RESEARCH ARTICLE

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Bumblebee colony density on farmland is influenced by late-summer nectar supply and garden cover

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Abstract

1. Floral resources are important in limiting pollinator populations, but they are often highly variable across time and space and the effect of this variation on pollinator population dynamics is not well understood. The phenology (timing) of floral resources is thought to be important in structuring pollinator populations, but few studies have directly investigated this.

2. Our study quantifies the landscape composition, seasonal nectar and pollen supply and *Bombus terrestris* colony density of 12 farms in southwest UK to investigate how landscape composition influences the phenology of floral resources and how both these factors affect colony density. We use this information in a spatially explicit predictive model to estimate the effect of different farmland management scenarios on seasonal resource supplies and colony density.

3. We find that farmland nectar supply during September is a strong predictor of *B. terrestris* colony density in the following year, explaining over half of all the variation in colony density; no other period of resource availability showed a significant association. Semi-natural habitat cover was not a good proxy for nectar or pollen supply and showed no significant association with colony density. However, the proportional cover of gardens in the landscape was significantly associated with colony density.

4. The predictive model results suggest that increasing the area of semi-natural flowering habitat has limited effect on bumblebee populations. However, improving the quality of these habitats through Environmental Stewardship and other management options is predicted to reduce the late-summer resource bottleneck and increase colony density.

5. *Synthesis and Applications.* Our study demonstrates the importance of considering the phenology of resources, rather than just total resource availability, when designing measures to support pollinators. Late summer appears to be a resource bottleneck for bumblebees in UK farmland, and consequently management strategies which increase late-summer nectar availability may be the most effective. These include mowing regimes to delay flowering of field margins until September, planting late-flowering cover crops such as red clover and supporting late-flowering wild plant species such as *Hedera helix*. Our results also suggest that rural gardens may play an important role in supporting farmland bumblebee populations.

1 | INTRODUCTION

Floral resources are important in limiting pollinator populations, with a loss of floral resources cited as one of the main factors driving pollinator declines (Goulson et al., 2015; Roulston & Goodell, 2011). However, floral resources are highly variable across time and space and it is not well understood how this variation affects pollinator populations. Different plant species have distinct flowering times (phenology), and at a landscape-level this gives rise to a seasonal pattern of floral resources, which is likely to shape pollinator communities (Ogilvie & Forrest, 2017). Heavily simplified landscapes such as farmlands, with low plant diversity, are likely to have high variation in their supply of floral resources through the year and the ensuing temporal gaps in resource availability may be limiting pollinator populations in these environments (Timberlake et al., 2019). Such gaps are likely to be especially detrimental to pollinators such as bumblebees, whose long flight seasons make them particularly reliant upon a season-long supply of floral resources (Williams et al., 2012).

The addition of floral resources to farmland can increase bumblebee colony growth, the production of queens and drones, and nest density (Carvell et al., 2015, 2017). Resources are particularly important in the early spring, when queens are establishing and provisioning new colonies (Carvell et al., 2017; Malfi et al., 2019) and in the late summer, when new queens and males are produced (Rundlof et al., 2014). Indeed, the widespread loss of late-season floral resources is believed to be one of the major drivers of bumblebee decline in the UK (Balfour et al., 2018; Fitzpatrick et al., 2007). However, to our knowledge, no studies have directly compared the relative importance of floral resources in different periods of the year on wild bumblebee populations, though this information should be considered when designing agri-environment schemes for pollinators.

Aside from floral resources, a variety of landscape factors can influence bumblebee colony density, including proximity to gardens (Goulson et al., 2010) and the proportion of semi-natural habitat (Goulson et al., 2010; Knight et al., 2009). Semi-natural habitats on farmland may offer valuable nesting sites and floral resources for pollinators and some studies even use semi-natural habitat cover as a proxy for food availability or nesting sites, assuming a strong relationship between the two (Roulston & Goodell, 2011). However, this relationship is not simple, as different semi-natural habitat types can have very different resource provisioning value and their value can change dramatically through the year (Mallinger et al., 2016; Timberlake et al., 2019).

Whilst previous studies have shown the importance of various landscape factors and floral resources on bumblebee colony density, none have directly measured the relationship between these three

variables. The aim of our study is to understand how habitat composition influences the seasonality of floral resources and how both these factors affect *Bombus terrestris* colony density. This information is crucial if we are to understand the temporal component of floral resource limitation and the ways in which farming landscapes can be modified to increase the temporal continuity of floral resources for pollinators. There are three objectives to our study: (a) to quantify the landscape-scale floral resources available to pollinators on 12 farms during the four main flowering periods (early spring, late spring, summer and late summer); (b) to estimate bumblebee colony density on each farm and investigate which season of resource supply, type of floral resource (pollen or nectar) and habitat types have influenced colony density; (c) to use this information to predict the effect of common habitat improvement and habitat degradation scenarios on seasonal floral resources and bumblebee colony density, doing this via a spatially explicit predictive model. We hypothesise that a greater coverage of semi-natural flowering habitat will increase landscape floral resources, with positive effects on colony density. We predict that floral resources in early spring and late summer will be most strongly associated with colony density, as these are periods of low resource availability (Timberlake et al., 2019), coinciding with key stages in the bumblebee life cycle. We also expect the presence of gardens to be important, due to their abundant and long-lived floral resources.

2 | MATERIALS AND METHODS

The underlying rationale for the study was to understand how landscape composition and floral resources in different periods of the year affect bumblebee colony density. To do this, we measured the seasonal floral resource supply and landscape composition of 12 farms and investigated their relationship with the colony density of the commonly used model species, *B. terrestris*, estimated using a molecular approach. Once this relationship was understood, a spatially explicit model was used to predict the effect of six pollinator-friendly Environmental Stewardship (ES) options, six other habitat improvement scenarios and six habitat degradation scenarios on seasonal resource supplies and bumblebee colony density.

2.1 | Study sites

The study was undertaken in 2017 and 2018 on 12 medium-sized (mean 182 ha), mixed farms (dairy, sheep and arable) in the West of England (Figure S1). Farms were selected if they were >5 km from a major urban area with boundaries >3 km apart (greater than bumblebee foraging range—see Table S3); and representative of the wider

landscape, assessed using Google Earth. At each site, the centre of the farm was identified and a circle with a 1 km radius was drawn from this central point (Figure S2). All surveying took place within this circle. All study circles consisted of a mixture of pasture and arable land, with fields separated by hedgerows, field margins or semi-natural woodland. Arable crops included maize, wheat, barley and some oats. Most study sites also contained small areas of rural gardens and rough ground (waste areas such dumping grounds; Table S1).

