forage to bees, and tree cover also affects ground flora communities.	(Augusto et al. 2003; Macivor et al. 2014; Hausmann et al. 2015)
Agricultural land	Includes any land being used for agricultural purposes, which may affect bees by pesticide usage, disturbance and forage availability.	(Krupke et al. 2012; Couvillon et al. 2014b)
Open land	All land classes not containing obstructions above ground level such as trees and buildings that may impact bee flight, communication and nesting.	(Plowright and Galen 1985; Esch and Burns 1996; Taylor and Macivor 2015; Crall et al. 2015)
Road	Road surface, not including vegetated verges. Roads affect bees by being non-vegetated and through collision mortality.	(Baxter-Gilbert et al. 2015)

Table 1. Description and justification of eight land-use categories used in the land classification with associated references.

Principal Components Analysis

The resulting eight land-use variables are too numerous to use for statistical analysis and are likely to be highly collinear; for example, proportion open land is likely to be correlated with proportion agricultural land. Principal Components Analysis (PCA) is a statistical tool that reduces dimensionality in a set of correlated variables by identifying a primary set of independent axes (or "Principal Components") that explain the majority of the variation in the explanatory variables (Ringnér 2008). It is particularly well-suited to land-use data, and is often used as a step to refine multiple correlated land-use variables in land classification protocols (Hahs and Mcdonnell 2006; Owen et al. 2006).

A separate PCA was performed for each of the four radii using the *prcomp* function in R version 3.2.1 (R Core Team 2018). The principal components that together captured 85% of the variation were selected as the land-use variables for further analysis. The eigenvector scores (the weighting of a variable on a principal component; scores that depart from zero indicate increasing importance of that variable to the component (Hahs and Mcdonnell 2006) for each of the eight initial land-use categories were extracted. Variables with scores greater than 0.4 or less than -0.4 were considered to show a strong association with the principle component. The types of variables strongly associated with a principal component were used to interpret the axis likely to be represented by the component (see Table 2).

Cluster analysis

It is possible to use the principal components themselves as a final land-use variable for subsequent analysis of the effect of land-use on the response variables. This is appropriate if continuous variables are desired, and if the data suggest an evenly distributed, linear land-use gradient. However, if a clustered land-use structure is suspected, as in the present data (see Fig 5), an additional step of cluster analysis is recommended (Owen et al. 2006). This also has the advantage of combining all of the principal components into a single categorical land-use variable, which can simplify analyses involving several covariates.

We performed a separate cluster analysis on the principal components for each radius (*hclust* function in R package *cluster*; Maechler et al. 2015). Hierarchical agglomerative clustering is a technique that examines distances between observations in the n-dimensional space occupied by the principal components and sequentially pairs together the two closest observations (and later clusters) to form a new cluster (Zepeda-Mendoza and Resendis-Antonio 2013). The exact outcome of the clustering depends on the method used to determine the distance between an observation and an existing cluster (e.g. taking the mean of the distance of all observations within a cluster as opposed to the minimum or maximum); here we use Ward's method, which tends to produce clusters with more equal size (Ward

1963). Similar land classification methods typically select optimum numbers of clusters using an ad hoc minimum group size based on practicality and geographical relevance (Bunce et al. 1996; Hall and Arnberg 2002; Owen et al. 2006); following this approach we split clusters so that each group contained a minimum of five sites. This produced a single categorical land-use variable at each of the four radii (hereafter called R750, R500, R250 and R100).

Radius selection

As previously mentioned, the spatial scale at which an animal responds to the surrounding land-use depends on numerous factors and cannot necessarily be determined *a priori* (Steffan-Dewenter et al. 2002). A more data-driven approach to determining spatial scale consists of conducting an initial analysis using the primary response variable or all response variables and using model selection to determine to which spatial scale the response variable(s) respond most strongly, and hence which land-use radius to use for subsequent analysis.

We employed a model selection approach using Akaike's Information Criterion corrected for small sample sizes (AICc). We built a full model for each of the four radii containing all covariates (in this case, weather and time covariates) and the relevant land-use variable (R750, R500, R250 or R100) against the primary dependent variable (in this case, *peak colony size*). The land-use variable contained in the model with the lowest AICc value (Johnson and Omland 2004) was selected as the spatial scale to which the response variable responds most strongly and thus used for subsequent full analysis. If the best two or more models are within <2ΔAICc of each other, biological relevance (e.g. known foraging range) may be used to select the final radius from the best set. Alternative approaches for datasets with more than one dependent variable include performing this initial analysis for all dependent variables and selecting the spatial scale most commonly supported, or selecting the relevant spatial scale for each dependent variable.

The final categorical land-use variable at the appropriate spatial scale may now be included in a full analysis using standard statistical methods.

Results

Land cover map

The manual land cover classification step using satellite imagery in QGIS produced land cover maps for the area surrounding each of the 38 sites at a 750m radius (Fig 4). The most common land cover class was woodland, making up 21.2% of the total land area in the landscape surrounding the sites at a 750m radius, followed by roads at 14.4% and housing at 12.1%. Site maps consisted of 36 to 845 (mean: 368) land cover patches.

Principal Components Analysis

The results of PCAs carried out on the proportion of eight land-use categories at each of the four radii are shown in Table 2. Approximately 85% of the variance was captured by two principal components (PCs) at the 750m, 500m and 250m radius and three PCs at the 100m radius. By examining the eigenvalues of the land-use categories in each of the PCs, the PCs were interpreted for all radii as PC1: urban to rural axis; PC2: covered to open axis and (at the 100m radius only) PC3: flower-rich to flower-poor axis.

The sites varied considerably in scores for each of the PCs at each radius (Fig 5), and the grouping of scores when all PCs were considered indicated clustering of the sites. For example, at the 500m radius, a group with positive scores in PC1 and near-zero scores in PC2, a group with negative in PC1 and positive in PC2, and a group with negative scores in both PCs was indicated (Fig 5b). As PC1 was interpreted as “urbanness” and PC2 as “openness” this suggested a group that was built-up and moderately open, a less built-up and less open group and a less built-up but open group. This supported the employment of a formal cluster analysis.

Cluster Analysis

Hierarchical cluster analysis using Ward’s method on the principal components, with a minimum cluster size set to five, produced one categorical land-use variable for each site, with two clusters at R750, three at R500, two at R250 and four at R100 (Fig 6). These were given descriptive names based on dominant land cover features of the sites in each cluster, ranging from the landscape to the local scale as follows:

R750: Urban, Rural; R500: City, Village, Agricultural; R250: Built-up, Open; R100: Dense housing, Sparse housing, Wooded, Fields.

Radius selection

Model selection of full models for each radius containing all covariates and the relevant categorical land-use variable (R750, R500, R250 or R100) against the primary dependent variable *peak colony size* showed the model containing R500 to have the lowest AICc (ΔAICc to next best model: 2.7; Table 3). This suggests that *peak colony size* responds most strongly to land-use at a 500m radius surrounding the sites, and thus that land-use at the 500m radius should be used in subsequent analysis.

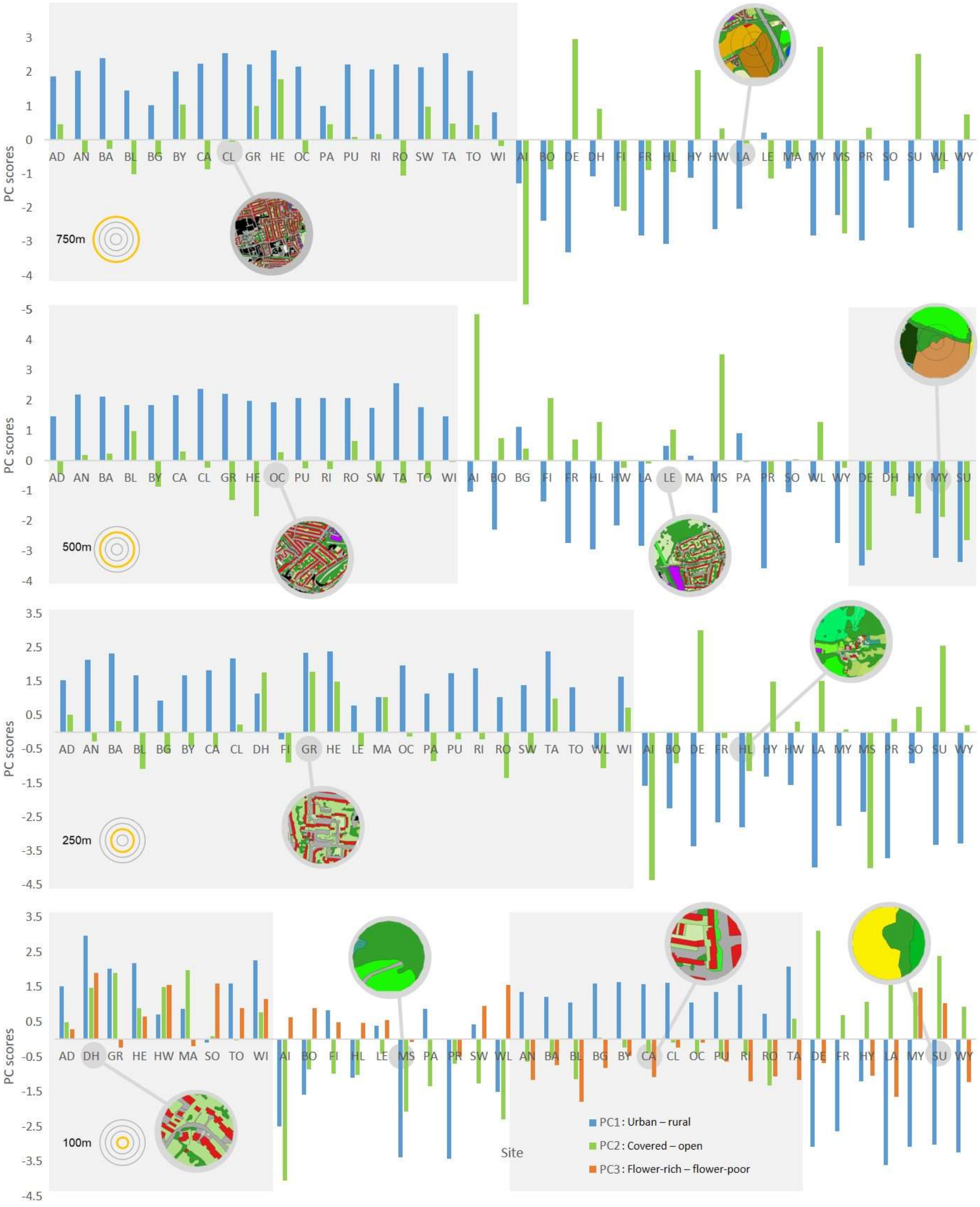

Figure 5. Eigenvector scores on principal components that captured approximately 85% of the variation in a principle component analysis (PCA) performed on land-use variables classified at each of four radii around each study site (two-letter codes). The clustering of the land-use types generated from subsequent cluster analysis (Fig 6) is illustrated in the grouping of PC scores, shown here separated by shaded boxes (determined by the later cluster analysis). For example, at the 500m radius (b), the "City" cluster (far left) is typified by a positive score on PC1 and neutral score on PC2, "Village" by positive to neutral PC1 and negative PC2, and "Agricultural" (far right) by negative PC1 and PC2. Inset circles show land cover maps at the relevant radius for representative sites for each group.

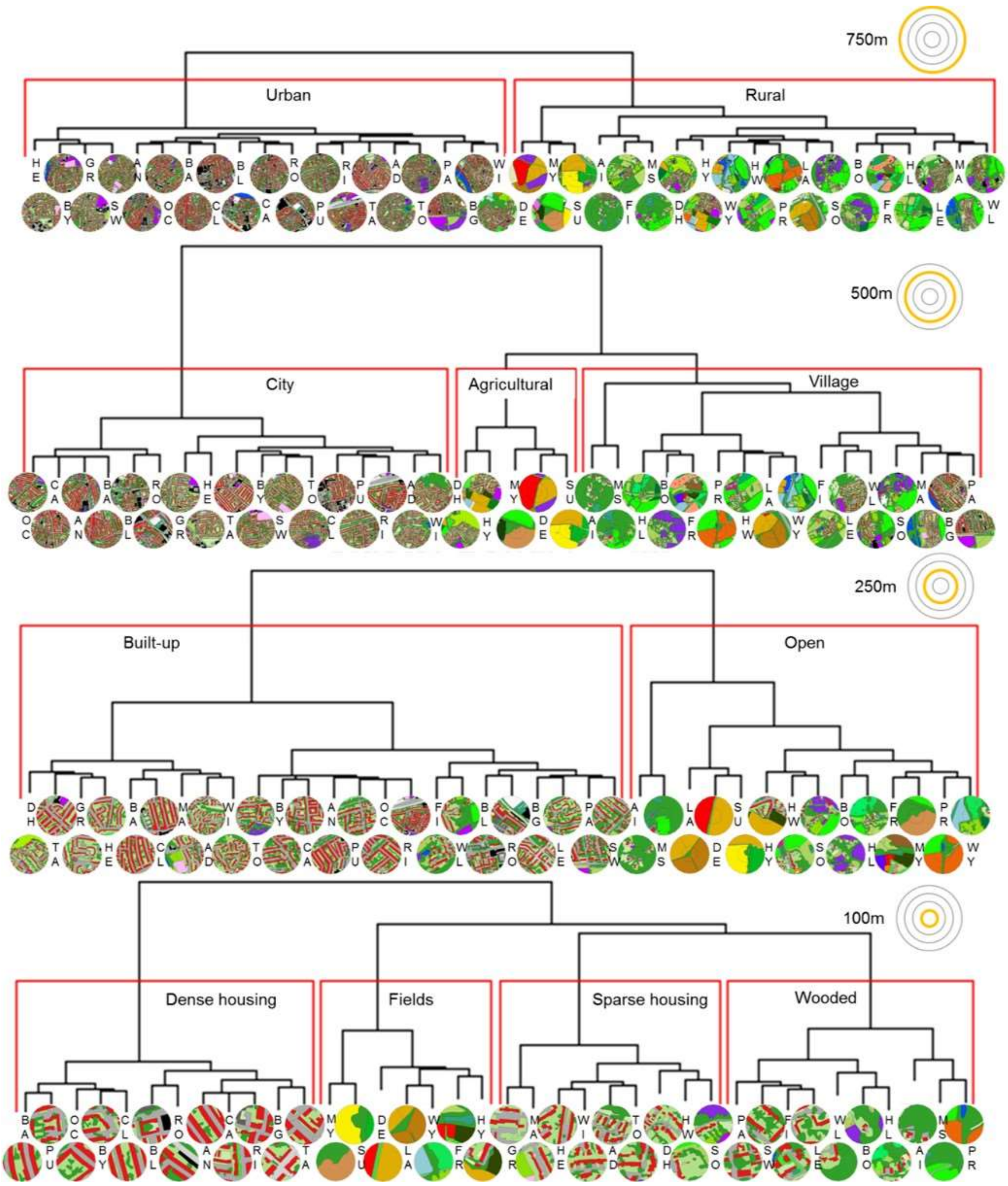

Figure 6. Cluster dendrograms of land-use of 38 sites at a 750m, 500m, 250m and 100m radii. Cluster analyses using Ward's method were performed on a set of principle components describing land-use to group sites into categorical land-use types (red boxes) which were given descriptive names from the landscape to the local scale. At the terminus of each branch the two-letter site name is given with an image of the GIS land cover map (see Appendix S1 for colour legend).

Radius		**PC1**	**PC2**	**PC3**
750m	Standard deviation	2.154	1.467	-
	Proportion of Variance	0.580	0.269	-
	Cumulative Proportion	0.580	0.849	-
	Eigenvector scores			-
	Proportion impervious surface	**0.440**	0.000	-
	Proportion flower-rich habitat	0.147	**0.512**	-
	Proportion domestic infrastructure	**0.458**	0.037	-
	Proportion open land	-0.247	**0.560**	-
	Proportion tree cover	-0.156	**-0.578**	-
	Proportion agricultural land	**-0.415**	0.258	-
	Proportion gardens	0.349	0.142	-
	Proportion road	**0.441**	0.032	-
500m	Standard deviation	2.133	1.463	**-**
	Proportion of Variance	0.569	0.268	**-**
	Cumulative Proportion	0.569	0.836	**-**
	Eigenvector scores			-
	Proportion impervious surface	**0.442**	-0.054	-
	Proportion flower-rich habitat	0.066	**-0.515**	-
	Proportion domestic infrastructure	**0.461**	-0.085	-
	Proportion open land	-0.289	**-0.515**	-
	Proportion tree cover	-0.085	**0.610**	-
	Proportion agricultural land	**-0.433**	-0.222	-
	Proportion gardens	0.338	-0.176	-
	Proportion road	**0.443**	-0.082	-
250m	Standard deviation	2.141	1.440	-
	Proportion of Variance	0.573	0.259	-
	Cumulative Proportion	0.573	0.832	-
	Eigenvector scores			-
	Proportion impervious surface	**0.440**	-0.011	-
	Proportion flower-rich habitat	0.157	**0.426**	-
	Proportion domestic infrastructure	**0.462**	0.033	-
	Proportion open land	-0.226	**0.583**	-
	Proportion tree cover	-0.139	**-0.599**	-
	Proportion agricultural land	**-0.418**	0.284	-
	Proportion gardens	0.373	0.188	-
	Proportion road	**0.429**	0.047	-
100m	Standard deviation	2.019	1.407	1.007
	Proportion of Variance	0.509	0.248	0.127
	Cumulative Proportion	0.509	0.757	0.884
	Eigenvector scores			
	Proportion impervious surface	**0.424**	-0.115	**-0.428**
	Proportion flower-rich habitat	0.174	**0.411**	**0.614**
	Proportion domestic infrastructure	**0.484**	-0.041	-0.151
	Proportion open land	-0.016	**0.673**	-0.165

	Proportion tree cover	-0.314	**-0.454**	0.416
	Proportion agricultural land	**-0.411**	0.367	-0.118
	Proportion gardens	**0.406**	0.121	**0.420**
	Proportion road	0.352	-0.093	0.163
Interpretation		Urban to rural	Open to covered	Flower-rich to flower-poor

Table 2. Results of Principal Components Analyses on proportion land-use categories at each of four radii. The principle components (PCs) that together capture approximately 85% of the variation were selected for subsequent analysis. Eigenvector scores for each of the land-use variables at each PC are shown and scores greater than 0.4 or less than -0.4 highlighted in bold and interpreted as having a strong relationship to that PC. The axes of each PC were interpreted based on these associated variables.

Model	AICc	ΔAICc	w_i
500m land-use + all covariates	93.3	0.00	0.65
750m land-use + all covariates	96.0	2.70	0.17
250m land-use + all covariates	96.8	3.52	0.11
100m land-use + all covariates	97.6	4.34	0.07

Table 3. Model selection table to compare the response of the primary dependent variable, *peak colony size* to land-use at four different radii (750m, 500m, 250m, and 100m) surrounding sites. “All covariates” refers to temperature, humidity and rainfall. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i).

Discussion

We describe a protocol for comprehensive classification of land-use surrounding study sites suitable for pollinator ecology research. Our method employs a multi-stage approach that allows flexibility in adapting the specific steps to the data or research question involved. We start by manually mapping land cover using visual identification of land cover patches from satellite imagery at a resolution appropriate to the taxon studied. This is supplemented by ground surveys for land cover patches where habitats or resources are transient. Land-use classes are then defined with specific reference to how the taxon interacts with the landscape based on knowledge of pollinator ecology, and the dimensionality of these variables is reduced using Principal Components Analysis (PCA). If the data suggest clustering of land-use types, cluster analysis is performed to incorporate the principal components into a categorical variable. Finally, model selection is carried out to determine the appropriate spatial scale for further analysis. The final land-use variable is a simple categorical variable at a single spatial scale, which contains information from multiple steps of refinement to generate a robust land classification from the pollinator's perspective.

In the case study presented here, the sites were selected to represent a gradient of urbanisation. Interestingly, the land classification elucidated that, rather than forming a linear gradient, land-use types clustered into relatively discrete categories that were not apparent from initial qualitative inspection. For example, at the 500m radius sites clustered into three distinct groups: agricultural, village and city. Land surrounding agricultural sites was dominated by fields, while village sites were characterised by housing in the immediate vicinity of the colony within a rural landscape, typically with extensive tree cover, and city sites consisted of dense inner-city urban land. Without this approach, village sites may have not been distinguished from agricultural sites as both groups are predominantly surrounded by rural land. Our analysis identified the importance of the covered to open axis (PC2) in addition to the urban to rural axis, and showed that agricultural and village sites differed sufficiently in how open they were to group separately following cluster analysis. Incidentally, analysis of the primary dependent variable in the dataset used in this case study, peak colony size, showed that colonies in the two land-use types containing built-up areas- village and city- grouped together, performing differently to agricultural colonies (Samuelson et al. 2018), supporting the land-use clustering generated by our land classification protocol. To compare our classification with a commonly used variable in single-step classification, we calculated percentage impervious surface for our sites: city sites contained mean 56.2% (± SE: 4.0%) impervious surface cover while village and agricultural sites contained 13.8 (±3.7)% and 8.6 (±4.5)% impervious surface respectively. This suggests our classification broadly agrees with the gradient described by this variable.

The primary value of our approach is in its flexibility to adapt to the focal study system and research question, due to the nature of building a land classification from the bottom up rather than co-opting existing classifications (Owen et al. 2006). The ability to differentiate land cover patches at relatively fine resolution (up to 1:2,300 scale using the Bing Aerial QGIS layer) is more appropriate to pollinator spatial scales than many existing land cover maps (e.g. CORINE in Europe; scale: 1:100,000; minimum width of linear elements: 100m; Büttner et al. 2004). Finer resolution data layers are available only for certain geographical areas (Troy and Wilson, 2006) and are often expensive, so our protocol may represent a good option for lower budget studies as the software used is open source. Existing land classifications that do contain data at a resolution relevant to pollinators such as the CEH Countryside Survey Land Cover Maps (e.g. LCM2015; Rowland et al. 2017) for the UK often combine land cover types that are extremely different from a pollinator perspective (e.g. "built up areas and gardens" in LCM2015 covers as varied land types as industrial estates, urban parkland and domestic gardens; Rowland et al. 2017), which can be separated using our approach. An additional advantage comprises the accommodation of transient habitats and resources through combining satellite imagery with ground survey, with initial land classification allowing targeted ground surveys of only the necessary land-use patches. This may also be used to track seasonal differences in foraging resources, by supplementing a base map with regular ground surveys. There may also be value in further manipulation of the data generated from the land classification presented here. Many pollinator ecology studies are interested in landscape metrics such as landscape diversity (Boscolo et al. 2017) which can be calculated from these data (Yeh and Huang 2009), or individual land-use elements such as the proportion area of a specific crop can be extracted for follow-up questions. The method also allows qualitative uses of the land cover map, such as identifying foraging hotspots in honeybee waggle dance studies (Couvillon et al. 2014a). Finally, refining the classification using statistical tools rather than directly using individual land cover variables in subsequent analysis allows the incorporation of an extensive set of land-use information within a single variable.

The most obvious limitation of our protocol is that it is time-consuming. The most labour-intensive step is generation of the land cover map. The time required depends on the complexity of the landscape and the resolution and upper radius selected; for example it took an experienced researcher c. 4 hours to generate a map and classify the land cover patches for each site in our case study, which was at relatively high resolution in complex urban landscapes. Radius and resolution should therefore be selected at the minimum required for ecological relevance. Although the advantages of this step have been outlined above, where necessary, existing data layers may be used if they are available and relevant to pollinator ecology, and the later land-use category definition, PCA and cluster analysis steps applied to these data. These latter step remain important because land-use categories from existing data layers present the same problems with collinearity as study-specific maps generated using the protocol described here. Ground truthing can also be time consuming, depending on landscape complexity and patch accessibility (here 2-3 hours per site). Ground truthing time can be minimised by targeting only relevant patches (e.g. arable fields), or using UAV (drone) surveys if the crop of interest is identifiable from a distance (e.g. oilseed rape).

Another limitation relates to errors introduced in classifying land cover patches from satellite imagery, particularly in cases where similar land cover types are hard to distinguish. This can be mitigated by verification with ground surveys and/or re-analysis of a subset of the data by an additional researcher to quantify error. Finally, manually drawing polygons to separate land cover patches can be subjective in terms of whether to separate or combine a patch. This highlights the importance of selecting a scale at which to view the satellite imagery at the start of the work, and it is important to note that "number of patches" is not an accurate measure of landscape heterogeneity for this reason.

The technique described here has potential applications in both pollinator ecology and other fields. While our case study is based upon *B. terrestris,* land-use has been shown to impact numerous other pollinator taxa (Baldock et al. 2015; Senapathi et al. 2015), to which our approach may be applied. In pollinator ecology, our protocol may be combined with existing models to assess effects of resource availability with reference to land-use (Lonsdorf et al. 2009; Williams et al. 2012; Kennedy et al. 2013), or with methods designed to evaluate landscape quality for pollinators (Couvillon and Ratnieks 2015). Our method can also be applied to the studies of the interactions between land-use and agricultural pest control, for which (as in pollinator ecology) land classification at a finer resolution than available data layers or with separation of specific land types (e.g. fallows, field margins) is often required (Bianchi et al. 2006), and varied responses of pest species and natural enemies to land-use necessitate flexibility in the spatial scales of analysis (Thies et al. 2005). Calculations of secondary landscape metrics that are known to affect natural pest control (Thies and Tscharntke 1999), including landscape heterogeneity, may also be relevant to this field. Our protocol may be extended to other systems for which human-focussed land classifications are not a suitable fit, as the spatial scale, land-use categories and resolution at which the land is classified may be adapted to the study system and research aims in question. Sometimes, human-focussed land classifications may be desirable, where the goal is to inform policy or convince governing bodies of conservation recommendations; in this case relevant factors can be incorporated into the classification at the "defining land-use variables" stage.

Conclusions

The aim of this paper was to develop a land classification protocol for use in pollinator ecology research, from a pollinator rather than human perspective. Our protocol builds on the existing array of land classification techniques used in studies of land-use effects on pollinators, expanding on methods employed in the pollinator literature (Banaszak-Cibicka and Zmihorski 2012; Verboven et al. 2014; Sponsler and Johnson 2015) and adapting techniques developed in geographical research (Hahs and Mcdonnell 2006). We have shown that bottom-up land classification is feasible for studies such as that described in our case study, and that useful land-use data may be generated from doing so. Future research should expand on and refine this approach, and we suggest that land-use studies in pollinator ecology should broadly adopt GIS-based multi-step land classification techniques.

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Author contributions

A.E.S and E.L. conceived the study. A.E.S. developed the protocol, led the statistical analysis and collected the empirical data used in the case study, with input and support from E.L. A.E.S wrote the manuscript draft and both authors produced the final edit.

Data accessibility

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.60s6123.

A land classification protocol for pollinator ecology research: an urbanisation case study

Supplementary Material

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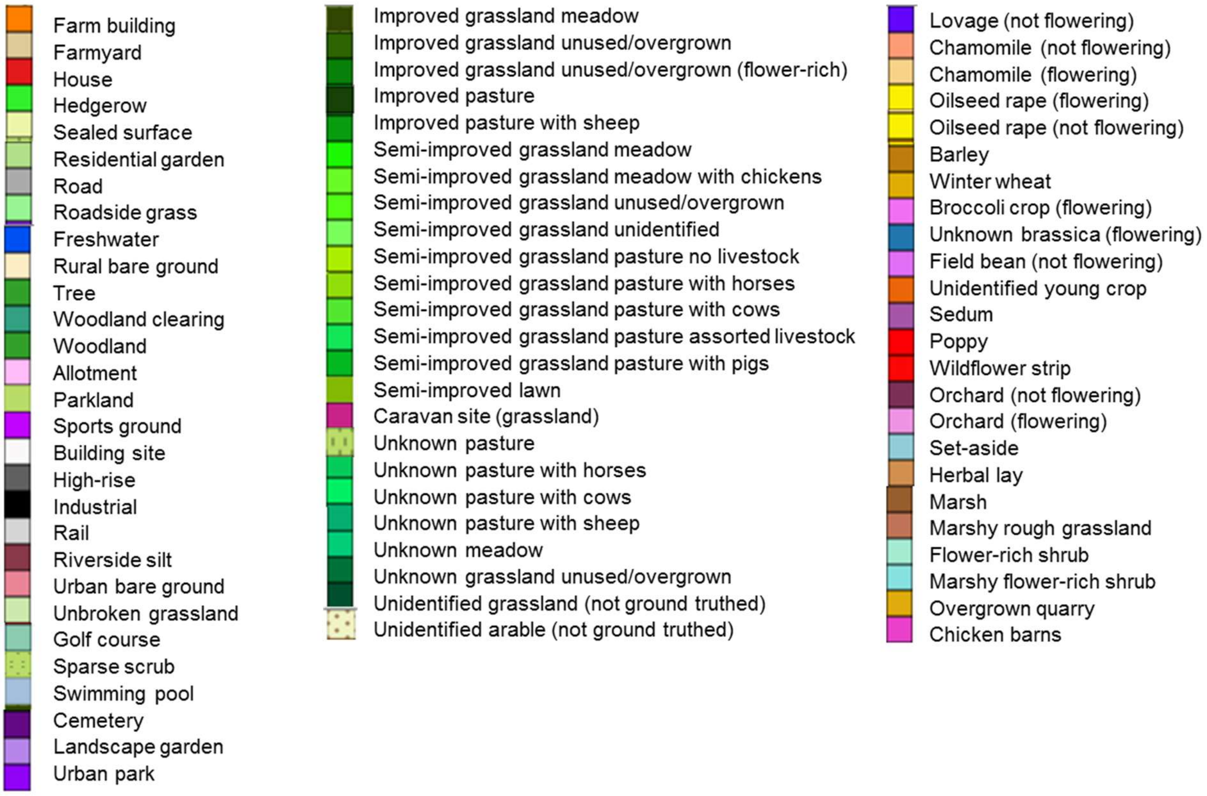
Appendix S1

List of land-use classes with assigned colours in GIS figures.



- Farm building
- Farmyard
- House
- Hedgerow
- Sealed surface
- Residential garden
- Road
- Roadside grass
- Freshwater
- Rural bare ground
- Tree
- Woodland clearing
- Woodland
- Allotment
- Parkland
- Sports ground
- Building site
- High-rise
- Industrial
- Rail
- Riverside silt
- Urban bare ground
- Unbroken grassland
- Golf course
- Sparse scrub
- Swimming pool
- Cemetery
- Landscape garden
- Urban park
- Improved grassland meadow
- Improved grassland unused/overgrown
- Improved grassland unused/overgrown (flower-rich)
- Improved pasture
- Improved pasture with sheep
- Semi-improved grassland meadow
- Semi-improved grassland meadow with chickens
- Semi-improved grassland unused/overgrown
- Semi-improved grassland unidentified
- Semi-improved grassland pasture no livestock
- Semi-improved grassland pasture with horses
- Semi-improved grassland pasture with cows
- Semi-improved grassland pasture assorted livestock
- Semi-improved grassland pasture with pigs
- Semi-improved lawn
- Caravan site (grassland)
- Unknown pasture
- Unknown pasture with horses
- Unknown pasture with cows
- Unknown pasture with sheep
- Unknown meadow
- Unknown grassland unused/overgrown
- Unidentified grassland (not ground truthed)
- Unidentified arable (not ground truthed)
- Lovage (not flowering)
- Chamomile (not flowering)
- Chamomile (flowering)
- Oilseed rape (flowering)
- Oilseed rape (not flowering)
- Barley
- Winter wheat
- Broccoli crop (flowering)
- Unknown brassica (flowering)
- Field bean (not flowering)
- Unidentified young crop
- Sedum
- Poppy
- Wildflower strip
- Orchard (not flowering)
- Orchard (flowering)
- Set-aside
- Herbal lay
- Marsh
- Marshy rough grassland
- Flower-rich shrub
- Marshy flower-rich shrub
- Overgrown quarry
- Chicken barns

Appendix S2

Land cover classes included in each of eight land-use categories

Code	Description	Impervious Surface	Flower-rich	Domestic Infrastructure	Garden	Tree Cover	Open	Agricultual	Road
FA	Farm building	1	0	0	0	0	0	1	0
FY	Farmyard	0	0	0	0	0	1	1	0
H	House	1	0	1	0	0	0	0	0
HR	Hedgerow	0	1	0	0	1	0	0	0
IS	Sealed surface	1	0	1	0	0	1	0	0
RG	Residential garden	0	1	1	1	0	1	0	0
RO	Road	1	0	1	0	0	1	0	1
ROG	Roadside grass	0	0	1	0	0	1	0	0
FW	Freshwater	0	0	0	0	0	1	0	0
RBG	Rural bare ground	0	0	0	0	0	1	0	0
T	Tree	0	0	0	0	1	0	0	0
WC	Woodland clearing	0	0	0	0	0	1	0	0
WL	Woodland	0	0	0	0	1	0	0	0
AL	Allotment	0	1	1	0	0	1	0	0
PL	Parkland	0	0	0	0	0	1	0	0

SG	Sports ground	0	0	1	0	0	1	0	0
BS	Building site	1	0	1	0	0	0	0	0
HI	High rise	1	0	1	0	0	0	0	0
IN	Industrial	1	0	1	0	0	0	0	0
R	Rail	1	0	1	0	0	1	0	0
TRS	Riverside silt	0	0	1	0	0	1	0	0
UBG	Urban bare ground	0	0	1	0	0	1	0	0
UG	Unbroken grassland	0	0	1	0	0	1	0	0
GC	Golf course	0	0	0	0	0	1	0	0
SS	Sparse scrub	0	0	0	0	0	1	0	0
SW	Swimming pool	0	0	1	0	0	1	0	0
LG	Landscape garden	0	1	0	1	0	1	0	0
GH	Cemetery	0	0	1	0	0	1	0	0
RHG	Manicured turf	0	0	1	1	0	1	0	0
UP	Urban Park	0	1	1	0	0	1	0	0
IGM	Improved grassland meadow	0	0	0	0	0	1	1	0
IGU	Improved grassland unused/overgrown	0	1	0	0	0	1	1	0
IGUF	Improved grassland unused/overgrown (flower-rich)	0	1	0	0	0	1	1	0
IP	Improved pasture	0	0	0	0	0	1	1	0
IPS	Improved pasture with sheep	0	0	0	0	0	1	1	0
SIGM	Semi-improved grassland meadow	0	0	0	0	0	1	1	0
SIGMC	Semi-Improved grassland meadow with chickens	0	0	0	0	0	1	1	0
SIGMH	Semi-Improved grassland meadow with horses	0	0	0	0	0	1	1	0
SIGU	Semi-Improved grassland unused/overgrown	0	1	0	0	0	1	1	0
SIG	semi-improved grassland (unidentified)	0	0	0	0	0	1	1	0
SIP	Semi-improved pasture no livestock	0	0	0	0	0	1	1	0
SIPH	Semi-improved pasture with horses	0	0	0	0	0	1	1	0
SIPC	Semi-improved pasture cows	0	0	0	0	0	1	1	0
SIPBS	Semi-improved pasture assorted livestock	0	0	0	0	0	1	1	0
SIPP	Semi-improved pasture pigs	0	0	0	0	0	1	1	0
SIL	Semi-improved lawn	0	0	0	0	0	1	1	0

CG	Caravan site (grassland)	0	0	0	0	0	1	1	0
UPA	Unknown pasture	0	0	0	0	0	1	1	0
UPH	Unkown pasture horses	0	0	0	0	0	1	1	0
UPC	Unkown pasture cows	0	0	0	0	0	1	1	0
UPS	Unkown pasture sheep	0	0	0	0	0	1	1	0
UM	unknown meadow	0	0	0	0	0	1	1	0
UGU	Unkown grassland unused/overgrown	0	0	0	0	0	1	1	0
PF	Unidentified grassland (not ground truthed)	0	0	0	0	0	1	1	0
AF	Unidentified arable (not ground truthed)	0	0	0	0	0	1	1	0
LVNF	Lovage (not flowering)	0	0	0	0	0	1	1	0
CHNF	Chamomile (not flowering)	0	0	0	0	0	1	1	0
CHF	Chamomile (flowering)	0	1	0	0	0	1	1	0
OSR	Oilseed rape (flowering)	0	1	0	0	0	1	1	0
BA	Barley	0	0	0	0	0	1	1	0
WW	Winter wheat	0	0	0	0	0	1	1	0
BRF	Broccoli (flowering)	0	1	0	0	0	1	1	0
UBF	Unknown brassica (flowering)	0	1	0	0	0	1	1	0
BNF	Beans (not flowering)	0	0	0	0	0	1	1	0
YUC	Unidentified young crop	0	0	0	0	0	1	1	0
SE	Sedum	0	1	0	0	0	1	1	0
PO	Poppy	0	1	0	0	0	1	1	0
WS	wildflower strip	0	1	0	0	0	1	1	0
ORNF	Orchard not flowering	0	0	0	0	0	1	1	0
ORF	Orchard flowering	0	1	0	0	0	1	1	0
SA	Set aside	0	0	0	0	0	1	1	0
HL	Herbal lay	0	1	0	0	0	1	1	0
MA	Marsh	0	0	0	0	0	1	0	0
RMA	Marshy rough grassland	0	0	0	0	0	1	0	0
FRS	Flower-rich shrub	0	1	0	0	0	1	0	0
MFRS	Marshy flower-rich shrub	0	1	0	0	0	1	0	0
OQ	Overgrown quarry	0	1	0	0	0	1	0	0
CB	Chicken barns	0	0	0	0	0	0	1	0

Chapter Four

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Lower bumblebee colony fitness in agricultural compared to urban environments

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Abstract

Urbanisation represents a rapidly growing driver of land-use change. While it is clear that urbanisation impacts species abundance and diversity, direct effects of urban land-use on animal reproductive success are rarely documented. Here we show that urban land-use is linked to long-term colony reproductive output in a key pollinator. We reared colonies from wild-caught bumblebee *(Bombus terrestris)* queens, placed them at sites characterised by varying degrees of urbanisation from inner city to rural farmland, and monitored the production of sexual offspring across the entire colony cycle. Our land-use cluster analysis identified three site categories, and this categorization was a strong predictor of colony performance. Crucially, colonies in the two clusters characterized by urban development produced more sexual offspring than those in the cluster dominated by agricultural land. These colonies also reached higher peak size, had more food stores, encountered fewer parasite invasions and survived for longer. Our results show a link between urbanisation and bumblebee colony fitness, supporting the theory that urban areas provide a refuge for pollinator populations in an otherwise barren agricultural landscape.

Keywords

Urbanisation, *Bombus terrestris,* fitness, land-use, pollinator ecology, bee

Background

We are living in the "Urban Age" (Elmqvist et al. 2013): over half the world's human population currently resides in cities (United Nations 2018) and an estimated three-fold increase in global urban land cover is predicted between 2000 and 2030 (Seto et al. 2012). Although urbanization has been shown to impact negatively upon species abundance and diversity for many taxa (Aronson et al. 2014), some groups successfully exploit anthropogenic habitats (Kark et al. 2007) and there is evidence to place wild bees among this number. For example, areas subject to urban expansion have lost fewer pollinator species than agricultural areas over the past 80 years (Senapathi et al. 2015) and species richness has been found to be higher in urban than agricultural areas (Baldock et al. 2015). These community level studies give reason to view urban environments as a potential refuge within barren agricultural landscapes, which have been associated with reduced floral resources (Carvell et al. 2006) and exposure to environmental contaminants (Desneux et al. 2007). Yet, the crucial question of whether land-use directly affects fitness – the ultimate driver of ecological success and evolutionary change – remains a largely neglected missing link in the correlations between urbanisation and species abundance in both bees and other taxa (Coleman and Barclay 2011; Crone and Williams 2016; Carvell et al. 2017).

Bumblebees comprise an important part of the pollinator community, but are currently subject to a multitude of threats that include changes in forage availability associated with land-use change (Carvell et al. 2006) and pressure from emerging parasites and disease (Fürst et al. 2014). Alteration of floral resources is likely to be an important driver of urban effects on bees (Harrison and Winfree 2015b), with cities and towns often offering high floral abundance and diversity in the form of gardens and parks (Loram et al. 2008). However, many horticultural plant varieties are unattractive to bees or invest energy in visual displays at the expense of reward provision (Garbuzov and Ratnieks 2014a) and competition may also affect forage availability: increased popularity of urban beekeeping has increased honeybee hive densities in urban areas (Alton and Ratnieks 2013), possibly increasing competitive interactions with wild bees (Goulson and Sparrow 2009). Parasite prevalence has also been linked to urbanisation, with higher parasite loads in urban areas reported in bumblebees (Goulson et al. 2012; Theodorou et al. 2016). Pesticide use has been identified as a threat to bees (Desneux et al. 2007) and exposure may vary across degrees of urbanisation (Botías et al. 2017). In the context of this array of potentially interacting drivers of urban effects, it is not clear how inhabiting urban areas affects bumblebee success at the colony level. This is because it has not been possible to study bumblebee colonies placed at outdoor sites beyond the very beginning of the period when reproductive offspring begin to emerge, because ethical concerns preclude the release of reproductive offspring from commercially-obtained bumblebee colonies (Ings et al. 2005, 2006; Colla et al. 2006). Thus, while there is evidence that bumblebee colony early weight gain may be enhanced in suburban compared to agricultural areas based on studies of pre-reproductive colonies (Goulson et al. 2002), to date no study has monitored the critical, extensive reproductive period of the colony life-cycle and thus assessed the effects of urbanisation on fitness itself.

Here, we addressed this gap by rearing colonies from wild-caught queens to investigate the effect of urbanisation on life-history and reproductive output in the bumblebee *Bombus terrestris audax.* This approach overcomes concerns associated with the use of commercial bees, including negative environmental impacts such as hybridisation (Ings et al. 2005), pathogen spillover (Colla et al. 2006) and competition (Ings et al. 2006). It also avoids the use of bees that have been subject to artificial selection (Velthuis and van Doorn 2006) and may differ from locally-adapted natural populations (Ings et al. 2006). The crucial outcome is that colonies can be monitored for their entire reproductive lifetime. We selected 38 sites across central London, its suburbs and the surrounding agricultural land (Fig. 1a), and categorized each site based on land-use through cluster analysis of principle components derived from 80 land-use variables. Through frequent censusing and sampling of colonies placed at these sites, we tracked for the first time the growth, reproductive output, nutritional status, and parasite prevalence of each colony from eclosion of the first cohort of workers until the end of the colony life cycle. To our knowledge, this represents the first experimental study in any taxon to demonstrate a direct relationship between urbanisation and fitness, with previous research typically employing an observational approach (e.g; Newhouse et al. 2008; Coleman and Barclay 2011).

Methods

Bumblebee colonies

We collected 176 foraging *Bombus terrestris audax* queens in Windsor Great Park, Surrey, UK during March and April 2016. Queens were chilled and transported to the lab where they were immediately screened microscopically for the endoparasites *Nosema* spp., *Apicystis bombi, Sphaerularia bombi* and *Crithidia bombi,* by collecting faeces in a microcapillary (Baubrand Intramark, Wertheim, Germany) and examining the sample under x400 magnification. Parasitised queens (n=6) were excluded from the experiment. Queens were kept in clear acrylic rearing boxes until colony founding (see Supplementary Methods for rearing protocol), after which they were re-screened and transferred to a wooden nest box (W 280 x L 320 x D 160mm) with a clear Perspex lid. Our final sample for placement in the field consisted of 43 colonies.

Field placement

We recruited 114 gardens and farms in South-East England (between central London and Basingstoke), of which 38 sites were selected across a region spanning inner city to countryside on the basis of distribution (> 1.5km apart), land-use type representativeness and accessibility (Fig.1a). This includes a range of urban and rural land-use types typical of Western Europe (Levers et al. 2018), from Central Business District, to suburban, to villages and medium-intensity agriculture containing a mixture of grassland and arable fields. Predominant crop types in the agricultural areas were cereals and brassica crops. The wide range of urban land types contained within London means that it is representative of a range of different urban types displayed by smaller cities (Schwarz 2010). We placed colonies in the field in protective plastic field boxes during the first week of May 2016, randomised to land-use type according to initial colony size (see Supplementary Methods). Colony placement was staggered over six days, with six or seven sites visited each day during daylight hours (8:00-20:00). Colony monitoring continued until moribundity (see below), which occurred for the last colony on 11th July.

