PROCEEDINGS OF
THE ROYAL SOCIETY B
BIOLOGICAL SCIENCES

Lower bumblebee colony reproductive success in agricultural compared to urban environments

Journal:	*Proceedings B*
Manuscript ID	RSPB-2018-0807.R1
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Samuelson, Ash; Royal Holloway University of London, Biological Sciences Gill, Richard; Imperial College London, Life Sciences Brown, Mark; Royal Holloway University of London, School of Biological Sciences Leadbeater, Ellouise; Royal Holloway University of London,
Subject:	Ecology < BIOLOGY, Environmental Science < BIOLOGY
Keywords:	Bombus terrestris, fitness, land-use, pollinator ecology, bee, urbanisation
Proceedings B category:	Ecology

Lower bumblebee colony reproductive success in agricultural compared to urban environments

Ash E. Samuelson[a*], Richard J. Gill[b], Mark J. F. Brown[a] & Ellouise Leadbeater[a]

[a] School of Biological Sciences, Royal Holloway University of London, Egham, United Kingdom

[b] Department of Life Sciences, Imperial College London, Silwood Park campus, Ascot, United Kingdom

*Corresponding author: ash.samuelson.2014@live.rhul.ac.uk

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

Urbanisation, *Bombus terrestris,* reproductive success, land-use, pollinator ecology, bee

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 important driver of urban effects on bees (14), with cities and towns often offering high floral abundance and diversity in the form of gardens and parks (15). However, many horticultural plant varieties are unattractive to bees or invest energy in visual displays at the expense of reward provision (16), and competition may also affect forage availability: increased popularity of urban beekeeping has increased honeybee hive densities in urban areas (17), possibly increasing competitive interactions with wild bees (18). Parasite prevalence has also been linked to urbanisation, with higher parasite loads in urban areas reported in bumblebees (19, 20). Pesticide use has been identified as a threat to bees (9) and exposure may vary across degrees of urbanisation (21). In the context of this array of potentially interacting drivers of urban effects, it is not clear how inhabiting urban areas affects bumblebee success at the colony level. This is because ethical concerns preclude the release of reproductive offspring from commercially-obtained bumblebee colonies (24-26), meaning that previous experiments have studied commercial colonies placed into the field only up to the very beginning of the period when reproductive offspring begin to emerge. Thus, while there is evidence that bumblebee colony early weight gain may be enhanced in suburban compared to agricultural areas based on studies of pre-reproductive colonies (22) (but cf. ref. 26), to date no study has monitored the critical, extensive reproductive period of the colony life-cycle and thus assessed the effects of urbanisation on lifetime reproductive success itself.

Here, we addressed this gap by rearing colonies from wild-caught queens to investigate the effect of urbanisation on life-history and reproductive output in the bumblebee *Bombus terrestris audax.* Using locally sourced queens allows ecologically relevant quantification of the impact of land-use on locally-adapted populations, rather than commercial bees that have been subject to artificial selection (27) and may differ from locally-adapted natural populations (26). It also overcomes concerns associated with the use of commercial bees, including negative environmental impacts such as hybridisation (24), pathogen spillover (25) and competition (26). A crucial outcome is that colonies can be monitored for their entire reproductive lifetime. We selected 38 sites across central London, its suburbs and the surrounding agricultural land (Fig. 1a), and categorized each site based on land-use through cluster analysis of principle components derived from 80 land-use variables. Through frequent censusing and sampling of colonies placed at these sites, we tracked for the first time the growth, reproductive output, nutritional status, and parasite prevalence of each colony from eclosion of the first cohort of workers until the end of the colony life cycle. To our knowledge, this represents the first experimental study in any taxon to demonstrate a direct relationship between urbanisation and reproductive success, with previous research typically employing an observational approach (e.g. 11, 28).

Methods

Bumblebee colonies

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

Field placement

We recruited 114 gardens and farms in South-East England (between central London and Basingstoke), of which 38 sites were selected across a region spanning inner city to countryside on the basis of distribution (> 1.5km apart), land-use type representativeness and accessibility (Fig.1a). This includes a range of urban and rural land-use types typical of Western Europe (29), from Central Business District, to suburban, to villages and medium-intensity agriculture containing a mixture of grassland and arable fields. Predominant crop types in the agricultural areas were cereals and brassica crops. The wide range of urban land types contained within London means that it is representative of a range of different urban types displayed by smaller cities (30). We placed colonies in the field in protective plastic field boxes during the first week of May 2016, randomised to land-use type according to initial colony size (see Supplementary Methods). Colony placement was staggered over six days, with six or seven sites visited each day during daylight hours (8:00-20:00). Colony monitoring continued until moribundity (see below), which occurred for the last colony on 11th July.

Data collection

Site visits followed approximately the same order as the colony placement, with each site visited weekly during the hours of darkness (21:30-4:30) at the same time each week. We recorded the following data (see Supplementary Methods for additional data): number of bees (average of three counts); queen status (alive, dead or absent); presence of nectar and pollen stores and presence and status of *Bombus vestalis* brood parasites (alive, injured or dead), which we removed to minimise *B. terrestris* queen death. To assess reproductive success, gynes were removed until one minute had passed with no gyne seen, and stored for later analysis. The same procedure was repeated for males, with sampling time capped at 15 minutes. Males are considered to leave the nest at two to four days old and gynes at two to eight days old (31) so our weekly removal of males and gynes reflects natural conditions and is unlikely to have impacted the colony's production of future males and gynes. Weekly removal of reproductives allows calculation of total reproductive output over the colony life cycle rather than a snapshot as obtained from traditional colony dissection methods that are carried out at the earliest sign of reproductive emergence (22). We removed one, three or five workers for later parasite analysis depending on colony size (<35, 35-50, >50 workers respectively), which were stored alive in vials for a maximum of 5 hours before freezing at -20°C.

