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Interactions among global change pressures act in a non-additive way on bumblebee individuals and colonies

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Abstract

1. Insect-mediated pollination is fundamental for terrestrial ecosystems as well as for crop production. However, bee populations are declining as a consequence of different global change pressures. Even though these pressures do not act in isolation, their interactive effects have received less attention.
2. Through a cross-treatment field experiment with *Bombus terrestris* colonies, we analysed the joint effect of climate warming, exposure to pesticides and landscape transformation on bumblebee development variables related to individual and colony fitness.
3. Our results show that an average increase of 1.62°C in the presence of high resource availability in the landscape increases the number of individuals of all colony castes. However, temperature increase is only favourable when foraging resources are not limiting. This is partly because under elevated temperatures, colonies can develop faster but also need to invest more in wax and silk material to protect the offspring and to cool the colony. We also found a decrease in the number of male production with exposure to pesticides.
4. At the individual level, colonies exposed to increased temperatures produced larger queens and workers, and at the same time, all castes presented less variability in body size. In addition, we observed a reduction in queen size with increasing resource availability.
5. Our experiment indicates that the interaction between global change pressures can be non-additive and that colony-level emergent properties of social species could buffer some of the individual impacts of these pressures. Hence, it is necessary to consider not only the joint effects of global change pressures but also the plasticity of the organisms' responses. This would help us to predict how populations will respond to these stressors in a complex real world and how species life-history traits could modulate their adverse effects through complex phenotypic plastic responses.

KEYWORDS
antagonisms, *Bombus terrestris*, climate change, homeostasis, landscape transformation, neonicotinoid, pollinator, synergies

1 | INTRODUCTION

During the last century, ecosystems throughout the world have faced an unprecedented level of rapid human-induced environmental change (MEA, 2005). Landscape transformation, climate change, pollution, overexploitation and alien species are components of global change with increasing impacts on species conservation and their functions (Cardinale et al., 2012).

These components of global change do not act in isolation, but can interact to accelerate biodiversity loss (Sala et al., 2000) and magnify their individual adverse impacts (Chapin et al., 2000). While these interactive effects are often perceived as synergistic, (i.e. the accumulated result of several stressors is greater than the additive sum of the results produced by each in isolation), they can also be antagonistic, with the accumulated result being less than additive (Darling & Côté, 2008; Folt et al., 1999; Galic et al., 2018). However, in spite of some evidence mainly from controlled experiments of selected pairwise interactive effects between global change components (Dance et al., 2017; Hoover et al., 2012; Leza et al., 2018; Tylianakis et al., 2008), the interactions among most global change pressures have been scarcely explored in realistic field conditions, especially for mobile organisms (Didham et al., 2007; Mora et al., 2007). Overall, there is a need for experimental studies on how species are affected simultaneously by multiple pressures (Côté et al., 2016; Darling & Côté, 2008).

There is a rising concern that some pollinator populations are declining due to global change pressures (Goulson et al., 2015). Pollinators play a key ecosystem role, with 87% of the world's angiosperm plant species relying at least partially on animal pollination (Ollerton et al., 2011). Furthermore, they provide a crucial ecosystem service for human welfare through the pollination of 75% of food crops (Klein et al., 2007). Pollinator population decreases have been associated with different global change pressures (Brown & Patxon, 2009; Potts et al., 2010) such as climate warming (Kerr et al., 2015; Memmott et al., 2007), biological invasions (Abe et al., 2010; Moron et al., 2009), the spread of pathogens (Cameron et al., 2011) and landscape alteration (Garibaldi et al., 2011; Winfree et al., 2011). The latter includes agricultural intensification (Kremen et al., 2002; Tscharntke et al., 2005) and the consequent exposure to pesticides such as neonicotinoids (Whitehorn et al., 2012; Woodcock et al., 2017). However, the combined effects of global change pressures on animal-mediated pollination remain elusive (González-Varo et al., 2013).

Bumblebees are important crop and wild plant pollinators, and are a taxon of major conservation concern worldwide (Cameron & Sadd, 2019). Declines in their populations have been mainly related to a reduction in nesting sites and preferred foraging resource availability in transformed agricultural landscapes (Knight et al., 2009). Climate warming is also affecting the phenology and distribution of bumblebee populations (Bartomeus et al., 2013; Kerr et al., 2015; Ogilvie et al., 2017) with potential consequences for their persistence, with an increasing risk of local extinction rates, colonisation and site occupancy with extreme heat events (Soroye et al., 2020).

For species with eusocial life histories such as bumblebees, increases in temperature may have effects both at the colony level and at the individual level. For example, Holland and Bourke (2015) found that increasing rearing temperatures by a few degrees increased *Bombus terrestris* colony sizes, queen production and overall colony lifespan but had no effect at all on individual longevity. Finally, the interaction of pollinating insects with human-dominated landscapes exposes them to agrochemicals such as herbicides, fungicides and insecticides, many of which are toxic to pollinators (Goulson et al., 2015). Of the pesticides to which bees are likely to be exposed, neonicotinoids have attracted most attention because numerous studies have indicated sub-lethal effects of neonicotinoid insecticides on bumblebees including a reduction in colony development (Gill et al., 2012), decreased foraging activity (Gill & Raine, 2014) and impaired reproductive success (Laycock et al., 2014; Whitehorn et al., 2012).

Although several theoretical frameworks have been developed to understand the complex interactions among global change pressures on bumblebees acting at different spatial and temporal scales (Becher et al., 2018; Cresswell, 2017; Crone & Williams, 2016), we still need experimental studies under realistic field conditions testing the interaction of more than two pressures. For this purpose, we conducted a three-crossed factorial field experiment to test the interactive effects of landscape transformation, climate change and pesticide exposure on bumblebees at the colony and at the individual level. We selected *B. terrestris* as a model species due to the common and widespread distribution of this taxon, as well as the extensive research attention it has generated. The main objectives of the study were (a) to quantify the combined effects of these three global change pressures on an eusocial pollinator species, (b) to examine whether these combined effects differ among castes and larval development stages and (c) to explore the combined effects on the body size of different castes. We predicted that on average these global change pressures would have a synergistic effect both at the individual level and at the colony level due the cumulative effects on individuals scaling up at the colony level.

