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Evans, AN, Llanos, JEM, Kunin, WE orcid.org/0000-0002-9812-2326 et al. (1 more author) (2018) Indirect effects of agricultural pesticide use on parasite prevalence in wild pollinators. Agriculture, Ecosystems & Environment, 258. pp. 40-48. ISSN 0167-8809

https://doi.org/10.1016/j.agee.2018.02.002

Indirect effects of agricultural pesticide use on parasite prevalence in wild pollinators

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ABSTRACT

Insect pollinators appear to be experiencing worldwide declines, a phenomenon that has been correlated both with exposure to chemical pesticides and disease prevalence. These factors have been found to have strong and often interacting negative effects on multiple pollinator species in laboratory based studies, however their interactions in the field are less clear. To try and understand the link between pesticide use on pollinator communities, and how this might impact on disease transmission, we took two complementary approaches. First, we undertook a series of pollinator surveys to assess the abundance and diversity of pollinator groups across British agricultural field sites subject to varying levels of pesticide use. We then screened the offspring of two taxa of tube nesting solitary bees (Osmia bicornis and Megachile spp.) for three parasite groups commonly associated with pollinators. We found lower pollinator abundance, group richness and diversity across agricultural sites associated with higher pesticide use. Specifically, there were fewer honey bees, hoverflies, solitary bees and wasps. Surprisingly, we found a lower prevalence of all three parasite groups in O. bicornis offspring reared in sites associated with higher pesticide use compared to lower pesticide use. We also found a lower prevalence of Ascosphaera but a higher prevalence of Microsporidia in Megachile offspring reared sites associated with higher pesticide use compared to lower pesticide use. Together, our results suggest that farm sites associated with higher pesticide use may be affecting pollinators indirectly by disrupting community structure and influencing disease epidemiology and vectoring opportunities. This highlights the importance of understanding the interactions between pesticide use and disease in both managed and wild bee populations for the future mitigation of pollinator declines.

Key words: Neonicotinoid; agriculture; pollinator decline; parasite; pollination

1. INTRODUCTION

Animal pollinators provide ecosystem services of environmental, agricultural and economic importance by pollinating an estimated 90% of all plant species, including essential agricultural crops (Kearns et al. 1998). European honey bees (Apis mellifera) are often cited as the most valuable agricultural pollinator. However, wild pollinators, such as wild bumblebees (Bombus spp), solitary bees, flies, wasps and Lepidoptera appear to pollinate certain (and prevalent) crops such as oilseed rape and orchard fruits more effectively (Velthuis 2001; Breeze et al. 2011), by for example doubling fruit setting rates compared to the equivalent visitation rate by managed honey bees (Garibaldi et al. 2013). Indeed, wild bees contribute approximately the same value towards crop production as managed bees do (Kleijn et al. 2016). The increasingly evident role of wild insects in crop pollination has led to the suggestion that maintaining both the diversity and abundance of wild pollinators is crucial in meeting the mounting demands on the agricultural industry (Klein et al. 2003; Greenleaf and Kremen 2006; Hoehn et al. 2008; Winfree et al. 2015). Unfortunately, multiple pollinator taxa are currently experiencing contracting ranges and reductions in species richness (Biesmeijer et al. 2006; Aizen and Harder 2009; Potts et al. 2010). This appears to be the result of a complex interaction between multiple stressors (Goulson et al. 2008; Bacandritsos et al. 2010; Ellis et al. 2010; vanEngelsdorp and Meixner 2010). Understanding how stressors responsible for pollinator declines interact is therefore a key target both for improving their conservation in the wild and in supporting future global crop production.

64 A key driver of pollinator decline is believed by many to be the environmental stressors generated via agricultural intensification.
65 For example, habitat fragmentation and landscape homogeneity in large-scale farm systems have been linked to reduced forage
66 and nesting habitats required for wild bees as well as general biodiversity loss (Weibull and Östman 2003). However, several
67 studies suggest it is the combination of reduced quantity and diversity of flowering plants and exposure to high levels
68 agrochemicals that is driving pollinator declines (Nazzi and Pennacchio 2014; Schmehl et al. 2014; Baude et al. 2016). While
69 significant lethal and sub-lethal effects of certain agrochemicals, such as neonicotinoid insecticides, have been found in laboratory
70 experiments (e.g. Cresswell 2011; Lundin et al. 2015), there has been less evidence of such detrimental effects on pollinators by
71 field-realistic exposure levels (Rundlöf et al. 2015). Some studies indicate no negative effects (Blacquiere et al. 2012; Nicholls et al.
72 2017), others indicate inconsistent sub-lethal effects (Woodcock et al. 2017), supporting the idea that prevailing environmental
73 conditions are a key factor determining the lethality of agrochemicals in the field. As of the 1st December 2013, the European
74 Commission initiated a restriction on the application of three major neonicotinoids (imidacloprid, clothianidin and thiamethoxam) on
75 animal-pollinated crops throughout the European Union until there is more conclusive evidence as to whether these pesticides are
76 causing unacceptable pollinator losses (European Commission 2013). The effect of the memorandum on neonicotinoids is currently
77 under review, but the general consensus remains that farming practises that involve high levels of their use pose a considerable
78 threat to all wild pollinators (Wood and Goulson 2017). Despite this consensus, the majority of studies on the effects of pesticides

79 on pollinators have focused on honey bees and bumblebees, leaving a gap in knowledge on the effects of agrochemicals on wild
80 pollinators (Blacquiere et al. 2012; Thompson 2010; FERA 2013; Lundin et al. 2015; Wood and Goulson 2017).
81
82 Several studies have also correlated pollinator declines with the spread of pathogens and parasites (Goka et al. 2001; Otterstatter
83 and Thomson 2008; Meeus et al. 2011; Arbetman et al. 2012; Szabo et al. 2012). Again, the focus of research has largely centred
84 on honey bees, and to a lesser extent bumblebee species. However, honey bees are generalist pollinators, which share their
85 foraging sites with wild pollinators (Hudewenz and Klein 2015). They are host to more than 70 different parasites (Morse and
86 Flottum 1997), and provide a significant reservoir of disease and potential for inter-species transmission, for example though
87 shared flower patches (Graystock et al. 2015a). Indeed, several non-Apis UK pollinator species have been associated with a
88 multitude of 'traditional' honey bee parasites (Evison et al. 2012; Fürst et al. 2014; Tehel et al. 2016; Villalobos 2016). Disease
89 associations between honey bees and bumblebees (Fürst et al. 2014), and parasite spillover between commercially reared and wild
90 pollinators (Graystock et al. 2013; Tehel et al. 2016) together suggest that inter-species transmission and/or novel vectoring routes
91 are exacerbating the effects of disease driven pollinator decline. For example, co-infection in bumblebees by their neogregarine
92 parasite Apicystis bombi and deformed wing virus (DWV), which is usually associated with honey bees, were shown to severely
93 increase mortality (Graystock et al. 2015b). Damaging epidemics resulting from parasites switching between honey bee species,
94 such as Varroa destructor (Mondet et al. 2014; Wilfert et al. 2016) and Nosema ceranae (Natsopoulou et al. 2015), are well

95 documented and have taught us a great deal about emerging infectious diseases (EIDs) of honey bees, but their interactions with
96 non-Apis species requires much more investigation.
97
98 The way in which parasites and pesticides interact may be a key reason for the contrasting results of studies investigating the effect
99 of pesticides on pollinator health (e.g. Woodcock et al. 2017). Laboratory studies consistently suggest that exposure to pesticides
100 increases the susceptibility of honey bees to disease, increasing mortality (e.g. Vidau et al. 2011; Wood and Goulson 2017), as well
101 as causing harmful sub-lethal effects such as a reduced ability to sterilize colony and brood food (e.g. Alaux et al. 2010). There
102 have also been reports of some insecticides, such as the carbamate Carbofuran, and the organophosphate Dimethoate, reducing
103 the peak larval weights of honey bee larvae (Davis et al. 1988), which may have knock on effects in terms of
104 immunocompromisation of adult honey bees (Yearsley et al. 2004). When adult workers of social species of bee are
105 immunocompromised through exposure to pesticides, an increased susceptibility to disease, particularly to those that are
106 commonly spread through shared foraging patches (Pettis et al. 2012; Wu et al. 2012; Pettis et al. 2013), is likely to exaccerbate its
107 spread. For example, long range generalist foraging habits of honey bees, and high levels of intra-colony transmission predispose
108 social species like these as superspreaders of disease, particularly if those hosts are already infected with other parasites (Vidau et
109 al. 2011). Consequently, synergistic interactions between emerging infectious diseases (Natsopoulou et al. 2015) and pesticide

110 exposure (e.g. Doublet et al. 2015) are likely to have serious consequences for wild pollinators such as solitary bees, but there is a
111 dearth of information on how these factors might interact in wild populations.
112
113 Based on this information, here we aimed to start to disentangle the mechanisms underlying the documented pollinator declines by
114 assessing, first, how differing levels of agricultural pesticide use impacts on the abundance, diversity and reproductive success of
115 populations of British pollinators, and second, how this might influence the prevalence of parasites across wild bees in the same
116 populations. We assessed the effect of level of pesticide use on wild pollinators using field surveys to measure general pollinator
117 abundance, group richness and diversity. As an additional measure to the flying pollinator activity, we also measured the
118 reproductive success of tube-nesting pollinator species, and the larval weight of their offspring (as an indicator of stable
119 development and the production of healthy adults; Bosch and Vicens 2002). Collecting tube-nesting pollinators as a method of
120 assessing pollinator biodiversity is useful because they provide a small, interacting and reproducing community within the wider
121 pollinator community (Tscharntke et al. 1998), and provide a more robust assessment of the local pollinator community than flying
122 insect surveys alone can. We then measured the prevalence of three parasites previously associated with pollinators (Evison et al.
123 2012) across the same landscape, using tube-nesting solitary bees of the genus Megachile as a consistent way to sample the
124 environment. These bees share a similar ecological niche to honey bees, as generalist pollinators (Hudewenz and Klein 2015), so
125 are a useful tool for detecting inter-species disease transmission across pollinator communities. Considering the potential impact of

parasites on pollinator health, a deeper understanding of how pesticide use influences their prevalence in wild pollinators is invaluable.

2. MATERIALS AND METHODS

2.1 Field site selection and method overview

Twenty-three agricultural sites across Cambridgeshire and East Anglia were used in the study (Fig. 1), which was performed during 2012. This set of sites were selected from a larger database of field sites (Fig. S1) originally identified by the IPI AgriLand project (Linking agriculture and land use change to pollinator populations, BB/I000364/1; Supplementary Material section S4; Gillespie et al. 2017). The farms in this database are a randomised selection of farms that were chosen to encompass variation in four specific variables thought to be important in driving pollinator declines, yet were otherwise comparable (Gillespie et al. 2017). These variables were pesticide use, habitat diversity, floral resource availability, and managed honey bee colony density (see Gillespie et al. 2017 and Supplementary Materials, section S3 for specific details on how these were calculated). From the farms in the Cambridgeshire and East Anglia regions of this database, we selected the 23 sites used in this study from conventional farms only, based on their pesticide use figure. Pesticide use was estimated based on information from the UK Pesticide Survey, and was calculated by multiplying areas of different crop cover by recommended insecticide application, weighted by toxicity to honey bees

142 (Supplementary Materials, section S4.1). We chose sites that differed in extremes of their pesticide use, and categorised 13 sites
143 as high and 10 as low pesticide use, based on whether their estimated pesticide application levels fell above or below the mean
144 pesticide use estimation figure. We used a series of survey protocols to assess abundance, richness and diversity of pollinators
145 (section 2.2) at 12 of the sites (which we refer to as Group A sites; Fig. 1, Table S1.1.1). At these sites, abiotic conditions were
146 recorded during flying pollinator surveys, and local flowering plant surveys were taken in the immediate area surrounding survey
147 sites, both of which were included as co-variates in analyses on the effect of the level of pesticide use (high or low) on local
148 pollinator abundance, richness and diversity. We used a separate sampling protocol to assess the prevalence of parasites amongst
149 two species of tube-nesting bees (section 2.3) at the remaining 11 sites (which we refer to as Group B sites; Table S1.2). No local
150 information was recorded at these sampling sites, but the remaining three landscape scale variables provided by the AgrilLand data
151 set (Gillespie et al. 2017) that were associated with each site (habitat diversity values derived from land cover maps [section S4.2],
152 floral resource availability calculated from published values of nectar production [section S4.3], and honey bee colony density
153 estimated from UK Governmental 'BeeBase' records [section S4.4]), were instead used as co-variates in analyses on the effect of
154 pesticide use (high or low) on parasite prevalence.
155
156 **2.2 Pollinator and flowering plant surveys (Group A sites)**

157 We used a series of surveys to assess how the abundance, group richness and diversity of pollinators across the Cambridgeshire
158 and East Anglia area differed across sites associated with high and low pesticide use. Flying insect surveys allowed us to assess
159 local pollinator presence, and placement of tube-nests (Fig. S2) around the sites allowed us to assess the reproductive success of
160 a variety of species of solitary tube-nesting species across these sites by providing nesting cavities to collect their brood. The tube-
161 nest arrays consisted of 33 cardboard tubes of five different aperture sizes (4, 5, 6, 8 and 10 mm diameter) which accommodate
162 multiple nesting species. During May, three tube-nests were placed at each of the 12 Group A sites and were collected in July. This
163 time period allowed for an adequate assessment of species with variable breeding season lengths to be collected. Between
164 placement and retrieval, tube-nests were left undisturbed, apart from two monitoring visits, during which flying pollinator surveys
165 were conducted. The monitoring visits were approximately 18 days apart, but were adjusted to correspond with the most suitable
166 weather to observe pollinator foraging activity, including low wind speeds and minimum mean daily temperatures of 13℃ (Po llard
167 and Yates 1994). Flying pollinator surveys were conducted by taking counts of all bumblebees, honey bees, hoverflies,
168 lepidopterans, solitary bees and wasps that were observed foraging within a 1 x 5 metre area surrounding the tube-nest during a 20
169 minute period (Brittain et al. 2010a). Temperature, wind speed and a ‘weather’ variable (weather conditions were classed as either
170 raining, overcast or sunny) were also recorded. Counts were taken while the surveyor stood in a location that allowed the area
171 surrounding the tube-nest to be observed in all directions. Recorded pollinators were categorised into the six groups using Field
172 Identification Guides (O’Toole and Shields 2007), and those that could not be identified on site were captured, photographed and

stored in ethanol for categorisation later. The species richness of animal-pollinated flowering plants within the same 1 x 5 metre area was also surveyed (Ebeling et al. 2008). After 72 days in the field, all these Group A tube-nests (36) were removed from the field sites and returned to the lab to assess the reproductive success of the species using the tube-nests, by counting the number of developing brood items and calculating their peak larval weight (calculations described in section 2.4).