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

Sample analysis

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

Land-use classification

Following best practice in the field (32, 33) we classified land-use at multiple radii surrounding each site using GIS analysis, based on satellite imagery with additional ground-truthing for agricultural sites.Agricultural sites were surveyed because mass crop blooms may not be detected by satellite images taken outside the bloom period The land-use classification protocol is described in full in (37) and is available as Supplementary Methods. Briefly, buffers at radii of 750m (*B. terrestris* typical foraging range (34-36)), 500m, 250m and 100m (representing steps of spatial scales at which bees may interact with the surrounding land (12, 37)) were generated around each site. Preliminary analysis showed that the majority of the response variables responded most strongly to land-use at a 500m radius so this was selected as our primary land-use variable. Land-use patches were defined by drawing polygons in QGIS v2.16 and categorised visually to one of 80 land-use classes (Table S14) from satellite imagery and ground surveys carried out in May 2016.

We refined the classification to produce a single categorical land-use variable via an established three-step process (32): 1) definition of land-use categories, 2) Principle Components Analysis (PCA) on the categories and 3) cluster analysis based on the PCA output (Fig. S2). Briefly, each land-use class was coded to one of eight categories (e.g. impervious surface, flower-rich habitat; Table S14) and the total area of each category within each site calculated. A PCA was then performed to reduce the dimensionality of the land-use variables, and cluster analysis (Ward's method) was performed on the first two principle components, which in combination captured approximately 85% of the variation (see below for loadings). Following (32, 33, 38), each cluster contained a minimum of five sites. Three clear clusters emerged (Fig. S3a), comprising a group characterised by dense urban development (henceforth named "city"; n=17), a group characterised by patches of housing surrounded by rural land ("village"; n=16) and a group dominated by agricultural fields ("agricultural"; n=5; Fig. 1b). Exploration of model fit confirmed that use of the clustered land-use categories to predict our main response variables explained more of the variance in our data than use of the PCs alone (Fig S3b), and comparison of models containing combinations of the PCs with those containing the clustered variable showed that for all response variables the clustered variable improved model fit (see Supplementary Methods and Table S11 for AIC values), justifying the necessity of the clustering step. Sites in the city cluster contained mean 56.2% (± SE: 4.0%) impervious surface and 0.1 (±0.1)% agricultural land cover while village and agricultural sites contained 13.8 (±3.7)% and 8.6 (±4.5)% impervious surface and 34.6 (±7.1)% and 71.2 (±11.5)% agricultural land cover respectively.

Statistical analysis

For each analysis, we built a comparison set of models including the full model (for predictors, see below) and all subsets, including the basic model containing only the constant and residual variance ("all-subset approach"). We selected the model or set of models with the lowest AICc as the best fitting model(s) (39). Where several models were within two AICc units of the best model, model averaging was carried out to obtain parameter estimates derived from the best set of models including the basic model if applicable (40). Final models were examined for spatial autocorrelation by using a Moran's I test on the residuals and graphically assessing the spatial pattern of residuals.

To analyse peak colony size linear regression was carried out on log-transformed data. Total production of sexuals (gynes and males) was analysed using zero-altered negative binomial hurdle models, where the response is modelled as a binary process (production of sexuals) and a zero-truncated count process (total sexuals in colonies that produced sexuals) (41). Binomial GAMs (allowing for a non-linear effect of week) with site as a random effect were used to analyse presence of nectar and pollen and ovary development. Queen survival, colony survival and onset of reproduction were subjected to survival analyses using non-parametric Cox proportional hazards models. Proportion of worker samples in each colony containing *Apicystis* and *Crithidia* were analysed using binomial GLMs. Male and worker fat content were analysed using Gaussian GAMs allowing for a non-linear effect of week with site as a random effect. *Bombus vestalis* invasion as a binary response was modelled using binomial GLMs. One factor level (city) for this variable had perfect separation (only zeroes); to deal with this three dummy observations were added for each land-use category with *B. vestalis* invasion set to one and weather variables set to whole-dataset means.

To investigate whether our results may have been driven by floral resource availability, we re-analysed the response variables that were found to be significantly affected by land-use (reproductive output, peak colony size, colony survival, queen survival, presence of nectar stores and presence of pollen stores) using proportion of flower-rich habitat as a predictor. We coded each land-use class as described above as flower-rich or flower-poor, based on reference to the literature (e.g. domestic gardens have been shown to support high floral diversity (15) and provide considerable resources to bees (42)) and on ground surveys in agricultural land to identify crop types and wildflower strips, and summed the area of flower-rich land-use patches to generate the proportion of flower-rich habitat at a 500m radius for each site. Each response variable was analysed using this predictor as described in the paragraph above. All analyses were conducted in R version 3.2.1 (43); for packages see Supplementary Methods.