2 | MATERIALS AND METHODS

2.1 | Study system and experimental design

The study was located in Sevilla and Huelva provinces (SW Spain) from mid-March to early May 2016. In all, 12 sites were selected within a 65 km landscape human transformation gradient (Figure 1), first based on the degree of urban or agricultural intensity, and then refined to an estimation of foraging resource availability by a quantification of flower abundance and richness at the landscape scale (see *Foraging resource availability* below). Land use was quantified using QGIS (Lyon 2.12 version) software, using as a reference the land use map 'Sistema de Información de Ocupación del Suelo en España (SIOSE011)' from the Spanish National Geographic Institute (SIOSE, 2011). In this region, the landscape is characterised by an

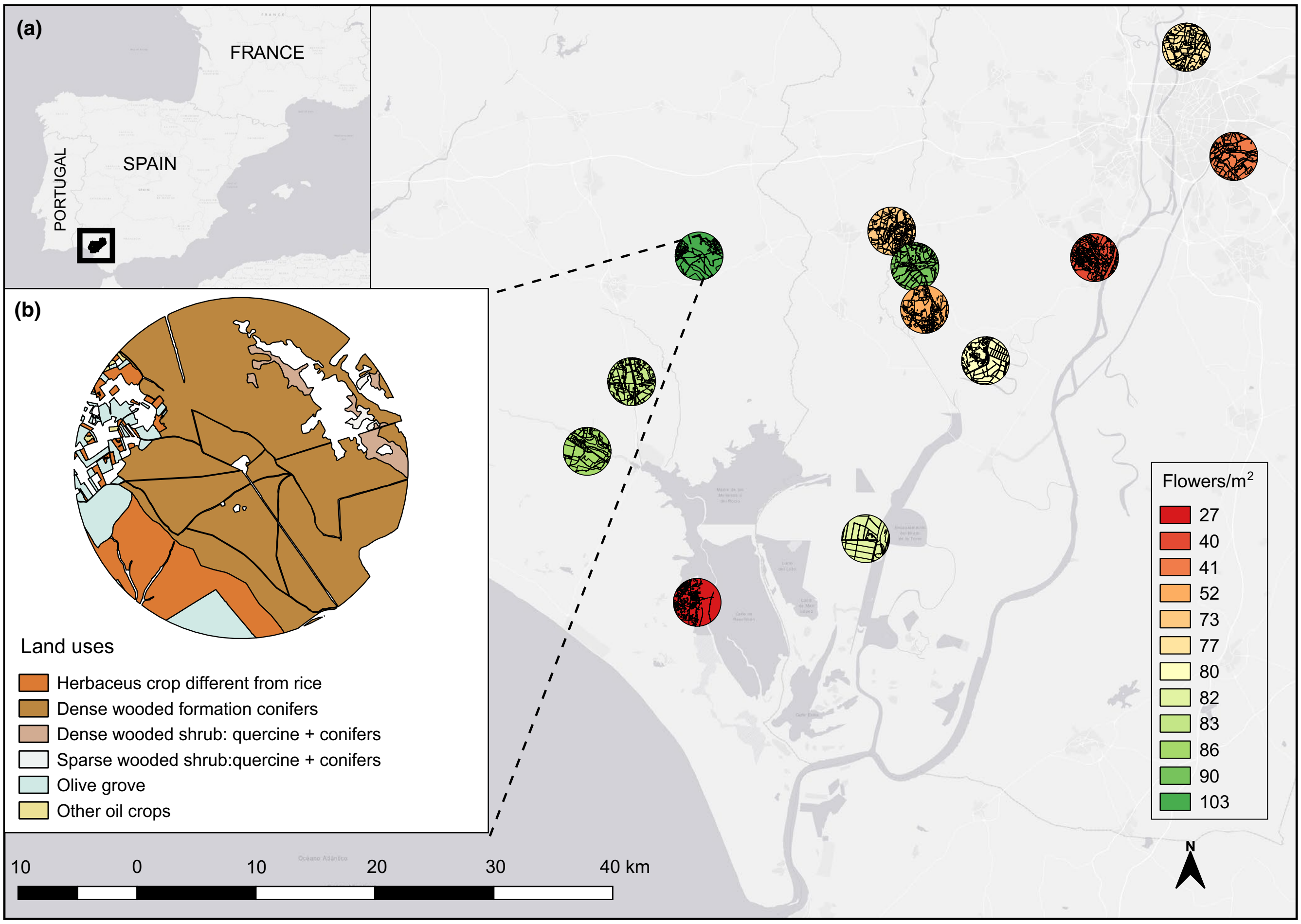

FIGURE 1 Map of the study area in Southern Spain. (a) Circles depict each of the 12 two-km radius buffer areas where *Bombus terrestris* colonies were allocated. The colour gradient goes from green (high mean flower density) to red (low mean flower density); (b) In the insert, different colours indicate different land uses

agricultural mosaic mainly composed of olive groves, vineyards, rice, citrus and oil crops with patches of shrublands, grasslands and open oak, eucalyptus and pine forests. Although the Mediterranean area is characterised as hot and dry, water sources were present in the 12 sites (*M* ± *SD*: 1.234 ± 2.248 km^2; range: 0.028–5.91 km^2).

In each of the 12 sites, two pairs of *B. terrestris* colonies were allocated. All colonies were purchased from Koppert Biological System (Natupol®) at the same time and came from the same breeding line preventing large genetic and age differences that could influence our results. Prior to the experiment, we checked that all colonies were in good conditions and had an active queen. Each colony and the corresponding attached feeding solution (in its plastic bag) were weighed to account for colony differences at the start of the experiment.

To avoid animal or weather damage to colonies, each colony pair was placed in a wooden box with the colony entrances situated at the opposite ends of the box. We left a space of ~10 cm between the two colonies. Due to the high abundance of ants in the study area, we had to coat the exterior of the boxes with a solution to deter ants from accessing the boxes (NEUDORFF®, a.i. 4.59 g/L pyrethrin extracted from *Chrysanthemum cinerariaefolium* flowers, and 825.3 g/L oil from organic rapeseed). Only the bottom of the boxes was coated, and hence bumblebees had no contact with the product while foraging. In any case, this product is compatible with organic farming and according to the manufacturer, it is safe for bees at the dosages applied on our boxes. Colonies were closely monitored and fanning behaviours (i.e. workers cooling the nest by moving the air with their wings at the entrance) were noted.

2.2 | Colony and individual bumblebee sampling

Newly purchased colonies were placed in the field in the second week of March and the experiment was completed in the first week of May, concurring with the Spring season. All colonies but one (see below) had reached queen production stage by that time, but still had more than 25 workers, indicating a comparable stage of development. Individuals could freely exit and enter the colonies during the experiment, but the size of the exit holes was small enough to prevent new queens to exit. At the end of the experiment, we closed the exit hole for 48 hr before collecting all the colonies from the field to capture all individuals from the colony. We collected all the colonies at the same time from the field, when they were all reaching the natural end of their lives (indicated by the production of reproductives). When collected, colonies were frozen immediately at −20°C in an industrial freezer for 48 hr. Afterward, each colony was dissected with forceps. We measured the following 16 colony variables: number of workers, males and queens; number of empty and full worker/queen cocoons; mites (absence/presence); pollen (absence/presence); number of deformed individuals (i.e. wing or leg deformities); number of newly emerged individuals; larval weight and weight of empty and full worker/queen cocoons.