The tube-nests were dismantled in the lab. The inner cardboard tubes were removed from the outer structure and any occupied cardboard tubes were dissected to reveal the brood cells. Developing brood were removed from the brood cells using soft forceps and placed individually in Petri dishes, along with any remaining food provisions and a sample of the partitioning material constructed by the insect. Weight measurements were taken following a similar protocol to Bosch and Vicens (2002): first, an empty 1.5 ml Eppendorf tube was weighed using a high-precision Mettler Toledo AX26 DeltaRange microbalance and the egg, larva, pupa or cocoon was then added to the Eppendorf tube. If present, the remaining food provisions from the brood cell were added. From these measurements, it was possible to calculate individual weights for the brood and remaining food. Once weighed, the Eppendorf tubes containing the brood and remaining food were pierced to provide an air hole and stored in a temperature-controlled room at 24℃ (Abbott et al. 2008) to continue development into adulthood in case of further need of identification.

2.3 Molecular screening for parasites (Group B sites only)

Alongside our surveys of the Group A sites, a separate sampling protocol was used to assess how different levels of pesticide use might affect disease transmission amongst the same populations of pollinators. To do this, a separate set of tube-nest arrays were placed at the 11 Group B sites. These arrays consisted of a single cardboard tube size (8 mm) and each were seeded with 10 pupae of the Megachilid solitary bee species Osmia bicornis. Megachilid bees show natal nest preference (e.g. Pitts-Singer 2007), so this technique allowed us to effectively use the bees to sample the environment for any parasites that they might acquire via their natural foraging for nectar and pollen, which they collect to mass provision their offspring. This way we could assess the prevalence of parasites picked up during foraging (i.e. via a horizontal transmission route) and spread amongst their offspring (i.e. via a vertical parasite transmission route). Being generalist pollinators (Hudewenz and Klein 2015), this meant we were effectively sampling their entire foraging range (~ 2km diameter around each tube-nest [Gathmann and Tscharntke 2002]). During April, four tube-nests were placed at each of the 11 Group B sites and collected in September. This time period maximised our sampling over the breeding season of Megachilid bees. These 44 tube-nests were left undisturbed the entire time they were in the field, and upon retrieval were stored at 4℃ for subsequent parasite screening (detailed below). Despite being seeded with Osmia bicornis, some tube-nests attracted other solitary tube-nesting species. The two solitary bee taxa that were collected most frequently and consistently from the surveyed Group A sites were O. bicornis and a Megachile leafcutting bee spp. (see results), the parasite screen was therefore performed only on these two groups. This also removed bias in low numbers of hosts per species, which may have skewed our assessment of parasite prevalence (Jovani and Tella 2006). All the developing O. bicornis and Megachile

individuals extracted from the Group B tube-nests were first weighed to assess if level of pesticide use in the area they were reared may have influenced larval development. The O. bicornis offspring (which overwinter as pupae) had their entire abdomen removed. The abdomen only was used to extract DNA for screening as the parasites being assessed in this study were most likely to be found in the gut (Evison et al. 2012). The Megachile offspring overwinter as larvae, so the entire body was used to extract DNA for screening.

We screened each individual for Wolbachia, Ascosphaera and Microsporidia. Wolbachia is a genus of intracellular bacteria that is thought to infect over half of all insect species (Hilgenboecker et al. 2008) and has the potential to disrupt the colony dynamics for social bees and population dynamics for solitary bees by manipulating the sex ratios of its hosts, or by negatively affecting host survival (Werren 1997). Ascosphaera and Microsporidia are commonly associated with bees, particularly honey bees, and have been implicated in colony losses across the globe (e.g. Cox-Foster et al. 2007; Higes et al. 2009). Ascosphaera apis is an obligate fungal brood parasite of Apis mellifera, causing a common disease known as chalkbrood (Aronstein and Murray 2010), but solitary bees are also associated with Ascosphaera infections (Anderson et al. 1998). The Microsporidia include the genus Nosema, which causes dysentery in the workers of several bee species (Paxton et al. 1997; Otti and Schmid-Hempel 2007; Plischuk et al. 2009), and important EIDs such as Nosema ceranae (Fürst et al. 2014).

The sample was homogenized and total DNA and RNA was extracted in 300µl 10% Chelex by heating to 95℃ for 20 min and centrifuged for 8 min at 4000 rpm. PCR amplification was carried out using ABI 3700 thermal cyclers in 10µl volumes containing 1µl Chelex supernatant, 0.2µl of each forward and reverse primer, 2µl PCR buffer and 0.05µl of 5U/µl Taq (Promega). Reactions contained primer specific quantities of 25mM $MgCl_2$ and 10mM dNTPs and made up to 10µl with ddH_20. To check the quality of the extraction, each sample was amplified at the CO1 gene using LCO-Hym/HCOout primers (Folmer et al. 1994; Prendini et al. 2005) with 1.5µl $MgCl_2$ and 1µl dNTPs, with an initial denaturation of 2 min at 94℃ followed by 35 cycles of 30s at 94℃, 45s at 50℃ and 2 min at 72℃, and a final extension step of 72℃ for 7 min. All extractions that amplified successfully were then screened for the presence/absence of 1) Ascosphaera using the AscoAll1/AscoAll2 primers (James and Skinner, 2005) with 1µl $MgCl_2$ and 1.5µl dNTPs, with an initial denaturation of 10 min at 94℃ followed by 30 cycles of 45s at 94℃, 45s at 62℃ and 1 minute at 72℃, and a final extension step of 72℃ for 5 min. 2) Microsporidia using the V1f/530r primers (Terry et al. 2004) with 1.5µl $MgCl_2$ and 0.5µl dNTPs, with an initial denaturation of 1 min at 95℃ followed by 35 cycles of 1 min at 95℃, 1 min at 60℃ and 1 min at 72℃, and a final extension step of 72℃ for 7 min. 3) Wolbachia using CoxA f/r primers (Baldo et al. 2006) with 1µl $MgCl_2$ and 1µl dNTPs, with an initial denaturation of 2 min at 94℃ followed by 30 cycles of 30s at 94℃, 45s at 55℃ and 2 min at 72° C, and ending with a final extension step of 72℃ for 7 min. PCR products were visualised under UV using 1% agarose gels stained with ethidium bromide and compared to a 100bp size ladder. Positive and negative controls were included in every PCR.

237 **2.4 Statistical analyses**

238 All statistical analyses were performed using R v3.1.3 (R Core Team 2013) and all averages reported are mean ± standard error.
239 We used mixed models that allowed us to account for sample size bias and complex structuring within the data set (Paterson and
240 Lello 2003). All the fixed effects within the models were assessed using stepwise model comparisons from the full model to assess
241 their importance for the model fit, but the final significance effect of pesticide level reported is derived from the full model including
242 all the fixed terms (no interactions). Supplementary material (section S3) lists details and results of every test performed.

243

244 During surveys performed at the Group A sites, fewer pollinators were recorded on survey days where rainy conditions prevailed
245 compared to survey days when overcast and sunny conditions prevailed ($\chi^2_2 = 22.9$; $P < 0.001$). As such, any data collected during
246 rainy conditions were removed prior to performing statistical analyses. This left a total of 90 pollinator surveys (30 surveys at low
247 pesticide sites and 60 surveys at high pesticide sites) across the three visits. The Simpson's index was used to calculate a
248 pollinator diversity value for each site, Simpson's [1-]D = $1-\sum(n/N)^2$, where n is the abundance of a specific pollinator group, and N
249 is the abundance of all pollinators per site. Simpson's D was analysed using a linear mixed effects model implemented using the
250 lmer function, and pollinator group richness and abundance were both analysed using generalised linear mixed effects models
251 implemented using the glmer function, fitted with a Poisson error distribution, both from the lme4 package (Bates et al. 2007). Visit
252 number nested within Site ID was included as a random effect to account for the repeated surveys taken from each tube-nest

across the three visits. We were interested in understanding the effect of the categorical variable pesticide use level (low or high) associated with the sites on our pollinator abundance, diversity and richness measures, but the categorical variables pollinator group (only in the abundance model) and weather (sun or overcast), and the continuous variables temperature (℃), plant species richness and wind speed (m/s) were all included as fixed effects. These analyses showed higher pollinator group richness (χ^2_1 = 9.60, P = 0.002) and diversity (χ^2_1 = 6.38, P = 0.012), during warmer temperatures. However, there were no effects of weather, plant diversity or wind speed in either model (Table S3). There were higher overall levels of pollinator visitations observed at sites with a higher plant diversity (χ^2_1 = 58.88, P < 0.001). Wind, weather and temperature did not have any overall effects on pollinator abundance (Table S3). Because our model of overall pollinator abundance showed a significant interaction between pesticide use and pollinator group (χ^2_5 = 48.17, P < 0.001), we then used the same generalised linear mixed effects model structure to assess abundance within each pollinator group (i.e. bumblebees, honey bees, hoverflies, lepidopterans, solitary bees and wasps) separately. The importance of temperature, plant diversity, wind speed, and weather for explaining the effect of pesticide use varied by pollinator group (Table S3).

For tube-nests collected from the Group A sites only, a generalised linear mixed effects model implemented using the glmer function fitted with a Poisson error distribution was used to analyse the effects of pesticide level on tube-nest occupancy rates (i.e. how many inner cardboard tubes contained brood, per tube-nest). We fitted tube size as a fixed factor to assess whether there

were differing occupancy rates per cardboard tube size, and tested for its interaction with pesticide use level (because differences in developing brood numbers between different tube sizes might indicate differing effects of pesticide use on different species collected). To circumvent the effect of differences in larval age when assessing the effect of pesticide use level on larval weight, linear regressions were used to produce coefficients from the relationship between larval weight and the weight of the unconsumed food provisions. These coefficient values represent the Feed Conversion Efficiency (FCE) and were produced for each species recorded nesting within the tube-nests. Estimates of FCE were similar for all species and agreed with published estimates of the FCE for the solitary bee Megachile pacifica that are between 38.5% and 58.5% (Wightman and Rogers 1978). The species-pooled mean FCE was 40.8% and this value was applied to all species. The remaining food of any individual larvae that still had food provisions upon collection was multiplied by the FCE and added to the larval weight to produce a projected peak larval weight. The residuals of these projected larval weights exhibited a normal distribution and were compared between low and high pesticide use sites for the two species found at both site types using a general linear mixed effect model, with species included as a fixed factor, and here we tested for its interaction with pesticide use level to again identify whether different species differed in their response to pesticide use level. In both analyses we included the plant diversity and pollinator diversity (Simpson's [1-]D) determined from the survey data as fixed effects, and the individual cardboard tube number nested within site ID was fitted as the random effect to account for the non-independence of larvae within these arrays, as they were likely to be siblings.

For the developing O. bicornis and Megachile spp. collected from Group B sites only, differences in the proportion of hosts testing positive for each parasite between sites of high and low pesticide use was analysed for each host species separately, using a generalised linear mixed effects model implemented using the glmer function fitted with a binomial error structure. Here we also included the original variables provided from the Agriland data set as co-variates (honey bee colony density, floral resource availability and habitat diversity; see supplementary material sections S4.2-S4.4 for details of how these variables were calculated), because this allowed us to account for how their variation may have influenced parasite prevalence across sites associated with different levels of pesticide use. We also fitted cardboard tube ID nested within Tube nest ID within Site ID as the random effect to account for shared nesting tubes influencing the likelihood of parasite detection. Finally, the weight of the developing O. bicornis and Megachile spp. were compared between sites of high and low pesticide use using a linear mixed effects model implemented using the lmer function, and fitted with the same parameters as above. In these analyses, more Osmia tested positive for Ascosphaera where floral resource availability was higher ($\chi^2_1 = 7.21$, $P = 0.007$), for Microsporidia where honey bee colony density was lower ($\chi^2_1 = 6.17$, $P = 0.013$), and for Wolbachia where habitat diversity was higher ($\chi^2_1 = 5.43$, $P = 0.02$). However, none of these variables were important in detecting parasites in Megachile. Again, there was no effect of any of these variables on the weight of cocoons.

3. RESULTS

3.1 Pollinator abundance, diversity and reproductive success

Pollinator abundance (χ^2_1 = 19.8, P < 0.001), group richness (χ^2_1 = 6.10, P = 0.014) and Simpson's diversity Index (χ^2_1 = 4.36, P = 0.037) were all lower across the Group A sites associated with high compared to low pesticide use (fig. 2). The abundance of honey bees (χ^2_1 = 21.48, P < 0.001), hoverflies (χ^2_1 = 9.00, P = 0.003), solitary bees (χ^2_1 = 9.53, P < 0.002), and wasps (χ^2_1 = 6.68, P = 0.009) were all lower across sites associated with high compared to low pesticide use. However, there was no difference in the abundance of bumblebees (χ^2_1 = 0.46, P = 0.496), or lepidopterans (χ^2_1 = 1.82, P = 0.178; fig. 2) between sites associated with high or low pesticide use.

