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Evans, AN, Llanos, JEM, Kunin, WE [orcid.org/0000-0002-9812-2326](http://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>

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

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4

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22 **ABSTRACT**

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

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

48 **1. INTRODUCTION**

49 Animal pollinators provide ecosystem services of environmental, agricultural and economic importance by pollinating an estimated  
50 90% of all plant species, including essential agricultural crops (Kearns et al. 1998). European honey bees (*Apis mellifera*) are often  
51 cited as the most valuable agricultural pollinator. However, wild pollinators, such as wild bumblebees (*Bombus* spp), solitary bees,  
52 flies, wasps and Lepidoptera appear to pollinate certain (and prevalent) crops such as oilseed rape and orchard fruits more  
53 effectively (Velthuis 2001; Breeze et al. 2011), by for example doubling fruit setting rates compared to the equivalent visitation rate  
54 by managed honey bees (Garibaldi et al. 2013). Indeed, wild bees contribute approximately the same value towards crop  
55 production as managed bees do (Kleijn et al. 2016). The increasingly evident role of wild insects in crop pollination has led to the  
56 suggestion that maintaining both the diversity and abundance of wild pollinators is crucial in meeting the mounting demands on the  
57 agricultural industry (Klein et al. 2003; Greenleaf and Kremen 2006; Hoehn et al. 2008; Winfree et al. 2015). Unfortunately, multiple  
58 pollinator taxa are currently experiencing contracting ranges and reductions in species richness (Biesmeijer et al. 2006; Aizen and  
59 Harder 2009; Potts et al. 2010). This appears to be the result of a complex interaction between multiple stressors (Goulson et al.  
60 2008; Bacandritsos et al. 2010; Ellis et al. 2010; vanEngelsdorp and Meixner 2010). Understanding how stressors responsible for  
61 pollinator declines interact is therefore a key target both for improving their conservation in the wild and in supporting future global  
62 crop production.

63

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 1<sup>st</sup> 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 through  
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 exacerbate 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

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

128

129

## 130 **2. MATERIALS AND METHODS**

### 131 **2.1 Field site selection and method overview**

132 Twenty-three agricultural sites across Cambridgeshire and East Anglia were used in the study (Fig. 1), which was performed during  
133 2012. This set of sites were selected from a larger database of field sites (Fig. S1) originally identified by the IPI AgriLand project  
134 (Linking agriculture and land use change to pollinator populations, BB/I000364/1; Supplementary Material section S4; Gillespie et  
135 al. 2017). The farms in this database are a randomised selection of farms that were chosen to encompass variation in four specific  
136 variables thought to be important in driving pollinator declines, yet were otherwise comparable (Gillespie et al. 2017). These  
137 variables were pesticide use, habitat diversity, floral resource availability, and managed honey bee colony density (see Gillespie et  
138 al. 2017 and Supplementary Materials, section S3 for specific details on how these were calculated). From the farms in the  
139 Cambridgeshire and East Anglia regions of this database, we selected the 23 sites used in this study from conventional farms only,  
140 based on their pesticide use figure. Pesticide use was estimated based on information from the UK Pesticide Survey, and was  
141 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°C (Pollard  
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

173 stored in ethanol for categorisation later. The species richness of animal-pollinated flowering plants within the same 1 x 5 metre  
174 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  
175 field sites and returned to the lab to assess the reproductive success of the species using the tube-nests, by counting the number  
176 of developing brood items and calculating their peak larval weight (calculations described in section 2.4).

177

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

187

188 **2.3 Molecular screening for parasites (Group B sites only)**

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

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

210

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

220

221 The sample was homogenized and total DNA and RNA was extracted in 300µl 10% Chelex by heating to 95°C for 20 min and  
222 centrifuged for 8 min at 4000 rpm. PCR amplification was carried out using ABI 3700 thermal cyclers in 10µl volumes containing 1µl  
223 Chelex supernatant, 0.2µl of each forward and reverse primer, 2µl PCR buffer and 0.05µl of 5U/µl Taq (Promega). Reactions  
224 contained primer specific quantities of 25mM MgCl<sub>2</sub> and 10mM dNTPs and made up to 10µl with ddH<sub>2</sub>O. To check the quality of the  
225 extraction, each sample was amplified at the CO1 gene using LCO-Hym/HCOout primers (Folmer et al. 1994; Prendini et al. 2005)  
226 with 1.5µl MgCl<sub>2</sub> and 1µl dNTPs, with an initial denaturation of 2 min at 94°C followed by 35 cycles of 30s at 94°C, 45s at 50°C and  
227 2 min at 72°C, and a final extension step of 72°C for 7 min. All extractions that amplified successfully were then screened for the  
228 presence/absence of 1) *Ascosphaera* using the *AscoAll1/AscoAll2* primers (James and Skinner, 2005) with 1µl MgCl<sub>2</sub> and 1.5µl  
229 dNTPs, with an initial denaturation of 10 min at 94°C followed by 30 cycles of 45s at 94°C, 45s at 62°C and 1 minute at 72°C, and a  
230 final extension step of 72°C for 5 min. 2) *Microsporidia* using the *V1f/530r* primers (Terry et al. 2004) with 1.5µl MgCl<sub>2</sub> and 0.5µl  
231 dNTPs, with an initial denaturation of 1 min at 95°C followed by 35 cycles of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C, and a  
232 final extension step of 72°C for 7 min. 3) *Wolbachia* using *CoxA f/r* primers (Baldo et al. 2006) with 1µl MgCl<sub>2</sub> and 1µl dNTPs, with  
233 an initial denaturation of 2 min at 94°C followed by 30 cycles of 30s at 94°C, 45s at 55°C and 2 min at 72° C, and ending with a final  
234 extension step of 72°C for 7 min. PCR products were visualised under UV using 1% agarose gels stained with ethidium bromide  
235 and compared to a 100bp size ladder. Positive and negative controls were included in every PCR.