Results

Land-use category strongly predicted the number of live sexual offspring (gynes and males) produced over the colony life cycle (Fig. 2a, Table S1a). Village colonies were significantly more likely to produce sexual offspring than agricultural colonies (model averaged estimate (MAE): 2.853, 95% CIs: [0.327 – 5.378], Table S2a), and both city (MAE: 2.789 [0.799 – 4.778]) and village (MAE: 2.566 [0.579 – 4.552]) colonies produced significantly higher numbers of sexuals than their agricultural counterparts. Our data suggest that this effect may reflect both the build-up of a larger workforce and, relatedly, longer queen lifespans in village and city colonies. Both village and city colonies displayed significantly higher peak size (number of bees) than agricultural colonies (Table S1b, Fig. 2b; City MAE: 0.918 [0.194 – 1.641], village MAE: 1.047 [0.319 – 1.774], Table S2b), and founding queens survived for longer (Table S1c, S2c; Fig. 3a; MAE of Hazard Ratios (HR) relative to agricultural colonies: City: 0.149 [0.041 – 0.542]); Village: 0.137 [0.039 – 0.488]. City and village colonies also took significantly longer to become moribund than agricultural colonies (City HR: 0.111 [0.031 – 0.396], village HR: 0.073 [0.019 – 0.271]; Table S1d; Fig. 3b). There was no significant effect of land-use on ovary development (see Supplementary Results).

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

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

Our land classification protocol (44) allows investigation into the aspects of the land-use that may underlie the effects found, by examining the variables that contribute to the clustering of land-use types. High domestic infrastructure, impervious surface and road cover, and low agricultural land cover, contributed strongly to Principle Component (PC) 1 (eigenvalue score >0.4 or >-0.4 (45)), while PC2 was defined by high tree cover and low open and flower-rich habitat cover (Table S10). The city cluster was characterised by positive scores on PC1 (mean 2.00 ± SE 0.07) and near-zero scores on PC2 (-0.27±0.17), suggesting a highly urbanised semi-open land type; the village cluster had medium negative scores on PC1 (-1.40±0.37) and positive PC2 scores (0.94±0.37), suggesting low intensity urbanisation with moderate tree cover; the agricultural cluster had low PC1 scores (-2.33±0.64) and low PC2 scores (-2.08±0.32), suggesting open land with very little urbanisation and high agricultural cover (Fig. S3a). Analysis of the PCs suggested it was the combination of both attributes of the land-use that drove the effects seen (see Supplementary Methods and Table S12 & S13 for results of these analyses). Investigation of the effect of the proportion of flower-rich habitat on the response variables as a possible key driver of the results showed no significant effect (Supplementary methods; Tables S3 & S4).

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 (e.g. 56). Alternatively, stronger colonies in city sites may have been more able to resist invasion (57), or volatiles from colonies may have been masked by air pollution (58), rendering them more difficult to locate (59). Reductions in forage availability in modern agricultural landscapes have been identified as a potential major driver of bee population declines (8). Accordingly, we found less stored pollen and nectar in agricultural colonies than in city or village colonies, suggesting forage availability may be a contributing factor to poor performance at agricultural sites. This is consistent with evidence from honeybees, where urbanisation has been shown to have a positive effect on food storage (60) (but cf. ref. 38), and supports research suggesting modern agricultural land provides insufficient forage resources for bees (8)

Investigation into the underlying attributes of our land-use classification indicates that it appears to be the shared attributes of high agricultural cover and low urbanisation that group the poor performing colonies in our study. A reasonable hypothesis from previous research showing higher colony weight gain in suburban areas than agricultural (22) would be that low intensity urban areas are most valuable to bee populations due to the combination of abundant gardens and proximity to semi-natural habitat; our finding that colonies in densely urbanised areas performed similarly to those in lower intensity urbanisation nonetheless fails to support this. We found no direct effect of the proportion of flower-rich habitat surrounding colonies on colony success. However, this may reflect the fact that fine resolution floral abundance surveys, taking into account floral density and species identity, are not possible in urban areas due to access restrictions to gardens. Future research could aim to investigate forage provision in urban areas using modelling approaches (62) to further assess floral availability as a driver in urban habitats. Floral factors differing between agricultural and built-up areas that may have contributed to a reduced ability to collect food may include the spatial distribution and composition of flower-rich patches (16, 63), the duration for which they are available (63), or potential effects of environmental contaminants on foraging behaviour (64).

Exposure to agrochemicals has been shown to impact on colony function and success in bumblebees (50, 64), including reproductive output (50) and parasite prevalence (65), and high levels of pesticide contamination are often found in both crop and wildflower resources in agricultural areas (66). There is evidence that bees in urban areas may be subject to lower pesticide exposure (21, but cf. ref. 67) offering another possible mechanism for our findings of lower colony success in agricultural areas. Ground surveys of the agricultural sites in this experiment showed a variety of crops in the surrounding farmland, with one site near a field of oilseed rape. This may represent a route of pesticide exposure (68), although the study took place after the EU moratorium restricting neonicotinoid use in flowering crops (69). However, neonicotinoids may remain in the soil and the nectar and pollen of non-target plants for prolonged periods following use on nearby crops (70), and other pesticides may also negatively affect bees (64). In general, fields around the agricultural sites were more commonly arable than pasture, compared to the village sites which more often contained pasture and woodland in undeveloped areas, providing the potential for different pesticide exposure between these land-use types, and the high incidence of gardens and parks in city areas may expose bees to a different suite of horticultural pesticide applications, about which little is known (70). Our findings highlight that the question of how bee exposure to pesticides varies with urbanisation is a major knowledge gap that requires exploration.