As the first stages of larvae development are covered by a wax bulk in the upper part of the colony, it was difficult to separate the wax coat from larvae during the dissection. Thus, we decided to measure the weight of the whole mass including wax as a proxy of larval weight. To measure individual empty (after adult emergence) and full workers/queen cocoon weight, we selected up to 10 cocoons (carefully removing any surplus pieces of wax) and averaged their weight per colony. The weight of empty cocoons was measured as a proxy for silk and wax colony investment in cocoon covering. For each caste, we sampled 10 individuals per colony when possible, and measured their intertegular distances with a digital caliper (0.01 mm precision) to estimate their body size (Kendall et al., 2019). For each colony, we calculated the mean and the coefficient of variation (CV) of the intertegular distance for each caste. Overall, in the 48 colonies dissected, we measured a total of 533 intertegular distances for workers (*M* ± *SD*; 11.14 ± 1.82 individuals per colony), 375 intertegular distances for males (8.29 ± 3.60) and 424 intertegular distances for queens (8.73 ± 3.61).

A correlation test analysis with the 'rcorr' function in the Hmisc R package (4.1-1 version) followed by a principal component analysis (PCA) with varimax rotation with the abovementioned 16 variables was run to select for uncorrelated variables. When two variables were highly collinear, we selected the one with a clearer biological interpretation (see Figure S1). For instance, the number of queens was highly correlated with the number of queen cocoons, so we selected only the number of queens as a proxy of colony fitness. Note that this further indicates that no new queens escaped the nest. Similarly, the number of males was highly correlated with the total number of empty male cocoons; hence, we selected the number of males as a dependent variable. The number of closed worker and males cocoons was highly correlated with larvae weight, so we took larvae weight as a proxy of the colony's growth activity. Finally, we decided to base our colony analysis on the following five variables: number of workers, queens and males; queen empty cocoon weight and larvae weight. While at the individual level, we took intertegular mean distance (body size hereafter) of males, workers and queens. We considered individual-level variables as measures performed on each individual (e.g. body size), and colony-level variables those that inform us of the fitness of the colony: number of workers, males, queens and larvae weight. Finally, variation coefficient should be consider as a colony-level product of the individual measures.

2.3 | Temperature treatment

In each site, a pair of colonies were randomly located <20 m apart at N/NW and S/SE orientation to simulate contrasting climatic conditions throughout the day. All sites had small shading constructions to maximise shadow/sun exposure between orientations (see Figure S2). Temperature and humidity were recorded with an iButton® (AO-2513-ON) data-logger located inside every wood box that contained each pair of colonies. The data-logger registered data every 20 min during the 7 weeks of the experiment. Maximum and minimum temperatures reached at the sites were on average 31.58 and 21.31°C, respectively.

A PCA with varimax rotation function followed by a correlation test analysis with the 'rcorr' function in the Hmisc R package (4.1-1 version) was used to select the best variables measured by the data-logger as a proxy of climate warming. The first eigenvector (PC1 56.1% variance explained) was mainly related to temperature patterns while the second eigenvector (PC2 17.8% variance explained) was mainly related to humidity patterns. Daily mean, median and maximal temperatures during the 7 weeks of the experiment were highly correlated; therefore, we selected the mean temperature in each orientation treatment as the independent climatic variable. On average, among the 48 colonies, the mean temperature difference between N/NW and S/SE sites was 1.62°C (ranging from 0.8 to 2.6°C; see Figure S3), a temperature well within the current 2050 climate change predictions by the IPCC panel (IPCC, 2000). Nevertheless, note that maximal temperatures ranges between 26.65 and 40.57°C; therefore, warmer treatments also experienced hotter extreme events. Whereas in temperate regions colony nest placement could seek southward locations for warmer microclimate, in Mediterranean areas with extreme temperature events, bumblebees are expected not to follow this pattern. Due to the low variability observed in humidity between the two orientations, we discarded this variable from further analyses (see Figure S4).

2.4 | Landscape foraging resource availability

For each site, we quantified the level of foraging resource availability offered by each landscape surrounding each site by an estimation of flower abundance and number of plant families (hereafter plant richness) within a 2-km-radius buffer around each site.

First, during spring, in each of the six most common land uses per site, we sampled flower richness and abundance in 10 one-m^2 random plots. In total, we measured flower resources in 34 different land uses (Table S1) representing 76.16 ± 3.4% of all land uses of each study buffer area. For the remaining uncommon land use types per landscape, we interpolated flower abundance and richness estimations with the most similar surveyed land use based on our expertise (Table S1). For example, buildings or industries were set equivalent to zero mean flower abundances; firewall flower abundances were set equivalent to mean values for harvested tree areas;

touristic campgrounds were set equivalent to garden and parks areas, etc.

Second, while bumblebees are polylectic, they tend to prefer foraging on only a few plant families. Hence, we calculated two flower resource metrics, one including all flower families and another where we discarded the families that are not commonly visited by bumblebees in the study area based on a large and extensive plant–pollinator interaction network collected in the same area (Magrach et al., 2019). The families discarded were *Aristolochiaceae*, *Geraniaceae*, *Euphorbiaceae*, *Anacardiaceae*, *Polygonaceae*, *Plantaginaceae*, *Oxalidaceae* and *Resedaceae*.

Third, the floral resources offered by each land use were estimated as the mean flower abundance and mean flower richness per square metre in each land use type, multiplied by the percentage occupied by each land use within a 2-km buffer. Finally, we calculated the mean total abundance in each 2-km buffer area as the sum of all land use types divided by the number of the total land uses present in the landscape. The same procedure was applied for plant richness. Because there was a strong significant correlation between family richness and flower abundance (Pearson correlation = 0.73; $p < 0.05$), we selected flower abundance as a proxy of landscape foraging resource availability to include in the analysis of the interactions among global change pressures.

Although a 2-km-radius landscape represents the typical foraging distance for *B. terestris* (Walther-Hellwig & Frankl, 2000), if resources are available, they tend to concentrate their activity within the first km or less (Walther-Hellwig & Frankl, 2000). Therefore, as a fourth step, we calculated resource availability both in 1- and 2-km-radii buffers around each site. Overall, the estimations of flower abundance at 1- and 2-km radii including all flowers or only the bumblebee's preferred floral families were highly correlated (Pearson correlation in all pairs $R > 0.54$; $p < 0.05$; see Table S2). Due to this correlation and to be conservative, we selected the abundance of all flowers in a '2-km-radius buffer' including all families as a proxy for the foraging resources available in the landscape for *B. terrestris*. Choosing other proxies did not alter the conclusions of the study.