236



## 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  $\pm$  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

253 across the three visits. We were interested in understanding the effect of the categorical variable pesticide use level (low or high)  
254 associated with the sites on our pollinator abundance, diversity and richness measures, but the categorical variables pollinator  
255 group (only in the abundance model) and weather (sun or overcast), and the continuous variables temperature (°C), plant species  
256 richness and wind speed (m/s) were all included as fixed effects. These analyses showed higher pollinator group richness ( $\chi^2_1 =$   
257 9.60,  $P = 0.002$ ) and diversity ( $\chi^2_1 = 6.38$ ,  $P = 0.012$ ), during warmer temperatures. However, there were no effects of weather,  
258 plant diversity or wind speed in either model (Table S3). There were higher overall levels of pollinator visitations observed at sites  
259 with a higher plant diversity ( $\chi^2_1 = 58.88$ ,  $P < 0.001$ ). Wind, weather and temperature did not have any overall effects on pollinator  
260 abundance (Table S3). Because our model of overall pollinator abundance showed a significant interaction between pesticide use  
261 and pollinator group ( $\chi^2_5 = 48.17$ ,  $P < 0.001$ ), we then used the same generalised linear mixed effects model structure to assess  
262 abundance within each pollinator group (i.e. bumblebees, honey bees, hoverflies, lepidopterans, solitary bees and wasps)  
263 separately. The importance of temperature, plant diversity, wind speed, and weather for explaining the effect of pesticide use varied  
264 by pollinator group (Table S3).

265

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

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

284

285 For the developing *O. bicornis* and *Megachile* spp. collected from Group B sites only, differences in the proportion of hosts testing  
286 positive for each parasite between sites of high and low pesticide use was analysed for each host species separately, using a  
287 generalised linear mixed effects model implemented using the `glmer` function fitted with a binomial error structure. Here we also  
288 included the original variables provided from the Agriland data set as co-variables (honey bee colony density, floral resource  
289 availability and habitat diversity; see supplementary material sections S4.2-S4.4 for details of how these variables were calculated),  
290 because this allowed us to account for how their variation may have influenced parasite prevalence across sites associated with  
291 different levels of pesticide use. We also fitted cardboard tube ID nested within Tube nest ID within Site ID as the random effect to  
292 account for shared nesting tubes influencing the likelihood of parasite detection. Finally, the weight of the developing *O. bicornis*  
293 and *Megachile* spp. were compared between sites of high and low pesticide use using a linear mixed effects model implemented  
294 using the `lmer` function, and fitted with the same parameters as above. In these analyses, more *Osmia* tested positive for  
295 *Ascospaera* where floral resource availability was higher ( $\chi^2_1 = 7.21$ ,  $P = 0.007$ ), for *Microsporidia* where honey bee colony density  
296 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  
297 these variables were important in detecting parasites in *Megachile*. Again, there was no effect of any of these variables on the  
298 weight of cocoons.

299

300

### 301 3. RESULTS

#### 302 3.1 Pollinator abundance, diversity and reproductive success

303 Pollinator abundance ( $\chi^2_1 = 19.8$ ,  $P < 0.001$ ), group richness ( $\chi^2_1 = 6.10$ ,  $P = 0.014$ ) and Simpson's diversity Index ( $\chi^2_1 = 4.36$ ,  $P =$   
304  $0.037$ ) were all lower across the Group A sites associated with high compared to low pesticide use (fig. 2). The abundance of  
305 honey bees ( $\chi^2_1 = 21.48$ ,  $P < 0.001$ ), hoverflies ( $\chi^2_1 = 9.00$ ,  $P = 0.003$ ), solitary bees ( $\chi^2_1 = 9.53$ ,  $P < 0.002$ ), and wasps ( $\chi^2_1 = 6.68$ ,  
306  $P = 0.009$ ) were all lower across sites associated with high compared to low pesticide use. However, there was no difference in the  
307 abundance of bumblebees ( $\chi^2_1 = 0.46$ ,  $P = 0.496$ ), or lepidopterans ( $\chi^2_1 = 1.82$ ,  $P = 0.178$ ; fig. 2) between sites associated with  
308 high or low pesticide use.

309

310 The average number of tubes occupied by brood within the mixed species tube-nests across Group A sites did not differ ( $\chi^2_1 =$   
311  $0.66$ ,  $P = 0.418$ ) between sites associated with high ( $3.17 \pm 1.03$  %) or low ( $4.04 \pm 1.56$  %) pesticide use, and there was no effect of  
312 tube size on the occupancy of tubes ( $\chi^2_1 = 8.82$ ,  $P = 0.066$ ). However, there was an interaction between tube size and site pesticide  
313 use level ( $\chi^2_1 = 15.05$ ,  $P = 0.005$ ), which likely reflected differing species composition at sites associated with high and low  
314 pesticide use (Table 1) occupying different tube-sizes within the tube-nests. In total, 162 developing brood items from seven  
315 different species were removed from the occupied tube-nests (91 high, 71 low; Table 1). Two species were found occupying nests  
316 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 *Ascospaera*,  
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 *Ascospaera* ( $\chi^2_1 = 4.35$ ,  $P = 0.037$ ; 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

333 Host DNA was successfully extracted and amplified in 77 developing Megachile bees. Of these, 63 tested positive for Ascospaera,  
334 10 tested positive for Microsporidia, and 7 tested positive for Wolbachia (Fig. 3b). Overall there was no difference in the proportion  
335 of parasites detected across sites associated with high or low pesticide use ( $\chi^2_1 = 0.023$ ,  $P = 0.881$ ), but the proportion of  
336 individuals testing positive differed between the three parasite types ( $\chi^2_2 = 120.7$ ,  $P < 0.001$ ; Fig. 3b), and there was an interaction  
337 between parasite type and site pesticide use level ( $\chi^2_2 = 13.79$ ,  $P = 0.001$ ; Fig. 3b). Cocoons collected from sites associated with  
338 high pesticide use were heavier ( $\chi^2_1 = 4.24$ ,  $P = 0.039$ ). Individual analyses of each parasite separately showed again that more  
339 individuals tested positive for Ascospaera across sites associated with low compared to high pesticide use ( $\chi^2_1 = 12.34$ ,  $P < 0.001$ ;  
340 Fig. 3b), but in contrast to the Osmia findings, more Megachile individuals tested positive for Microsporidia in across sites  
341 associated with high compared to low pesticide use ( $\chi^2_1 = 3.94$ ,  $P = 0.047$ ). However, there was no difference between high and  
342 low pesticide use sites ( $\chi^2_1 = 0.01$ ,  $P = 0.917$ ) in the prevalence of Wolbachia.