Data collection

Site visits followed approximately the same order as the colony placement, with each site visited weekly during the hours of darkness (21:30-4:30) at the same time each week. We recorded the following data (see Supplementary Methods for additional data): number of bees (average of three counts); queen status (alive, dead or absent); presence of nectar and pollen stores and presence and status of *Bombus vestalis* brood parasites (alive, injured or dead), which we removed to minimise *B. terrestris* queen death. To assess reproductive success, gynes were removed until one minute had passed with no gyne seen, and stored for later analysis. The same procedure was repeated for males, with sampling time capped at 15 minutes. Males are considered to leave the nest at two to four days old and gynes at two to eight days old (Free and Butler 1959) so our weekly removal of males and gynes reflects natural conditions and is unlikely to have impacted the colony's production of future males and gynes. Weekly removal of reproductives allows calculation of total reproductive output over the colony life cycle rather than a snapshot as obtained from traditional colony dissection methods that are carried out at the earliest sign of reproductive emergence (Goulson et al. 2002). We removed one, three or five workers for later parasite analysis depending on colony size (<35, 35-50, >50 workers respectively), which were stored alive in vials for a maximum of 5 hours before freezing at -20°C.

For the first two weeks, colonies in which the queen died (n=5 of 43; 1 city, 1 village, 3 agricultural) were replaced with new colonies. Following this, colonies were removed from the field when moribund, defined as <10 workers remaining and queen death or <3 workers remaining with no queen death. Remaining workers were frozen at – 20°C and dissected (see below). We obtained daily data for average temperature, average humidity and total rainfall for each site by downloading data from the weather station nearest each site that had data for the full study period (www.wunderground.com).

Sample analysis

Up to three workers per colony per week were dissected. For each bee, the abdomen was placed in Ringer's solution and examined for the presence of conopid fly and braconid wasp larvae and the larger tracheae for the tracheal mite *Locustacarus buchneri.* Sections of the Malpighian tubules, hindgut and fat body were removed, crushed and examined under x400 magnification for the presence of the endoparasites *Crithidia bombi, Nosema* spp. and *Apicystis bombi.* Each slide was examined by two researchers. In addition, the ovary development of all collected workers (n=393) and the body fat content of all workers, gynes (n=46), and a random sample of max. 20 males per colony to limit workload (total n=418) were assessed (see Supplementary Methods).

Land-use classification

Following best practice in the field (Bunce et al. 1996; Owen et al. 2006) we classified land-use at multiple radii surrounding each site using GIS analysis, based on satellite imagery with additional ground-truthing for agricultural sites. Agricultural sites were surveyed because mass crop blooms may not be detected by satellite images taken outside the bloom period. The land-use classification protocol is described in full in (37) and is available as Supplementary Methods. Briefly, buffers at radii of 750m (*B. terrestris* typical foraging range; Osborne et al. 1999; Darvill et al. 2004; Knight et al. 2005), 500m, 250m and 100m (representing steps of spatial scales at which bees may interact with the surrounding land; Moreira et al. 2015; Carvell et al. 2017) were generated around each site. Preliminary analysis showed that the majority of the response variables responded most strongly to land-use at a 500m radius so this was selected as our primary land-use variable. Land-use patches were defined by drawing polygons in QGIS v2.16 and categorised visually to one of 80 land-use classes (Table S14) from satellite imagery and ground surveys carried out in May 2016.

We refined the classification to produce a single categorical land-use variable via an established three-step process (Owen et al. 2006; Samuelson and Leadbeater 2018): 1) definition of land-use categories, 2) Principle Components Analysis (PCA) on the categories and 3) cluster analysis based on the PCA output (Fig. S2). Briefly, each land-use class was coded to one of eight categories (e.g. impervious surface, flower-rich habitat; Table S14) and the total area of each category within each site calculated. A PCA was then performed to reduce the dimensionality of the land-use variables, and cluster analysis (Ward's method) was performed on the first two principle components, which in combination captured approximately 85% of the variation (see below for loadings). Following Bunce et al. (1996) and Owen et al. (2006), each cluster contained a minimum of five sites. Three clear clusters emerged (Fig. S3a), comprising a group characterised by dense urban development (henceforth named "city"; n=17), a group characterised by patches of housing surrounded by rural land ("village"; n=16) and a group dominated by agricultural fields ("agricultural"; n=5; Fig. 1b). Exploration of model fit confirmed that use of the clustered land-use categories to predict our main response variables explained more of the variance in our data than use of the PCs alone (Fig S3b), and comparison of models containing combinations of the PCs with those containing the clustered variable showed that for all response variables the clustered variable improved model fit (see Supplementary Methods and Table S11 for AIC values), justifying the necessity of the clustering step. Sites in the city cluster contained mean 56.2% (± SE: 4.0%) impervious surface and 0.1 (±0.1)% agricultural land cover while village and agricultural sites contained 13.8 (±3.7)% and 8.6 (±4.5)% impervious surface and 34.6 (±7.1)% and 71.2 (±11.5)% agricultural land cover respectively.

Statistical analysis

For each analysis, we built a comparison set of models including the full model (for predictors, see below) and all subsets, including the basic model containing only the constant and residual variance ("all-subset approach"). We selected the model or set of models with the lowest AICc as the best fitting model(s) (Johnson and Omland 2004). Where several models were within two AICc units of the best model, model averaging was carried out to obtain parameter estimates derived from the best set of models including the basic model if applicable (Symonds and Moussalli 2011). Final models were examined for spatial autocorrelation by using a Moran's I test on the residuals and graphically assessing the spatial pattern of residuals.

To analyse peak colony size linear regression was carried out on log-transformed data. Total production of sexuals (gynes and males) was analysed using zero-altered negative binomial hurdle models, where the response is modelled as a binary process (production of sexuals) and a zero-truncated count process (total sexuals in colonies that produced sexuals; Zuur and Ieno 2011). Binomial GAMs (allowing for a non-linear effect of week) with site as a random effect were used to analyse presence of nectar and pollen and ovary development. Queen survival, colony survival and onset of reproduction were subjected to survival analyses using non-parametric Cox proportional hazards models. Proportion of worker samples in each colony containing *Apicystis* and *Crithidia* were analysed using binomial GLMs. Male and worker fat content were analysed using Gaussian GAMs allowing for a non-linear effect of week with site as a random effect. *Bombus vestalis* invasion as a binary response was modelled using binomial GLMs. One factor level (city) for this variable had perfect separation (only zeroes); to deal with this three dummy observations were added for each land-use category with *B. vestalis* invasion set to one and weather variables set to whole-dataset means.

To investigate whether our results may have been driven by floral resource availability, we re-analysed the response variables that were found to be significantly affected by land-use (reproductive output, peak colony size, colony survival, queen survival, presence of nectar stores and presence of pollen stores) using proportion of flower-rich habitat as a predictor. We coded each land-use class as described above as flower-rich or flower-poor, based on reference to the literature (e.g. domestic gardens have been shown to support high floral diversity (Loram et al. 2008) and provide considerable resources to bees (Goulson et al. 2010)) and on ground surveys in agricultural land to identify crop types and wildflower strips, and summed the area of flower-rich land-use patches to generate the proportion of flower-rich habitat at a 500m radius for each site. Each response variable was analysed using this predictor as described in the paragraph above. All analyses were conducted in R version 3.2.1 (R Core Team 2018); for packages see Supplementary Methods. Raw data are archived in Dryad (entry doi: 10.5061/dryad.9120fb3).

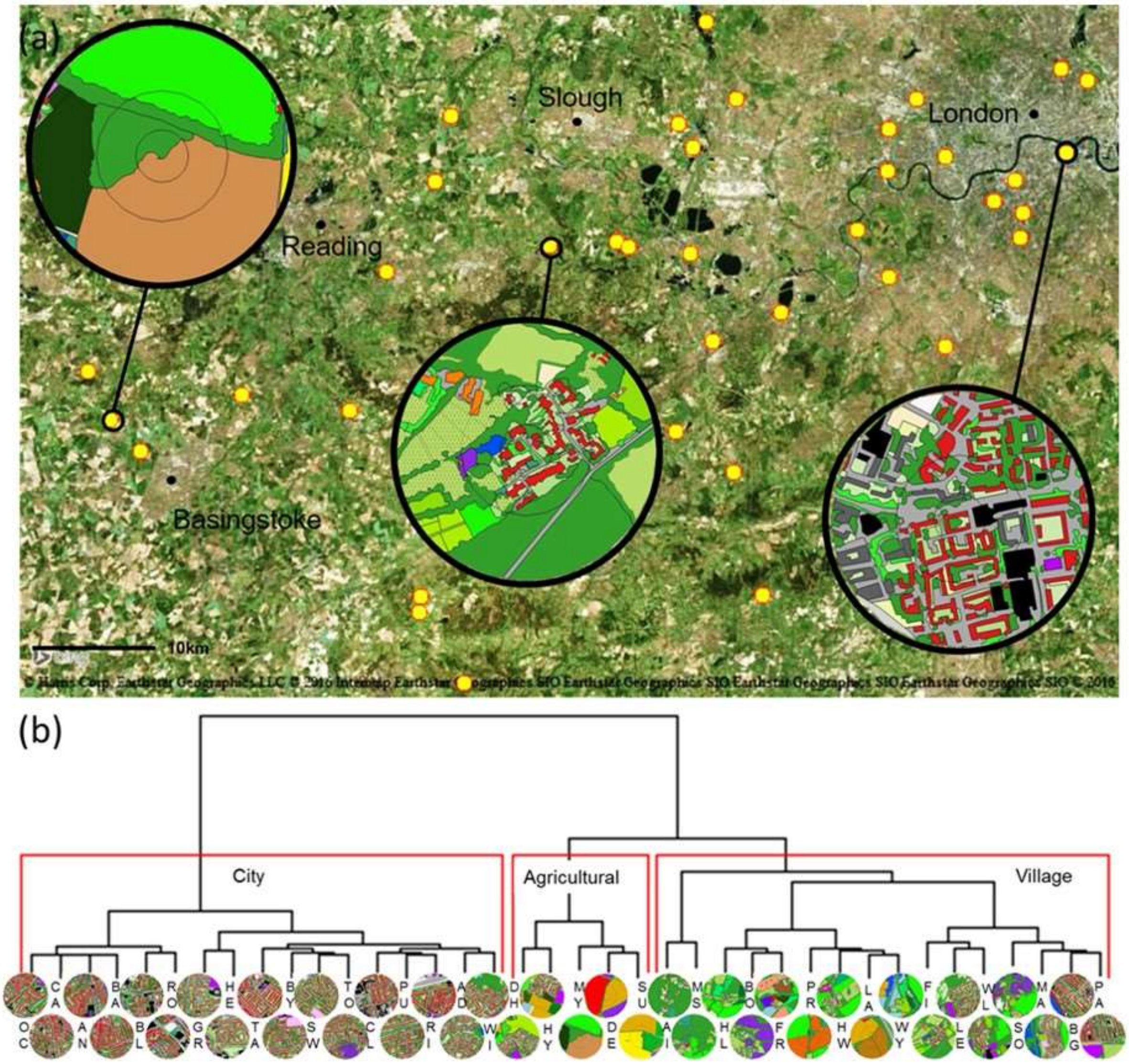

Figure 1. a) Location of 38 sites in South-East England at which a *B. terrestris* colony was placed for up to 10 weeks from May to July. Inset circles show land-use classification at a 500m radius for three typical sites (left to right: agricultural, village, city). b) Cluster dendrograms of land-use of 38 sites at a 500m radius. Cluster analyses using Ward's method were performed on a set of principle components describing land-use to group sites into categorical land-use types (red boxes). At the terminus of each branch the two-letter site name is given with an image of the GIS land classification (see Table S14 for colour key).

Results

Land-use category strongly predicted the number of live sexual offspring (gynes and males) produced over the colony life cycle (Fig. 2a, Table S1a). Village colonies were significantly more likely to produce sexual offspring than agricultural colonies (model averaged estimate (MAE): 2.853, 95% CIs:

[0.327 – 5.378], Table S2a), and both city (MAE: 2.789 [0.799 – 4.778]) and village (MAE: 2.566 [0.579 – 4.552]) colonies produced significantly higher numbers of sexuals than their agricultural counterparts. Our data suggest that this effect may reflect both the build-up of a larger workforce and, relatedly, longer queen lifespans in village and city colonies. Both village and city colonies displayed significantly higher peak size (number of bees) than agricultural colonies (Table S1b, Fig. 2b; City MAE: 0.918 [0.194 – 1.641], village MAE: 1.047 [0.319 – 1.774], Table S2b), and founding queens survived for longer (Table S1c, S2c; Fig. 3a; MAE of Hazard Ratios (HR) relative to agricultural colonies: City: 0.149 [0.041 – 0.542]); Village: 0.137 [0.039 – 0.488]. City and village colonies also took significantly longer to become moribund than agricultural colonies (City HR: 0.111 [0.031 – 0.396], village HR: 0.073 [0.019 – 0.271]; Table S1d; Fig. 3b). There was no significant effect of land-use on ovary development (see Supplementary Results).

Agricultural colonies were found to contain less stored food than their city or village equivalents. Colonies in city (nectar MAE: 2.015 [0.520 – 3.509], Table S1f, S2f; pollen MAE: 2.109 [1.045 – 3.173], Table S1g, S2g) and village (nectar MAE: 1.902 [0.410 – 3.394]; pollen MAE: 2.038 [0.973 – 3.102]) land-use clusters were significantly more likely to contain nectar (Fig. 4a) and pollen (Fig. 4b) stores than agricultural colonies, in which we found almost no nectar stores and limited pollen after four weeks of development. We found no effect of land-use on the fat content of workers or males (Tables S5b & c).

Land-use had no effect on the prevalence of *Apicystis bombi* in colonies, although further analysis suggested that land-use in the immediate area surrounding the colony may have an effect (see supplementary results, Table S5g). Similarly, there was no effect of land-use on *Crithidia bombi* presence (Table S5f). Only three bees were parasitised by *Syntretus* sp. (one city site and one village site), and no *Nosema bombi* or *Locustacaris buchneri* was found in any of our samples. The brood parasite *Bombus vestalis* was present in our study area, and hence we carefully monitored colonies to detect attempted parasite invasions. We recorded 14 invasion attempts by *B. vestalis* queens (max. 4 in a single colony). Land-use category was a significant predictor of the probability of an invasion attempt (Table S2h), with city (MAE: -3.776 [-6.304 – -1.249]) and village (MAE: -2.943 [-5.444 – -0.442]) colonies being less likely to be invaded than agricultural colonies (Table S2h, Fig. 3c). Accordingly, we investigated the possibility that increased brood parasite invasions explain the poorer development of colonies in agricultural sites by performing a separate analysis in which three models were compared for each response variable: 1) the best model from the original analysis, 2) the same model but with parasite invasion events replacing land-use as a predictor and 3) a model with both parasite invasion and land-use. For all variables, the model containing land-use only or land-use and invasions fit the data better than the model containing invasions alone (i.e. had a lower AICc value, Table S9). In other words, although parasite invasions explain some of the variance in our data, land-use influences colony performance irrespective of invasion status.

Our land classification protocol (Samuelson and Leadbeater 2018) allows investigation into the aspects of the land-use that may underlie the effects found, by examining the variables that contribute to the clustering of land-use types. High domestic infrastructure, impervious surface and road cover, and low agricultural land cover, contributed strongly to Principle Component (PC) 1 (eigenvalue score >0.4 or >-0.4; Hahs and Mcdonnell 2006), while PC2 was defined by high tree cover and low open and flower-rich habitat cover (Table S10). The city cluster was characterised by positive scores on PC1 (mean 2.00 ± SE 0.07) and near-zero scores on PC2 (-0.27±0.17), suggesting a highly urbanised semi-open land type; the village cluster had medium negative scores on PC1 (-1.40±0.37) and positive PC2 scores (0.94±0.37), suggesting low intensity urbanisation with moderate tree cover; the agricultural cluster had low PC1 scores (-2.33±0.64) and low PC2 scores (-2.08±0.32), suggesting open land with very little urbanisation and high agricultural cover (Fig. S3a). Analysis of the PCs suggested it was the combination of both attributes of the land-use that drove the effects seen (see Supplementary Methods and Table S12 & S13 for results of these analyses). Investigation of the effect of the proportion of flower-rich habitat on the response variables as a possible key driver of the results showed no significant effect (Supplementary methods; Tables S3 & S4).

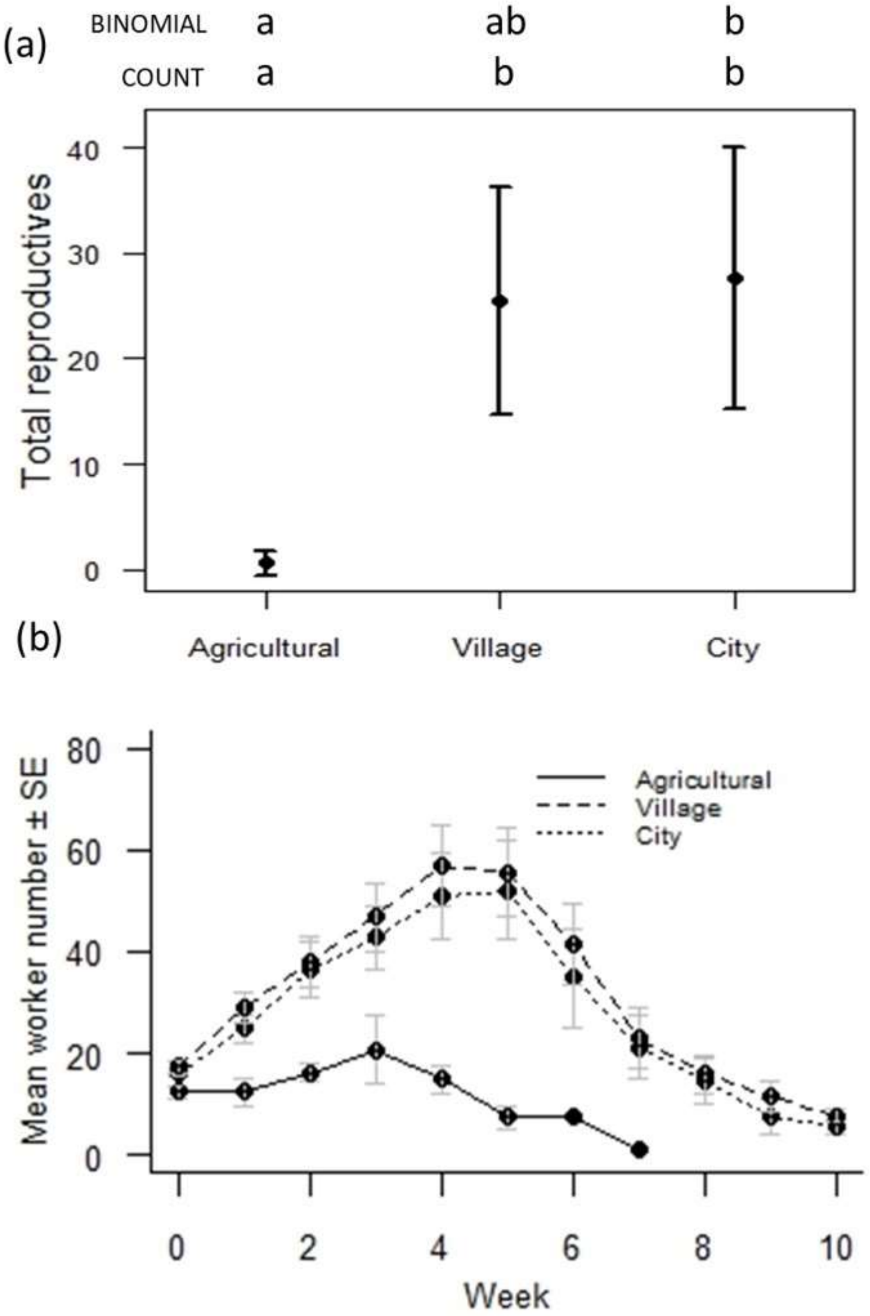

Figure 2. a) Mean number of sexual offspring (gynes + males) with 95% confidence intervals (CIs) for colonies of *B.terrestris* in agricultural, village and city sites based on land-use at a 500m radius. Letters indicate significant differences between land-use types based on 95% CIs on parameter estimates from both the binomial (presence/absence of sexuals) and count (number of sexuals produced) components of a zero-inflated hurdle model. b) Mean (± SE) colony size (number of bees) from weekly night-time bumblebee colony censuses. To analyse peak colony size linear regression was carried out on log-transformed data.

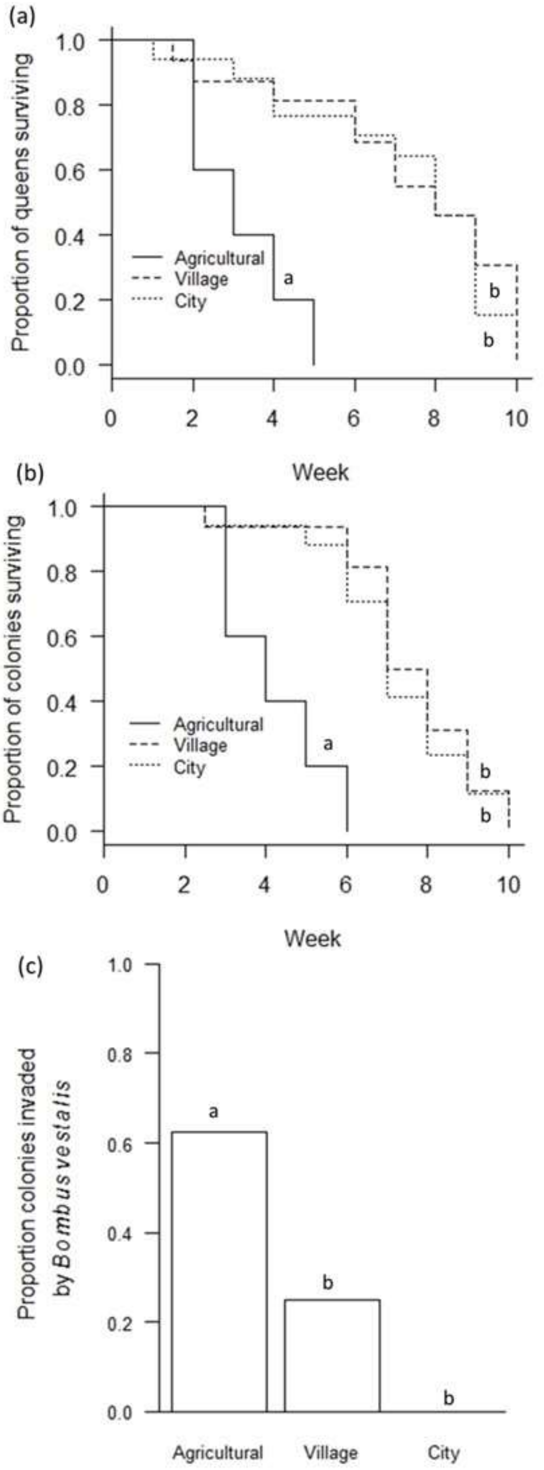

Figure 3. Kaplan-Meier curves of a) queen survival and b) colony survival for colonies of *B.terrestris* in agricultural, village and city sites based on land-use at a 500m radius. Each step in the Kaplan-Meier curves represents the week at which (a) queens died or (b) colonies were removed from the field; for example, all queens in agricultural sites had died by week 5. c) Proportion of colonies invaded by *Bombus vestalis* in agricultural, village and city sites, analysed as a binary response. Letters indicate significant differences between land-use types based on 95% CIs on model-averaged parameter estimates from a) and b) Cox proportional hazards models and c) binomial GLMs.

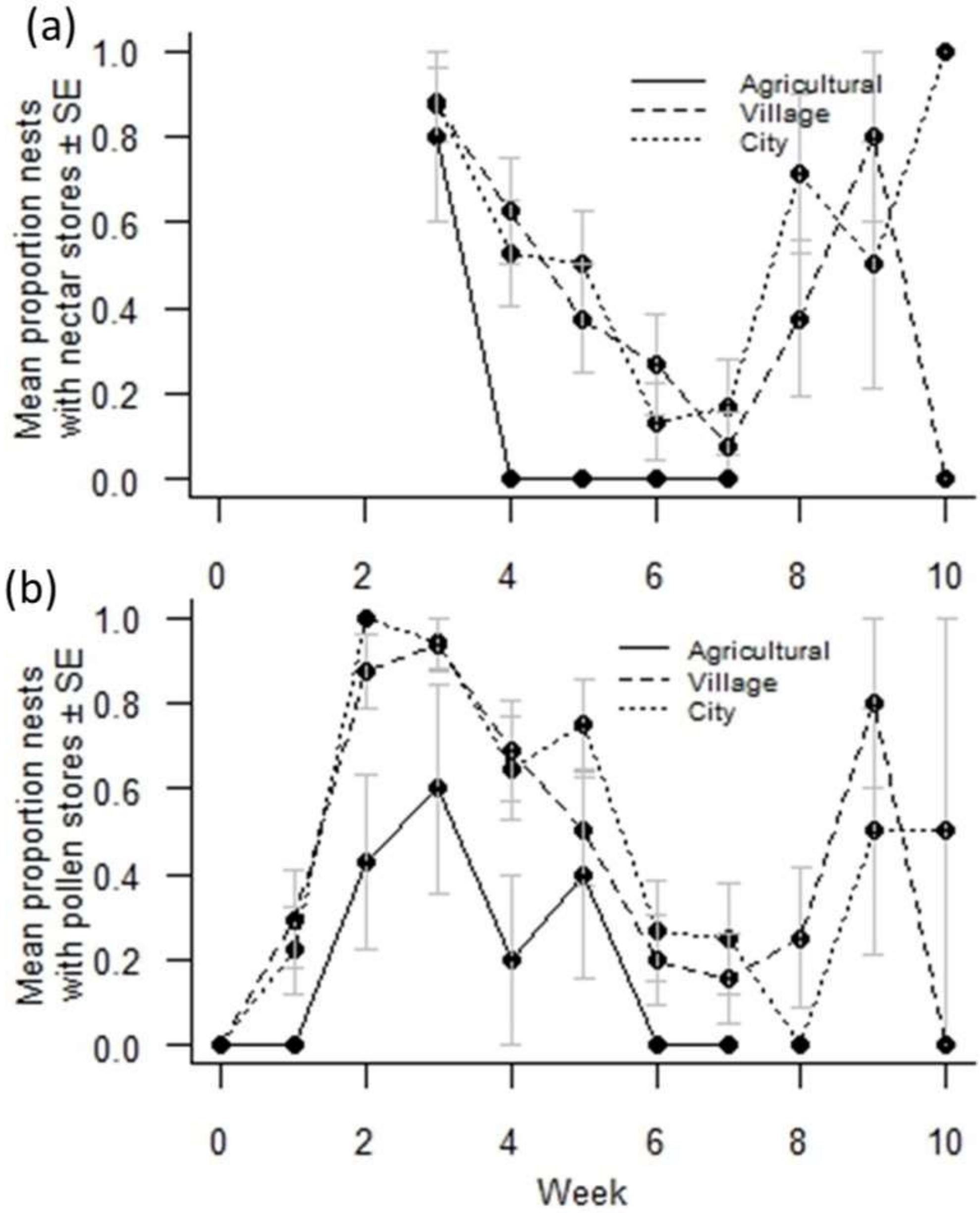

Figure 4. Mean (± SE) proportion of *B. terrestris* colonies containing a) nectar and b) pollen stores over 10 weeks in agricultural, village and city sites based on land-use at a 500m radius. Binomial GAMs allowing for a non-linear effect of week with site as a random effect were used to analyse presence of nectar and pollen. Nectar data were collected from week 3 due to provision of sucrose during week 1.

Discussion

Our results demonstrate a direct association between urbanisation and higher fitness in a key insect pollinator, *B. terrestris*. We found increases in reproductive output, colony growth and food stores as well as lower brood parasitism by *B. vestalis* in colonies placed in urbanised areas compared to sites dominated by agriculture. Previous research has described correlative evidence for higher abundance of bees (e.g; Sirohi et al. 2015) and higher bumblebee nest densities (Osborne et al. 2008; Goulson et al. 2010) in urban areas, but whether this may be driven by migration between land-use types or effects of land-use on population dynamics has remained unclear (Gill et al. 2016). Our experimental design, whereby colonies reared from wild-caught queens were placed in different land-use types over the full colony life cycle in order to measure reproductive output, provides evidence for a causal link between reproductive success and urbanisation, elucidating a potential mechanism behind these observed differences in pollinator populations between urban and rural areas.

We employed a high-resolution approach to measuring reproduction, collecting almost all males and gynes present in the nest at weekly night-time inspections, over the entire colony life cycle from first worker emergence to moribundity. This builds on traditional methods of dissecting nests at the very onset of reproduction (Goulson et al. 2002; Whitehorn et al. 2012; Williams et al. 2012), capturing a higher proportion of the total reproductive output and allowing worker and male production to be distinguished (Crone and Williams 2016), which may explain our detection of a strong effect of urban land-use on reproduction in contrast to previous studies (Goulson et al. 2002; Parmentier et al. 2014; Kämper et al. 2016). Furthermore, consideration of asymmetrical reproductive investment in gynes and males means our results are potentially conservative. Gyne production requires greater resource investment than male production (Duchateau and Velthuis 1988), and in our study, agricultural colonies failed to produce even a single gyne. Gyne production is likely to have a particularly strong effect on population dynamics, as queens hibernate and found new colonies (Sladen 1912), so our findings suggest that agricultural populations may not be self-sustaining (Savard et al. 2000). Queens of common bumblebee species may migrate long distances (Lepais et al. 2010), raising the possibility that cities may act as a source of new queens to replenish such agricultural population sinks and therefore support the pollination of crops in agriculturally intense landscapes.

Parasite pressure presents a significant emerging threat to wild bee populations (Fürst et al. 2014) and previous research has provided evidence for a link between land-use and parasite prevalence in bumblebees (Goulson et al. 2012; Theodorou et al. 2016). However, no effect of land-use was found on *Crithidia bombi* presence and levels of *Nosema bombi, Syntretus* sp. and *Locustacaris buchneri* were either zero or too low for analysis. Conversely, invasions by the brood parasite *B. vestalis* were strongly affected by land-use, with higher invasion rates in agricultural and village colonies than city. This may reflect lower *B. vestalis* abundance or even complete absence in the urban areas studied, although surveys have recorded the species in cities (e.g. Archer 2012). Alternatively, stronger colonies in city sites may have been more able to resist invasion (Goulson and Brown 2003), or volatiles from colonies may have been masked by air pollution (Lusebrink et al. 2015), rendering them more difficult to locate (Fisher 1983). Reductions in forage availability in modern agricultural landscapes have been identified as a potential major driver of bee population declines (Carvell et al. 2006). Accordingly, we found less stored pollen and nectar in agricultural colonies than in city or village colonies, suggesting forage availability may be a contributing factor to poor performance at agricultural sites. This is consistent with evidence from honeybees, where urbanisation has been shown to have a positive effect on food storage (Lecocq et al. 2015; but cf. Sponsler and Johnson 2015), and supports research suggesting modern agricultural land provides insufficient forage resources for bees (Carvell et al. 2006).

Investigation into the underlying attributes of our land-use classification indicates that it appears to be the shared attributes of high agricultural cover and low urbanisation that group the poor performing colonies in our study. A reasonable hypothesis from previous research showing higher colony weight gain in suburban areas than agricultural (Goulson et al. 2002) would be that low intensity urban areas are most valuable to bee populations due to the combination of abundant gardens and proximity to semi-natural habitat; our finding that colonies in densely urbanised areas performed similarly to those in lower intensity urbanisation nonetheless fails to support this. We found no direct effect of the proportion of flower-rich habitat surrounding colonies on colony success. However, this may reflect the fact that fine resolution floral abundance surveys, taking into account floral density and species identity, are not possible in urban areas due to access restrictions to gardens. Future research could aim to investigate forage provision in urban areas using modelling approaches (Lonsdorf et al. 2009) to further assess floral availability as a driver in urban habitats. Floral factors differing between agricultural and built-up areas that may have contributed to a reduced ability to collect food may include the spatial distribution and composition of flower-rich patches (Garbuzov and Ratnieks 2014a; Kallioniemi et al. 2017), the duration for which they are available (Kallioniemi et al. 2017), or potential effects of environmental contaminants on foraging behaviour (Gill et al. 2012).

Exposure to agrochemicals has been shown to impact on colony function and success in bumblebees (Gill et al. 2012; Whitehorn et al. 2012), including reproductive output (Whitehorn et al. 2012) and parasite prevalence (McArt et al. 2017), and high levels of pesticide contamination are often found in both crop and wildflower resources in agricultural areas (David et al. 2016). There is evidence that bees in urban areas may be subject to lower pesticide exposure (Botías et al. 2017, but cf. Johnson and Pettis 2014) offering another possible mechanism for our findings of lower colony success in agricultural areas. Ground surveys of the agricultural sites in this experiment showed a variety of crops in the surrounding farmland, with one site near a field of oilseed rape. This may represent a route of pesticide exposure (Rundlöf et al. 2015), although the study took place after the EU moratorium restricting neonicotinoid use in flowering crops (European Commission 2013). However, neonicotinoids may remain in the soil and the nectar and pollen of non-target plants for prolonged periods following use on nearby crops (Bonmatin et al. 2015), and other pesticides may also negatively affect bees (Gill et al. 2012). In general, fields around the agricultural sites were more commonly arable than pasture, compared to the village sites which more often contained pasture and woodland in undeveloped areas, providing the potential for different pesticide exposure between these land-use types, and the high incidence of gardens and parks in city areas may expose bees to a different suite of horticultural pesticide applications, about which little is known (Bonmatin et al. 2015). Our findings highlight that the question of how bee exposure to pesticides varies with urbanisation is a major knowledge gap that requires exploration.

We show for the first time that the reproductive output of *B. terrestris* colonies placed in built-up areas is higher than in agricultural areas, suggesting that the current urban expansion may have positive consequences for generalist bumblebee species. Our findings suggest that abundance and diversity differences found in previous studies (Winfree et al. 2011) may be driven by a direct impact of land-use on fitness, rather than migration between land-use types, and support the growing evidence that some types of agricultural land represent a barren landscape for pollinators (Carvell et al. 2006, 2017). Given than agricultural land is the most common primary land-use in Europe (Eurostat 2016), our finding that urban areas are linked to higher reproductive success suggests that developed land may provide a refuge for populations of *B. terrestris* and potentially other generalist bumblebee species within a landscape dominated by intensive farming.

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Author Contributions

A.E.S. and E.L. conceived the initial idea; A.E.S., E.L. and M.J.F.B designed the experiments; R.J.G. provided feedback on the experimental design; A.E.S. performed the experiment and the statistical analyses; A.E.S. wrote the manuscript draft and E.L., R.J.G., M.J.F.B. and A.E.S. provided the final edit.

Lower bumblebee colony fitness in agricultural compared to urban environments

Supplementary Material

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Supplementary Methods

Bumblebee colony rearing

Queens were kept in clear acrylic rearing boxes (W 67 x L 127 x D 50; Allied Plastics, Kingston, UK) with a plastic perforated mesh base (The Plastic Shop, Coventry, UK). Each queen was provided with a gravity feeder (Savic, Kortrijk, Belgium) containing 25% inverted sucrose solution (Thorne, Windsor, UK) and a pollen ball formed of finely ground pollen (Biobest, Westerlo, Belgium) and 25% sucrose solution. These were changed weekly unless the queen was incubating the pollen ball or eggs were present, in which case an additional pollen ball was added. Gravity feeders were refilled every two days. Bees were kept in the dark or under red light at 26°C and 50-60% RH.

We monitored colony founding, excluding queens that had not laid eggs after 8 weeks from the experiment (n=51). From the initial 176 queens, 122 produced eggs and 53 produced >10 workers. Colonies that hatched 10 workers were transferred into a wooden nest box (W 280 x L 320 x D 160mm) with a clear Perspex lid. During transfer, queens again underwent faecal screening for parasites and colonies with parasitized queens excluded from the experiment (n=2). Colonies were kept in the wooden nest boxes in the lab for 7-10 days before field placement; mean time (± SE) between queen collection and placement in the field was 35.45 ± 0.58 days.

Field placement

We advertised the study on social media, requesting gardens and farms in South-East England (between central London and Basingstoke) with side access and not accessed regularly by children under 16 or dogs. For the purposes of colony randomisation, sites were initially classified to four crude land-use types based on visual classification using Google Earth: urban (U), suburban (S), village (V) or agricultural (A); these classifications were not used for further analysis. Colonies containing between 10 and 25 workers were ordered by worker number and assigned first to land-use type in cyclical order (USVASVAUVAUSAUSV...)., and then to individual sites in visit order, with colonies containing more workers assigned to earlier visited sites to maximise equality of colony size at time of placement.

We installed colonies in the presence of the site owner, who was given an information sheet and asked to sign a consent form before colony placement. Wooden nest boxes were placed inside protective field boxes, consisting of a plastic storage box (W 440 x L 710 x D 310mm; Really Useful Box, Kingston, UK) lined with insulation wrap (Thermawrap, Creswell, UK) inside the walls and lid and aluminium foil on the base (Fig. S1), and connected to the outside via a 10cm black plastic pipe (diameter: 32mm) inserted through a 40mm hole in the field box. To protect against badger damage, field boxes were fixed to the ground using screw or groundhog pegs and secured with a ratchet strap. We placed field boxes under a tree or bush for shade, away from paths or benches and in a location receiving morning sun and midday shade as indicated by the site owner. No sucrose or pollen was fed to the colonies throughout the experiment, except that each colony initially received a small gravity feeder containing 150ml 25%

inverted sucrose solution to reduce any risk of mortality following transfer, which was removed after one week.

Data collection and analysis

The field boxes contained EM-30KAM scales connected to AD-1688 weighing data loggers (A&D Instruments, Abingdon, UK), which stood on a ceramic floor tile to provide a hard surface. Nest boxes were placed on the scales to allow continuous weight recording. On arrival at each visit, we replaced the scale head unit containing the display and battery with a charged unit, weighed a known 1kg weight and then recorded the weight of the nest box before carrying out any manipulations. The weight data are not presented here, as humidity from the colonies caused the measurements to be unreliable.

In addition to the data reported in the main text, we also collected the following data: photograph of the nest before and after removing the wax cover, number of gyne larvae and pupae, wax cover (full, partial or absent), presence of open or closed egg towers as an indicator of competition point (Duchateau and Velthuis 1988) and presence of dead *B. terrestris* queens, indicating usurpation attempts. A pollen sample was taken from one pollen pot if present.

During dissection of collected workers, ovaries were removed and the presence of developed oocytes was recorded. If present, the length of the terminal oocyte of each of the 8 ovarioles was measured to assess ovary development (Amsalem and Hefetz 2010; Evans et al. 2016). Ovary development was modelled as a binary response (presence/absence of at least one developed oocyte) using binomial GAMs allowing for a non-linear effect of week with site as a random effect. The effect of queen presence on ovary development was analysed with binomial GLMMs with site as a random effect (week was not included as it was highly collinear with queen presence).

The body fat content of all collected workers (n=393) and gynes (n=46), and a random sample of max. 20 males per colony to limit workload (total n=418), was assessed (Brown et al. 2000). For each bee, we measured thorax width (between tegulas (wing joints)) as the mean of three digital calliper measurements (accuracy = 0.01mm) and wet mass using a precision balance (accuracy = 0.0001g) before drying for 72h at 70°C. The dry mass of the whole bee and abdomen alone were measured before immersing the abdomen in 4ml diethyl ether for 24h to extract the fat. The abdomen was rinsed with fresh ether, dried for 72h and weighed again. The difference between the weights before and after ether extraction represents the amount of fat; relative fat content is expressed as the ratio of fat (mg) to thorax width (mm). Only two males were collected from agricultural colonies (compared to 179 in village and 189 in city) so these were excluded from analysis of the effect of land-use at a 500m radius on male size and fat content.

Land-use classification

The full land-use classification protocol is as follows. Sites were located on Google Earth version 7.1.5.1557 and imported into QGIS version 2.16. Buffers were generated around each site at four radii (see below). Polygons were drawn around each visually-identified land-use patch at 1:5000m (agricultural areas) or 1:2500m (built-up areas) zoom at a resolution separating individual buildings, fields and gardens. Each polygon was assigned to one of 34 initial land-use classes (e.g. house, residential garden, arable field, hedgerow; for full list see Appendix S3) using Bing Aerial satellite imagery. Ground surveys were carried out in May 2016 at all sites which contained agricultural land within a 750m radius (n=19) to further classify fields by crop grown, bloom stage and presence of wildflower strips and other floral resources. This resulted in a total of 80 land-use classes.

To refine the classification to produce the categorical land-use variables used in the analysis, we carried out a three-step process following Owen et al. (2006): 1) definition of land-use categories, 2) PCA on the categories and 3) cluster analysis to generate a categorical land-use variable (Fig. S2). To do this, the total area of each land-use class was calculated around each site. Eight land-use categories were defined: impervious surface (including built area), flower-rich habitat, domestic infrastructure, garden, tree cover, agricultural land, open land and road (excluding vegetated verges). Each of the 80 land-use classes was coded according to whether it belonged to each category based on *a priori* knowledge and reference to the literature (e.g. "house" was coded as belonging to impervious surface and domestic infrastructure, and "garden" to flower-rich habitat (Loram et al. 2008; Goulson et al. 2010), domestic infrastructure and open land; Table S14), allowing calculation of the proportion of each category at each radius. For example, the total area of woodland, hedgerow and freestanding trees was summed and divided by the total area of the circle to generate the proportion of tree cover. A PCA was then performed to reduce the dimensionality of the land-use variables. Two principle components captured approximately 85% of the variation, "PC1" and "PC2" (Table S10).

A cluster analysis using Ward's method was carried out on the principle components to group sites with similar land-use attributes, to allow for non-linearity in the effect of degree of urbanisation and to incorporate land-use information into a single variable capturing both principle components. Similar land classification methods typically select optimum numbers of clusters using an ad hoc minimum group size based on practicality and geographical relevance (Bunce et al. 1996; Hall and Arnberg 2002; Owen et al. 2006); following this approach we split clusters so that each cluster contained a minimum of five sites.

We investigated whether the clustered land-use variable generated by our land classification protocol or the principle components (PCs) themselves better fit our data by performing an additional analysis on our main response variables (reproductive output, colony size, colony survival, queen survival, nectar stores and pollen stores) in which we compared a model containing the clustered land-use variable with models containing all possible combinations of the PCs including their interaction. We found that for all response variables, the model with the clustered land-use variable had the lowest AIC (Table S11), justifying the use of the clustered variable in the main analysis.

To investigate whether one or both of the PCs were driving the effects of land-use on the response variables, we performed a full analysis on each of the main response variables (see *Statistical Analysis* section in main text for regressions used) using a model selection approach to compare models containing combinations of the PCs and the weather covariates and performing model averaging on the optimal model set (Tables S12 & S13). No clear pattern emerged, with little evidence that one PC is more important than the other, with the combination of both often key. It is the combination of the PCs which drives the clustering, as suggested by the grouping of the PC scores (Fig S3a), so these findings further support use of the clustered land-use variable.

Radius selection

Land-use was initially classified for four radii around each site: 750m, 500m, 250m and 100m; hereafter R750, R500, R250 and R100. This allows for the analysis to detect the nearest spatial scale at which colonies respond, which may vary depending on the response (Moreira et al. 2015). A PCA was carried out for all radii; for R750, R500 and R250, two principle components captured 85% of the variation and three for R100. Cluster analysis on the principle components produced one categorical land-use variable for each site ranging from the landscape to the local scale: R750: Urban, Rural; R500: City, Village, Agricultural (Fig. 1b in main text); R250: Built-up, Open; R100: Dense housing, Sparse housing, Wooded, Fields.

