No detectable impact of parasite-infected commercial bumblebees on wild bees in areas adjacent to greenhouses despite diet overlap

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A B S T R A C T

Over two million commercial bumblebee colonies are used on an annual basis to pollinate around 20 crop types worldwide. Despite their use, especially with crops grown in greenhouses, there is mounting evidence that many individuals also forage outside of them. Hence, the use of commercial bumblebees poses a risk to wild pollinators, especially to those who share floral resources and pathogens. To date, however, there is little evidence about the impact of commercial bumblebees on pollinator communities in Europe. We surveyed the abundance of commercial *Bombus terrestris* and the prevalence of four of its parasites in natural areas at increasing distances from vegetable crops in Cabo de Gata-Níjar (Almería, SE Spain), the most extensive greenhouse cultivation area in the world. We also estimated resource niche overlap (i.e., shared plants used) between commercial bumblebees and the native pollinator community. Finally, we explored whether the abundance and diversity of pollinators in natural habitats were influenced by agricultural expansion (i.e., distance to greenhouses) and bumblebee abundance. We found a sharp reduction in commercial bumblebee densities at increasing distances from greenhouses, with most bumblebees (95%) foraging within a radius of less than 200 m from them. However, these commercial bumblebees had high parasite prevalence (41% of individuals infected) of trypanosomatids, microsporidians and neogregarines. Moreover, their diet particularly overlapped with honeybees and large wild bees. Yet, pollinator density and diversity were not related to the distance from greenhouses or to bumblebee abundance. Although our results suggest that commercial bumblebees do not significantly harm wild pollinators, actions like preventing their escape from greenhouses, monitoring their health and optimising their use should be considered so as to minimise future risks.

1. Introduction

About one-third of the total ice-free surface of the planet is currently cultivated (Ellis et al., 2010). Since the second half of the last century, the area cultivated by flowering crops has increased significantly (Potts et al., 2016). Indeed, nowadays, the human population mainly consumes, directly or indirectly, fruits and seeds from flowering crops, most of which are dependent on pollination mediated by insects that enhance their quantity and quality (e.g., cocoa, almond, soybean and oilseed rape) (Klein et al., 2007).

Agricultural lands are highly connected to natural habitats through the movement of pollinators. Because flowering crops produce highly rewarding floral blooms, many insect pollinators that live in nearby natural areas spill over into crops to fulfil their feeding needs (Garibaldi et al., 2013). However, pollination services by wild pollinators are often not sufficient to ensure full crop pollination. This is for two main reasons: first, agricultural expansion and the associated loss of natural habitat impair wild pollinator species (Winfree et al., 2011). Second, some cultivation practices, e.g., large monoculture intensive crops with massive flowering blooms, crops flowering in winter when wild pollinators are scarce and greenhouse crops, exacerbate the difficulties of pollinators in reaching the crop. Therefore, commercial pollinators are

frequently used to secure crop pollination. In fact, the use of these pollinators has experienced an increase in the past few decades in parallel with the increase in the area devoted to flowering crops (Potts et al., 2016; Velthuis and van Doorn, 2006).

Honeybees (*Apis* spp.) are the most used managed pollinator species worldwide (Garibaldi et al., 2013; Klein et al., 2007), but bumblebees (*Bombus* spp.) are commonly used in some vegetable (e.g., tomato, pepper and watermelon) and berry (e.g., strawberry, blueberry and raspberry) greenhouse crops (Velthuis and van Doorn, 2006). Bumblebees were first commercially produced in the late nineteen-eighties in the Netherlands for the pollination of tomato crops. Nowadays, it is roughly estimated that around two million bumblebee colonies are produced on an annual basis to aid in the pollination of 20 crop types worldwide (Velthuis and van Doorn, 2006).

While crops usually provide monospecific but extremely abundant floral resources, natural habitats provide higher floral diversities. In general, polylectic bees and, in particular, bumblebees, need a diverse diet to maintain the health and proper development of their colonies (Brunner et al., 2014; Tasei and Aupinel, 2008). Thus, despite the high abundance of crop flowers, many bumblebee workers constantly spill over to outside of crops to feed on wild plants (Murray et al., 2013; Trillo et al., 2020; Whittington et al., 2004). Even queens can escape from agricultural facilities and become naturalised in the surrounding area (Trillo et al., 2019b).

