Lower bumblebee colony reproductive success in agricultural compared to urban environments

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Manuscripts

1   **Lower bumblebee colony reproductive success in agricultural**

2   **compared to urban environments**

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Abstract

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

Keywords

Background

We are living in the "Urban Age" (1): over half the world's human population currently resides in cities (2) and an estimated three-fold increase in global urban land cover is predicted between 2000 and 2030 (3). Although urbanization has been shown to impact negatively upon species abundance and diversity for many taxa (4), some groups successfully exploit anthropogenic habitats (5) and there is evidence to place wild bees among this number. For example, areas subject to urban expansion have lost fewer pollinator species than agricultural areas over the past 80 years (6) and species richness has been found to be higher in urban than agricultural areas (7). These community level studies give reason to view urban environments as a potential refuge within barren agricultural landscapes, which have been associated with reduced floral resources (8) and exposure to environmental contaminants (9). Yet, the crucial question of whether land-use directly affects fitness – the ultimate driver of ecological success and evolutionary change – remains a largely neglected missing link in the correlations between urbanisation and species abundance in both bees and other taxa (10-12).

Bumblebees comprise an important part of the pollinator community, but are currently subject to a multitude of threats that include changes in forage availability associated with land-use change (8) and pressure from emerging parasites and disease (13). Alteration of floral resources is likely to be an

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45   important driver of urban effects on bees (14), with cities and towns often offering high floral

46   abundance and diversity in the form of gardens and parks (15). However, many horticultural plant

47   varieties are unattractive to bees or invest energy in visual displays at the expense of reward provision

48   (16), and competition may also affect forage availability: increased popularity of urban beekeeping

49   has increased honeybee hive densities in urban areas (17), possibly increasing competitive interactions

50   with wild bees (18). Parasite prevalence has also been linked to urbanisation, with higher parasite

51   loads in urban areas reported in bumblebees (19, 20). Pesticide use has been identified as a threat to

52   bees (9) and exposure may vary across degrees of urbanisation (21). In the context of this array of

53   potentially interacting drivers of urban effects, it is not clear how inhabiting urban areas affects

54   bumblebee success at the colony level. This is because ethical concerns preclude the release of

55   reproductive offspring from commercially-obtained bumblebee colonies (24-26), meaning that

56   previous experiments have studied commercial colonies placed into the field only up to the very

57   beginning of the period when reproductive offspring begin to emerge. Thus, while there is evidence

58   that bumblebee colony early weight gain may be enhanced in suburban compared to agricultural areas

59   based on studies of pre-reproductive colonies (22) (but cf. ref. 26), to date no study has monitored the

60   critical, extensive reproductive period of the colony life-cycle and thus assessed the effects of

61   urbanisation on lifetime reproductive success itself.

62   Here, we addressed this gap by rearing colonies from wild-caught queens to investigate the effect of

63   urbanisation on life-history and reproductive output in the bumblebee *Bombus terrestris audax*. Using

64   locally sourced queens allows ecologically relevant quantification of the impact of land-use on

65   locally-adapted populations, rather than commercial bees that have been subject to artificial selection

66   (27) and may differ from locally-adapted natural populations (26). It also overcomes concerns

67   associated with the use of commercial bees, including negative environmental impacts such as

68   hybridisation (24), pathogen spillover (25) and competition (26). A crucial outcome is that colonies

69   can be monitored for their entire reproductive lifetime. We selected 38 sites across central London, its

70   suburbs and the surrounding agricultural land (Fig. 1a), and categorized each site based on land-use

71   through cluster analysis of principle components derived from 80 land-use variables. Through

72   frequent censusing and sampling of colonies placed at these sites, we tracked for the first time the

73   growth, reproductive output, nutritional status, and parasite prevalence of each colony from eclosion

74   of the first cohort of workers until the end of the colony life cycle. To our knowledge, this represents

75   the first experimental study in any taxon to demonstrate a direct relationship between urbanisation and

76   reproductive success, with previous research typically employing an observational approach (e.g. 11,

77   28).

78   **Methods**

79   **Bumblebee colonies**

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80   We collected 176 foraging *Bombus terrestris audax* queens in Windsor Great Park, Surrey, UK during

81   March and April 2016. Queens were chilled and transported to the lab where they were immediately

82   screened microscopically for the endoparasites *Nosema* spp., *Apicystis bombi, Sphaerularia bombi*

83   and *Crithidia bombi,* by collecting faeces in a microcapillary (Baubrand Intramark, Wertheim,

84   Germany) and examining the sample under x400 magnification. Parasitised queens (n=6) were

85   excluded from the experiment. Queens were kept in clear acrylic rearing boxes until colony founding

86   (see Supplementary Methods for rearing protocol), after which they were re-screened and transferred

87   to a wooden nest box (W 280 x L 320 x D 160mm) with a clear Perspex lid. Our final sample for

88   placement in the field consisted of 43 colonies.