2.5 | Pesticide treatment

The colonies were placed in the field 1 week before applying the pesticide treatments to allow them to adapt to the field conditions before performing any handling.

The pesticide treatment consisted of the application of a field-realistic dose (3.5 ng/g) of the neonicotinoid insecticide imidacloprid (Botías et al., 2015; Dively & Kamel, 2012; Stoner & Eitzer, 2012). To obtain the target concentration, we first prepared a stock solution of imidacloprid (Pestanal® analytical standard, Sigma-Aldrich) at 1 mg/ml in acetone (ACS reagent, ≥99.5%, Sigma Aldrich). From this stock solution, we prepared a 0.001 mg/ml solution with acetone and then a 0.0001 mg/ml solution with distilled water as a solvent to avoid acetone detection by bumblebees. All solutions were prepared in tubes covered with aluminium foil to avoid imidacloprid photolysis degradation. Finally, we applied a solution of 60% concentration of sucrose diluted in water, and added the pesticide solution to obtain a 3.5 ng/g concentration. We also prepared a control solution with the same concentration of acetone but without any pesticide.

To feed the bumblebees with the prepared solution, three tubes containing 47.5 g of sucrose solution with 3.5 ng/g of imidacloprid were added directly to the syrup bag attached at the bottom of the colony box. Before adding the solution, we measured the weight of the syrup bag to calculate the amount of ingested solution and thus of pesticide consumed. Syrup with either pesticide or control treatments was weighed. Since the syrup bags contained some syrup when the treated solution was added, the concentration of imidacloprid decreased, with the effective concentration of exposure in the colonies being 0.88 ng/g in average (range = 0.41–1.82 ng/g; Table S3).

We applied the solution twice in all of the 48 colonies to mimic the length of the flowering season of a crop treated with pesticides. Half of the colonies received the control solution and half received the pesticide treatment solution on two different nights with an interval of 2 weeks between them. To avoid stressing the colonies with our presence and to mimic the conditions of agrochemical applications in agricultural environments, the first application contained one dosage and the second contained two dosages. The treatment was applied at night to minimise colony stress and disturbance. Note that none of the colonies were located close to potentially treated flowering crops (i.e. oilseed rape, sunflower, strawberries).

To obtain a continuous variable of pesticide total consumption per colony (see Figure S3), we used the following formula: $[(CI*1C) + (C2*2C)]$, with CI being the initial concentration of imidacloprid applied in the syrup solution bag, 1C the total consumption of syrup weighed 2 weeks after the first treatment was applied in the colonies, C2 the concentration of imidacloprid calculated in the second application and 2C the total consumption of syrup weighed 2 weeks after the second application was performed. Finally, we ran a GLM analysis to analyse the differences in total syrup consumed between pesticide and non-pesticide-treated colonies. We found no significant differences in nectar consumed between treated and untreated colonies (estimate ± *SE* = −43.88 ± 127.13; $p = 0.73$; Figure S5).

2.6 | Statistical analysis

All statistical analyses were performed in R v. 3.3.3 (R Core Team, 2017). Prior to analysis, all predictors were scaled by subtracting the mean and dividing by the standard deviation with the 'scale' base function in R.

We analysed the interaction among global change pressures at the colony level with GLM with pesticide consumed, foraging resource availability, mean temperature and all the pairwise interactions as fixed factors. We ran parallel models for number of queens, number of workers, number of males and larvae weight as response variables. All models included the initial colony weight as a covariable to control for differences in the development stage of the colonies. Due to large statistical power needed to model three-way interactions, we did not test for a three-way interaction among all global change pressures.

The individual body size of the different castes was analysed following the same criteria using generalised linear mixed models (glmer) in R using the LME4 package (Bates et al., 2015; v.1.1-15) with colony as a random factor to account for the non-independence of the different individuals measured within the same colony. Because size variation of bumblebee workers could be up to 10-fold on mass (Goulson et al., 2002), and it has been hypothesised that this may be related to task differentiation (Goulson et al., 2002), we also calculated the variance between individuals of the same caste by calculating the coefficient of variation (standard deviation/mean) as the response variable with the same model structure explained above.

We used a negative binomial errors distribution to model the number of workers, number of queens and queen body size. For the number of males and the intertegular distance of queens and workers, we used a Gaussian error distribution. We always tested the full models with all the specified interactions and with no model simplification. While we focus our interpretation of the results on the effect sizes observed (Greenland et al., 2016), *p* values were provided for completeness and computed using lmerTest (Kuznetsova et al., 2017) based on Statterhwaite's estimation and with no other corrections.

To test if there was an increase in nesting material investment in queen cocoons with temperature, we ran a GLM between weight of empty queen cocoons and mean temperature. All model outputs were visualised using 'jtools' (Long, 2019; v. 0.9.4) and 'ggplot2's (Wickham, 2016; v. 2.2.1).

Models error fit were evaluated visually, and zero-inflation and over-dispersion were tested for using the DHARMA package (Hartig, 2017; v.0.1.5). We also tested for potential multicollinearity by calculating variance inflation factors (VIF), which were lower than two in all response variables. In addition, we tested for potential spatial autocorrelation on model residuals using the Moran Index, which were low and non-significant for all models (Moran's *I* ranges between − 0.04 and 0.18; *p* value > 0.05).

Finally, we calculated the magnitude and the direction of the effects for single and combined stressors comparing those with