The average number of tubes occupied by brood within the mixed species tube-nests across Group A sites did not differ (χ^2_1 = 0.66, P = 0.418) between sites associated with high (3.17 ± 1.03 %) or low (4.04 ± 1.56 %) pesticide use, and there was no effect of tube size on the occupancy of tubes (χ^2_1 = 8.82, P = 0.066). However, there was an interaction between tube size and site pesticide use level (χ^2_1 = 15.05, P = 0.005), which likely reflected differing species composition at sites associated with high and low pesticide use (Table 1) occupying different tube-sizes within the tube-nests. In total, 162 developing brood items from seven different species were removed from the occupied tube-nests (91 high, 71 low; Table 1). Two species were found occupying nests at sites associated with both high and low pesticide use: a potter wasp Ancistrocerus nigricornis (5 high, 54 low) and the red mason

317 bee Osmia bicornis (3 high, 11 low). Four more species were found only at sites associated with high pesticide use: the leafcutter
318 bees Megachile willughbiella (48) and Megachile centuncularis (16), the blue mason bee Osmia caerulescens (10) and one species
319 of the solitary bee, genus Hylaeus (9). One more species was found at only sites associated with low pesticide use: a species of
320 the spider-hunting wasp family Pompilidae (6). There was no difference in the mean projected weights of the brood between sites
321 associated with high and low pesticide use, irrespective of species (table S3).
322
323 **3.2 Parasite prevalence**
324 Host DNA was successfully extracted and amplified in 55 developing O. bicornis bees. Of these, 13 tested positive for Ascosphaera,
325 7 tested positive for Microsporidia, and 18 tested positive for Wolbachia (Fig. 3a). Overall there were more parasites detected
326 across sites associated with low pesticide use ($\chi^2_1 = 8.57$, P = 0.003; Fig. 3a). The proportion of individuals testing positive differed
327 between the three parasite types ($\chi^2_2 = 7.58$, P = 0.02; Fig. 3a), but there was no interaction between parasite type and site
328 pesticide use level ($\chi^2_2 = 0.696$, P = 0.706; Fig. 3a). There was no difference in the weight of cocoons between sites associated
329 with high and low pesticide use. Individual analyses of each parasite separately backed up the main result and showed more
330 individuals testing positive for Ascosphaera ($\chi^2_1 = 4.35$, P = 0.037; Fig. 3a), Microsporidia ($\chi^2_1 = 5.85$, P = 0.016; Fig. 3a) and
331 Wolbachia ($\chi^2_1 = 4.34$, P = 0.037; Fig. 3a) across sites associated with low compared to high pesticide use.
332

Host DNA was successfully extracted and amplified in 77 developing Megachile bees. Of these, 63 tested positive for Ascosphaera, 10 tested positive for Microsporidia, and 7 tested positive for Wolbachia (Fig. 3b). Overall there was no difference in the proportion of parasites detected across sites associated with high or low pesticide use (χ^2_1 = 0.023, P = 0.881), but the proportion of individuals testing positive differed between the three parasite types (χ^2_2 = 120.7, P < 0.001; Fig. 3b), and there was an interaction between parasite type and site pesticide use level (χ^2_2 = 13.79, P = 0.001; Fig. 3b). Cocoons collected from sites associated with high pesticide use were heavier (χ^2_1 = 4.24, P = 0.039). Individual analyses of each parasite separately showed again that more individuals tested positive for Ascosphaera across sites associated with low compared to high pesticide use (χ^2_1 = 12.34, P < 0.001; Fig. 3b), but in contrast to the Osmia findings, more Megachile individuals tested positive for Microsporidia in across sites associated with high compared to low pesticide use (χ^2_1 = 3.94, P = 0.047). However, there was no difference between high and low pesticide use sites (χ^2_1 = 0.01, P = 0.917) in the prevalence of Wolbachia.

4. DISCUSSION

Our pollinator surveys support mounting evidence that agricultural sites associated with higher levels of pesticide use exhibit lower pollinator abundance and pollinator group richness and diversity than those associated with lower levels of pesticide use. However, we found no evidence of any detrimental effects of nesting in sites associated with higher pesticide use on the reproductive effort in

terms of brood numbers or projected larval weight of multiple solitary species of pollinator, including O. bicornis. Contrary to what we expected, our parasite screen of developing solitary bees revealed that the prevalence of Ascosphaera fungal parasites amongst both O. bicornis and Megachile spp. was lower in agricultural sites associated with higher levels of pesticide use compared to those associated with lower levels of pesticide use. In O. bicornis the prevalence of both Microsporidia and Wolbachia also followed this pattern, however a different pattern was found for Megachile spp. with more Microsporidia detected at agricultural sites associated with higher levels of pesticide use, and no difference in the prevalence of Wolbachia. Our results together suggest that when it comes to parasite prevalence, the indirect effects of pesticide use in an agricultural area, via impacts on pollinator population abundances, dynamics and vectoring (i.e. ecological effects on disease transmission), may be more important than the direct detrimental effects of rearing offspring in areas of high pesticide use, highlighting an important interaction that may be contributing to pollinator declines.

Our results corroborate similar studies that have found a negative relationship between pesticide use and pollinator abundance, richness and diversity (Alston et al. 2007; Brittain et al. 2010b; Biesmeijer 2012; Rundlöf et al. 2015). The interaction between the level of pesticide use and pollinator group abundance suggests that the pollinator groups we assessed were affected differently by the level of pesticide use associated with an agricultural area. Honey bees, solitary bees, hoverflies and wasps were more abundant in sites associated with lower pesticide compared to higher pesticide use, whereas the abundance of bumblebees and

lepidopterans was affected not by the level of pesticide use associated with the area, but instead by the weather and local plant species diversity at the time of the survey. Although our site selection protocol, and inclusion of abiotic and landscape variables in our analyses aimed to limit site bias in our data collection, it remains important to emphasise that areas of low pesticide application rate are still likely to differ in a variety of aspects to areas of high pesticide application rate. For example, factors that farmers must consider before deciding on a growing system, such as water content in root zones, soil type and microclimate may differ, which will affect the structure and abundance of (particularly floral resources in) semi-natural habitats at a local scale, all of which will influence how attractive the area is to different types of pollinator. This, in part, is reflected in the pattern of how our sampling sites fell across the Cambridgeshire and East Anglia area, with some spatial clustering of higher or lower pesticide use areas across the landscape. Despite this caveat, we believe our findings show an important and underappreciated aspect the drivers of pollinator decline that requires further attention.

Despite the visitation surveys revealing differences in pollinator abundance between agricultural sites associated with differing levels of pesticide use, no differences were found in the occupancy rate of the mixed-species tube-nests, which suggests that the level of pesticide residue on nearby crops and wildflowers has little impact on nest site selection, at least for the species we recorded occupying the tube-nests. However, the differences in species composition between agricultural sites associated with differing levels of pesticide use could suggest more subtle effects of pesticide use in the local area on nest site preference. For

example, O. bicornis were more prevalent at sites associated with low pesticide use and Megachile spp. were only found at sites associated with high pesticide use when sampled using the mixed-species tube nests. Of the species collected nesting within the tube-nests at sites associated with both high and low pesticide use (A. nigricornis and O. bicornis), there were no differences in their mean projected peak larval weights. Indeed, the Megachile spp. cocoons collected using the single size tube-nests (for parasite screening at the Group B sites) were heavier in sites associated with high compared to low pesticide use. This is in line with previous studies that propose there are no significant sub-lethal effects of pollen contamination by pesticides at field-realistic doses on the development of solitary bees (Abbott et al. 2008; Nicholls et al. 2017). Other studies have reached similar conclusions for bumblebees (Franklin et al. 2004; Woodcock et al. 2017) and honey bees (Cutler and Scott-Dupree 2007; Cutler et al. 2014). There is also some evidence to suggest that the use of pesticides on farms can have a positive effect on reproductive success in solitary bees. For example, Williams and Kremen (2007) found that O. lignaria produced and provided for more offspring on farms using pesticides compared to farms not using pesticides, as long as they had access to floral resources from semi-natural habitats. The use of some pesticides could therefore be affecting population dynamics in subtler ways by influencing nest site preference and provisioning rates. For example, if pollen availability is higher due to fewer pests or competitiors, that might have a beneficial effect on the reproductive success of pollen foragers, particularily species such as Megachile spp. that use leaf material to line their brood cells. However, brood weight is not the only viable indicator of stable and healthy development. It is therefore important that the effect of field-realistic levels of pesticide use on larval or pupal mortality, or other factors such as pupal head width and

development time, is investigated across multiple taxa in response to multiple pesticides to understand whether these effects occur through direct toxicity or via more complex behavioural pathways.

We found evidence that the prevalence of Ascosphaera fungal parasites amongst both O. bicornis and Megachile spp. was lower in agricultural sites associated with higher pesticide use compared to those associated with lower pesticide use. Hosts and vectors of Ascosphaera include honey bees, hoverflies, solitary bees and wasps (Evison et al. 2012; Wynns et al. 2013). Considering that our surveys showed that the abundance of all these groups were lower across agricultural sites associated with higher pesticide use, this suggests that such sites support more limited vectoring opportunities for some parasites and pathogens. The prevalence of Microsporidia and Wolbachia also followed this pattern in O. bicornis, but interestingly the pattern was not the same for the Megachile spp. with higher prevalence of Microsporidia across sites associated with higher pesticide use, and no difference in prevalence of Wolbachia. This suggests that the biology of the host, rather than these parasites may be more important in influencing their vectoring patterns. Microsporidia can cause nosemosis, a form of dysentery, in their hosts, and sub-lethal exposure to neonicotinoids increases the susceptibility of honey bees to the microsporidion Nosema ceranae (Pettis et al. 2012; Wu et al. 2012; Pettis et al. 2013) and causes increased mortality in individuals already infected with N. ceranae (Vidau et al. 2011). Ladas (1970) found a similar interaction between the presence of N. ceranae spores in honey bees and the insecticide dichlorodiphenyltrichloroethane (DDT). This might explain the higher prevalence of Microsporidia in Megachile spp. in sites

413 associated with higher pesticide use; they will be foraging for leaf material to line their nests, which is more likely to be
414 contaminated with Microsporidia spores. Even if honey bee abundance is lower in areas associated with higher pesticide use, a
415 higher potential for horizontal transmission due to a change in disease pathology would negate the lower vectoring potential as a
416 result of there being fewer hosts. Similarly, Wolbachia is thought to primarily transmit vertically (Werren 1997), however recent
417 evidence suggests common horizontal transmission routes in Lepidoptera (Ahmed et al. 2016). Our surveys suggested that
418 Lepidoptera abundance was not influenced by the level of pesticide use associated with the area, again suggesting that the higher
419 incidence of Wolbachia in Megachile cocoons (relative to Osmia) from higher pesticide use sites could be due to transmission via
420 leaf foraging. All three of the parasites screened for in this study have been found in bumblebees (Evison et al. 2012; Blaker et al.
421 2014), which were the most commonly observed pollinator group during our surveys and are likely to be acting as important hosts
422 and/or vectors of many parasites (Graystock et al. 2015a). Co-infection by these parasites is known to exacerbate disease
423 outbreaks in honey bees (Hedtke et al. 2011) and co-infection by other parasites can cause increased virulence effects in
424 bumblebees (Graystock et al. 2015b). Despite the evidence that pesticides also compound disease virulence in some pollinators
425 (Vidau et al. 2011; Pettis et al. 2012; Wu et al. 2012; Pettis et al. 2013) the complex interaction between co-infection, pesticide
426 effects on virulence, and host mortality influencing vectoring opportunity is vastly underappreciated, particularly in wild pollinators.
427

Our results highlight the complex nature of the interactions between diverse stressors on pollinator health, however they do not resolve targets for action. Agricultural sites associated with higher pesticide use appeared support a reduced abundance of some pollinator groups, which may result in reduced or altered vectoring opportunities for parasites of those pollinators. Laboratory studies that show increased virulence of parasites, and higher mortality of hosts after pesticide exposure (Alaux et al. 2010; Aufauvre et al. 2012; Pettis et al. 2012; Wu et al. 2012) do not determine how pesticide exposure influences the biological relationship between virulence and transmission. Understanding how pesticide use influences natural parasite transmission routes requires field (or semi-field) studies that incorporate natural foraging by pollinators. If direct exposure to pesticides increases the susceptibility to parasites, a consequent higher mortality will lead to reductions in detectible infections as fewer bees survive to provision their nests. Again, how this influences parasite virulence in wild populations, and the subsequent impact on the number of foundresses surviving to provision nests is unknown. Our results do not allow us to separate out these effects; because our methods relied on collecting pollinators healthy enough to fly and provision a nest, our results are therefore skewed towards collecting either benign infections or more resistant hosts; virulent infections would remove hosts from the sampling pool. The results of our farmer questionnaires (Table S1.1.2) showed that fungicides and organophosphates were only applied in the high pesticide sites, and neonicotinoids were more frequently applied, which again introduced an element of bias in our data collection because the effects of pesticide exposure on parasite virulence and transmission may differ between functional types of chemicals. For example, fungicides may directly kill fungal pathogens such as Ascosphaera and Microsporidia present on forage (Parker 1984).

444 Pesticides can also target different life stages of insects and the application of larval-targeted pesticides to adults may produce
445 skewed results of lethal and sub-lethal effects (Cutler and Scott-Dupree 2007); such as the fungicide Captan, which was previously
446 thought to be relatively harmless to honey bees but has been found to have lethal effects on larvae at the recommended field dose
447 (Mussen et al. 2004). In addition, the toxicity of some agrochemicals varies with body size, surface-area-to-volume ratio and mass-
448 specific metabolic rate, so larger bees such as bumblebees will be affected differently to smaller bees such as Hylaetus
449 (Valdovinos-Nunez et al. 2009). The mounting evidence that pesticides and fungicides may affect pollinators of different sizes and
450 life-stages differently underlines the importance of acquiring data regarding dissimilarities in risk factors for pollinator groups to
451 better inform policy makers about the impact of pesticides and parasites on non-Apis pollinators. The mechanisms behind the
452 patterns found in this study and others urgently require more attention, particularly with regards to understanding how the
453 synergistic effects of multiple agrochemical use and multiple parasite infections play out in the field via large-scale surveys, on both
454 managed and wild non-Apis pollinators.
455

456 **ACKNOWLEDGEMENTS**

457 This work was supported by the University of Leeds and from a grant awarded by Behavioural Ecology and the Environment:
458 Education and Research. Christopher Douglas Moore and Charlotte Howard provided essential data collection. Robin Dean

459 assisted with solitary bee species identification. Farmers and landowners gave access to land. Data collection was supported by
460 the UK Insect Pollinators Initiative grant AgriLand: Linking agriculture and land use change to pollinator populations (BB/I000364/1).
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463 **REFERENCES**
464 Abbott, V. A., Nadeau, J. L., Higo, H. A. and Winston, M. L. 2008. Lethal and sublethal effects of imidacloprid on Osmia lignaria and
465 clothianidin on Megachile rotundata (Hymenoptera: Megachilidae). J. Econ. Entomol. 10, 784-796.
466 Ahmed, M. Z., Breinholt, J. W. and Kawahara, A. Y. 2016. Evidence for common horitontal transmission of Wolbachia among
467 butterflies and moths. BMC Evol. Biol. 1,118.
468 Alaux, C., Brunet, J.L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M. and Le Conte, Y. 2010. Interactions between
469 nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera). Environ. Microbiol. 1, 774-782.
470 Alston, D. G., Tepedino, V. J., Bradley, B. A., Toler, T. R., Griswold, T. L. and Messinger, S. M. 2007. Effects of the Insecticide
471 Phosmet on Solitary Bee Foraging and Nesting in Orchards of Capitol Reef National Park, Utah. Environ. Entomol. 3, 811-816.
472 Anderson, D. L., Gibbs, A. J. and Gibson, N. L. 1998. Identification and phylogeny of spore-cyst fungi (Ascosphaera spp.) using
473 ribosomal DNA sequences. Mycol. Res. 10, 541-547.