89   **Field placement**

90   We recruited 114 gardens and farms in South-East England (between central London and

91   Basingstoke), of which 38 sites were selected across a region spanning inner city to countryside on the

92   basis of distribution (> 1.5km apart), land-use type representativeness and accessibility (Fig.1a). This

93   includes a range of urban and rural land-use types typical of Western Europe (29), from Central

94   Business District, to suburban, to villages and medium-intensity agriculture containing a mixture of

95   grassland and arable fields. Predominant crop types in the agricultural areas were cereals and brassica

96   crops. The wide range of urban land types contained within London means that it is representative of a

97   range of different urban types displayed by smaller cities (30). We placed colonies in the field in

98   protective plastic field boxes during the first week of May 2016, randomised to land-use type

99   according to initial colony size (see Supplementary Methods). Colony placement was staggered over

100   six days, with six or seven sites visited each day during daylight hours (8:00-20:00). Colony

101   monitoring continued until moribundity (see below), which occurred for the last colony on 11th July.

102   **Data collection**

103   Site visits followed approximately the same order as the colony placement, with each site visited

104   weekly during the hours of darkness (21:30-4:30) at the same time each week. We recorded the

105   following data (see Supplementary Methods for additional data): number of bees (average of three

106   counts); queen status (alive, dead or absent); presence of nectar and pollen stores and presence and

107   status of *Bombus vestalis* brood parasites (alive, injured or dead), which we removed to minimise *B.*

108   *terrestris* queen death. To assess reproductive success, gynes were removed until one minute had

109   passed with no gyne seen, and stored for later analysis. The same procedure was repeated for males,

110   with sampling time capped at 15 minutes. Males are considered to leave the nest at two to four days

111   old and gynes at two to eight days old (31) so our weekly removal of males and gynes reflects natural

112   conditions and is unlikely to have impacted the colony's production of future males and gynes.

113   Weekly removal of reproductives allows calculation of total reproductive output over the colony life

114   cycle rather than a snapshot as obtained from traditional colony dissection methods that are carried

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115   out at the earliest sign of reproductive emergence (22). We removed one, three or five workers for

116   later parasite analysis depending on colony size (<35, 35-50, >50 workers respectively), which were

117   stored alive in vials for a maximum of 5 hours before freezing at -20°C.

118   For the first two weeks, colonies in which the queen died (n=5 of 43; 1 city, 1 village, 3 agricultural)

119   were replaced with new colonies. Following this, colonies were removed from the field when

120   moribund, defined as <10 workers remaining and queen death or <3 workers remaining with no queen

121   death. Remaining workers were frozen at – 20°C and dissected (see below). We obtained daily data

122   for average temperature, average humidity and total rainfall for each site by downloading data from

123   the weather station nearest each site that had data for the full study period (www.wunderground.com).

Sample analysis

125   Up to three workers per colony per week were dissected. For each bee, the abdomen was placed in

126   Ringer's solution and examined for the presence of conopid fly and braconid wasp larvae and the

127   larger tracheae for the tracheal mite *Locustacarus buchneri*. Sections of the Malpighian tubules,

128   hindgut and fat body were removed, crushed and examined under x400 magnification for the presence

129   of the endoparasites *Crithidia bombi, Nosema* spp. and *Apicystis bombi*. Each slide was examined by

130   two researchers. In addition, the ovary development of all collected workers (n=393) and the body fat

131   content of all workers, gynes (n=46), and a random sample of max. 20 males per colony to limit

132   workload (total n=418) were assessed (see Supplementary Methods).

Land-use classification

134   Following best practice in the field (32, 33) we classified land-use at multiple radii surrounding each

135   site using GIS analysis, based on satellite imagery with additional ground-truthing for agricultural

136   sites.Agricultural sites were surveyed because mass crop blooms may not be detected by satellite

137   images taken outside the bloom period The land-use classification protocol is described in full in (37)

138   and is available as Supplementary Methods. Briefly, buffers at radii of 750m (*B. terrestris* typical

139   foraging range (34-36)), 500m, 250m and 100m (representing steps of spatial scales at which bees

140   may interact with the surrounding land (12, 37)) were generated around each site. Preliminary

141   analysis showed that the majority of the response variables responded most strongly to land-use at a

142   500m radius so this was selected as our primary land-use variable. Land-use patches were defined by

143   drawing polygons in QGIS v2.16 and categorised visually to one of 80 land-use classes (Table S14)

144   from satellite imagery and ground surveys carried out in May 2016.

145   We refined the classification to produce a single categorical land-use variable via an established three-

146   step process (32): 1) definition of land-use categories, 2) Principle Components Analysis (PCA) on

147   the categories and 3) cluster analysis based on the PCA output (Fig. S2). Briefly, each land-use class

148   was coded to one of eight categories (e.g. impervious surface, flower-rich habitat; Table S14) and the

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149   total area of each category within each site calculated. A PCA was then performed to reduce the

150   dimensionality of the land-use variables, and cluster analysis (Ward's method) was performed on the

151   first two principle components, which in combination captured approximately 85% of the variation

152   (see below for loadings). Following (32, 33, 38), each cluster contained a minimum of five sites.