We show for the first time that the reproductive output of *B. terrestris* colonies placed in built-up areas is higher than in agricultural areas, suggesting that the current urban expansion may have positive consequences for generalist bumblebee species. Our findings suggest that abundance and diversity differences found in previous studies (71) may be driven by a direct impact of land-use on fitness, rather than migration between land-use types, and support the growing evidence that some types of agricultural land represent a barren landscape for pollinators (8, 12). Given than agricultural land is the most common primary land-use in Europe (72), our finding that urban areas are linked to higher reproductive success suggests that developed land may provide a refuge for bumblebee populations within a landscape dominated by intensive farming.

Acknowledgements

We are grateful to the participants in this study for hosting bumblebee colonies on their properties. We thank Alexis Gkantiragas for help with GIS land-use classification and parasite analysis; Margaret Fitzherbert for conducting ground surveys of agricultural land; Judit Bagi and Kel Liu for help with bumblebee rearing; Luke D'Addiego for help with data collection; Natural England and The Crown Estate for permission to collect bumblebee queens from Windsor Great Park and the Leadbeater and Brown labs for advice and technical assistance. We would like to thank two anonymous reviewers and the editor for insightful comments that improved the manuscript.

Funding

A.E.S.'s research is supported by the ICL-RHUL BBSRC DTP BB/M011178/1 and by donations from High Wycombe Beekeepers' Association and Essex Beekeepers' Association. E.L.'s research is supported by ERC Starting Grant BeeDanceGap and her contribution was also partly funded by a Leverhulme Trust Early Career Fellowship. R.J.G.'s research is supported by NERC grant NE/L00755X/1 and the Grand Challenges in Ecosystems and the Environment Initiative at Silwood Park. M.J.F.B.'s research is supported by BBSRC grant BB/N000668/1.

Author Contributions

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

Data Accessibility

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

References

1. Elmqvist T, Fragkias M, Goodness J, Güneralp B, Marcotullio PJ, McDonald RI, et al. Urbanization, Biodiversity and Ecosystem Services: Challenges and Opportunities: A Global Assessment: Springer; 2013.
2. United Nations. World urbanization prospects: the 2014 revision, Highlights. New York; 2014. Contract No.: ST/ESA/SER.A/352.
3. Seto KC, Güneralp B, Hutyra LR. Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. Proceedings of the National Academy of Sciences. 2012;109(40):16083-8.
4. Aronson MF, La Sorte FA, Nilon CH, Katti M, Goddard MA, Lepczyk CA, et al. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proceedings of the Royal Society B. 2014;281(1780):20133330.
5. Kark S, Iwaniuk A, Schalimtzek A, Banker E. Living in the city: can anyone become an ‘urban exploiter'? Journal of Biogeography. 2007;34(4):638-51.
6. Senapathi D, Carvalheiro LG, Biesmeijer JC, Dodson C-A, Evans RL, McKerchar M, et al. The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England. Proceedings of the Royal Society B: Biological Sciences. 2015;282(1806):20150294.
7. Baldock KC, Goddard MA, Hicks DM, Kunin WE, Mitschunas N, Osgathorpe LM, et al. Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. Proceedings of the Royal Society of London B: Biological Sciences. 2015;282(1803):20142849.
8. Carvell C, Roy DB, Smart SM, Pywell RF, Preston CD, Goulson D. Declines in forage availability for bumblebees at a national scale. Biological Conservation. 2006;132(4):481-9.
9. Desneux N, Decourtye A, Delpuech J-M. The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology. 2007;52:81-106.
10. Crone EE, Williams NM. Bumble bee colony dynamics: quantifying the importance of land use and floral resources for colony growth and queen production. Ecology Letters. 2016;19(4):460-8.
11. Coleman JL, Barclay RM. Influence of urbanization on demography of little brown bats (*Myotis lucifugus*) in the prairies of North America. PloS ONE. 2011;6(5):e20483.
12. Carvell C, Bourke AFG, Dreier S, Freeman SN, Hulmes S, Jordan WC, et al. Bumblebee family lineage survival is enhanced in high-quality landscapes. Nature. 2017;543(7646):547-9.
13. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature. 2014;506(7488):364-6.
14. Harrison T, Winfree R. Urban drivers of plant-pollinator interactions. Functional Ecology. 2015;29(7):879-88.
15. Loram A, Thompson K, Warren PH, Gaston KJ. Urban domestic gardens (XII): the richness and composition of the flora in five UK cities. Journal of Vegetation Science. 2008;19(3):321-30.
16. Garbuzov M, Ratnieks FL. Quantifying variation among garden plants in attractiveness to bees and other flower-visiting insects. Functional Ecology. 2014;28(2):364-74.
17. Alton K, Ratnieks F. To bee or not to bee. Biologist. 2013;60(4):12-5.
18. Goulson D, Sparrow KR. Evidence for competition between honeybees and bumblebees; effects on bumblebee worker size. Journal of insect conservation. 2009;13(2):177-81.