474 Arbetman, M. P., Meeus, I., Morales, C. L., Aizen, M. A. and Smagghe, G. 2012. Alien parasite hitchhikes to Patagonia on invasive
475 bumblebee. Biol. Invas. 1, 489-494.
476 Aronstein, K. A. and Murray, K. D. 2010. Chalkbrood disease in honey bees. J. Invert. Pathol. 10, Supplement, S20-S29.
477 Aufauvre, J., Biron, D. G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M., Viguès, B., Belzunces, L. P., Delbac, F. and Blot, N.
478 2012. Parasite-insecticide interactions: a case study of Nosema ceranae and fipronil synergy on honeybee. Sci. Rep. 2, 326.
479 Bacandritsos, N., Granato, A., Budge, G., Papanastasiou, I., Roinioti, E., Caldon, M. et al. 2010. Sudden deaths and colony
480 population decline in Greek honey bee colonies. J. Invert. Pathol. 10, 335-340.
481 Baldo, L., Dunning Hotopp, J. C., Jolley, K. A., Bordenstein, S. R., Biber, S. A., Choudhury, R. R. et al. 2006. Multilocus Sequence
482 Typing System for the Endosymbiont Wolbachia pipientis. Appl. Environ. Microbiol. 7, 7098-7110.
483 Bates, D., Maechler, M. and Bolker, B. 2007. lme4: Fit linear and generalized linear mixed-effects models. R package version 14.
484 Baude, M., Kunin, W. E., Boatman, N. D., Conyers, S., Davies, N., Gillespie, M. A. K. et al. 2016. Historical nectar assessment
485 reveals the fall and rise of floral resources in Britain. Nature 53, 85-88.
486 Biesmeijer, J. C. 2012. Causes of the decline of wild bees in North-West Europe. Entomologische Berichten (Amsterdam) 7, 14-20.
487 Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T. et al. 2006. Parallel Declines in
488 Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science 31, 351-354.

489 Blacquiere, T., Smagghe, G., van Gestel, C. A. M. and Mommaerts, V. 2012. Neonicotinoids in bees: a review on concentrations,
490 side-effects and risk assessment. Ecotox. 2, 973-992.
491 Blaker, E. A., Strange, J. P., James, R. R., Monroy, F. P. and Cobb, N. S. 2014. PCR reveals high prevalence of non/low
492 sporulating Nosema bombi (Microsporidia) infections in bumble bees (Bombus) in Northern Arizona. J. Invert. Pathol. 12, 25-33.
493 Bosch, J. and Vicens, N. 2002. Body size as an estimator of production costs in a solitary bee. Ecol. Entomol. 2, 129-137.
494 Breeze, T. D., Bailey, A. P., Balcombe, K. G. and Potts, S. G. 2011. Pollination services in the UK: How important are honeybees?
495 Agricul. Ecosys. Environ. 14, 137-143.
496 Brittain, C., Bommarco, R., Vighi, M., Barmaz, S., Settele, J. and Potts, S. G. 2010a. The impact of an insecticide on insect flower
497 visitation and pollination in an agricultural landscape. Agricul. Forest Entomol. 1, 259-266.
498 Brittain, C. A., Vighi, M., Bommarco, R., Settele, J. and Potts, S. G. 2010b. Impacts of a pesticide on pollinator species richness at
499 different spatial scales. Basic and Applied Ecology 1, 106-115.
500 Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D. et al. 2007. A metagenomic survey of microbes in honey
501 bee Colony Collapse Disorder. Science 3, 283-287.
502 Cresswell, J. E. 2011. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey
503 bees. Ecotoxicol. 2, 149-157.

504 Cutler, C. G. and Scott-Dupree, C. D. 2007. Exposure to Clothianidin Seed-Treated Canola Has No Long-Term Impact on Honey
505 Bees. J. Econ. Entomol. 10, 765-772.
506 Cutler, G. C., Scott-Dupree, C. D., Sultan, M., McFarlane, A. D. and Brewer, L. 2014. A large-scale field study examining effects of
507 exposure to clothianidin seed-treated canola on honey bee colony health, development, and overwintering success. PeerJ ,
508 e652.
509 Davis, A. R., Solomon, K. R. and Shuel, R. W. 1988. Laboratory Studies of Honeybee Larval Growth and Development as Affected
510 by Systemic Insecticides at Adult-Sublethal Levels. J. Apicul. Res. 2, 146-161.
511 Doublet, V., Labarussias, M., de Miranda, J. R., Moritz, R. F. A. and Paxton, R. J. 2015. Bees under stress: sublethal doses of a
512 neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environ. Microbiol. 1, 969–
513 983.
514 Ebeling, A., Klein, A.-M., Schumacher, J., Weisser, W. W. and Tscharntke, T. 2008. How does plant richness affect pollinator
515 richness and temporal stability of flower visits? Oikos 11, 1808-1815.
516 Ellis, J. D., Evans, J. D. and Pettis, J. 2010. Colony losses, managed colony population decline, and Colony Collapse Disorder in
517 the United States. J. Apicul. Res. 4, 134-136.
518 European Commission. 2013. EU Regulation No 485/201, Official Journal of the European Union.

519 Evison, S. E. F., Roberts, K. E., Laurenson, L., Pietravalle, S., Hui, J., Biesmeijer, J. C. et al. 2012. Pervasiveness of Parasites in
520 Pollinators. PLoS ONE , e30641.
521 FERA. 2013. Neonicotinoid Pesticides and Bees: Report to Syngenta Ltd. York, UK.
522 Morse, R. A. and Flottum, K. 1997. Honey Bee Pests, Predators, and Diseases. The A.I. Root Co., OH.
523 Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c
524 oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. , 294-299.
525 Franklin, M. T., Winston, M. L. and Morandin, L. A. 2004. Effects of Clothianidin on Bombus impatiens (Hymenoptera: Apidae)
526 Colony Health and Foraging Ability. J. Econ. Entomol. 9, 369-373.
527 Fürst, M. A., McMahon, D. P., Osborne, J. L., Paxton, R. J. and Brown, M. J. F. 2014. Disease associations between honeybees
528 and bumblebees as a threat to wild pollinators. Nature 50, 364-366.
529 Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A. et al. 2013. Wild Pollinators
530 Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science 33, 1608-1611.
531 Gathmann, A and Tscharntke, T. 2002. Foraging ranges of solitary bees. J. Anim. Ecol. 71, 757-764.
532 Gillespie, M. A. K., Baude, M., Biesmeijer, J., Boatman, N., Budge, G. E. et al. 2017. A method for the objective selection of
533 landscape-scale study regions and sites at the national level. Methods. Ecol. Evol. 8, 1468-1476.

534 Goka, K., Okabe, K., Yoneda, M. and Niwa, S. 2001. Bumblebee commercialization will cause worldwide migration of parasitic
535 mites. Mol. Ecol. 1, 2095-2099.
536 Goulson, D., Lye, G. C. and Darvill, B. 2008. Decline and Conservation of Bumble Bees. Ann. Rev. Entomol. 5, 191-208.
537 Graystock, P., Goulson, D. and Hughes, W. O. H. 2015a. Parasites in bloom: flowers aid dispersal and transmission of pollinator
538 parasites within and between bee species. Proc. Roy. Soc. Lond. B: Biol. Sci. 28, 20151371
539 Graystock, P., Meeus, I., Smagghe, G., Goulson, D. and Hughes, W. O. H. 2015b. The effects of single and mixed infections of
540 Apicystis bombi and deformed wing virus in Bombus terrestris. Parasitol. 14, 358-365.
541 Graystock, P., Yates, K., Evison, S. E. F., Darvill, B., Goulson, D. and Hughes, W. O. H. 2013. The Trojan hives: Pollinator
542 pathogens, imported and distributed in bumblebee colonies. J. Appl. Ecol. 5, 1207-1215.
543 Greenleaf, S. S. and Kremen, C. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. 10,
544 13890-13895.
545 Hedtke, K., Jensen, P. M., Jensen, A. B. and Genersch, E. 2011. Evidence for emerging parasites and pathogens influencing
546 outbreaks of stress-related diseases like chalkbrood. J. Invert. Pathol. 10, 167-173.
547 Higes, M., Martin-Hernandez, R., Martinex-Salvador, A., Garrido-Bailon, E., Gonzalez-Porto, A.V. et al. 2009. A preliminary study of
548 the epidemiological factors related to honey bee colony loss in Spain. Environ. Microbiol. Rep. , 243-250.

549 Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. and Werren, J. H. 2008. How many species are infected with
550 Wolbachia? a statistical analysis of current data. FEMS Microbiol. Lett. 28, 215-220.
551 Hoehn, P., Tscharntke, T., Tylianakis, J. M. and Steffan-Dewenter, I. 2008. Functional group diversity of bee pollinators increases
552 crop yield. Proc. Roy. Soc. Lond. B: Biol. Sci. 27, 2283-2291.
553 Hudewenz, A. and Klein, A.-M. 2015. Red mason bees cannot compete with honey bees for floral resources in a cage experiment.
554 Ecol. Evol. 5, 5049-5056.
555 James, R. R. and Skinner, J. S. 2005. PCR diagnostic methods for Ascosphaera infections in bees. J. Invertebr. Pathol. 9, 98-103.
556 Jovani, R. and Tella, J. L. 2006. Parasite prevalence and sample size: misconceptions and solutions. Trends Parasitol. 22, 214-
557 218.
558 Kearns, C. A., Inouye, D. W. and Waser, N. M. 1998. Endangered Mutualisms: The Conservation of Plant-Pollinator Interactions.
559 Ann. Rev. Ecol. Sys. 2, 83-112.
560 Kleijn, D., Winfree, R. Bartomeus, I., Carvalheiro, L. G., Henry, M. et al. 2016. Delivery of crop pollination services is an insufficient
561 argument for wild pollinator conservation. Nat. Comms. , doi:10.1038/ncomms8414.
562 Klein, A. M., Steffan–Dewenter, I. and Tscharntke, T. 2003. Fruit set of highland coffee increases with the diversity of pollinating
563 bees. Proc. Roy. Soc. Lond. B: Biol. Sci. 27, 955-961.

564 Ladas, A. 1970. Der Einfluss verschiedener Konstitutions- und Umweltfaktoren auf die Anfalligkeit der Honigbiene(Apis mellifica L.)
565 gegenuber zwei insektiziden Pflanzenschutzmitteln. University of Bonn.
566 Lundin, O., Rundlöf, M., Smith, H. G., Fries, I. and Bommarco, R. 2015. Neonicotinoid Insecticides and Their Impacts on Bees: A
567 Systematic Review of Research Approaches and Identification of Knowledge Gaps. PLoS ONE 1, e0136928.
568 Meeus, I., Brown, M. J. F., De Graaf, D. C. and Smagghe, G. U. Y. 2011. Effects of Invasive Parasites on Bumble Bee Declines.
569 Conserv. Biol. 2, 662–71.
570 Mondet, F., de Miranda, J. R., Kretzschmar, A., Le Conte, Y. and Mercer, A. R. 2014. On the Front Line: Quantitative Virus
571 Dynamics in Honeybee (Apis mellifera) Colonies along a New Expansion Front of the Parasite Varroa destructor. PLoS. Pathog.
572 1, e1004323.
573 Mussen, E. C., Lopez, J. E. and Peng, C. Y. S. 2004. Effects of Selected Fungicides on Growth and Development of Larval Honey
574 Bees, Apis mellifera L. (Hymenoptera: Apidae). Environ. Entomol. 3, 1151-1154.
575 Natsopoulou, M. E., McMahon, D. P., Doublet, V., Bryden, J. and Paxton, R. J. 2015. Interspecific competition in honeybee
576 intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease. Proc. Roy. Soc. Lond. B:
577 Biol. Sci. 28, 20141896.
578 Nazzi, F. and Pennacchio, F. 2014. Disentangling multiple interactions in the hive ecosystem. Trends Parasitol. 3, 556-561.

579 Nicholls, E., Fowler, R., Niven, J. E., Gilbert, J. D. and Goulson, D. 2017. Larval exposure to field realistic concentrations of
580 clothianidin has no effect on development rate, over-winter survival or adult metabolic rate in a solitary bee, Osmia bicornis.
581 PeerJ , e3417
582 O'Toole, C. and Shields, C. 2007. Guide to bees of Britain. Field Studies Council
583 Otterstatter, M. C. and Thomson, J. D. 2008. Does Pathogen Spillover from Commercially Reared Bumble Bees Threaten Wild
584 Pollinators? PLoS ONE , e2771.
585 Otti, O. and Schmid-Hempel, P. 2007. Nosema bombi: A pollinator parasite with detrimental fitness effects. J. Invert. Pathol. 9, 118-
586 124.
587 Parker, F. D. 1984. Effect of fungicide treatments on incidence of chalkbrood disease in nests of the Alfafa leafcutting bee
588 (Hymenoptera: Megachilidae). J. Econ. Entomol. 7, 113-117.
589 Paterson, S. and Lello, J. 2003. Mixed models: getting the best use of parasitological data. Trends Parasitol. 8, 370-375.
590 Paxton, R., Fries, I., Pieniazek, N. and Tengö, J. 1997. High incidence of infection of an undescribed microsporidium (Microspora)
591 in the communal bee Andrena scotica (Hymenoptera, Andrenidae). Apidologie 2, 129-142.
592 Pettis, J. S., Lichtenberg, E. M., Andree, M., Stitzinger, J., Rose, R. and vanEngelsdorp, D. 2013. Crop Pollination Exposes Honey
593 Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae. PLoS ONE , e70182.

Pettis, J. S., vanEngelsdorp, D., Johnson, J. and Dively, G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwiss. 9, 153-158.

Pitts-Singer, T. L. 2007. Olfactory response of Megachilid bees, Osmia lignaria, Megachile rotunda, and M. pugnata, to individual cues from old nest cavities. Environ. Entomol. 3, 402-408.

Plischuk, S., Martín-Hernández, R., Prieto, L., Lucía, M., Botías, C., Meana, A. et al. 2009. South American native bumblebees (Hymenoptera: Apidae) infected by Nosema ceranae (Microsporidia), an emerging pathogen of honeybees (Apis mellifera). Environ. Microbiol. Rep. , 131-135.