343

344

#### 345 **4. DISCUSSION**

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

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

359

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



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

375

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

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

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

399

400 We found evidence that the prevalence of *Ascospaera* fungal parasites amongst both *O. bicornis* and *Megachile* spp. was lower in  
401 agricultural sites associated with higher pesticide use compared to those associated with lower pesticide use. Hosts and vectors of  
402 *Ascospaera* include honey bees, hoverflies, solitary bees and wasps (Evison et al. 2012; Wynns et al. 2013). Considering that our  
403 surveys showed that the abundance of all these groups were lower across agricultural sites associated with higher pesticide use,  
404 this suggests that such sites support more limited vectoring opportunities for some parasites and pathogens. The prevalence of  
405 Microsporidia and *Wolbachia* also followed this pattern in *O. bicornis*, but interestingly the pattern was not the same for the  
406 *Megachile* spp. with higher prevalence of Microsporidia across sites associated with higher pesticide use, and no difference in  
407 prevalence of *Wolbachia*. This suggests that the biology of the host, rather than these parasites may be more important in  
408 influencing their vectoring patterns. Microsporidia can cause nosemosis, a form of dysentery, in their hosts, and sub-lethal  
409 exposure to neonicotinoids increases the susceptibility of honey bees to the microsporidion *Nosema ceranae* (Pettis et al. 2012;  
410 Wu et al. 2012; Pettis et al. 2013) and causes increased mortality in individuals already infected with *N. ceranae* (Vidau et al. 2011).  
411 Ladas (1970) found a similar interaction between the presence of *N. ceranae* spores in honey bees and the insecticide  
412 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

428 Our results highlight the complex nature of the interactions between diverse stressors on pollinator health, however they do not  
429 resolve targets for action. Agricultural sites associated with higher pesticide use appeared support a reduced abundance of some  
430 pollinator groups, which may result in reduced or altered vectoring opportunities for parasites of those pollinators. Laboratory  
431 studies that show increased virulence of parasites, and higher mortality of hosts after pesticide exposure (Alaux et al. 2010;  
432 Aufauvre et al. 2012; Pettis et al. 2012; Wu et al. 2012) do not determine how pesticide exposure influences the biological  
433 relationship between virulence and transmission. Understanding how pesticide use influences natural parasite transmission routes  
434 requires field (or semi-field) studies that incorporate natural foraging by pollinators. If direct exposure to pesticides increases the  
435 susceptibility to parasites, a consequent higher mortality will lead to reductions in detectible infections as fewer bees survive to  
436 provision their nests. Again, how this influences parasite virulence in wild populations, and the subsequent impact on the number of  
437 foundresses surviving to provision nests is unknown. Our results do not allow us to separate out these effects; because our  
438 methods relied on collecting pollinators healthy enough to fly and provision a nest, our results are therefore skewed towards  
439 collecting either benign infections or more resistant hosts; virulent infections would remove hosts from the sampling pool. The  
440 results of our farmer questionnaires (Table S1.1.2) showed that fungicides and organophosphates were only applied in the high  
441 pesticide sites, and neonicotinoids were more frequently applied, which again introduced an element of bias in our data collection  
442 because the effects of pesticide exposure on parasite virulence and transmission may differ between functional types of chemicals.  
443 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/1000364/1).

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657 **Figures and tables**

658

659 **Figure legends**

660

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

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

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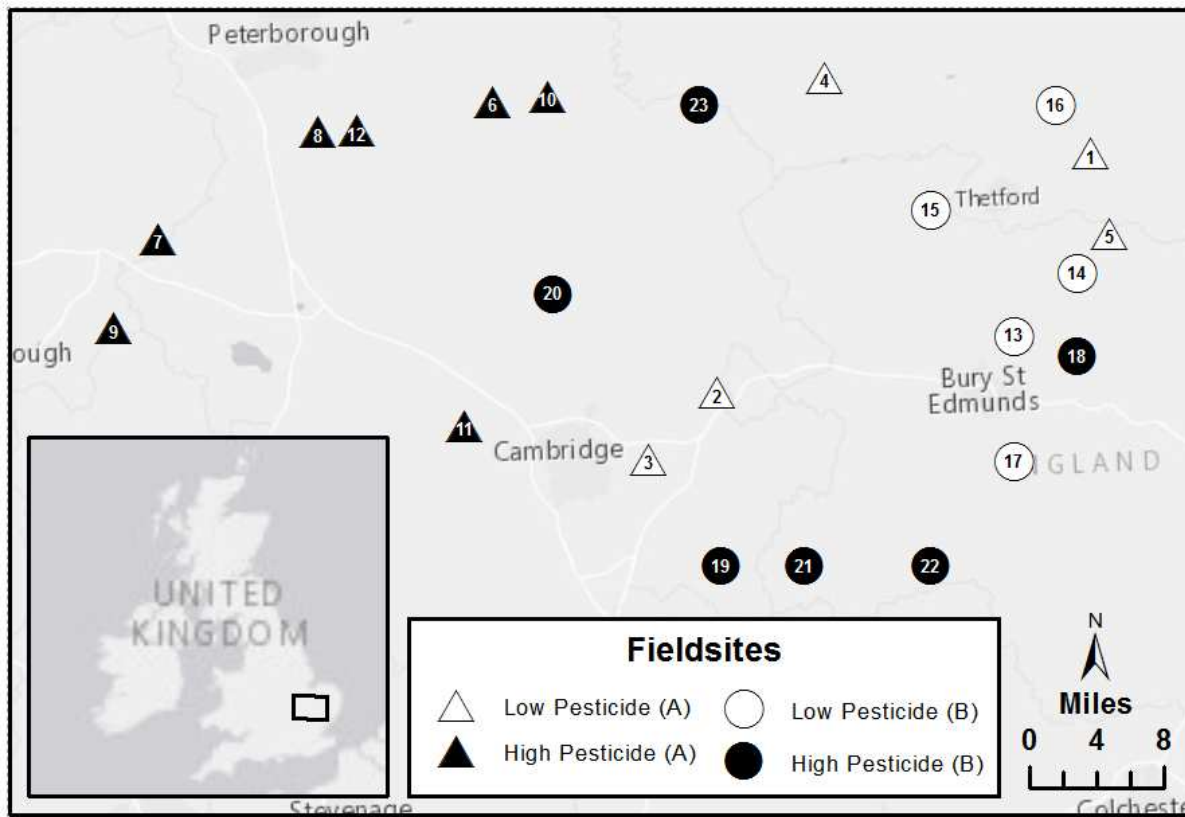
669 **Figure 3.** The proportion of a) *O. bicornis* and b) *Megachile* solitary bees testing positive for each of the three screened parasites,  
670 grouped by pesticide load. Asterisks indicate statistical significance of: \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ .

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674 **Figure 1**



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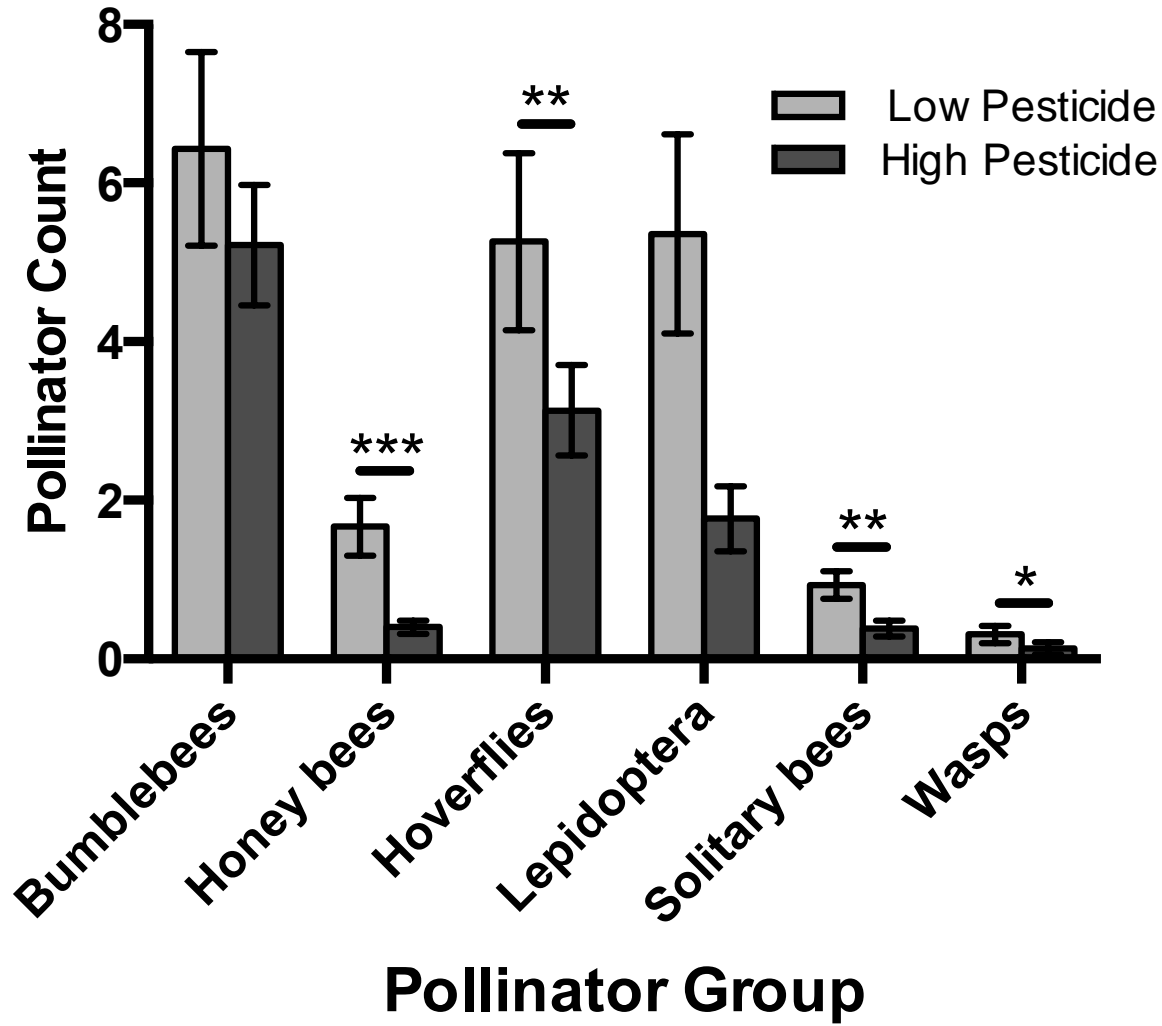
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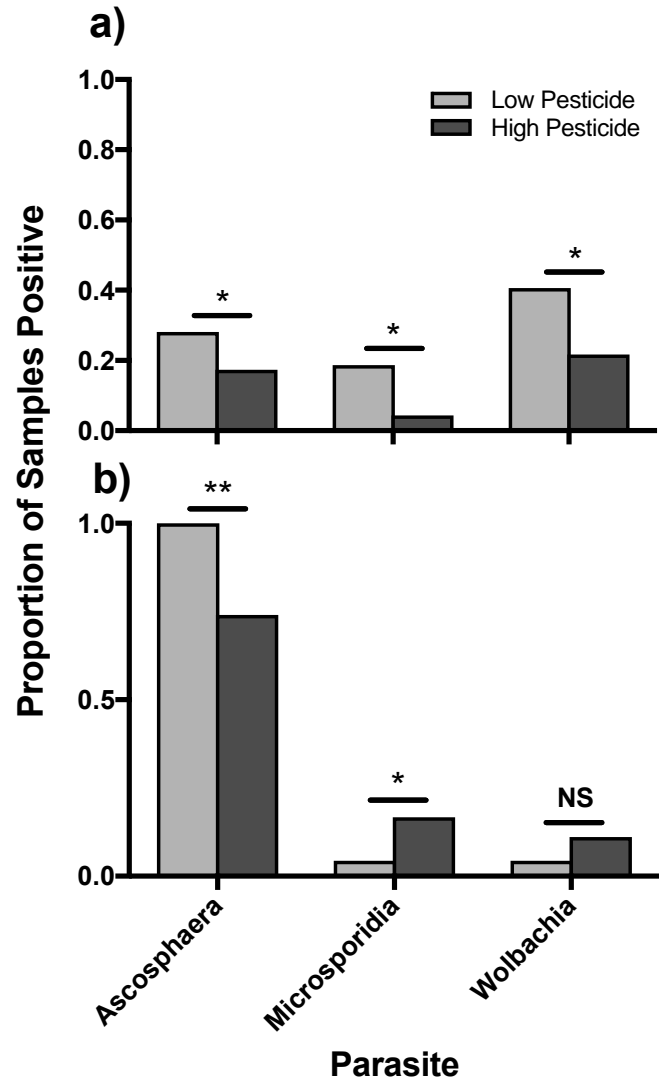
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680 Figure 2