We carried out an initial analysis of each dependent variable by fitting a full model for each radius containing the relevant land-use variable (R750, R500, R250 or R100), weather covariates (temperature, humidity and rainfall) and week (for time series) and compared the models using Akaike Information Criteria corrected for small sample sizes (AICc). Models containing R500 fit the data best for the majority of the variables (Table S8) suggesting that colonies responded most strongly to landscape at a 500m radius. We therefore selected this as our main land-use variable for the full analysis.

Statistical analysis

Dependent variables were either analysed as multiple observations per colony over time (e.g. male size) with site as a random effect, or at the colony level (e.g. total number of sexual offspring). Daily weather records were averaged (temperature and humidity) or summed (rainfall) over each week of the experiment. At five sites colonies became moribund within the first two weeks and were replaced; to ensure data for both the original and replacement colonies were included in the analysis data for these sites were pooled and an average taken. Final models were validated graphically to assess fit and check that assumptions had been met (Zuur et al. 2013). For the statistical analysis the R packages survival (Therneau 2015), MuMIn (Barton 2018), plotrix (Lemon 2006), lattice (Sarkar 2008), mgcv (Wood 2011), VGAM (Yee 2010), nlme (Pinheiro et al. 2015), lme4 (Bates et al. 2015), effects (Fox 2003), pscl (Zeileis et al. 2008), ade4 (Dray and Dufour 2007), spdep (Bivand and Piras 2015), ncf (Bjornstad 2018) were used for model fitting and producing graphs.

Supplementary Results & Discussion

Classification of land-use around the colonies showed that sites clustered into three distinct groups: agricultural, village and city. Land surrounding agricultural sites was dominated by fields, while village sites were characterised by housing in the immediate vicinity of the colony within a rural landscape and city sites consisted of dense inner-city urban land. Colonies in the two land-use types containing built-up areas- village and city- grouped together, performing better overall than agricultural colonies. This suggests a positive impact of local urban patches on colony success, with wider landscape context contributing less to the effect of land-use.

Land-use at the 500m radius did not affect the size of males produced (Table S5d), but further analysis including land-use at other radii showed that local habitat at a 100m radius did affect male size (Table S7a, Fig. S5a), with significantly larger males in colonies in wooded land than fields (MAE: 0.279 [0.050 – 0.508], Table S7a) and a trend towards larger males in sparse housing (MAE: 0.192 [-0.052 – 0.436]) and dense housing (MAE: 0.185 [-0.037 – 0.406]), an effect potentially mediated by resource availability (Sutcliffe and Plowright 1988; Ribeiro 1994). However, we found no effect of land-use on size of workers (Table S5e). Onset of reproduction was not significantly affected by land-use (Table S5a).We dissected samples of workers to assess ovary development, and found the optimal model set for ovary development contained models including land-use (Table S1e), with a non-significant trend (i.e. 95% CIs cross zero) towards a higher proportion of bees in agricultural colonies displaying developed ovaries than city (MAE: -1.739 [-3.830 – 0.351]) or village (MAE: -1.912 [-4.002 – 0.178], Fig. S4; Table S2e). There was a strong effect of queen presence on ovary development (ΔAICc to basic model = 12.71), with bees in colonies where the queen was alive being less likely to have developed oocytes (MAE: -1.241 [-1.877 – -0.604]).

In general, parasite levels were low compared to other studies (Imhoof and Schmid-Hempel 1999; Rutrecht and Brown 2008; Whitehorn et al. 2011; Goulson et al. 2012), possibly due to an unusually wet spring, which may have impeded transmission of pathogens on flowers (Durrer and Schmid-

Hempel 1994; Graystock et al. 2015). Land-use at the 500m radius did not affect the presence of the parasite *Apicystis bombi* in colonies (Table S5g). Further analysis showed that local habitat at the 100m radius did have an effect (Table S6b), but did not show a consistent response to increasing urbanisation, with a lower proportion of colonies containing the parasite in dense housing (MAE: -2.756 [-4.882 – -0.630]) or wooded (MAE: -2.147 [-3.790 – -0.505]) sites than in sparse housing sites (Table S7b, Fig. S5b). Little is known about *A. bombi* transmission (Meeus et al. 2011), but the effect of land-use on its prevalence may be mediated by bumblebee densities (Goulson et al. 2012) or spillover from commercial colonies (Graystock et al. 2013).

Total invasion rates by *Bombus vestalis* (21% of colonies) were lower than reported rates in previous field studies which ranged from 30%-100% (Sladen 1912; Müller and Schmid-Hempel 1992; Pelletier and McNeil 2003; Carvell et al. 2008; Erler and Lattorff 2010), although several field studies report no brood parasite invasions (Goulson et al. 2002; Williams et al. 2012) suggesting high natural variation in this response. Although invasions by *B. vestalis* explain some of the variance in our data, our analysis suggests land-use influences colony performance irrespective of invasion status. Invasions were not included in the main analysis to generate parameter estimates as this was highly collinear with land-use.

Rainfall positively affected male size (MAE: 0.006 [0.002 – 0.010], Table S2b), peak colony size (MAE: 0.013 [0.000 – 0.026], Table S2c.) and marginally increased colony survival (Hazard ratio: 0.977 [0.956 – 0.999], Table S2e). Rainfall also marginally negatively affected presence of pollen in the nest (MAE: -0.026 [-0.053 – 0.000]).

Lower reproductive output and colony growth in agricultural colonies may have been partially driven by the fact that agricultural colonies lost their queen earlier on average, halting gyne and worker production and restricting male production to workers (Goulson 2010). Correspondingly, there was a trend towards bees in agricultural colonies being more likely to have developed ovaries, which was strongly linked to queen absence. Queen loss may have been driven in turn by nutritional stress or parasitism (Frehn and Schwammberger 2001; Rutrecht and Brown 2008; Goulson 2010).

Supplementary Figures

Figure S1. Insulated field box containing a young *Bombus terrestris* colony at time of placement in the field. Boxes contained a data logger connected to scales on which the colony box was placed; the colony was connected to the outside by a plastic pipe.

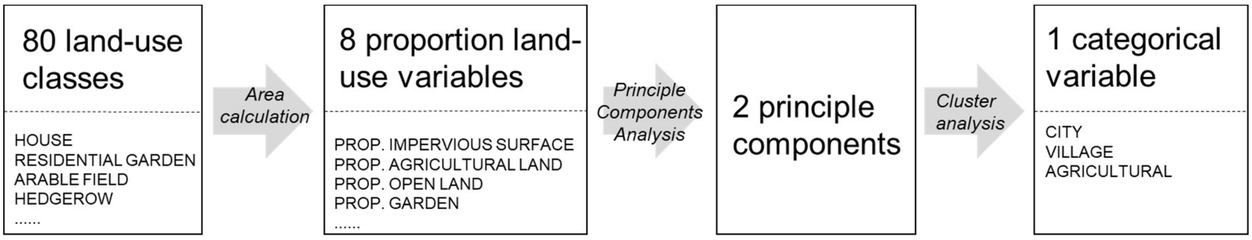

Figure S2: Overview of the methods used to classify land-use at 38 sites across a gradient of urbanisation in SE England.

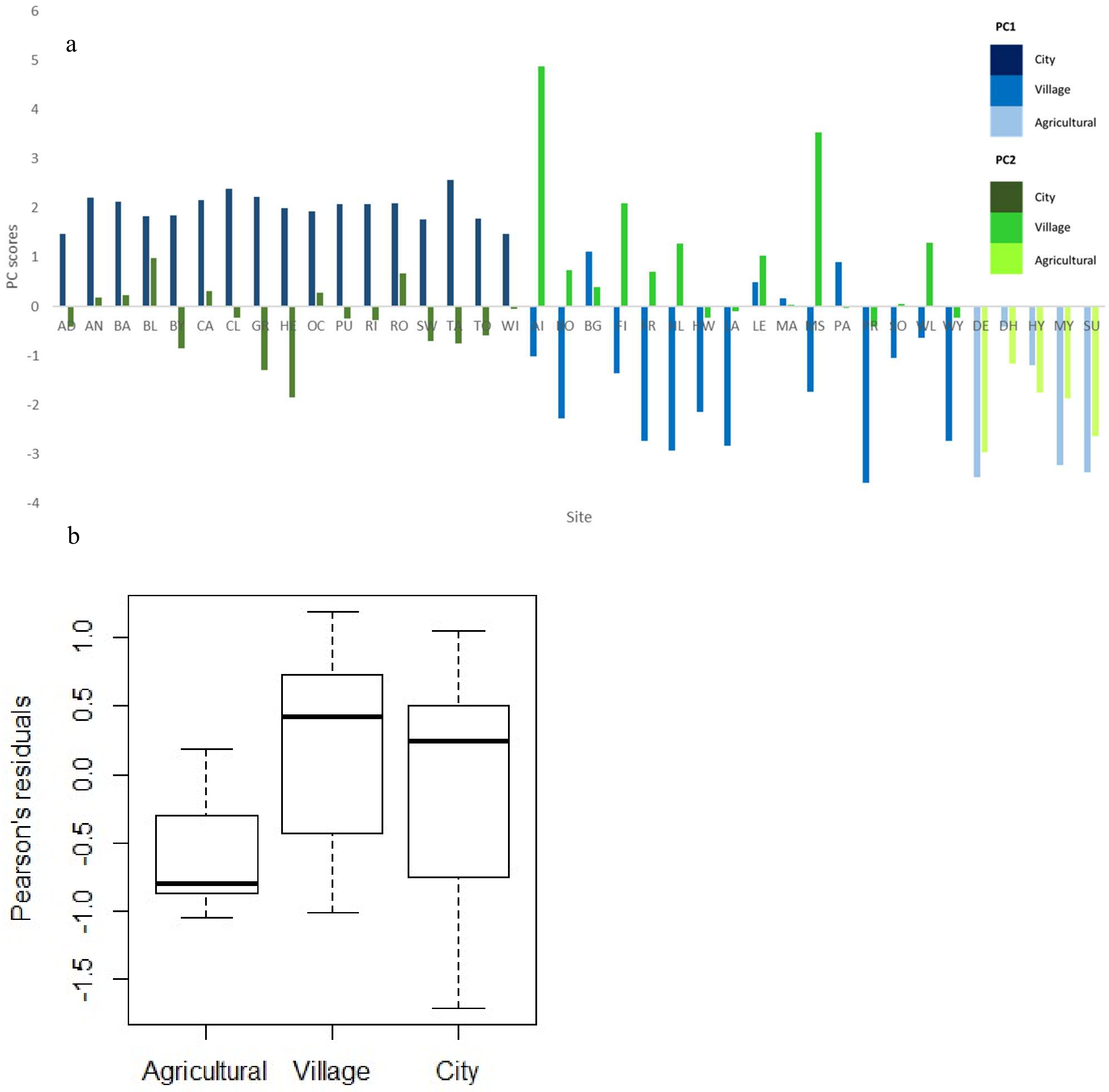

Figure S3. a) Scores on two principal components, PC1 and PC2, that captured approximately 85% of the variation in a principle component analysis (PCA) performed on land-use variables classified in a 500m radius around each study site (two-letter codes). The clustering of the three land-use types generated from subsequent cluster analysis ("City", "Village" and "Agricultural"), is illustrated in the grouping of PC scores (City: dark blue/green; Village: bright blue/green; Agricultural: pale blue/green). The "City" cluster is typified by a positive score on PC1 and neutral score on PC2, "Village" by positive to neutral PC1 and negative PC2, and "Agricultural" by negative PC1 and PC2. b) Plot of Pearson's residuals for model validation purposes of a model including PC1 and PC2 as predictors of colony size by land-use cluster from the original land classification (not included in this model). This shows variation following the clusters that is not explained by the model using the PCs alone, justifying the further step of cluster analysis.

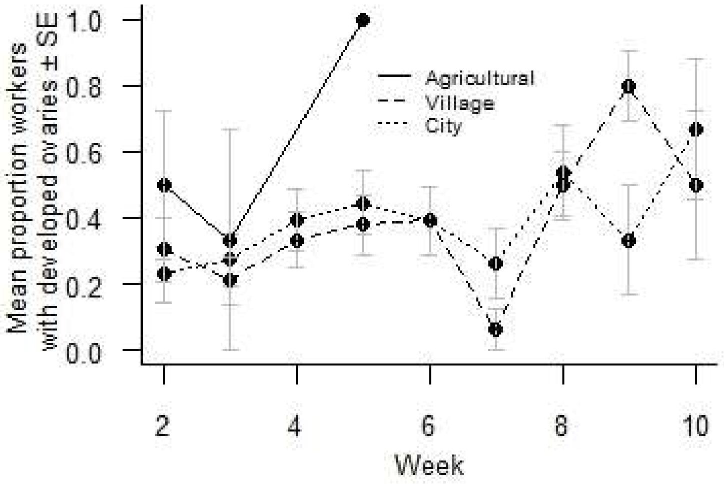

Figure S4. Mean (± SE) proportion of workers with developed ovaries by week from samples of workers collected for dissection.

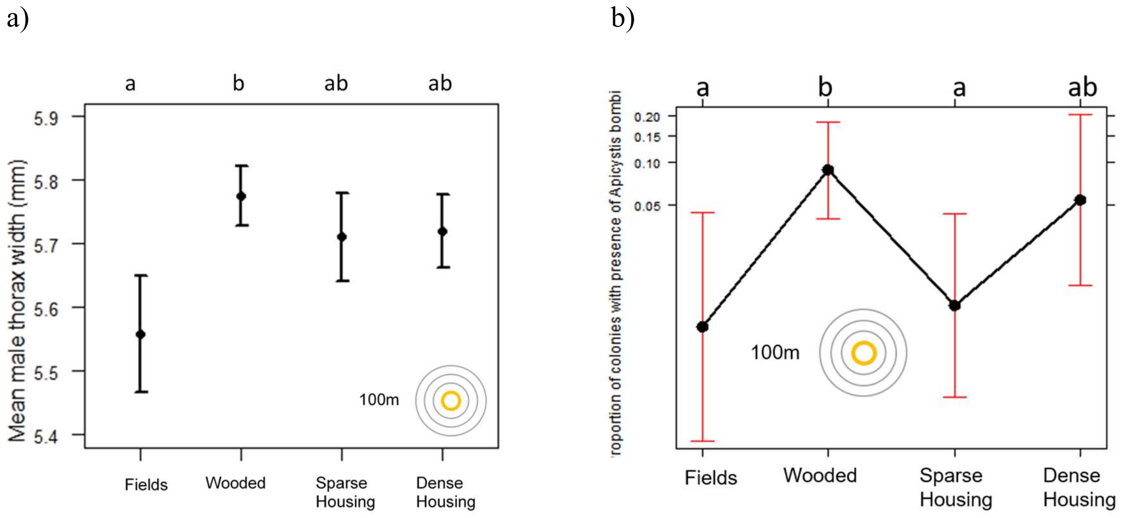

Figure S5 a). Mean thorax width of males with 95% CIs for colonies of *B.terrestris* in fields, wooded, sparse housing and dense housing sites based on land-use at a 100m radius. Letters indicate significant differences between land-use types based on 95% CIs on model-averaged parameter estimates. b) Effects plot from a binomial GLM showing the effect of land-use at a 100m radius on presence of the parasite *Apicystis bombi* in colonies of *B. terrestris* placed in sites across an urbanisation gradient. Error bars indicate 95% confidence intervals and letters indicate significant differences between land-use types based on 95% confidence intervals on parameter estimates.

Supplementary Tables

See Appendix 3.

Chapter Five

Samuelson, AE; Gill, RJ and Leadbeater, E. *submitted.* Urbanisation is associated with reduced disease pressure, larger colonies and more species-rich pollen forage for honeybees.

Submitted to *Apidologie*

Urbanisation is associated with reduced disease pressure, larger colonies and more species-rich pollen forage for honeybees

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Abstract

Bees are vital pollinators but are faced with numerous threats that include habitat loss, emerging parasites and pesticide exposure. Urbanisation represents a rapidly expanding driver of land-use change and is likely to interact with known threats to bees. Honeybees (*Apis mellifera*) are one of the most widespread and important pollinator species, but despite extensive speculation, it is unclear how urbanisation affects honeybee colony success. Here we investigated effects of urbanisation on food store quality and colony health by sampling 51 honeybee hives in four different land-use types: urban, suburban, rural open and rural wooded during two seasons (spring and autumn). We found positive effects of urban land-use on colony strength and species richness (but not Shannon diversity) of stored pollen, alongside lower late-season *Nosema* sp. infection in urban and suburban colonies. Rural open colonies contained significantly fewer bees than colonies in other landscapes, while *Varroa* mite counts followed no consistent urbanization pattern. Spring-collected pollen species composition was strongly driven by land-use type with pollen samples grouping into urban, suburban and rural clusters.Our results suggest that honeybees may be able to thrive in cities, while lower colony performance in strength,

parasitisation and pollen foraging in rural areas adds to the growing evidence that modern agricultural landscapes constitute poor habitat for pollinators.

Introduction

Honeybees (*Apis mellifera* L.) provide a significant proportion of the world's pollination services (Grünewald 2010), contributing to the pollination of almost 70% of the most important crop plants worldwide (Klein et al. 2007). Thus, documented environmental threats to honeybee populations, including habitat loss and the associated lack of forage (Couvillon et al. 2014b; Alaux et al. 2017), parasites and disease (Brosi et al. 2017), and environmental contaminants such as pesticides (Henry et al. 2012; Wood and Goulson 2017), have led to widespread concern. In some areas of the world a significant reduction in the number of managed hives is evident: in the UK this number has decreased from 300,000 to 135,000 in the last 60 years (Alton and Ratnieks 2013), and hives in Europe as a whole declined by 16% between 1985-2005, although hive numbers have increased elsewhere (Potts et al. 2010b).

Urban areas represent an exception to the overall decline in honeybee populations in Europe (Alton and Ratnieks 2013; Lorenz and Stark 2015), and indeed, there is evidence to suggest that urban habitats may offer considerable resources for pollinators in general (Hall et al. 2016; Samuelson et al. 2018; Baldock et al. 2019). Several studies have found greater abundance and diversity of wild pollinators in urban than rural areas (McFrederick and LeBuhn 2005; Winfree et al. 2007c; Baldock et al. 2015), and it has been suggested that urban land provides a refuge from the threats of forage scarcity (Garbuzov et al. 2015b) and pesticide exposure (Nicholls et al. 2018). Honeybees are important bioindicators of landscape provision for pollinators (Couvillon et al. 2014b) due to their large foraging ranges (Seeley 1995)and generalist foraging behaviour (Thomson 2004) but research to date has generated mixed results regarding the effect of urbanisation on honeybee colony success (Naug 2009; Sponsler and Johnson 2015; Garbuzov et al. 2015b; Lecocq et al. 2015; Youngsteadt et al. 2015a).

Forage availability is a strong driver of bee populations (Carvell et al. 2006; Couvillon et al. 2014a). Urban areas may offer more abundant forage than intensive agricultural areas in the form of flowering plants in gardens and parks (Goddard et al. 2010; Samuelson et al. 2018); however, it is unclear whether this is sufficient to support honeybees at the high densities found in urban areas (Alton and Ratnieks 2013). Indeed, evidence from waggle dance studies (Garbuzov et al. 2015b; Sponsler et al. 2017) and food store experiments (Naug 2009; Sponsler and Johnson 2015; Lecocq et al. 2015) give varying conclusions on the effect of urban land on honeybee foraging success. In addition, not all urban flowers are attractive to bees: a survey of flower-visiting insects in a public garden in Lewes, UK found that only 4% of the varieties in bloom were highly attractive to insects (Garbuzov et al. 2014). Forage availability may also depend on season, with some rural colonies experiencing gaps in forage availability in summer (Couvillon et al. 2014a) and evidence suggesting a longer season of available forage in urban hives (Lecocq et al. 2015). Pollen is an important food source for a honeybee colony, providing protein, lipids and micronutrients essential for colony development (Keller et al. 2005). However, most research on the effects of land-use on honeybee nutrition focusses on nectar (e.g. Lecocq et al. 2015). Because the nutritional quality of pollen diet varies widely depending on the contribution of different plant species (Keller et al. 2005), land-use is likely to have a strong effect on pollen diet quality. Urban areas may provide a diverse range of pollen sources (Garbuzov and Ratnieks 2014a), which may have implications for colony health (Di Pasquale et al. 2013; Smart et al. 2016; Dolezal and Toth 2018), but to our knowledge no study to date has demonstrated whether urbanisation affects the diversity of pollen collected by honeybees.

Urbanisation also interacts with parasite and disease stressors (Dolezal and Toth 2018). This may be mediated by higher hive densities (Alton and Ratnieks 2013; Brosi et al. 2017), resource patchiness (Youngsteadt et al. 2015a), temperature differences (Gago et al. 2013) and differences in beekeeper experience and practices in urban and rural areas (Alton and Ratnieks 2013). Initial evidence indicates that some honeybee (Youngsteadt et al. 2015a) and bumblebee (Goulson et al. 2012; Theodorou et al. 2016) diseases may be more prevalent in urban areas. However, research has yet to investigate a link between urban land-use and arguably the greatest current parasite threat to honeybee populations (Genersch 2010; Brosi et al. 2017), the *Varroa destructor* mite, which, usually in combination with its associated viruses, causes colonies to rapidly die out in the absence of treatment (Brettell and Martin 2017).

Urban areas are expanding, with an estimated three-fold increase in global urban land cover predicted between 2000 and 2030 (Seto et al. 2012). At the same time, urban beekeeping is on the rise: in London, the number of beekeepers rose from 464 to 1,237 between 2008 and 2013 and the number of hives doubled to over 3,500 (Alton and Ratnieks 2013) while in Berlin the number of beekeepers increased by 53% and hives by 44% between 2006 and 2012 (Lorenz and Stark 2015). This combination of growing interest in urban beekeeping and global urban expansion means that honeybees are likely to come into increasing contact with cities in the future. Here we investigate the effects of urbanisation on a suite of colony-level measures in honeybees, using an extensive network of 51 honeybee hives located across a gradient of urbanisation in South-East England at two time points during the foraging season. To investigate the relationship between urbanisation and parasitisation, we measure *Varroa* infestation as well as *Nosema* sp. prevalence, a common microsporidian gut parasite of the honeybee. To investigate effects on foraging, we analyse species composition and diversity of pollen collected by bees in different land-use types.

Methods

Site selection

We used existing honeybee colonies in 51 apiaries located across a gradient of urbanisation in South-East England. Apiaries were selected from an initial 123 beekeeper applications with the aim to maximise spatial independence and land-use type representativeness and minimise collinearity of covariates. Apiaries using hive types other than National, Commercial, Langstroth and WBC hives were excluded from the study, as were commercial beekeepers. Preliminary data exploration showed collinearity between apiary size (number of hives) and land-use, and beekeeper experience and land-use, with larger apiaries and more experienced beekeepers in rural areas. Furthermore, several sites were non-independent (<6000m apart; foraging ranges likely to overlap (Samuelson, Schuerch, Gill and Leadbeater, unpublished data)). To eliminate both issues, the following site selection protocol was carried out. Where two or more sites were less than 6000m apart, only one site was chosen to be in the study based on the following objectives (in order): 1) maximising number of sites, 2) balanced representation of land-use types 3) minimising collinearity (e.g. rural apiaries with few hives were preferred to those with many) and 4) maximising geographical spread.

Land-use classification

We classified land-use at a radius of 3000m around each site (radius based on the 99th percentile of waggle dance communicated distances from a separate study; Samuelson, Schuerch and Leadbeater, unpublished data). Classification was carried out in QGIS v2.16 following methods outlined in Samuelson & Leadbeater (2018). Briefly, land-use patches were defined by drawing polygons in QGIS over a satellite imagery baselayer (Bing Maps) and categorised visually to one of 29 land-use classes. Each land-use class was then coded to one of seven categories (e.g. impervious surface, tree cover) and the total area of each category within each site calculated. A PCA was performed to reduce the dimensionality of the land-use variables, and cluster analysis (Ward's method with a minimum cluster size of five; Bunce et al. 1996; Hall and Arnberg 2002; Owen et al. 2006) was performed on the first two principle components (defined as urban-ness and openness), which in combination captured 82.2% of the variation. Four clear clusters emerged (Fig. 1), comprising a group characterised by high urban-ness scores with mid-level openness scores (Urban, n=13), a group with high urban-ness and openness scores (Suburban, n=13), a group with low urban-ness and high openness (Rural Open, n=13) and a group with low urban-ness and openness (Rural Wooded, n=12). This grouping was used as a categorical land-use variable in all subsequent analyses.

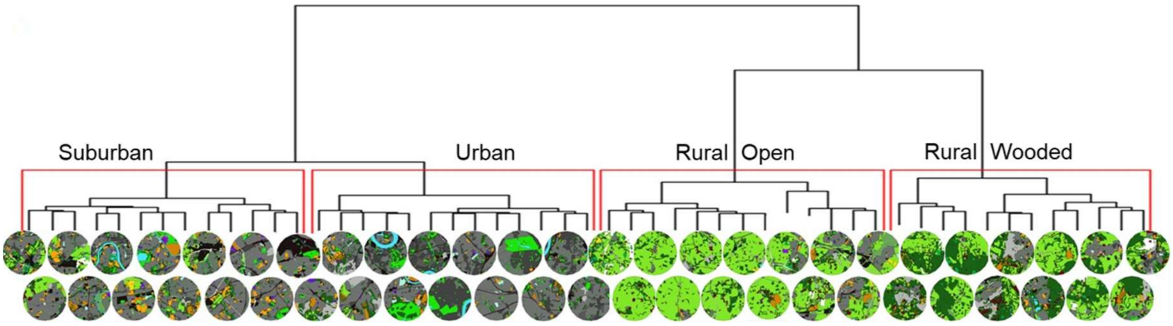

Figure 1. Cluster analysis (Ward's method) of land-use types of 51 sites located in SE England; branch terminals show land-use maps of individual sites generated from GIS classification.

Sample collection

Two periods of sample collection were carried out: one in the early season (1st-27th May 2015; Spring) to coincide with the oilseed rape bloom (Free and Ferguson 1980), and one in the late season (18th August – 14th September 2015; Autumn) to coincide with late summer resource scarcity (Couvillon et al. 2014a) . Each site was visited once in each period, in approximately the same order. Sample collection was carried out between 10:00 and 17:00 on suitable days (>12°C, wind speed <20kmh), with a maximum of three apiaries visited each day. Site data for each apiary included GPS co-ordinates, weather, number of hives, site description, and number of colonies overwintered. Samples were collected from one (queenright) hive per apiary. Hive data included hive type and disease treatment history since 2013. Colony strength (bee covered surface) was calculated by counting the number of sides of frames covered (>50%) with bees and recording the size (deep/shallow) and type (National, Commercial etc.) of frame (Delaplane et al. 2013b).

30 returning foragers were collected to test for *Nosema* sp. (foragers are more likely to harbour the parasite; Fries et al. 2013). *Varroa destructor* mite samples were taken using the "icing sugar shake method" (see Supplementary Methods) with 300 nurse bees per colony (Macedo et al. 2002). 30 cells of freshly stored pollen were collected from the same frame as the Varroa samples using a spatula (Tsvetkov et al. 2017). Cells were selected on the basis of freshness (powdery texture, no nectar seal). This pollen was likely to have been collected within 2 weeks prior to sampling (Vásquez and Olofsson 2009). All samples were placed immediately into dry ice and then into storage at -80°C within a maximum of two days.

Pollen analysis and parasite screening

A small portion of each pollen sample (n=2746) was mounted on a slide with water, basic fuchsin and glycerine jelly (Brunel Microscopes, Chippenham, UK) on a hotplate set to 80°C. These were examined using a light microscope at 400× magnification and each pollen morphotype was given a unique number. Because each pollen sample came from a single cell, samples were almost always homogenous. On the rare occasion that a sample contained more than one pollen type, the dominant type was recorded. Pollen types were identified to family, genus or species where possible, on the basis of pollen morphology and colour using a combination of identification guides (Sawyer and Pickard 1981; Moore et al. 1991; Pollen-Wiki 2016; AutPal 2017). We screened pooled samples of 30 bees per colony microscopically for *Nosema* sp. following Fries et al. (2013) and Human et al. (Human et al. 2013) (see Supplementary Methods). Varroa mites obtained from a sample of 300 bees (see above and Supplementary Methods) were counted three times and the mode taken.

Statistical analysis

Pollen species composition was analysed using PERMANOVA to investigate whether communities differed between land-use types in spring and autumn. For all other analyses we followed an information theoretic approach to model selection (Grueber et al. 2011) (see Supplementary Methods). Final models were validated graphically to assess fit and check that assumptions had been met (Zuur and Ieno 2011), and examined for spatial autocorrelation by using a Moran's I test on the residuals and graphically assessing the spatial pattern of residuals.

Binomial GLMMs were performed to analyse proportion of pollen collected from woody species. Linear mixed models were used to analyse pollen species evenness (logit-transformed). A single extreme observation where a single species (*Impatiens glandulifera*) made up all thirty pollen samples was removed for this analysis; including it did not change the outcome. Pollen species richness was analysed using GLMMs with Poisson error structure, and pollen Shannon diversity was analysed with linear mixed models after scaling of the dependent variable. Colony strength (bee covered surface) was analysed using linear mixed models. Overwintering success, reported by beekeepers as a proportion of hives surviving the previous winter, was modelled using GLMs (binomial error structure). *Nosema* infection and *Varroa* infestation were analysed using zero-altered poisson hurdle models (*hurdle* function in R package *pscl*), where the response is modelled as a binary process (infection) and a zero-truncated count process (number of spores/mites; Zuur and Ieno 2011). *Nosema* spore counts were cuberoot transformed prior to analysis. Final models containing categorical variables were rerun with each factor level coded as the baseline variable to investigate pairwise differences between factor levels (Grotenhuis and Thijs 2015; see Table S3). Table 1 in the main text displays outputs with Land-use = Urban and Season = Autumn as the baselines. We carried out additional analyses to test the relationships between *Varroa* treatment, land-use and *Varroa* infestation (see Supplementary Methods).

All analyses were conducted in R version 3.2.1 (R Core Team 2018) using packages *MuMIn* (Barton 2018), *lme4* (Bates et al. 2015), *pscl* (Zeileis et al. 2008), *vegan* (Oksanen et al. 2018), *betapart* (Baselga et al. 2018), *beeswarm* (Eklund 2016) and *Hmisc* (Harrell Jr and Dupont 2018).

Results

Colony health

Land-use had a significant effect on colony strength (bee covered surface), with the best model retaining only land-use as a predictor (Fig 2a; ΔAICc to next best model = 3.05, Table S1a). The main driver of this effect was poor performance by colonies in rural open landscapes, which contained significantly fewer bees than all other land-use types (rural open parameter estimate with urban as baseline [95% CIs]: -1.101[-1.784- -0.481]; Table 1a; for full pairwise comparisons see Table S3c). Colonies from rural wooded sites were stronger, but still contained significantly fewer bees than those from suburban sites (rural wooded parameter estimate with suburban as baseline: -0.722[-1.411- -0.034]) although this difference did not reach statistical significance for urban sites (rural wooded parameter estimate with urban as baseline: -0.377[-1.073- 0.319]). Only apiary size affected overwintering success, with a positive relationship between apiary size and success (Table S2b).

Nosema infection was analysed through hurdle models in which the count process models spore count, and the binomial process probability of infection. In the autumn, both rural open and wooded sites showed significantly higher spore count than urban and suburban sites; (rural open parameter estimate with urban as baseline: 0.397[0.275-0.519]; rural wooded parameter estimate with urban as baseline: 0.310[0.186-0.433]; Table S3e & Fig 2b); accordingly, land-use, season and their interaction were all retained within the count process of every model in the candidate set (Table S1c). These differences dwarfed differences between site types in the spring (Fig 2b), although at this stage small but significant differences could still be detected between sites, with suburban displaying lower *Nosema* prevalence than urban and rural open (urban parameter estimate with suburban as baseline: 0.190[0.072-0.307]; rural open estimate with suburban as baseline: 0.218[0.102-0.334]; Table S3e & Fig 2b). The probability of infection did not differ significantly between sites (only "Apiary size" was retained in the binomial process within the final model set; Table 1).

For *Varroa,* land-use, season and their interaction along with apiary size were all retained in the count process of the best model (ΔAICc to next best model= 2.3). Unlike for *Nosema,* hives in all land-use types showed a rise in *Varroa* mite counts between spring and autumn (autumn parameter estimate with urban and spring as baseline: 2.295[1.773-2.816], Table S3d & Fig. 2). There was no consistent effect of urbanisation, with the highest autumn counts in urban and rural wooded colonies (suburban parameter estimate with urban as baseline: -0.594[-0.780- -0.407]; rural open parameter estimate with urban as baseline: -0.187[-0.358- -0.017]; Table S3d & Fig 2c). In contrast, in spring, *Varroa* counts were lower in urban than all other land-use types (suburban parameter estimate with urban as baseline: 0.954[0.349-1.559]; rural open parameter estimate with urban as baseline: 1.025[0.462-1.587]; rural wooded parameter estimate with urban as baseline: 0.960[0.405-1.515]; Table S3d & Fig 2c). In terms of the probability of infection, only season was retained in the binomial process of the best model (ΔAICc to next best model= 2.3; Table S1a).

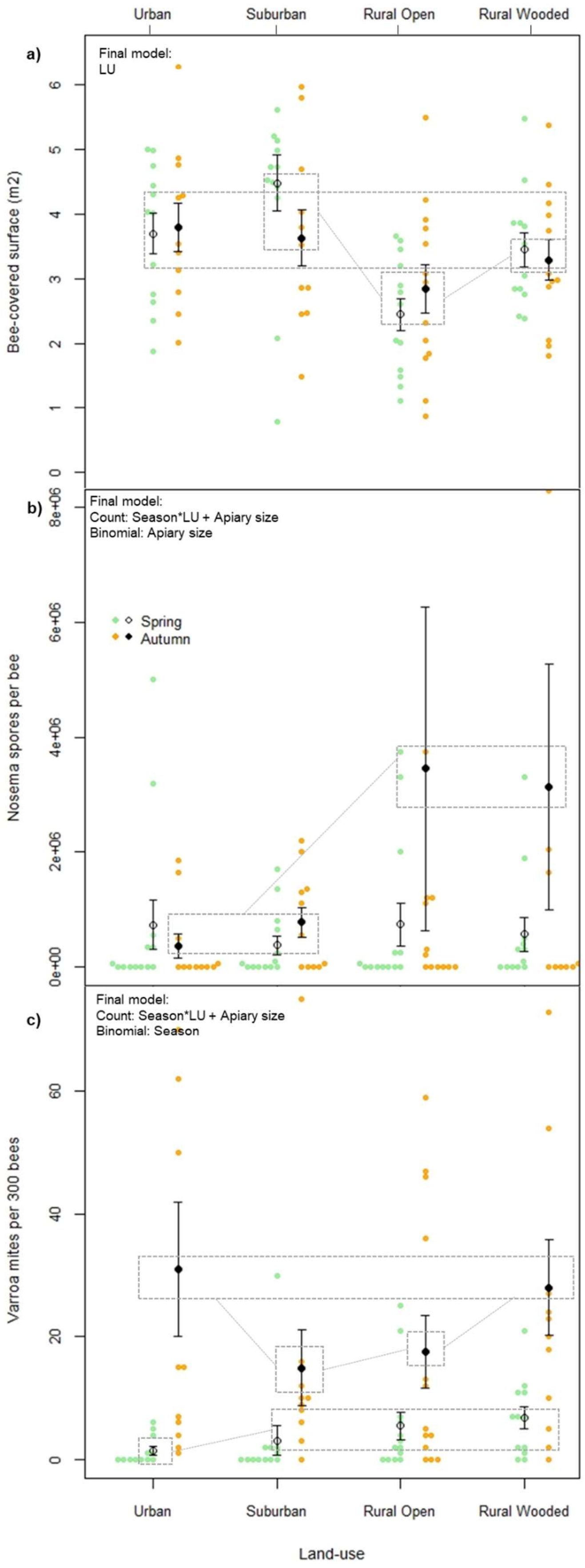
Urban
Suburban
Rural Open
Rural Wooded
a)
Final model:
LU
Bee-covered surface (m2)
0
1
2
3
4
5
6
b)
Final model:
Count: Season*LU + Apiary size
Binomial: Apiary size
◇ Spring
◆ Autumn
Nosema spores per bee
0e+00
2e+06
4e+06
6e+06
8e+06
c)
Final model:
Count: Season*LU + Apiary size
Binomial: Season
Varroa mites per 300 bees
0
20
40
60
Urban
Suburban
Rural Open
Rural Wooded
Land-use

Figure 2. Means and standard errors for a) colony strength, b) *Varroa* mite count and c) *Nosema* spore count across four land-use types in two periods, spring and autumn. Raw data are displayed as green (spring) and orange (autumn) points. Important significant pairwise differences with large effect sizes (see Table S3) are highlighted in grey boxes and variables included in the final model or model set are inset. LU = land use.

Pollen

Pollen Diversity

Land-use, season and their interaction along with apiary size were all included in the best model set for pollen species richness (Table S1e), with higher overall richness in urban than rural open colonies (spring rural open parameter estimate with urban as baseline: -0.320[-0.586- -0.054]; autumn rural open parameter estimate with urban as baseline: -0.316[-0.581- -0.051]; Table S3b & Fig. 3b). There was no effect on any of the measured variables on Shannon diversity (Fig. 3c) or evenness, with the null model showing the lowest AICc (ΔAICc to next best model; evenness: 3.93, Shannon diversity: 3.26; Tables S1f & S1g).

Species composition

In spring, the five most common pollen types in each land-use type made up on average 75.7 ± SE 3.0% of samples across land-use types compared to 48.8 ± 2.6% in autumn (Fig. 4c). Spring-collected pollen composition differed significantly between land-use types (PERMANOVA, df = 3, F =3.6, p = 0.005). Rural wooded and rural open species composition did not significantly differ, but composition in these land-use types differed significantly from those in urban and suburban, which also differed from each other (Fig. 5a; Table 1f). In the autumn, collected pollen composition was not significantly affected by land-use (PERMANOVA, df = 1, F =1.2, p = 0.160; Fig. 5b). These differences were reflected in the differences between land-use types in proportion of pollen collected from woody plants. Land-use, season and their interaction along with apiary size affected the proportion of pollen collected from woody plants (ΔAICc to next best model: 7.64; Fig. 3a & Table S1d). A significantly greater proportion of pollen was collected from woody plants in spring (mean ± SE: 81.9 ± 2.9%) than in autumn (mean: 10.5 ± 1.9%; autumn parameter estimate with spring and urban as baseline: 4.700[4.004-5.395]; Tables 1d & S3a). In spring, woody plants made up a greater proportion of collected pollen in urban and suburban than either rural land-use type (rural open parameter estimate with suburban as baseline: -2.220[-3.062- -1.379]; rural wooded parameter estimate with suburban as baseline: -1.728[-2.591- -0.865]) while in autumn there were no significant differences between land-use types (Tables 1d & S3a).

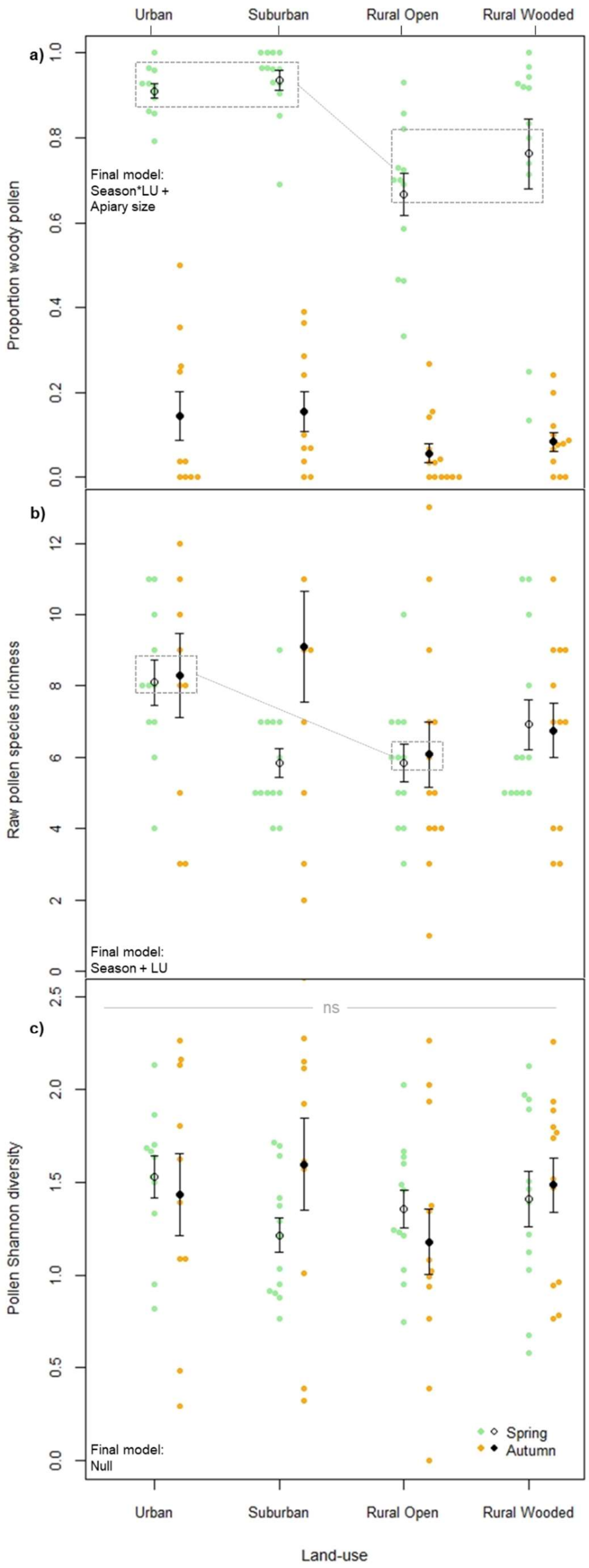
Urban
Suburban
Rural Open
Rural Wooded
a)
Proportion woody pollen
0.0
0.2
0.4
0.6
0.8
1.0
Final model:
Season*LU +
Apiary size
b)
Raw pollen species richness
0
2
4
6
8
10
12
Final model:
Season + LU
c)
ns
Pollen Shannon diversity
0.0
0.5
1.0
1.5
2.0
2.5
Final model:
Null
Spring
Autumn
Urban
Suburban
Rural Open
Rural Wooded
Land-use

Figure 3. Means and standard errors for a) proportion woody pollen, b) pollen species richness and c) pollen Shannon diversity across four land-use types in two periods, spring and autumn. Raw data are displayed as green (spring) and orange (autumn) points. Important significant pairwise differences with large effect sizes (see Table S3) are highlighted in grey boxes and variables included in the final model or model set are inset.