Pollard, E. and Yates, T. J. 1994. Monitoring butterflies for ecology and conservation: the British butterfly monitoring scheme: Springer Science & Business Media.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 2, 345-353.

Prendini, L., Weygoldt, P. and Wheeler, W. C. 2005. Systematics of the Damon variegatus group of African whip spiders (Chelicerata: Amblypygi): Evidence from behaviour, morphology and DNA. Organisms Diversity & Evolution , 203-236.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rundlöf, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederstrom, V., Herbertsson, L. and Smith, H.G. 2015. Seed coating with

610 a neonicotinoid insecticide negatively affects wild bees. Nature 52, 77-80.
611 Schmehl, D. R., Teal, P. E. A., Frazier, J. L. and Grozinger, C. M. 2014. Genomic analysis of the interaction between pesticide
612 exposure and nutrition in honey bees (Apis mellifera). J. Insect Physiol. 7, 177-190.
613 Szabo, N. D., Colla, S. R., Wagner, D. L., Gall, L. F. and Kerr, J. T. 2012. Do pathogen spillover, pesticide use, or habitat loss
614 explain recent North American bumblebee declines? Conserv. Letters , 232-239.
615 Terry, R. S., Smith, J. E., Sharpe, R. G., Rigaud, T., Littlewood, D. T. J., Ironside, J. E. et al. 2004. Widespread vertical
616 transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. P. Roy. Soc. B 27, 1783-1789.
617 Thompson, H. M. 2010. Risk assessment for honey bees and pesticides - recent developments and 'new issues'. Pest Man. Sci. 6,
618 1157-1162.
619 Tscharntke, T, Gathmann, A. and Steffan-Dewenter, I. 1998. Bioindication using trap-nesting bees and wasps and their natural
620 enemies: community structure and interactions. J Appl, Ecol. 35, 708-719.
621 Valdovinos-Nunez, G. R., Quezada-Euan, J. J. G., Ancona-Xiu, P., Moo-Valle, H., Carmona, A. and Sanchez, E. R. 2009.
622 Comparative Toxicity of Pesticides to Stingless Bees (Hymenoptera: Apidae: Meliponini). J. Econ. Entomol. 10, 1737-1742.
623 vanEngelsdorp, D. and Meixner, M. D. 2010. A historical review of managed honey bee populations in Europe and the United Staes
624 and the factors that may affect them. J. Invert. Pathol. 103, S80-S95.

625 Velthuis, H. H. W. 2001. [Review of the book Crop Pollination by Bees. K.S. Delaplane & D.F. Mayer, 2000.] Entomol. Exp. Appl. 9,
626 127-129.
627 Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Vigues, B., Brunet, J. L. et al. 2011. Exposure to Sublethal Doses of Fipronil
628 and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by Nosema ceranae. PLoS ONE , e21550.
629 Villalobos, E. M. 2016. The mite that jumped, the bee that traveled, the disease that followed. Science 35, 554-556.
630 Weibull, A.-C. and Östman, Ö. 2003. Species composition in agroecosystems: The effect of landscape, habitat, and farm
631 management. Basic Appl. Ecol. , 349-361.
632 Werren, J. H. 1997. Biology of Wolbachia. Ann. Rev. Entomol. 4, 587-609.
633 Wightman, J. and Rogers, V. 1978. Growth, energy and nitrogen budgets and efficiencies of the growing larvae of Megachile
634 pacifica (Panzer) (Hymenoptera: Megachilidae). Oecologia 3, 245-257.
635 Wilfert, L., Long, G., Leggett, H. C., Schmid-Hempel, P., Butlin, R., Martin, S. J. M. et al. 2016. Deformed wing virus is a recent
636 global epidemic in honeybees driven by Varroa mites. Science 35, 594-597.
637 Williams, N. M. and Kremen, C. 2007. Resource distributions among habitats determine solitary bee offspring production in a
638 mosaic landscape. Ecol. Appl. 1, 910-921.
639 Winfree, R., Fox, J.W., Williams, N.M., Reilly, J.R. and Cariveau, D.P. 2015. Abundance of common species, not species richness,
640 drives delivery of a real-world ecosystem service. Ecol. Lett. 1, 626-635.

641 Wood, T., J. and Goulson, D. 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013.
642 Environ. Sci. Poll. Res. 21, 17285-17325.
643 Woodcock, B.A., Bullock, J.M., Shore, Heard, M.S., Pereira, M.S., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P.,
644 Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S. and Pywell, R.F. 2017.
645 Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science. 634, 1393-1395
646 Wu, J. Y., Smart, M. D., Anelli, C. M. and Sheppard, W. S. 2012. Honey bees (Apis mellifera) reared in brood combs containing
647 high levels of pesticide residues exhibit increased susceptibility to Nosema (Microsporidia) infection. J. Invert. Pathol. 10, 326-
648 329.
649 Wynns, A. A., Jensen, A. B. and Eilenberg, J. 2013. Ascosphaera callicarpa, a New Species of Bee-Loving Fungus, with a Key to
650 the Genus for Europe. PLoS ONE , e73419.
651 Yearsley, J.M., Kyriazakis, I. and Gordon I.J. 2004. Delayed costs of growth and compensatory growth rates. Funct. Ecol. 1, 563–
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Figures and tables

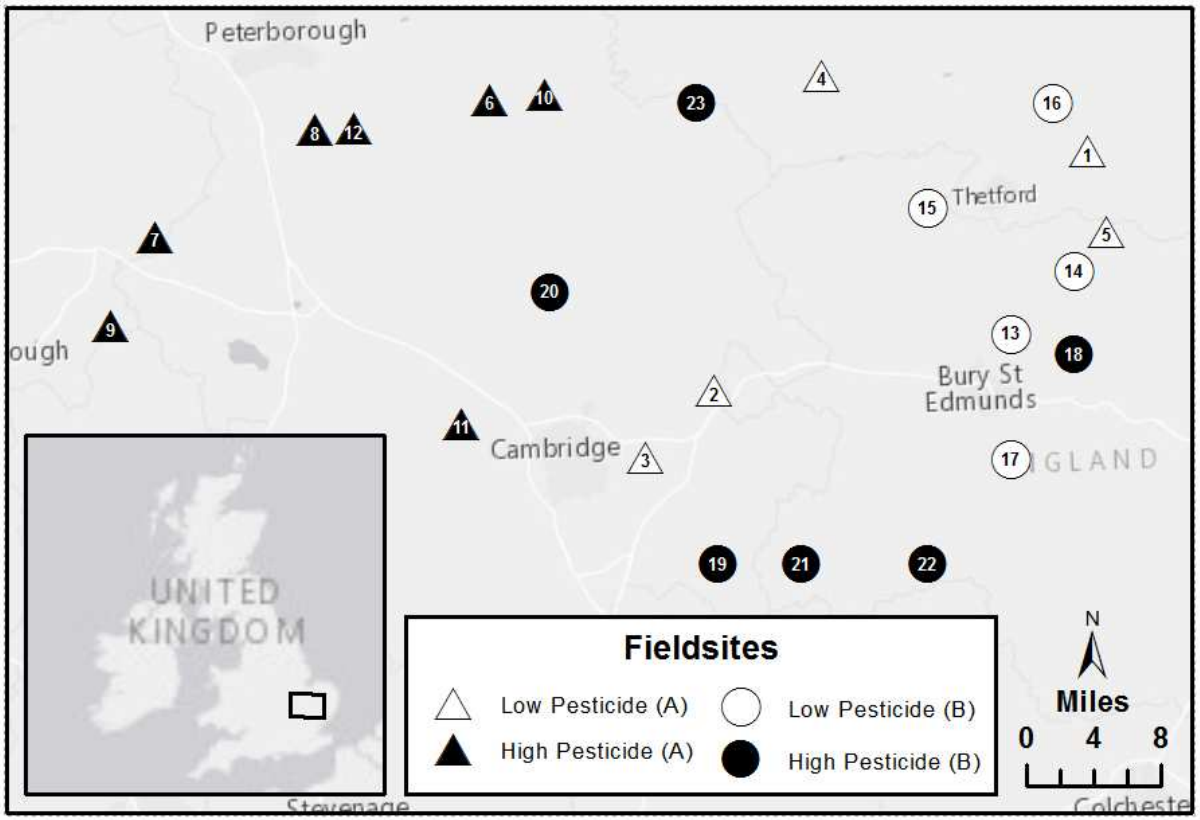
Figure legends

Figure 1. The location of the 23 field sites used in the study across Cambridgeshire and East Anglia (inset map shows the location within UK). Group A sites (detailed in Table S1) are represented by triangular markers, and Group B sites (detailed in Table S3) are represented by circular markers. Low pesticide sites are represented by open markers, and high pesticide sites by filled markers.

Figure 2. Mean (± S.E.) abundance of pollinators in each pollinator group at low and high pesticide sites. Asterisks indicate statistical significance of: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Figure 3. The proportion of a) O. bicornis and b) Megachile solitary bees testing positive for each of the three screened parasites, grouped by pesticide load. Asterisks indicate statistical significance of: * = $P < 0.05$; ** = $P < 0.01$.

Figure 1


Peterborough
Thetford
Bury St Edmunds
Cambridge
ough
GLAND
Stevenage
Colcheste
UNITED KINGDOM
Fieldsites
Low Pesticide (A)
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High Pesticide (A)
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Miles
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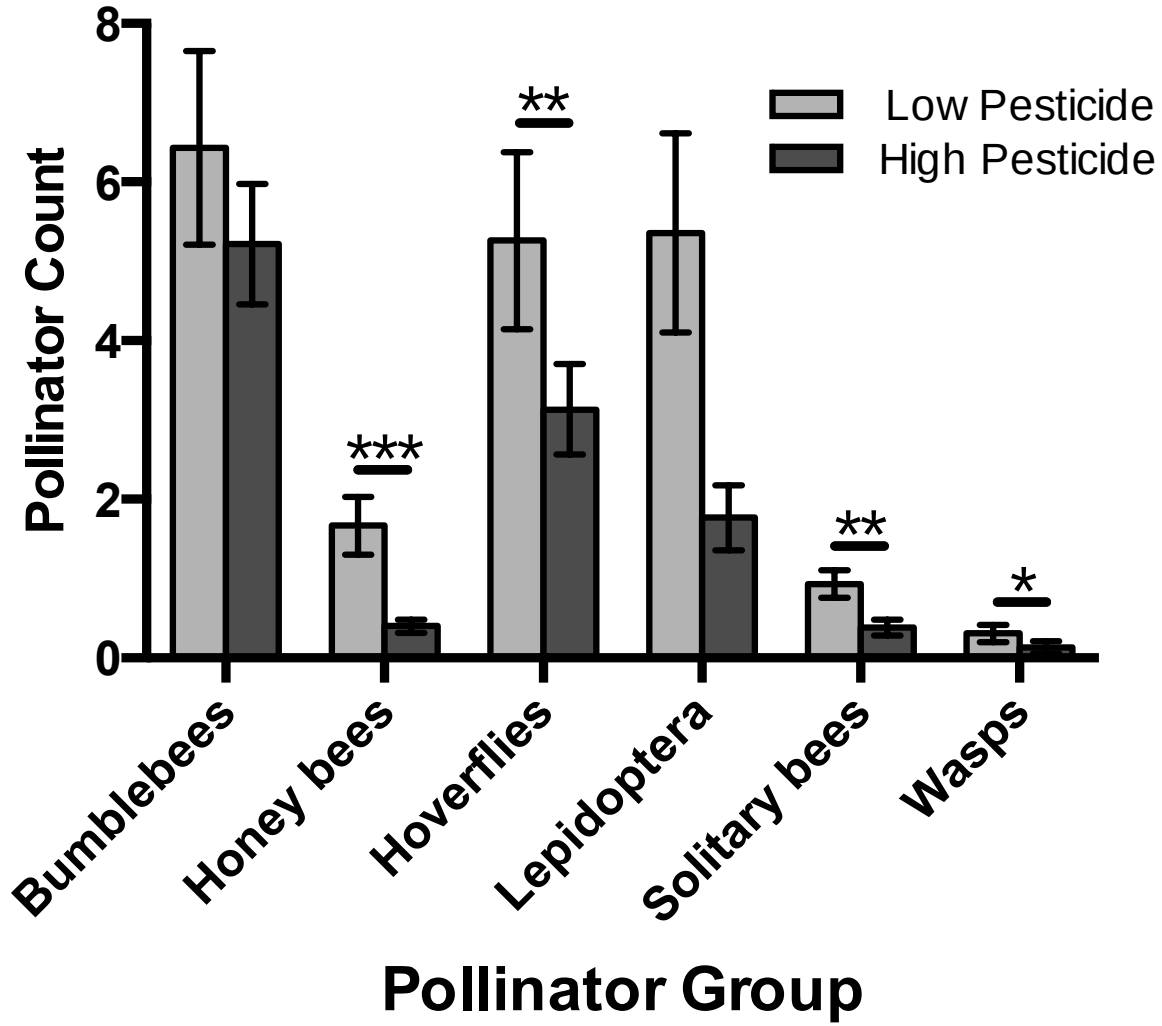
680 **Figure 2**



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682 **Figure 3**

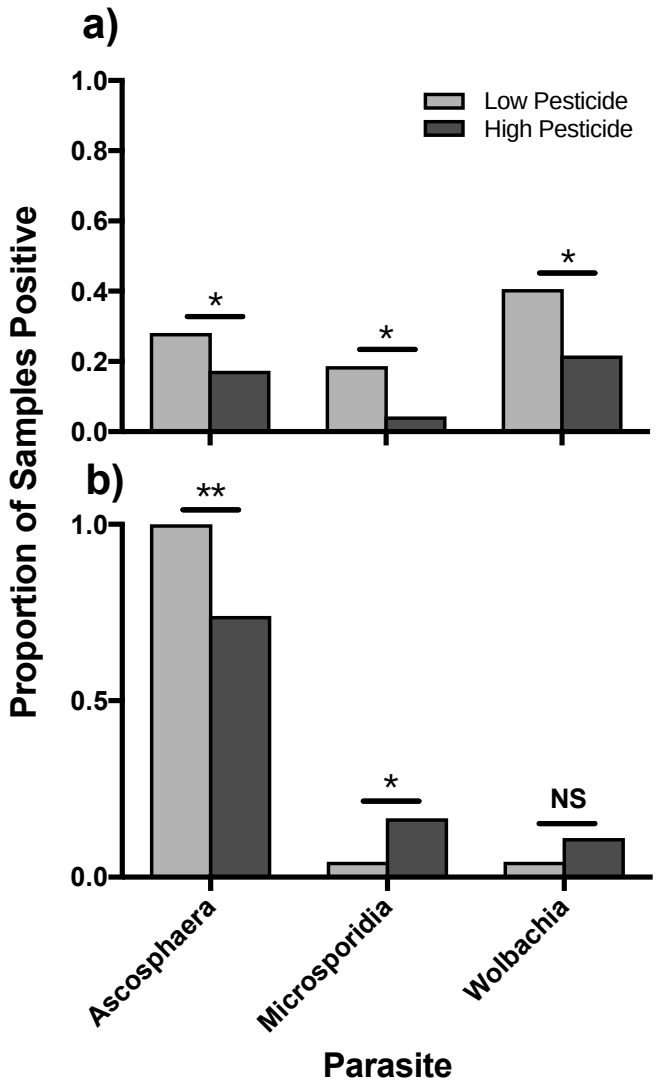

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Table 1

Species occupancy of mixed-species solitary tube-nests placed in low and high pesticide sites.