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682 Figure 3



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685 **Table 1**

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

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**Table 1**

<b>Solitary Bee and Solitary Wasp Species</b>								688
	Ancistrocerus nigricornis	Osmia bicornis	Megachile centuncularis	Megachile willughbiella	Osmia caerulescens	Hylaeus sp.	Pomilid sp.	689
<b>Low pest. n (%)</b>	54 (91.53)	11 (78.57)	-	-	-	-	6 (100)	690
<b>High pest. n (%)</b>	5 (8.47)	3 (21.43)	16 (100)	48 (100)	10 (100)	9 (100)	-	691
<b>Total n</b>	59	14	16	48	10	9	6	692
<b>Low pest. expected mass in mg (<math>\pm</math>SE)</b>	44.89 (2.02)	57.15 (5.96)	-	-	-	-	37.51 (2.24)	693
<b>High pest. expected mass in mg (<math>\pm</math>SE)</b>	52.7 (17.34)	57.8 (7.07)	164.01 (11.82)	177.87 (16.37)	70.02 (4.36)	4.47 (0.256)	-	694

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706 **Supplementary material**

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

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

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711 Here we provide supplementary material that covers the following areas:

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

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

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

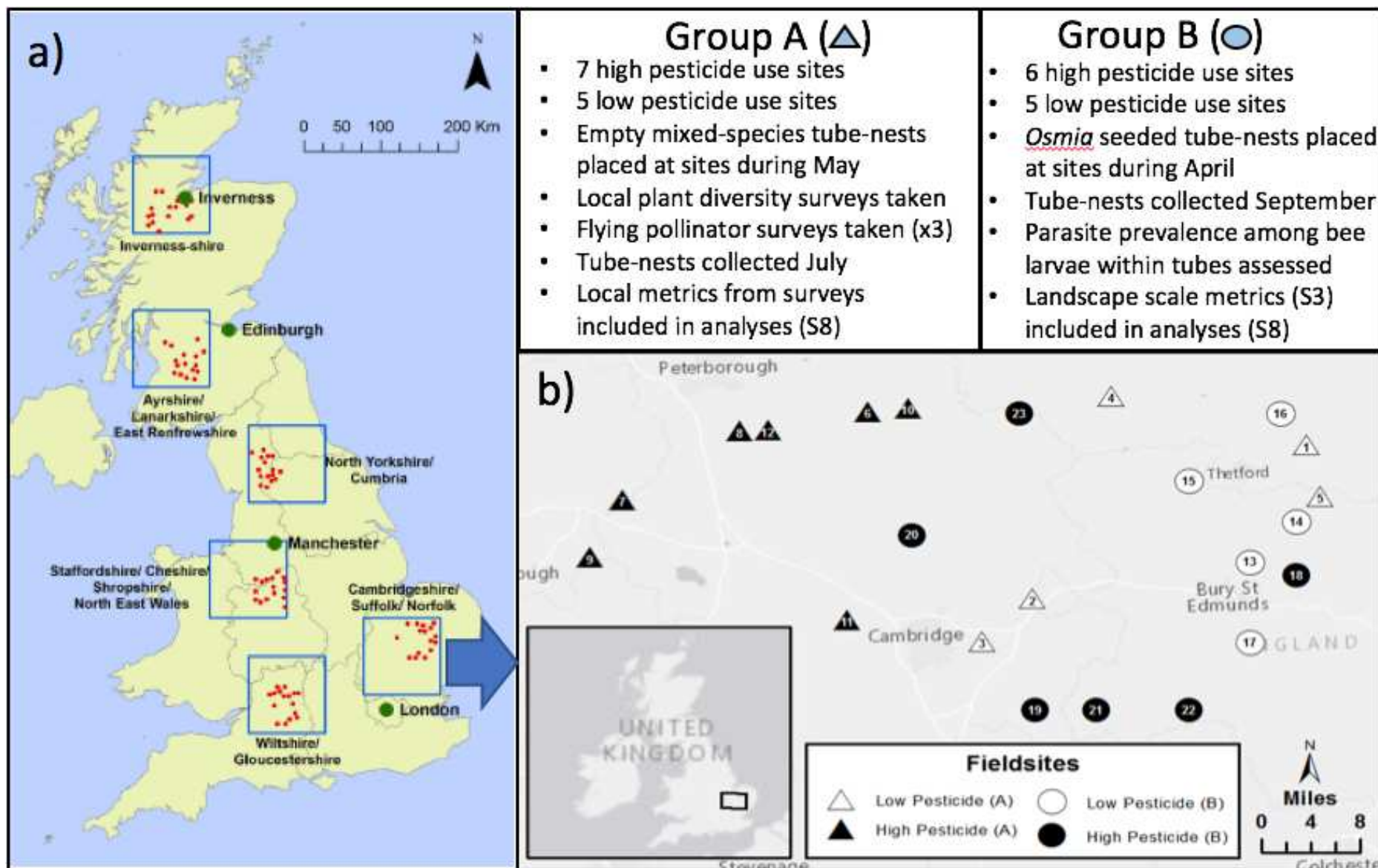
719 **S4** – Details on the sources of data used for site selection protocol used by the IPI Agriland project and the processing steps used  
720 to convert them to landscape scale variables. The landscape scale variable “Pesticide use” was the defining feature used in the  
721 selection of the sites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