153   Three clear clusters emerged (Fig. S3a), comprising a group characterised by dense urban

154   development (henceforth named "city"; n=17), a group characterised by patches of housing

155   surrounded by rural land ("village"; n=16) and a group dominated by agricultural fields

156   ("agricultural"; n=5; Fig. 1b). Exploration of model fit confirmed that use of the clustered land-use

157   categories to predict our main response variables explained more of the variance in our data than use

158   of the PCs alone (Fig S3b), and comparison of models containing combinations of the PCs with those

159   containing the clustered variable showed that for all response variables the clustered variable

160   improved model fit (see Supplementary Methods and Table S11 for AIC values), justifying the

161   necessity of the clustering step. Sites in the city cluster contained mean 56.2% (± SE: 4.0%)

162   impervious surface and 0.1 (±0.1)% agricultural land cover while village and agricultural sites

163   contained 13.8 (±3.7)% and 8.6 (±4.5)% impervious surface and 34.6 (±7.1)% and 71.2 (±11.5)%

164   agricultural land cover respectively.

165   **Statistical analysis**

166   For each analysis, we built a comparison set of models including the full model (for predictors, see

167   below) and all subsets, including the basic model containing only the constant and residual variance

168   ("all-subset approach"). We selected the model or set of models with the lowest AICc as the best

169   fitting model(s) (39). Where several models were within two AICc units of the best model, model

170   averaging was carried out to obtain parameter estimates derived from the best set of models including

171   the basic model if applicable (40). Final models were examined for spatial autocorrelation by using a

172   Moran's I test on the residuals and graphically assessing the spatial pattern of residuals.

173   To analyse peak colony size linear regression was carried out on log-transformed data. Total

174   production of sexuals (gynes and males) was analysed using zero-altered negative binomial hurdle

175   models, where the response is modelled as a binary process (production of sexuals) and a zero-

176   truncated count process (total sexuals in colonies that produced sexuals) (41). Binomial GAMs

177   (allowing for a non-linear effect of week) with site as a random effect were used to analyse presence

178   of nectar and pollen and ovary development. Queen survival, colony survival and onset of

179   reproduction were subjected to survival analyses using non-parametric Cox proportional hazards

180   models. Proportion of worker samples in each colony containing *Apicystis* and *Crithidia* were

181   analysed using binomial GLMs. Male and worker fat content were analysed using Gaussian GAMs

182   allowing for a non-linear effect of week with site as a random effect. *Bombus vestalis* invasion as a

183   binary response was modelled using binomial GLMs. One factor level (city) for this variable had

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184   perfect separation (only zeroes); to deal with this three dummy observations were added for each

185   land-use category with *B. vestalis* invasion set to one and weather variables set to whole-dataset

186   means.

187   To investigate whether our results may have been driven by floral resource availability, we re-

188   analysed the response variables that were found to be significantly affected by land-use (reproductive

189   output, peak colony size, colony survival, queen survival, presence of nectar stores and presence of

190   pollen stores) using proportion of flower-rich habitat as a predictor. We coded each land-use class as

191   described above as flower-rich or flower-poor, based on reference to the literature (e.g. domestic

192   gardens have been shown to support high floral diversity (15) and provide considerable resources to

193   bees (42)) and on ground surveys in agricultural land to identify crop types and wildflower strips, and

194   summed the area of flower-rich land-use patches to generate the proportion of flower-rich habitat at a

195   500m radius for each site. Each response variable was analysed using this predictor as described in the

196   paragraph above. All analyses were conducted in R version 3.2.1 (43); for packages see

197   Supplementary Methods.

Results

199   Land-use category strongly predicted the number of live sexual offspring (gynes and males) produced

200   over the colony life cycle (Fig. 2a, Table S1a). Village colonies were significantly more likely to

201   produce sexual offspring than agricultural colonies (model averaged estimate (MAE): 2.853, 95%

202   CIs: [0.327 – 5.378], Table S2a), and both city (MAE: 2.789 [0.799 – 4.778]) and village (MAE:

203   2.566 [0.579 – 4.552]) colonies produced significantly higher numbers of sexuals than their

204   agricultural counterparts. Our data suggest that this effect may reflect both the build-up of a larger

205   workforce and, relatedly, longer queen lifespans in village and city colonies. Both village and city

206   colonies displayed significantly higher peak size (number of bees) than agricultural colonies (Table

207   S1b, Fig. 2b; City MAE: 0.918 [0.194 – 1.641], village MAE: 1.047 [0.319 – 1.774], Table S2b), and

208   founding queens survived for longer (Table S1c, S2c; Fig. 3a; MAE of Hazard Ratios (HR) relative to

209   agricultural colonies: City: 0.149 [0.041 – 0.542]); Village: 0.137 [0.039 – 0.488]. City and village

210   colonies also took significantly longer to become moribund than agricultural colonies (City HR: 0.111

211   [0.031 – 0.396], village HR: 0.073 [0.019 – 0.271]; Table S1d; Fig. 3b). There was no significant

212   effect of land-use on ovary development (see Supplementary Results).