19. Theodorou P, Radzevičiūtė R, Settele J, Schweiger O, Murray TE, Paxton RJ. Pollination services enhanced with urbanization despite increasing pollinator parasitism. Proceedings of the Royal Society B: Biological Sciences. 2016;283(1833):20160561.
20. Goulson D, Whitehorn P, Fowley M. Influence of urbanisation on the prevalence of protozoan parasites of bumblebees. Ecological Entomology. 2012;37(1):83-9.
21. Botías C, David A, Hill EM, Goulson D. Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes. Environmental Pollution. 2017;222:73-82.
22. Goulson D, Hughes W, Derwent L, Stout J. Colony growth of the bumblebee, *Bombus terrestris*, in improved and conventional agricultural and suburban habitats. Oecologia. 2002;130(2):267-73.
23. Kämper W, Werner PK, Hilpert A, Westphal C, Blüthgen N, Eltz T, et al. How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*. Landscape Ecology. 2016;31(10):2245-58.
24. Ings TC, Raine NE, Chittka L. Mating preference in the commercially imported bumblebee species Bombus terrestris in Britain (Hymenoptera: Apidae). Entomologia Generalis. 2005;28(3):233.
25. Colla SR, Otterstatter MC, Gegear RJ, Thomson JD. Plight of the bumble bee: pathogen spillover from commercial to wild populations. Biological Conservation. 2006;129(4):461-7.
26. Ings T, Ward N, Chittka L. Can commercially imported bumble bees out-compete their native conspecifics? Journal of Applied Ecology. 2006;43(5):940-8.
27. Velthuis HH, Van Doorn A. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie. 2006;37(4):421.
28. Newhouse MJ, Marra PP, Johnson LS. Reproductive success of House Wrens in suburban and rural landscapes. The Wilson Journal of Ornithology. 2008;120(1):99-104.
29. Levers C, Müller D, Erb K, Haberl H, Jepsen MR, Metzger MJ, et al. Archetypical patterns and trajectories of land systems in Europe. Regional Environmental Change. 2015:1-18.
30. Schwarz N. Urban form revisited—Selecting indicators for characterising European cities. Landscape and Urban Planning. 2010;96(1):29-47.
31. Free JB, Butler CG. Bumblebees. London: Collins; 1959.
32. Owen S, MacKenzie A, Bunce R, Stewart H, Donovan R, Stark G, et al. Urban land classification and its uncertainties using principal component and cluster analyses: A case study for the UK West Midlands. Landscape and Urban Planning. 2006;78(4):311-21.
33. Bunce RGH, Barr CJ, Clarke RT, Howard DC, Lane AMJ. Land Classification for Strategic Ecological Survey. Journal of Environmental Management. 1996;47(1):37-60.
34. Osborne J, Clark S, Morris R, Williams I, Riley J, Smith A, et al. A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. Journal of Applied Ecology. 1999;36(4):519-33.
35. Darvill B, Knight ME, Goulson D. Use of genetic markers to quantify bumblebee foraging range and nest density. Oikos. 2004;107(3):471-8.
36. Knight ME, Martin AP, Bishop S, Osborne JL, Hale RJ, Sanderson RA, et al. An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Molecular Ecology. 2005;14(6):1811-20.
37. Moreira EF, Boscolo D, Viana BF. Spatial heterogeneity regulates plant-pollinator networks across multiple landscape scales. PloS ONE. 2015;10(4):e0123628.
38. Hall O, Arnberg W. A method for landscape regionalization based on fuzzy membership signatures. Landscape and Urban Planning. 2002;59(4):227-40.
39. Johnson JB, Omland KS. Model selection in ecology and evolution. Trends in Ecology & Evolution. 2004;19(2):101-8.
40. Symonds MR, Moussalli A. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology. 2011;65(1):13-21.

41. Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed Effects Models and Extensions in Ecology with R: Springer New York; 2011.

42. Goulson D, Lepais O, O'Connor S, Osborne JL, Sanderson RA, Cussans J, et al. Effects of land use at a landscape scale on bumblebee nest density and survival. Journal of Applied Ecology. 2010;47(6):1207-15.

43. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015.

44. Samuelson AE, Leadbeater E. A land classification protocol for pollinator ecology research: An urbanization case study. Ecol Evol 2018;In Press.

45. Hahs AK, McDonnell MJ. Selecting independent measures to quantify Melbourne's urban–rural gradient. Landscape and urban planning. 2006;78(4):435-48.

46. Sirohi MH, Jackson J, Edwards M, Ollerton J. Diversity and abundance of solitary and primitively eusocial bees in an urban centre: a case study from Northampton (England). Journal of Insect Conservation. 2015;19(3):487-500.

47. Osborne JL, Martin AP, Shortall CR, Todd AD, Goulson D, Knight ME, et al. Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. Journal of Applied Ecology. 2008;45(3):784-92.

48. Gill RJ, Baldock KC, Brown MJ, Cresswell JE, Dicks LV, Fountain MT, et al. Protecting an ecosystem service: approaches to understanding and mitigating threats to wild insect pollinators. Advances in Ecological Research. 2016;54:135-56.

49. Williams NM, Regetz J, Kremen C. Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. Ecology. 2012;93(5):1049-58.

50. Whitehorn PR, O'Connor S, Wackers FL, Goulson D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science. 2012;336(6079):351-2.

51. Parmentier L, Meeus I, Cheroutre L, Mommaerts V, Louwye S, Smagghe G. Commercial bumblebee hives to assess an anthropogenic environment for pollinator support: a case study in the region of Ghent (Belgium). Environmental Monitoring and Assessment. 2014;186(4):2357-67.

52. Duchateau MJ, Velthuis HHW. Development and reproductive strategies in *Bombus terrestris* colonies. Behaviour. 1988;107(3/4):186-207.

53. Sladen FWL. The Humble-Bee. Cambridge: Cambridge University Press; 1912.

54. Savard J-PL, Clergeau P, Mennechez G. Biodiversity concepts and urban ecosystems. Landscape and Urban Planning. 2000;48(3):131-42.