722

723 **S1. Site details and associated measurements**

724 The complete set of 23 agricultural sites in Cambridgeshire and East Anglia used in the study were identified for use from a  
725 database of field sites selected by the IPI AgriLand project (Linking agriculture and land use change to pollinator populations,  
726 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  
727 study were from just one of these six regions, which covered the Cambridgeshire and East Anglia area (detailed in fig. S1). The  
728 field sites were used to collect different response variables related to pollinators. Group A sites were used to assess abundance,  
729 richness and diversity of pollinators via flying insect surveys and using tube-nests (section S2) to collect the offspring of tube-  
730 nesting species. Local plant surveys were taken in the area around placement of these tube-nests and weather conditions recorded  
731 during flying insect surveys. These data were then included as co-variates in analyses on the effect of the level of pesticide use in  
732 the local area (high or low) on pollinator abundance, richness and diversity. Group B sites were used to sample the prevalence of  
733 parasites commonly associated with pollinators amongst species of tube-nesting bees who share a similar ecological niche to  
734 honey bees. No local information was recorded about the sites around the tube-nest placement t the Group B sites, instead  
735 landscape scale variables (calculated in section S3) were used as co-variates in analyses on the effect of the level of pesticide use  
736 in the local area (high or low) on parasite prevalence.

737  
738 **Figure S1.** Site locations and details showing a) The AgriLand project selected six 100 km<sup>2</sup> regions that were as representative as possible of the British  
739 landscape across vegetation and environmental gradients (blue squares). 96 field sites (sixteen 2 x 2 km<sup>2</sup> sites per region) within these were chosen using  
740 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  
741 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  
742 markers, and Group B sites (detailed in Table S3) are represented by circular markers. Low pesticide sites are represented by open markers, and high  
743 pesticide sites by filled markers.



745 **S1.1 Group A sites**

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

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

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756 Landowners of each of the Group A sites were sent a questionnaire relating to the use of pesticides on their land, the results from  
 757 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  
 758 study were considered by the landowners to be 'conventional' as opposed to 'organic'. The high pesticide farms tended to be more  
 759 intensified large-scale cereal producers compared to the low pesticide farms that tended to be smaller and grew a more even mix of  
 760 cereals and vegetable crops. Four of the seven high pesticide sites had been applied with neonicotinoids by seed dressing  
 761 compared to only one of the low pesticide sites. Additionally, fungicides and organophosphates were only mentioned in responses  
 762 from high pesticide sites. The majority of insecticides, except neonicotinoids, in both high and low sites were sprayed rather than  
 763 coated directly on the seeds. All farmers in the study were aware of the importance of honey bees and bumblebees as pollinators,  
 764 but the majority of farmers were not aware of the role that solitary bees played in pollination.

765 **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.  
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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 <sup>†</sup> -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 <sup>†</sup> -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

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782 **S1.2 Group B sites**

783 The remaining 11 of our 23 sites (Group B sites) were also selected based on extremes of the pesticide use estimation values  
 784 (calculated in section S3.1), and designated as high pesticide or low pesticide according to whether their associated value fell  
 785 above or below the mean use value. These sites were not subject to any local pollinator or plant surveys or farmer questionnaires.  
 786 However, as each were explicitly linked to the four variables used in their original selection we used these in the statistical analyses  
 787 outlined in table S8 to assess how they may have influenced parasite abundance in our samples.  
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792 **Table S1.2** Summary of variables associated with Group B sites and corresponding points on map (figure S1).  
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 794

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

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817 **S2. Tube nest design**

818 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  
819 cardboard tubes of five different aperture sizes (4, 5, 6, 8 and 10 mm diameter) to accommodate multiple nesting species. In  
820 contrast, the tube-nest arrays placed at the remaining 11 Group B sites consisted of a single cardboard tube size (8 mm) and each  
821 were seeded with 10 *Osmia bicornis* pupae. The tube-nests were painted with circles of white UV-reflective paint, which is attractive  
822 to pollinators (Westphal et al. 2008), and were attached securely with plastic cable-ties to stable and visible linear field boundaries,  
823 such as hedgerows and fence posts. Tube-nests were fixed horizontally, with their plastic peak covering the nest entrance to  
824 reduce rain exposure, and facing between South and East to maximise morning sun exposure during peak pollinator foraging hours  
825 (Everaars et al. 2011).