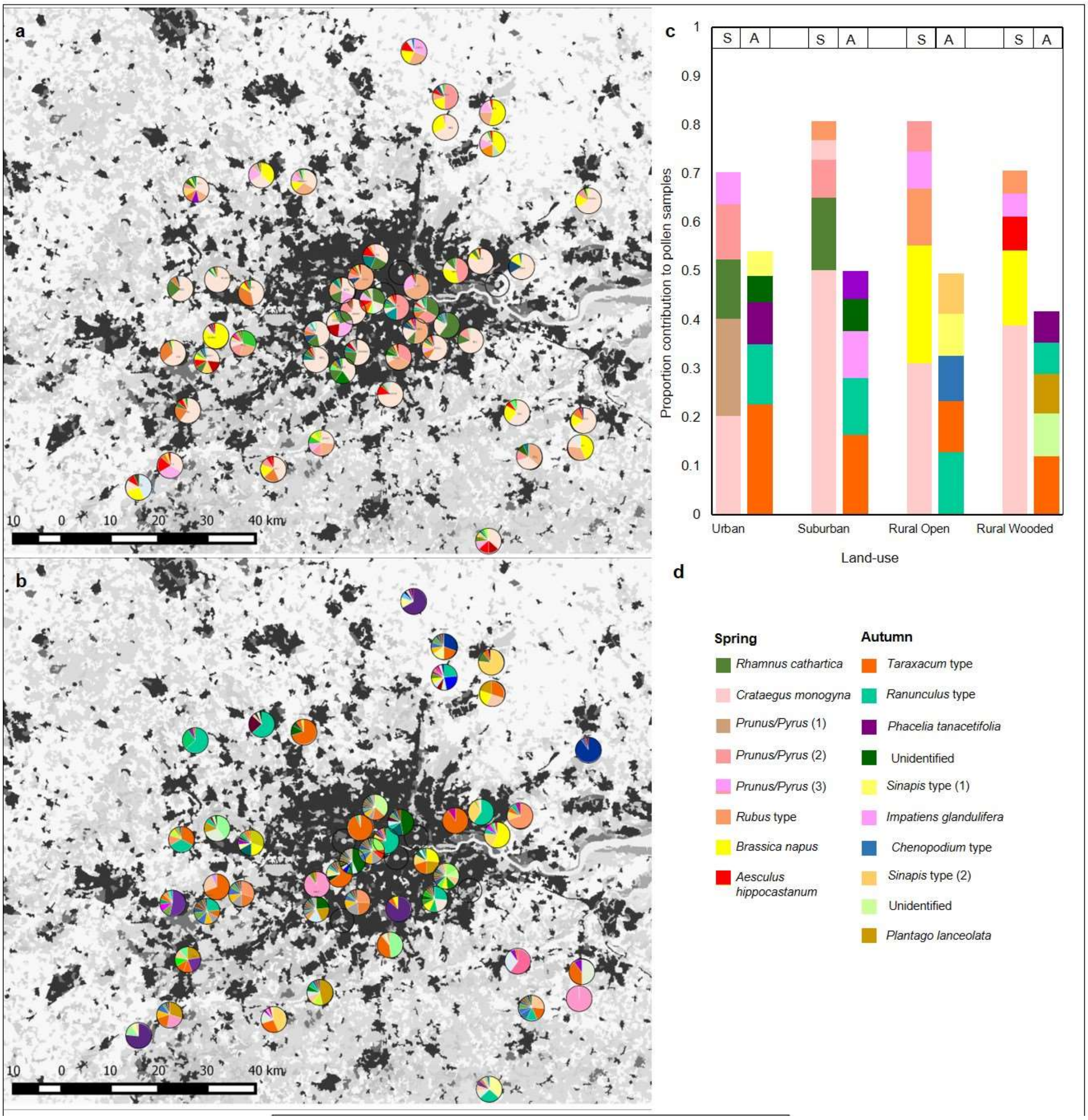

Figure 4. Contribution of different pollen types to pollen samples in each of 51 sites across SE England collected in a) spring and b) autumn. c) Five most important pollen types (greatest contribution) in each of four land-use types in spring and autumn. d) Colour legend for five most important pollen types in each land-use type. Additional pollen types and their colours are shown in the supplementary material.

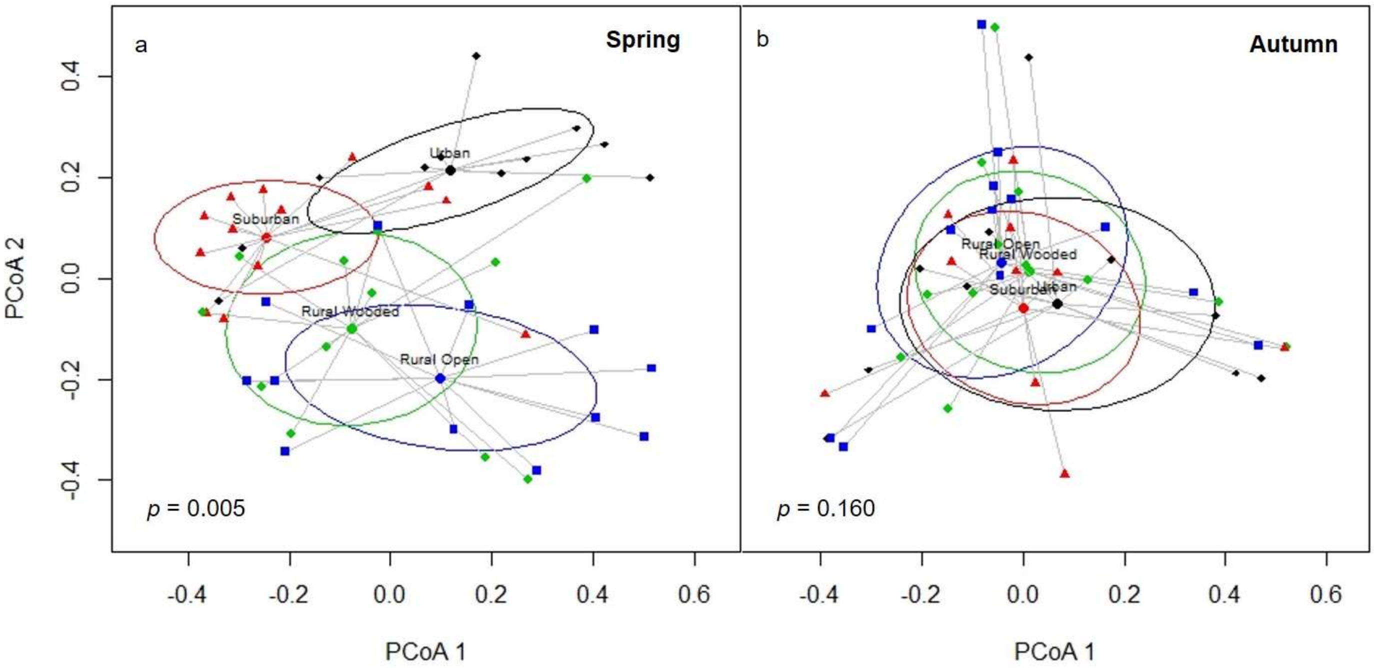

Figure 5. Principal co-ordinate analysis (PCoA) plots to visualise differences in pollen species composition in four land-use types in a) spring and b) autumn using Bray-Curtis distances (Beals 1984). Ellipses represent one standard deviation from the centroid and *p* values from PERMANOVA analyses are inset. Points represent individual sites: urban (black diamonds), suburban (red triangles), rural wooded (green diamonds), rural open (blue squares).

Table 1. a-e) Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable; see Table S1) for analyses where land-use was included in the optimal model(s). Parameters highlighted in bold are considered important to the model (continuous variables) or significantly different from the baseline (categorical variables) based on 95% CIs not crossing zero. All analyses shown have urban as the baseline for land-use and autumn as the baseline for season; for other baseline combinations see Table S3. f) Results from PERMANOVA tests to analyse the effect of land-use on pollen species composition in spring and autumn, and pairwise comparisons between land-use types in the spring.

a) Colony strength				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	3.742	0.254	3.244	4.239
Land-use (Suburban)	0.345	0.355	-0.351	1.041
Land-use (Rural Open)	**-1.101**	**0.349**	**-1.784**	**-0.418**
Land-use (Rural Wooded)	-0.377	0.355	-1.073	0.319

b) *Nosema*				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	4.499	0.057	4.387	4.611
Apiary Size	-0.015	0.018	-0.050	0.021
Season (Spring)	0.126	0.069	-0.010	0.262
Land-use (Suburban)	0.109	0.065	-0.019	0.237
Land-use (Rural Open)	**0.397**	**0.062**	**0.275**	**0.520**
Land-use (Rural Wooded)	**0.309**	**0.063**	**0.186**	**0.433**
Land-use (Suburban) : Season (Spring)	**-0.350**	**0.090**	**-0.528**	**-0.173**
Land-use (Rural Open) : Season (Spring)	**-0.422**	**0.087**	**-0.592**	**-0.252**
Land-use (Rural Wooded) : Season (Spring)	**-0.467**	**0.087**	**-0.638**	**-0.295**
Binomial process				
(Intercept)	-0.251	0.495	-1.222	0.720
Apiary Size	0.440	0.283	-0.115	0.994

c) *Varroa*				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	3.809	0.069	3.674	3.944
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Spring)	**-2.295**	**0.266**	**-2.816**	**-1.773**
Land-use (Suburban)	**-0.594**	**0.095**	**-0.780**	**-0.407**
Land-use (Rural Open)	**-0.187**	**0.087**	**-0.358**	**-0.017**
Land-use (Rural Wooded)	0.074	0.078	-0.079	0.227
Land-use (Suburban) : Season (Spring)	**1.548**	**0.323**	**0.915**	**2.181**
Land-use (Rural Open) : Season (Spring)	**1.212**	**0.299**	**0.626**	**1.798**
Land-use (Rural Wooded) : Season (Spring)	**0.886**	**0.294**	**0.311**	**1.462**
Binomial process				
(Intercept)	2.128	0.473	1.201	3.055
Season (Spring)	**-1.757**	**0.555**	**-2.845**	**-0.669**

d) Proportion woody pollen				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-1.823	0.447	-2.699	-0.947
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Spring)	**4.684**	**0.353**	**3.991**	**5.376**
Land-use (Suburban)	0.259	0.480	-0.682	1.200

Land-use (Rural Open)	-0.653	0.491	-1.615	0.309
Land-use (Rural Wooded)	-0.159	0.482	-1.104	0.785
Land-use (Suburban) : Season (Spring)	0.280	0.487	-0.675	1.234
Land-use (Rural Open) : Season (Spring)	**-1.030**	**0.450**	**-1.912**	**-0.148**
Land-use (Rural Wooded) : Season (Spring)	**-1.014**	**0.449**	**-1.895**	**-0.134**
e) Pollen species richness				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	2.041	0.115	1.814	2.267
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Spring)	-0.102	0.101	-0.299	0.095
Land-use (Suburban)	-0.076	0.158	-0.386	0.233
Land-use (Rural Open)	**-0.316**	**0.135**	**-0.581**	**-0.051**
Land-use (Rural Wooded)	-0.186	0.134	-0.448	0.076
Land-use (Suburban) : Season (Spring)	-0.422	0.220	-0.854	0.010
Land-use (Rural Open) : Season (Spring)	-0.019	0.226	-0.461	0.424
Land-use (Rural Wooded) : Season (Spring)	0.045	0.220	-0.386	0.477
f) Pollen species composition (PERMANOVA)				
Overall		F	R^2	P value
Spring		**3.653**	**0.199**	**0.005**
Autumn		1.269	0.029	0.159
Pairs (spring)				
Suburban vs Urban		**3.742**	**0.145**	**0.003**
Suburban vs Rural Open		**6.609**	**0.223**	**0.001**
Suburban vs Rural Wooded		**2.821**	**0.109**	**0.008**
Urban vs Rural Open		**4.578**	**0.179**	**0.001**
Urban vs Rural Wooded		**3.114**	**0.129**	**0.007**
Rural Open vs Rural Wooded		1.464	0.062	0.190

Discussion

Our results contribute to a growing body of evidence suggesting that many pollinator taxa may be positively associated with cities (Baldock et al. 2015; Hall et al. 2016; Samuelson et al. 2018). We found positive effects of urban land-use on honeybee colony strength and the raw species richness of collected pollen, alongside lower late-season counts for *Nosema* sp. infection in hives from urban and suburban sites. Colony strength was lower in rural open colonies than all other land-use types, complementing recent research demonstrating lower bumblebee colony growth in agricultural areas (Samuelson et al. 2018). Higher *Nosema* sp. infection in rural sites may have affected colony growth (Genersch 2010) or lower *Varroa* counts in urban colonies in the spring may have increased brood expansion (Rosenkranz et al. 2010), or forage availability may have limited colony growth (Dolezal and Toth 2018). There is evidence to suggest that honeybees have lower foraging success in rural than urban areas (Garbuzov, Schürch and Ratnieks, 2015; Lecocq *et al.*, 2015 although cf. Naug, 2009) and the nutritional quality (protein content) of pollen has been shown to be higher in built-up areas (Donkersley et al. 2017), despite the lack of difference in diet diversity demonstrated in the present study. In addition, colony growth may be affected by exposure to pesticides (Woodcock et al. 2017), which is likely to be higher in rural than urban areas (Botías et al. 2017). Although oilseed rape surrounding colonies in this study was planted after the EU moratorium came into effect (European Commission 2013) and therefore not treated with neonicotinoids, residues remain in the soil and in the nectar and pollen of wildflowers (Bonmatin et al. 2015) and treatment with other pesticides such as pyrethroids can also negatively affect honeybees (Ingram et al. 2015).

Our finding of higher autumn *Nosema sp.* spore counts in rural than urban and suburban sites may reflect greater prevalence of *Nosema* sp. in the environment in rural landscapes, possibly driven by transmission of the parasite from commercial bumblebee colonies in use in agricultural environments (Graystock et al. 2013), or more concentrated resource patches in agricultural areas such as mass-flowering crops leading to increased localised bee densities (Requier et al. 2015). Our results contradict findings from a USA study which showed higher *Nosema ceranae* loads in honeybee colonies (n=39) in more urbanised areas (Youngsteadt et al. 2015a), but this difference may reflect an urban heat island effect (Gago et al. 2013) as *N. ceranae* is cold-sensitive (Gisder et al. 2010). *Nosema apis,* which does not exhibit the same cold sensitivity (Youngsteadt et al. 2015a), is more prevalent than *N. ceranae* in UK populations (Klee et al. 2007).

Varroa mite counts were higher in autumn (mean: 22.7 mites per 300 bees) than spring (mean: 4.2), following typical patterns of build-up over the season (Rosenkranz et al. 2010). In autumn, *Varroa* counts were highest in urban and rural wooded colonies, a result not driven by *Varroa* treatment (see Supplementary Results). High *Varroa* mite counts in urban colonies may reflect increased parasite transmission due to higher hive densities (Alton and Ratnieks 2013; Brosi et al. 2017) associated with higher rates of drone drifting (Seeley and Smith 2015), or increased pressure on urban beekeepers to prevent swarming (Park 1990) as swarming introduces brood-rearing gaps that halt *Varroa* build-up (Loftus et al. 2016). Increased *Varroa* infestation in rural wooded areas may be due to concentrated foraging resources (Dornhaus and Chittka 2004; Peck et al. 2016) or knock-on effects of poor nutrition (Janmaat and Winston 2000; Dolezal and Toth 2018).

Identification of the pollen collected by colonies highlights important forage plants in urban and rural areas and suggests that a richer variety of floral resources are available in cities, with raw species richness (but not Shannon diversity) higher in urban than rural open colonies. This may be due to agricultural intensification reducing the available pollen sources in farmland areas (Lecocq et al. 2015) in contrast to the highly diverse array of exotic and native flowering plants available in urban gardens (Garbuzov and Ratnieks 2014a). Pollen diet richness has implications for honeybee health (Dolezal and Toth 2018), supporting immune function and glucose oxidase activity (an enzyme involved in food store sterilisation; Alaux et al. 2010), reducing disease mortality (Di Pasquale et al. 2013) and promoting acquisition of beneficial gut microorganisms (Anderson et al. 2013; Corby-Harris et al. 2016).

Spring pollen composition was strongly differentiated by land-use type, with distinct urban, suburban and rural (open + wooded) groups. In autumn there was no difference in composition across land-use, possibly because important pollen sources in autumn samples consisted of generalist plants such as *Taraxacum* (dandelion) and *Ranunculus* (buttercup) which are common weeds in both agricultural and urban landscapes (Sterry 2008; Hicks et al. 2016). In contrast, important spring sources were more likely to consist of agricultural or urban specialists, such as *Rhamnus cathartica* in urban (buckthorn, a common hedge plant; Kurylo and Endress 2007) and *Brassica napus* in rural areas (oilseed rape, a widespread agricultural crop; Garbuzov et al. 2015a). This is intensified by the fact that the five most important species made up a larger contribution (mean across land-use types: 76%) in spring than in autumn (mean: 49%), suggesting that colonies focussed collection more on these common plant sources in the spring than in the autumn. Spring pollen sources were dominated by woody plants (reflecting results from previous research; Keller et al. 2005), and urban and suburban colonies collected a significantly higher proportion of woody pollen than rural colonies. This reliance on pollen from trees and shrubs in the spring, when demand for protein is high while colonies are building up brood (Keller et al. 2005), highlights the importance of urban trees in otherwise high-density built-up areas (Macivor et al. 2014). In rural areas, *B. napus* contributed a substantial proportion of spring-collected pollen. However, no colony fed exclusively on *B. napus* when it was available, with the highest proportion of *B.napus* pollen at 53% and the mean (excluding colonies containing no *B. napus* pollen) at 22.6 ± 4%

(reflecting the 14% average found in a study by Garbuzov *et al*., (2015a)). This has implications for colony exposure to pesticides. Many studies calculate exposure assuming exclusive foraging on *B. napus* while it is in bloom (Whitehorn et al. 2012; Henry et al. 2012); our findings suggest that this would result in an overestimation of pesticide exposure. However, it is important to note that nectar collection may rely more heavily on mass-flowering crops than pollen (Samuelson et al.; Requier et al. 2015).

Our study demonstrates largely positive effects of urbanisation on honeybee colony success, supporting results from recent studies showing the benefits of urban areas for both domesticated (Garbuzov et al. 2015b; Lecocq et al. 2015) and wild (Baldock et al. 2015; Senapathi et al. 2015; Samuelson et al. 2018) bees. Lower *Nosema* infection and larger colony sizes in urban areas suggest honeybees can thrive in this anthropogenic habitat, while adding to the growing evidence that agricultural landscapes constitute poor habitat for pollinators. These results contribute to a growing body of research employing a "landscape physiology" approach to pollinator ecology, illuminating the interactions between land-use and pollinator health (Alaux et al. 2017). Future research should attempt to elucidate the drivers of the benefits of urban areas for bees to ensure these remain suitable habitats as urbanisation continues to contribute to land-use change in the future.

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Author Contributions

A.E.S. and E.L. conceived the initial idea and designed the experiment. R.J.G. provided feedback on the experimental design; A.E.S. performed the experiment and the statistical analyses; A.E.S. wrote the manuscript draft and E.L., R.J.G., and A.E.S. provided the final edit.

Urbanisation is associated with reduced disease pressure, larger colonies and more species-rich pollen forage for honeybees

Supplementary Material

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Supplementary Methods

Varroa destructor mite samples were taken using the icing sugar shake method (Macedo et al. 2002). A frame from the lowest brood box containing brood at all stages and freshly stored pollen was shaken into a washtub and the flying bees allowed to leave. A cup of c.300 bees (100ml) was collected and tipped into a jar containing 1tbsp icing sugar (Tate & Lyle, London, UK). This was rolled to ensure all bees were covered in sugar and left for 5 minutes in the shade, after which the icing sugar was shaken through the lid of the jar (size 8 hardware mesh) into a resealable plastic food bag. This was later dissolved in water and the number of mites counted three times and the mode taken.

We screened pooled samples of 30 bees per colony microscopically for *Nosema* sp. following Fries et al. (2013) and Human et al. (2013). We removed the abdomens of 30 frozen bees per colony and ground them with 30ml distilled water. 14µl of suspension was transferred to a haemocytometer, and the number of spores in five squares was counted. We did not identify the spores to species as *Nosema apis* and *Nosema ceranae* cannot be reliably differentiated microscopically. To obtain the *Nosema* sp. load for 30 bees, the following formula was applied:

$$sample\ volume\ (ml) \times \left(\frac{total\ no.counted\ particles\ \times dilution\ factor\ (\partial)}{area\ of\ squares\ counted\ (mm^2) \times chamber\ depth\ (mm)} \right)$$

We employed an information theoretic approach for all statistical analyses apart from pollen species composition. We used an "all-subset" approach to build a comparison set that comprised the basic model containing only the constant and residual variance and subset models containing combinations of the variables *land-use, season* and *log-transformed apiary size (number of hives)*. We also included a two-way interaction between land-use and season. We selected the model or set of models with the lowest AICc as the best fitting model(s) (Johnson and Omland 2004). Where several models were within two AICc units of the best model, model averaging was carried out to obtain parameter estimates derived from the best set of models including the basic model if applicable (Symonds and Moussalli 2011) and the conditional average reported as this excludes parameters not included in the models (Grueber et al. 2011). Where mixed models were appropriate, site was included as a random effect.

We carried out additional analyses to test the relationships between *Varroa* treatment, land-use and *Varroa* infestation. To test the effect of recent *Varroa* treatment on *Varroa* infestation, we reran the analysis for both seasons separately including the categorical covariate *Varroa treatment* as this was collinear with season. We retained the variable *land-use* as a chi-square test suggested this was not collinear with treatment. We grouped treatments carried out in the previous 6 months into "none", "physical" (including shook swarm and icing sugar), "thymol", "oxalic acid", "formic acid" and "pyrethroid", and within each season excluded treatment groups with <3 observations, resulting in the groups "none" and "oxalic" in spring and "thymol", "formic acid" and "none" in autumn. We carried out an additional analysis to investigate whether land-use affected beekeepers' decision to use *Varroa* treatment. We ran binomial GLMMs on *Varroa* treatment as a binary response with data from winter and summer treatments over the last three years for each site, with site as a random effect.

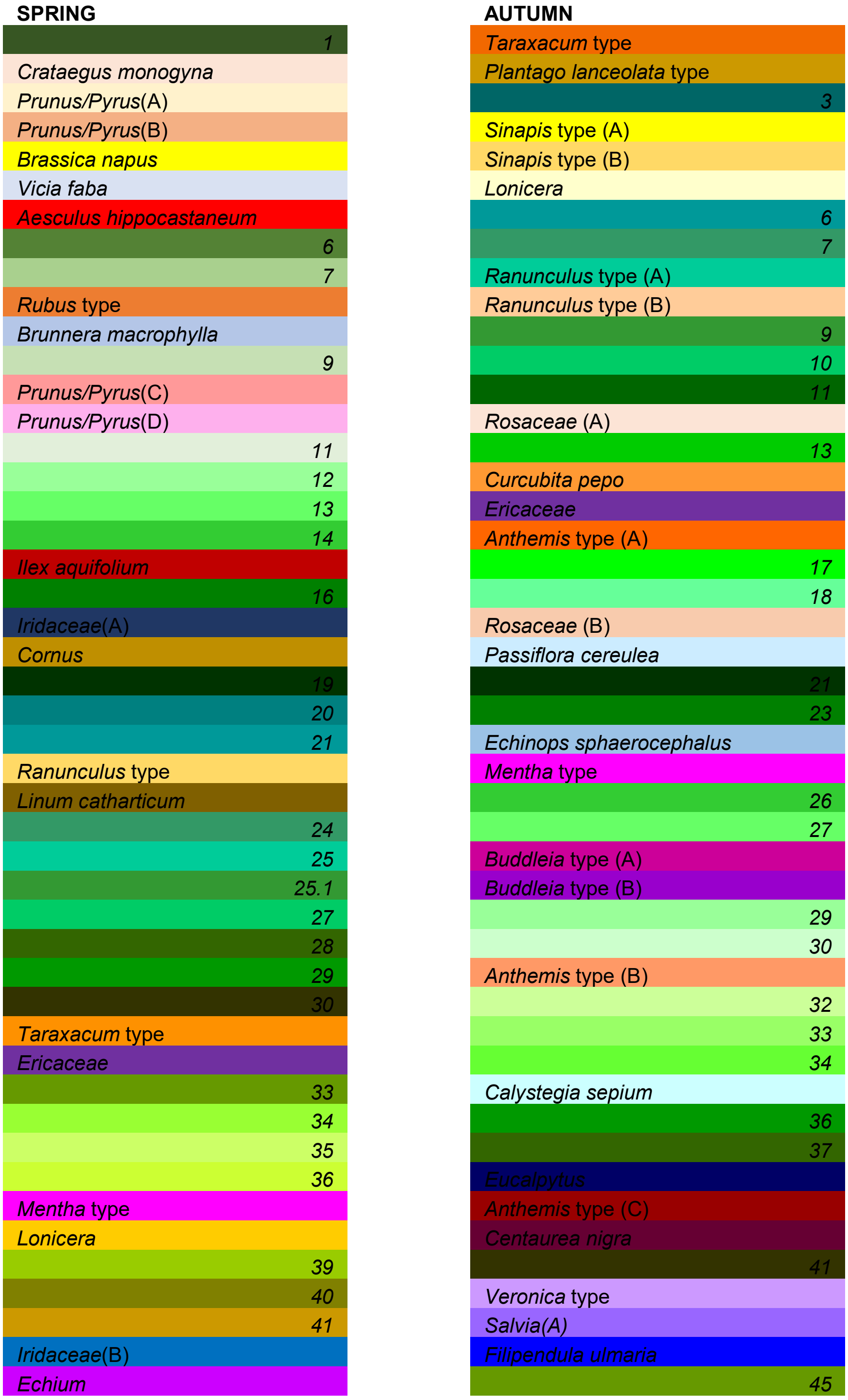
Supplementary Results and Discussion

Analysis of the effect of recent (≤ 6 months) *Varroa* treatment on mite count (separate models for each season due to seasonal differences in treatment practices) found that treatment in addition to land-use had an effect on *Varroa* infestation (Tables S1c & S1d). In spring, colonies that had been treated with oxalic acid had lower mite counts than those that had not been treated in the last six months, while in autumn colonies that had been treated with formic acid had fewer mites than those treated with thymol, which in turn had fewer than those not treated at all (Fig S2; Tables S2d & S2e). A formal analysis of the association between land-use and treatment practice found that there was no effect of land-use on the likelihood to use *Varroa* treatment; in other words beekeepers in one land-use type were no less likely to treat their hives than those in another (Tables S1b & S2c), suggesting that differences in mite counts between land-use types do not reflect differing treatment between land-use types. Apiary size also had a small negative association with *Varroa* infestation (independently of land-use as the apiary size of our sites was balanced across land-use types), meaning that larger apiaries had slightly lower mite counts.

Overall, the unbalanced pollen composition with a high contribution of common plants (overall mean: 62%) reflect findings from previous research, with the identity of important species also matching those found in other studies (review: Keller et al. 2005).

Supplementary Figures

Figure S1. List of pollen types and colour legend for Figs 1a and b. Where it was not possible to visually identify pollen types to species/genus/family, morphotypes were given a unique number.



SPRING

1
Crataegus monogyna
Prunus/Pyrus(A)
Prunus/Pyrus(B)
Brassica napus
Vicia faba
Aesculus hippocastaneum
6
7
Rubus type
Brunnera macrophylla
9
Prunus/Pyrus(C)
Prunus/Pyrus(D)
11
12
13
14
Ilex aquifolium
16
Iridaceae(A)
Cornus
19
20
21
Ranunculus type
Linum catharticum
24
25
25.1
27
28
29
30
Taraxacum type
Ericaceae
33
34
35
36
Mentha type
Lonicera
39
40
41
Iridaceae(B)
Echium

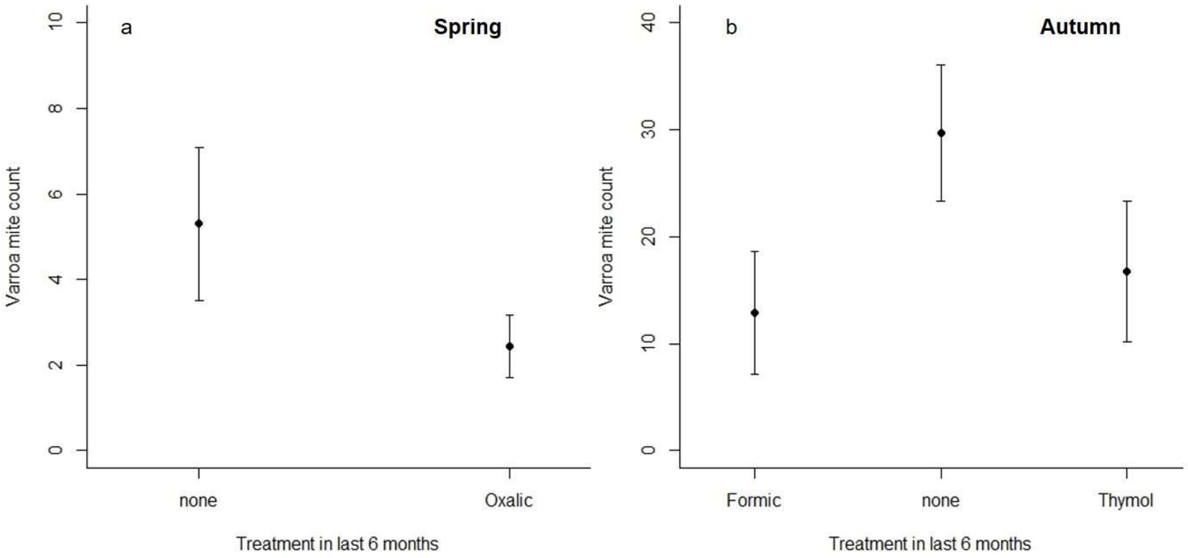
AUTUMN

Taraxacum type
Plantago lanceolata type
3
Sinapis type (A)
Sinapis type (B)
Lonicera
6
7
Ranunculus type (A)
Ranunculus type (B)
9
10
11
Rosaceae (A)
13
Curcubita pepo
Ericaceae
Anthemis type (A)
17
18
Rosaceae (B)
Passiflora cereulea
21
23
Echinops sphaerocephalus
Mentha type
26
27
Buddleia type (A)
Buddleia type (B)
29
30
Anthemis type (B)
32
33
34
Calystegia sepium
36
37
Eucalyptus
Anthemis type (C)
Centaurea nigra
41
Veronica type
Salvia(A)
Filipendula ulmaria
45

44
Compositae type
Malvaceae type
47
48
Phacelia tanacetifolia

46
Impatiens glandulifera
48
Gladiolus type
50
51
52
53
Phacelia tanacetifolia
Zea mays
Umbelliferaceae (A)
Umbelliferaceae (B)
Geranium/Pelargonium
Salvia(B)
61
Epilobium parviflorum
Borago officianalis
Chenopodium type
Veronica serpyllifolia
Iridaceae type
Oenothera biennis
68
69
Compositae type
Iridaceae
72
Asparagus
Begonia
Vicia faba
76
77
78
80
81
82
83

Figure S2. Mean (± SE) *Varroa* mite count for colonies treated with different *Varroa* treatments in the last six months. a) In spring colonies were either treated with no treatment or oxalic acid (sublimation or trickle). b) In autumn colonies were treated either with formic acid, thymol or no treatment. A small number of colonies were treated with a pyrethroid (n=1) or a physical method e.g. icing sugar or shook swarm (n=3); these were excluded from the analysis.



Supplementary Tables

See Appendix 2

Chapter Six

Samuelson, AE; Schürch, R and Leadbeater, E. *submitted.* Waggle dance analysis demonstrates greater forage availability for honeybees in urban than rural areas.

Submitted to *Nature Ecology & Evolution*

Waggle dance analysis demonstrates greater forage availability for honeybees in urban than rural areas

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Abstract

Urban areas are predicted to expand in the coming decades, bringing both domesticated and wild bees into increasing contact with anthropogenic habitats. Recent evidence suggests that modern agricultural land provides poor foraging resources for bees; could cities, with their diverse and abundant flora, represent refuges within a barren agricultural landscape? Waggle dance decoding is a unique and potentially sensitive method for investigating honeybee foraging behaviour whereby the dances performed by foragers are "translated" to identify foraging locations. Here we present the first large-scale dance-decoding investigation of differences between urban and rural foraging. We decoded 3378 waggle dances over two years at twenty sites in urban and agricultural land in SE England to investigate differences in foraging distance as a proxy for forage availability. Foraging trip distance was consistently lower at urban sites across the season, suggesting a higher availability of forage in the city. This was not compensated for by higher quality forage in rural areas; indeed, urban bees collected nectar with a higher mean sugar content. Land-use preference analysis highlighted residential gardens as important sources of urban forage, while mass-flowering crops represented preferred foraging resources in the countryside. These findings provide a potential mechanism for recent studies showing positive

effects of urbanisation on bee populations, and highlight the insufficient and transient resources provided by modern agricultural landscapes.

Introduction

Bees are extremely important pollinators of both crops and wild plants (Grünewald 2010). It is of concern, therefore, that bee populations are under threat from environmental stressors, including from emerging and invasive parasites and disease (Fürst et al. 2014; Brosi et al. 2017) and pesticide use (Wood and Goulson 2017; Siviter et al. 2018b). The most pressing threat, however, is habitat loss and the associated reduction in available forage (Potts et al. 2010a). Agricultural intensification over the last century has resulted in the widespread conversion of flower-rich habitat such as wildflower meadows and hedgerows to typically nutritionally-poorer land such as cereal crops (Williams et al. 2015). As a result, forage availability for bees has drastically declined in recent years (Carvell et al. 2006; Biesmeijer et al. 2006; Roulston and Goodell 2010).

One land-use type potentially deviating from this trend is urban land. Urbanisation is a rapidly growing driver of land-use change, with an estimated three-fold increase in global urban land cover predicted between 2000 and 2030 (Seto et al. 2012). Although urbanisation negatively impacts many animal taxa (Adams 2005; Aronson et al. 2014), there is growing evidence to suggest that cities and towns may support bee populations (Baldock et al. 2015, 2019; Hall et al. 2016; Samuelson et al. 2018). Urban land potentially offers high floral abundance and diversity in the form of gardens and parks: 87% of UK homes have a garden, with a total area of 432,924 ha (Davies et al. 2009). In contrast to agricultural resources such as mass-flowering crops, gardens and parks are often designed to encourage blooming across the season (Plascencia and Philpott 2017) and in recent years there has been an emphasis on bee-friendly planting in urban areas (Garbuzov and Ratnieks 2014b; Threlfall et al. 2015). As such, foraging success may be higher in urban bees: Lecocq *et al.* (2015) showed that honeybee (*Apis mellifera L.)* hives in urban areas gained more weight than hives in agricultural or mixed landscapes, and a recent study showed increased food stores in urban compared to agricultural bumblebee colonies (Samuelson et al. 2018). However, the vast array of flowering plants on display in cities may not always be beneficial for bees. Horticultural plants vary widely in their attractiveness to bees, with some artificially selected ornamental varieties displaying inaccessible nectaries or exotic varieties possessing adaptations for vertebrate pollination (Garbuzov and Ratnieks 2014a), meaning that only a small proportion of the flowers available in urban land are attractive to insects (Garbuzov and Ratnieks 2014a; Garbuzov et al. 2014). In line with this, a number of studies have found negative effects of urbanisation on honeybee food collection (Naug 2009; Sponsler and Johnson 2015); it is unclear from the current available evidence, therefore, whether urbanisation supports or constrains bee foraging.

Here, we survey the floral resources available to honeybees in urban and agricultural environments by capitalizing upon the unique communication behaviour of this generalist pollinator. Honeybees collect food from a broad range of forage resources that incorporate many species also visited by wild bee communities (Thomson 2004), across a vast foraging range (up to a 10km radius; Seeley 1995). Successful foragers communicate locations of profitable resources to their nestmates, by performing a figure-of-eight “waggle dance” on the comb that encodes the distance to the resource (in the duration of the “waggle” run) and the angle from the sun’s azimuth (in the angle of the dance relative to gravity; von Frisch 1967). By decoding these dances, it is possible to obtain filtered real-time information about the forage sites that have been found by the hive’s workforce (Grüter et al. 2010) that is relatively less affected by proximity to local hotspots than traditional surveying, with no access limitations (a key hurdle in surveying urban areas). Because honeybees are economical foragers that are unlikely to travel to distant resources when near ones of similar quality are available, the distance of these sites from the hive acts as a proxy for forage availability (Visscher and Seeley 1982; Waddington et al. 1994; Couvillon et al. 2014a, 2015; Couvillon and Ratnieks 2015), while the quality of forage can be independently verified by non-destructive assay of the sucrose content of forager-collected nectar (Couvillon et al. 2014a).

Initial research using waggle dance decoding in single-site experiments has suggested mixed impacts of urbanisation on forage availability. Garbuzov *et al.* (2015b) found that distances communicated by bees in a single urban location were typically lower and showed less seasonal variation than those communicated by bees in a rural site in a comparable study in the same region (Couvillon et al. 2014a).

Waggle dance communicated distances in suburban Florida and California were found to be shorter than those in a comparable experiment in temperate deciduous forest (Visscher and Seeley 1982; Waddington et al. 1994). In contrast a study analysing dance frequency for different land-use types surrounding an apiary found no evidence for a preference for urban areas; indeed, there was a non-significant trend towards avoidance of urban land (Couvillon et al. 2014b), and analysis of dances from a single hive located on a boundary between urban and rural land showed a preference for rural foraging (Sponsler et al. 2017). However, no study to date has compared waggle dances from urban and rural hives in the same experiment.

Here, in the most geographically extensive waggle dance study to date, we decoded 2827 dances from ten urban and ten rural hives in SE England over 24 weeks from April-September 2017 and an additional 551 dances in 2016 to investigate differences in foraging patterns between urban and rural hives. We compared foraging distance as a proxy for forage availability between the two landscape types alongside analysis of nectar sugar concentration to investigate whether differences in foraging distance might be energetically compensated for by differences in quality. We also investigated preferences for specific land-use types within the urban and rural landscapes across the season by simulating visits to habitat patches surrounding hives and analysing the probability of visitation once distance is taken into account. Our findings have implications for our understanding of urban and rural resource provision not only for honeybees but also potentially for some other pollinator taxa that rely on similar forage sources.

Methods

Sites

We selected ten urban and ten rural sites in SE England that either had existing observation hives (n=10) or an existing apiary where it was possible to situate an observation hive for the duration of the experiment (n=10) (Fig. 1). Sites were located at least 5000m apart to minimise overlapping of foraging ranges and were selected to be representative of the extremes of the urbanisation gradient in the region. We classified the land at a 2500m radius (incorporating the 95th percentile of recorded dances) around each site using QGIS v3.0.2 following methods outlined in Samuelson & Leadbeater (2018). Briefly, we generated land-use maps by drawing polygons around habitat patches on a satellite imagery (Bing Maps) base layer and classifying these patches into 33 land-use categories, which were used both to confirm the similarity of sites in urban and rural groups and for later land-use preference analysis. Land classification using satellite imagery does not account for transient crops (Samuelson and Leadbeater 2018) such as oilseed rape (OSR), which may be an important driver of honeybee foraging patterns (Requier et al. 2015), so we additionally performed aerial surveys to locate OSR fields at each rural site during May (the OSR bloom period). Using a drone (DJI Phantom 4; DJI, Shenzhen, China) we recorded a 360° video of the landscape around the hive from 120m directly above the hive. This video was then used to fill in the location of OSR fields (seen as bright yellow patches) in the GIS maps.

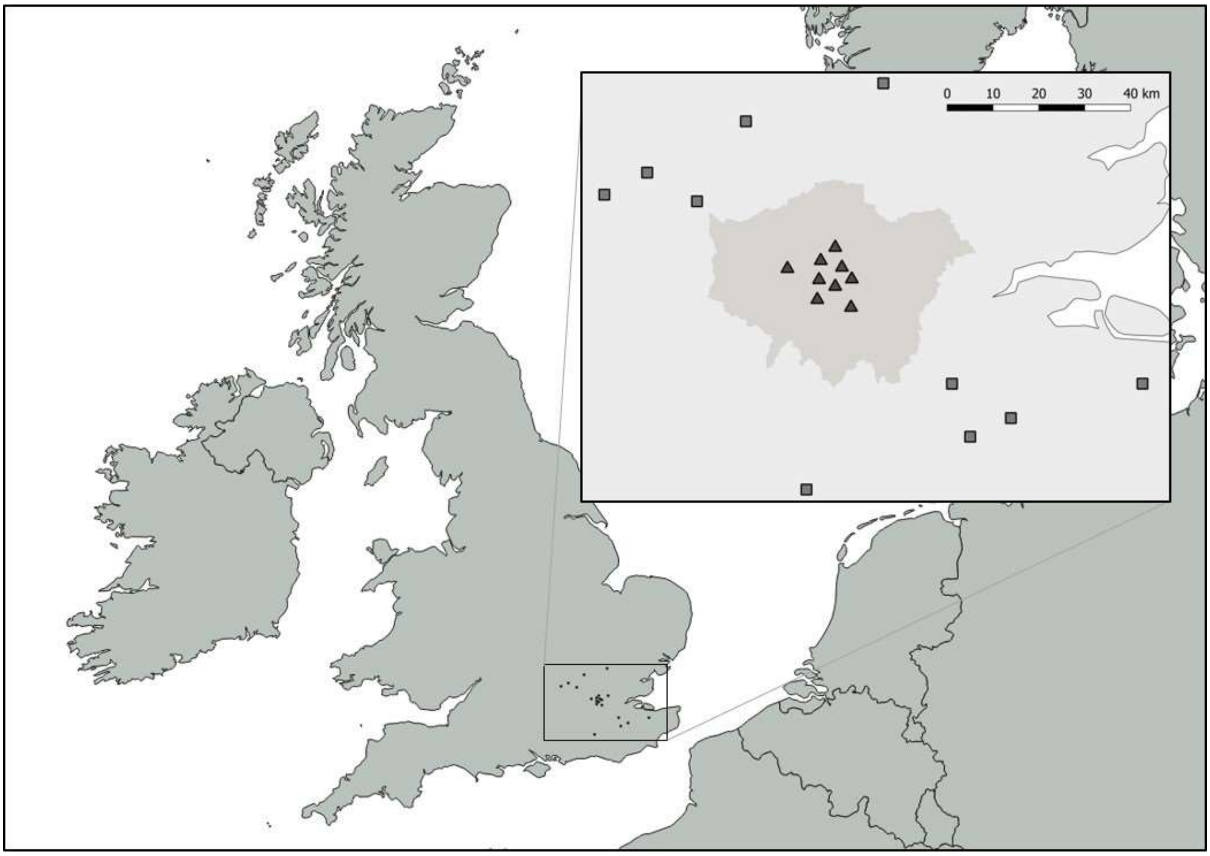

Figure 1. Location of ten urban (triangles) and ten rural (squares) observation hives in SE England. In the inset map the Greater London area is indicated by dark grey shading. Hives were located in the highly urbanised centre of London and the agricultural areas around London to represent extremes of an urbanisation gradient.

Honeybee colonies

An observation hive containing a honeybee colony with workers, brood and a queen was placed at each site. Observation hives are hives with glass or Perspex typically covering a set of frames one comb deep so that bee behaviour on the comb can be observed from both sides. Because some sites (n=10) had existing hives, hive size varied slightly from 3 to 8 occupied frames. At sites that did not have existing observation hives we used standard three frame hives (two shallow and one deep) (Thorne, Windsor, UK). At sites where we set up the hives, hives were situated in plastic storage sheds (130 x 74 x 110 cm; Keter, Birmingham, UK) with access to the outside through a clear PVC tube (25mm diameter; RS Components, Corby, UK). Colonies were supplied with supplementary sugar syrup (50% Brix) in a gravity feeder at the top of the hive only when no nectar was visible in the storage cells or when a period of poor weather was expected to avoid starvation. If colonies died they were replaced immediately with a new nucleus from the same apiary if possible or from the university apiary (n=5). Colonies were checked every few days by apiary managers to ensure adequate stores were available and the pipe was not blocked, and thoroughly every two weeks before data collection. Swarm control was carried out between April and July by removing a frame of brood from hives that had become crowded (all frames covered with bees) and by removing queen cells; no colony swarmed during the experiment. At the end of the experiment colonies were merged with other colonies at the same apiary or transferred to nucleus hives at the university.

Data collection

Each site was visited once every two weeks for 24 weeks between April and September 2017 (a total of 12 visits to each site). Two sites were visited in a day in the morning (between 8:00 and 12:00; "AM") and afternoon (between 12:00 and 17:00; "PM") in nearby pairs, alternating between urban and rural pairs each day. The order of visits was kept approximately consistent throughout the experiment (weather permitting) and the period (AM or PM) of the visits alternated each fortnight. Sites were visited only on sunny, warm (>12°C) and calm (wind speed <15km/h) days to ensure bees were foraging. Site data recorded included weather and colony strength (number of frames covered with bees to nearest 0.5). Temperature data was taken from the London Heathrow weather station (wunderground.com). Daily average temperature was used rather than maximum or minimum as this correlated most strongly with temperature data recorded by us inside the hive housings (buildings/sheds) at the time of visits.