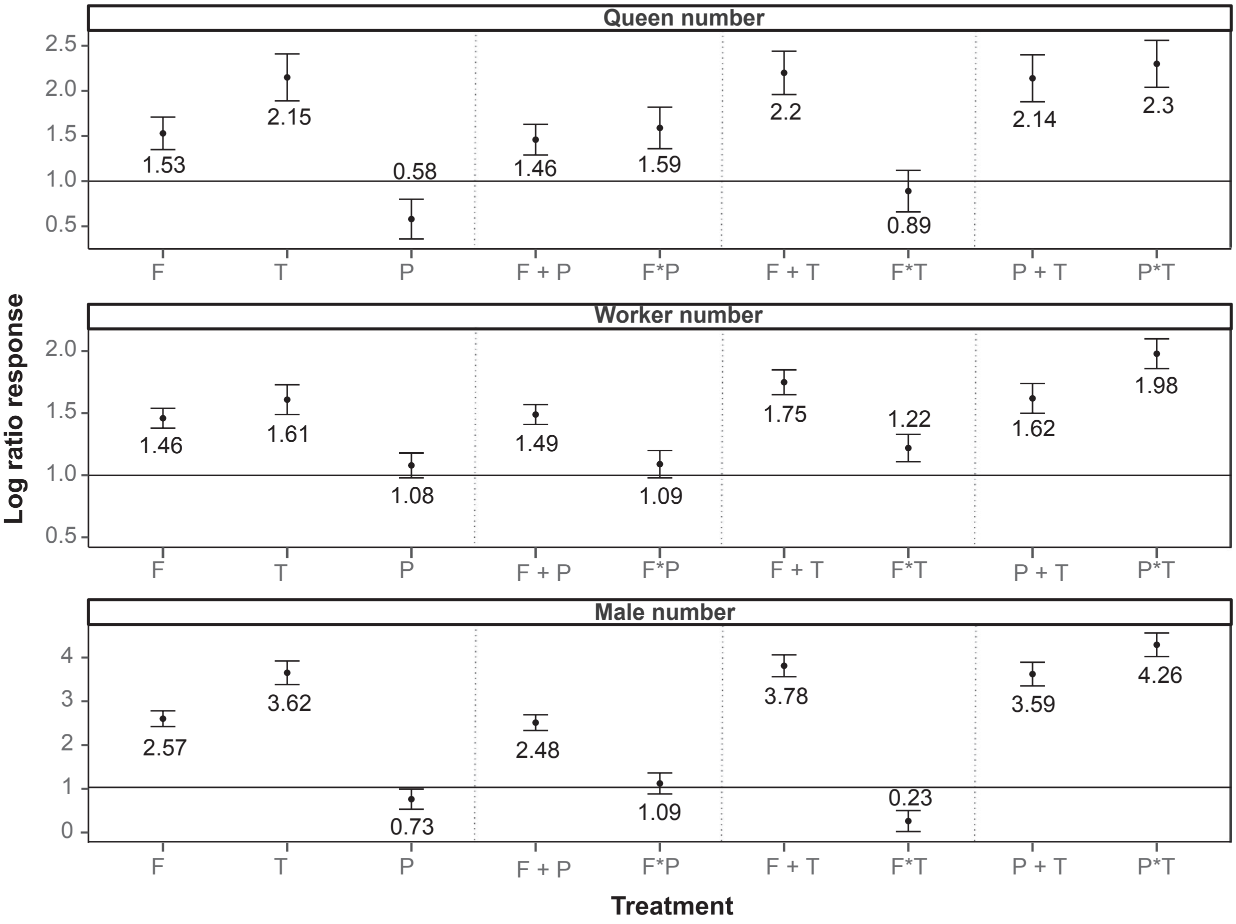

FIGURE 2 Mean ± *SE* log ratio responses of different single stressor treatments (left), simulate additive and interaction effects regarding control on colony number in different castes. Response ratios (RR) were calculated as RR = ln(Stress treatment/Control), where Stress treatments and Controls were calculated with values predicted by the full model. Treatments are: P = Pesticide; T = Temperature; F = Foraging resource availability. Symbols: '+' represents additive combination of two stressors, '*' means interaction effect of both stressors in combination

the expected additive effect. We follow Darling & Côté, 2008 to calculate the response ratios. An advantage of the use of these response ratios is that they are also proportionally symmetrical and have a sampling distribution that approximates normality (Hedges et al., 1999; Lajeunesse & Forbes, 2003). Response ratios (RR) were calculated as RR = ln(Stress treatment/Control); where Stress treatments and Controls were calculated with values predicted by the model. Using the predicted values of the full model, we can isolate the single effects of each stressor, when the other stressors are fixed to the Control values and compare them with the predicted responses of the stressors in combination. Control values used to perform model predictions corresponds with the 0 values of pesticide, the lower mean temperature and maximum mean flower abundance, while Stress treatment values used corresponds to the maximum pesticide consumed, the highest mean temperature and minimum mean flower abundance. In a second step, we also calculated the simulated additive effect as the sum of the predicted results of the individual stressors. Then, we applied the ln-transformed response ratio, a metric usually used in meta-analyses (see Darling & Côté, 2008; Hedges et al., 1999) of the predicted effects of single, interactive and simulated additive treatment effects versus the control (i.e. colonies with low temperature exposure, pesticide absence and high foraging resource availability). To calculate the standard error, we first calculated the variance as Hedges et al. (1999) proposed for meta-analysis:

$$\frac{(SD_E)^2}{n_E\overline{X}_E^2} + \frac{(SD_C)^2}{n_C\overline{X}_C^2}.$$

$\overline{X}_E$ and SD_E represent mean and standard deviation of experimental treatment, $\overline{X}_C$ and SD_C, mean and standard deviation of the results in the control treatment. Finally, n_C and n_E represent sample size of control and experimental treatment, respectively. We then obtained the standard error of the mean, calculated through the root square of the variance divided by sampling number root square.

We plotted the results following the representation by Galic et al. (2018) of synergistic interactions (Figure 2; Figure S6) and proceeded to interpret them based on the classification of Piggott et al. (2015) where both negative and positive synergistic and antagonistic interactions are presented.

3 | RESULTS

On average, colonies had 80 ± 46.77 (*M* ± *SD*) worker individuals, 33 ± 43.58 male individuals and 55 ± 70.40 queen individuals. The number of individuals per colony was highly variable. For instance, males ranged from 0 to 151 and the number of queens from 1 to 329. On average, the proportion of males over total reproductives was: 0.39 ± 0.32. We only found mites in six colonies belonging to different treatments. Bumblebee workers fanning the colony were only observed in 7 of the 12 south-facing colonies. Only one colony collapsed at one north + pesticide treatment. However, removing this colony from the analysis did not alter the results.

We found a consistent interactive effect of temperature and foraging resource availability on the number of workers, queens and males (Figure 3; Table 1). There was an increase in the number of individuals in all castes with temperature but only when resource availability was high. In contrast, when resource availability was low, there was a decrease in the production of queens and males at high temperatures. Hence, low flower resource availability diminished the positive effect of high temperatures in reproductive individuals. Interestingly, we found a significant effect of mean temperature on the weight of empty queen cocoons (Figure 4; Table 1).

Pesticide exposure had a weak interaction with resource availability for the number of workers (Table 1), with more workers produced under high pesticide consumed only in the presence of high foraging resource availability. However, the number of males decreased by 27% at high levels of colony pesticide consumption (Table 1). There were no significant main effects or interactions between treatments on larvae weight.