There is a growing concern that commercial bumblebees pose risks to wild pollinators (reviewed by Mallinger et al. (2017)) by competing for floral resources (e.g., Ishii et al. (2008)) by hybridising with native congeners (e.g., Bartomeus et al. (2020)) and by spreading their pathogens into wild populations (e.g., Meeus et al. (2011)). These impacts might be highly pervasive in areas where the propagule pressure of commercial pollinators is very high, such as in landscapes with extensive greenhouse cover areas that produce several annual cycles of vegetables (Potts et al., 2016; Velthuis and van Doorn, 2006). However, robust evidence of the adverse impacts of commercial bumblebees on native wild pollinator populations in the field is scarce, at least in Europe (Trillo et al., 2019b; Whitehorn et al., 2013; although see Bartomeus et al. (2020) for genetic introgression processes).

With the aim to investigate the risks that commercial bumblebees (*B. terrestris*) pose to pollinators, we surveyed the abundance and distance at which they forage from greenhouses as well as their parasite loads. We conducted the study in Cabo de Gata-Níjar (Almería, SE Spain), the most extensive greenhouse vegetable production area in the world, where commercial bumblebees are frequently and intensively used. We aimed to answer the following questions: (1) To what distance do commercial bumblebees escaping from greenhouse crops travel to forage on wild plants? (2) Do these commercial bumblebees carry internal parasites able to be transmitted to other pollinators? (3) What floral resources do bumblebees use compared with other pollinators? (4) Do agricultural expansion (i.e., distance to greenhouses) and the presence of bumblebees affect pollinator abundance and diversity in natural habitats? We expect a large number of bumblebees to forage especially close to greenhouses and to be infected by several parasites. Due to the generalist behaviour of bumblebees, we expect their floral preferences to overlap to some degree with other pollinators. Finally, we expect wild pollinators to be negatively affected by both the presence of bumblebees and the loss of natural habitat due to the large cover of greenhouses.

2. Materials and methods

2.1. Study area

The study was conducted in the Cabo de Gata-Níjar area in the province of Almería (SE Spain, see Fig. 1) in 2020. The climate is semiarid with average annual minimum and maximum temperatures of 14.7 °C and 23.4 °C, respectively, and an average annual precipitation of 200 mm (AEMET, 2020). Almería has the highest concentration of