213   Agricultural colonies were found to contain less stored food than their city or village equivalents.

214   Colonies in city (nectar MAE: 2.015 [0.520 – 3.509], Table S1f, S2f; pollen MAE: 2.109 [1.045 –

215   3.173], Table S1g, S2g) and village (nectar MAE: 1.902 [0.410 – 3.394]; pollen MAE: 2.038 [0.973 –

216   3.102]) land-use clusters were significantly more likely to contain nectar (Fig. 4a) and pollen (Fig. 4b)

217   stores than agricultural colonies, in which we found almost no nectar stores and limited pollen after

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218   four weeks of development. We found no effect of land-use on the fat content of workers or males

219   (Tables S5b & c).

220   Land-use had no effect on the prevalence of *Apicystis bombi* in colonies, although further analysis

221   suggested that land-use in the immediate area surrounding the colony may have an effect (see

222   supplementary results, Table S5g). Similarly, there was no effect of land-use on *Crithidia bombi*

223   presence (Table S5f). Only three bees were parasitised by *Syntretus* sp. (one city site and one village

224   site), and no *Nosema bombi* or *Locustacaris buchneri* was found in any of our samples. The brood

225   parasite *Bombus vestalis* was present in our study area, and hence we carefully monitored colonies to

226   detect attempted parasite invasions. We recorded 14 invasion attempts by *B. vestalis* queens (max. 4

227   in a single colony). Land-use category was a significant predictor of the probability of an invasion

228   attempt (Table S2h), with city (MAE: -3.776 [-6.304 – -1.249]) and village (MAE: -2.943 [-5.444 – -

229   0.442]) colonies being less likely to be invaded than agricultural colonies (Table S2h, Fig. 3c).

230   Accordingly, we investigated the possibility that increased brood parasite invasions explain the poorer

231   development of colonies in agricultural sites by performing a separate analysis in which three models

232   were compared for each response variable: 1) the best model from the original analysis, 2) the same

233   model but with parasite invasion events replacing land-use as a predictor and 3) a model with both

234   parasite invasion and land-use. For all variables, the model containing land-use only or land-use and

235   invasions fit the data better than the model containing invasions alone (i.e. had a lower AICc value,

236   Table S9). In other words, although parasite invasions explain some of the variance in our data, land-

237   use influences colony performance irrespective of invasion status.

238   Our land classification protocol (44) allows investigation into the aspects of the land-use that may

239   underlie the effects found, by examining the variables that contribute to the clustering of land-use

240   types. High domestic infrastructure, impervious surface and road cover, and low agricultural land

241   cover, contributed strongly to Principle Component (PC) 1 (eigenvalue score >0.4 or >-0.4 (45)),

242   while PC2 was defined by high tree cover and low open and flower-rich habitat cover (Table S10).

243   The city cluster was characterised by positive scores on PC1 (mean 2.00 ± SE 0.07) and near-zero

244   scores on PC2 (-0.27±0.17), suggesting a highly urbanised semi-open land type; the village cluster

245   had medium negative scores on PC1 (-1.40±0.37) and positive PC2 scores (0.94±0.37), suggesting

246   low intensity urbanisation with moderate tree cover; the agricultural cluster had low PC1 scores (-

247   2.33±0.64) and low PC2 scores (-2.08±0.32), suggesting open land with very little urbanisation and

248   high agricultural cover (Fig. S3a). Analysis of the PCs suggested it was the combination of both

249   attributes of the land-use that drove the effects seen (see Supplementary Methods and Table S12 &

250   S13 for results of these analyses). Investigation of the effect of the proportion of flower-rich habitat

251   on the response variables as a possible key driver of the results showed no significant effect

252   (Supplementary methods; Tables S3 & S4).

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Discussion

Our results demonstrate a direct association between urbanisation and higher reproductive output in a key insect pollinator, *B. terrestris*. We found increases in reproductive output, colony growth and food stores as well as lower brood parasitism by *B. vestalis* in colonies placed in urbanised areas compared to sites dominated by agriculture. Previous research has described correlative evidence for higher abundance of bees (e.g. 46) and higher bumblebee nest densities (42, 47) in urban areas, but whether this may be driven by migration between land-use types or effects of land-use on population dynamics has remained unclear (48). Our experimental design, whereby colonies reared from wild-caught queens were placed in different land-use types over the full colony life cycle in order to measure reproductive output, provides evidence for a causal link between reproductive success and urbanisation, elucidating a potential mechanism behind these observed differences in pollinator populations between urban and rural areas. Our use of colonies established from locally-sourced queens gives our findings direct ecological relevance to the impacts of land-use change on wild bumblebee populations.

We employed a high-resolution approach to measuring reproduction, collecting almost all males and gynes present in the nest at weekly night-time inspections, over the entire colony life cycle from first worker emergence to moribundity. This builds on traditional methods of dissecting nests at the very onset of reproduction (22, 49, 50), capturing a higher proportion of the total reproductive output and allowing worker and male production to be distinguished (10), which may explain our detection of a strong effect of urban land-use on reproduction in contrast to previous studies (22, 23, 51). Furthermore, consideration of asymmetrical reproductive investment in gynes and males means our results are potentially conservative. Gyne production requires greater resource investment than male production (52), and in our study, agricultural colonies failed to produce even a single gyne. Gyne production is likely to have a particularly strong effect on population dynamics, as queens hibernate and found new colonies (53), so our findings suggest that agricultural populations may not be self-sustaining (54). Queens of common bumblebee species may migrate long distances (55), raising the possibility that cities may act as a source of new queens to replenish such agricultural population sinks and therefore support the pollination of crops in agriculturally intense landscapes.