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827 **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  
828 hedgerows and fence posts.  
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835 **S3. Statistical analyses performed, and results from each test**

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837 **Table S3:** Model parameters and test values for all statistical tests. NS, \*, \*\*, \*\*\* indicates no significance or significance of the term  
 838 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;  
 839 **Response variable** – the dependent variable in the model; **Function** – the name of the model function used in R to fit the model;  
 840 **Error family** – the type of probability distribution assumed by the model; **Random term** – nested terms and repeated measures  
 841 accounted for in the model; **Fixed terms** – the independent variables that are included in the model, and dropped from the full  
 842 model to assess their significance to the fit of the data; **Test statistics** – the output of the statistical test and the significance of the  
 843 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	$\chi^2_5 = 48.17, P < 0.001$ *** $\chi^2_1 = 19.80, P < 0.001$ *** $\chi^2_5 = 957.4, P < 0.001$ *** $\chi^2_1 = 3.193, P = 0.074$ NS $\chi^2_1 = 58.88, P < 0.001$ *** $\chi^2_1 = 2.959, P = 0.085$ NS $\chi^2_1 = 3.340, P = 0.068$ 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	$\chi^2_1 = 6.096, P = 0.014$ ** $\chi^2_1 = 9.604, P = 0.002$ ** $\chi^2_1 = 3.136, P = 0.077$ NS $\chi^2_1 = 3.058, P = 0.080$ NS $\chi^2_1 = 0.059, P = 0.808$ 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	$\chi^2_1 = 4.362, P = 0.037$ * $\chi^2_1 = 6.383, P = 0.012$ * $\chi^2_1 = 2.200, P = 0.138$ NS $\chi^2_1 = 0.543, P = 0.461$ NS $\chi^2_1 = 0.067, P = 0.796$ 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	$\chi^2_1 = 0.463, P = 0.496$ NS $\chi^2_1 = 0.359, P = 0.549$ NS $\chi^2_1 = 61.79, P < 0.001$ *** $\chi^2_1 = 0.395, P = 0.530$ NS $\chi^2_1 = 0.985, P = 0.321$ 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	$\chi^2_1 = 21.48, P < 0.001$ *** $\chi^2_1 = 7.679, P = 0.006$ ** $\chi^2_1 = 0.384, P = 0.536$ NS $\chi^2_1 = 0.001, P = 0.976$ NS $\chi^2_1 = 1.224, P = 0.269$ 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	$\chi^2_1 = 9.528, P < 0.002$ ** $\chi^2_1 = 0.198, P = 0.656$ NS

determined by surveys					Plant Diversity Wind speed Weather	$\chi^2_1 = 0.799$ , P = 0.371 NS $\chi^2_1 = 1.812$ , P = 0.178 NS $\chi^2_1 = 1.072$ , 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	$\chi^2_1 = 6.684$ , P = 0.009 ** $\chi^2_1 = 2.505$ , P = 0.114 NS $\chi^2_1 = 2.758$ , P = 0.097 NS $\chi^2_1 = 1.102$ , P = 0.294 NS $\chi^2_1 = 5.881$ , 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	$\chi^2_1 = 1.816$ , P = 0.178 NS $\chi^2_1 = 15.74$ , P < 0.001 *** $\chi^2_1 = 20.57$ , P < 0.001 *** $\chi^2_1 = 5.484$ , P = 0.019 * $\chi^2_1 = 8.096$ , 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	$\chi^2_1 = 9.000$ , P = 0.003 ** $\chi^2_1 = 0.122$ , P = 0.727 NS $\chi^2_1 = 0.261$ , P = 0.609 NS $\chi^2_1 = 1.853$ , P = 0.173 NS $\chi^2_1 = 1.953$ , 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	$\chi^2_1 = 15.05$ , P = 0.005 ** $\chi^2_1 = 0.657$ , P = 0.418 NS $\chi^2_4 = 8.817$ , P = 0.066 NS $\chi^2_1 = 0.534$ , 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	$\chi^2_2 = 0.995$ , P = 0.319 NS $\chi^2_1 = 0.442$ , P = 0.506 NS $\chi^2_2 = 0.473$ , P = 0.492 NS $\chi^2_1 = 2.942$ , P = 0.086 NS
Proportion of developing <i>Osmia bicornis</i> 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	$\chi^2_2 = 0.696$ , P = 0.706 NS $\chi^2_1 = 8.574$ , P = 0.003 ** $\chi^2_2 = 7.576$ , P = 0.023 * $\chi^2_1 = 6.572$ , P = 0.010 ** $\chi^2_1 = 0.029$ , P = 0.864 NS $\chi^2_1 = 7.639$ , P = 0.006 **
Proportion of developing <i>Osmia bicornis</i> testing positive for <i>Ascospaera</i> at Group B sites	Total counts of number of developing brood where <i>Ascospaera</i> DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 4.349$ , P = 0.037 * $\chi^2_1 = 0.813$ , P = 0.367 NS $\chi^2_1 = 3.560$ , P = 0.060 NS $\chi^2_1 = 7.211$ , P = 0.007 **
Proportion of developing <i>Osmia bicornis</i> testing positive for <i>Microsporidia</i> at Group B sites	Total counts of number of developing brood where <i>Microsporidia</i> DNA was detected via PCR	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density Floral Resources	$\chi^2_1 = 5.852$ , P = 0.016 * $\chi^2_1 = 0.151$ , P = 0.698 NS $\chi^2_1 = 6.174$ , P = 0.013 * $\chi^2_1 = 2.360$ , P = 0.125 NS
Proportion of developing <i>Osmia bicornis</i> testing positive for <i>Wolbachia</i> at	Total counts of number of developing brood where <i>Wolbachia</i> DNA was	glmer	Binomial	Site ID/Tube nest ID/Tube ID	Pesticide Level Habitat Diversity Honey bee Density	$\chi^2_1 = 4.344$ , P = 0.037 * $\chi^2_1 = 5.427$ , P = 0.020 * $\chi^2_1 = 0.000$ , P = 0.990 NS

Group B sites	detected via PCR				Floral Resources	$\chi^2_1 = 1.918, P = 0.166$ NS
Weight of developing <i>Osmia bicornis</i> 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 <i>Megachile</i> 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 <i>Megachile</i> testing positive for <i>Ascospaera</i> at Group B sites	Total counts of number of developing brood where <i>Ascospaera</i> 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 <i>Megachile</i> 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 <i>Megachile</i> testing positive for <i>Wolbachia</i> at Group B sites	Total counts of number of developing brood where <i>Wolbachia</i> 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 <i>Megachile</i> 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