Table 1

	Solitary Bee and Solitary Wasp Species						
	Ancistrocerus nigricornis	Osmia bicornis	Megachile centuncularis	Megachile willughbiella	Osmia caerulescens	Hylaeus sp.	Pomilid sp.
Low pest. n (%)	54 (91.53)	11 (78.57)	-	-	-	-	6 (100)
High pest. n (%)	5 (8.47)	3 (21.43)	16 (100)	48 (100)	10 (100)	9 (100)	-
Total n	59	14	16	48	10	9	6
Low pest. expected mass in mg (±SE)	44.89 (2.02)	57.15 (5.96)	-	-	-	-	37.51 (2.24)
High pest. expected mass in mg (±SE)	52.7 (17.34)	57.8 (7.07)	164.01 (11.82)	177.87 (16.37)	70.02 (4.36)	4.47 (0.256)	-

Supplementary material

Indirect effects of agricultural pesticide use on parasite prevalence in wild pollinators

Alexander N Evans, Joseph E M Llanos, William E Kunin, Sophie E F Evison

Here we provide supplementary material that covers the following areas:

S1 – Further details on how both Group A and Group B sites we used in this study were selected from a larger database, including qualitative validation of pesticide use at Group A sites. And detail on what the co-variables associated with each site were that we used during the main analysis on how the level of pesticide use in the local area affected our response variables across the sites.

S2 – Details on the design of the tube-nests used at each of the field sites to collect information on the abundance, diversity and reproductive success of tube-nesting species (at Group A sites) and prevalence of pollinator associated parasites amongst two species of Megachild bee (at Group B sites).

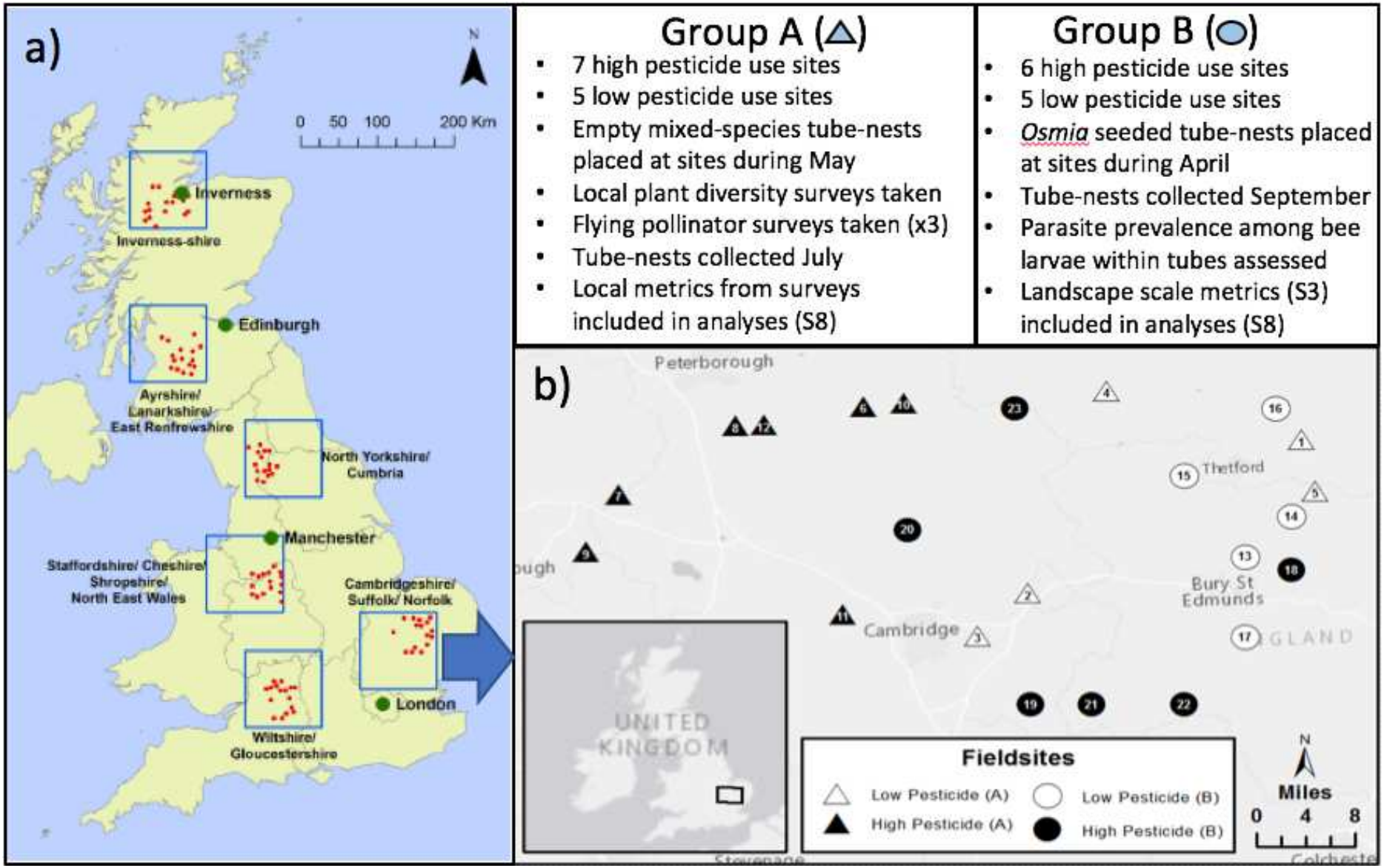
S3 – Details of every statistical analysis used in the study, including model structure, and output values for every variable.

S4 – Details on the sources of data used for site selection protocol used by the IPI Agriland project and the processing steps used to convert them to landscape scale variables. The landscape scale variable “Pesticide use” was the defining feature used in the selection of the sites used in this study.

S1. Site details and associated measurements

The complete set of 23 agricultural sites in Cambridgeshire and East Anglia used in the study were identified for use from a database of field sites selected by the IPI AgriLand project (Linking agriculture and land use change to pollinator populations, BB/I000364/1). The full set of Agriland field sites covered six 100 x 100km regions across the UK, but all the field sites used in our study were from just one of these six regions, which covered the Cambridgeshire and East Anglia area (detailed in fig. S1). The field sites were used to collect different response variables related to pollinators. Group A sites were used to assess abundance, richness and diversity of pollinators via flying insect surveys and using tube-nests (section S2) to collect the offspring of tube-nesting species. Local plant surveys were taken in the area around placement of these tube-nests and weather conditions recorded during flying insect surveys. These data were then included as co-variates in analyses on the effect of the level of pesticide use in the local area (high or low) on pollinator abundance, richness and diversity. Group B sites were used to sample the prevalence of parasites commonly associated with pollinators amongst species of tube-nesting bees who share a similar ecological niche to honey bees. No local information was recorded about the sites around the tube-nest placement t the Group B sites, instead landscape scale variables (calculated in section S3) were used as co-variates in analyses on the effect of the level of pesticide use in the local area (high or low) on parasite prevalence.

Figure S1. Site locations and details showing a) The AgriLand project selected six 100 km2 regions that were as representative as possible of the British landscape across vegetation and environmental gradients (blue squares). 96 field sites (sixteen 2 x 2 km^2 sites per region) within these were chosen using the Agriland site selection protocol (red circles) detailed in section S3 (further detail in Gillespie et al. 2017) and b) Location of the 23 field sites used in this study taken from within the Cambridgeshire and East Anglia region of the AgriLand set. Group A sites (detailed in Table S1) are represented by triangular markers, and Group B sites (detailed in Table S3) are represented by circular markers. Low pesticide sites are represented by open markers, and high pesticide sites by filled markers.


a)
N
0 50 100 200 Km
Inverness
Inverness-shire
Edinburgh
Ayrshire/
Lanarkshire/
East Renfrewshire
North Yorkshire/
Cumbria
Manchester
Staffordshire/ Cheshire/
Shropshire/
North East Wales
Cambridgeshire/
Suffolk/ Norfolk
London
Wiltshire/
Gloucestershire
Group A (△)
7 high pesticide use sites
5 low pesticide use sites
Empty mixed-species tube-nests placed at sites during May
Local plant diversity surveys taken
Flying pollinator surveys taken (x3)
Tube-nests collected July
Local metrics from surveys included in analyses (S8)
Group B (○)
6 high pesticide use sites
5 low pesticide use sites
Osmia seeded tube-nests placed at sites during April
Tube-nests collected September
Parasite prevalence among bee larvae within tubes assessed
Landscape scale metrics (S3) included in analyses (S8)
b)
Peterborough
Thetford
Bury St
Edmunds
Cambridge
UNITED
KINGDOM
Fieldsites
Low Pesticide (A)
High Pesticide (A)
Low Pesticide (B)
High Pesticide (B)
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Miles
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S1.1 Group A sites

From a list of potential sites identified from the Agriland database within the Cambridgeshire and East Anglia region, the 12 Group A sites were selected based on extremes of the pesticide use estimation values (calculated in section S3.1), and designated as high pesticide or low pesticide according to whether their associated value fell above or below the mean use value. Following site identification, each of the Group A sites underwent animal pollinated flowering plant surveys to obtain the plant diversity scores listed in table S1. These scores, along with data on weather conditions collected during pollinator surveys (wind speed, weather and temperature), were used in the statistical analyses outlined in table S8.

Table S1.1.1 Summary of variables associated with Group A sites and corresponding points on map (Fig. S1).

Site number on map	Pesticide level	Total pesticide score	Plant diversity score
1	Low	17250.44	2.00
2	Low	19251.88	1.00
3	Low	682227.52	1.00
4	Low	998045.86	1.00
5	Low	1216086.72	4.00
6	High	17235301.37	2.00
7	High	17435192.34	3.00
8	High	17625641.66	2.00
9	High	18263659.28	1.00
10	High	18349003.72	4.00
11	High	19302067.76	4.00
12	High	21220906.25	2.00

Landowners of each of the Group A sites were sent a questionnaire relating to the use of pesticides on their land, the results from which are outlined in table S2. This was to collect qualitative data on the level of pesticide use on these farms. All farms in this study were considered by the landowners to be 'conventional' as opposed to 'organic'. The high pesticide farms tended to be more intensified large-scale cereal producers compared to the low pesticide farms that tended to be smaller and grew a more even mix of cereals and vegetable crops. Four of the seven high pesticide sites had been applied with neonicotinoids by seed dressing compared to only one of the low pesticide sites. Additionally, fungicides and organophosphates were only mentioned in responses from high pesticide sites. The majority of insecticides, except neonicotinoids, in both high and low sites were sprayed rather than coated directly on the seeds. All farmers in the study were aware of the importance of honey bees and bumblebees as pollinators, but the majority of farmers were not aware of the role that solitary bees played in pollination.

Table S1.1.2 results of farmer questionnaires relating to Group A sites. Sites 1 to 5 are low pesticide sites and sites 6 to 12 are high pesticide sites.

Site	Organic / Traditional	Current Crops	Natural / Synthetic Fertiliser	Pesticides (neonicotinoids marked with *, fungicides marked with †)	Application: Spraying / Seed Dressing	Frequency of Applications	Agricultural-Environmental Stewardship scheme
1	Traditional	Asparagus, corn, parsnips, onions, carrots	Both	Cyhalothrin	Spraying	Once a year	HLS and ELS
2	Traditional	Winter wheat, sugar beet, winter barley, spring barley	Both	Primicarb	Spraying	Once a year	ELS
3	Traditional	Oilseed rape, winter wheat, barley	Both	Thiamethoxam*, cypermethrin	Both	Once a year	No
4	Traditional	Onions, sugarbeet, wheat, maize, lettuce	Both	Cyhalothrin	Both	Once a year	ELS
5	Traditional	Winter wheat, sugar beet, potatoes, parsnips	Both	Cyhalothrin	Spraying	Once a year	HLS
6	Traditional	Oilseed rape, winter wheat	Both	Cypermethrin	Spraying	Twice a year	ELS

7	Traditional	Wheat, sugrabeet, oilseed rape, mustard	Synthetic	Cyhalothrin, cypermethrin, prothioconazole†-clothianidin*	Both	Once a year	HLS and ELS
8	Traditional	Winter corn	Both	Primicarb	Spraying	Once a year	ELS
9	Traditional	Wheat, oilseed rape	Synthetic	Thiamethoxam*, cypermethrin, prothioconazole†-clothianidin*, cyhalothrin, fluvalinate, thiacloprid*	-	-	ELS
10	Traditional	Winter wheat, oilseed rape, spring wheat	Synthetic	Thiamethoxam*	Both	Once a year	No
11	Traditional	Winter wheat, oilseed rape, spring barley	Both	Thiamethoxam*	Both	Once a year	HLS and ELS
12	Traditional	Potatoes, onions, winter wheat	Both	Cyhalothrin	Spraying	Once a year	No

S1.2 Group B sites

The remaining 11 of our 23 sites (Group B sites) were also selected based on extremes of the pesticide use estimation values (calculated in section S3.1), and designated as high pesticide or low pesticide according to whether their associated value fell above or below the mean use value. These sites were not subject to any local pollinator or plant surveys or farmer questionnaires. However, as each were explicitly linked to the four variables used in their original selection we used these in the statistical analyses outlined in table S8 to assess how they may have influenced parasite abundance in our samples.

Table S1.2 Summary of variables associated with Group B sites and corresponding points on map (figure S1).