Parasite pressure presents a significant emerging threat to wild bee populations (13) and previous research has provided evidence for a link between land-use and parasite prevalence in bumblebees (19, 20). However, no effect of land-use was found on *Crithidia bombi* presence and levels of *Nosema bombi, Syntretus* sp. and *Locustacaris buchneri* were either zero or too low for analysis. Conversely, invasions by the brood parasite *B. vestalis* were strongly affected by land-use, with higher invasion rates in agricultural and village colonies than city. This may reflect lower *B. vestalis* abundance or even complete absence in the urban areas studied, although surveys have recorded the species in cities

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288 (e.g. 56). Alternatively, stronger colonies in city sites may have been more able to resist invasion (57),

289 or volatiles from colonies may have been masked by air pollution (58), rendering them more difficult

290 to locate (59). Reductions in forage availability in modern agricultural landscapes have been

291 identified as a potential major driver of bee population declines (8). Accordingly, we found less stored

292 pollen and nectar in agricultural colonies than in city or village colonies, suggesting forage

293 availability may be a contributing factor to poor performance at agricultural sites. This is consistent

294 with evidence from honeybees, where urbanisation has been shown to have a positive effect on food

295 storage (60) (but cf. ref. 38), and supports research suggesting modern agricultural land provides

296 insufficient forage resources for bees (8)

297 Investigation into the underlying attributes of our land-use classification indicates that it appears to be

298 the shared attributes of high agricultural cover and low urbanisation that group the poor performing

299 colonies in our study. A reasonable hypothesis from previous research showing higher colony weight

300 gain in suburban areas than agricultural (22) would be that low intensity urban areas are most valuable

301 to bee populations due to the combination of abundant gardens and proximity to semi-natural habitat;

302 our finding that colonies in densely urbanised areas performed similarly to those in lower intensity

303 urbanisation nonetheless fails to support this. We found no direct effect of the proportion of flower-

304 rich habitat surrounding colonies on colony success. However, this may reflect the fact that fine

305 resolution floral abundance surveys, taking into account floral density and species identity, are not

306 possible in urban areas due to access restrictions to gardens. Future research could aim to investigate

307 forage provision in urban areas using modelling approaches (62) to further assess floral availability as

308 a driver in urban habitats. Floral factors differing between agricultural and built-up areas that may

309 have contributed to a reduced ability to collect food may include the spatial distribution and

310 composition of flower-rich patches (16, 63), the duration for which they are available (63), or

311 potential effects of environmental contaminants on foraging behaviour (64).

312 Exposure to agrochemicals has been shown to impact on colony function and success in bumblebees

313 (50, 64), including reproductive output (50) and parasite prevalence (65), and high levels of pesticide

314 contamination are often found in both crop and wildflower resources in agricultural areas (66). There

315 is evidence that bees in urban areas may be subject to lower pesticide exposure (21, but cf. ref. 67)

316 offering another possible mechanism for our findings of lower colony success in agricultural areas.

317 Ground surveys of the agricultural sites in this experiment showed a variety of crops in the

318 surrounding farmland, with one site near a field of oilseed rape. This may represent a route of

319 pesticide exposure (68), although the study took place after the EU moratorium restricting

320 neonicotinoid use in flowering crops (69). However, neonicotinoids may remain in the soil and the

321 nectar and pollen of non-target plants for prolonged periods following use on nearby crops (70), and

322 other pesticides may also negatively affect bees (64). In general, fields around the agricultural sites

323 were more commonly arable than pasture, compared to the village sites which more often contained

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324 pasture and woodland in undeveloped areas, providing the potential for different pesticide exposure

325 between these land-use types, and the high incidence of gardens and parks in city areas may expose

326 bees to a different suite of horticultural pesticide applications, about which little is known (70). Our

327 findings highlight that the question of how bee exposure to pesticides varies with urbanisation is a

328 major knowledge gap that requires exploration.

329 We show for the first time that the reproductive output of *B. terrestris* colonies placed in built-up

330 areas is higher than in agricultural areas, suggesting that the current urban expansion may have

331 positive consequences for generalist bumblebee species. Our findings suggest that abundance and

332 diversity differences found in previous studies (71) may be driven by a direct impact of land-use on

333 fitness, rather than migration between land-use types, and support the growing evidence that some

334 types of agricultural land represent a barren landscape for pollinators (8, 12). Given than agricultural

335 land is the most common primary land-use in Europe (72), our finding that urban areas are linked to

336 higher reproductive success suggests that developed land may provide a refuge for bumblebee

337 populations within a landscape dominated by intensive farming.

338

354 **Author Contributions**

355 A.E.S. and E.L. conceived the initial idea; A.E.S., E.L. and M.J.F.B designed the experiments; R.J.G.

356 provided feedback on the experimental design; A.E.S. performed the experiment and the statistical

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357   analyses; A.E.S. wrote the manuscript draft and E.L., R.J.G., M.J.F.B. and A.E.S. provided the final
358   edit.