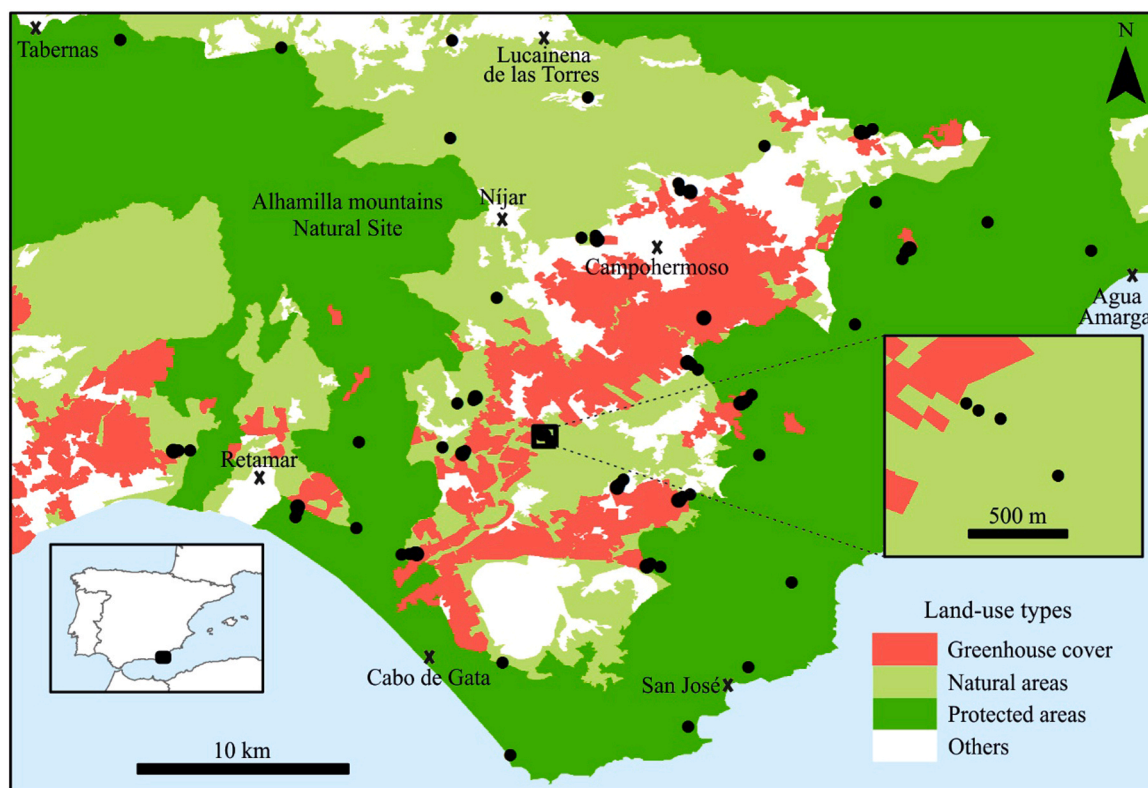**Fig. 1.** Map of the study area in Cabo de Gata-Níjar (Almería, SE Spain). Points represent the 80 sampling plots located in natural areas at 30 m, 100 m, 200 m, 500 m, 1 km, 5 km and 7 km from greenhouses. Crosses denote main towns in the area.

greenhouses in the world, with ~30,000 ha mainly producing tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*), watermelon (*Citrullus lanatus*) and zucchini (*Cucurbita pepo*) (Cajamar, 2019; De Rafael and Fernández-Prados, 2018). These crops are harvested throughout most of the year and their production relies on insect-mediated pollination (Klein et al., 2007). Thus, farmers place honeybee hives (*Apis mellifera*) or bumblebee colonies (*Bombus terrestris*) in greenhouses. Despite the fact that greenhouses have closed ends and sides, they are not airtight and it is common to observe their walls with torn plastic and large holes. This makes greenhouses highly permeable to the spillover of insects, as seen in other countries (e.g., Murray et al. (2013), Whittington et al. (2004)).

The natural areas of the region are mainly dominated by esparto grass (*Macrochloa tenacissima*) intermixed with shrubs and herbs, some of which are endemic (i.e., *Antirrhinum charidemi*, *Dianthus charidemi* and *Ulex canescens*). Among the most representative and widespread entomophilous species are the shrubs *Anthyllis cytisoides*, *Helianthemum almeriense*, *Lavandula multifida* and *Thymus hyemalis* and the herbs *Asphodelus tenuifolius*, *Echium creticum*, *Reichiardia tangitana* and *Zygophyllum creticum*. Most plant species bloom from January to April (Blanca et al., 2009).

Cabo de Gata-Níjar harbours a great diversity of wild pollinators. They are well represented, for instance, by solitary bee species of the families Andrenidae and Halictidae, flies of the families Syrphidae and Bombyliidae, and butterflies of the family Nymplalidae (González-Robles et al., 2020). However, it is important to note that native bumblebees do not occur in the study area (Ortiz-Sánchez and Aguirre-Segura, 1991).

2.2. Pollinator and floral sampling design

The study was conducted across 36 km^2 within the Cabo de Gata-Níjar area where we selected 80 plots (20 m^2) in natural areas at increasing distances from greenhouses. Specifically, we selected 16 plots at 30 m, 15 at 100 m, 15 at 200 m, 15 at 500 m, 9 at 1 km, 6 at 5 km and 4 at 7 km (Fig. 1 and Table A.1). In 15 sites, we set up groups of four plots at distances of 30 m, 100 m, 200 m and 500 m from greenhouses. These sites (hereafter landscapes) were at least two kilometres apart from each other and the rest of the plots (all in different landscapes) to ensure independence in our sampling (Osborne et al., 1999). Plot distances to greenhouses were calculated using Google Earth (accessed January 2020). To create the map in Fig. 1 we used ArcGIS (ESRI, 2016) based on the CORINE 2018 Land Cover Map (https://datos.gob.es/es/catalogo/e00125901-spaignclc2018).