Regarding body size, we found that neither body size nor the CV of any caste show interactions between stressors (Tables 2 and 3). Both

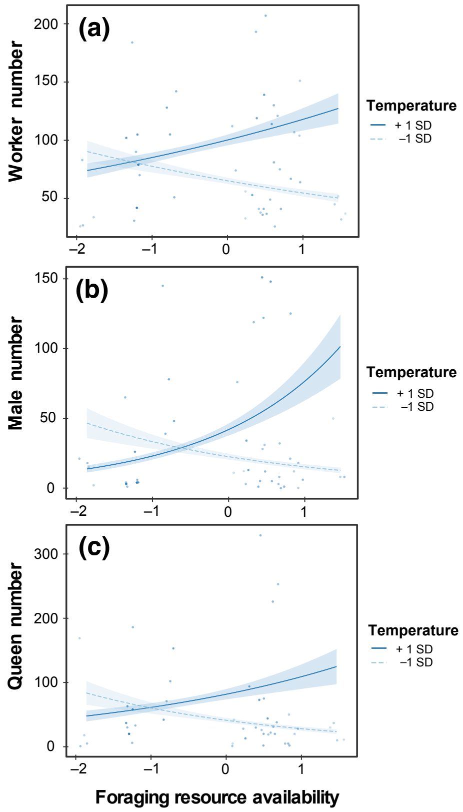

FIGURE 3 Scatterplot showing the interaction between temperature and foraging resource availability in the landscape on (a) number of workers, (b) males and (c) queens. Dashed light blue line and continuous dark blue lines show the predicted effects at the mean temperature −1 *SD* and +1 *SD*, respectively. Dots represent raw data. Light and dark blue bands represent 95% confidence intervals. *X*-axis represents a gradient of foraging resource availability from lowest foraging resource availability (negative values) to highest (positive values) resource availability. Both temperature and foraging resource availability are scaled

TABLE 1 Summary of GLM models obtained for different colony response variables. Large effect sizes variables are indicated in bold

Model	Terms	Estimate	*SE*	Statistic	*p* value
Queen number	Intercept	4.06	0.16	24.78	0.00
	Initial weight	0.16	0.21	0.77	0.44
	Temperature	**0.34**	**0.18**	**1.88**	**0.06**
	Foraging resource availability	−0.05	0.20	−0.24	0.81
	Pesticide	0.00	0.16	−0.03	0.98
	Temperature × foraging resource availability	**0.33**	**0.18**	**1.86**	**0.06**
	Foraging resource availability × pesticide	−0.15	0.17	−0.89	0.37
	Temperature × pesticide	0.14	0.21	0.67	0.51
Worker number	Intercept	4.39	0.08	57.44	0.00
	Initial weight	**0.27**	**0.10**	**2.75**	**0.01**
	Temperature	**0.21**	**0.08**	**2.54**	**0.01**
	Foraging resource availability	−0.01	0.09	−0.07	0.94
	Pesticide	−0.02	0.08	−0.22	0.83
	Temperature × foraging resource availability	**0.17**	**0.08**	**2.02**	**0.04**
	Foraging resource availability × pesticide	**0.15**	**0.08**	**1.88**	**0.06**
	Temperature × pesticide	0.07	0.10	0.76	0.45
Male number	Intercept	3.42	0.17	20.18	0.00
	Initial weight	**0.49**	**0.22**	**2.26**	**0.02**
	Temperature	0.31	0.19	1.64	0.10
	Foraging resource availability	0.10	0.20	0.51	0.61
	Pesticide	**−0.39**	**0.17**	**−2.35**	**0.02**
	Temperature × foraging resource availability	**0.49**	**0.19**	**2.65**	**0.01**
	Foraging resource availability × pesticide	−0.04	0.18	−0.21	0.83
	Temperature × pesticide	0.28	0.22	1.30	0.20
Larvae weight	Intercept	2.47	0.12	19.74	0.00
	Initial weight	**0.37**	**0.16**	**2.33**	**0.02**
	Temperature	0.22	0.14	1.60	0.11
	Foraging resource availability	−0.21	0.15	−1.42	0.15
	Pesticide	0.21	0.12	1.70	0.09
	Temperature × foraging resource availability	−0.06	0.13	−0.48	0.63
	Foraging resource availability × pesticide	−0.03	0.13	−0.27	0.79
	Temperature × pesticide	0.00	0.16	0.03	0.98
Empty cocoons	Intercept	0.42	0.02	22.82	0.00
	Temperature	**0.05**	**0.02**	**2.27**	**0.03**
	Pesticide	0.03	0.02	1.70	0.10
	Foraging resource availability	0.00	0.02	−0.21	0.83
	Initial weight	0.03	0.02	1.53	0.13

worker and queen size increased with temperature by 10% and 3%, respectively (Table 2; Figure S6) and we also observed a 3% decrease in queen size when foraging resource availability increased. Interestingly, body size variation decreased in queens, workers and males when exposed to high temperatures. We found no consistent effect of pesticides or foraging resource availability on CV of any caste (Table 3).

We found that interactive effects were lower than the expected additive effects in both reproductive castes (queens and males).

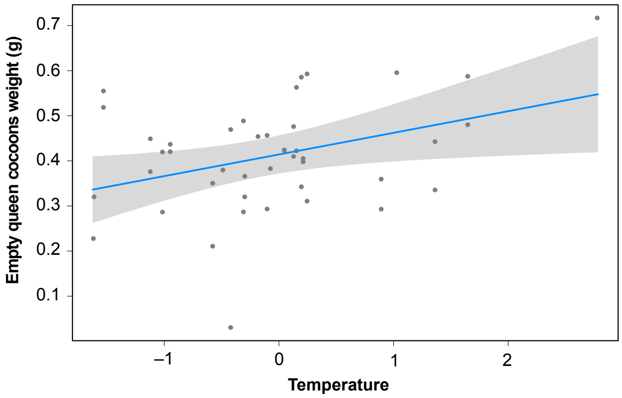

FIGURE 4 Scatterplot showing the positive relationship between temperature and empty queen cocoon weight. Cocoons are composed of silk and wax. The dots represent partial residuals. Also shown is the significant predicted GLM line (blue solid) and the 95% confidence intervals (grey band). The *X*-axis represents scaled mean temperature (variable mean subtraction divided by the standard deviation).

Interactive effects in the presence of low levels of resources and high temperature triggered a decrease in queen production by 11% in comparison to controls, therefore showing a synergistic negative effect (i.e. more negative than the additive effect of these stressors). For males, the decrease was 77%, also with a synergistic negative effect. The number of workers increased by 22% and the interactive effect was lower than the additive effect of these pressures (antagonism positive effect). The interaction between foraging resource availability and pesticide on the number of workers increased the number of individuals by 9% when compared to controls, also showing a positive antagonistic effect (i.e. less positive than predicted additively).