55. Lepais O, Darvill BEN, O'Connor S, Osborne JL, Sanderson RA, Cussans J, et al. Estimation of bumblebee queen dispersal distances using sibship reconstruction method. Molecular Ecology. 2010;19(4):819-31.

56. Archer M. The wasps, ants and bees (Hymenoptera: Aculeata) of the'Green Spaces' of urban York. Entomologists Monthly Magazine. 2012;148(1780):173.

57. Goulson D, Brown MJF. Natural Enemies. In: Goulson D, editor. Bumblebees: their behaviour and ecology. Oxford: OUP; 2003.

58. Lusebrink I, Girling RD, Farthing E, Newman TA, Jackson CW, Poppy GM. The Effects of Diesel Exhaust Pollution on Floral Volatiles and the Consequences for Honey Bee Olfaction. Journal of Chemical Ecology. 2015;41(10):904-12.

59. Fisher RM. Recognition of host nest odour by the bumblebee social parasite *Psithyrus ashtoni* (Hymenoptera: Apidae). Journal of the New York Entomological Society. 1983;91(4):503-7.

60. Lecocq A, Kryger P, Vejsnæs F, Jensen AB. Weight watching and the effect of landscape on honeybee colony productivity: Investigating the value of colony weight monitoring for the beekeeping industry. PloS ONE. 2015;10(7):e0132473.

61. Sponsler DB, Johnson RM. Honey bee success predicted by landscape composition in Ohio, USA. PeerJ. 2015;3:e838.

62. Lonsdorf E, Kremen C, Ricketts T, Winfree R, Williams N, Greenleaf S. Modelling pollination services across agricultural landscapes. Annals of botany. 2009;103(9):1589-600.

63. Kallioniemi E, Åström J, Rusch GM, Dahle S, Åström S, Gjershaug JO. Local resources, linear elements and mass-flowering crops determine bumblebee occurrences in moderately intensified farmlands. Agriculture, ecosystems & environment. 2017;239:90-100.

64. Gill RJ, Ramos-Rodriguez O, Raine NE. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature. 2012;491(7422):105-8.

65. McArt SH, Urbanowicz C, McCoshum S, Irwin RE, Adler LS. Landscape predictors of pathogen prevalence and range contractions in US bumblebees. Proceedings of the Royal Society B: Biological Sciences. 2017;284(1867).

66. David A, Botías C, Abdul-Sada A, Nicholls E, Rotheray EL, Hill EM, et al. Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. Environment international. 2016;88:169-78.

67. Johnson J, Pettis J. A survey of imidacloprid levels in water sources potentially frequented by honeybees (Apis mellifera) in the eastern USA. Water, Air, & Soil Pollution. 2014;225(11):1-6.

68. Rundlöf M, Andersson GK, Bommarco R, Fries I, Hederström V, Herbertsson L, et al. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature. 2015;521(7550):77-80.

69. EC. Commission implementing regulation (EU) No 485/2013 of 24 May 2013 amending Implementing Regulation EU) No 540/2011, as regards the conditions of approval of the active substances clothianidin, thiamethoxam and imidacloprid, and prohibiting the use and sale of seeds treated with plant protection products containing those active substances. Official Journal of the European Union. 2013;139:12-26.

70. Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, et al. Environmental fate and exposure; neonicotinoids and fipronil. Environ Sci Pollut Res. 2015;22(1):35-67.

71. Winfree R, Bartomeus I, Cariveau DP. Native pollinators in anthropogenic habitats. Annual Review of Ecology, Evolution, and Systematics. 2011;42.

72. Eurostat. Land cover, land use and landscape. Eurostat yearbook [Internet]. 2016 27/04/2017. Available from: http://ec.europa.eu/eurostat/statistics-explained/index.php/Land_cover,_land_use_and_landscape.