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

859 The information included in this section is modified from Gillespie et al. 2017. The AgriLand dataset provided specific measures of  
 860 pesticide use estimation (S3.1), habitat biodiversity (S3.2), floral resource availability (S3.3) and honey bee colony density (S3.4)  
 861 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;  
 862 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  
 863 for each of the four variables was calculated from comprehensive national datasets as follows:  
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865 **S4.1 Pesticide use** (values given as “Insecticide Loadings”) was estimated based on information from the UK Pesticide Usage  
 866 Survey (PUS; Table S4.1) and cropping data derived from the 2010 Defra June Agricultural Survey for England and the 2010 IACS  
 867 (Integrated Administration and Control System) data held by the Welsh and Scottish devolved administrations. The crop types listed  
 868 in each dataset were assigned to 36 crop groups and the area under each crop group summarised to the site level.  
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870 **Table S4.1** Crop type and year of survey for Pesticide Usage Survey data  
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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%

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 873 The Pesticide Usage Survey data contained individual records of the mass of active ingredient and area of crop to which it has  
 874 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  
 875 crop groups previously created from the cropping data, and the proportional representation of that crop type within the crop group  
 876 was calculated. Toxicity data for *A. mellifera* came from two sources; the Pesticide Properties DataBase (PPDB; University of

877 Hertfordshire (2013)) and Agritox (www.dive.afssa.fr/agritox/index.php; viewed 15/10/12). The PPDB records are primarily sourced  
 878 from EFSA (European Food Safety Authority) reports. Agritox sources most its data from applications for chemical authorisation  
 879 which have been validated by European experts. Where possible, both oral and contact LD<sub>50</sub> were obtained. The active ingredient  
 880 in the PUS data was linked to the lowest LD<sub>50</sub> recorded for the compound and this data was used to calculate hazard quotients  
 881 (eqn. 1) for each PUS record. The hazard quotients were then multiplied by the treated crop area and summed to produce a total  
 882 hazard score for each PUS crop type and region combination. This was converted to a value representing the hazard per hectare  
 883 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  
 884 proportional representation that the PUS crop type makes to the crop group, and summing the weighted scores within crop group.  
 885 The insecticide loading for each of the study sites was then calculated by multiplying the area of each crop group within the site by  
 886 the hazard score of that crop group in the region in which the site falls.  
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$$\text{Hazard Quotient} = \frac{\text{Application Rate}}{LD_{50}} \quad [\text{eqn.1}]$$

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**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:

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

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where  $p_i$  is the proportion of the area of the site in m<sup>2</sup> belonging to the  $i$ th 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	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.
	Bog (Grass dominated)	
	Bog (Heather dominated)	
Inland Rock	Inland rock	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.
	Despoiled land	

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**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:

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

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where  $a_i$  is the area in  $m^2$  of the  $i$ th sub-broad habitat category,  $c_{j,i}$  is the regional average cover of the  $j$ th flowering plant species occurring in habitat  $i$  taken from the CS2007 and  $s_j$  is the sugar potential in kg/ha/year of the  $j$ th 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:

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

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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)),  $A_{aes-j}$  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 Bioflor (Klotz et al. 2002; [www.bioflor.de](http://www.bioflor.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 ( $F_{11, 91}=10.24$ ,  $p < 0.0001$ ,  $R^2 = 0.55$ ) 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 Müller (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	Po
		F, D	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 Gynodioecious = 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	Gynomoecious, Andromonoecious, Trimonoecious	Same
		Gynodioecious, Androdioecious, Trioecious	Different
		Hermaphroditic* Monoecious*	Same*
		Dioecious*	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)

954 \* 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.  
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956 **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  
 957 variable.  
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	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		

959 \*\*“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  
 960 nectaries, and plants pollinated by specific species groups).

961 † “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).

962 ‡ 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  
 963 separated on “different individuals”).  
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972 **S4.4 Managed honey bee density.** The English, Welsh and Scottish Governments sponsor honey bee apiary inspection  
973 programmes and collate inspection data in a database known as 'BeeBase'. Colony assessment data were queried for the years  
974 2001-2010 and the number of bees present in mid-summer for an average colony estimated. The number of adult bees was  
975 estimated using the brood and assuming an 87.5% survival across all life stages (Winston 1991). The number of colonies present  
976 in each apiary was calculated for three apiary classes: 1) apiary owned by a single amateur beekeeper (39 colonies or less); 2)  
977 shared apiary of one or more amateur beekeeper; and 3) apiary owned by a professional beekeeper (40 or more colonies owned).  
978 Observations of foraging behaviour were gathered for ten site/season combinations from the published literature (Waddington et al.  
979 1994; Beekman & Ratnieks 2000). Foraging observations were grouped into 200m bins representing different foraging ranges for  
980 each site/year combination and a distribution model fitted to the sum of all foraging observations. A Gamma distribution was found  
981 to account for the short distance flights and a lognormal distribution for the longer flights. The significance of the lognormal part of  
982 the model (compared to the Gamma distribution) was tested using an F-test for nested models (Genstat V15). The final model was  
983 used to estimate the proportion of the foraging force likely to be active in radiating 200 m bands up to the maximum foraging  
984 distance reported for honey bees (13 km; Eckert 1933). The honey bee density map was completed by rendering foraging models  
985 and apiary sizes for all registered apiaries across England, Wales and Scotland (ArcMap 10.0; Esri 2011). Honey bee forager  
986 density around each apiary was calculated for a set of 200m concentric circular bands out to a distance of 13km. The bands were  
987 intersected with each other and the forager densities for intersecting bands were summed to give the expected density of honey  
988 bee foragers. These polygons were then intersected with the selected 2km site squares and the total expected number of honey  
989 bee foragers calculated by multiplying the densities by the area of the intersected polygons within the selected 2km squares.

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