4 | DISCUSSION

Our results show that interactions between global change pressures act in a non-additive way on *B. terrestris*. We detected both

TABLE 2 Summary of GLMM models obtained with different individual-level response variables. Large effect sizes variables are indicated in bold

Model	Term	Estimate	*SE*	Statistic	*p* value
Queen body size	Intercept	7.58	0.05	164.36	0.00
	Initial weight	0.08	0.06	1.28	0.21
	Temperature	**0.1**	**0.05**	**2.05**	**0.05**
	Foraging resource availability	**−0.11**	**0.05**	**−2.02**	**0.05**
	Pesticide	0.03	0.05	0.56	0.58
	Temperature × foraging resource availability	0.07	0.05	1.49	0.15
	Foraging resource availability × pesticide	0.02	0.05	0.42	0.68
	Temperature × pesticide	0.1	0.06	1.7	0.10
Worker body size	Intercept	4.84	0.06	82.48	0.00
	Initial weight	0.02	0.07	0.29	0.78
	Temperature	**0.13**	**0.06**	**1.96**	**0.06**
	Foraging resource availability	0.02	0.07	0.24	0.81
	Pesticide	0.01	0.06	0.22	0.83
	Temperature × foraging resource availability	0	0.06	−0.03	0.97
	Foraging resource availability × pesticide	0.08	0.06	1.37	0.18
	Temperature × pesticide	0.02	0.07	0.31	0.76
Male body size	Intercept	5.43	0.07	82.6	0.00
	Initial weight	0.07	0.09	0.81	0.42
	Temperature	0.09	0.07	1.29	0.21
	Foraging resource availability	−0.1	0.08	−1.18	0.25
	Pesticide	−0.1	0.07	−1.45	0.15
	Temperature × foraging resource availability	0.03	0.07	0.46	0.65
	Foraging resource availability × pesticide	0.05	0.07	0.76	0.45
	Temperature × pesticide	0.04	0.08	0.53	0.60

TABLE 3 Summary of GLM models obtained with different variation coefficients of different castes. Strong effect sizes or marginally significant variables are indicated in bold

Model	Term	Estimate	*SE*	Statistic	*p* value
Queen CV	Intercept	0.04	0	14.69	0
	Initial weight	−0.01	0	−1.35	0.19
	Temperature	**−0.01**	**0**	**−2.5**	**0.02**
	Foraging resource availability	0	0	−0.11	0.91
	Pesticide	0	0	0.97	0.34
	Temperature × foraging resource availability	0	0	−1.03	0.31
	Foraging resource availability × pesticide	0	0	−0.07	0.94
	Temperature × pesticide	0	0	−0.68	0.5
Worker CV	Intercept	0.12	0.01	18.72	0
	Initial weight	0	0.01	0.24	0.81
	Temperature	**−0.01**	**0.01**	**−2.02**	**0.05**
	Foraging resource availability	0	0.01	−0.52	0.61
	Pesticide	−0.01	0.01	−0.9	0.37
	Temperature × foraging resource availability	0	0.01	−0.54	0.59
	Foraging resource availability × pesticide	0	0.01	0.38	0.71
	Temperature × pesticide	0	0.01	0.22	0.83
Male CV	Intercept	0.07	0.01	9.64	0
	Initial weight	−0.01	0.01	−1.15	0.26
	Temperature	**−0.02**	**0.01**	**−2.06**	**0.05**
	Foraging resource availability	0	0.01	0.33	0.75
	Pesticide	0	0.01	0.18	0.86
	Temperature × foraging resource availability	0	0.01	−0.59	0.56
	Foraging resource availability × pesticide	0	0.01	0.59	0.56
	Temperature × pesticide	−0.01	0.01	−0.64	0.53

synergistic and antagonistic interactions among global change pressures in agreement with the trend reported in other studies (Côté et al., 2016; Darling & Côté, 2008) and while most interactive effects were lower than the expected additive effects, other interactive effects produced larger responses. At the colony level, the strongest pattern we found was a negative synergistic interaction between temperature and foraging resource availability in reproductive castes. The number of individuals increased at high temperatures only when the landscape offered high foraging resources, but not when resource availability was low. Interestingly, we also found that the observed impacts at the colony level differed from those at the individual level. Hence, the scale at which pressures operate is important for understanding the long-term fitness consequences in species with social life histories. Nevertheless, it is also important to take into account the temporal scale in which these pressures interact because as Reich et al. (2006) found, interactions could switch from antagonistic to synergistic after years of interplay in nature. This could be especially pronounced in scenarios where different generations and developmental stages overlap within a colony, such as in *B. terrestris*.

Structural stability confers ecological systems the capacity to return to their performance regime after a stress (Landi et al., 2018). In eusocial insects, social homeostasis has been defined as the capacity of a social organism to go back to the initial state prior to a particular distress (Emerson, 1956). In addition, social insects could have bottom-up and top-down effects hierarchically integrated across different levels of organisation, producing non-additive responses in each level as reflected in our results. This suggests that response to environmental stresses could be modulated through multidimensional phenotypic plasticity (Stillwell et al., 2007; Westneat et al., 2019). Three lines of evidence indicate that this occurs in bumblebee colonies. First, despite the many combination of impacts applied to our colonies, only one colony collapsed, while all other colonies were still active at the end of the experiment and most of them produced new queens. Second, we observed differences in investment in male and female reproduction, as well as in the size of the individuals depending on the external conditions, indicating different strategies to cope with different stresses. For instance, potential strategies could be to produce sex ratios biased to the cheaper sex in presence of lower resource availability (Beekman & Stratum, 1998) or alternatively, to reduce the investment per individual producing smaller progeny. Finally, we observed some buffering mechanisms related to particular stressors. For instance, temperature is regulated actively by most social insects (Seeley & Heinrich, 1981) through nest design and behavioural mechanisms, providing a certain degree of independence from climatic conditions. Bumblebees are able to boost nest air circulation to diminish increases in temperature by fanning their wings (Vogt, 1986a). This is crucial for the maintenance of brood temperature in the nest and to avoid metamorphosis disruptions and lower eclosion rates (Heinrich, 1979; Himmer, 1927; Vogt, 1986b). This behaviour was observed in our colonies, with fanning workers allocated at the entrance of the colony during the hottest time of the day. This ability is bolstered by the size of the colony conferring less variation in brood temperature (Seeley & Henrich, 1981). Our hypothesis is that the colonies exposed to higher temperatures produced a higher work force, and thus, a higher amount of workers were available to invest in queen rearing, larvae insulation and other colony tasks. Nevertheless, in small colonies, this behaviour can lead to a reduction of larvae care (Gardner et al., 2007). Additionally, we found a significant positive relationship between empty queen cocoon weight and temperature (Figure 4), suggesting that nest design capabilities could lead to a major investment in wax isolation and hence mitigate temperature effects. Some authors have reported an impairment in the ability to build a wax canopy upon imidacloprid consumption (Crall et al., 2018), but we did not observe any difference in our treatments. Overall, these mechanisms may be reinforced by the fact that an increase in the colony size makes it more resilient to stresses. This is the case observed in our colonies, with a positive correlation between the number of workers and the number of new queens (see Figure S7).