2.2 | Objective 1: Characterising and quantifying floral resources for pollinators in the four main flowering periods

Floral resources were measured during four main flowering periods: summer (July 2017), late summer (September 2017), early spring (March 2018) and late spring (May 2018); these periods being identified as the four distinct phases of farmland floral phenology in this region (Timberlake et al., 2019). Floral resources in the summer and late summer affect the number of new queens produced by a colony (Crone & Williams, 2016) and the fat reserves needed for queen hibernation (Goulson, 2010), whilst spring floral resources affect colony establishment and survival (Carvell et al., 2017). Thus, all four floral resource sampling periods had the potential to influence colony density by summer 2018, when worker bees were sampled on each farm to estimate colony density (Figure 1).

To quantify floral resources, all flowering habitats with an overall landscape cover >1% were sampled for floral abundance, from which their nectar production could then be calculated using published data. These were: permanent pasture, semi-natural woodland, hedgerows and field margins. Gardens comprised a mean of 2% coverage of the study circles but were not sampled due to a lack of data on the nectar production of garden plants. The arable crops on these study farms were non nectar-producing, so were also not sampled in this study.

On each of the four sampling occasions, six 50-m transects were randomly placed in each of the sampled habitat types (i.e. 24 transects for a farm with all four habitat types). On each transect, the number of floral units of each forb species was recorded in 1 m^2 quadrats at 5-m intervals along its entire length (i.e. 10 quadrats per transect). For trees and shrubs, all floral units in a 5-m vertical column above

the quadrat were counted. Above this, the tree's height within the vertical column was estimated with a clinometer and the floral abundance values were multiplied up accordingly (Baude et al., 2016). A floral unit was defined as one or multiple flowers that can be visited by insects without flying (Carvalheiro et al., 2008). Values for the nectar sugar production of each species were from Baude et al. (2016) and Timberlake et al. (2019); these were multiplied by the number of open floral units per square metre, per habitat, to provide an estimate of the grams of sugar per unit area per 24-hr period for each habitat. This was multiplied by the habitat area in the sampling circle to provide an estimate of total nectar sugar availability during that period.

Pollen values were recorded by measuring the volume of individual pollen grains, the number of pollen grains per stamen, the number of stamens per flower and the mean number of flowers per floral unit to scale up pollen volume to the floral unit (Appendix S1). These data were collected prior to this study in 2011 and 2012. Pollen data were available for all the species collectively contributing 99% of floral abundance in each flowering period, with the exception of *Allium ursinum*. A substitute value for *A. ursinum* was input from a close taxonomic relative (*Allium cepa*). A sensitivity analysis was conducted by doubling and halving the pollen values from *A. cepa* and no effect on overall results was observed. Total landscape pollen supply could not be predicted due to a lack of data on the longevity of individual flowers from each species (unlike nectar, pollen is not replenished each day, so knowing floral longevity is crucial). However, we assumed that the *relative* pollen values among farms would be conserved, despite absolute values not being available, and we used these values to test for the influence of pollen availability on colony density (Objective 2). Finally, to investigate whether flowering habitat cover was a good proxy for floral resources, we regressed the overall area of semi-natural flowering habitat against the supply of nectar and pollen in each of the four flowering seasons.

2.3 | Objective 2: Estimating bumblebee colony density on study farms and factors driving its variation

Colony density was estimated using a molecular approach. Samples for analysis were from bee tarsi which were collected nonlethally from the right mid-leg of *B. terrestris* workers following Holehouse et al. (2003).

FIGURE 1 The stages in the lifecycle of a typical *Bombus terrestris* colony over the course of the study (2017–2018). Floral abundance surveys are marked with red arrows and coincide with the major lifecycle stages in the lead-up to bumblebee sampling for molecular colony density analysis in summer 2018 (black arrow)
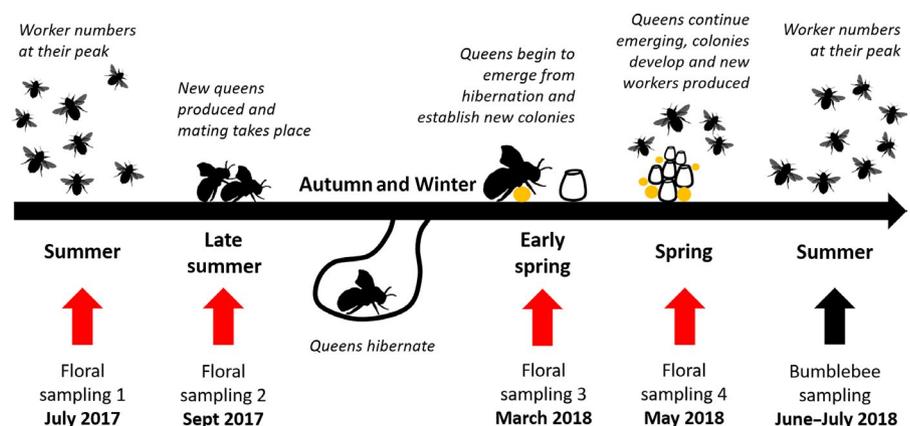
Pilot data confirmed that *B. terrestris* was the most abundant species on our study farms. Sampling took place in June and July 2018 with each farm sampled twice (once in June and once in July). Following Wood et al. (2015), the sampling area on each farm was divided into quarters (see Figure S2) and during each visit, the four quarters were sampled for 2 hr each by walking them haphazardly and catching all *B. terrestris* individuals encountered (Appendix S2).

2.3.1 | Molecular methods

DNA was extracted from the tarsal samples following the HotSHOT extraction protocol (Truett et al., 2000) and individuals genotyped at 14 *B. terrestris* microsatellite loci (see Appendix S3 and Table S2) following Dreier et al. (2014). PCR products were diluted and visualised on an ABI3730 Automated Capillary Sequencer (Applied Biosystems) using a ROX 500 size standard. Allele sizes were assigned using GeneMapper v4.1 and the genotype of each individual was recorded (Appendix S4). For each locus, the number of alleles, allele frequencies, observed and expected heterozygosity and estimated null allele frequency were calculated in Cervus version 3.0.7. Genepop 4.2 was used to check Hardy–Weinberg equilibrium and linkage disequilibrium (Appendix S5 and Table S2).