359   **Data Accessibility**

360   Raw data are archived in Dryad (entry doi: 10.5061/dryad.c68cj62).

361

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535   **Figure 1**. a) Location of 38 sites in South-East England at which a *B. terrestris* colony was placed for up to 10 weeks from

536   May to July. Inset circles show land-use classification at a 500m radius for three typical sites (left to right: agricultural,

537   village, city). b) Cluster dendrograms of land-use of 38 sites at a 500m radius. Cluster analyses using Ward's method were

538   performed on a set of principle components describing land-use to group sites into categorical land-use types (red boxes). At

539   the terminus of each branch the two-letter site name is given with an image of the GIS land classification (see Table S14 for

540   colour key).

541   **Figure 2.** a) Mean number of sexual offspring (gynes + males) with 95% confidence intervals (CIs) for colonies of

542   *B.terrestris* in agricultural, village and city sites based on land-use at a 500m radius. Letters indicate significant differences

543   between land-use types based on 95% CIs on parameter estimates from both the binomial (presence/absence of sexuals) and

544   count (number of sexuals produced) components of a zero-inflated hurdle model. b) Mean (± SE) colony size (number of

545   bees) from weekly night-time bumblebee colony censuses. To analyse peak colony size linear regression was carried out on

546   log-transformed data.

547   **Figure 3.** Kaplan-Meier curves of a) queen survival and b) colony survival for colonies of *B.terrestris* in agricultural, village

548   and city sites based on land-use at a 500m radius. Each step in the Kaplan-Meier curves represents the week at which (a)

549   queens died or (b) colonies were removed from the field; for example, all queens in agricultural sites had died by week 5. c)

550   Proportion of colonies invaded by *Bombus vestalis* in agricultural, village and city sites, analysed as a binary response.

551   Letters indicate significant differences between land-use types based on 95% CIs on model-averaged parameter estimates

552   from a) and b) Cox proportional hazards models and c) binomial GLMs.

553   **Figure 4.** Mean (± SE) proportion of *B. terrestris* colonies containing a) nectar and b) pollen stores over 10 weeks in

554   agricultural, village and city sites based on land-use at a 500m radius. Binomial GAMs allowing for a non-linear effect of

555   week with site as a random effect were used to analyse presence of nectar and pollen. Nectar data were collected from week

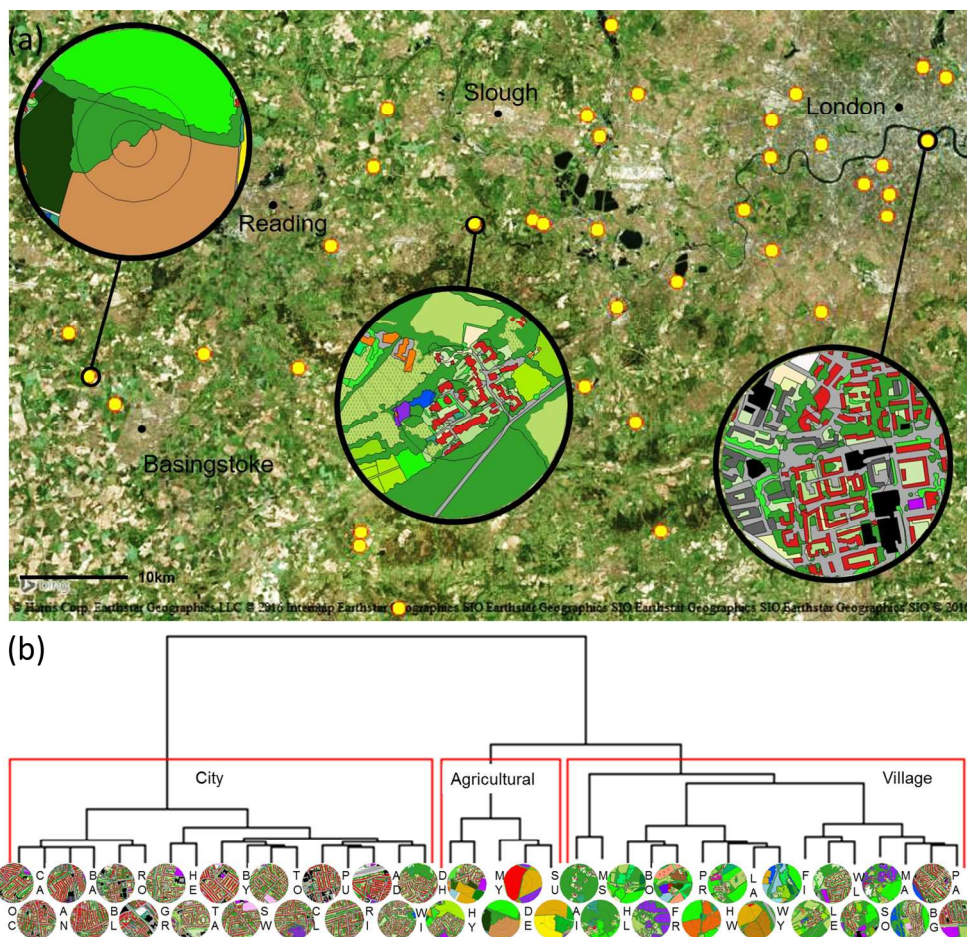556   3 due to provision of sucrose during week 1.