Plots were selected in areas without esparto grass to obtain greater densities of entomophilous flowering plants. In order to ensure similar entomophilous plant species identities among plots, all of them shared an average of five (ranges from 2 to 8, plant species) of the most representative taxa: *A. cytisoides*, *A. tenuifolius*, *E. creticum*, *H. almeriense*, *L. multifida*, *R. tangitana*, *T. hyemalis* and *Z. creticum*.

We conducted pollinator censuses during two periods (hereafter rounds): the first round was from 29th January to 26th February and the second round was from 29th February to 28th March. Due to the COVID-19 pandemic lockdown, ten plots (one at 30, 100, 200, 500 m and 1 km, two at 5 km and three at 7 km from greenhouses) were not sampled during the second round, however, note that those were not a biased subsample, and all distances are well represented in our analysis (Table A.1). Each round was conducted by walking five parallel transects (20 m×2.5 m each) for 30 minutes per plot. More specifically, we walked each transect for six minutes, stopping every 45 seconds to observe plant-pollinator interactions in an area measuring 2.5 m^2 each time. The total accumulated observation time was 75 h.

All transect walks were conducted between 10:30 h and 17:15 h. The order of sampling between plots was partially random during each round and throughout the study area. That is, for logistical reasons, in most cases, either the plots belonging to the same landscape or the

nearby ones were sampled on the same day. However, the sampling order of each plot within the same landscape was randomly selected between landscapes. Sampling was conducted on sunny or partly cloudy and non-windy days with temperatures in the shade ranging in the first round between 14 °C and 25 °C and in the second round between 16 °C and 30 °C.

During the transect walks, we recorded every individual insect observed apparently feeding on a flower and the identity of the plant species. Pollinators were assigned to one of the 14 taxonomic categories defined in Table 1. When possible, specimens were identified to the lowest taxonomical level within each category.

To estimate floral resource availability per plot, we also walked five 20 m long parallel transects, just after the pollinator censuses. In each transect walk, we used a 40 cm^2 square to identify and count all open flowers. The floral unit for the Asteraceae family was the inflorescence. The square was placed every two metres along the transect (i.e., 10 times per transect walk).

Overall, we recorded 83 flowering plant taxa (61 identified to species level). Specifically, we recorded 62 (10.1±0.4, mean±SE, hereafter) taxa per plot in the first sampling round and 71 (11.8±0.5) in the second round. The average floral density was 132.7±15.2 flowers m^2 in the first round and 121.1±12.8 flowers m^2 in the second round (Table A.2). There were significantly more plant taxa in the second round than in the first round (Wilcoxon signed-rank paired test: n=70, p=0.006), but floral density was not significantly different between rounds (Wilcoxon signed-rank paired test: n=70, p=0.205).

2.3. Pollinator pollen loads

In order to amplify the resolution of the identity of flowering plant taxa visited by insects through their pollen loads, we captured 60 commercial bumblebee workers and 73 honeybees during the first sampling round and 149 bumblebee workers and 36 honeybees during the second round. In addition, during the second round, we captured females of five native bee species. Specifically, four individuals of *Anthophora hispanica* (native bee extra-large size), nine of *Rhodanthidium sticticum* (native bee large size), 16 of *Eucera notata* (native bee large size), four of *A. leucophaea* (native bee large size) and four of *E. elongatula* (native bee medium size). We selected these native bee species because they were relatively abundant, easy to identify at first sight in the field and similar in size to bumblebees and honeybees. Additionally, several bee individuals from the categories specified in Table 1 were captured in order to partially identify the pollinator species

Table 1
Taxonomic categories assigned to pollinators.