Workers were assigned to colonies using the program *Colony*, version 2.0.6.5 (Wang, 2013), which uses a maximum likelihood approach to determine sibship or parent–offspring relationships, based upon their multi-locus genotypes (Appendix S6). The number of colonies present on each farm were estimated using a DNA-based capture–recapture model (Two Innate Rate Model) implemented in CAPWIRE (Miller et al., 2005; Appendix S6).

Bumblebees forage over large distances, so many workers detected in the sampling area were likely to originate from colonies based outside this area. Thus, the original sampling area (radius 1 km) was extended by 1973 m—the maximum estimated foraging range of *B. terrestris*, taken as the mean of a range of published values (Table S3), and all colony density values were divided by this new total sampling area ($27.8\ \text{km}^2$) to give density estimates per km^2.

2.3.2 | Estimating the relationship between colony density, floral resources and habitat composition

We were interested in finding the combination of landscape and seasonal floral resource factors which best predicted bumblebee colony density. Linear regression models were used to investigate these relationships, but due to the limited sample size (12 farms), we included no more than two variables in a single model. Landscape and floral resource variables were therefore regressed against colony density in separate models and the top-ranking models from each component of the analysis were subsequently merged to find the overall top-ranking predictive model. Top-ranking models were identified as those with the highest adjusted R^2 value, lowest *p*-values and lowest akaike information criterion (AIC) value (differing by more than 2 points).

To test which period of the year showed the strongest association between floral resources and colony density, nectar supply in each main flowering period (March, May, July and September) was regressed against bumblebee colony density in four separate linear models. To investigate whether the availability of pollen was influential, estimates of pollen supply in each season were added as a factor to each of the four linear models, and the models with and without pollen, as well as pollen individually (i.e. without nectar) were compared (Table S4). The total richness of flowering species was also regressed against colony density. For the analysis of landscape factors, the total area of semi-natural flowering habitat, as well as the area of each semi-natural habitat individually (pasture, hedgerow, field margin and woodland), and gardens (measured using QGIS v.2.12.3), were regressed against colony density. Landscape factors significantly associated with colony density were added to the top-ranking floral resource model to test whether they improved its fit to the data. Finally, a piecewise structural equation modelling (SEM) approach was used to investigate the direct (e.g. nesting sites) and indirect (via floral resources) effects of landscape composition on colony density and confirm our choice of predictive model (Appendix S7).

All analyses were carried out in R, version 3.5.1 (R Core Team, 2018). The normality of residuals, homoscedasticity, heteroscedasticity and linearity of each model were checked using diagnostic plots and variables were log-transformed, as necessary. Models were run with and without outliers to test their influence on the results. Collinearity between variables was checked using variance inflation factors (VIF) and variables were standardised (divided by the mean) if VIFs exceeded 5; this reduced them below 5 in all cases.

2.4 | Objective 3: Modelling the effect of common habitat improvement and habitat degradation scenarios on bumblebee colony density

Habitat manipulation scenarios were applied in-silico to the 12 farms to investigate the effect of different management options on seasonal nectar supplies and *B. terrestris* populations. Using field data on the nectar productivity of different habitats and plant species (see Objective 1 results), alongside data on the nectar productivity of Environmental Stewardship (ES) Scheme flowering margins (Baude et al., 2016; Boatman & Conyers unpublished), we modelled the impact of six common ES options (Natural-England, 2013) and six other pollinator-friendly management scenarios. We also modelled six scenarios of habitat or plant species loss to investigate the sensitivity of *B. terrestris* populations to different types of habitat degradation. All 18 scenarios are listed in Table 1. The simulated scenarios were applied to each farm individually, taking into account their existing habitat composition and seasonal nectar production, thus each simulation was a bespoke treatment for each farm, these being then treated as 12 independent replicates for the manipulation in question. The impact of each scenario was modelled in three stages:

TABLE 1 Simulated scenarios used in the habitat manipulation model. Scenarios are grouped into Environmental Stewardship options (ES), other habitat improvement options (HI) and habitat degradation scenarios (HD). Where relevant, the ES code is given (Natural-England, 2013), along with the standard area to which this is applied (units), and the number of points associated with the option. See Figure S7 for examples of 'high' and 'low quality' habitats on the study farms

	Scenario code	ES code	Units	Points	Manipulation details
Environmental Stewardship options	ES1	EF4	5 ha	2,250	All existing field-margins (mean of 5 ha/km^2) replaced with EF4 pollen and nectar mix (standard cut)
	ES2	EF4	1 ha	450	1 ha of pasture replaced with EF4 pollen and nectar mix (standard cut)
	ES3	EF4	1 ha	450	1 ha of pasture replaced with EF4 pollen and nectar mix (late June cut)
	ES4	EB3	500 m	210	500 m of hedgerow raised to highest recorded nectar provisioning quality (simulating management for wildlife)
	ES5	EK2	1 ha	85	1 ha of pasture raised to highest recorded nectar provisioning quality (simulating reduced chemical inputs)
	ES6	EJ13	1 ha	65	1 ha red clover cover crop added to an arable area of each farm
Habitat improvement scenarios	HI1	NA	All	NA	Pasture area expanded by 10% onto arable land
	HI2	NA	All	NA	Woodland area expanded by 10% onto arable land
	HI3	NA	NA	NA	Hedgerow area expanded by 10% onto arable land
	HI4	NA	NA	NA	Margin area expanded by 10% onto arable land
	HI5	NA	NA	NA	All below average hedgerows raised to mean recorded nectar provisioning quality
	HI6	NA	NA	NA	All below average pasture raised to mean recorded nectar provisioning quality
Habitat degradation scenarios	HD1	NA	NA	NA	10% of pasture area lost and replaced with arable land
	HD2	NA	NA	NA	10% of woodland area lost and replaced with arable land
	HD3	NA	NA	NA	10% of hedgerow area lost and replaced with arable land
	HD4	NA	NA	NA	10% of margin area lost and replaced with arable land
	HD5	NA	NA	NA	Complete loss of *Hedera helix* from farms
	HD6	NA	NA	NA	Complete loss of *Cirsium arvense* from farms

1. For each habitat or plant species that was 'added' or 'removed' in the manipulation scenario, its nectar productivity value was added (or subtracted) from the total nectar supply of each individual farm to give a predicted post-simulation nectar value for each of the 12 farms.
2. The linear regression model which best explained bumblebee colony density, based upon nectar productivity and habitat composition (see Objective 2 results), was used to predict changes in bumblebee colony density as a result of the altered nectar productivity on each farm.
3. The percentage change in bumblebee colony density from pre-simulation density to post-simulation density was then calculated for each farm, this providing a standardised metric by which to measure the effectiveness of each manipulation scenario among farms.