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Figure 1. a) Location of 38 sites in South-East England at which a B. terrestris colony was placed for up to 10 weeks from May to July. Inset circles show land-use classification at a 500m radius for three typical sites (left to right: agricultural, village, city). b) Cluster dendrograms of land-use of 38 sites at a 500m radius. Cluster analyses using Ward's method were performed on a set of principle components describing land-use to group sites into categorical land-use types (red boxes). At the terminus of each branch the two-letter site name is given with an image of the GIS land classification (see Supplementary Material for colour key).
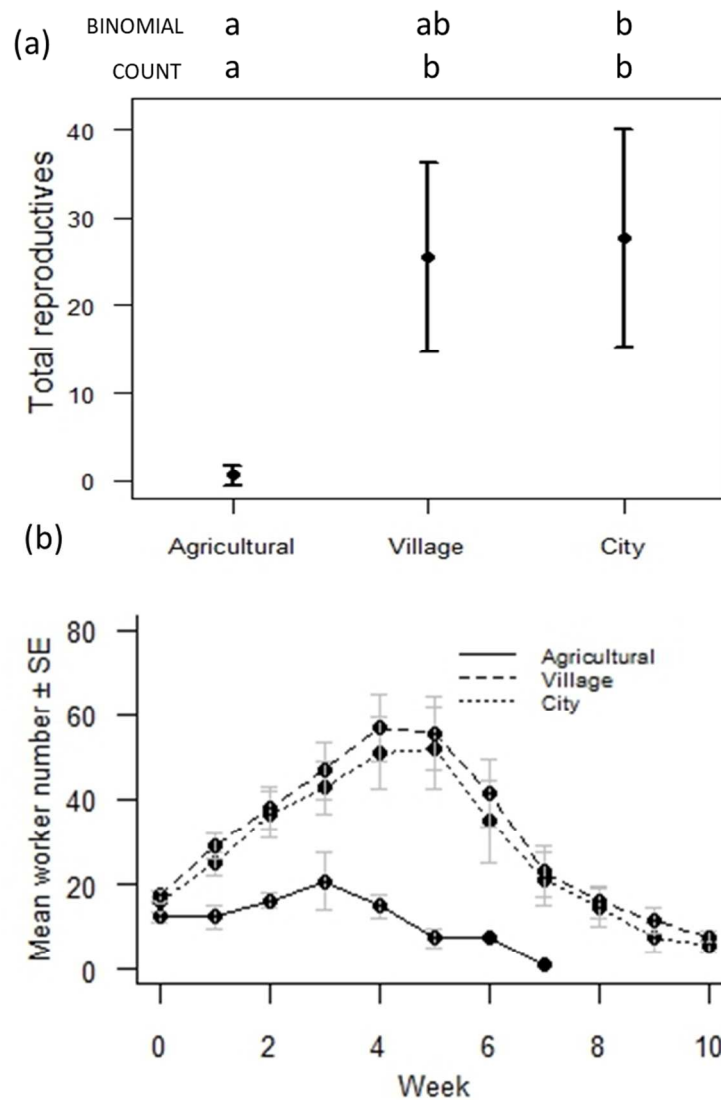
170x158mm (300 x 300 DPI)

Figure 2. a) Mean number of sexual offspring (gynes + males) with 95% confidence intervals (CIs) for colonies of B.terrestris in agricultural, village and city sites based on land-use at a 500m radius. Letters indicate significant differences between land-use types based on 95% CIs on parameter estimates from both the binomial (presence/absence of sexuals) and count (number of sexuals produced) components of a zero-inflated hurdle model. b) Mean (± SE) colony size (number of bees) from weekly night-time bumblebee colony censuses. To analyse peak colony size linear regression was carried out on log-transformed data.
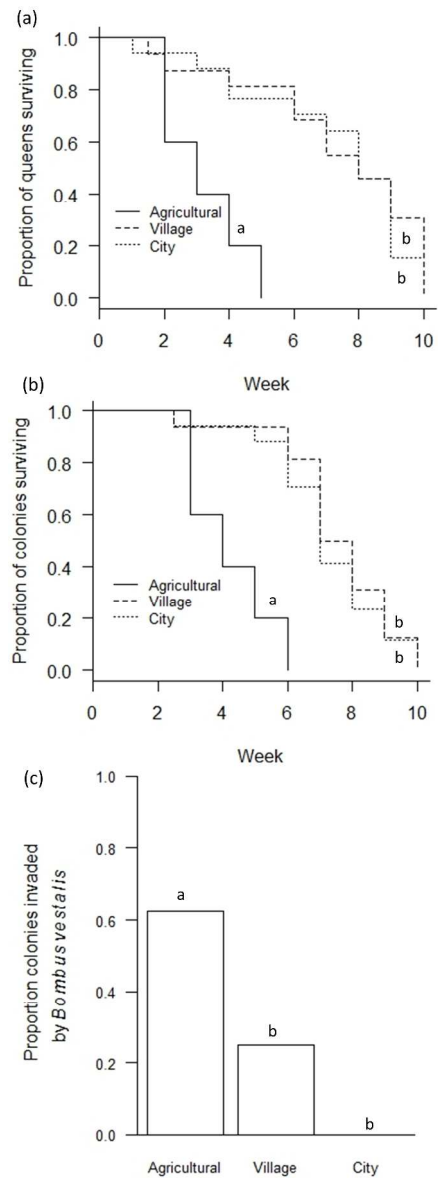
190x281mm (300 x 300 DPI)