Site number on map	Pesticide level	Total pesticide score	Habitat Diversity (Shannon Index)	Honey Bee Density	Floral Resources score
13	Low	6161453.97	0.32	228790.19	1123885.49
14	Low	3215006.59	0.20	8367.60	365887.57
15	Low	0.00	0.25	9190.12	1113628.21
16	Low	3609812.57	0.70	100289.85	689768.32
17	Low	1766273.00	0.28	185692.56	943446.99
18	High	12704885.49	0.41	27342.83	260700.97
19	High	13120327.80	0.22	237068.84	1687761.34
20	High	9168271.59	0.46	211818.11	5533815.51
21	High	11815088.44	0.47	27839.77	13309544.85
22	High	17963177.10	0.31	105611.10	239376.93
23	High	11433555.15	0.02	3895.57	1232891.62

S2. Tube nest design

Tube-nests consisted of a plastic tube with a peaked edge (Fig. S2). Tube-nest arrays used at 12 Group A sites consisted of 33 cardboard tubes of five different aperture sizes (4, 5, 6, 8 and 10 mm diameter) to accommodate multiple nesting species. In contrast, the tube-nest arrays placed at the remaining 11 Group B sites consisted of a single cardboard tube size (8 mm) and each were seeded with 10 Osmia bicornis pupae. The tube-nests were painted with circles of white UV-reflective paint, which is attractive to pollinators (Westphal et al. 2008), and were attached securely with plastic cable-ties to stable and visible linear field boundaries, such as hedgerows and fence posts. Tube-nests were fixed horizontally, with their plastic peak covering the nest entrance to reduce rain exposure, and facing between South and East to maximise morning sun exposure during peak pollinator foraging hours (Everaars et al. 2011).

Figure S2. The mixed species tube nest. All tube-nests were attached securely with plastic cable-ties to stable and visible linear field boundaries, such as hedgerows and fence posts.

S3. Statistical analyses performed, and results from each test

Table S3: Model parameters and test values for all statistical tests. NS, *, **, *** indicates no significance or significance of the term at the level of 0.05, 0.01 and 0.001, respectively. Table includes details on **Test for** – the purpose of the model and statistical tests; **Response variable** – the dependent variable in the model; **Function** – the name of the model function used in R to fit the model; **Error family** – the type of probability distribution assumed by the model; **Random term** – nested terms and repeated measures accounted for in the model; **Fixed terms** – the independent variables that are included in the model, and dropped from the full model to assess their significance to the fit of the data; **Test statistics** – the output of the statistical test and the significance of the selected term's effect on the response variable.

Test for:	Response variable	Function	Error family	Random term	Fixed terms	Test statistics
Overall pollinator abundance across all Group A sites as determined by surveys	Total counts of each species at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level*Pollinator Species Pesticide Level Pollinator Group Temperature Plant Diversity Wind speed Weather	χ^2_5 = 48.17, P < 0.001 *** χ^2_1 = 19.80, P < 0.001 *** χ^2_5 = 957.4, P < 0.001 *** χ^2_1 = 3.193, P = 0.074 NS χ^2_1 = 58.88, P < 0.001 *** χ^2_1 = 2.959, P = 0.085 NS χ^2_1 = 3.340, P = 0.068 NS
Overall pollinator richness across all Group A sites as determined by surveys	Total number of species recorded at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 6.096, P = 0.014 ** χ^2_1 = 9.604, P = 0.002 ** χ^2_1 = 3.136, P = 0.077 NS χ^2_1 = 3.058, P = 0.080 NS χ^2_1 = 0.059, P = 0.808 NS
Overall pollinator diversity across all Group A sites as determined by surveys	Simpson's D calculated for each site	lmer	n/a	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 4.362, P = 0.037 * χ^2_1 = 6.383, P = 0.012 * χ^2_1 = 2.200, P = 0.138 NS χ^2_1 = 0.543, P = 0.461 NS χ^2_1 = 0.067, P = 0.796 NS
Bumblebee abundance at Group A sites as determined by surveys	Total counts of bumblebees at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 0.463, P = 0.496 NS χ^2_1 = 0.359, P = 0.549 NS χ^2_1 = 61.79, P < 0.001 *** χ^2_1 = 0.395, P = 0.530 NS χ^2_1 = 0.985, P = 0.321 NS
Honey bee abundance at Group A sites as determined by surveys	Total counts of honey bees at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 21.48, P < 0.001 *** χ^2_1 = 7.679, P = 0.006 ** χ^2_1 = 0.384, P = 0.536 NS χ^2_1 = 0.001, P = 0.976 NS χ^2_1 = 1.224, P = 0.269 NS
Solitary bee abundance at Group A sites as	Total counts of solitary bees at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature	χ^2_1 = 9.528, P < 0.002 ** χ^2_1 = 0.198, P = 0.656 NS

determined by surveys					Plant Diversity Wind speed Weather	χ^2_1 = 0.799, P = 0.371 NS χ^2_1 = 1.812, P = 0.178 NS χ^2_1 = 1.072, P = 0.300 NS
Wasp abundance at Group A sites as determined by surveys	Total counts of wasps at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 6.684, P = 0.009 ** χ^2_1 = 2.505, P = 0.114 NS χ^2_1 = 2.758, P = 0.097 NS χ^2_1 = 1.102, P = 0.294 NS χ^2_1 = 5.881, P = 0.015 *
Lepidoptera abundance at Group A sites as determined by surveys	Total counts of lepidoptera at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 1.816, P = 0.178 NS χ^2_1 = 15.74, P < 0.001 *** χ^2_1 = 20.57, P < 0.001 *** χ^2_1 = 5.484, P = 0.019 * χ^2_1 = 8.096, P = 0.004 **
Hoverfly abundance at Group A sites as determined by surveys	Total counts of hoverflies at each site	glmer	Poisson	Site ID/Visit number	Pesticide Level Temperature Plant Diversity Wind speed Weather	χ^2_1 = 9.000, P = 0.003 ** χ^2_1 = 0.122, P = 0.727 NS χ^2_1 = 0.261, P = 0.609 NS χ^2_1 = 1.853, P = 0.173 NS χ^2_1 = 1.953, P = 0.162 NS
Tube uptake rate at Group A sites as determined by tube nest content analysis	Total counts of number of tubes with evidence of developing brood at each site	glmer	Poisson	Site ID/Tube ID	Pesticide Level*Tube Size Pesticide Level Tube Size Plant Diversity	χ^2_1 = 15.05, P = 0.005 ** χ^2_1 = 0.657, P = 0.418 NS χ^2_4 = 8.817, P = 0.066 NS χ^2_1 = 0.534, P = 0.465 NS
Projected peak weights of developing brood at Group A sites as determined from species food conversion efficiencies	Estimated final weight of developing brood within tube nests at each site	lmer	n/a	Site ID/ Tube ID	Pesticide Level*Species Pesticide Level Species Plant Diversity	χ^2_2 = 0.995, P = 0.319 NS χ^2_1 = 0.442, P = 0.506 NS χ^2_2 = 0.473, P = 0.492 NS χ^2_1 = 2.942, P = 0.086 NS
Proportion of developing Osmia bicornis testing positive for a parasite at Group B sites	Total counts of number of developing brood where parasite DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level*Parasite Type Pesticide Level Parasite Type Habitat Diversity Honey bee Density Floral Resources	χ^2_2 = 0.696, P = 0.706 NS χ^2_1 = 8.574, P = 0.003 ** χ^2_2 = 7.576, P = 0.023 * χ^2_1 = 6.572, P = 0.010 ** χ^2_1 = 0.029, P = 0.864 NS χ^2_1 = 7.639, P = 0.006 **
Proportion of developing Osmia bicornis testing positive for Ascosphaera at Group B sites	Total counts of number of developing brood where Ascosphera DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	χ^2_1 = 4.349, P = 0.037 * χ^2_1 = 0.813, P = 0.367 NS χ^2_1 = 3.560, P = 0.060 NS χ^2_1 = 7.211, P = 0.007 **
Proportion of developing Osmia bicornis testing positive for Microsporidia at Group B sites	Total counts of number of developing brood where Microsporidia DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	χ^2_1 = 5.852, P = 0.016 * χ^2_1 = 0.151, P = 0.698 NS χ^2_1 = 6.174, P = 0.013 * χ^2_1 = 2.360, P = 0.125 NS
Proportion of developing Osmia bicornis testing positive for Wolbachia at	Total counts of number of developing brood where Wolbachia DNA was	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density	χ^2_1 = 4.344, P = 0.037 * χ^2_1 = 5.427, P = 0.020 * χ^2_1 = 0.000, P = 0.990 NS

Group B sites	detected via PCR				Floral Resources	$\chi^2_1 = 1.918$, P = 0.166 NS
Weight of developing Osmia bicornis at Group B sites	Measured weight of developing brood within tube nests at each site	lmer	n/a	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 3.834$, P = 0.050 NS $\chi^2_1 = 0.044$, P = 0.835 NS $\chi^2_1 = 0.452$, P = 0.502 NS $\chi^2_1 = 2.897$, P = 0.089 NS
Proportion of developing Megachile testing positive for a parasite at Group B sites	Total counts of number of developing brood where parasite DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level*Parasite Type Pesticide Level Parasite Type Habitat Diversity Honey bee Density Floral Resources	$\chi^2_2 = 13.79$, P = 0.001 ** $\chi^2_1 = 0.023$, P = 0.881 NS $\chi^2_2 = 120.7$, P < 0.001 *** $\chi^2_1 = 0.299$, P = 0.585 NS $\chi^2_1 = 0.013$, P = 0.910 NS $\chi^2_1 = 0.346$, P = 0.556 NS
Proportion of developing Megachile testing positive for Ascosphaera at Group B sites	Total counts of number of developing brood where Ascosphaera DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 12.34$, P = 0.001 *** $\chi^2_1 = 0.506$, P = 0.477 NS $\chi^2_1 = 0.633$, P = 0.426 NS $\chi^2_1 = 0.110$, P = 0.740 NS
Proportion of developing Megachile testing positive for Microsporidia at Group B sites	Total counts of number of developing brood where Microsporidia DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 3.935$, P = 0.047 * $\chi^2_1 = 0.889$, P = 0.346 NS $\chi^2_1 = 0.118$, P = 0.731 NS $\chi^2_1 = 2.417$, P = 0.120 NS
Proportion of developing Megachile testing positive for Wolbachia at Group B sites	Total counts of number of developing brood where Wolbachia DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 0.011$, P = 0.917 NS $\chi^2_1 = 0.082$, P = 0.775 NS $\chi^2_1 = 0.050$, P = 0.823 NS $\chi^2_1 = 0.050$, P = 0.823 NS
Weight of developing Megachile at Group B sites	Measured weight of developing brood within tube nests at each site	lmer	n/a	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 4.237$, P = 0.039 * $\chi^2_1 = 0.569$, P = 0.451 NS $\chi^2_1 = 0.185$, P = 0.667 NS $\chi^2_1 = 1.913$, P = 0.200 NS

S4. The sources of data used for site selection protocol used by the IPI Agriland project and details of the processing steps used to convert them to landscape variables

The information included in this section is modified from Gillespie et al. 2017. The AgriLand dataset provided specific measures of pesticide use estimation (S3.1), habitat biodiversity (S3.2), floral resource availability (S3.3) and honey bee colony density (S3.4) for a series of sites across six regions of the UK. Datasets were compiled using the UK National Grid at the "tetrad" scale (2 x 2km; 4 x 1km grid cells on OS 1:25000 maps). For each potential site (total potential sites per region = 2500) within each region, a value for each of the four variables was calculated from comprehensive national datasets as follows:

S4.1 Pesticide use (values given as "Insecticide Loadings") was estimated based on information from the UK Pesticide Usage Survey (PUS; Table S4.1) and cropping data derived from the 2010 Defra June Agricultural Survey for England and the 2010 IACS (Integrated Administration and Control System) data held by the Welsh and Scottish devolved administrations. The crop types listed in each dataset were assigned to 36 crop groups and the area under each crop group summarised to the site level.

Table S4.1 Crop type and year of survey for Pesticide Usage Survey data

Survey Type	Year	Holdings Visited	Percentage Area Visited
Arable	2010	1,187	5%
Bulbs and Flowers	2009	111	34%
Fodder crops and Grassland	2009	1,394	9% of fodder area 2% of grassland area
Hardy Ornamental Nursery Stock	2009	272	12%
Hops	2008	36	50%
Orchards	2008	235	49%
Soft fruit	2010	315	49%
Vegetables	2007	623	29%

The Pesticide Usage Survey data contained individual records of the mass of active ingredient and area of crop to which it has been applied, grouped by crop type, region and month of application. Each of the PUS crop types was also linked to one of the 36 crop groups previously created from the cropping data, and the proportional representation of that crop type within the crop group was calculated. Toxicity data for A. mellifera came from two sources; the Pesticide Properties DataBase (PPDB; University of

Hertfordshire (2013)) and Agritox (www.dive.afssa.fr/agritox/index.php; viewed 15/10/12). The PPDB records are primarily sourced from EFSA (European Food Safety Authority) reports. Agritox sources most its data from applications for chemical authorisation which have been validated by European experts. Where possible, both oral and contact LD_{50} were obtained. The active ingredient in the PUS data was linked to the lowest LD_{50} recorded for the compound and this data was used to calculate hazard quotients (eqn. 1) for each PUS record. The hazard quotients were then multiplied by the treated crop area and summed to produce a total hazard score for each PUS crop type and region combination. This was converted to a value representing the hazard per hectare for each crop group by dividing the summed hazard score by the total area of the crop grown in the region, weighting this by the proportional representation that the PUS crop type makes to the crop group, and summing the weighted scores within crop group. The insecticide loading for each of the study sites was then calculated by multiplying the area of each crop group within the site by the hazard score of that crop group in the region in which the site falls.

$$Hazard\ Quotient = \frac{Application\ Rate}{LD_{50}} \qquad \text{[eqn.1]}$$

S4.2 Habitat diversity values were derived from the Land Cover Map, LCM2007. An adapted Shannon diversity index was calculated for each potential site using the following equation:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i \qquad \text{[eqn.2]}$$

where p_i is the proportion of the area of the site in m^2 belonging to the ith sub-broad habitat category, and R is the number of sub-broad habitat categories. The sub-broad habitat categories of the LCM2007 are listed in Table S4.2

Table S4.2 Descriptions of Broad habitat sub-classes LCM 2007 used to calculate habitat diversity indices and to proportionately allocate transects for the collection of flower data.