We made the assumption that the relationship between nectar supply and colony density remains linear within the range of values investigated, rather than levelling off at a certain point as other factors become limiting. The true effects of the habitat manipulations are therefore unlikely to be as great as those predicted by the model; nevertheless, the model provides a way to directly compare different ES schemes and other habitat changes in a spatially explicit manner.

3 | RESULTS

3.1 | Objective 1: Characterising and quantifying floral resources for pollinators in the four main flowering periods

During 48 visits to the 12 farms, a total of 158,127 individual floral units from 133 flowering plant species were counted. A two-way ANOVA showed the supply of nectar at the landscape scale differed significantly between flowering periods ($F = 4.181$, df: 3, $p = 0.01$, Figure S3), with highest mean nectar production in May, followed by July, September and March (Table 2). Within each flowering period, habitats varied greatly in their nectar production (Figure 2). Pasture was the dominant source of nectar in March (40% ±12 of total supply) and July (50% ±6), largely because of its extensive coverage (48.3% ±6.1) of the study area. Woodland, when present, covered an average of just 5.8% (±0.04) of farms but produced most of the nectar in May (37% ±12 *SE*). Hedgerows produced the majority of nectar in September (65% ±8 *SE*), despite covering only 5.89% (±0.02 *SE*) of the study sites. While nectar was produced by 133 plant species, a few species dominated the supply (Figure S6), with three species providing >75% of the total nectar supply in each

TABLE 2 Seasonal nectar values (all habitats merged) and bumblebee colony sampling results for each of the 12 study farms. The number of bumblebee workers collected and genotyped from each farm are shown alongside the molecular-based estimates of colony density

| Farm code	Seasonal nectar production (g km^{-2} day^{-1})				Workers genotyped	*Bombus terrestris* colony density (nests/km^2)
	March	May	July	Sept		
B	0.45	3,832.82	1,103.91	1,589.44	60	11.8
CHH	4.96	3,025.49	2,790.36	867.15	71	20.2
CHM	0.22	982.54	7,073.12	1,569.29	71	72.0
CN	30.57	1,559.33	5,408.98	2,425.66	87	21.1
CW	2.73	1,097.41	1,564.03	693.90	73	8.5
EM	2.65	22,969.40	1,589.38	775.57	55	9.0
ET	1.73	2,521.58	3,992.38	763.41	78	12.1
LH	0.04	2,025.18	1,400.37	3,014.37	88	72.0
LM	0.43	34,478.18	1,090.83	767.58	81	11.7
MN	4.02	2,002.24	3,019.04	1,346.26	62	15.4
OB	4.34	6,301.29	5,302.82	3,064.66	80	38.5
PH	0.08	807.13	2,901.22	670.49	80	12.0
Mean	4.35	6,800.22	3,103.04	1,462.31	73.83	25.4
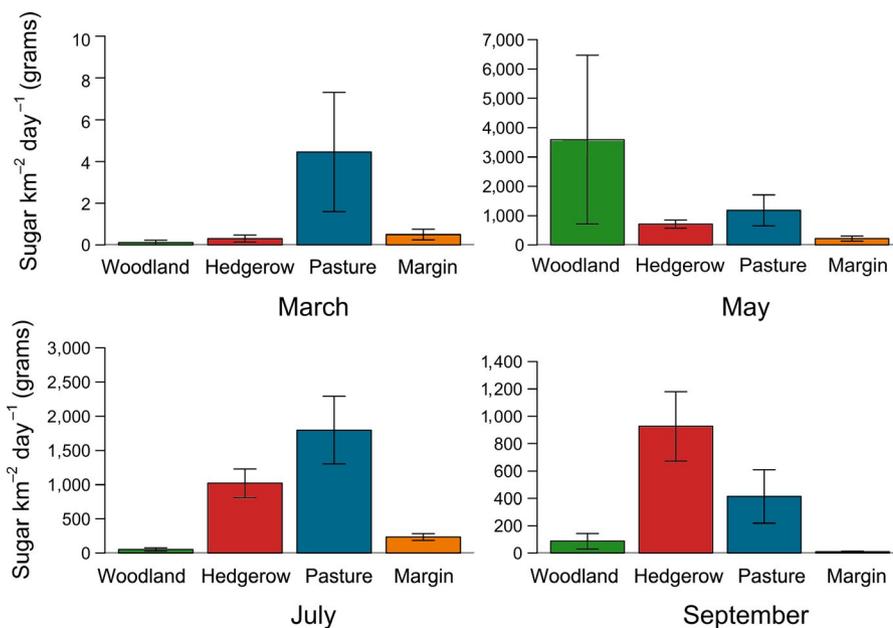
FIGURE 2 Total daily sugar production of each habitat type (represented by different colours) in each of the four sampling periods. Values displayed are the mean of the 12 study farms, $\pm SE$. Note the different *y*-axis scale on each graph

period. In March these were: *Bellis perennis*, *Lamium purpureum* and *Cardamine flexuosa*; in May: *Allium ursinum*, *Taraxacum officinale* and *Anthriscus sylvestris*; in July: *Trifolium repens*, *Rubus fruticosus* and *Cirsium arvense* and in September: *Hedera helix*, *Cirsium arvense* and *Taraxacum officinale*.

Contrary to our predictions, the proportional area of semi-natural flowering habitat was not associated with nectar supply in any period of the year, implying flowering habitat coverage is not always a good proxy for floral resources (March: Adj. R^2: 0.18, $F = 3.48$, $p = 0.092$; May: Adj. R^2: −0.07, $F = 0.31$, $p = 0.592$; July: Adj. R^2: 0.09, $F = 2.11$, $p = 0.176$; Sept: Adj. R^2: 0.20, $F = 3.80$, $p = 0.080$). The proportional area of semi-natural flowering habitat was associated with pollen supply only in September (Sept: Adj. R^2: 0.45, $F = 10.06$,

$p = 0.010$); there was no significant relationship between these two variables in March (Adj. R^2: 0.02, $F = 1.23$, $p = 0.293$), May (Adj. R^2: −0.08, $F = 0.16$, $p = 0.694$) or July (Adj. R^2: −0.07, $F = 0.29$, $p = 0.605$).