Category	Description
Bombyliidae	Diptera from the Bombyliidae family
Bumblebee	Workers of the species *Bombus terrestris*
Bumblebee males	Males of the species *Bombus terrestris*
Coleoptera	Individuals of the Coleoptera order
Diptera	Diptera other than the Syrphidae family
Honeybee	Workers of the species *Apis mellifera*
Lepidoptera	Individuals of the Lepidoptera order
Native bee extra-large size	Hymenoptera of bee families, similar in size to *Bombus terrestris* (>150 mm length). E.g., *Anthophora hispanica*
Native bee large size	Hymenoptera of bee families, similar in size to *Apis mellifera* (around 120–150 mm length). E.g., *Rhodanthidium sticticum* and *Eucera notata*
Native bee medium size	Hymenoptera of bee families, similar in size to *Panurgus banksianus* and *Eucera elongatula* (around 90–119 mm length)
Native bee small size	Hymenoptera of bee families, similar in size to *Hoplitis adunca* and *Panurgus calcaratus* (<90 mm length)
Parasitoid	Individuals of the Parasitica infraorder of the Hymenoptera order
Syrphidae	Diptera of the Syrphidae family
Vespidae	Hymenoptera of the Vespidae family

present in the study area (Table A.3). Wild pollinators were not captured during the first sampling round because they were scarce (see results). All specimens are deposited at the EBD-CSIC.

Bees were captured while foraging using aerial nets throughout the transect walks, just after completing the plant-pollinator survey or on different days within the sampling period. Pollen loads were preferably collected from specialised structures of bees (i.e., corbiculae, scopae) to capture pollen that is actively collected or from their bodies (i.e., pollen passively transported) when the former was not observed.

Bee individuals were placed in individual clean vials in a chilled box for 5 min and their pollen was removed from their specialised structures using clean tweezers or by rubbing their bodies with a cube ($\sim$0.3 cm^3) of fuchsin jelly (Beattie, 1971). After that, the bees were released. Bumblebees, honeybees and the wild bee individuals that we could not identify in the field to species level were placed in individual clean vials in a chilled box and frozen at $-20\ ^\circ$C at the end of the day. Once in the lab, the specimens were identified and their pollen loads sampled using the same methods as in the field.

In the lab, each cube of fuchsin with body pollen was placed on a slide, slightly heated and covered with a cover slide. The pollen samples from specialised structures were first placed in Eppendorfs and mixed for 30 s with 300 µl ethanol or with 600 µl when the solution was too dense. Then, we added 15 µl of each solution to a droplet of hot fuchsin jelly and mounted it on a slide. In these cases, we diluted the pollen sample because of the high density of pollen grains, which would hinder their identification.

For each slide, we identified to the lowest taxonomic level possible the first 300 pollen grains observed in random fields at $\times$200 magnification under the light microscope. This amount of pollen has been frequently used as a diet estimator (e.g., Jha et al. (2013)). For pollen identification, we used a reference pollen collection of 68 plant species from the area obtained during the field sampling campaigns and, when needed, we asked for the help of experts. In total, we counted 103,068 pollen grains that belonged to 40 plant taxa. Of these, we could identify 23 and 10 to the species and genus levels, respectively (Table A.4). For brevity, we hereafter use the term "species" instead of "taxa" to refer to the lowest taxonomic level of both the recorded plants in the field and pollen identification in the lab.

In total, 55% of bumblebees and 73% of honeybees captured during the first sampling round had pollen from specialised structures, while in the second round it was 54% of bumblebees and 50% of honeybees. In the case of wild bees captured during the second round, 50% of the individuals of *A. hispanica*, 50% of *A. leucophaea*, 75% of *E. elongatula*, 31% of *E. notata* and 22% of *R. sticticum* had pollen from specialised structures. Although only the pollen actively collected by bees will be used for feeding, the pollen passively collected represents the flowers that the bee has visited (to gather nectar and/or pollen) over a long period of time, which is also a good proxy of floral resource use (Bosch et al., 2009). Thus, due to the low sample size particularly for native bees and the variable percentage of bees with pollen actively collected vs. pollen passively transported, we decided to merge both types of pollen and not to consider their origin in presenting the results.