Two hours of waggle dance data were recorded by training a camcorder (Canon Legria HF R606, Amstelveen, NL) onto the dancefloor (the location where most dance activity is seen; Couvillon et al. 2012). Plumb lines to provide a reference for gravity and a radio-controlled clock were attached to the glass in the field of view. At the end of filming we collected nectar sugar concentration data following Couvillon *et al.* (2014a) by blocking the entrance to the hive and collecting ten returning foragers not carrying pollen. Following anaesthesia in a cool bag containing ice blocks we stimulated regurgitation by massaging the bees' abdomens with forceps. Using a microcapillary tube (Blaubrand Intramark, Wertheim, Germany), crop contents were transferred to a 0-80% Brix refractometer (Kern, Balingen, Germany) to measure sugar concentration. Sugar concentration is an important axis of nectar quality as honeybees calculate the energetic efficiency of a foraging trip to evaluate a forage site and therefore higher sugar concentration may compensate for longer foraging distance (Seeley 1994). However, a caveat of this measure is that it does not account for increased viscosity associated with higher sugar concentration, which above a threshold concentration reduces the energetic efficiency of a resource by increasing the time taken to consume it (Bailes et al. 2018). It also does not include other axes of forage quality including nectar micronutrient and amino acid composition and pollen quality (Vaudo et al. 2015).

Dances were also recorded weekly for two months (July-August) in 2016 at four of the sites, two urban (ZSL & HOR) and two rural (HER & YAL), to investigate whether results were consistent across years.

Waggle dance decoding

Using video analysis with QuickTime 7.1 (frame-by-frame playback at 25fps) we decoded up to 40 dances per session (mean = 15.5) following methods outlined in Couvillon *et al.* (2012). Briefly, we decoded four waggle runs for each dance, excluding the first and last runs, as these have been shown to exhibit more variation than middle runs. For each run we recorded the angle from vertical by connecting two dots drawn on the bee's thorax early and late in the run on acetate overlaid on the computer screen and measuring the angle of the intersection of this trajectory and the plumb line. This was converted into an azimuth by adding the angle to the sun's azimuth at the time of the dance (http://www.esrl.noaa.gov/gmd/grad/solcalc/). We measured run duration by recording the first frame in which the bee started vibrating its body and the first frame after the vibration had finished. When a run was interrupted (e.g. by colliding with another bee), that run and the next were skipped to avoid an effect of incoming angle on angle from vertical. When high dance activity was observed resulting in difficulty differentiating individual dancers over time, each dance at a single timepoint was decoded and the video skipped forward six minutes to avoid recording the same dance twice.

Statistical analysis

To analyse waggle run duration (as a proxy for foraging distance) and nectar sugar content we built GAMMs allowing for a non-linear effect of our time variable, fortnight, on the response, and including site as a random effect. To incorporate an interaction between land-use and fortnight we allowed separate smoothers. For waggle run duration, the response variable was log transformed median run duration per video. We combined data within videos (representing a single sample session for each hive) because although data collection was designed to minimise the possibility of recording the same bee twice (see *Waggle dance decoding)* it is not possible to identify individual foragers and so we cannot rule out the possibility of a small proportion of dances being carried out by previously recorded individuals, which would cause an analysis of the raw data to violate the assumptions of independence. Analysis of the raw data resulted in the same results as our main analysis using video medians. Due to concurvity (non-linear collinearity) between fortnight and temperature, we used sequential regression (Graham 2003) to generate the variable residual temperature by regressing temperature against fortnight using a GAM and extracting the residuals to produce a variable containing the variation in temperature not explained by fortnight. Decoder was included to test for an effect of which researcher decoded the dances, and was split into the lead researcher (69% of dances) and trained research assistants (n=9, 31% of dances). The full model for waggle run duration contained the covariates land-use, fortnight, residual temperature, filming period (AM or PM) and decoder.

Model selection was carried out using a "full subset" approach with a set of models containing all combinations of covariates plus a basic model containing the intercept and random effect. We selected the model with the lowest AICc (Akaike's Information Criterion corrected for small sample sizes) as our best fitting model(s). Where one or more models were within 2 ΔAIC of the best model, we performed model averaging on the best model set (Symonds and Moussalli 2011). Nectar

sugar content (% Brix) was analysed using the same approach, with the covariates land-use, fortnight, residual temperature, period (AM or PM) and colony strength. We excluded zero values as these indicate bees that were collecting water (Couvillon et al. 2014a). Colony strength (bee-covered surface) was calculated by multiplying the number of frames covered with bees by the surface area of the relevant frames, depending on hive type (National or Commercial) and frame size (Deep or Shallow). We collected additional data in 2016 from a subset of hives (two urban, two rural) over eight weeks to confirm that our results were consistent across years. Because this dataset was smaller (n=551 dances) we pooled dances across the study period and instead of using median durations we accounted for non-independence of dances by including *video ID* as a random effect. Final models were validated graphically to assess fit and check that assumptions had been met (Zuur and Ieno 2011), and examined for spatial autocorrelation by using a Moran's I test on the residuals and graphically assessing the spatial pattern of residuals.

Land-use preference analysis

The strong difference found in foraging distance between urban and rural hives led us to investigate which land-use types within these differing landscapes received most attention by foraging bees during different seasons (spring: April-May, summer: June-July, autumn: August-September). For each site, we produced a land-use raster of radius 2500m (incorporating the 95th percentile of recorded dances) and resolution 25m. The raster separated land-use patches into broad categories selected for ecological relevance to pollinator use of the landscape (Samuelson and Leadbeater 2018), combined from land-use classes in our initial GIS classification. In rural landscapes the categories were: built-up, non-agricultural, woodland, arable, pasture, fruit, oilseed rape and other agricultural; in urban landscapes the categories were: continuous urban, dense residential, sparse residential, parks, amenity grassland, railway, woodland and water (including riverbanks).

To convert waggle run durations to foraging trip distances we used a universal calibration derived from waggle dance data from multiple honeybee populations worldwide (Schürch *et al.* in prep.) which relates waggle run duration to distance using a linear equation and incorporates variation in distance communication. For a single site-season combination we simulated a single foraging location for each recorded dance (mean n=47.86 ±SE 3.22), following methods outlined in Schürch *et al.* (2013b) to incorporate variability in angle and distance communication. Each land-use patch was recorded as visited or not visited by one or more of the simulated foraging visits, along with its land-use type, area and distance of nearest edge to the hive. This was repeated for each site in a landscape-season combination, e.g. spring data for all sites in urban landscapes. This generated a data frame containing the variables land-use patch ID, land-use type, visited (0/1), distance from hive, site ID. A binomial GLMM was constructed with visited as the response and fixed effects of land-use, distance (allowing for an inverse relationship between distance and visit probability; Schürch et al. 2013b), log-transformed area and a random effect of site ID. The adjusted odds ratios (AORs) for visitation of each land-use type relative to the baseline type (selected as the most urban land-use type: "continuous urban" in urban landscapes, "built-up" in rural landscape), corrected for distance to the hive, were extracted from the model and stored. This procedure was simulated 1000 times, so that foraging locations reflected the distribution of probabilities defined by the calibration described above. Each iteration generated AORs from the model; the median AOR and 95% confidence intervals (CIs) were extracted. The entire procedure was repeated for each of the six landscape-season combinations. All analyses were conducted in R version 3.2.1 (R Core Team 2018) using packages *MuMIn* (Barton 2018)*, circular* (Agostinelli and Lund 2017)*, ascii* (Hajage 2011)*, raster* (Hijmans 2017)*, sp* (Pebesma and Bivand 2005; Bivand et al. 2013)*, rgdal* (Bivand et al. 2018)*, rgeos* (Bivand and Rundel 2017)*, FSA* (Ogle 2018)*, lme4* (Bates et al. 2015)*, beeswarm* (Eklund 2016) and *Hmisc* (Harrell Jr and Dupont 2018).

Results

Waggle run duration

We decoded 2827 waggle dances (1428 urban and 1399 rural) in 183 site-fortnight combinations in 2017 (see Fig. S1 for all dance plots). Waggle run duration ranged from 0.12 to 12.44 sec, which translates to a minimum distance of 56m and a maximum distance of 9523m. Median distance across all sites and fortnights was 897m. We found a strong overall effect of land-use on waggle run duration, with the optimal model set containing land-use and its interaction with fortnight captured in separate smooth terms for each land-use type, showing the relationship between fortnight and duration differed between urban and rural sites (Table 1a). Overall, waggle run durations were significantly longer in rural sites than urban sites, with the rural median higher than the urban median at each fortnightly time point (Fig. 3a). Translated to foraging distance, the urban median was 708m (mean: 979m; maximum: 9523m) and the rural median was 1108m (mean: 1402m; maximum: 8599m). There was no effect of residual temperature (additional to its contribution to the effect of fortnight, Table 2a) or of filming period (AM or PM; Table 2a). Whether a dance was decoded by the lead researcher or a trained assistant was not included in the optimal model set and had no effect on recorded duration (Table 1a).

In rural hives duration did not follow a strong seasonal pattern, whereas urban hives followed a pattern of shorter durations in the spring and longer durations for the remaining season (Fig 3a). Investigation of seasonal patterns at separate sites shows that this is likely to be due to peaks of availability differing across individual rural sites, consistent with the existence of transient resource patches such as mass-flowering crops (Fig. 4). Fortnightly variation (standard deviation) in median log-transformed durations across sites was significantly higher in rural hives than urban (ΔAIC to null model: 2.63; effect size ± 95% CIs: 0.122 [0.019-0.224]).

Data from 551 dances decoded from a subset of hives (two urban and two rural) collected in 2016 produced similar results, with a strong effect of land-use on duration (Fig 3b, Tables 1b & 2b). Translated to foraging distance, the urban median was 475m (mean: 540m; maximum: 2517m) and the rural median was 927m (mean: 1288m; maximum: 3979m), indicating strong consistency across years.

Nectar sugar content

Land-use and its interaction with season affected nectar sugar concentration, with overall higher sugar content in urban land than rural (Tables 1b & 2b). In both land-uses nectar sugar content declined over the season, although this decline was less smooth in urban land (Fig. 3c). Mean sugar content (excluding zero samples which indicate water collection and made up 2.6% of all samples) across all sites was 39.35(± 0.59)% Brix (urban mean: 41.38(±0.99)%; rural mean: 38.02(±0.73)%).

Land-use preference

In urban areas across the season bees showed a large significant preference for sparse residential land (spring odds ratio (OR) [95% CIs]: 5.4 [3.0-10.0]; summer: 4.6 [2.9-7.2]; autumn: 4.2 [2.6-6.8]) and a small significant preference for dense residential land (spring OR: 2.4 [1.3-4.3]; summer: 3.4 [2.1-5.8]; autumn: 3.3 [1.9-5.5]) relative to the baseline of continuous urban (Fig. 5a-c). Visitation to woodland, water, railway, parks and urban grassland was not different to the baseline. In rural areas in spring (Fig. 5d), bees showed a strong preference for oilseed rape fields (OR: 24.8 [13.0-46.0] relative to the baseline of built-up land. In summer and autumn, bees showed a small significant preference for non-agricultural land (summer OR: 1.6 [1.0-2.6]; autumn: 2.1 [1.2-3.6]). In all seasons, rural bees showed a small significant preference for pasture (spring OR: 3.0 [2.0-4.7]; summer: 3.4 [2.4-4.9]; autumn: 2.8 [1.7-4.5]), fruit (spring OR: 5.7 [2.4-11.1]; summer: 4.4 [2.0-8.7]; autumn: 4.9 [1.5-11.0]) and arable (spring OR: 6.4 [3.9-11.1]; summer: 9.3 [6.4-13.7]; autumn: 7.6 [4.5-12.2]). Visitation to woodland and other agricultural was not significantly different to the baseline (Fig 5d-f).

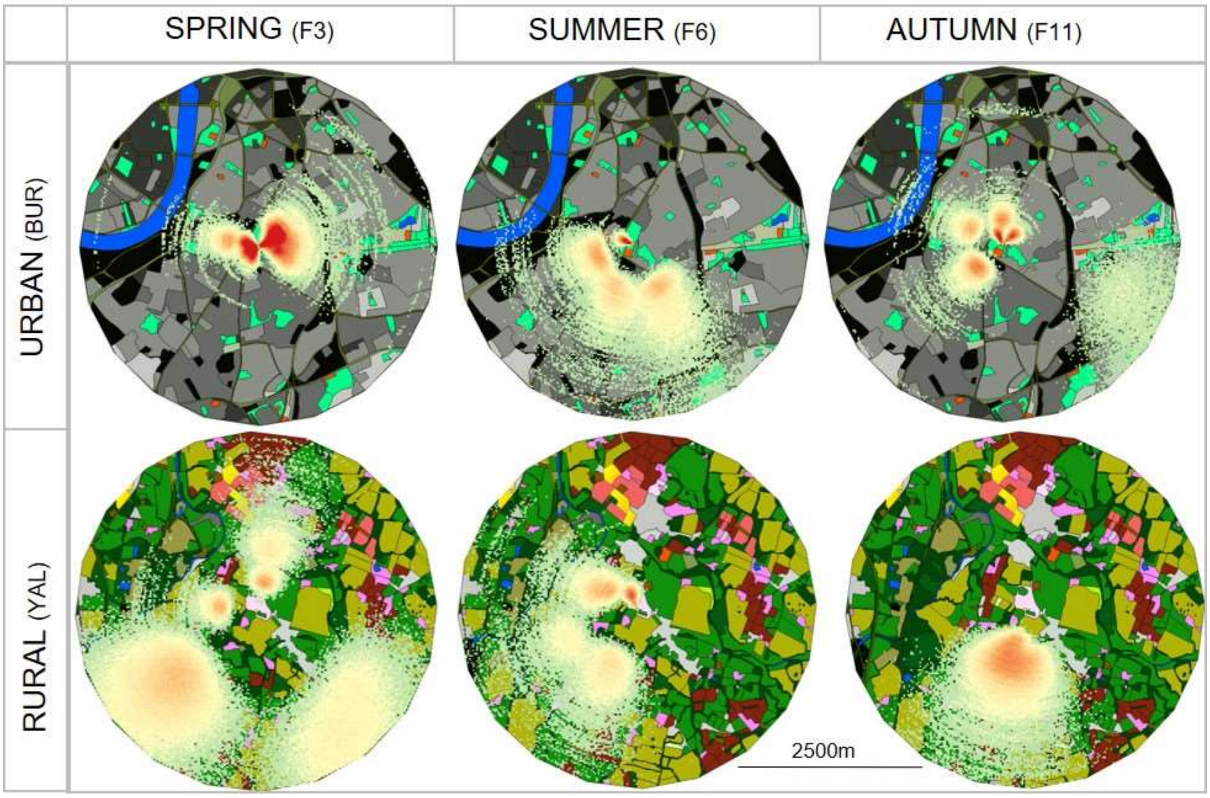

Figure 2. Example waggle dance plots from one urban site (BUR) and one rural site (YAL). Each circle shows the dances recorded on a single filming period (up to 3 hours) during spring (fortnight 3), summer (fortnight 6) and autumn (fortnight 11). Waggle dances are displayed as probability heatmaps generated from 1000 simulations of each dance allowing incorporation of variability in distance and angle communication (Schürch et al. 2013b). Dance plots are overlaid on GIS land-use maps (radius 2500m) produced for land-use preference analysis. For waggle dance plots for all 183 site-fortnight combinations see Fig S1.

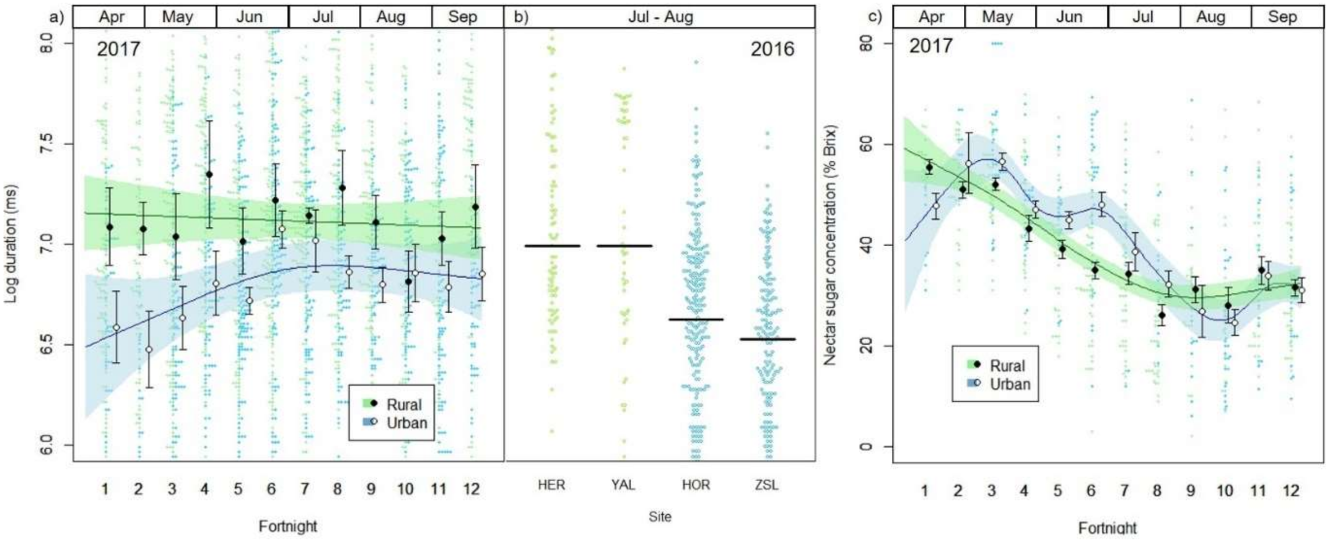

Figure 3. a) Median log-transformed waggle run duration ± SE for urban (open circles) and rural (filled circles) colonies over twelve fortnightly timepoints between April and September 2017. Lines are fitted from GAMs allowing a non-linear relationship between fortnight and duration, with shaded areas indicating 95% CIs. Raw data are shown in the background beeswarm plot (blue: urban, green: rural). b) Beeswarm plot of log-transformed waggle run durations for dances recorded over eight weeks in July and August 2016 at a subset of two urban (blue) and two rural (green) sites. Black lines indicate median values. c) Median nectar sugar concentration (% Brix) ± SE from nectar collected from returning foragers immediately after waggle dance data collection in 2017 (see (a) for information on plot features).

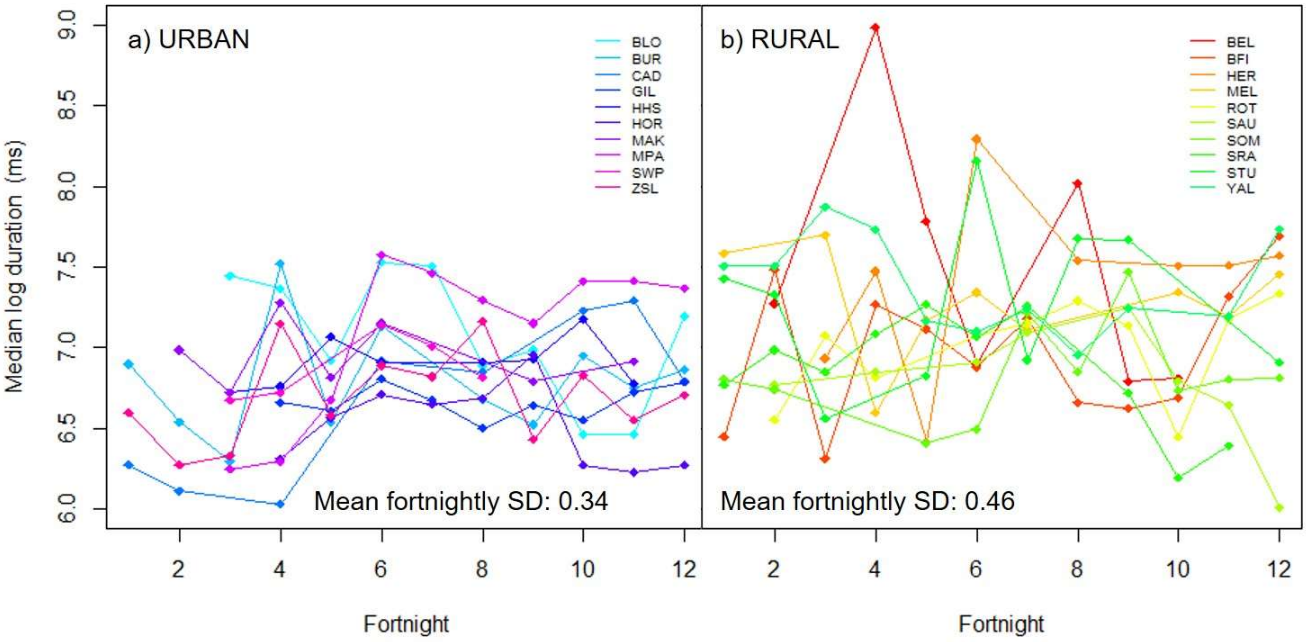

Figure 4. Median log-transformed waggle run durations for individual a) urban and b) rural sites over twelve fortnightly timepoints, showing greater variation in foraging distance across rural sites than urban sites (mean standard deviation is inset). Variation in peaks of forage availability in rural sites suggest reliance on a few ephemeral crop resources, the identity of which differ between sites. In contrast urban sites follow a consistent seasonal pattern suggesting exploitation of a larger number of resources.

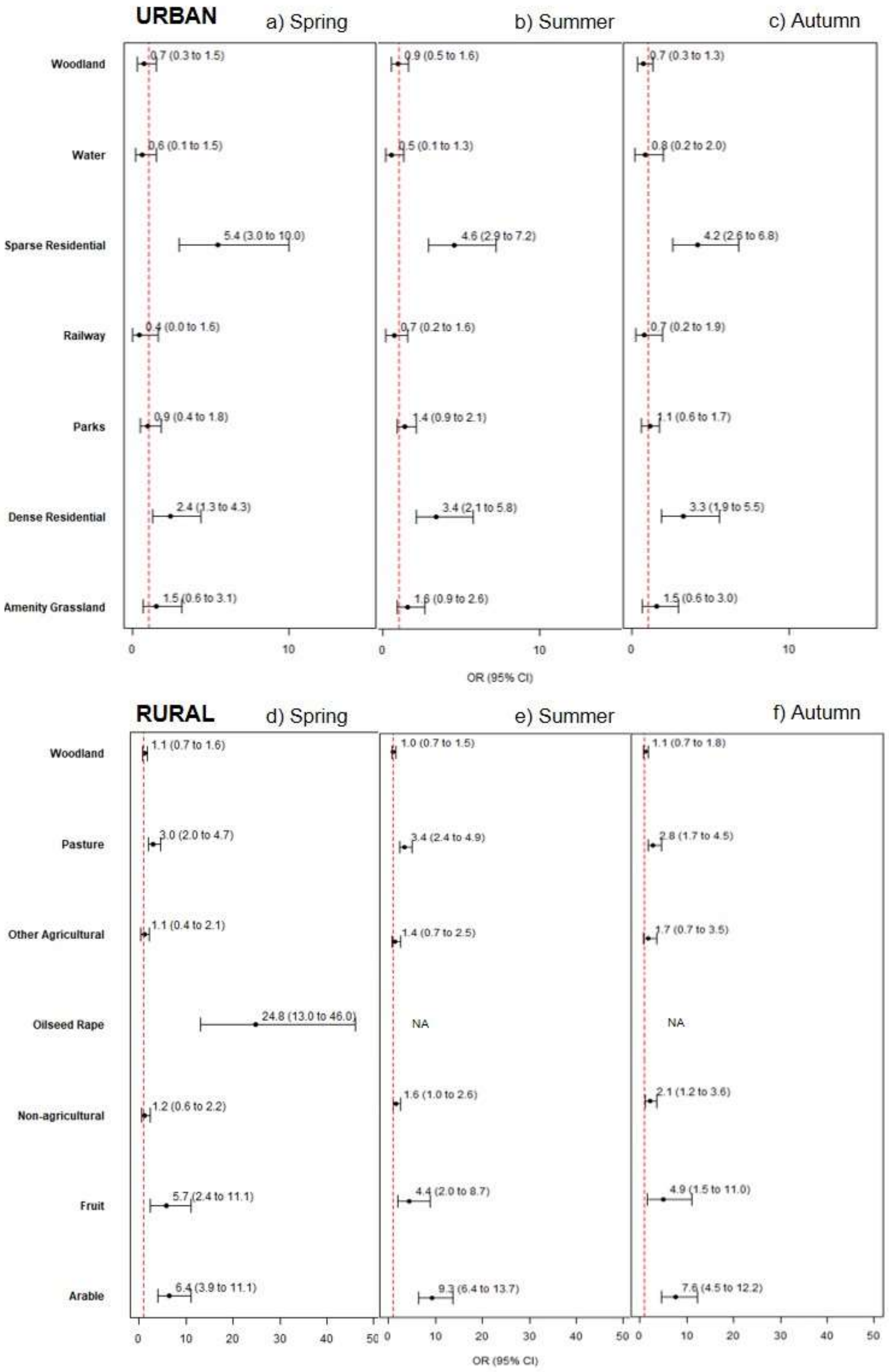

Figure 5. Adjusted odds ratios (± 95% CIs) for visitation to land-use types within urban (a-c) and rural (d-f) landscapes derived from simulated foraging visits using 2017 waggle dance data. An odds ratio of 1 is indicated by the red dashed line. Error bars that do not cross 1 indicate significant differences from the baseline land-use type ("Continuous Urban" in Urban and "Built-Up" in Rural). Data from fortnights 1-4 (spring), 5-8 (summer) and 9-12 (autumn) are pooled.

	df	AICc	ΔAICc	w_i	Land-use	s(Fortnight): Land-use	s(Fortnight)	Decoder	Period	Residual Temperature
a) Waggle run duration (2017)										
Model 8	**19**	**188.7**	**0**	**0.178**	+	+				+
Model 9	**20**	**188.9**	**0.2**	**0.16**	+	+			+	
Model 5	**20**	**188.9**	**0.23**	**0.158**	+	+			+	+
Model 3	**18**	**189**	**0.25**	**0.157**	+	+				
Model 2	**18**	**190.4**	**1.69**	**0.076**	+		+			
Model 6	20	191	2.31	0.056	+	+		+		+

	df	AICc	ΔAICc	w_i	Land-use	s(Fortnight): Land-use	s(Fortnight)	Colony Strength	Period	Residual Temperature
b) Nectar sugar content (2016)										
Model 2	**25**	**5481.5**	**0**	**0.349**	+	+				+
Model 8	**26**	**5483**	**1.49**	**0.166**	+	+				
Model 9	**26**	**5483.2**	**1.73**	**0.147**	+	+		+		
Model 7	26	5483.5	2.01	0.128	+	+			+	
Model 4	26	5484.7	3.21	0.07	+	+		+		+
Model 6	26	5485	3.51	0.06	+	+			+	+

	df	AICc	ΔAICc	w_i	Land-use	Week	Land-use:Week
c) Waggle run duration (2016)							
Model 3	**4**	**627.8**	**0**	**0.524**	+		
Model 1	**6**	**628.2**	**0.49**	**0.41**	+	+	+
Model 2	5	633.1	5.35	0.036	+	+	
Basic model	3	633.7	5.94	0.027			
Model 4	4	638.7	10.93	0.002		+	

Table 1. Tables of candidate models using GAMMs to analyse a) 2017 waggle run median duration and b) nectar sugar content. Models containing the interaction between fortnight and land-use contained a separate smoother for each land-use; all models contained the random effect “site”. c) Table of candidate models using linear mixed models to analyse 2016 waggle run duration with recording session included as a random effect. In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (wi); only the first six candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

<table>
<tr><td colspan="5">a) Waggle run duration (2017)</td></tr>
<tr><td rowspan="2">Parameters</td><td rowspan="2">Estimate</td><td rowspan="2">Std. Error</td><td colspan="2">95% CIs</td></tr>
<tr><td>Lower</td><td>Upper</td></tr>
<tr><td>(Intercept)</td><td>4.551</td><td>0.883</td><td>2.820</td><td>6.282</td></tr>
<tr><td>Land-use (Urban)</td><td>1.180</td><td>0.516</td><td>0.168</td><td>2.192</td></tr>
<tr><td>Residual Temperature</td><td>0.011</td><td>0.016</td><td>-0.019</td><td>0.042</td></tr>
<tr><td>Period (PM)</td><td>-0.033</td><td>0.054</td><td>-0.138</td><td>0.072</td></tr>
<tr><td colspan="2">Smooth terms</td><td>edf</td><td>F</td><td><i>p</i> value</td></tr>
<tr><td colspan="2">s (Fortnight) [Urban]</td><td>1.600</td><td>1692.741</td><td>< 0.001</td></tr>
<tr><td colspan="2">s (Fortnight) [Rural]</td><td>2.453</td><td>72.273</td><td>< 0.001</td></tr>
<tr><td colspan="2">s (Site)</td><td>12.386</td><td>2.211</td><td>< 0.001</td></tr>
<tr><td colspan="5">b) Nectar sugar content (2017)</td></tr>
<tr><td rowspan="2">Parameters</td><td rowspan="2">Estimate</td><td rowspan="2">Std. Error</td><td colspan="2">95% CIs</td></tr>
<tr><td>Lower</td><td>Upper</td></tr>
<tr><td>(Intercept)</td><td>30.680</td><td>4.918</td><td>21.041</td><td>40.319</td></tr>
<tr><td>Land-use (Urban)</td><td>22.020</td><td>7.384</td><td>7.547</td><td>36.493</td></tr>
<tr><td>Residual Temperature</td><td>0.057</td><td>0.156</td><td>-0.250</td><td>0.363</td></tr>
<tr><td>Colony strength</td><td>0.000</td><td>0.000</td><td>0.000</td><td>0.000</td></tr>
<tr><td colspan="2">Smooth terms</td><td>edf</td><td>F</td><td><i>p</i> value</td></tr>
<tr><td colspan="2">s (Fortnight) [Urban]</td><td>5.562</td><td>29.907</td><td>< 0.001</td></tr>
<tr><td colspan="2">s (Fortnight) [Rural]</td><td>8.003</td><td>20.127</td><td>< 0.001</td></tr>
<tr><td colspan="2">s (Site)</td><td>10.175</td><td>1.533</td><td>0.002</td></tr>
<tr><td colspan="5">c) Waggle run duration (2016)</td></tr>
<tr><td rowspan="2">Parameters</td><td rowspan="2">Estimate</td><td rowspan="2">Std. Error</td><td colspan="2">95% CIs</td></tr>
<tr><td>Lower</td><td>Upper</td></tr>
<tr><td>(Intercept)</td><td>7.364</td><td>0.349</td><td>6.680</td><td>8.047</td></tr>
<tr><td>Land-use (Urban)</td><td>-0.836</td><td>0.423</td><td>-1.664</td><td>-0.007</td></tr>
<tr><td>Week</td><td>-0.075</td><td>0.089</td><td>-0.250</td><td>0.100</td></tr>
<tr><td>Land-use (Urban):Week</td><td>0.088</td><td>0.106</td><td>-0.119</td><td>0.295</td></tr>
</table>

Table 2. Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable; see Table 1) for analyses of waggle run duration and nectar sugar content. Parameters highlighted in bold are considered significantly different from the baseline based on 95% CIs that do not cross zero.

Discussion

Our results show a significant increase in waggle dance duration and therefore foraging distance in rural compared to urban hives. Duration was greater at urban hives at every fortnightly time-point over a six-month foraging season, suggesting the effect of land-use on foraging distance is consistent through the year. Waggle dances recorded at a subset of hives in 2016 over two months follow a similar pattern, suggesting the effect is repeatable across years. Greater foraging distances in rural areas were not compensated for energetically by increased nectar sugar content; indeed, sugar content was lower overall in rural areas. Within the urban landscape, bees showed a preference for foraging in sparse residential areas typified by gardens, while in rural areas bees disproportionately visited oilseed rape fields in the spring. This study demonstrates a positive effect of urbanisation on pollinator forage availability using waggle dance data from multiple observation hives in urban and rural areas for the first time.

Honeybees are sensitive to foraging economics, maximising net energy gain by preferring nearer and higher quality (sugar concentration) forage sources (Schmid-Hempel 1987) and as such waggle dance communicated distance is a reliable proxy for forage availability (Couvillon et al. 2014a). Because honeybees take into account the other forage available when deciding whether to advertise a foraging site (Samuelson and Leadbeater 2017), dance data also represents filtered information about the foraging landscape, indicating resources that bees are actively exploiting (Grüter et al. 2010).

Communicated foraging distance was significantly higher in rural than urban sites across the season, suggesting urban sites consistently provide more available forage than rural sites. This supports recent research showing that hives in urban areas gained more weight over a year than those in agricultural or mixed areas (Lecocq et al. 2015), although cf. (Naug 2009; Sponsler and Johnson 2015). Although no study to date has compared waggle dance data from urban and rural hives in the same experiment, our findings support results from single-site studies that typically show short foraging distances in urban and suburban landscapes (Waddington et al. 1994; Garbuzov et al. 2015b) and longer distances in rural landscapes (Visscher and Seeley 1982; Couvillon et al. 2014a). In contrast, analysis of dances from a site on a boundary between urban and rural land found a preference for foraging in agricultural land

(Sponsler et al. 2017); this may be explained by the presence of an abundant agricultural weed, goldenrod, providing an important resource during the seven week study period. Our finding that forage availability is higher in urban areas is likely to be due to increased floral provision from intentionally planted flowering plants in gardens and parks (Davies et al. 2009) and urban trees, which have been shown to be particularly important in urban areas in the spring (Samuelson et al. *in prep*). This indicates that despite many horticultural plants being inaccessible or unattractive to bees (Garbuzov and Ratnieks 2014a; Garbuzov et al. 2014) the resources available are sufficient to provide a benefit over agricultural areas. Recent emphasis on bee-friendly planting in cities (Threlfall et al. 2015) may have contributed to this. Hive density in cities is typically very high (Alton and Ratnieks 2013; Lorenz and Stark 2015); our results suggest that resource provision in urban areas may be sufficient to support these high densities. In contrast to urban areas, agricultural intensification has reduced floral provision in rural land through removal of hedgerows and eradication of wildflowers via herbicide use (Williams et al. 2015). While mass-flowering crops such as oilseed rape (OSR) provide large nectar influxes at important periods in the colony season, these resources are ephemeral and often are followed by dearth in the post-bloom period (Requier et al. 2015).

Our findings of local seasonal patterns in foraging distance provide evidence for reliance on these mass-flowering crops. We did not find a strong overall seasonal pattern in foraging distance in rural hives, in contrast to previous research at a single rural site (Couvillon et al. 2014a). However, a major strength of our study is the inclusion of hives at multiple rural sites ranging over a wide area containing different crop types. Fortnightly variation (SD) in foraging distance was significantly higher in rural sites than urban, suggesting that regional peaks and troughs in forage availability balanced out across the sites and that local seasonal variation was in fact high. This indicates that periods of dearth may vary across different rural landscapes depending on the crops available, supported by recent research showing peaks in the mass of pollen and nectar collected by rural honeybee colonies during mass-flowering crop blooms (Requier et al. 2015). For example, sites such as "SRA" that showed short distances in spring were often those where the bees visited OSR fields during this period (see Fig S1 for full dance plots), while other sites such as "YAL" with shorter distances in summer months may have been relying on other crops (in the case of "YAL", lavender and fruit farming was common in the area). This variation in peaks of availability also points at a greater reliance on single or smaller numbers of plant taxa in rural areas. As well as resulting in a forage landscape with decreased redundancy and less resilience to loss of one or two resources (Memmott et al. 2004), reliance on a smaller number of resources has implications for diet diversity and consequently colony health (Di Pasquale et al. 2013). In contrast in urban areas foraging distance was shortest for almost all sites in early spring and remained constant across the rest of the season. This suggests that urban sites supported consistently high floral provision in the spring (a pattern likely driven by exploitation of urban trees; Samuelson et al. *in prep.*; Keller et al. 2005; Macivor et al. 2014) and maintained a consistent provision across the year, indicating reliance on a greater number of species, the composition of which overlaps between sites.

Foraging economics dictates that both nectar sugar concentration and flight distance must be taken into account when deeming a flower patch economically profitable to visit (Schmid-Hempel 1987; Seeley 1994). We have shown that honeybees fly further in rural than urban areas, but if this had been compensated for by higher quality nectar the two land-use types may be economically equivalent. However, nectar sugar content data collected from returning foragers at each site visit alongside dance data collection shows that this is not the case. In fact, sugar content was greater in urban than rural hives across the season overall. This may indicate a greater overall availability of forage, allowing bees to focus on collection of higher quality nectar (Seeley 1995). We also found that in both urban and rural areas, sugar content declined across the season. This reflects results from a similar study at a rural location in SE England, where nectar sugar content decreased from May to August and started to climb again in September (Couvillon et al. 2014a). This may be due to direct seasonal effects such as temperature on nectar production by plants (Pacini et al. 2003), effects resulting from plant-pollinator interactions (Rathcke 1983) such as increased abundance of other pollinators later in the season affecting nectar concentrations produced or overall forage availability forcing bees to be less choosy (Seeley 1995).

Analysis of preference for small-scale land-use types within the broader urban or rural landscapes by simulating visits to land-use patches allows identification of foraging hotspots (Couvillon et al. 2014b).

In urban bees we found a significant preference for visiting sparse residential areas and a smaller significant preference for visiting dense residential areas relative to the baseline land-use of continuous urban. These residential areas are typified by large areas of gardens, which have been shown to provide important resources for bees (Levé et al. 2019). This suggests that it is the presence of gardens that drive the increased forage availability and related lower foraging distances in urban land. To illustrate this, our highest-density urban site, "BLO", located in the City of London CBD, showed anomalously high foraging distances compared to other urban sites. Examination of foraging plots at this site (see Fig. S1) show that bees flew long distances (on average 1158m) to reach lower-density residential land. All other land-use types showed no significant difference to the baseline, indicating that bees did not prefer or avoid these compared to continuous urban. In rural areas, during the oilseed rape bloom bees showed an extremely strong preference for foraging in oilseed rape fields. This supports conclusions from this study and other work (e.g. Requier et al. 2015) suggesting a reliance on mass-flowering crops in rural areas. Fruit, pasture and arable all showed a small significant preference in all seasons, indicating improved resource provision in these areas relative to woodland and non-agricultural, and to the baseline of built-up land. In rural areas built-up land consisted of isolated patches of housing or industrial land, which may explain the lower preference for this land-use type in rural areas.

Our findings demonstrate that overall, urban areas provide abundant foraging resources for honeybees and potentially other pollinator species with similar foraging requirements and behaviour. Despite being present at higher densities in cities, urban honeybee colonies were able to collect nectar and pollen significantly closer to the hive and the quality of the nectar collected was higher. Variation in peaks of forage availability was higher in rural areas, suggesting that bees in these areas rely on specific mass-flowering crops that are abundant in each region, and suffer periods of dearth in between. As such, conservation efforts should be directed towards increasing non-crop floral provision in agricultural areas (such as wildflower strips; Williams et al. 2015) to increase consistency of forage availability at a local scale across the season and to minimise reliance on small numbers of ephemeral plant taxa. Our findings emphasise the benefit of low-density residential urbanisation containing gardens and parks and suggest that bee-friendly planting in these areas should be encouraged. While the positive effects of urbanisation on forage provision for pollinators should be acknowledged, urban land remains a small percentage of total land cover and these islands of abundant forage may not be sufficient to support bee populations across a landscape dominated by intensive agriculture.

Acknowledgements

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Author Contributions

A.E.S. and E.L. conceived the initial idea and designed the experiment; A.E.S. performed the experiment; A.E.S. & R.S. performed the statistical analyses; A.E.S. wrote the manuscript draft and E.L., R.S., and A.E.S. provided the final edit.

Waggle dance analysis demonstrates greater foraging success in honeybees in urban than rural areas

Supplementary Material

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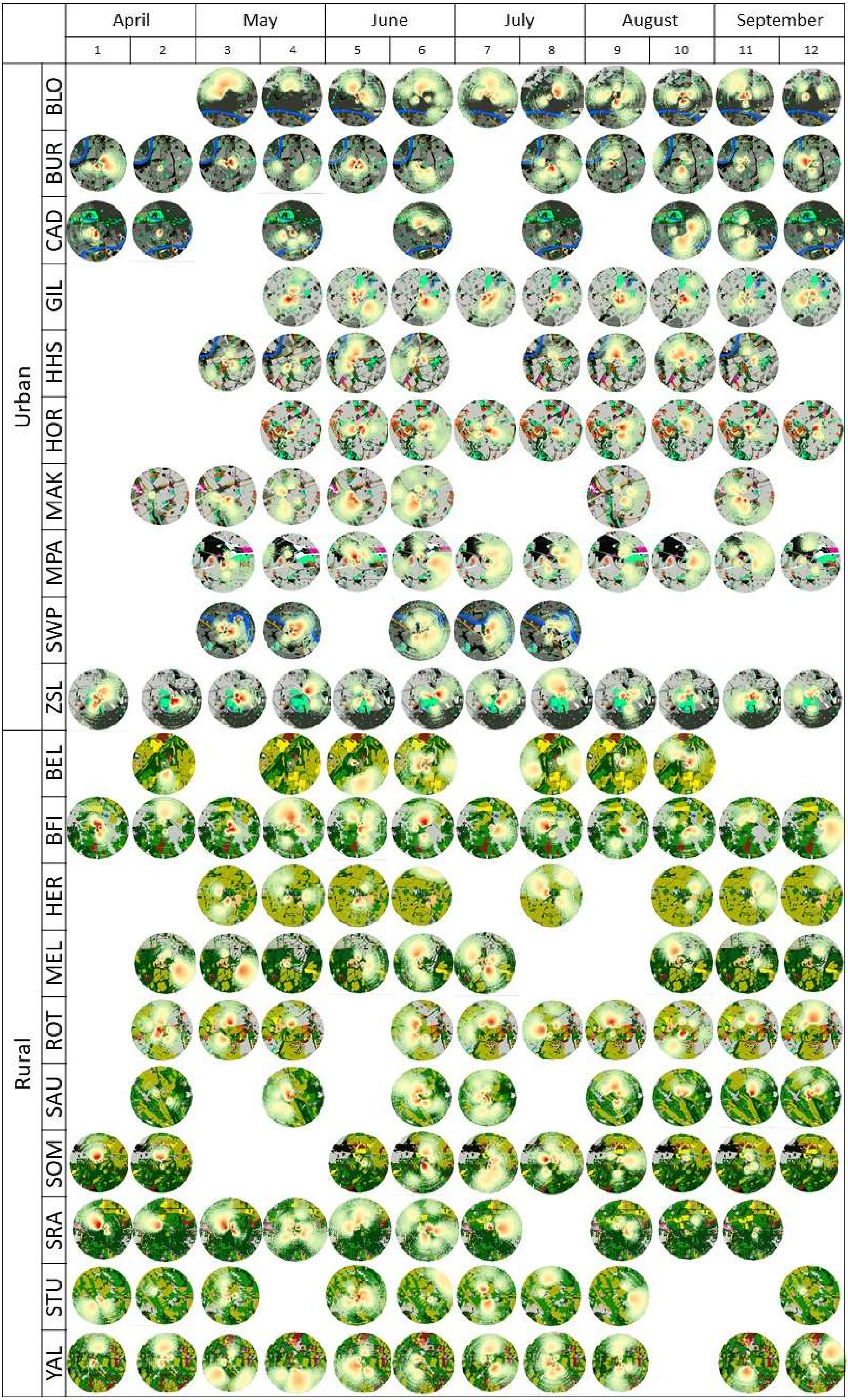
Figure S1. Waggle dance plots from ten urban sites and ten rural sites over six months (12 fortnightly timepoints). Each circle shows the dances recorded on a single filming period (up to 3 hours) at a single location. Waggle dances are displayed as probability heatmaps generated from 1000 simulations of each dance allowing incorporation of variability in distance and angle communication (Schürch et al. 2013b). Dance plots are overlaid on GIS land-use maps (radius 2500m) produced for land-use preference analysis.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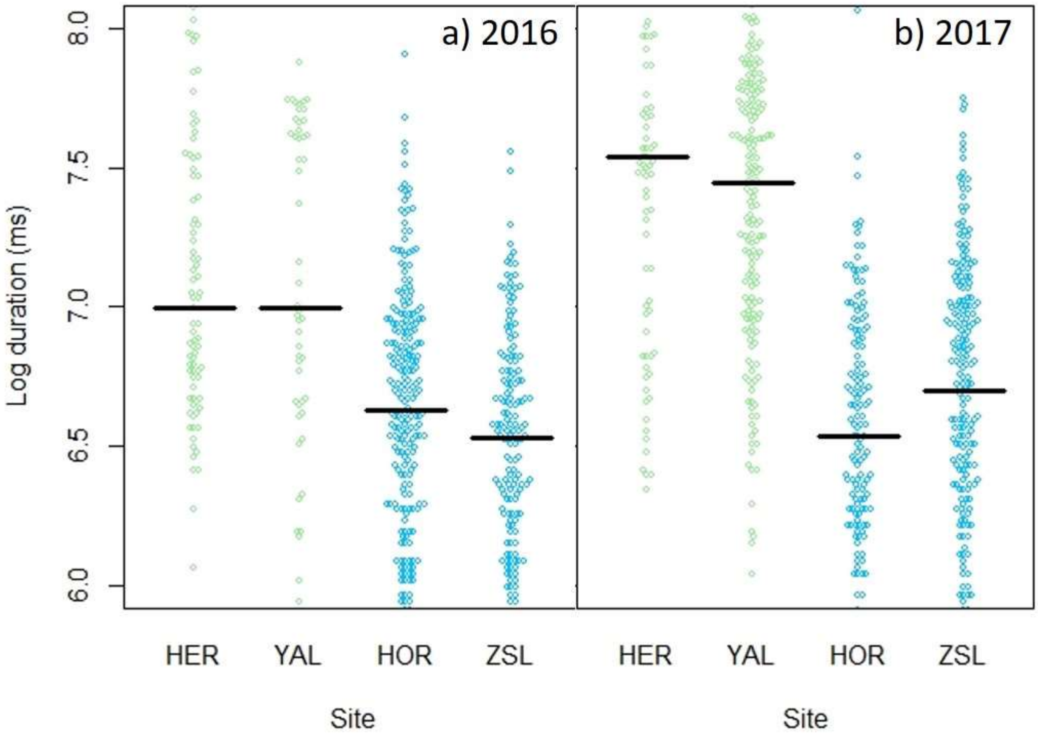
BUR
CAD
GIL
HHS
HOR
MAK
MPA
SWP
ZSL
Rural
BEL
BFI
HER
MEL
ROT
SAU
SOM
SRA
STU
YAL

Figure S2. Beeswarm plots of log-transformed waggle run durations for a subset of two urban (blue) and two rural (green) sites at which dances were recorded in both a) 2016 and b) 2017. Black lines indicate median values.