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

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

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

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

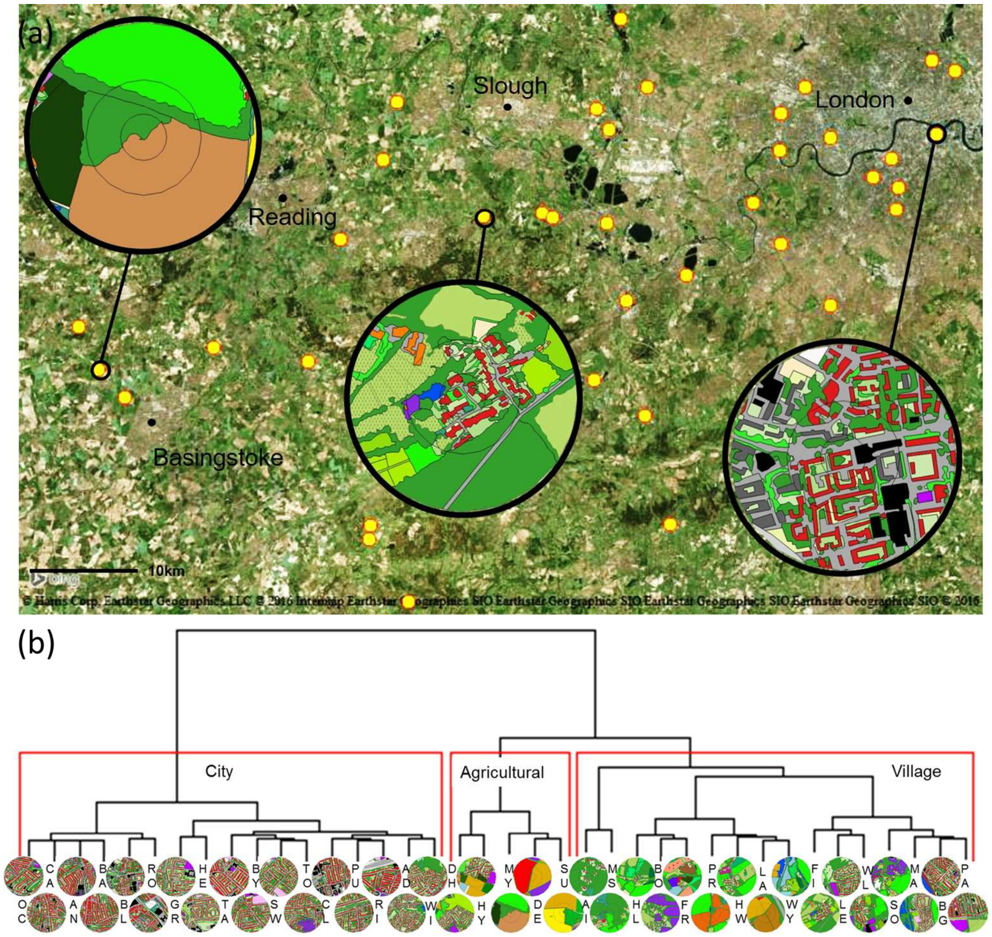

Figure 1. a) Location of 38 sites in South-East England at which a B. terrestris colony was placed for up to 10 weeks from May to July. Inset circles show land-use classification at a 500m radius for three typical sites (left to right: agricultural, village, city). b) Cluster dendrograms of land-use 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)

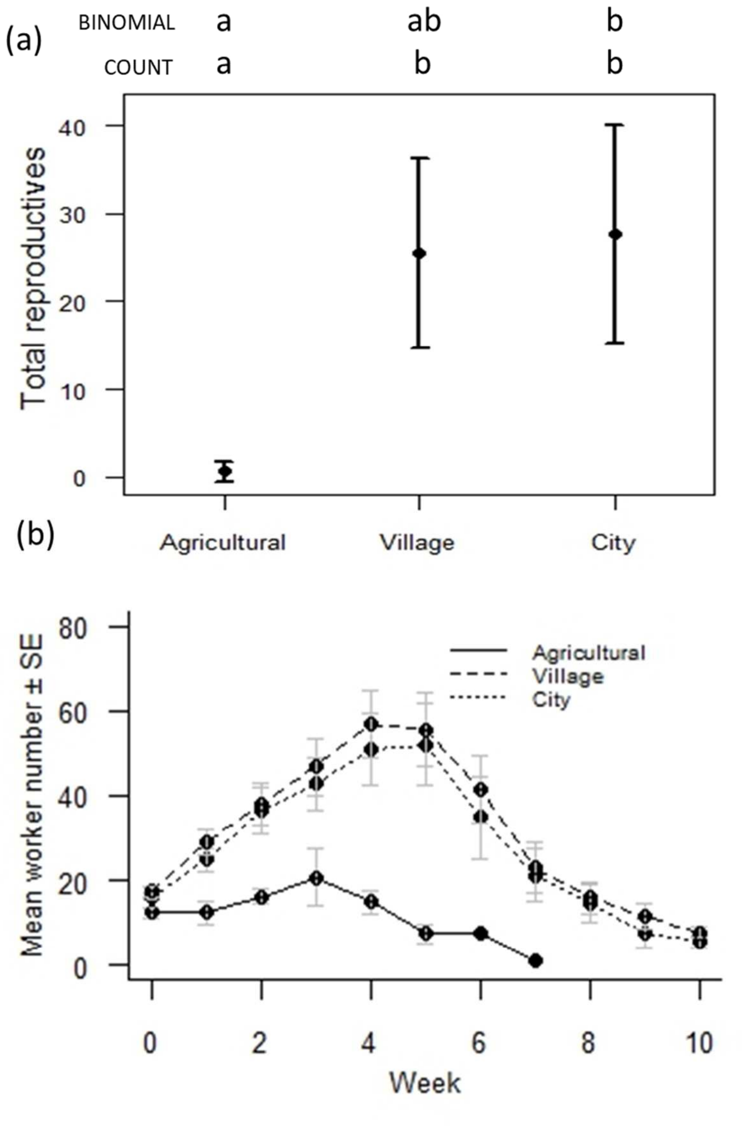

Figure 2. a) Mean number of sexual offspring (gynes + males) with 95% confidence intervals (CIs) for colonies of B.terrestris in agricultural, village and city sites based on land-use at a 500m radius. Letters indicate significant differences between land-use types based on 95% CIs on parameter estimates 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)