3.2 | Objective 2: Estimating bumblebee colony density on study farms and factors driving its variation

A total of 886 *B. terrestris* individuals, with a mean of 74 ($\pm$10 *SE*) per farm, were genotyped at 12–14 microsatellite loci. Two individuals were shown by molecular analysis to be incorrectly identified (both *Bombus lucorum*) and these were excluded from further

analysis. Molecular data revealed 304 unique colonies on the 12 farms (Table 2) and an estimated colony density ranging from 9 to 72 nests/km^2, with a mean density of 25 nests/km^2 (± 6 SE).

Colony density was significantly associated with the supply of nectar in September (Adj. R^2: 0.52, $F = 12.91$, $p = 0.005$), but not in March, May or July (March: Adj. R^2: 0.01, $F = 1.11$, $p = 0.318$; May: Adj. R^2: -0.01, $F = 0.90$, $p = 0.366$; July: Adj. R^2: 0.12, $F = 2.57$, $p = 0.140$, Figure 3, Table S4). However, when September floral abundance, rather than September nectar was used as an explanatory variable, it was not a significant predictor of colony density (Adj. R^2: -0.09, $F = 0.046$, $p = 0.834$), implying floral abundance alone may not always be a good pro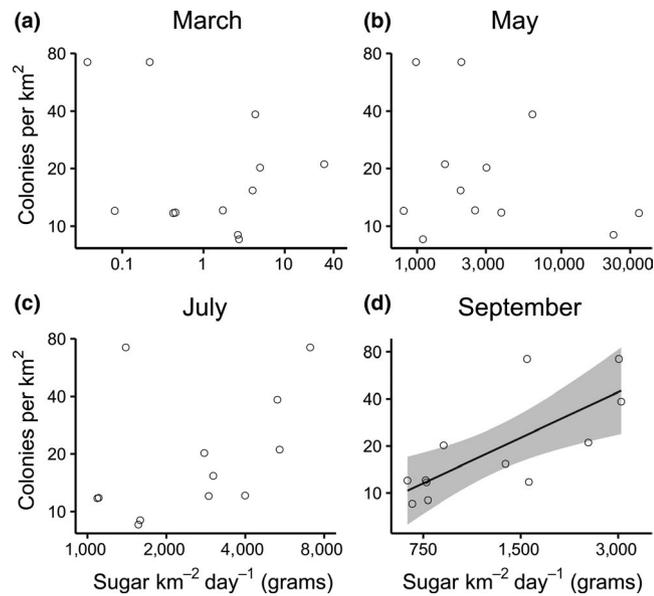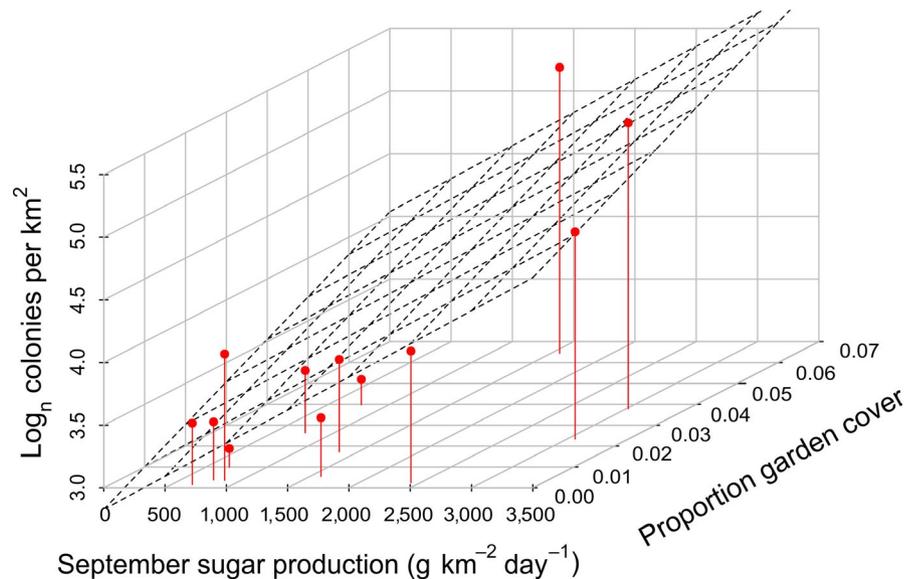xy for resource availability. Pollen supply in September was significantly associated with colony density, though it did not improve the fit of the September nectar-colony density model (Table S4), implying this relationship was merely a result of the collinearity between pollen and nectar supply (Figure S4). Indeed, adding pollen availability as an additional variable in the nectar-colony density models did not improve their fit in any case (Table S4). The species richness of flowering plants was also not associated with colony density (Table S4).

No significant correlation was found between the proportional area of any semi-natural flowering habitats and colony density (Table S4). However, the proportional coverage of gardens was significantly associated with colony density (Adj. R^2: 0.29, $F = 5.58$, $p = 0.040$), despite gardens only covering a mean of 2% (± 0.5 SE) of the study sites. The multiple linear regression model with both September nectar supply and proportional garden cover as explanatory variables explained more of the variation in colony density among farms and had a lower AIC score and p-value than any other model (Adj. R^2: 0.68, $F = 12.94$, September nectar $p = 0.005$, garden cover $p = 0.029$, Figure 4, Table S4). This multiple linear regression model was used in Objective 3 to predict changes in B. terrestris colony density in response to habitat manipulations. The piecewise SEM corroborated this choice of predictive model, as garden cover and September nectar were the only significant causal paths to colony density in the SEM and semi-natural flowering habitat cover showed no significant direct or indirect effects on colony density (Figure S5).