Chapter Seven: General Discussion

Key findings

Honeybees and bumblebees thrive in urban areas

The overarching conclusion from the three experimental chapters of this thesis is that honeybees and bumblebees may be able to not only thrive in urban areas but may also be more successful in these areas than in agricultural land in a wide array of measures. In Chapter Four, bumblebee colonies in agricultural areas produced fewer reproductives and workers, survived for less time and were less likely to contain food stores than city or village colonies. In Chapter Five, honeybee colonies in urban areas were found to contain more workers, have lower *Nosema* spore counts and have access to a richer pollen diet than those in rural areas. In Chapter Six, I show that honeybees in urban areas have to travel less far throughout the season to find food, show less variation in foraging distance across the season and have access to nectar with a higher average sugar concentration than those in rural areas.

These findings contribute to a growing body of evidence highlighting the benefit of urban areas for pollinators. Abundance and diversity of pollinator species have frequently been shown to be higher in urban than rural areas (McFrederick and LeBuhn 2005; Baldock et al. 2015; Hall et al. 2016). However, it has remained unclear whether these differences are driven by effects of land-use on colony reproductive success or migration between habitat types (Gill et al. 2016), and which mechanisms are behind these effects. The findings generated by this thesis highlight the effect of urban land on social bees at the colony-level, elucidating potential drivers behind bee population responses to land-use change.

Forage availability is a key driver of these differences

Forage availability emerged as the key driver of the success of bees in urban environments. In Chapter Four, bumblebee colonies were less likely to contain pollen and nectar stores in agricultural sites than in city and village sites. In Chapter Five, honeybee colonies' pollen diets had a higher species richness than those in rural areas. In Chapter Six, honeybees had to travel less far to find forage and had less seasonal variation in forage availability in the city. As habitat loss and the associated decline in forage has been highlighted as the most important current threat facing pollinator populations (Carvell et al. 2006; Potts et al. 2010a), the contribution of this factor to urban bee success is not surprising. These results support recent research suggesting that cities and towns can provide abundant resources for wild pollinators (Hall et al. 2016; Baldock et al. 2019). In honeybees, evidence to date has been more equivocal: while some studies demonstrate abundant forage available for honeybees in urban areas (Garbuzov et al. 2015b; Lecocq et al. 2015) others find that forage availability is lower in urban than rural areas (Naug 2009; Sponsler and Johnson 2015; Sponsler et al. 2017). These contradicting results may be due to specific habitat attributes that differ between the regions studied; for example, two studies were conducted in an area where an abundant agricultural wildflower, goldenrod, was available during the study period, which increases the available rural forage (Sponsler and Johnson 2015; Sponsler et al. 2017).

Consequently, it is important to investigate which attributes of the urban landscape contribute to the foraging success of colonies. In Chapter Six, I investigated in more detail which aspects of urban and rural land-use support honeybee foraging by analysing preferences for small-scale land-use types within the two broader landscapes. Bees showed a strong preference for foraging in sparse residential land-use in urban areas. This land-use type consisted of typically terraced housing with large areas of gardens and was preferred to other urban land-use types such as dense residential that contained less garden area, highlighting the importance of this habitat for bee forage. This supports previous research showing the benefits of gardens for bees (Goulson et al. 2010; Samnegård et al. 2011; Normandin et al. 2017; Levé et al. 2019). Research has suggested many horticultural plant varieties are unattractive or inaccessible to bees (Molumby and Przybylowicz 2012; Garbuzov and Ratnieks 2014a; Martins et al. 2017); the preference for gardens shown here suggests that despite this there are sufficient resources in the form of utilisable horticultural varieties or "weed" species to result in high visitation compared to other urban land classes. . The low seasonal variation in foraging distance is likely to reflect the fact that gardens are often designed to have flowers in bloom throughout the season (Harrison et al. 2018). One site (BLO) was an exception to the rule of short foraging distances in urban areas; this was a densely urbanised site with little access to gardens. Bees at this site travelled relatively long distances to reach residential areas containing gardens, again highlighting the importance of this habitat type. The strong contribution of woody plants to pollen resources in urban honeybee colonies in Chapter Five suggests that abundant trees in residential areas also contribute to the attractiveness of this land-use type.

In rural areas, by far the most attractive land-use type in Chapter Six was oilseed rape (OSR), and in Chapter Five, a large proportion of rural colonies (83%) collected pollen from OSR in the spring. The high seasonal variation in foraging distance in rural bees in Chapter Six also suggests a focus on small numbers of transient resources, reflecting the reliance of bees in rural areas on mass-flowering crops (Westphal et al. 2003). This raises a conservation concern if economic trends cause mass-flowering crops to be less extensively grown in the future, for example if the financial viability of OSR is affected by the recent neonicotinoid ban (Dewar 2017). However, pollen analysis from colonies in Chapter Five found that no colony foraged exclusively on OSR, demonstrating at least some provision from non-crop sources. The discrepancy between the extreme preference for OSR in Chapter Six and the relatively low contribution of OSR to pollen samples in Chapter Five may reflect a higher reliance on mass-flowering crops for nectar than for pollen (Requier et al. 2015). The findings from these experiments on the contribution of OSR to honeybee diet may be used to inform future studies modelling pesticide exposure to honeybees, which often assume bees exclusively forage on mass-flowering crops during bloom (e.g. Henry et al. 2012).

Parasites and pesticide exposure present additional threats to bee populations, and these may have also contributed to the success of bees in urban compared to rural areas. I found lower *Nosema* infection in urban bees in Chapter Five, although the effect of urbanisation on *Varroa* levels varied by season and no effect of urbanisation was found on endoparasite prevalence in bumblebee colonies in Chapter Four. However, there were fewer invasions by the brood parasite *Bombus vestalis* in bumblebee colonies at city sites. Previous research has similarly found mixed effects of urbanisation on parasitisation, suggesting complicated factors mediate this relationship. Although I did not directly investigate pesticide exposure in this thesis, lower colony success in honeybees (Chapter Five) and bumblebees (Chapter Four) in rural areas may have been driven in part by increased exposure to agricultural pesticides (David et al. 2016; Nicholls et al. 2018).

Are urban areas good, or are agricultural areas bad?

The finding that the two bee species studied here thrive in urban areas suggests that developed land may contribute towards protecting these species and other similar taxa from pressures implicated in the global pollinator decline (Vanbergen et al. 2013). The ability of honeybees and bumblebees to adapt to these novel environments that are considerably different to their evolutionary context suggests that the continuing trend of urban expansion (Seto et al. 2011) is unlikely to harm generalist pollinators, and may even have positive consequences. However, there are several major caveats to this conclusion.

First, cities and towns represent an extremely small proportion of total land cover (Grimm et al. 2008). While these islands of flower-rich and low-pesticide habitat may provide refuges for bees within a wider inhospitable landscape, urban land is far from sufficient to support the global population of pollinators. Urban land is an extreme form of fragmented habitat, consisting of disconnected patches of urbanisation at the landscape scale (Poelmans and Van Rompaey 2009), within which suitable habitat is further fragmented into patches (Levé et al. 2019). Fragmentation presents genetic, disease and resource-related problems for pollinator populations inhabiting cities (Steffan-Dewenter and Schiele 2008), which are likely to increase if the surrounding agricultural land becomes less able to support pollinators in the future with increasing agricultural intensification (Carvell et al. 2006). The provision of abundant floral resources and high density of managed beehives in cities are likely to increase overall density of pollinators, which may increase competitive interactions (Plascencia and Philpott 2017) and disease transmission (Youngsteadt et al. 2015a; Brosi et al. 2017). High human disturbance and areas of impervious surface may also make urban areas less hospitable to pollinators with specific nesting requirements, such as ground-nesting species (Threlfall et al. 2015). Greater proportions of highly bred and exotic horticultural flowering plants are also less likely to provide food for specialist pollinator species (Molumby and Przybylowicz 2012). The conclusion of the findings of this thesis is therefore not that urban land represents the ideal habitat for bees, but that modern agricultural land provides insufficient habitat resources and as such the bee species studied here are better able to survive in cities, particularly residential urban land, an anthropogenic habitat far removed from the environment in which they evolved.

The positive characteristics of urban land serve to highlight the shortcomings of modern agricultural land. Agricultural land is associated with negative impacts on several aspects of foraging success in *A. mellifera* and *B. terrestris*,as evidenced by the long foraging distances and the vulnerability to seasonal peaks and troughs displayed in rural honeybees in Chapter Six, the less rich pollen diet experienced by honeybees in Chapter Five and the lower likelihood of containing food stores in rural bumblebee colonies in Chapter Four. While mass-flowering crops clearly provide an important food source for bees in farmland, my results support previous research showing bees suffer from forage scarcity during gaps between crop blooms (Requier et al. 2015), suggesting consistent and varied sources of forage such as wildflower meadows and hedgerows that were available in historical agricultural systems are no longer available (Carvell et al. 2006). Although not directly tested here, other research has suggested that bees are protected from high exposure to pesticides in urban areas (David et al. 2016; Nicholls et al. 2018), again highlighting a negative aspect of intensive agriculture rather than a specific benefit of urban land. From an economic perspective, the finding that agricultural areas may be less able to support pollinator populations raises concerns regarding the delivery of crop pollination services. In addition, pollinator attraction to areas of high floral availability may dilute densities of pollinators in the wider landscape (Holzschuh et al. 2016); higher bee densities in urban areas (Osborne et al. 2008; Goulson et al. 2010) may therefore have negative consequences for agricultural pollination.

In addition, there is a danger in isolating a single taxon or guild; while pollinators may thrive in cities due largely to the fact that their food resource, flowering plants, is incidentally favoured and propagated by humans in many urban areas, many other animals and plants do not benefit from this provision and do not thrive in urban areas. Many taxa are sensitive to human disturbance, noise and light pollution (Coleman and Barclay 2011); others rely on food sources not found in urban areas (Croci et al. 2008); others are affected by urban heat islands resulting in shifting phenologies (Harrison et al. 2018).

Focussing on the positive impact of urban land on pollinators mistakenly suggests that anthropogenic land-use change may have universal positive impacts for wildlife in general and avoids the conclusion that even for pollinators cities may represent the "least-worst" option in an almost entirely anthropogenic landscape such as Western Europe.

Implications for other pollinators

I used *A. mellifera* and *B. terrestris* as study systems for this thesis in part because they are generalist pollinators, so impacts of urbanisation on these taxa may be indicative of impacts of urbanisation on some other pollinators with shared ecological requirements , although there are considerable differences in foraging behaviour even amongst generalists (Petanidou et al. 2008). While honeybees are domesticated animals and are not limited by nesting resources, they forage in the wild on resources shared with wild pollinators. Use of the waggle dance to track foraging behaviour has been suggested to represent a biological indicator of landscape quality for other pollinators i (Couvillon et al. 2014a); my finding of higher forage availability in urban areas and the identification of residential areas containing gardens as preferred foraging sites may therefore be representative of some other pollinator species' experience of the landscape. I also used bumblebees as a model organism to confirm whether results are consistent in a wild species with a foraging range more representative of other wild pollinators. The broad agreement of results suggest that these findings may reflect the responses of some other = pollinator taxa to urbanisation, particularly other social, generalist, central-place foraging species.

However, the results here may be primarily relevant to generalist pollinators and those not sensitive to human disturbance (Williams et al. 2010; Collado et al. 2018). Specialist pollinators, such as some long-tongued bumblebee species, may be less able to adapt to exploit exotic or ornamental floral varieties present in gardens (Goulson et al. 2005; Desaegher et al. 2018). Pollinators with specific nesting requirements may also be unable to thrive in cities, and those with life-history traits adapted to specific habitats such as forest have been shown to be replaced in urban habitats by open-adapted species

(Harrison et al. 2018). Specialist species include some of the rarest and most threatened species in Europe (Goulson et al. 2005). As such, those pollinators unable to adapt to exploit urban land may represent the most important focus of conservation.

Can these results be extrapolated to other cities?

A major advantage of the land classification protocol developed in Chapter Three and used throughout this thesis is that it uses characteristics of the land itself to classify land-use type and degrees of urbanisation rather than defining all London sites as "urban" and all sites outside London as "rural", and therefore the definition of urban land is not dependent on which city is being studied. London is a large city with a broad extent of different degrees of urbanisation (Schwarz 2010); the use of a bottom-up land classification protocol allows a representative range of urban habitats to be represented in a single city, affording several benefits including controlling for broader geographical factors (such as latitude) and minimising travel time allowing increased sample size. While some urban attributes such as pollution, pesticide use and human disturbance are not captured in the land classification, major habitat attributes relevant to pollinator forage provision are incorporated, making the results comparable to results of studies in other cities using a similar classification approach (Hahs and Mcdonnell 2006).

Land cover in the UK is broadly representative of Western Europe, with the proportion of urban and agricultural land falling within the centre of the ranges for the region as a whole (Eurostat 2017). However, the structure and composition of cities vary across the region; for example, northern European cities typically have more available urban green space than southern European cities (Kabisch et al. 2016). It is therefore fundamental to identify which characteristics of urban land contribute to effects on pollinators if extrapolation of results between cities is desired. In contrast to straightforward comparisons of urban and rural pollinator communities (Hernandez et al. 2009), the field of urban pollinator ecology is moving towards this approach of identifying underlying attributes (Matteson and Langellotto 2010; Baldock et al. 2015, 2019). Similarly, the results from this thesis identify land-use types within the broader urban and rural landscapes that contribute to colony success, such as gardens in urban areas and mass-flowering crops in rural areas. Emphasising examination of underlying variables is an important direction for land-use ecology studies, as it allows generalisation by showing that it is not urbanisation itself that supports bee populations but aspects of urbanisation that can be identified in different cities. Furthermore, it allows generalisation to wider questions about pollinator conservation. For example, my findings suggest that natural habitat is not a requirement for honeybee success providing sufficient forage is available. Understanding the importance of forage in pollinator success in urban areas affords insights into the requirements for pollinators in all land-use types and helps elucidate the drivers behind pollinator declines.

Conservation recommendations

The primary recommendation arising from these findings is that agricultural areas should be made more hospitable for pollinators in order that urban areas do not become the only habitat capable of supporting pollinators in the future. A large body of research suggests that bees are failing to thrive in agricultural areas (Kremen et al. 2002; Potts et al. 2010a), and the results of this thesis support this. Specific focus should be directed towards improving forage, particularly in the provision of consistent resources to fill the gaps between mass-flowering crop blooms (Requier et al. 2015). Research has shown that wildflower strips can be successful in achieving this goal (Williams et al. 2015), alongside hedgerows and other woody elements that also support pollinator resource provision and increase habitat connectivity (Van Den Berge et al. 2018). Habitat restoration to historic vegetation types (Tonietto and Larkin 2018) and ecological intensification of agriculture (Kovács-Hostyánszki et al. 2017) have also been shown to benefit bee populations. This is particularly relevant in the face of reported reductions in the financial viability of mass-flowering crops (Dewar 2017) following the neonicotinoid ban in Europe (European Commission 2013). Significant reductions in the area of OSR grown, such as a 14% decrease in the UK between 2014 (pre-ban) and 2016 (post-ban), is likely to have a considerable impact on agricultural forage provision for bees (Dewar 2017).

In addition, the positive aspects of urban habitats should be highlighted and encouraged if cities are to remain important refuges for pollinator populations (Hall et al. 2016). The finding that urban land is not universally good for bees, and that flower-rich urban green spaces and gardens are required for this to be the case should be emphasised (Davis et al. 2017; New 2018; Banaszak-Cibicka et al. 2018). Recent initiatives to encourage planting of bee-friendly plants are likely to have contributed to the high forage availability demonstrated in urban land (Simao et al. 2018); these should therefore be encouraged. However, previous research has suggested that information on which plants are attractive to bees that is disseminated to the public is highly varied and often inaccurate (Garbuzov and Ratnieks 2014b) and in order to be an effective conservation measure efforts should be made to consolidate and refine this information based on empirical evidence. In addition, care should be taken that intentional planting of non-native species does not negatively impact native urban plant populations (Johnson et al. 2017). The high preference for foraging in residential areas with gardens suggests that gardens are an important part of city resource provision for pollinators and conservation efforts should focus on highlighting individuals' ability to have an impact on pollinator success by maintaining private gardens to be suitable for pollinators (Lerman et al. 2018; Desaegher et al. 2018; Levé et al. 2019). It is well known that the public is often keen to support pollinator populations (Garbuzov and Ratnieks 2014b; Mwebaze et al. 2018); this tendency may be particularly effectively utilised in cities, where large areas of habitat are controlled by individuals' preferences rather than economic or policy-related drivers as in agricultural land (Goddard et al. 2010; Iuliano et al. 2017).

A common response by individuals to learning of threats to pollinators is to take up beekeeping as a conservation measure to help bee populations (Lorenz and Stark 2015; Brown 2018). This is potentially a misguided action: while research in this thesis demonstrates that urban land provides sufficient forage resources for honeybees, increasing honeybee populations without increasing forage provision is likely to lead to increased competition with wild pollinators (Colla and MacIvor 2017; Brown 2018) and to intraspecific disease transmission (Fürst et al. 2014). While urban beekeeping should not necessarily be discouraged, as increasing honeybee stocks helps to reverse the downward trend in Europe over the last century (Potts et al. 2010b), information about the limits of beekeeping as a conservation measure should be more readily available (Colla and MacIvor 2017) and policy implemented to mitigate potential impacts such as regulation of hive placement (Stange et al. 2017). Many members of the public lack knowledge of non-*Apis* pollinator diversity (Ratnieks et al. 2016), so education may help direct conservation efforts to more universally beneficial activities such as increasing floral provision. In addition, as a domesticated animal, it can be argued that a major motivation for conserving honeybee populations is an economic one and therefore increasing honeybee populations in urban areas has little conservation value as the majority of the crop and wildflower pollination service demand exists in agricultural areas (Breeze et al. 2011). Conversely, maintenance of large populations including different native subspecies is important to preserve genetic diversity in *A. mellifera* as a species (De La Rúa et al. 2009), which is currently primarily possible through domestication due to the impact of the *Varroa* epidemic on wild populations (Brettell and Martin 2017).

Focussing on promoting the benefits of urban land for honeybees and a generalist bumblebee may come at the cost of ignoring requirements of other pollinator taxa that are not positively associated with cities. The distributions of many pollinator taxa are driven by specialism on specific food plants, both at the adult and larval stages (Biesmeijer et al. 2006), nesting requirements (Cariveau and Winfree 2015), parasite pressure (Potts et al. 2010a) and competition with other pollinators (Goulson and Sparrow 2009). Indeed, previous research has shown differing responses in native bees to urbanisation based on nesting requirements and dietary breadth (Cane et al. 2006a). These factors may preclude urban land as a suitable habitat for many of the most threatened pollinators, Therefore, conservation programmes should take care to avoid excluding these taxa when improving habitat for pollinators.

Future research

The experiments in this thesis focussed on the breadth of habitats available in a typical Western European landscape, which contain very small areas of semi-natural habitat and almost no pristine habitat (Levers et al. 2018), and as such semi-natural habitat was not included in the land-use types investigated. The findings in this thesis therefore represent a comparison of the major land-use types available to pollinators, agricultural and urban. To investigate the absolute response of bees to urban land, comparison would need to be made against a baseline of the natural environment in which bees evolved. Although this would be less relevant for conservation in the current environmental context, it would be interesting to investigate whether bees are more successful in an anthropogenic habitat, indicating a taxon with high levels of adaptability and habitat flexibility.

Future research should also investigate the possible negative effects of urbanisation. Honeybees are kept at considerably higher densities in cities than the countryside (Alton and Ratnieks 2013) and bumblebees have been shown to exist at higher densities in cities (Goulson et al. 2010). This may have implications for competition for resources both between colonies and between species (Plascencia and Philpott 2017). Particular concerns have been raised about honeybees outcompeting wild pollinators (Colla and MacIvor 2017); this would be interesting to investigate in an urban context. High population density may also increase intra- and inter-specific disease transmission (Fürst et al. 2014; Brosi et al. 2017). Environmental pollution such as exhaust (Lusebrink et al. 2015; Jürgens and Bischoff 2017) or light (Knop et al. 2017) may affect urban pollination by bees and other pollinators, while urban warming (Hamblin et al. 2018) may present an additional threat.

Social factors involved in urban beekeeping should also be investigated, as these are likely to have effects on success of both honeybees and the wild pollinators with which they co-exist. Beekeeper practice varies widely and is often culturally driven by the practices taught by local associations; it would be interesting to investigate whether there is a spatial pattern in beekeeping practices because of this phenomenon and whether land-use has an effect. In addition to arbitrary differences driven by location, urban beekeeping often necessitates specific beekeeping practices such as smaller apiary sizes, more rigorous swarm control, reduced emphasis on honey yields and preference for less aggressive strains (Park 1990). It would be interesting to investigate possible implications for disease transmission and establishment, and regional population structures. Similarly, socio-economic factors are likely to have a strong impact on resource provision for both domesticated and wild pollinators, relating to housing density, conservation motivation and education (Iuliano et al. 2017; Baldock et al. 2019); this is an area that would benefit from further research.

Research into the contribution of pesticide exposure to differences between urban and rural bee success is strongly lacking, with only a handful of studies investigating urban pesticide exposure in honeybees and bumblebees (Johnson and Pettis 2014; David et al. 2016; Nicholls et al. 2018). While it is often taken for granted that cities provide a refuge from agricultural pesticides (Colla and MacIvor 2017), these studies suggest bees may in fact come into contact with high levels of pesticides in towns and cities either from horticultural use (Larson et al. 2013) or non-plant use such as ant or flea control (David et al. 2016; Nicholls et al. 2018). The extent to which this affects bee populations represents a significant research gap in our understanding of urban bee ecology.

As described above, the land classification protocol followed here maximises the possibility of generalisation between cities. However, future research should investigate how consistent the results found here are between different cities, particularly cities with a lower area of gardens and tree cover. Cities may also differ in pesticide use policies in public green spaces, proximity to rural land resulting in exposure to agricultural pesticides and honeybee hive density. These characteristics are likely to drive pollinator response to urban land and future research should investigate the extent to which this is the case in different cities. In addition, investigation of how cities differ from each other in pollinator success would further illuminate the underlying attributes of urban land that contribute to pollinator provision.

Concluding remarks

The findings in this thesis highlight the success of two key pollinator species in urban environments. However it is important to emphasise that the poor performance of honeybee and bumblebee colony performance in agricultural areas reveals the need for conservation efforts to focus on improving these areas, which represent the most common primary land-use in Europe (Eurostat 2016). While urban areas may provide a refuge within this broader impoverished landscape, these habitat islands are insufficient to support pollinator populations and are unlikely to be suitable for the rarest and most threatened pollinator taxa that often rely on specialist food or nesting resources. Future research should aim to test the efficacy of measures to improve agricultural areas for pollinators and further examine the specific aspects of urban land that support pollinator success.

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Appendix One

Samuelson, A.E. and Leadbeater, E. (2017). Foraging by Honeybees. In: Vonk, J. and Shackleford, T.K. (eds.), *Encyclopedia of Animal Cognition and Behavior*. Springer, New York

Foraging by Honeybees

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Introduction

The foraging behavior of a honeybee colony (*Apis mellifera.* L) represents one of the most sophisticated examples of cooperative behavior in the animal kingdom and has arisen as the evolutionary product of an unusual and highly complex social system. Honeybees are advanced eusocial insects, defined by overlapping generations, cooperative brood care, and reproductive division of labor. In each colony a single queen is almost always responsible for the vast majority of the reproductive output, with the functionally sterile workers (her daughters) helping to rear their sisters (other workers and new queens) and brothers (drones). The colony therefore functions as a single reproductive unit, with the workers sharing a common goal: to optimize foraging effort in a manner that allows the colony to grow in size until the entire group can reproduce by splitting, in a process known as swarming. Seeley (1989) and others have thus described the colony as a "superorganism" – an individual entity with many of the attributes of an organism, but itself composed of individual organisms. Consequently, a discussion of foraging behavior in this species must include both the behavior of the individual units, the workers, and the colony-level behavior of the "superorganism" as a whole as it efficiently exploits the surrounding resources to sustain itself.

Honeybees are central place foragers. Although domestic colonies reside in hives that are managed by beekeepers, such hives mimic the hollow tree cavities that are the species' natural nest sites. Inside the nest, the workers build sheets of vertical wax comb made up of hexagonal cells in which food is stored and brood (young bees) are reared. From that base, a subset of the workers, the foragers, leave to collect resources from the environment and bring them back to the nest. Tasks in the colony are allocated by age, with younger bees performing less risky tasks inside the nest such as nursing brood and food processing and switching to the more hazardous task of foraging toward the end of the lifespan. Foragers can travel up to approximately 14 km to collect food, although typically 95% of foraging trips are within 6 km in a temperate environment. This represents a foraging area of 100 km^2, partially reflecting the enormous requirements of a colony typically comprising around 30,000 animals. This demand represents a considerable foraging challenge: a honeybee colony in a temperate climate must collect approximately 120 kg of nectar, 20 kg of pollen, 25 l of water, and 100 g of resin each year (Seeley 1995).

Nectar is the energy currency of the colony and is collected both to fuel the current activity of the

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bees and to store for the winter and other times of dearth (Abou-Shaara 2014). Nectar is concentrated by evaporating off much of the water content, then mixed with salivary enzymes and stored as honey. If nectar is freely available, a colony will continue to collect and store as much as its labor and space resources allow. Pollen provides the protein source, as well as containing lipids and vitamins, but can only be processed by nurse bees. These young bees digest the pollen collected by foragers and convert it to a protein-rich secretion called brood food from the hypopharyngeal glands in the head and fed to other bees by trophyllaxis (transfer of liquid via the proboscis). In contrast to nectar, the colony does not collect pollen indefinitely, but instead maintains a stable amount that is sufficient to buffer the colony through periods when forage is unavailable. Water is collected for honey dilution, particularly early in the season when the colony is utilizing stored honey and pollen, and for nest cooling by evaporation. Collected in much smaller quantities, resin is mixed with saliva to form a sticky substance called propolis and used to seal cracks in the nest cavity.

The main forage substances, nectar and pollen, are collected primarily from flowers (although sugar may also be collected from fruit, honeydew, or robbing other honeybee colonies). Floral resources are transitory, characterized by a constantly varying patchwork of profitable locations that change over time. Some species of plant only produce nectar at certain times of the day, different species come into bloom at different times, and patches may be depleted and replenished over varying periods. To efficiently exploit the resources available to it, a colony must track the locations and profitability of the forage landscape as it changes over time. Patches that have ceased to be rewarding must be abandoned, additional foragers must be recruited to more rewarding patches and a scouting effort must be maintained to gather information on new forage sites. In addition, a colony must preserve a sufficient amount of stored food as a buffer during times of dearth such as bad weather or poor nectar flow, balancing this against current nutritional demands of brood and adults and available storage space and processing labor. How honeybees have evolved to respond to this foraging challenge, both at the individual and colony level, is discussed below.

Individual Foraging Behavior

In one foraging trip, a bee collects up to 15 mg of pollen or 30 mg of nectar (or a combination of both), often requiring visits to over 100 flowers. Efficient foraging at the individual level requires the integration of learning, sensory perception, adaptable flower handling behavior, and navigation.

Locating and Collecting Forage Resources

Honeybees rely primarily on visual and olfactory perception to locate flowers in the environment. Bees have compound eyes made up of several thousand functional units called ommatidia, resulting in visual resolution 100 times poorer than that of the human eye (Chittka and Raine 2006). Bee color vision is determined by the presence of three different color receptors in the ommatidia (ultraviolet [UV], green, and blue), allowing bees to perceive a visible spectrum spanning 300–650 nm, encompassing UV but not red light. Green light is particularly important for motion vision and detection of small targets. The polarization pattern of light can also be detected by bees using a narrow band of photodetectors on the dorsal region of the eyes (Kraft et al. 2011). Honeybee antennae contain at least 130 olfactory receptors, resulting in highly developed odor perception.

Learning about the visual and olfactory cues that identify the most rewarding flowers plays a critical role in maximizing honeybee foraging efficiency (Menzel and Muller 1996). Bees are able to quickly learn to associate the characteristics of flowers, such as color, shape, pattern, and odor, with reward, and will preferentially visit flower types that they learn to be more rewarding. In the laboratory, it has been shown that individuals are capable of learning not just about the physical properties of these cues, but also about the relationships between them. For example, individuals can learn concepts such as “the same as,” or “above/below.” Although the extent to which such abilities are put to use in the field has not yet been fully explored, it is clear that such "nonelemental learning" abilities have potential applications in optimizing foraging efficiency. Individuals also respond to information provided inadvertently by conspecifics in the field, including the visual presence of other feeding bees, which they use to identify both rewarding inflorescences and rewarding flower species. To minimize visits to flowers that other bees have recently drained, individuals typically avoid inflorescences where they detect cuticular hydrocarbons that have been inadvertently deposited by other foragers. Finally, there is some evidence that volatiles produced by stressed conspecifics might lead bees to avoid dangerous areas where other bees have been injured, since such volatiles have been shown to induce both aversion responses and future avoidance of contextual stimuli in laboratory settings.

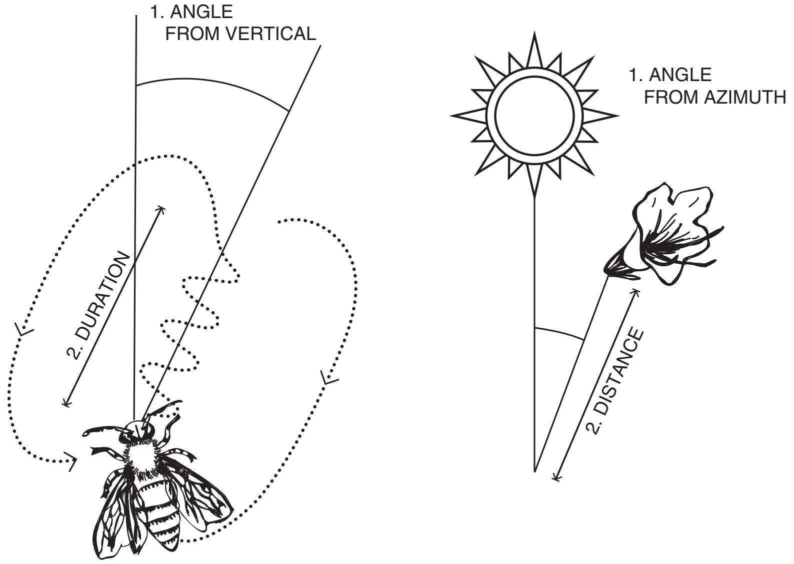
Although honeybees are generalist foragers, feeding from a broad range of species, a single individual commonly prefers one species during a foraging trip (Grüter and Ratnieks 2011). This may clearly be beneficial for the plant, increasing the probability that pollen from a conspecific will be transferred to its stigma resulting in pollination, but flower constancy may also be advantageous for the bee, increasing flower handling efficiency through honing skills on a specific floral morphology, or distributing a colony's foragers across different resources. However, visiting only one species in a mixed patch of flowers may have energetic costs associated with increased flight distance between flowers, and it has been suggested that flower constancy is a constraint due to limited memory capacity for floral characteristics. Nevertheless, the behavioral flexibility of flower constancy – for example, increasing fidelity when rewards are higher – suggests an adaptive basis.

As central place foragers, individual bees must find their way home at the end of a foraging bout. Foragers use a combination of path integration, celestial compass orientation, and landmark learning to navigate to resource patches and back to the nest (De Marco and Menzel 2005). Path integration involves integrating each successive vector of travel during a flight to produce a continuously updated distance and direction from the hive. Distance is measured by the bee using optic flow, i.e., the amount of visual information that passes the eye per unit time, on the outbound flight to the resource (Si et al. 2003), while the direction is measured using celestial cues from the sun and polarized light. Bees also use memory of landmarks as a spatial reference of nest location or as checkpoints along a route.

Communicating Forage Information

In 1946, the Austrian ethologist Karl von Frisch made a discovery that would later lead him to be awarded a Nobel Prize in Physiology or Medicine (shared with Niko Tinbergen and Konrad Lorenz): that honeybee foragers can communicate to their nestmates the location of the resource which they have visited, in order to recruit other foragers to exploit the same resource (von Frisch 1967). This highly evolved behavior, known as the waggle dance, represents perhaps the only known example of symbolic communication in a nonprimate species. On returning to the nest with pollen, nectar, or water from a particularly profitable source, a forager navigates to a part of the comb near the entrance, the "dance floor," where unemployed foragers are waiting for information on the day's forage. Here she performs a distinctive figure-of-eight dance on the comb, vibrating her body laterally 12–15 times per second as she walks in a straight line (the waggle phase), then ceases vibrating and veers alternately to the left or right, looping back to the start (the return phase) to repeat the waggle run (Fig. 1). This dance encodes three pieces of information about the resource: distance, direction, and quality (Couvillon 2012).

The vector (distance and direction) information is contained in the waggle phase. The distance of the resource from the nest is encoded in the length of time that the bee vibrates her body, with a linear relationship between duration and distance. Dances for locations that are very close to the hive elicit such short waggle runs that often no vibration is present, resulting in an erratic truncated dance called the round dance, now widely accepted as an extremely short waggle dance rather than a separate behavior (Gardner et al. 2008). Bees have a gravity-proprioceptive



Foraging by Honeybees, Fig. 1 The honeybee waggle dance. A forager returning from a profitable resource such as a flower patch informs her nestmates of the location of the resource by performing a distinctive figure-of-eight dance on the comb (*left*). Two pieces of information are encoded in the dance (*right*): (*1*) the angle from the solar azimuth, encoded in the angle of the waggle run with respect to gravity; and (*2*) the distance from the nest, encoded in the length of time for which the bee vibrates her body. This forms a vector allowing unemployed foragers following the dance to locate the resource

system involving sensory neurons in the neck hair plates, allowing the bee to direct her waggle run at a specific angle relative to gravity. This corresponds to the angle from the current solar azimuth at which the resource is located, measured from celestial compass information. A bee may continue dancing over sufficient time for the sun's position to have moved, rendering the original dance angle inaccurate; however, the integration of endogenous circadian clock information allows a dancer to extrapolate the current azimuth without leaving the nest, modifying her angle to track the sun's position over time.

A combination of tactile input via antennation of the dancer's abdomen and perception of directional jet air flows produced by the oscillation of the dancer's wings allow bees following the dance to determine the angle and duration in the darkness of the hive (Gil and De Marco 2010; Michelsen 2003). These two pieces of information form a vector that is theoretically sufficient for nestmates to locate the resource. However, there is considerable variation between different runs within a single dance, meaning that the dance does not signify a precise location. Could this error be adaptive? Floral resources rarely consist of a single point in space, and researchers have theorized that the error has evolved to optimally spread foragers across large patches of flowers. Accordingly, angular variation decreases with increasing communicated distance, consistent with recruits being spread across even-sized patches at near and far distances. Yet there is also considerable evidence to the contrary, suggesting that dance angle variation is driven by biophysical constraints: for example, error is reduced when dancers have a direct view of the sun, when dancing parallel to gravity or when return phases are longer (Preece and Beekman 2014). To minimize the effects of this error, it appears dance followers take an average of several runs, with recruits that follow more runs locating the site more accurately.

Not all foraging resources are of equal quality: nectar concentration and volume, distance from the nest, floral density, and risk of predation or competition all contribute to the profitability of a resource (Abbott and Dukas 2009). The third piece of information encoded in the dance concerns resource quality, primarily in the form of the number of waggle runs performed. Bees may perform between 1 and 100 runs within a single dance, with a linear relationship between number of runs and resource quality. The rate of waggle runs per unit time, modified by altering the duration of the return phase, is also correlated with quality. A forager's measure of profitability is based on two sets of variables: features of the resource itself and the current forage supply and demand of the colony. For nectar sources, resource variables include sugar concentration; while all bees show a linear relationship (up to the point where viscosity limits profitability) between runs and sugar concentration, individual assessment varies widely between bees, reflected in differences in the slope of the relationship and the concentration threshold that elicits dancing. Sugar concentration is combined with the energetic cost of the flight (related to distance, but not measured using optic flow) to calculate the energy efficiency of exploiting a resource and it is this in addition to risk that informs a bee's assessment of the objective quality of a resource.

In contrast to nectar, it is likely that bees cannot assess the protein content of pollen (Beekman et al. 2016). Assessment of relative profitability in the context of current overall forage influx into the colony and processing capacity is discussed below (see "▶ Regulating Nectar Influx"). Combining these variables results in a final integrated assessment of quality encoded in the run number and intensity. However, unlike the vector information, this is not directly perceived by dance followers, who only follow a small proportion of the runs of any dance. Instead, this forms part of the elegant system of feedback loops regulating forage intake at the colony level described below (see "▶ Spreading Labor Across the Foraging Landscape").

Colony-Level Foraging Behavior

We have seen that the individual forager possesses a suite of complex behavioral traits that allow her to efficiently locate flower patches, collect resources, and communicate their location on return to the nest. How are these individual endeavors integrated at the colony level, to spread labor across the foraging landscape, tightly control the influx of resources and regulate the balance between collection and processing of food? Like the human body, a honeybee colony must regulate its energy intake and expenditure, maintaining a complex array of homeostatic processes that depend upon extensive information flow between its units, the individual bees. No single individual or group possesses global information about all the processes occurring in the colony. Rather, each individual responds to local information according to a set of simple rules, resulting in a self-organized system that is capable of responding flexibly to environmental conditions and making adaptive decisions in its own right. Information flow in the colony consists of a combination of signals (behaviors that have evolved specifically to convey information) and cues (provided inadvertently, as a byproduct of other behavior) (Seeley 1998). One example of a signal, the waggle dance, has already been discussed; in the following section, we present a further array of signals and cues involved in the regulation of foraging at the colony level.

Spreading Labor Across the Foraging Landscape

At its strongest, a honeybee colony may contain up to 60,000 workers. This labor market must be effectively distributed across the major behavioral roles – cleaners, nurses, food storers, and foragers – and even across subdivisions within those groups, such as pollen or nectar foraging, scouting, or dance following. The proportion of workers involved in each task must be balanced with the supply and demand of brood and forage, and coordinated with other roles; it does not work to have large numbers of successful nectar foragers returning if there are insufficient food storers to receive their loads. At the highest level, behavioral tasks are allocated by age: newly emerged bees clean cells, the second youngest care for brood, middle-aged bees process food and perform other in-nest tasks and the oldest bees forage. This age polyethism potentially maximizes worker longevity, with the task involving the highest associated mortality, foraging, allocated to bees close to the end of their natural lifespans. The age of transition is flexible and can respond to changes in colony requirements. Within the forager caste, an individual's propensity to collect pollen, nectar, or water is associated with sensitivity to sucrose; bees with low, medium, and high sucrose response thresholds preferentially forage for water, pollen, and nectar respectively. This variation is thought to be adaptive at the colony level, facilitating division of labor.

At any one time, around a quarter of the colony workforce are foragers. Those not currently employed in exploiting a resource, due to the abandonment of a no longer rewarding patch or just beginning their foraging career, may be allocated to a patch in a number of ways. A small proportion of foragers will be scouts, exploring the environment in search of novel food sources. The remaining foragers may use memory of previously visited patches, or social information to locate resources. Social information about foraging locations comes in the form of waggle dances or via olfactory information from floral odor carried on dancers' bodies or in nectar samples transferred to followers (trophallaxis). At any time on the dance floor, several dancers may be advertising multiple different locations. How does a forager choose which location to visit? As previously described, the dancer encodes the profitability of the resource in the number of waggle runs performed. One solution would be for a follower to survey many of the dances and select the one communicating the highest quality. However, such a system would not only require extensive dance sampling effort on the part of dance followers, but also necessitate measurement and comparison of the number of runs that each dancer performs. Instead, a much simpler self-organized system has evolved to achieve the same end, based on each worker responding to a small amount of local information rather than collecting global information. A dance follower has no perception of the quality encoded in the dance, and thus she may well select and follow a dance not advertising the most profitable site available. However, because dances for higher quality sites contain more runs, the number of bees that encounter these dances is higher. As such, the number of recruits to a resource is proportional to the number of waggle runs performed for it. In addition, more profitable resources are more likely to elicit dances in the first place. This ensures an appropriate distribution of foragers across sites, relative to their quality, and allows the colony to rapidly track changes in profitability.

A surprisingly low proportion of dance followers visit the advertised site. The location encoded in the waggle dance (a signal) is not the only piece of information provided by dancing bees, and the floral odor carried by dancers (a cue) may take precedence over the location information conveyed by the dance (Grüter and Farina 2009). If the odor is from a flower species a follower has experience with, this can reactivate the memory of a previously visited forage patch, creating a conflict between personal memory and social information about forage locations encoded in the dance (Grüter et al. 2008). There is evidence that in this scenario bees preferentially rely upon their own location memories, but increase their use of waggle dance information when those sites become unrewarding. As foragers age, they rely less on waggle dance information and more on private memory. Unemployed foragers may also follow a dance for confirmation of the continued profitability of a previously visited site. In practice, only 12–25% of dances are followed for the discovery of new sites, with the remaining associated with reactivation to known resources (Biesmeijer and Seeley 2005).

Regulating Nectar Influx

Collection of resources must be coordinated with the colony's current requirements. Returning nectar foragers must decide whether to recruit additional foragers to a patch, given the current availability of other sources. Rather than collecting direct, global information about the entire colony's nectar influx, returning foragers use an indirect cue in the form of the time it takes to unload their nectar load. If nectar influx is currently low, there will be several unemployed food-storer bees waiting to receive nectar, so unloading time will be short. If nectar influx is high, a returning forager may have to queue to unload. The precision of this measurement is increased by making multiple transfers of portions of the load; the average unloading delay informs the forager about the colony's nectar influx. Although her objective assessment of the quality (sugar concentration, distance, and risk) of the resource does not change, this information about nectar influx alters the quality threshold that elicits a dance and the intercept of the relationship between quality and number of runs. Therefore, a low-quality resource that would elicit many waggle runs during a time of dearth may not elicit any dancing at all during peak nectar flow.