Broad Habitat class	Broad Habitat sub-classes	Description
Broadleaved woodland	Deciduous Recent (<10yrs)	Broadleaved woodlands are characterised by stands >5 m high with tree cover >20%; scrub (<5 m) = cover >30%. Recent woodland =

	Mixed Scrub	plantations created less than 10 years ago.
Coniferous Woodland	Conifer Recent (<10yrs) Felled	Includes semi-natural stands and plantations, with cover >20%. This includes new plantation and recently felled areas. Recent woodland = plantations created less than 10 years ago.
Arable and Horticulture	Arable bare Arable Orchard	Includes annual crops, perennial crops such as berries and orchards and freshly ploughed land.
Improved Grassland	Improved grassland Hay	Improved grassland is distinguished from semi-natural grasslands based on its higher productivity, lack of winter senescence and location and/or context.
Neutral Grassland	Neutral	Neutral Grassland is determined based on botanical composition and it also includes semi-improved grasslands managed for silage, hay or pasture
Calcareous Grassland	Calcareous	The same methods apply as for Neutral Grassland (see above).
Acid Grassland	Acid Bracken	The same methods apply to Acid grassland as for Neutral Grassland (see above).
Rough Grassland	Rough / unmanaged grassland	The grass that remains as Rough grassland is a mix of areas of managed, low productivity grassland, plus some areas of semi-natural grassland, which could not be assigned Neutral, Calcareous or Acid grassland with confidence
Fen, Marsh and Swamp	Fen / swamp	Includes fen, fen meadows, rush pasture, swamp, flushes and springs.
Heather Heather grassland	Heather & dwarf shrub Burnt heather Gorse Dry heath Heather grass	Dwarf Shrub Heath is divided into two classes, depending on the density of Heather, producing Heather and Heather grassland classes respectively. Note, the Broad Habitat classification treats ericaceous vegetation on peat > 0.5 m depth as Bog.
Bog	Bog	Bog includes ericaceous, herbaceous and mossy swards in areas with a peat depth > 0.5 m. Bog forms part of an ecological continuum

	Blanket bog Bog (Grass dominated) Bog (Heather dominated)	covering Acid Grassland, Dwarf Shrub Heath and some types of Fen, Marsh and Swamp and the separation of these habitats can be difficult, as the surface vegetation (i.e. land cover) maybe very similar and the division rests on the depth of peat. The division in the field can account for species presence, plus peat depth.
Inland Rock	Inland rock Despoiled land	Covers both natural and artificial exposed rock surfaces which are >0.25ha, such as inland cliffs, caves, screes and limestone pavements, as well as various forms of excavations and waste tips such as quarries and quarry waste.

S4.3 Floral resources availability in kg of sugar per ha per year was initially derived by combining information from the LCM2007, the National Countryside Survey 2007 (CS2007; Carey et al. (2008)) and published values of nectar production for 124 species. The first step was to estimate regionally appropriate estimates for the aerial features mapped for each site, using the following equation:

$$F = \sum_{i,j} a_i (c_{j,i} s_j) \qquad \text{[eqn. 3]}$$

where a_i is the area in m^2 of the ith sub-broad habitat category, $c_{j,i}$ is the regional average cover of the jth flowering plant species occurring in habitat i taken from the CS2007 and s_j is the sugar potential in kg/ha/year of the jth flowering plant species. F therefore represents the regional mean sugar potential of flowering plants occurring within sub-broad habitat categories included in the LCM2007. Regionally appropriate plant covers were estimated using all CS “X”, “U” and “Y” plot samples. These vegetation plot samples were all 2 x 2m in size and are stratified to sample all habitats (“X plots”), unenclosed upland habitats (“U plots”) and priority habitats (“Y plots”) respectively. They are a stratified random sample of the plant species composition of broad and priority habitats occurring in the random 1x1 km survey squares that are the foundation of the Countryside Survey (Norton et al. 2012). Thus, estimates of F specific to each focal region and sub-broad habitat class were derived from vegetation plots within those 1x1 km squares coinciding with the focal 100 x 100 km region square and a buffer of 50 km on all sides. This equation was further modified to take account of the higher density of “weeds” on organic agricultural land and agri-environment schemes that were not covered by CS2007, and the extraordinary contribution that mass-flowering crops make to the overall floral resource availability of a landscape. The final calculation is therefore represented by:

$$F_T = F + (A_O \times F_A \times 6.26) + (A_{aes-j} \times W_j) + F_{MFC} \quad \text{[eqn. 4]}$$

where A_o is the area of organic arable land multiplied by the locally appropriate arable resource value F_A but upweighted to reflect the higher weed densities in organic arable fields and (calculated from raw data used in Gabriel et al. (2010)), $A_{aes\text{-}j}$ is the area of relevant management options in each national agri-environment scheme (Environmental Stewardship in England, Glastir in Wales and Land Manager Options and Rural Priorities schemes in Scotland (from FERA records), weighted by the relative value of each to pollinators as judged by an expert assessment (Breeze et al. 2014), and F_{MFC} is the floral resources for mass flowering crops (assessed from Defra June Agricultural Survey data, Defra 2010).

The goal was to estimate nectar production for a large fraction of Britain's animal-pollinated plants. While there are >2500 spp. of plants in the flora (Preston et al. 2002), CS data showed that the commonest 440 species together account for 99% of the total cover, and less than half of these are potentially rewarding to pollinators and are likely to contribute substantially to floral resources on a large scale (Baude et al. 2016). Published values of sugar production (s) were only available for 124 species at the time of the study site selection. It was therefore necessary to estimate these values for the remaining plants on the list of the most common and most rewarding insect-pollinated British plants. This was achieved through linear modelling (using R 2.15.1 (R Core Team 2011)) with published sugar (kg/ha/year) as a response variable and various plant traits as explanatory variables. Plant traits for all species were collated from online databases Biolflor (Klotz et al. 2002; www.biolflor.de) and EcoFlora (Fitter & Peat 1994; http://www.ecoflora.co.uk/), with supporting information from Crane & Walker (1984), Crane et al. (1984), Grime et al. (1988), Stace (2010) and Crawford (2000). Where information on a trait could not be found in any published sources for a plant, the value was estimated from the scores of other plants in that genus. When most plants within the genus shared the same score or trait, that value was used for the missing plant. When the plants within the genus were widely differing in the trait, the missing plant was given the value of the most similar or closely related species.

The linear model was fitted with as many plant trait variables as possible (no interactions) and then a backward selection protocol using AIC to compare models was employed to derive the most important plant traits in explaining sugar production. Due to a limited number of published sugar values, subsequent prediction for all 220 species was problematic because of a lack of representation of all plant trait values. For example, there were no sugar production values for certain plant families meaning that subsequent prediction of sugar production could not be made for missing plant families. Some of the plant trait categories required amalgamation therefore and this was performed ensuring that new categories made biological sense. Important reclassifications are described in Table S4.3.1. The final linear model ($F_{11, 91}$=10.24, $p < 0.0001$, $R2 = 0.55$) had six single terms (Table S4.3.2) and was used to make predictions of sugar production (kg/ha/year) for 96 species. The subsequent estimates were then used in eqn. 3.

952 **Table S4.3.1** Reclassifications of plant trait categories for inclusion in linear modelling and subsequent predictions.
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Plant trait	Description and categories	Category without published sugar values	Reclassified category
Müller class	After Műller (1881), a classification into categories according to depth of nectar display or pollinator groups. Relevant categories: A = open nectar display AB = part hidden nectar source B = totally hidden nectar source H = Hymenoptera pollinated F = Lepidoptera pollinated D = Diptera pollinated Po = pollen is main reward W= wind pollinated O = occasionally insect pollinated	O, W F, D	Po H
Dicliny	Based on the category of Dicliny: the spatial separation of sexes on flowers. Hermaphroditic = all flowers bisexual Monoecious = male and female flowers on same plant Dioecious = male and female on different plant Gynomonoecious = female and bisexual on same plant Gynodioecious = female and bisexual on different plants Andromonoecious = male and bisexual on same plant Androdioecious = male and bisexual on different plants Trioecious = female, male and bisexual on different plants Trimonoecious = female, male and bisexual on same plant	Gynomonoecious, Andromonoecious, Trimonoecious Gynodioecious, Androdioecious, Trioecious Hermaphroditic* Monoecious* Dioecious*	Same Different Same* Different*
Strategy	Ecological strategy following the system of Grime et al. (1988). c – competitors (highly competitive plants) r – ruderals (Usually annual, weedy plant species which produce many seeds and can easily colonize pioneer habitats)	s sr	Assigned to the closest ecological category for each species

s – stress-tolerators (Species with slow relative growth rates and morphological and/or physiological adaptations to conditions of resource scarcity and climatic severity).
cr – competitors/ruderals (Intermediate between these two types)
cs – competitors/stress-tolerant (Intermediate between these two types)
sr – stress-tolerant/ruderals (Intermediate between these two types)
csr – competitors/stress-tolerant/ruderals (Intermediate between all three types, usually rosette plants or small, perennial species which can utilize spatio-temporal niches very well and have an intermediate life span)

* These categories do have representatives with published values, but were still reclassified as “Same” or “Different” as above to maintain a two-level categorical variable.

Table S4.3.2. Analysis of variance table of the final linear model used to predict sugar production (kg/ha/year) using published values as the response variable.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Müller class*	4	748.4	187.0	18.92	<0.0001
Breeding system†	3	83.7	27.9	2.82	<0.05
log(Maximum Height (mm))	1	126.1	126.1	12.75	<0.001
Same or Different‡	1	73.4	73.4	7.42	<0.01
Corolla Depth (mm)	1	14.5	14.5	1.46	0.229
Mean Bee Index	1	68.6	68.6	6.93	<0.01
Residuals	91	899.9	9.9		

*“Müller class” refers to the Müller classification system of flower shape and in this dataset, there were five classes (pollen (pollen is main reward), open nectaries, partly-hidden nectaries, hidden nectaries, and plants pollinated by specific species groups).
† “Breeding system” is defined by the origin of the gametes and this dataset had five classes (allogamous, facultative allogamous, autogamous, facultative autogamous and mixed mating systems).
‡The “same or different” term refers to relative location of male and female flowers on an individual plant (both sexes were on the “same” plant (including hermaphroditic plants) or the sexes were separated on “different individuals).

S4.4 Managed honey bee density. The English, Welsh and Scottish Governments sponsor honey bee apiary inspection programmes and collate inspection data in a database known as 'BeeBase'. Colony assessment data were queried for the years 2001-2010 and the number of bees present in mid-summer for an average colony estimated. The number of adult bees was estimated using the brood and assuming an 87.5% survival across all life stages (Winston 1991). The number of colonies present in each apiary was calculated for three apiary classes: 1) apiary owned by a single amateur beekeeper (39 colonies or less); 2) shared apiary of one or more amateur beekeeper; and 3) apiary owned by a professional beekeeper (40 or more colonies owned). Observations of foraging behaviour were gathered for ten site/season combinations from the published literature (Waddington et al. 1994; Beekman & Ratnieks 2000). Foraging observations were grouped into 200m bins representing different foraging ranges for each site/year combination and a distribution model fitted to the sum of all foraging observations. A Gamma distribution was found to account for the short distance flights and a lognormal distribution for the longer flights. The significance of the lognormal part of the model (compared to the Gamma distribution) was tested using an F-test for nested models (Genstat V15). The final model was used to estimate the proportion of the foraging force likely to be active in radiating 200 m bands up to the maximum foraging distance reported for honey bees (13 km; Eckert 1933). The honey bee density map was completed by rendering foraging models and apiary sizes for all registered apiaries across England, Wales and Scotland (ArcMap 10.0; Esri 2011). Honey bee forager density around each apiary was calculated for a set of 200m concentric circular bands out to a distance of 13km. The bands were intersected with each other and the forager densities for intersecting bands were summed to give the expected density of honey bee foragers. These polygons were then intersected with the selected 2km site squares and the total expected number of honey bee foragers calculated by multiplying the densities by the area of the intersected polygons within the selected 2km squares.

References

Baude, M. et al. 2016. Historical nectar assessment reveals the fall and rise of floral resources in Britain. Nature 530**:** 85-88.

Beekman, M. and Ratnieks, F.L.W. 2000. Long-range foraging by the honey-bee, Apis mellifera L. Func. Ecol. 14: 490-496.

Breeze, T.D. et al. 2014. Costing conservation: an expert appraisal of the pollinator habitat benefits of England's entry level stewardship. Biodiv. Conserv. 23: 1193-1214.

Carey, P.D. et al. 2008. Countryside Survey: UK Results from 2007. Centre for Ecology & Hydrology.

Crane, E. and Walker, P. 1984. Pollination Directory of World Crops. IBRA.

Crane, E. et al. 1984. Directory of important world honey sources. International Bee Research Association, London.

Crawford, M. 2000. Bee Plants, 2nd edn. Agroforestry Research Trust.

Eckert, J.E. 1933. The flight range of the honeybee. J. Agri. Res. 47: 0257-0285.

ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Everaars, J. et al. 2011. Microsite conditions dominate habitat selection of the red mason bee (Osmia bicornis, Hymenoptera: Megachilidae) in an urban environment: A case study from Leipzig, Germany. – Landscape and Urban Planning 103: 15-23.

1005 Fitter, A.H. and Peat, H.J. 1994. The Ecological Floral Database. J. Ecol. 82: 415-425.
1006 Gabriel, D. et al. 2010. Scale matters: the impact of organic farming on biodiversity at different spatial scales. Ecol. Lett. 13: 858-
1007 869.
1008 Grime, J. et al. 1988. Comparative Plant Ecology: A Functional Approach to Common British Species. Springer.
1009 Klotz, A.H. et al. 2002. BIOLFLOR - Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland.
1010 Bundesamt für Naturschutz.
1011 Norton, L.R. et al. 2012 Measuring stock and change in the GB countryside for policy - Key findings and developments from the
1012 Countryside Survey 2007 field survey. J. Envir. Man. 113: 117-127.
1013 Preston, C.D. et al. 2002. New Atlas of the British and Irish Flora. Oxford University Press.
1014 R Core Team 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna,
1015 Austria.
1016 Stace, C. 2010. New Flora of the British Isles. Cambridge University Press.
1017 Waddington, K.D. et al. 1994. Comparisions of forager distributions from matched honey bee colonies in suburban environments.
1018 Behav. Ecol. Sociobiol. 35: 423-429.
1019 Westphal, C. et al. 2008. Measuring bee diversity in different European habitats and biogeographical regions. – Ecol. Monograph
1020 78: 653-671.
1021 Winston M.L. 1991. The biology of the honey bee - Harvard University Press
1022
1023