FIGURE 3 Relationships between farmland nectar supply in (a) March, (b) May, (c) July and (d) September and *Bombus terrestris* colony density. Where significant, a regression line and 95% confidence intervals (grey shading) are fitted to the data. Note the logarithmic scale on each plot

3.3 | Objective 3: Modelling the effect of common habitat improvement and habitat degradation scenarios on bumblebee colony density

The greatest predicted increase in bumblebee colony density (413% ± 190 SE) was achieved by adding 1 ha of the standard Environmental Stewardship (ES) pollen and nectar (EF4) mix to the margins of arable fields and cutting this in June to extend its flowering period until September (scenario ES3, Table 1; Figure 5; Table S5). In contrast,
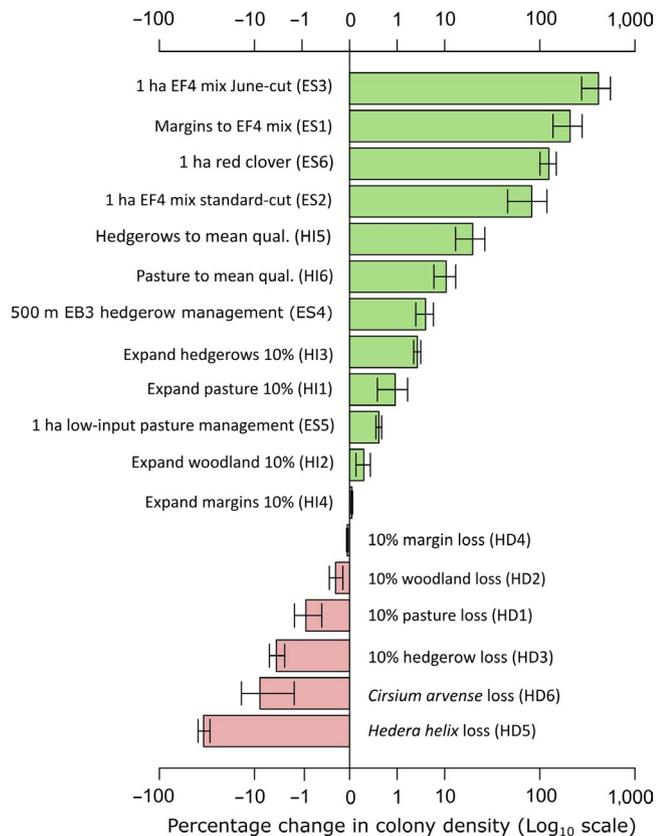

FIGURE 4 Three-dimensional scatterplot showing the relationship between September sugar supply ($p = 0.005$), proportional garden cover ($p = 0.029$) and Log$_n$*Bombus terrestris* colony density on the 12 study farms. A regression plane (dotted black lines) based upon the top-ranking linear regression model (Adj. R^2: 0.68, $p = 0.002$, akaike information criterion: 17.97) is fitted to the datapoints for individual farms (shown in red)

FIGURE 5 Output of the habitat manipulation models showing percentage change in *Bombus terrestris* colony density resulting from each scenario. Values shown are the mean result ($\pm SE$) for the 12 study farms to which each manipulation scenario was applied individually. Note the logarithmic scale on the *x*-axis. Habitat improvement scenarios are shown in green, whilst habitat or species loss scenarios are shown in red. See Table 1 for more details on each manipulation

adding 1 ha of EF4 mix (scenario ES2), or even sowing *all* margins with EF4 mix (scenario ES1), but cutting these at the standard time of August/September, predicted an increase in colony density of 81% (± 46 *SE*) and 207% (± 71 *SE*) respectively. Adding a 1 ha cover crop of red clover to each square kilometre of farmland (scenario ES6) predicted an increase in September nectar production of 141% (± 21 *SE*) and bumblebee colony density of 124% (± 27 *SE*). Increasing the area of semi-natural flowering habitats on farmland (scenarios HI1-HI4) had minimal effect on September nectar supply (<7% increase) and colony density (<6% increase) because the September nectar supply of these habitats is already so low that increasing their area has little effect. However, raising the quality of below-average hedgerows and permanent pasture fields to the mean nectar provisioning quality recorded for these habitats (scenarios HI5–HI6), was predicted to increase September nectar production by 31% (± 12 *SE*) and 19% (± 6.2 *SE*) respectively, and bumblebee colony density by 19% (± 6.7 *SE*) and 9.4% (± 2.7 *SE*) respectively. Of all the plant species flowering on farmland in September, losing ivy *Hedera helix*, was predicted to have the most negative effect, followed by *Cirsium arvense* (a decrease in September nectar production of 64% ± 8.1 and 11% ± 4.9,

respectively and a decrease in colony density of 33.2% ± 5.1 and 7.8% ± 5.2 respectively; Figure 5).

4 | DISCUSSION

Farmland nectar supplies varied substantially among habitats and sampling periods on the 12 farms with less than three plant species providing the majority of nectar in each period. Late-summer (September) nectar resources were strongly associated with *B. terrestris* colony density in the following year, with garden cover also important. The habitat manipulation model demonstrated the potential for different farmland management scenarios to substantially increase or decrease the supply of late-summer nectar resources, with corresponding effects on colony density. Overall, our results suggest that the seasonality of resource supply could be as important as total resource availability in limiting pollinator populations. They also highlight the importance of directly measuring floral resources, such as pollen and nectar, rather than just inferring them from floral abundance or flowering habitat cover. In what follows, we discuss the role that seasonal floral resources and landscape factors play in driving bumblebee population dynamics, highlighting the management implications of these findings and discussing the limitations of our data.

4.1 | The effect of floral resources on bumblebee colony density

The availability of nectar during September was a strong predictor of *B. terrestris* colony density in the following year, explaining over half of the variation in colony density and suggesting it may be an important factor limiting bumblebee populations. This is likely due to the limited availability of farmland nectar during September (Baude et al., 2016; Timberlake et al., 2019), coinciding with the production of new queens and males (Figure 1) and a time when queens begin their physiological preparations for hibernation, which requires a nectar-rich diet (Costa et al., 2020). Our findings are consistent with Rundlof et al. (2014) who report significantly higher bumblebee queen densities in landscapes with late-flowering red clover fields. Though our study was limited to *B. terrestris*, the results are likely to be applicable to many other farmland bumblebee species, especially those with similar phenologies and diet breadth.

Contrary to our predictions, the inclusion of pollen data did not improve our ability to predict colony density values in any season and there was no effect of either pollen or nectar availability on colony density in the early spring, despite this being a period of resource deficit (Timberlake et al., 2019). Pollen is a crucial protein and lipid-rich resource for developing colonies in early spring (Goulson, 2010; Kämper et al., 2016), so the lack of any effect of early spring pollen availability was surprising. It is possible that our use of relative pollen values rather than absolute values, or the collinearity between pollen and nectar, limited our ability to detect its effect on colony density. Pollen from non-forbs (e.g. grasses) were also not included in

our estimates, despite being utilised by bumblebees (Timberlake *et al.* unpublished). Furthermore, variation in floral resource supply among farms in March was low and only 20% of *B. terrestris* queens were recorded emerging in March in this region (BeeWalk, 2018), thus floral resources in March would not have influenced the survival of the majority of bees. Given the importance of September floral resources recorded in this study, it is concerning that very few studies record pollinators or floral resources beyond August. Though most British pollinators and flowering plant species are at their peak in mid-summer (Balfour et al., 2018), studies limited to this period of the year are likely to miss important population dynamics such as the production of males and new queens in late summer which must mate and forage prior to hibernation (Goulson, 2010).