In addition to the waggle dance, several other movement signals have evolved to regulate the balance between nectar collection and processing. Unloading delay is also used by returning foragers to perceive a need for increased food processing effort. A long delay increases the probability that the returning forager will subsequently perform a "tremble dance," which stimulates nest bees to become food-storers and inhibits waggle dances in other returning foragers (Biesmeijer 2003b). A bee shaking a nestmate by grasping her with her legs and vibrating her body forms a more general, context-dependent signal to encourage reassessment of current activity (Biesmeijer 2003a). An inhibitory signal, the stop signal, is also now known to be used by foragers and dance followers to interrupt dancers advertising a site known to be associated with danger from predation or competitive aggression or to be no longer rewarding (Nieh 2010).

Homestatic Control of Pollen Stores

As discussed above, the only limits to the amount of nectar a honeybee colony extracts from its environment are the labor available to collect and process it and the space available for storage. In contrast, a colony maintains a limited stable pollen store that requires precise homeostatic control (Schmickl and Crailsheim 2004). Thus, instead of being driven primarily by supply, pollen foraging is also driven by demand. Pollen demand changes with the amount of brood present and supply changes with the foraging conditions; regulation needs to integrate both these variables. How pollen stores are homeostatically controlled has been subject to considerable debate and research effort, with two main theories posited: (1) the direct, independent hypothesis, and (2) the brood food hypothesis.

The direct, independent hypothesis suggests that the global status of pollen supply and demand are measured directly by returning foragers. Quantity of stored pollen (supply) may be measured by the time taken to find an empty cell; unlike nectar, pollen is deposited directly into cells by foragers. Amount of brood (pollen demand) may be measured by brood pheromone levels in the nest, which may be either an evolved signal or an incidental cue. According to this theory, foragers integrate these pieces of information to assess the need to collect pollen. Some evidence in support of this exists: brood pheromone has been shown to affect pollen foraging activity and returning pollen foragers perform cell inspections (Dreller et al. 1999). The alternative theory suggests that an indirect cue – the protein content of the hypopharyngeal secretions ("brood food") fed by nurse bees to all workers – gives information on the combined pollen supply and demand. In times of protein scarcity, either due to increased brood or reduced pollen stores, low protein content in the brood food fed to foragers would stimulate increased pollen foraging. Research has shown that separating foragers from the pollen stores with a screen does not disrupt pollen store homeostasis (Camazine 1993), suggesting that direct contact with stores is not required, and an inhibitory effect of trophallaxis on pollen foragers has also been found (Camazine et al. 1998). However, experiments using a protease inhibitor to artificially lower brood food protein content have found no evidence for the brood food hypothesis (Sagili and Pankiw 2007). The two hypotheses are not mutually exclusive, and it is likely that a combination of direct and indirect cues are involved in regulation of pollen storage.

Conclusion

In any animal species, foraging behavior is under intense selection pressure. The ability of an individual to successfully extract energy and nutrition from its environment while minimizing hazards and metabolic costs is a strong driver of fitness. The honeybee is no exception: natural selection has shaped individual foraging behavior such that a bee can locate flower patches, efficiently collect nectar and pollen and remember the locations and characteristics of profitable resources. However, the honeybee differs from most other taxa in that selection has also acted strongly upon foraging behavior at another level of biological organization: the colony. The behavior of the "superorganism" has evolved such that the colony acts as a static reproductive unit from which individual parts can be deployed to collect resources from its surroundings. As such, selection has favored maximizing the energy balance and nutritional regulation of the colony as a whole. Intricate networks of information flow via communication signals and cues coupled with a suite of behavioral rules at the individual level in response to local information results in a highly complex self-organized foraging system. In this way, the honeybee colony provides an intriguing comparative model for nutritional regulation and sensory perception in analogous self-organized systems such as the human body, on a macroscopic scale.

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Cross-References

- ▶ Altruism
- ▶ Benefits of Group Living
- ▶ Central-Place Foraging
- ▶ Cooperation
- ▶ Entomology
- ▶ Feeding
- ▶ Food Patch
- ▶ Foraging
- ▶ Group-Living
- ▶ Hymenoptera Cognition
- ▶ Hymenoptera Communication
- ▶ Hymenoptera Diet
- ▶ Hymenoptera Life History
- ▶ Hymenoptera Navigation
- ▶ Hymenoptera Sensory Systems
- ▶ Insect Cognition
- ▶ Insect Communication
- ▶ Insect Diet
- ▶ Insect Life History
- ▶ Insect Navigation
- ▶ Insect Sensory Systems
- ▶ Karl von Frisch
- ▶ Landmark
- ▶ Learning
- ▶ Navigation
- ▶ Navigation by Honeybees
- ▶ Nest Construction
- ▶ Olfactory Cues
- ▶ Olfactory Discrimination
- ▶ Optic Flow
- ▶ Social Behavior
- ▶ Social Learning
- ▶ Species-Specific Behavior
- ▶ Superorganism
- ▶ Symbolic Communication
- ▶ The Waggle Dance
- ▶ Visual Recognition
- ▶ Visuo-Spatial Memory

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Appendix Two

Supplementary Tables for Chapter Four

Table S1. Tables of candidate models to analyse the effect of land-use on colony success measures using a) zero-inflated hurdle models to analyse reproductive production as a binary response and a count process; b) linear models to analyse log-transformed peak colony size; Cox proportional hazards models to analyse c) queen survival and d) colony survival; binomial GAMs to analyse e) ovary development and presence of f) nectar and g) pollen in the nest over time with *site* as a random effect; h) binomial GLMs to analyse *Bombus vestalis* invasions. In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i); only the first eight candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

a	Reproductive output			
Count process	Binomial process	AICc	ΔAICc	w_i
500m Land-use	**500m Land-use**	**284.4**	**0**	**0.277**
Basic	**500m Land-use**	**284.5**	**0.14**	**0.259**
500m Land-use	**Basic**	**284.9**	**0.5**	**0.216**
Basic	**Basic**	**285.7**	**1.32**	**0.143**
500m Land-use + Temperature	500m Land-use	287.3	2.93	0.064
500m Land-use + Temperature + Rainfall	500m Land-use	290.5	6.14	0.013
500m Land-use + Temperature	500m Land-use + Temperature	290.6	6.16	0.013
500m Land-use + Temperature + Rainfall	500m Land-use	292.7	8.3	0.004

b	Peak colony size		
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall	**88.7**	**0**	**0.333**
500m Land-use	**89.8**	**1.05**	**0.197**
500m Land-use + Rainfall + Humidity	**90.3**	**1.55**	**0.153**
500m Land-use + Humidity	91.7	3.04	0.073
Rainfall + Humidity	92.3	3.63	0.054
500m Land-use + Temperature	92.4	3.71	0.052
500m Land-use + Temperature + Rainfall + Humidity	93.3	4.57	0.034
Basic	94	5.28	0.024

c	Queen survival		
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall	**140.3**	**0**	**0.365**
500m Land-use	**141.4**	**1.11**	**0.21**
500m Land-use + Rainfall + Humidity	142.8	2.5	0.104
500m Land-use + Humidity	143.5	3.28	0.071
500m Land-use + Temperature	143.6	3.37	0.068
Rainfall	143.8	3.56	0.061
500m Land-use + Temperature + Rainfall + Humidity	145.4	5.17	0.028
Temperature + Rainfall	145.9	5.61	0.022

d	Colony survival		
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall	**195.9**	**0**	**0.472**
500m Land-use	197.9	2.02	0.172
500m Land-use + Rainfall + Humidity	198.1	2.19	0.158
500m Land-use + Temperature + Rainfall + Humidity	200.2	4.29	0.055
500m Land-use + Humidity	200.3	4.37	0.053

500m Land-use + Temperature	200.3	4.38	0.053
500m Land-use + Temperature + Humidity	202.8	6.88	0.015
Rainfall	204.1	8.17	0.008
e Ovary development			
Model	AICc	ΔAICc	w_i
500m Land-use + s(Week,5)	**474.2**	**0**	**0.108**
500m Land-use + Rainfall + Humidity + s(Week,5)	**474.2**	**0.06**	**0.105**
500m Land-use + Temperature + Humidity + s(Week,5)	**474.5**	**0.35**	**0.09**
500m Land-use + Temperature + s(Week,5)	**474.6**	**0.42**	**0.087**
500m Land-use + Rainfall + s(Week,5)	**474.6**	**0.45**	**0.086**
500m Land-use + Humidity + s(Week,5)	**474.8**	**0.66**	**0.077**
500m Land-use + Temperature + Rainfall + Humidity + s(Week,5)	**474.9**	**0.72**	**0.075**
500m Land-use + Temperature + Rainfall + s(Week,5)	**474.9**	**0.76**	**0.074**
f Presence of nectar in nest			
Model	AICc	ΔAICc	w_i
500m Land-use + s(Week,5)	**216.7**	**0**	**0.401**
500m Land-use + Rainfall + s(Week,5)	**218.1**	**1.4**	**0.199**
500m Land-use + Temperature + s(Week,5)	219	2.25	0.13
500m Land-use + Rainfall + Humidity + s(Week,5)	220.3	3.61	0.066
500m Land-use + Temperature + Rainfall + s(Week,5)	220.4	3.72	0.062
500m Land-use + Temperature + Humidity + s(Week,5)	221.2	4.48	0.043
s(Week,5)	221.7	5.04	0.032
500m Land-use + Temperature + Rainfall + Humidity + s(Week,5)	222.5	5.83	0.022
g Presence of pollen in nest			
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall + s(Week,5)	**290.1**	**0**	**0.302**
500m Land-use + Rainfall + Humidity + s(Week,5)	**290.1**	**0.02**	**0.299**
500m Land-use + Temperature + Rainfall + Humidity + s(Week,5)	**292**	**1.93**	**0.115**
500m Land-use + Temperature + Rainfall + s(Week,5)	292.1	2.02	0.11
500m Land-use + Temperature + Humidity + s(Week,5)	292.9	2.83	0.073
500m Land-use + s(Week,5)	293	2.86	0.072
500m Land-use + Temperature + s(Week,5)	295.1	4.99	0.025
500m Land-use + Temperature + Rainfall + Humidity	299.8	9.67	0.002
h *Bombus vestalis* invasions			
Model	AICc	ΔAICc	w_i
500m Land-use + Humidity	**53**	**0**	**0.247**
500m Land-use	**53.2**	**0.18**	**0.226**
500m Land-use + Rainfall	**53.8**	**0.74**	**0.171**
500m Land-use + Rainfall + Humidity	**54.4**	**1.35**	**0.126**
500m Land-use + Temperature + Humidity	55	2.02	0.09
500m Land-use + Temperature + Rainfall	55.1	2.1	0.087
500m Land-use + Temperature + Rainfall + Humidity	56.8	3.77	0.037
Temperature	61.3	8.25	0.004

Table S2. Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable) for analyses of the effect of land-use at a 500m radius on colony success measures. Parameters highlighted in bold are considered important to the model based on 95% CIs.

a	Reproductive output			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count: (Intercept)	2.0609	1.5099	-0.899	5.02
Count: 500m Land-use (Village)	2.5657	1.0136	0.579	4.552
Count: 500m Land-use (City)	2.7886	1.0149	0.799	4.778
Count: Log(theta)	0.6642	0.3215	0.034	1.294
Binomial: (Intercept)	-0.5195	1.3848	-3.234	2.195
Binomial: 500m Land-use (Village)	2.8526	1.2885	0.327	5.378
Binomial: 500m Land-use (City)	2.2618	1.2383	-0.165	4.689

b	Peak colony size			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.954273	1.245037	-0.486	4.395
500m Land-use (Village)	**1.046538**	**0.371272**	**0.319**	**1.774**
500m Land-use (City)	**0.917691**	**0.369246**	**0.194**	**1.641**
Rainfall	**0.012639**	**0.006641**	**0**	**0.026**
Humidity	0.023268	0.021884	-0.02	0.066

c	Queen survival				
Parameters	Regression coefficient (b)	d. Error (SE(b	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
500m Land-use (Village)	**-1.9862**	**0.64692**	**0.137216**	**0.038613**	**0.487611**
500m Land-use (City)	**-1.90358**	**0.65913**	**0.149034**	**0.040947**	**0.542436**
Rainfall	-0.02592	0.01395	0.974413	0.948132	1.001423

d	Colony survival				
Parameters	Regression coefficient (b)	d. Error (SE(b	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
500m Land-use (Village)	**-2.61347**	**0.66815**	**0.07328**	**0.019781**	**0.271472**
500m Land-use (City)	**-2.19901**	**0.64963**	**0.110913**	**0.031046**	**0.39624**
Rainfall	**-0.02301**	**0.01125**	**0.977253**	**0.95594**	**0.99904**

e	Ovary development			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	2.241878	5.220795	-7.991	12.475
500m Land-use (Village)	-1.91184	1.06624	-4.002	0.178
500m Land-use (City)	-1.739429	1.066493	-3.83	0.351
Humidity	-0.064702	0.063921	-0.19	0.061
Rainfall	-0.008717	0.017147	-0.042	0.025

Temperature	0.10983	0.242273	-0.365	0.585

f	Presence of nectar in nest			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-1.89874	0.709561	-3.289	-0.508
500m Land-use (Village)	**1.902089**	**0.761149**	**0.41**	**3.394**
500m Land-use (City)	**2.014523**	**0.762484**	**0.52**	**3.509**
Rainfall	-0.01196	0.013825	-0.039	0.015

g	Presence of pollen in nest			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	0.871551	2.783962	-4.585	6.328
500m Land-use (Village)	**2.03765**	**0.543247**	**0.973**	**3.102**
500m Land-use (City)	**2.108748**	**0.542887**	**1.045**	**3.173**
Rainfall	**-0.02663**	**0.013466**	**-0.053**	**0**
Humidity	-0.04209	0.028787	-0.099	0.014
Temperature	-0.10151	0.120004	-0.337	0.134

h	*Bombus vestalis* invasions			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-1.57201	6.43246	-14.18	11.036
500m Land-use (Village)	**-2.94303**	**1.27595**	**-5.444**	**-0.442**
500m Land-use (City)	**-3.77645**	**1.28965**	**-6.304**	**-1.249**
Humidity	0.12588	0.08739	-0.045	0.297
Rainfall	-0.0293	0.02472	-0.078	0.019

i	Onset of reproduction				
Parameters	Regression coefficient (b)	Std. Error (SE(b))	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
500m Land-use (Village)	1.389	1.039	4.010837	0.523384	30.7
500m Land-use (City)	1.155	1.042	3.174023	0.411758	24.5

j	Male fat content			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.339245	0.491812	0.375	2.303
500m Land-use (City)	-0.10054	0.105539	-0.307	0.106
Humidity	-0.00379	0.006483	-0.016	0.009
Rainfall	0.004544	0.003072	-0.001	0.011
Temperature	0.002222	0.024564	-0.046	0.05

k	Worker fat content			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	0.416772	0.16027	0.103	0.731
Humidity	0.00178	0.001914	-0.002	0.006
Rainfall	-0.00458	0.006904	-0.018	0.009

l	Worker thorax width			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	4.409565	0.790866	2.859	5.96
Humidity	0.01202	0.006308	0	0.024
500m Land-use (Village)	0.242615	0.171482	-0.093	0.579
500m Land-use (City)	0.247923	0.171305	-0.088	0.584
Temperature	-0.01903	0.024369	-0.067	0.029

m	Male thorax width			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	5.737185	0.497939	4.761	6.713
Humidity	0.001443	0.005363	-0.009	0.012
Rainfall	0.005706	0.002244	0.001	0.01
Temperature	-0.01748	0.018316	-0.053	0.018
500m Land-use (City)	-0.03755	0.07087	-0.176	0.101

n	*Crithidia bombi* presence			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-12.1946	3.374	-18.808	-5.582
Temperature	**0.545**	**0.2044**	**0.144**	**0.946**

o	*Apicystis bombi* presence			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-5.4916	3.73871	-12.819	1.836
Humidity	0.07823	0.06331	-0.046	0.202
Rainfall	0.01477	0.01542	-0.015	0.045

Table S3. Tables of candidate models to analyse the effect of proportion flower rich habitat on colony sucess measures using a) zero-inflated hurdle models to analyse reproductive production as a binary response and a count process; b) linear models to analyse log-transformed peak colony size; Cox proportional hazards models to analyse c) queen survival and d) colony survival; binomial GAMs to analyse presence of e) nectar and f) pollen in the nest over time with *site* as a random effect. In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i); only the first eight candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

a	Reproductive output			
Count process	Binomial process	AICc	ΔAICc	w_i
Basic	**Basic**	**285.7**	**0**	**0.446**
Proportion flower-rich	**Basic**	**287.2**	**1.53**	**0.207**
Basic	**Proportion flower-rich**	**287.6**	**1.86**	**0.176**
Proportion flower-rich	Proportion flower-rich	289.3	3.55	0.076
Proportion flower-rich + Temperature	Proportion flower-rich	290	4.3	0.052
Proportion flower-rich + Temperature	Proportion flower-rich + Temperature	292.5	6.79	0.015
Proportion flower-rich + Rainfall + Temp	Proportion flower-rich	292.8	7.04	0.013
Proportion flower-rich	Temperature + Rainfall + Humidity	294.9	9.18	0.005

b	Peak colony size		
Model	AICc	ΔAICc	w_i
Humidity + Rainfall	**92.3**	**0**	**0.22**
Proportion flower-rich + Rainfall	**92.5**	**0.17**	**0.202**
Proportion flower-rich + Rainfall + Humidity	**93.8**	**1.47**	**0.106**
Basic	**94**	**1.65**	**0.097**
Rainfall + Temperature	**94.2**	**1.88**	**0.086**
Humidity + Rainfall + Temperature	94.9	2.58	0.061
Proportion flower-rich + Rainfall	95.2	2.87	0.053
Humidity	95.2	2.88	0.052

c	Queen survival		
Model	AICc	ΔAICc	w_i
Rainfall	**143.8**	**0**	**0.345**
Proportion flower-rich + Rainfall	145.8	2	0.127
Rainfall + Temperature	145.9	2.05	0.124
Humidity + Rainfall	146.1	2.23	0.113
Basic	146.5	2.72	0.088
Proportion flower-rich + Rainfall + Humidity	148.2	4.34	0.039
Humidity + Rainfall + Temperature	148.2	4.39	0.038
Humidity	148.4	4.55	0.035

d	Colony survival		
Model	AICc	ΔAICc	w_i
Rainfall	**204.1**	**0**	**0.276**
Humidity + Rainfall	**205.3**	**1.28**	**0.145**
Rainfall + Temperature	**205.8**	**1.79**	**0.113**
Basic	**205.9**	**1.89**	**0.107**

Proportion flower-rich + Rainfall	206.3	2.22	0.091
Proportion flower-rich	207.4	3.32	0.052
Proportion flower-rich + Rainfall + Humidity	207.5	3.49	0.048
Proportion flower-rich + Rainfall	207.6	3.55	0.047
e Presence of nectar in nest			
Model	AICc	ΔAICc	w_i
Proportion flower-rich + s(Week,5)	**220.9**	**0**	**0.262**
s(Week,5)	**221.7**	**0.82**	**0.174**
Proportion flower-rich + Temperature + s(Week,5)	**222.8**	**1.84**	**0.105**
Proportion flower-rich + Rainfall + s(Week,5)	223	2.04	0.094
Rainfall + s(Week,5)	223.7	2.77	0.066
Humidity + s(Week,5)	223.8	2.92	0.061
Temperature + s(Week,5)	223.9	3	0.058
Proportion flower-rich + Temperature + Humidity + s(Week,5)	224.6	3.67	0.042
f Presence of pollen in nest			
Model	AICc	ΔAICc	w_i
Rainfall + s(Week,5)	**290.1**	**0**	**0.302**
Temperature + Rainfall + s(Week,5)	**290.1**	**0.02**	**0.299**
Proportion flower-rich + Rainfall + s(Week,5)	**292**	**1.93**	**0.115**
Humidity + s(Week,5)	292.1	2.02	0.11
Proportion flower-rich + Rainfall + Humidity + s(Week,5)	292.9	2.83	0.073
s(Week,5)	293	2.86	0.072
Temperature + Rainfall + Humidty + s(Week,5)	295.1	4.99	0.025
Proportion flower-rich + Temperature + Rainfall + s(Week,5)	299.8	9.67	0.002

Table S4. Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable) for analyses of the effect of proportion flower rich habitat on colony success measures. Parameters highlighted in bold are considered important to the model based on 95% CIs.

a	Reproductive output			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count: (Intercept)	3.591	0.2549	3.091396	4.090604
Count: Log(theta)	0.5569	0.3042	-0.039332	1.153132
Binomial: (Intercept)	0.8727	0.4859	-0.079664	1.825064
Count: Proportion flower-rich	-1.8742	1.8792	-5.557432	1.809032
Binomial: Proportion flower-rich	-2.5731	3.2161	-8.876656	3.730456

b	Peak colony size			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	2.033239	1.880911	-1.65334656	5.71982456
Humidity	0.029757	0.023192	-0.01569932	0.07521332
Rainfall	**0.016138**	**0.007091**	**0.00223964**	**0.03003636**
Proportion flower-rich	-1.388045	1.145582	-3.63338572	0.85729572
Temperature	-0.03032	0.087733	-0.20227668	0.14163668

c	Queen survival				
Parameters	Regression coefficient (b)	d. Error (SE(b	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
Rainfall	**-0.02674**	**0.01202**	**0.97361**	**0.9509**	**0.9968**

d	Colony survival				
Parameters	Regression coefficient (b)	d. Error (SE(b	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
Rainfall	**-0.02045**	**0.01014**	**0.97975768**	**0.96047781**	**0.999425**
Humidity	-0.03508	0.03593	0.96552817	0.899871953	1.035975
Temperature	0.09596	0.14425	1.10071503	0.82963453	1.46037

e	Presence of nectar in nest			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-0.082289	1.072149	-2.18370104	2.01912304
Proportion flower-rich	-3.02378	1.948696	-6.84322416	0.79566416
Temperature	0.073488	0.129147	-0.17964012	0.32661612
Rainfall	-0.0076597	0.0136529	-0.034419384	0.019099984

f	Presence of pollen in nest			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	0.85498	1.79459	-2.6624164	4.3723764
Rainfall	**-0.02602**	**0.01292**	**-0.0513432**	**-0.0006968**
Temperature	**0.06601**	**0.11279**	**-0.1550584**	**0.2870784**
Proportion flower-rich	**-0.49613**	**1.66022**	**-3.7501612**	**2.7579012**
Humidity	-0.0446	0.02833	-0.1001268	0.0109268

Table S5. Tables of candidate models using a) Cox proportional hazards models to analyse onset of reproduction; Gaussian GAMs to analyse b) male fat content, c) worker fat content, d) male size and e) worker size; and binomial GLMs to analyse f) *Crithidia bombi* and g) *Apicystis bombi* presence. In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i); only the first eight candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

a	Onset of reproduction		
Model	AICc	ΔAICc	w_i
Basic	**154.8**	**0**	**0.323**
500m Land-use	**156.5**	**1.67**	**0.14**
Humidity	156.9	2.09	0.114
Rainfall	156.9	2.11	0.113
500m Land-use + Humidity	158.4	3.52	0.056
500m Land-use + Temperature	158.8	3.98	0.044
500m Land-use + Rainfall	158.9	4.03	0.043
Temperature + Humidity	159	4.21	0.039

b	Male fat content		
Model	AICc	ΔAICc	w_i
500m Land-use + s(Week,)	**303.2**	**0**	**0.133**
s(Week)	**303.4**	**0.18**	**0.121**
500m Land-use + Rainfall + Humidity + s(Week)	**304.1**	**0.84**	**0.087**
Rainfall + Humidity + s(Week)	**304.2**	**0.93**	**0.083**
500m Land-use + Humidity + s(Week)	**304.2**	**1.02**	**0.079**
Humidity + s(Week)	**304.4**	**1.19**	**0.073**
500m Land-use + Temperature + s(Week)	**304.7**	**1.47**	**0.064**
500m Land-use + Temperature + Rainfall + s(Week)	**304.9**	**1.63**	**0.059**

c	Worker fat content		
Model	AICc	ΔAICc	w_i
s(Week)	**-319.6**	**0**	**0.185**
Humidity + s(Week)	**-319.4**	**0.22**	**0.166**
Temperature + s(Week)	**-318.9**	**0.68**	**0.131**
Temperature + Humidity + s(Week)	**-318.1**	**1.54**	**0.086**
Rainfall + Humidity + s(Week)	-317.5	2.07	0.066
500m Land-use + s(Week)	-317.4	2.24	0.06
Temperature + Rainfall + s(Week)	-317.2	2.4	0.056
500m Land-use + Humidity + s(Week)	-317	2.58	0.051

d	Male thorax width		
Model	AICc	ΔAICc	w_i
Rainfall + Humidity + s(Week)	**106**	**0**	**0.145**
Temperature + Rainfall + s(Week)	**106.1**	**0.07**	**0.14**
500m Land-use + Rainfall + Humidity + s(Week)	**106.4**	**0.37**	**0.12**
500m Land-use + Temperature + Rainfall + s(Week)	**106.4**	**0.42**	**0.118**
Temperature + Rainfall + Humidity + s(Week)	**106.9**	**0.87**	**0.094**
500m Land-use + Temperature + Rainfall + Humidity + s(Week)	**107.2**	**1.23**	**0.079**
s(Week)	**107.4**	**1.36**	**0.074**
500m Land-use + s(Week)	**107.6**	**1.62**	**0.065**

e	Worker thorax width		
Model	AICc	ΔAICc	w_i
Humidity + s(Week)	**450.3**	**0**	**0.175**
500m Land-use + Humidity + s(Week)	**451.1**	**0.83**	**0.116**
Temperature + s(Week)	**451.3**	**1.06**	**0.103**
Temperature + Humidity + s(Week)	**451.4**	**1.17**	**0.098**
500m Land-use + Temperature+ s(Week)	**451.8**	**1.52**	**0.082**
s(Week)	**452.1**	**1.83**	**0.07**
500m Land-use + Temperature + Humidity + s(Week)	**452.2**	**1.93**	**0.067**
Rainfall + Humidity + s(Week)	452.3	2.07	0.062

f	*Crithidia bombi* presence		
Model	AICc	ΔAICc	w_i
Temperature	**51.3**	**0**	**0.325**
Temperature + Rainfall	53.5	2.13	0.112
500m Land-use + Temperature + Rainfall	53.6	2.31	0.103
Temperature + Humidity	53.7	2.41	0.098
500m Land-use + Temperature + Humidity	54	2.69	0.085
500m Land-use	54.3	2.92	0.075
500m Land-use + Rainfall	55.3	3.94	0.045
Basic	56	4.66	0.032

g	*Apicystis bombi* presence		
Model	AICc	ΔAICc	w_i
Basic	**54**	**0**	**0.255**
Humidity	**54.6**	**0.66**	**0.183**
Rainfall	**55.3**	**1.35**	**0.13**
500m Land-use	56.2	2.22	0.084
Temperature	56.2	2.26	0.082
Temperature + Humidity	56.7	2.74	0.065
500m Land-use + Humidity	57.5	3.6	0.042
Temperature + Rainfall	57.7	3.7	0.04

Table S6. Tables of candidate models including land-use at a 100m radius around colonies using a) GAMs to analyse male thorax width over time with site as a random effect and b) binomial GLMs to analyse presence of *Apicystis bombi.* In both cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (wi); only the first eight candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

a	Male thorax width		
Model	AICc	ΔAICc	w_i
100m Land-use + Temperature + Rainfall + s(Week,5)	**104.8**	**0**	**0.158**
100m Land-use + Rainfall + Humidity + s(Week,5)	**105**	**0.18**	**0.145**
Rainfall + Humidity + s(Week,5)	**105.3**	**0.51**	**0.122**
100m Land-use + Temperature + Rainfall + Humidity + s(Week,5)	**105.4**	**0.56**	**0.119**
Temperature + Rainfall + s(Week,5)	**105.4**	**0.6**	**0.117**
Temperature + Rainfall + Humidity + s(Week,5)	**106.2**	**1.44**	**0.077**
100m Land-use + s(Week,5)	**106.6**	**1.74**	**0.066**
s(Week,5)	107.1	2.27	0.051

b	*Apicystis bombi* presence		
Model	AICc	ΔAICc	w_i
100m Land-use + Humidity	**47.8**	**0**	**0.329**
100m Land-use	**48.5**	**0.78**	**0.223**
100m Land-use + Rainfall + Humidity	**49.2**	**1.42**	**0.162**
100m Land-use + Rainfall	50.6	2.84	0.08
100m Land-use + Temperature + Humidity	50.7	2.92	0.076
100m Land-use + Temperature + Rainfall + Humidity	51.1	3.32	0.063
100m Land-use + Temperature + Rainfall + Humidity	53.2	5.44	0.022
Basic	54	6.19	0.015

Table S7. Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable; see Table S3 for optimal model sets) for variables where land-use at a 100m radius was found to have a significant effect: a) male size and b) *Apicystis bombi* presence. Parameters highlighted in bold are considered important to the model based on 95% CIs.

a	Male thorax width			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	5.71E+00	5.58E-01	4.618	6.806
100m Land-use (Dense housing)	1.85E-01	1.13E-01	-0.037	0.406
100m Land-use (Wooded)	**2.79E-01**	**1.17E-01**	**0.05**	**0.508**
100m Land-use (Sparse housing)	1.92E-01	1.24E-01	-0.052	0.436
Temperature	-2.11E-02	1.86E-02	-0.058	0.015
Rainfall	**5.97E-03**	**2.21E-03**	**0.002**	**0.01**
Humidity	-7.63E-06	5.85E-03	-0.011	0.011

b	*Apicystis bombi* presence			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-10.7731	8.38836	-27.214	5.668
100m Land-use (Fields)	-0.59468	0.84446	-2.25	1.06
100m Land-use (Dense housing)	**-2.75632**	**1.08475**	**-4.882**	**-0.63**
100m Land-use (Wooded)	**-2.14746**	**0.83782**	**-3.79**	**-0.505**
Humidity	0.16451	0.09411	-0.02	0.349

Table S8. Model selection tables to compare the response of dependent variables to land-use at four different radii (750m, 500m, 250m, and 100m) surrounding sites. “All covariates” refers to temperature, humidity and rainfall and where relevant, week and the random effect of site. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i).

a	Reproductive output		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	306.8	0	0.522
250m land-use + all covariates	308.3	1.54	0.241
750m land-use + all covariates	308.4	1.58	0.237
100m land-use + all covariates	324.4	17.62	0

b	Peak colony size		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	93.3	0	0.647
750m land-use + all covariates	96	2.7	0.168
250m land-use + all covariates	96.8	3.52	0.112
100m land-use + all covariates	97.6	4.34	0.074

c	Queen survival		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	141.4	0	0.823
250m land-use + all covariates	146	4.66	0.08
100m land-use + all covariates	146.3	4.89	0.071
750m land-use + all covariates	148.3	6.94	0.026

d	Colony survival		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	197.9	0	0.965
100m land-use + all covariates	206.5	8.64	0.013
250m land-use + all covariates	206.8	8.91	0.011
750m land-use + all covariates	206.9	8.99	0.011

e	Ovary development		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	474.9	0	0.355
100m land-use + all covariates	475.7	0.84	0.234
750m land-use + all covariates	475.9	1.07	0.208
250m land-use + all covariates	476	1.12	0.203

f	Presence of nectar in nest		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	222.5	0	0.591
750m land-use + all covariates	224	1.44	0.288
100m land-use + all covariates	226.3	3.8	0.088
250m land-use + all covariates	228.3	5.78	0.033

g	Presence of pollen in nest		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	285.3	0	0.997
250m land-use + all covariates	298.5	13.15	0.001
750m land-use + all covariates	298.7	13.39	0.001
100m land-use + all covariates	301.4	16.04	0

h	*Bombus vestalis* invasions		
Model	AICc	ΔAICc	w_i
750m land-use + all covariates	28.9	0	0.728
500m land-use + all covariates	31.2	2.32	0.228
250m land-use + all covariates	35.4	6.57	0.027
100m land-use + all covariates	36.4	7.54	0.017

i	Onset of reproduction		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	163.1	0	0.385
250m land-use + all covariates	163.6	0.48	0.302
750m land-use + all covariates	163.8	0.66	0.278
100m land-use + all covariates	167.9	4.83	0.035

j	Male fat content		
Model	AICc	ΔAICc	w_i
500m land-use + all covariates	305.2	0	0.31
250m land-use + all covariates	305.5	0.39	0.256
750m land-use + all covariates	305.6	0.45	0.247
100m land-use + all covariates	306.2	1.01	0.187

k	Male thorax width		
Model	AICc	ΔAICc	w_i
100m land-use + all covariates	105.9	0	0.39
750m land-use + all covariates	107.1	1.25	0.209
250m land-use + all covariates	107.2	1.32	0.202
500m land-use + all covariates	107.2	1.35	0.199

l	Worker fat content		
Model	AICc	ΔAICc	w_i
750m land-use + all covariates	-315.5	0	0.354
250m land-use + all covariates	-315.1	0.38	0.293
100m land-use + all covariates	-314.5	1.03	0.211
500m land-use + all covariates	-313.7	1.83	0.142

m	Worker thorax width		
Model	AICc	ΔAICc	w_i
250m land-use + all covariates	453.7	0	0.298
750m land-use + all covariates	453.8	0.15	0.276
500m land-use + all covariates	454.3	0.61	0.22
100m land-use + all covariates	454.4	0.73	0.207

n	*Crithidia bombi* presence		
Model	AICc	ΔAICc	w_i
250m land-use + all covariates	56.4	0	0.647
750m land-use + all covariates	58.5	2.12	0.224
500m land-use + all covariates	60.9	4.52	0.068
100m land-use + all covariates	61.1	4.73	0.061

o	*Apicystis bombi* presence		
Model	AICc	ΔAICc	w_i
100m land-use + all covariates	51.1	0	0.941
750m land-use + all covariates	57.9	6.84	0.031
250m land-use + all covariates	58.5	7.46	0.023
500m land-use + all covariates	61.4	10.36	0.005

Table S9. Model selection tables to investigate whether *Bombus vestalis* invasions are a better predictor than land-use for variables found to be affected by land-use in the main analysis. Each model contains the covariates included in the optimal model derived from the main analysis and either land-use, total *B. vestalis* invasions or both. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i).

a	Reproductive output			
Count process	Binomial process	AICc	ΔAICc	w_i
500m Land-use	500m Land-use	284.4	0	0.81
B. vestalis invasions	*B. vestalis* invasions	287.8	3.41	0.148
500m Land-use + *B. vestalis* invasions	500m Land-use + *B. vestalis* invasions	290.3	5.92	0.042

b Male thorax width			
Model	AICc	ΔAICc	w_i
100m Land-use + Rainfall + s (week)	104.8	0	0.531
100m Land-use + *B. vestalis* invasions + Rainfall + s (week)	106.2	1.39	0.265
B. vestalis invasions + Rainfall + s (week)	106.7	1.92	0.204

c Peak colony size			
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall	88.7	0	0.485
B. vestalis invasions + Rainfall	89.8	1.08	0.283
500m Land-use + *B. vestalis* invasions + Rainfall	90.2	1.47	0.232

d Queen survival			
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall	140.3	0	0.438
B. vestalis invasions + Rainfall	140.5	0.24	0.389
500m Land-use + *B. vestalis* invasions + Rainfall	142.1	1.85	0.173

e Colony survival			
Model	AICc	ΔAICc	w_i
500m Land-use + Rainfall	195.9	0	0.708
500m Land-use + *B. vestalis* invasions + Rainfall	197.7	1.86	0.279
B. vestalis invasions + Rainfall	203.9	8.05	0.013

f Ovary development			
Model	AICc	ΔAICc	w_i
500m Land-use + *B. vestalis* invasions + s(week)	471.6	0	0.737
500m Land-use + s(week)	474.2	2.56	0.205
B. vestalis invasions + s(week)	476.7	5.08	0.058

g Presence of nectar in nest			
Model	AICc	ΔAICc	w_i
500m Land-use + s(week)	216.7	0	0.713
500m Land-use + *B. vestalis* invasions + s(week)	218.8	2.1	0.249
B. vestalis invasions + s(week)	222.6	5.86	0.038

h Presence of pollen in nest			
Model	AICc	ΔAICc	w_i
500m Land-use + s(week)	290.1	0	0.746
500m Land-use + *B. vestalis* invasions + s(week)	292.3	2.18	0.251
B. vestalis invasions + s(week)	301.6	11.53	0.002

i *Apicystis bombi* presence			
Model	AICc	ΔAICc	w_i
100m Land-use + *B. vestalis* invasions + Humidity	47.2	0	0.562
100m Land-use + Humidity	47.8	0.54	0.43
B. vestalis invasions + Humidity	55.6	8.34	0.009

Table S10. Principle component analysis (PCA) performed on the land-use categories at a 500m radius around the study sites. a) Importance of components from the PCA. PC1 and PC2 captured approx. 85% of the variation so were used for subsequent cluster analysis. b) Contribution of land-use categories to PC1 and PC2.

a)

	PC1	**PC2**	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	**2.1334**	**1.4628**	0.9409	0.56175	0.22331	0.21199	0.10639	0.04403
Proportion of Variance	**0.5689**	**0.2675**	0.1107	0.03944	0.00623	0.00562	0.00141	0.00024
Cumulative Proportion	**0.5689**	**0.8364**	0.947	0.98649	0.99273	0.99834	0.99976	1

b)

	PC1	PC2
Proportion impervious surface	**0.44195973**	-0.05430851
Proportion flower-rich habitat	0.06611866	**-0.51498963**
Proportion domestic infrastructure	**0.46086551**	-0.08500644
Proportion open land	-0.28862927	**-0.51546033**
Proportion tree cover	-0.084578	**0.61002256**
Proportion agricultural land	**-0.43254879**	-0.22174299
Proportion gardens	0.33839798	-0.17557914
Proportion road	**0.44252792**	-0.08237299

Table S11. Tables of candidate models to compare land-use variables (clustered variable and principle components) using a) zero-inflated hurdle models to analyse reproductive production as a binary response and a count process; b) linear models to analyse log-transformed peak colony size; Cox proportional hazards models to analyse c) queen survival and d) colony survival; binomial GAMs to analyse presence of e) nectar and f) pollen in the nest over time with *site* as a random effect. In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i); only the first eight candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

a	Reproductive output			
Count process	Binomial process	AICc	ΔAICc	w_i
500m Land-use (clustered)	**500m Land-use (clustered)**	**284.4**	**0**	**0.608**
Basic	**Basic**	**285.7**	**1.32**	**0.314**
PC1	PC1	289.8	5.36	0.042
PC2	PC2	290.3	5.93	0.031
PC1 + PC2	PC1 + PC2	295	10.63	0.003
PC1*PC2	PC1*PC2	296.1	11.72	0.002

b	Peak colony size		
Model	AICc	ΔAICc	w_i
500m Land-use (clustered)	**89.8**	**0**	**0.759**
Basic	94	4.23	0.091
PC1	95.1	5.32	0.053
PC2	95.2	5.46	0.05
PC1 + PC2	96.4	6.65	0.027
PC1*PC2	97	7.28	0.02

c	Queen survival		
Model	AICc	ΔAICc	w_i
500m Land-use (clustered)	**141.4**	**0**	**0.804**
Basic	146.5	5.18	0.06
PC1	146.8	5.48	0.052
PC2	147.8	6.45	0.032
PC1 + PC2	147.9	6.49	0.031
PC1*PC2	148.7	7.36	0.02

d	Colony survival		
Model	AICc	ΔAICc	w_i
500m Land-use (clustered)	**197.9**	**0**	**0.953**
Basic	205.9	8.03	0.017
PC1	206.8	8.89	0.011
PC1*PC2	207.2	9.34	0.009
PC2	208	10.14	0.006
PC1 + PC2	208.9	11	0.004

e	Presence of nectar in nest		
Model	AICc	ΔAICc	w_i
500m Land-use (clustered)	**216.7**	**0**	**0.643**
PC1 + PC2	220.1	3.42	0.116
PC1	221	4.31	0.075

PC2	221.3	4.55	0.066
Basic	221.7	5.04	0.052
PC1*PC2	221.9	5.15	0.049
f	Presence of pollen in nest		
Model	AICc	ΔAICc	w_i
500m Land-use (clustered)	**293**	**0**	**0.65**
PC1*PC2	**294.3**	**1.33**	**0.335**
PC1 + PC2	302.3	9.3	0.006
PC1	303.2	10.19	0.004
PC2	304	11.01	0.003
Basic	304.8	11.82	0.002

Table S12. Tables of candidate models investigate the effects of two land-use variables, principle component (PC) 1 and PC 2, on colony sucess measures using a) zero-inflated hurdle models to analyse reproductive production as a binary response and a count process; b) linear models to analyse log-transformed peak colony size; Cox proportional hazards models to analyse c) queen survival and d) colony survival; binomial GAMs to analyse presence of e) nectar and f) pollen in the nest over time with *site* as a random effect. In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i); only the first eight candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

a	Reproductive output			
Count process	Binomial process	AICc	ΔAICc	w_i
Basic	**Basic**	**285.7**	**0**	**0.359**
Basic	**PC1**	**287.4**	**1.72**	**0.152**
Temperature	PC1	289.4	3.71	0.056
Basic	PC1 + Rainfall	289.6	3.89	0.051
Rainfall	PC1	289.7	4.02	0.048
Temperature	Temperature	289.9	4.13	0.045
Rainfall	Rainfall	289.9	4.16	0.045
Humidity	PC1	290	4.33	0.041

b	Peak colony size		
Model	AICc	ΔAICc	w_i
Rainfall	**91.8**	**0**	**0.135**
PC2 + Rainfall	**92.1**	**0.29**	**0.117**
PC1 + Humidity + Rainfall	**92.9**	**1.02**	**0.081**
PC1 + Rainfall	**93.4**	**1.59**	**0.061**
PC2 + Humidity + Rainfall	**93.5**	**1.65**	**0.059**
Basic	94	2.15	0.046
Temperature + Rainfall	94.2	2.38	0.041
PC1 + PC2 + Humidity + Rainfall	94.4	2.56	0.037

c	Queen survival		
Model	AICc	ΔAICc	w_i
PC2 + Rainfall	**142.8**	**0**	**0.19**
Rainfall	**143.8**	**1.01**	**0.115**
PC1 + Rainfall	**144.8**	**1.94**	**0.072**
PC2 + Temperature + Rainfall	145	2.21	0.063
PC2 + Humidity + Rainfall	145.1	2.24	0.062
PC1 + PC2 + Rainfall	145.6	2.77	0.048
Temperature + Rainfall	145.9	3.06	0.041
PC1 + PC2 + Temperature + Rainfall	145.9	3.13	0.04

d	Colony survival		
Model	AICc	ΔAICc	w_i
Rainfall	**204.1**	**0**	**0.139**
Humidity + Rainfall	**205.3**	**1.28**	**0.073**
PC2 + Rainfall	**205.5**	**1.42**	**0.068**
PC1 + PC2 + Rainfall	**205.6**	**1.54**	**0.065**
PC1 + Rainfall	**205.8**	**1.77**	**0.058**

Temperature + Rainfall	**205.8**	**1.79**	**0.057**
Basic	**205.9**	**1.89**	**0.054**
PC1 + Humidity + Rainfall	206.4	2.39	0.042
e	Presence of nectar in nest		
Model	AICc	ΔAICc	w_i
PC1 + PC2	**220.1**	**0**	**0.13**
PC1	**221**	**0.88**	**0.084**
PC2	**221.3**	**1.13**	**0.074**
PC1 + PC2 + Rainfall	**221.7**	**1.59**	**0.059**
Basic	**221.7**	**1.61**	**0.058**
PC1*PC2	**221.9**	**1.73**	**0.055**
PC1 + PC2 + Humidity	222.2	2.09	0.046
PC1 + PC2 + Temperature	222.5	2.33	0.041
f	Presence of pollen in nest		
Model	AICc	ΔAICc	w_i
PC1*PC2 + Rainfall	**291.9**	**0**	**0.327**
PC1*PC2 + Humidity + Rainfall	**292.6**	**0.69**	**0.232**
PC1*PC2 + Temperature + Rainfall	**293.2**	**1.3**	**0.17**
PC1*PC2	294.3	2.35	0.101
PC1*PC2 + Temperature + Humidity + Rainfall	294.7	2.72	0.084
PC1*PC2 + Temperature + Humidity	295.9	3.98	0.045
PC1*PC2 + Temperature	296.1	4.14	0.041
Basic	304.8	12.85	0.001