A 1.62°C average increase in temperature had the greatest impact on the production of individuals for all castes. This result is in agreement with Nasir et al. (2019), which reported that the greatest number of new queens was produced in the 30°C temperature treatments. In another laboratory experiment, Holland and Bourke (2015) also reported that an increase of 5°C improved the reproductive success of *B. terrestris* colonies, with an increase in the production of workers, males and queens. However, we show that a higher temperature is only favourable when foraging resources are not limiting. On the contrary, we observed that at low temperatures, irrespective of floral resources, colonies did not increase the number of reproductive individuals over the course of the season. This is in agreement with studies that found that some colonies failed to produce a single new queen despite the high food availability present in agricultural landscapes (Samuelson et al., 2018).

Contrary to expectations, we did not find any strong interaction of the pesticide application with other stressors. There are several possible explanations for this. First, the dose applied (3.5 ng/g) and the actual concentrations of exposure (M = 0.88 ng/g, range = 0.41–1.82 ng/g) may not have a great impact on the colony at the stage of development, when the treatment was applied. At that time, most colonies probably had more than 100 workers, with colony homeostasis buffering any detrimental effect produced by pesticide exposure at the individual level. Duckerin (2019) has also reported that *B. terrestris* colonies could modulate pesticide damage; although the behaviour of active foragers showed the toxic effect, at the colony level foraging performance continued with minimal changes. The last could suggest that colonies could have a higher than expected level of resilience than predicted from only individual responses. Nevertheless, we found an impact of pesticide as a single stressor reducing the number of males, concurring with the findings of Fauser-Misslin et al., (2014) of a negative effect of imidacloprid on male production. We also expected a reduction in the number of queens, given that the cost of producing queens is greater than the cost of males for the colony (Beekman & Stratum, 1998), and hence in stress conditions the expected sex ratio should be biased towards males. It is worth noting that although we applied field-relevant concentrations of imidacloprid to the bumblebee colonies (Botías et al., 2015; Nicholls et al., 2018), we may have underestimated field exposure, since only pesticide-treated syrup and not pollen was supplied to the colonies.

Body size variation of bumblebee workers, a proxy for foraging efficiency (Peat et al., 2005), could vary up to 10-fold (Alford, 1975; Goulson et al., 2002). We hypothesised that workers' size could be affected by the interactions among pressures. However, we did not detect any interacting effect other than a simple increase in worker and queen size with increasing temperature as found in other studies (Gérard et al., 2019; Ramírez-Delgado et al., 2016). An unexpected finding was that queen body size decreased with an increase in foraging resource availability. Larger bees are predicted to have greater foraging ranges (Greenleaf et al., 2007), with potentially greater dispersal abilities. In addition, in temperate climates the new queens diapause for 6–9 months after mating before emerging when temperature rises. The ability of overwintering of a queen depends on its size, and the latter depends on temperature (Vesterlund, 2015). However, in our case, it seems that the benefits of larger queens are not offset by a higher production cost due to the ad libitum disposal of available food. Another interesting finding was a decrease in the variation of body size with an increase in the temperature in all castes. As mentioned above, size variation could represent a strategy to optimise task allocation (Goulson et al., 2002). A decrease in body size variation in workers could enhance the recruitment inside the nest to tasks such as fanning or could be a result of a trade-off of resource distribution between the number and the size variation of colony individuals. These results are in agreement with Austin and Dunlap (2019), where they find that variation in body size is a key feature to resist the impact of altered environments and important for the maintenance of bumblebee populations.

One of the limitations of our study is that we lack information on the whole life cycle of our colonies. The development of colonies can be divided into three phases: an initial phase in which the colony is founded, a development growth phase and finally the mature reproductive phase (Oster & Wilson, 1978). Our study only captured the final stage, and although it is the most relevant to define the reproductive success of the colony, prior phases are likely to be far more vulnerable. So we tried to capture all colonies during their mature stage, but with the trade-off of capturing them all at the same time. Moreover, reared colonies for commercial purposes could differ from wild populations due to artificial selection (Velthuis & Doorn, 2006). In this regard, future research should analyse the success of the new overwintered queen in producing natural colonies, and thus their fitness contribution to fulfilling the whole life cycle.

The absence of some significant pairwise interactions in our models should be taken with caution as some stressors could have slower impacts than others, thus producing no additional effects when acting in combination with other stressors (Vinebrooke et al., 2004). This could have occurred in our experiment, where colonies were placed in the field 1 week before applying neonicotinoid dosages to allow acclimatisation. While this could have obscured some complex interactions that we did not observe, we followed the recommendations by Darling and Côte (2008) to avoid simultaneous exposure to pressures and hence, replicated as much as possible the realistic conditions of sequential exposure to pressures typically occurring at the field scale. Finally, our relatively small sample sizes due to the field-realistic experimental design would lead to a low statistical power to detect interactions, reducing the likelihood of finding some more subtle effects.

In summary, our results show that pairwise interactions among global change pressures act in a non-additive way on bumblebees at the colony level. Moreover, the flexibility in organisms fitness-related traits in front of multiple environmental stressors and its integration across different levels of organisation could have important consequences on the conservation of healthy species populations. Only a holistic view can reveal better insights into how organisms with complex social organisations exposed to complex environments will survive in the Anthropocene. However, non-social life forms, such as solitary bees, may be more vulnerable to the impact of these interactions, but this remains unexplored. Understanding how the life history of pollinators buffers the effects of global change pressures could shed light on the winners and losers in the face of the complexity of all components of global change.

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AUTHORS' CONTRIBUTIONS

C.Z.-T., M.V., C.B. and I.B. conceived the ideas and designed the methodology; C.Z.-T., C.B. and I.B. conducted fieldwork; C.Z.-T. and I.B. analysed the data; C.Z.-T. led the writing of the manuscript with contributions of all authors. All authors contributed critically to drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

The data underlying the study are deposited in Figshare https://figshare.com/s/ba0b6f89fc85bab0e498.

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