4.2 | The landscape factors driving bumblebee colony density

The proportion of garden cover in each sampling circle had a significantly positive effect on *B. terrestris* colony density, despite a very low landscape coverage. This corresponds with previous studies which report increased bumblebee nest density, growth rates and survival with proximity to gardens (Goulson et al., 2010; Samuelson et al., 2018). This may be a result of the more continuous and diverse floral resources in gardens, or their increased availability of nesting sites. However, our results, which show an even stronger association between colony density and September nectar supply, suggest that small areas of garden may not be sufficient to counteract the September resource bottleneck in this region.

Landscape context is important in determining pollinator populations in farmland and several studies have detected a positive relationship between the proportion of semi-natural habitat and bumblebee colony density e.g. Knight et al. (2009); but see Herrmann et al. (2007). In our study, it was not semi-natural habitat cover per se which explained the variation in colony density, rather it was the nectar provisioning of the later-flowering, nectar-rich plant species such as ivy *Hedera helix* and *Cirsium arvense* within these habitats, which proved important. Semi-natural habitat cover is sometimes used as a proxy for floral resource availability; however, it has been suggested that where possible, floral resources should be measured directly rather than inferred (Roulston & Goodell, 2011). Our study directly measured nectar and pollen resources in each main flowering period of the year, as well as the coverage of different habitats, and showed that semi-natural habitat cover does not serve as a good proxy for floral resources.

4.3 | Management implications

The importance of September nectar resources detected in this study reinforces the point that the common management practice of cutting flowering margins at the start of September is inappropriate for pollinators with long flight seasons such as bumblebees (Dicks et al., 2015). Instead, and as prescribed in the revised EF4 scheme

(Natural-England, 2013), it is advisable to mow at least some areas in late June, or in multiple phases, to delay flowering and thereby increase floral resource supplies in September (Pywell et al., 2011). Increasing the proportions of long-flowering or late-flowering forage plants in these mixes, would also be advisable. The use of cover or forage crops such as red clover, *Phacelia* spp. and borage have declined over time (Goulson et al., 2005), but our results suggest that including small areas of these crops on farmland, particularly later flowering species such as red clover, may significantly boost bumblebee populations. Earlier-flowering crops such as oilseed rape are likely to be less important, as their flowering coincides with the peak of farmland nectar supply in our study region (Timberlake et al., 2019). Managing semi-natural farmland habitats to increase the densities of late-flowering species (e.g. *Hedera helix* and *Cirsium arvense*) and long-flowering species (e.g. *Taraxacum officinale)*, will further reduce the late-summer resource bottleneck. These interventions are likely to be most effective in structurally and temporally simple landscapes such as arable farmland, as their influence will be proportionally greater. Interventions should be maintained year after year however, to ensure long-term benefits for bumblebee populations. Hedgerows provided most of the farmland nectar during September, highlighting their importance for pollinators. Field margins, however, were the least nectar-rich habitat type in September but could be made the most nectar-rich and theoretically support three times as many bumblebee colonies, if sown with the standard EF4 pollen and nectar mix (Figure 5). Finally, our results indicate that small areas of gardens within an agricultural landscape may play an important role in supporting bumblebee populations.

5 | CONCLUSIONS

Overall, our results demonstrate that by understanding the seasonality of floral resource supply and the effect of this on pollinator population dynamics, ecologists and land managers will be better able to design targeted management practices to support wild pollinator populations.

AUTHORS' CONTRIBUTIONS

T.P.T. and J.M. conceived the ideas and designed the methodology; T.P.T. collected the data; T.P.T. and I.P.V. analysed the data; M.B.

provided the nectar and pollen data; T.P.T. and J.M. led the writing of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

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REFERENCES

Balfour, N. J., Ollerton, J., Castellanos, M. C., & Ratnieks, F. L. W. (2018). British phenological records indicate high diversity and extinction rates among late-summer-flying pollinators. *Biological Conservation*, *222*, 278–283. https://doi.org/10.1016/j.biocon.2018.04.028

Baude, M., Kunin, W. E., Boatman, N. D., Conyers, S., Davies, N., Gillespie, M. A. K., Morton, R. D., Smart, S. M., & Memmott, J. (2016). Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature*, *530*, 85–88. https://doi.org/10.1038/nature16532

Beewalk Dataset. (2018). figshare. Dataset, https://doi.org/10.6084/m9.figshare.12280547.v1

Carvalheiro, L. G., Barbosa, E. R. M., & Memmott, J. (2008). Pollinator networks, alien species and the conservation of rare plants: *Trinia glauca* as a case study. *Journal of Applied Ecology*, *45*, 1419–1427.

Carvell, C., Bourke, A. F. G., Dreier, S., Freeman, S. N., Hulmes, S., Jordan, W. C., Redhead, J. W., Sumner, S., Wang, J., & Heard, M. S. (2017). Bumblebee family lineage survival is enhanced in high-quality landscapes. *Nature*, *543*, 547–549. https://doi.org/10.1038/nature21709

Carvell, C., Bourke, A. F. G., Osborne, J. L., & Heard, M. S. (2015). Effects of an agri-environment scheme on bumblebee reproduction at local and landscape scales. *Basic and Applied Ecology*, *16*, 519–530. https://doi.org/10.1016/j.baae.2015.05.006

Costa, C. P., Duennes, M. A., Fisher, K., Der, J. P., Watrous, K. M., Okamoto, N., Yamanaka, N., & Woodard, S. H. (2020). Transcriptome analysis reveals nutrition- and age-related patterns of gene expression in the fat body of pre-overwintering bumble bee queens. *Molecular Ecology*, *29*(4), 720–737. https://doi.org/10.1111/mec.15361

Crone, E. E., & Williams, N. M. (2016). Bumble bee colony dynamics: Quantifying the importance of land use and floral resources for colony growth and queen production. *Ecology Letters*, *19*, 460–468. https://doi.org/10.1111/ele.12581