Table S13. Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable) for analyses of the effects of two land-use variables, principle component (PC) 1 and PC 2, on colony success measures. Parameters highlighted in bold are considered important to the model based on 95% CIs.

a	Reproductive output			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count: (Intercept)	3.5152	0.1534	3.214536	3.815864
Count: Log(theta)	0.5469	0.3036	-0.048156	1.141956
Binomial: (Intercept)	0.7782	0.3508	0.090632	1.465768
Binomial: PC1	0.1461	0.1657	-0.178672	0.470872

b	Peak colony size			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.969041	1.605113	-1.17698048	5.11506248
Rainfall	0.015703	0.007107	0.00177328	0.02963272
PC2	**0.116651**	**0.086587**	**-0.05305952**	**0.28636152**
PC1	0.071603	0.061452	-0.04884292	0.19204892
Humidity	0.03433	0.024667	-0.01401732	0.08267732

c	Queen survival				
Parameters	Regression coefficient (b)	d. Error (SE(	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
PC2	-0.29103	0.16707	0.74749325	0.538758856	1.037099
Rainfall	**-0.0311**	**0.01384**	**0.96937863**	**0.943436329**	**0.996034**
PC1	-0.12021	0.10448	0.8867342	0.722534001	1.08825

d	Colony survival				
Parameters	Regression coefficient (b)	d. Error (SE(	Hazard Ratio (e^b)	95% CIs on Hazard Ratio	
				Lower	Upper
Rainfall	-0.02074	0.01056	0.97947359	0.959409201	0.999958
Humidity	-0.03508	0.03593	0.96552817	0.899871953	1.035975
PC2	-0.0516	0.20504	0.94970867	0.635414079	1.419463
PC1	**-0.03598**	**0.09274**	**0.96465959**	**0.804326176**	**1.156954**
PC1*PC2	0.19165	0.09981	1.21124651	0.9960303	1.472965
Temperature	0.09596	0.14425	1.10071503	0.82963453	1.46037

e	Presence of nectar in nest			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-0.195	0.224526	-0.63507096	0.24507096
PC1	0.163325	0.100082	-0.03283572	0.35948572
PC2	0.23431	0.15761	-0.0746056	0.5432256
Rainfall	-0.011536	0.014234	-0.03943464	0.01636264
PC1:PC2	0.059358	0.099606	-0.13586976	0.25458576

f	Presence of pollen in nest			
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.1021857	2.0791457	-2.972939872	5.177311272
PC1	0.0441291	0.0836861	-0.119895656	0.208153856
PC2	-0.1320626	0.1495473	-0.425175308	0.161050108
Rainfall	**-0.0267797**	**0.0132768**	**-0.052802228**	**-0.000757172**
PC1:PC2	**-0.2422267**	**0.0768928**	**-0.392936588**	**-0.091516812**
Humidity	-0.0340518	0.0278561	-0.088649756	0.020546156
Temperature	0.0772162	0.1120405	-0.14238318	0.29681558

Table S14. Initial land cover classes obtained from generating the land cover map for each site using GIS. Columns show which of the eight land categories they were assigned to and relevant colour in GIS figures

Code	Description	Impervious Surface	Flower-rich	Domestic Infrastructure	Garden	Tree Cover	Open	Agricultual	Road	Colour
FA	Farm building	1	0	0	0	0	0	1	0	🟫
FY	Farmyard	0	0	0	0	0	1	1	0	🟨
H	House	1	0	1	0	0	0	0	0	🟥
HR	Hedgerow	0	1	0	0	1	0	0	0	🟩
IS	Sealed surface	1	0	1	0	0	1	0	0	🟨
RG	Residential garden	0	1	1	1	0	1	0	0	🟩
RO	Road	1	0	1	0	0	1	0	1	⬜
ROG	Roadside grass	0	0	1	0	0	1	0	0	🟩
FW	Freshwater	0	0	0	0	0	1	0	0	🟦
RBG	Rural bare ground	0	0	0	0	0	1	0	0	🟥
T	Tree	0	0	0	0	1	0	0	0	🟩
WC	Woodland clearing	0	0	0	0	0	1	0	0	🟦
WL	Woodland	0	0	0	0	1	0	0	0	🟩
AL	Allotment	0	1	1	0	0	1	0	0	🟪
PL	Parkland	0	0	0	0	0	1	0	0	🟨
SG	Sports ground	0	0	1	0	0	1	0	0	🟪
BS	Building site	1	0	1	0	0	0	0	0	⬜
HI	High rise	1	0	1	0	0	0	0	0	⬛
IN	Industrial	1	0	1	0	0	0	0	0	⬛
R	Rail	1	0	1	0	0	1	0	0	⬜
TRS	Riverside silt	0	0	1	0	0	1	0	0	🟪
UBG	Urban bare ground	0	0	1	0	0	1	0	0	🟥
UG	Unbroken grassland	0	0	1	0	0	1	0	0	🟩
GC	Golf course	0	0	0	0	0	1	0	0	🟩
SS	Sparse scrub	0	0	0	0	0	1	0	0	🟩
SW	Swimming pool	0	0	1	0	0	1	0	0	🟦
LG	Landscape garden	0	1	0	1	0	1	0	0	🟪
GH	Cemetery	0	0	1	0	0	1	0	0	🟪
RHG	Manicured turf	0	0	1	1	0	1	0	0	🟪
UP	Urban Park	0	1	1	0	0	1	0	0	🟩
IGM	Improved grassland meadow	0	0	0	0	0	1	1	0	🟩
IGU	Improved grassland unused/overgrown	0	1	0	0	0	1	1	0	🟩
IGUF	Improved grassland unused/overgrown (flower-rich)	0	1	0	0	0	1	1	0	🟩
IP	Improved pasture	0	0	0	0	0	1	1	0	🟩
IPS	Improved pasture with sheep	0	0	0	0	0	1	1	0	🟩
SIGM	Semi-improved grassland meadow	0	0	0	0	0	1	1	0	🟩

SIGMC	Semi-Improved grassland meadow with chickens	0	0	0	0	0	1	1	0	🟩
SIGMH	Semi-Improved grassland meadow with horses	0	0	0	0	0	1	1	0	🟩
SIGU	Semi-Improved grassland unused/overgrown	0	1	0	0	0	1	1	0	🟩
SIG	semi-improved grassland (unidentified)	0	0	0	0	0	1	1	0	🟩
SIP	Semi-improved pasture no livestock	0	0	0	0	0	1	1	0	🟩
SIPH	Semi-improved pasture with horses	0	0	0	0	0	1	1	0	🟩
SIPC	Semi-improved pasture cows	0	0	0	0	0	1	1	0	🟩
SIPBS	Semi-improved pasture assorted livestock	0	0	0	0	0	1	1	0	🟩
SIPP	Semi-improved pasture pigs	0	0	0	0	0	1	1	0	🟩
SIL	Semi-improved lawn	0	0	0	0	0	1	1	0	🟨
CG	Caravan site (grassland)	0	0	0	0	0	1	1	0	🟪
UPA	Unknown pasture	0	0	0	0	0	1	1	0	🟩
UPH	Unkown pasture horses	0	0	0	0	0	1	1	0	🟩
UPC	Unkown pasture cows	0	0	0	0	0	1	1	0	🟩
UPS	Unkown pasture sheep	0	0	0	0	0	1	1	0	🟩
UM	unknown meadow	0	0	0	0	0	1	1	0	🟩
UGU	Unkown grassland unused/overgrown	0	0	0	0	0	1	1	0	🟩
PF	Unidentified grassland (not ground truthed)	0	0	0	0	0	1	1	0	🟩
AF	Unidentified arable (not ground truthed)	0	0	0	0	0	1	1	0	⬜
LVNF	Lovage (not flowering)	0	0	0	0	0	1	1	0	🟦
CHNF	Chamomile (not flowering)	0	0	0	0	0	1	1	0	🟧
CHF	Chamomile (flowering)	0	1	0	0	0	1	1	0	🟧
OSR	Oilseed rape (flowering)	0	1	0	0	0	1	1	0	🟨
BA	Barley	0	0	0	0	0	1	1	0	🟫
WW	Winter wheat	0	0	0	0	0	1	1	0	🟫
BRF	Broccoli (flowering)	0	1	0	0	0	1	1	0	🟪
UBF	Unknown brassica (flowering)	0	1	0	0	0	1	1	0	🟦
BNF	Beans (not flowering)	0	0	0	0	0	1	1	0	🟪
YUC	Unidentified young crop	0	0	0	0	0	1	1	0	🟧
SE	Sedum	0	1	0	0	0	1	1	0	🟪
PO	Poppy	0	1	0	0	0	1	1	0	🟥

WS	wildflower strip	0	1	0	0	0	1	1	0	🟥
ORNF	Orchard not flowering	0	0	0	0	0	1	1	0	🟫
ORF	Orchard flowering	0	1	0	0	0	1	1	0	🟥
SA	Set aside	0	0	0	0	0	1	1	0	⬜
HL	Herbal lay	0	1	0	0	0	1	1	0	🟫
MA	Marsh	0	0	0	0	0	1	0	0	🟫
RMA	Marshy rough grassland	0	0	0	0	0	1	0	0	🟫
FRS	Flower-rich shrub	0	1	0	0	0	1	0	0	⬜
MFRS	Marshy flower-rich shrub	0	1	0	0	0	1	0	0	🟦
OQ	Overgrown quarry	0	1	0	0	0	1	0	0	🟧
CB	Chicken barns	0	0	0	0	0	0	1	0	🟪

Appendix Three

Supplementary Tables for Chapter Five

Table S1. Tables of candidate models using a full-subset information theoretic approach . In all cases, the basic model included the constant and the residual variance, with all other models containing the basic model plus the indicated covariates. Models are presented in order of ΔAICc from the best model alongside their respective Akaike weights (w_i); only the first four candidate models are shown. The best sets of models which were averaged to obtain model averaged estimates (models <2 ΔAICc from the model with the lowest AICc) are highlighted in bold.

	df	AICc	ΔAICc	w_i	Land-use	Season	Land-use: Season	Apiary Size
a) Colony strength								
Model 7	**6**	**325.7**	**0**	**0.638**	+			
Model 6	7	328.7	3.05	0.138	+	+		
Model 3	7	329.3	3.67	0.102	+			+
Model 5	10	329.8	4.18	0.079	+	+	+	

					Count process				Binomial process			
	df	AICc	ΔAICc	w_i	Land-use	Season	Land-use: Season	Apiary Size	Land-use	Season	Land-use: Season	Apiary Size
b) *Varroa*												
Model 28	**11**	**1497.6**	**0**	**0.638**	+	+	+	+		+		
Model 78	12	1499.9	2.3	0.201	+	+	+	+		+		+
Model 27	14	1501.6	4.06	0.084	+	+	+	+	+	+		
Model 26	17	1503.2	5.68	0.037	+	+	+	+	+	+	+	
c) *Nosema*												
Model 55	**10**	**1823**	**0**	**0.261**	+	+	+					+
Model 5	**9**	**1823**	**0.02**	**0.259**	+	+	+					
Model 30	**10**	**1824.8**	**1.85**	**0.103**	+	+	+	+				
Model 80	**11**	**1824.9**	**1.89**	**0.101**	+	+	+	+				+

	df	AICc	ΔAICc	w_i	Land-use	Season	Land-use: Season	Apiary Size
d) Proportion woody pollen								
Model 6	**10**	**504.5**	**0**	**0.697**	+	+	+	+
Model 1	**9**	**506.4**	**1.85**	**0.276**	+	+	+	
Model 7	7	512.2	7.64	0.015	+	+		+
Model 2	6	512.7	8.19	0.012	+	+		
e) Pollen species richness								
Basic mode	**2**	**464.9**	**0**	**0.177**				
Model 3	**3**	**465**	**0.1**	**0.168**		+		
Model 2	**6**	**465.3**	**0.4**	**0.145**	+	+		
Model 4	**5**	**465.3**	**0.48**	**0.139**	+			
f) Pollen species evenness								
Basic mode	**3**	**226.4**	**0**	**0.779**				
Model 4	4	230.3	3.93	0.109		+		
Model 5	4	230.9	4.48	0.083				+
Model 3	6	234.5	8.12	0.013	+			
g) Pollen Shannon diversity								
Basic mode	**3**	**271**	**0**	**0.689**				
Model 9	4	274.3	3.26	0.135				+

Model 3	4	274.5	3.47	0.121		+		
Model 7	5	277.8	6.75	0.024		+		+

	df	AICc	ΔAICc	w_i	Land-use	Apiary Size	Land-use: Apiary
h) Overwintering success							
Model 4	**2**	**81.6**	**0**	**0.661**		**+**	
Null model	**1**	**84.5**	**2.89**	**0.156**			
Model 2	5	85.4	3.79	0.099	+	+	
Model 1	8	86.5	4.94	0.056	+	+	+
i) Effect of land-use on V*arroa* treatment use							
Null model	**2**	**281.5**	**0**	**0.584**			
Model 3	**3**	**282.5**	**0.97**	**0.36**		**+**	
Model 2	5	287.1	5.64	0.035	+		
Model 1	6	288.2	6.7	0.021	+	+	

					Count process			Binomial process		
	df	AICc	ΔAICc	w_i	Land-use	Recent *Varroa* treatment	Apiary Size	Land-use	Recent *Varroa* treatment	Apiary Size
j) *Varroa* treatment effect on *Varroa* mite count (spring)										
Model 20	**7**	**233.1**	**0**	**0.417**	**+**	**+**	**+**			
Model 19	10	235.5	2.38	0.127	+	+	+	+		
Model 36	8	235.6	2.44	0.123	+	+	+			+
Model 18	8	235.9	2.77	0.104	+	+	+		+	
k) *Varroa* treatment effect on *Varroa* mite count (autumn)										
Model 20	**8**	**970.2**	**0**	**0.671**	**+**	**+**	**+**			
Model 36	9	973.2	3.01	0.149	+	+	+			+
Model 18	10	973.8	3.59	0.111	+	+	+		+	
Model 19	11	975.9	5.69	0.039	+	+	+	+		

Table S2. a) Results from PERMANOVA tests to analyse the effect of land-use on pollen species composition in spring and autumn, and pairwise comparisons between land-use types in the autumn. b-e) Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable; see Table S1) for analyses of b) overwintering success, c) land-use effect on *Varroa* treatment and d & e) *Varroa* treatment effect on mite count. Parameters highlighted in bold are considered important to the model (continuous variables) or significantly different from the baseline (categorical variables) based on 95% CIs.

a) Pollen species composition (PERMANOVA)

Overall	F	R^2	P value
Spring	**3.653**	**0.199**	**0.005**
Autumn	1.269	0.029	0.159
Pairs (autumn)			
Suburban vs Urban	0.575	0.031	0.962
Suburban vs Rural Open	0.896	0.041	0.591
Suburban vs Rural Wooded	0.969	0.046	0.508
Urban vs Rural Open	0.681	0.031	0.861
Urban vs Rural Wooded	0.918	0.044	0.570
Rural Open vs Rural Wooded	1.075	0.045	0.346

b) Overwintering success

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	0.860	0.514	-0.148	1.867
Apiary Size	**0.618**	**0.278**	**0.073**	**1.163**

c) Land-use effect on *Varroa* treatment

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	0.487	0.338	-0.177	1.150
Apiary Size	0.251	0.241	-0.222	0.724

d) *Varroa* treatment effect on *Varroa* mite count (spring)

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	2.075	0.365	1.360	2.791
Apiary Size	**-0.322**	**0.121**	**-0.559**	**-0.084**
Land-use (Suburban)	-0.939	0.628	-2.169	0.291
Land-use (Rural Open)	0.633	0.347	-0.047	1.313
Land-use (Rural Wooded)	**0.967**	**0.342**	**0.298**	**1.637**
Recent *Varroa* treatment (Oxalic)	**-0.620**	**0.182**	**-0.975**	**-0.264**
Binomial process				
(Intercept)	0.234	0.307	-0.368	0.836

e) *Varroa* treatment effect on *Varroa* mite count (autumn)

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	3.121	0.106	2.913	3.329
Apiary Size	**-0.220**	**0.039**	**-0.297**	**-0.143**
Land-use (Suburban)	**-0.873**	**0.097**	**-1.062**	**-0.684**
Land-use (Rural Open)	**-0.505**	**0.092**	**-0.686**	**-0.324**
Land-use (Rural Wooded)	-0.143	0.079	-0.298	0.012
Recent *Varroa* treatment (none)	**1.073**	**0.097**	**0.883**	**1.262**
Recent *Varroa* treatment (Thymol)	**0.539**	**0.116**	**0.312**	**0.766**
Binomial process				
(Intercept)	2.079	0.474	1.150	3.009

Table S3. Coefficients and 95% confidence intervals (CIs) for the optimal model or model sets (model averaged where applicable; see Table 2 in main text) for all analyses where land-use was included in the optimal model. For each analysis the output is given with each combination of factor levels as the baseline to demonstrate pairwise comparisons. Parameters highlighted in bold are significantly different to the baseline based on 95% CIs.

a) Proportion woody pollen				
SEASON: AUTUMN / LAND-USE: URBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-1.693	0.417	-2.511	-0.875
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Spring)	**4.700**	**0.355**	**4.004**	**5.395**
Land-use (Suburban)	0.284	0.483	-0.663	1.231
Land-use (Rural Open)	-0.606	0.491	-1.568	0.357
Land-use (Rural Wooded)	-0.131	0.485	-1.080	0.819
Land-use (Suburban) : Season (Spring)	0.275	0.489	-0.684	1.233
Land-use (Rural Open) : Season (Spring)	**-1.056**	**0.451**	**-1.940**	**-0.172**
Land-use (Rural Wooded) : Season (Spring)	**-1.038**	**0.450**	**-1.921**	**-0.156**
SEASON: AUTUMN / LAND-USE: SUBURBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-1.409	0.429	-2.249	-0.568
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Spring)	**4.974**	**0.343**	**4.302**	**5.646**
Land-use (Urban)	-0.284	0.483	-1.231	0.663
Land-use (Rural Open)	-0.890	0.473	-1.816	0.036
Land-use (Rural Wooded)	-0.415	0.469	-1.334	0.504
Land-use (Urban) : Season (Spring)	-0.275	0.489	-1.233	0.684
Land-use (Rural Open) : Season (Spring)	**-1.330**	**0.440**	**-2.193**	**-0.467**
Land-use (Rural Wooded) : Season (Spring)	**-1.313**	**0.438**	**-2.171**	**-0.454**
SEASON: AUTUMN / LAND-USE: RURAL OPEN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	-2.299	0.456	-3.193	-1.404
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Spring)	**3.644**	**0.278**	**3.099**	**4.188**
Land-use (Suburban)	0.890	0.473	-0.036	1.816
Land-use (Urban)	0.606	0.491	-0.357	1.568
Land-use (Rural Wooded)	0.475	0.473	-0.452	1.402
Land-use (Suburban) : Season (Spring)	**1.330**	**0.440**	**0.467**	**2.193**
Land-use (Urban) : Season (Spring)	**1.056**	**0.451**	**0.172**	**1.940**
Land-use (Rural Wooded) : Season (Spring)	0.017	0.392	-0.750	0.785
SEASON: AUTUMN / LAND-USE: RURAL WOODED				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper

(Intercept)	-1.824	0.433	-2.672	-0.976
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Spring)	**3.661**	**0.278**	**3.116**	**4.207**
Land-use (Rural Open)	-0.475	0.473	-1.402	0.452
Land-use (Suburban)	0.415	0.469	-0.504	1.334
Land-use (Urban)	0.131	0.485	-0.819	1.080
Land-use (Rural Open) : Season (Spring)	**-0.017**	**0.392**	**-0.785**	**0.751**
Land-use (Suburban) : Season (Spring)	**1.313**	**0.438**	**0.454**	**2.171**
Land-use (Urban) : Season (Spring)	1.038	0.450	0.156	1.921

SEASON: SPRING / LAND-USE: URBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	3.007	0.422	2.180	3.833
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Autumn)	**-4.700**	**0.355**	**-5.395**	**-4.004**
Land-use (Rural Wooded)	**-1.169**	**0.434**	**-2.019**	**-0.319**
Land-use (Rural Open)	**-1.662**	**0.425**	**-2.494**	**-0.829**
Land-use (Suburban)	0.559	0.472	-0.366	1.483
Land-use (Rural Wooded) : Season (Autumn)	**1.038**	**0.450**	**0.156**	**1.921**
Land-use (Rural Open) : Season (Autumn)	**1.056**	**0.451**	**0.172**	**1.940**
Land-use (Suburban) : Season (Autumn)	-0.275	0.489	-1.233	0.684

SEASON: SPRING / LAND-USE: SUBURBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	3.565	0.457	2.669	4.461
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Autumn)	**-4.974**	**0.343**	**-5.646**	**-4.302**
Land-use (Urban)	-0.559	0.472	-1.483	0.366
Land-use (Rural Wooded)	**-1.728**	**0.440**	**-2.591**	**-0.865**
Land-use (Rural Open)	**-2.220**	**0.430**	**-3.062**	**-1.378**
Land-use (Urban) : Season (Autumn)	0.275	0.489	-0.684	1.233
Land-use (Rural Wooded) : Season (Autumn)	**1.313**	**0.438**	**0.454**	**2.171**
Land-use (Rural Open) : Season (Autumn)	**1.330**	**0.440**	**0.467**	**2.193**

SEASON: SPRING / LAND-USE: RURAL OPEN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.345	0.399	0.562	2.128
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Autumn)	**-3.644**	**0.278**	**-4.188**	**-3.099**
Land-use (Suburban)	**2.220**	**0.430**	**1.378**	**3.062**
Land-use (Urban)	**1.661**	**0.425**	**0.829**	**2.494**
Land-use (Rural Wooded)	0.492	0.390	-0.271	1.256
Land-use (Suburban) : Season (Autumn)	**-1.330**	**0.440**	**-2.193**	**-0.467**
Land-use (Urban) : Season (Autumn)	**-1.056**	**0.451**	**-1.940**	**-0.172**
Land-use (Rural Wooded) : Season (Autumn)	-0.017	0.392	-0.785	0.750

SEASON: SPRING / LAND-USE: RURAL WOODED

Parameters	Estimate	Std. Error	95% CIs

Parameters	Estimate	Std. Error	Lower	Upper
(Intercept)	1.838	0.392	1.069	2.606
Apiary Size	**-0.366**	**0.184**	**-0.727**	**-0.005**
Season (Autumn)	**-3.661**	**0.278**	**-4.207**	**-3.116**
Land-use (Rural Open)	-0.492	0.390	-1.256	0.271
Land-use (Suburban)	**1.728**	**0.440**	**0.865**	**2.591**
Land-use (Urban)	**1.169**	**0.434**	**0.319**	**2.019**
Land-use (Rural Open) : Season (Autumn)	0.017	0.392	-0.750	0.785
Land-use (Suburban) : Season (Autumn)	**-1.313**	**0.438**	**-2.171**	**-0.454**
Land-use (Urban) : Season (Autumn)	**-1.038**	**0.450**	**-1.921**	**-0.156**

b) Pollen species richness

SEASON: AUTUMN / LAND-USE: URBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	2.041	0.115	1.814	2.267
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Spring)	-0.102	0.101	-0.299	0.095
Land-use (Rural Wooded)	-0.186	0.134	-0.448	0.076
Land-use (Rural Open)	**-0.316**	**0.135**	**-0.581**	**-0.051**
Land-use (Suburban)	-0.076	0.158	-0.386	0.233
Land-use (Rural Wooded) : Season (Srping)	0.045	0.220	-0.386	0.477
Land-use (Rural Open) : Season (Spring)	-0.019	0.226	-0.461	0.424
Land-use (Suburban) : Season (Spring)	-0.422	0.220	-0.854	0.010

SEASON: AUTUMN / LAND-USE: SUBURBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	2.010	0.111	1.792	2.228
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Spring)	-0.166	0.151	-0.461	0.130
Land-use (Urban)	0.076	0.158	-0.233	0.386
Land-use (Rural Wooded)	-0.110	0.164	-0.431	0.211
Land-use (Rural Open)	-0.240	0.158	-0.550	0.070
Land-use (Urban) : Season (Spring)	0.422	0.220	-0.010	0.854
Land-use (Rural Wooded) : Season (Spring)	**0.468**	**0.221**	**0.034**	**0.901**
Land-use (Rural Open) : Season (Spring)	0.403	0.227	-0.042	0.848

SEASON: AUTUMN / LAND-USE: RURAL OPEN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.912	0.132	1.654	2.171
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Spring)	-0.105	0.101	-0.302	0.093
Land-use (Suburban)	0.240	0.158	-0.070	0.550
Land-use (Urban)	**0.316**	**0.135**	**0.051**	**0.581**
Land-use (Rural Wooded)	0.130	0.137	-0.138	0.398
Land-use (Suburban) : Season (Spring)	-0.404	0.227	-0.849	0.041
Land-use (Urban) : Season (Spring)	0.019	0.226	-0.424	0.461
Land-use (Rural Wooded) : Season (Spring)	0.064	0.227	-0.380	0.508

SEASON: AUTUMN / LAND-USE: RURAL WOODED				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.965	0.100	1.770	2.160
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Spring)	-0.095	0.107	-0.306	0.115
Land-use (Rural Open)	-0.130	0.137	-0.398	0.138
Land-use (Suburban)	0.110	0.164	-0.211	0.431
Land-use (Urban)	0.186	0.134	-0.076	0.449
Land-use (Rural Open) : Season (Spring)	-0.064	0.227	-0.508	0.380
Land-use (Suburban) : Season (Spring)	**-0.468**	**0.221**	**-0.901**	**-0.034**
Land-use (Urban) : Season (Spring)	-0.046	0.220	-0.477	0.386

SEASON: SPRING / LAND-USE: URBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.986	0.115	1.761	2.211
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Autumn)	0.102	0.101	-0.095	0.299
Land-use (Rural Wooded)	-0.177	0.133	-0.438	0.083
Land-use (Rural Open)	**-0.320**	**0.136**	**-0.586**	**-0.054**
Land-use (Suburban)	-0.161	0.156	-0.468	0.146
Land-use (Rural Wooded) : Season (Autumn)	-0.045	0.220	-0.477	0.386
Land-use (Rural Open) : Season (Autumn)	0.018	0.226	-0.424	0.461
Land-use (Suburban) : Season (Autumn)	0.422	0.220	-0.010	0.854

SEASON: SPRING / LAND-USE: SUBURBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.921	0.107	1.712	2.129
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Autumn)	0.166	0.151	-0.130	0.461
Land-use (Urban)	0.161	0.156	-0.146	0.468
Land-use (Rural Wooded)	-0.016	0.163	-0.336	0.303
Land-use (Rural Open)	-0.159	0.159	-0.471	0.152
Land-use (Urban) : Season (Autumn)	-0.422	0.220	-0.854	0.010
Land-use (Rural Wooded) : Season (Autumn)	**-0.467**	**0.221**	**-0.901**	**-0.034**
Land-use (Rural Open) : Season (Autumn)	-0.404	0.227	-0.848	0.041

SEASON: SPRING / LAND-USE: RURAL OPEN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.856	0.133	1.595	2.117
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Autumn)	0.105	0.101	-0.093	0.302
Land-use (Suburban)	0.159	0.159	-0.152	0.470
Land-use (Urban)	**0.320**	**0.136**	**0.054**	**0.586**
Land-use (Rural Wooded)	0.143	0.138	-0.127	0.413
Land-use (Suburban) : Season (Autumn)	0.403	0.227	-0.042	0.848
Land-use (Urban) : Season (Autumn)	-0.019	0.226	-0.461	0.424
Land-use (Rural Wooded) : Season (Autumn)	-0.064	0.227	-0.508	0.380

SEASON: SPRING / LAND-USE: RURAL WOODED

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	1.914	0.098	1.722	2.106
Apiary Size	-0.055	0.061	-0.174	0.064
Season (Autumn)	0.095	0.107	-0.115	0.306
Land-use (Rural Open)	-0.143	0.138	-0.413	0.127
Land-use (Suburban)	0.016	0.163	-0.303	0.336
Land-use (Urban)	0.177	0.133	-0.083	0.438
Land-use (Rural Open) : Season (Autumn)	0.064	0.227	-0.380	0.508
Land-use (Suburban) : Season (Autumn)	**0.467**	**0.221**	**0.034**	**0.901**
Land-use (Urban) : Season (Autumn)	0.045	0.220	-0.386	0.477

c) Colony strength

LAND-USE: URBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	3.742	0.254	3.244	4.239
Land-use (Suburban)	0.345	0.355	-0.351	1.041
Land-use (Rural Open)	**-1.101**	**0.349**	**-1.784**	**-0.418**
Land-use (Rural Wooded)	-0.377	0.355	-1.073	0.319

LAND-USE: SUBURBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	4.087	0.249	3.600	4.574
Land-use (Urban)	-0.345	0.355	-1.041	0.351
Land-use (Rural Open)	**-1.446**	**0.345**	**-2.121**	**-0.771**
Land-use (Rural Wooded)	**-0.722**	**0.351**	**-1.411**	**-0.034**

LAND-USE: RURAL OPEN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	2.641	0.239	2.173	3.109
Land-use (Suburban)	**1.446**	**0.345**	**0.771**	**2.121**
Land-use (Urban)	**1.101**	**0.349**	**0.418**	**1.784**
Land-use (Rural Wooded)	**0.724**	**0.345**	**0.048**	**1.399**

LAND-USE: RURAL WOODED

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
(Intercept)	3.365	0.249	2.878	3.852
Land-use (Rural Open)	**-0.724**	**0.345**	**-1.399**	**-0.048**
Land-use (Suburban)	**0.722**	**0.351**	**0.034**	**1.411**
Land-use (Urban)	0.377	0.355	-0.319	1.073

d) *Varroa*

SEASON: AUTUMN / LAND-USE: URBAN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				

(Intercept)	3.809	0.069	3.674	3.944
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Spring)	**-2.295**	**0.266**	**-2.816**	**-1.773**
Land-use (Suburban)	**-0.594**	**0.095**	**-0.780**	**-0.407**
Land-use (Rural Open)	**-0.187**	**0.087**	**-0.358**	**-0.017**
Land-use (Rural Wooded)	0.074	0.078	-0.079	0.227
Land-use (Suburban) : Season (Spring)	**1.548**	**0.323**	**0.915**	**2.181**
Land-use (Rural Open) : Season (Spring)	**1.212**	**0.299**	**0.626**	**1.798**
Land-use (Rural Wooded) : Season (Spring)	**0.886**	**0.294**	**0.311**	**1.462**
Binomial process				
(Intercept)	2.128	0.473	1.201	3.055
Season (Spring)	**-1.757**	**0.555**	**-2.845**	**-0.669**
SEASON: AUTUMN / LAND-USE: SUBURBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	3.216	0.092	3.035	3.396
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Spring)	**-0.747**	**0.183**	**-1.105**	**-0.388**
Land-use (Urban)	**0.594**	**0.095**	**0.407**	**0.780**
Land-use (Rural Open)	**0.406**	**0.103**	**0.205**	**0.608**
Land-use (Rural Wooded)	**0.668**	**0.095**	**0.480**	**0.855**
Land-use (Urban) : Season (Spring)	**-1.548**	**0.323**	**-2.181**	**-0.915**
Land-use (Rural Open) : Season (Spring)	-0.336	0.228	-0.783	0.111
Land-use (Rural Wooded) : Season (Spring)	**-0.662**	**0.221**	**-1.095**	**-0.228**
Binomial process				
(Intercept)	2.128	0.473	1.201	3.055
Season (Spring)	**-1.757**	**0.555**	**-2.845**	**-0.669**
SEASON: AUTUMN / LAND-USE: RURAL OPEN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	3.622	0.088	3.449	3.795
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Spring)	**-1.083**	**0.136**	**-1.350**	**-0.816**
Land-use (Suburban)	**-0.406**	**0.103**	**-0.608**	**-0.205**
Land-use (Urban)	**0.187**	**0.087**	**0.017**	**0.358**
Land-use (Rural Wooded)	**0.261**	**0.086**	**0.093**	**0.430**
Land-use (Suburban) : Season (Spring)	0.336	0.228	-0.111	0.783
Land-use (Urban) : Season (Spring)	**-1.212**	**0.299**	**-1.798**	**-0.626**
Land-use (Rural Wooded) : Season (Spring)	**-0.326**	**0.184**	**-0.687**	**0.035**
Binomial process				
(Intercept)	2.128	0.473	1.201	3.055
Season (Spring)	**-1.757**	**0.555**	**-2.845**	**-0.669**
SEASON: AUTUMN / LAND-USE: RURAL WOODED				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				

(Intercept)	3.883	0.078	3.731	4.035
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Spring)	**-1.408**	**0.124**	**-1.652**	**-1.165**
Land-use (Rural Open)	**-0.261**	**0.086**	**-0.430**	**-0.093**
Land-use (Suburban)	**-0.668**	**0.095**	**-0.855**	**-0.480**
Land-use (Urban)	-0.074	0.078	-0.227	0.079
Land-use (Rural Open) : Season (Spring)	0.326	0.184	-0.035	0.687
Land-use (Suburban) : Season (Spring)	**0.662**	**0.221**	**0.228**	**1.095**
Land-use (Urban) : Season (Spring)	**-0.886**	**0.294**	**-1.462**	**-0.311**
Binomial process				
(Intercept)	2.128	0.473	1.201	3.055
Season (Spring)	**-1.757**	**0.555**	**-2.845**	**-0.669**
SEASON: SPRING / LAND-USE: URBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	1.515	0.263	0.999	2.031
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Autumn)	**2.295**	**0.266**	**1.773**	**2.816**
Land-use (Rural Wooded)	**0.960**	**0.283**	**0.405**	**1.515**
Land-use (Rural Open)	**1.025**	**0.287**	**0.462**	**1.587**
Land-use (Suburban)	**0.954**	**0.309**	**0.349**	**1.559**
Land-use (Rural Wooded) : Season (Autumn)	**-0.886**	**0.294**	**-1.462**	**-0.311**
Land-use (Rural Open) : Season (Autumn)	**-1.212**	**0.299**	**-1.798**	**-0.626**
Land-use (Suburban) : Season (Autumn)	**-1.548**	**0.323**	**-2.181**	**-0.915**
Binomial process				
(Intercept)	0.372	0.291	-0.198	0.941
Season (Autumn)	**1.757**	**0.555**	**0.669**	**2.845**
SEASON: SPRING / LAND-USE: SUBURBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	2.469	0.173	2.130	2.808
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Autumn)	**0.747**	**0.183**	**0.388**	**1.105**
Land-use (Urban)	**-0.954**	**0.309**	**-1.559**	**-0.349**
Land-use (Rural Wooded)	0.006	0.199	-0.385	0.397
Land-use (Rural Open)	0.071	0.204	-0.329	0.470
Land-use (Urban) : Season (Autumn)	**1.548**	**0.323**	**0.915**	**2.181**
Land-use (Rural Wooded) : Season (Autumn)	**0.662**	**0.221**	**0.228**	**1.095**
Land-use (Rural Open) : Season (Autumn)	0.336	0.228	-0.111	0.783
Binomial process				
(Intercept)	0.372	0.291	-0.198	0.941
Season (Autumn)	**1.757**	**0.555**	**0.669**	**2.845**
SEASON: SPRING / LAND-USE: RURAL OPEN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				

(Intercept)	2.539	0.131	2.282	2.797
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Autumn)	**1.083**	**0.136**	**0.816**	**1.350**
Land-use (Suburban)	-0.071	0.204	-0.470	0.329
Land-use (Urban)	**-1.025**	**0.287**	**-1.587**	**-0.462**
Land-use (Rural Wooded)	-0.065	0.163	-0.385	0.256
Land-use (Suburban) : Season (Autumn)	-0.336	0.228	-0.783	0.111
Land-use (Urban) : Season (Autumn)	**1.212**	**0.299**	**0.626**	**1.798**
Land-use (Rural Wooded) : Season (Autumn)	0.326	0.184	-0.035	0.687
Binomial process				
(Intercept)	0.372	0.291	-0.198	0.941
Season (Autumn)	**1.757**	**0.555**	**0.669**	**2.845**
SEASON: SPRING / LAND-USE: RURAL WOODED				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	2.475	0.120	2.240	2.710
Apiary Size	**-0.304**	**0.038**	**-0.378**	**-0.230**
Season (Autumn)	**1.408**	**0.124**	**1.165**	**1.652**
Land-use (Rural Open)	0.065	0.163	-0.256	0.385
Land-use (Suburban)	-0.006	0.199	-0.397	0.385
Land-use (Urban)	**-0.960**	**0.283**	**-1.515**	**-0.405**
Land-use (Rural Open) : Season (Autumn)	-0.326	0.184	-0.687	0.035
Land-use (Suburban) : Season (Autumn)	**-0.662**	**0.221**	**-1.095**	**-0.228**
Land-use (Urban) : Season (Autumn)	**0.886**	**0.294**	**0.311**	**1.462**
Binomial process				
(Intercept)	0.372	0.291	-0.198	0.941
Season (Autumn)	**1.757**	**0.555**	**0.669**	**2.845**

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SEASON: AUTUMN / LAND-USE: URBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	4.495	0.056	4.386	4.605
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Spring)	0.073	0.068	-0.059	0.206
Land-use (Suburban)	0.110	0.065	-0.018	0.238
Land-use (Rural Open)	**0.397**	**0.062**	**0.275**	**0.519**
Land-use (Rural Wooded)	**0.310**	**0.063**	**0.186**	**0.433**
Land-use (Suburban) : Season (Spring)	**-0.299**	**0.089**	**-0.474**	**-0.125**
Land-use (Rural Open) : Season (Spring)	**-0.369**	**0.085**	**-0.537**	**-0.202**
Land-use (Rural Wooded) : Season (Spring)	**-0.414**	**0.086**	**-0.583**	**-0.246**
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978
SEASON: AUTUMN / LAND-USE: SUBURBAN				
Parameters	Estimate	Std. Error	95% CIs	

Parameters	Estimate	Std. Error	Lower	Upper
Count process				
(Intercept)	4.605	0.040	4.527	4.683
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Spring)	**-0.226**	**0.057**	**-0.338**	**-0.114**
Land-use (Urban)	-0.110	0.065	-0.238	0.018
Land-use (Rural Open)	**0.287**	**0.051**	**0.188**	**0.386**
Land-use (Rural Wooded)	**0.200**	**0.051**	**0.100**	**0.300**
Land-use (Urban) : Season (Spring)	**0.299**	**0.089**	**0.125**	**0.474**
Land-use (Rural Open) : Season (Spring)	-0.070	0.078	-0.223	0.083
Land-use (Rural Wooded) : Season (Spring)	-0.115	0.078	-0.268	0.038
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978

SEASON: AUTUMN / LAND-USE: RURAL OPEN

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	4.892	0.038	4.818	4.967
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Spring)	**-0.296**	**0.053**	**-0.399**	**-0.192**
Land-use (Rural Wooded)	-0.087	0.048	-0.180	0.006
Land-use (Suburban)	**-0.287**	**0.051**	**-0.386**	**-0.188**
Land-use (Urban)	**-0.397**	**0.062**	**-0.519**	**-0.275**
Land-use (Rural Wooded) : Season (Spring)	-0.045	0.075	-0.192	0.102
Land-use (Suburban) : Season (Spring)	0.070	0.078	-0.083	0.223
Land-use (Urban) : Season (Spring)	**0.369**	**0.085**	**0.202**	**0.537**
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978

SEASON: AUTUMN / LAND-USE: RURAL WOODED

Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	4.805	0.038	4.731	4.879
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Spring)	**-0.341**	**0.053**	**-0.445**	**-0.237**
Land-use (Rural Open)	0.087	0.048	-0.006	0.180
Land-use (Suburban)	**-0.200**	**0.051**	**-0.300**	**-0.100**
Land-use (Urban)	**-0.310**	**0.063**	**-0.433**	**-0.186**
Land-use (Rural Open) : Season (Spring)	0.045	0.075	-0.102	0.192
Land-use (Suburban) : Season (Spring)	0.115	0.078	-0.038	0.268
Land-use (Urban) : Season (Spring)	**0.414**	**0.086**	**0.246**	**0.583**
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978

SEASON: SPRING / LAND-USE: URBAN

Parameters	Estimate	Std. Error	95% CIs

Parameters	Estimate	Std. Error	Lower	Upper
Count process				
(Intercept)	4.569	0.043	4.484	4.654
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Autumn)	-0.073	0.068	-0.206	0.059
Land-use (Rural Wooded)	-0.104	0.058	-0.219	0.010
Land-use (Rural Open)	0.028	0.059	-0.087	0.143
Land-use (Suburban)	**-0.190**	**0.060**	**-0.307**	**-0.072**
Land-use (Rural Wooded) : Season (Autumn)	**0.414**	**0.086**	**0.246**	**0.583**
Land-use (Rural Open) : Season (Autumn)	**0.369**	**0.085**	**0.202**	**0.537**
Land-use (Suburban) : Season (Autumn)	**0.299**	**0.089**	**0.125**	**0.474**
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978
SEASON: SPRING / LAND-USE: SUBURBAN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	4.379	0.046	4.288	4.470
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Autumn)	**0.226**	**0.057**	**0.114**	**0.338**
Land-use (Urban)	**0.190**	**0.060**	**0.072**	**0.307**
Land-use (Rural Wooded)	0.085	0.059	-0.030	0.201
Land-use (Rural Open)	**0.218**	**0.059**	**0.102**	**0.334**
Land-use (Urban) : Season (Autumn)	**-0.299**	**0.089**	**-0.474**	**-0.125**
Land-use (Rural Wooded) : Season (Autumn)	0.115	0.078	-0.038	0.268
Land-use (Rural Open) : Season (Autumn)	0.070	0.078	-0.083	0.223
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978
SEASON: SPRING / LAND-USE: RURAL OPEN				
Parameters	Estimate	Std. Error	95% CIs	
			Lower	Upper
Count process				
(Intercept)	4.597	0.044	4.511	4.682
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Autumn)	**0.296**	**0.053**	**0.192**	**0.399**
Land-use (Rural Wooded)	**-0.132**	**0.058**	**-0.246**	**-0.019**
Land-use (Suburban)	**-0.218**	**0.059**	**-0.334**	**-0.102**
Land-use (Urban)	-0.028	0.059	-0.143	0.087
Land-use (Rural Wooded) : Season (Autumn)	0.045	0.075	-0.102	0.192
Land-use (Suburban) : Season (Autumn)	-0.070	0.078	-0.223	0.083
Land-use (Urban) : Season (Autumn)	**-0.369**	**0.085**	**-0.537**	**-0.202**
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978
SEASON: SPRING / LAND-USE: RURAL WOODED				
Parameters	Estimate	Std. Error	95% CIs	

Parameters	Estimate	Std. Error	Lower	Upper
Count process				
(Intercept)	4.464	0.043	4.379	4.550
Apiary Size	-0.009	0.018	-0.044	0.027
Season (Autumn)	**0.341**	**0.053**	**0.237**	**0.445**
Land-use (Rural Open)	**0.132**	**0.058**	**0.019**	**0.246**
Land-use (Suburban)	-0.085	0.059	-0.201	0.030
Land-use (Urban)	0.104	0.058	-0.010	0.219
Land-use (Rural Open) : Season (Autumn)	-0.045	0.075	-0.192	0.102
Land-use (Suburban) : Season (Autumn)	-0.115	0.078	-0.268	0.038
Land-use (Urban) : Season (Autumn)	**-0.414**	**0.086**	**-0.583**	**-0.246**
Binomial process				
(Intercept)	-0.241	0.482	-1.186	0.705
Apiary Size	0.431	0.279	-0.116	0.978