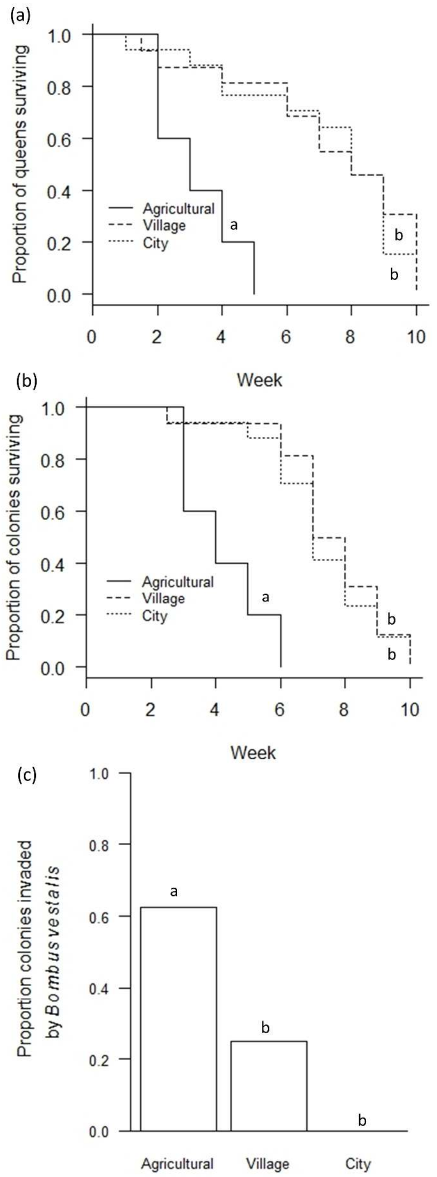

Figure 3. Kaplan-Meier curves of a) queen survival and b) colony survival for colonies of B.terrestris in agricultural, village and city sites based on land-use at a 500m radius. Each step in the Kaplan-Meier curves represents the week at which (a) queens died or (b) colonies were removed from the field; 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.

383x1025mm (300 x 300 DPI)

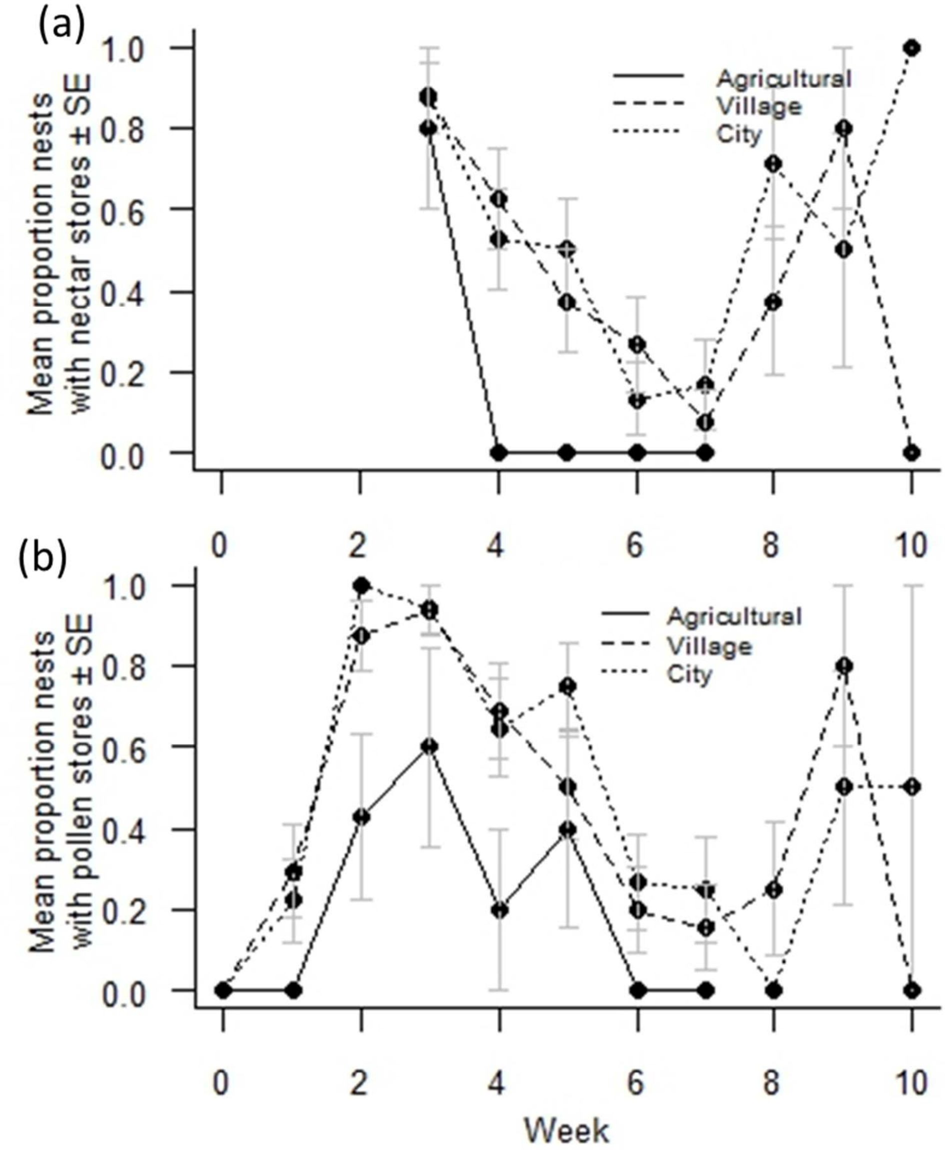

Figure 4. Mean (± SE) proportion of B. terrestris colonies containing a) nectar and b) pollen stores over 10 weeks in agricultural, village and city sites based on land-use at a 500m radius. Binomial GAMs allowing for a non-linear effect of week with site as a random effect were used to analyse presence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

168x207mm (300 x 300 DPI)