Dicks, L. V., Baude, M., Roberts, S. P. M., Phillips, J., Green, M., & Carvell, C. (2015). How much flower-rich habitat is enough for wild pollinators? Answering a key policy question with incomplete knowledge. *Ecological Entomology*, *40*, 22–35. https://doi.org/10.1111/een.12226

Dreier, S., Redhead, J. W., Warren, I. A., Bourke, A. F. G., Heard, M. S., Jordan, W. C., Sumner, S., Wang, J., & Carvell, C. (2014). Fine-scale spatial genetic structure of common and declining bumble bees across an agricultural landscape. *Molecular Ecology*, *23*, 3384–3395. https://doi.org/10.1111/mec.12823

Fitzpatrick, Ú., Murray, T. E., Paxton, R. J., Breen, J., Cotton, D., Santorum, V., & Brown, M. J. F. (2007). Rarity and decline in bumblebees – A test of causes and correlates in the Irish fauna. *Biological Conservation*, *136*, 185–194. https://doi.org/10.1016/j.biocon.2006.11.012

Goulson, D. (2010). *Bumblebees: Behaviour, ecology, and conservation* (2nd ed.). Oxford University Press.

Goulson, D., Hanley, M. E., Darvill, B., Ellis, J. S., & Knight, M. E. (2005). Causes of rarity in bumblebees. *Biological Conservation*, *122*, 1–8. https://doi.org/10.1016/j.biocon.2004.06.017

Goulson, D., Lepais, O., O'Connor, S., Osborne, J. L., Sanderson, R. A., Cussans, J., Goffe, L., & Darvill, B. (2010). Effects of land use at a landscape scale on bumblebee nest density and survival. *Journal of Applied Ecology*, *47*, 1207–1215. https://doi.org/10.1111/j.1365-2664.2010.01872.x

Goulson, D., Nicholls, E., Botias, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, *347*, 1255957. https://doi.org/10.1126/science.1255957

Herrmann, F., Westphal, C., Moritz, R. F. A., & Steffan-Dewenter, I. (2007). Genetic diversity and mass resources promote colony size and forager densities of a social bee (*Bombus pascuorum*) in agricultural landscapes. *Molecular Ecology*, *16*, 1167–1178.

Holehouse, K. A., Hammond, R. L., & Bourke, A. F. G. (2003). Non-lethal sampling of DNA from bumble bees for conservation genetics. *Insectes Sociaux*, *50*, 277–285. https://doi.org/10.1007/s00040-003-0672-6

Kämper, W., Werner, P. K., Hilpert, A., Westphal, C., Blüthgen, N., Eltz, T., & Leonhardt, S. D. (2016). How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*. *Landscape Ecology*, *31*, 2245–2258. https://doi.org/10.1007/s10980-016-0395-5

Knight, M. E., Osborne, J. L., Sanderson, R. A., Hale, R. J., Martin, A. P., & Goulson, D. (2009). Bumblebee nest density and the scale of available forage in arable landscapes. *Insect Conservation and Diversity*, *2*, 116–124. https://doi.org/10.1111/j.1752-4598.2009.00049.x

Malfi, R. L., Crone, E., & Williams, N. (2019). Demographic benefits of early season resources for bumble bee (*B. vosnesenskii*) colonies. *Oecologia*, *191*, 377–388. https://doi.org/10.1007/s00442-019-04472-3

Mallinger, R. E., Gibbs, J., & Gratton, C. (2016). Diverse landscapes have a higher abundance and species richness of spring wild bees by providing complementary floral resources over bees' foraging periods. *Landscape Ecology*, *31*, 1523–1535. https://doi.org/10.1007/s10980-015-0332-z

Miller, C. R., Joyce, P., & Waits, L. P. (2005). A new method for estimating the size of small populations from genetic mark-recapture data. *Molecular Ecology*, *14*, 1991–2005. https://doi.org/10.1111/j.1365-294X.2005.02577.x

Natural-England. (2013). *Environmental stewardship handbook: Entry level stewardship*. Natural England Peterborough.

Ogilvie, J. E., & Forrest, J. R. K. (2017). Interactions between bee foraging and floral resource phenology shape bee populations and communities. *Current Opinion in Insect Science*, *21*, 75–82. https://doi.org/10.1016/j.cois.2017.05.015

Pywell, R. F., Meek, W. R., Hulmes, L., Hulmes, S., James, K. L., Nowakowski, M., & Carvell, C. (2011). Management to enhance pollen and nectar resources for bumblebees and butterflies within intensively farmed landscapes. *Journal of Insect Conservation*, *15*, 853–864. https://doi.org/10.1007/s10841-011-9383-x

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Roulston, T. H., & Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annual Review of Entomology*, *56*(56), 293–312. https://doi.org/10.1146/annurev-ento-120709-144802

Rundlof, M., Persson, A. S., Smith, H. G., & Bommarco, R. (2014). Late-season mass-flowering red clover increases bumble bee queen and male densities. *Biological Conservation*, *172*, 138–145. https://doi.org/10.1016/j.biocon.2014.02.027

Samuelson, A. E., Gill, R. J., Brown, M. J. F., & Leadbeater, E. (2018). Lower bumblebee colony reproductive success in agricultural compared with urban environments. *Proceedings of the Royal Society B: Biological Sciences*, *285*. https://doi.org/10.1098/rspb.2018.0807

Timberlake, T. P., Vaughan, I. P., Baude, M., & Memmott, J. (2020). Data from: Bumblebee colony density on farmland is influenced by

late-summer nectar supply and garden cover. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.931zcrjjb

Timberlake, T. P., Vaughan, I. P., & Memmott, J. (2019). Phenology of farmland floral resources reveals seasonal gaps in nectar availability for bumblebees. *Journal of Applied Ecology*, *00*, 1–12.

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., & Warman, M. L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques*, *29*, 52–54. https://doi.org/10.2144/00291bm09

Wang, J. L. (2013). A simulation module in the computer program colony for sibship and parentage analysis. *Molecular Ecology Resources*, *13*, 734–739.

Williams, N. M., Regetz, J., & Kremen, C. (2012). Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. *Ecology*, *93*, 1049–1058. https://doi.org/10.1890/11-1006.1

Wood, T. J., Holland, J. M., Hughes, W. O. H., & Goulson, D. (2015). Targeted agri-environment schemes significantly improve the population size of common farmland bumblebee species. *Molecular Ecology*, *24*, 1668–1680. https://doi.org/10.1111/mec.13144

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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