Figure 3. Kaplan-Meier curves of a) queen survival and b) colony survival for colonies of B.terrestris in agricultural, village and city sites based on land-use at a 500m radius. Each step in the Kaplan-Meier curves represents the week at which (a) queens died or (b) colonies were removed from the field; for example, all queens in agricultural sites had died by week 5. c) Proportion of colonies invaded by Bombus vestalis in agricultural, village and city sites, analysed as a binary response. Letters indicate significant differences between land-use types based on 95% CIs on model-averaged parameter estimates from a) and b) Cox proportional hazards models and c) binomial GLMs.
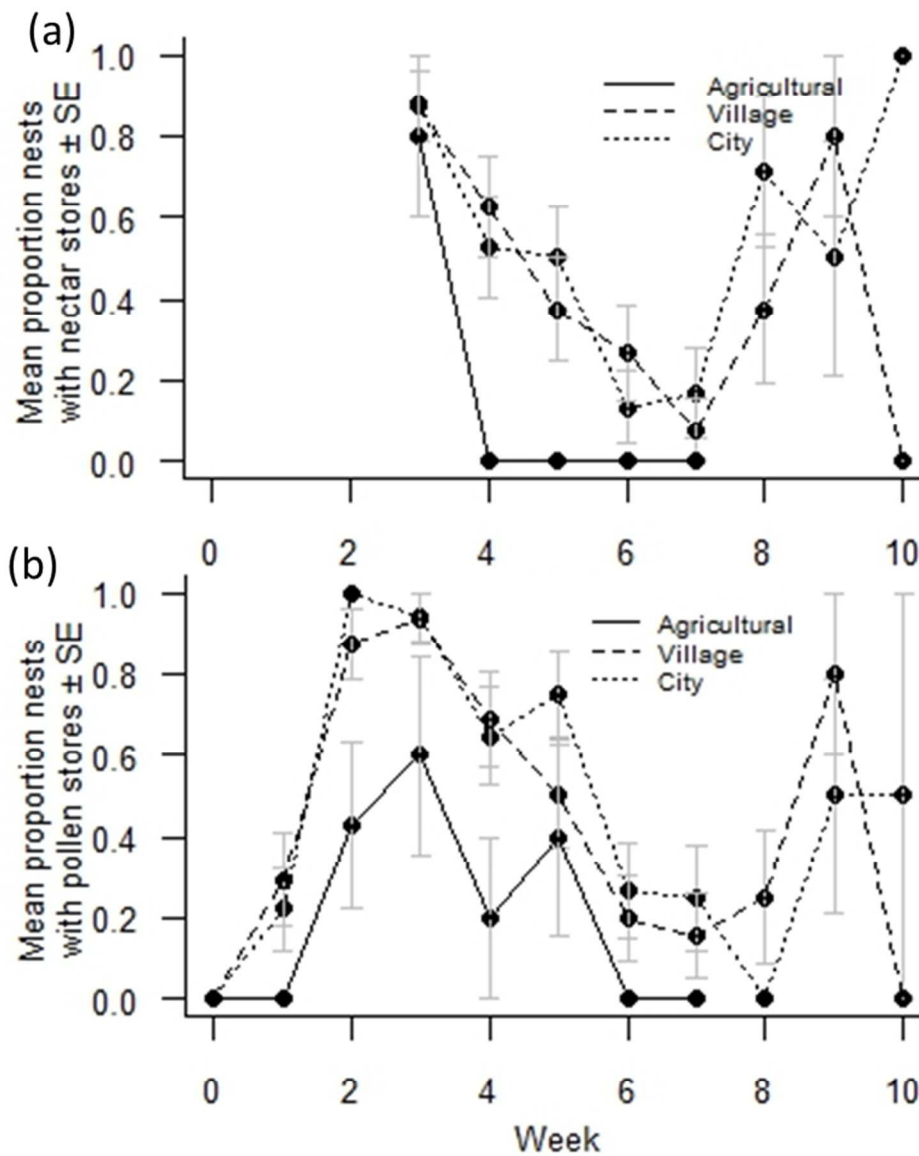
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Figure 4. Mean (± SE) proportion of B. terrestris colonies containing a) nectar and b) pollen stores over 10 weeks in agricultural, village and city sites based on land-use at a 500m radius. Binomial GAMs allowing for a non-linear effect of week with site as a random effect were used to analyse presence of nectar and pollen. Nectar data were collected from week 3 due to provision of sucrose during week 1.

168x207mm (300 x 300 DPI)