2.4. Bumblebee internal parasites

In order to explore whether commercial bumblebees harbour internal parasites, all the bumblebees captured for pollen load samples (209 individuals) were dissected in the lab. The air sacs of each bumblebee were inspected under a magnifying lens for the tracheal mite *Locustacarus buchneri* (Podapolipidae) (Yoneda et al., 2008). Then, 3 pieces (0.2 cm$\times$0.2 cm, approx.) of the fat body, hind gut and Malpighian tubules were dissected and mounted on a slide. We screened each slide at $\times$400 magnification for the presence of spores of the neogregarine *Apicystis bombi* (Lipotrophidae), the trypanosome *Crithidia* (Trypanosomatidae) and microsporidians of the genus *Nosema* (Nosematidae). All these parasites potentially affect bumblebee health (Macfarlane et al.,

1995; Otterstatter and Whidden, 2004; Otti and Schmid-Hempel, 2007) and have been reported to spread to other congeners, for instance, via shared flowers (Durrer and Schmid-Hempel, 1994; Goka et al., 2006; Graystock et al., 2015). Moreover, there is robust evidence of parasite transmission between the genera *Bombus* and *Apis* (Fürst et al., 2014; Graystock et al., 2013) as well as some signs of transmission between *Bombus* and other bee genera (Figueroa et al., 2020; Ngor et al., 2020; Tian et al., 2018). We estimated parasite prevalence (presence) instead of individual infection levels (abundance) because the latter is influenced by confounding factors that drive infection intensity (Rutrecht and Brown, 2009).

2.5. Statistical analyses

All statistical analyses were performed in R v. 3.6.1 (R Core Team, 2019). Bumblebee and honeybee observations were pooled over the two sampling rounds. We were not interested in the temporal effect, which would be caused only by phenological reasons or management actions. For analysis concerning wild pollinators, we only used data from the second round because they were scarce in the first round (see results). Consistently, data was expressed as the number of visitors per 100 m^2 (hereafter density).

To analyse whether bumblebee abundance in natural habitats was dependent on greenhouse distance, we built a generalised linear mixed model (GLMM) with gamma as the error distribution family and log as the link function. Bumblebee density (expressed as $x+1$ because the selected model family does not allow data with zero values) was included as the response variable, and the distance to greenhouses, floral density (flowers m^2) and floral richness (mean number of species between rounds per plot) were included as explanatory variables. All explanatory variables were scaled with the 'scale' base function in R. 'Landscape identity' was included in the GLMM as a random factor to account for the non-independence of plots located in the same landscapes. We checked for spatial autocorrelation through model residual maps in the geographical space and no signs of spatial autocorrelation were observed.

To explore the overlap in floral resource use of commercial bumblebees with the other pollinators, we separately analysed the observed plant-pollinator interactions and pollen loads. For the observed interactions, we compared the diet breadth and resource niche overlap between bumblebees and honeybees, and with the most abundant wild pollinator groups (see below for index calculations). Whereas for pollen loads, we compared the diet breadth and resource niche overlap between bumblebees and honeybees, and with the 5 native bee species captured. Comparisons between bumblebees and honeybees were performed per round, while that between bumblebees and wild pollinators were exclusively performed with data from the second round.

Diet breadth of pollinators was calculated using the inverse of Simpson's diversity index ($1/D = 1/\sum |n_i(n_i-1)/N(N-1)|$; where n_i is either the number of flowers of species i that were visited or the number of pollen grains counted and N is either the total number of flowers visited or the total number of pollen grains counted). Larger values indicate higher diversity. *Resource niche overlap* between bumblebees and the rest of the pollinators was calculated using the Hulbert proportional similarity index (Hurlbert, 1978). The proportional similarity index (PS) measures the degree to which frequency of interspecific encounter is higher or lower than it would be if each species used each floral resource state in proportion to its abundance. It is calculated as $PS = \sum_i min(p_{i,b}, p_{i,o})$; where $p_{i,b}$ is either the proportion of interactions on species i or the proportion of pollen grains of species i for bumblebees and $p_{i,o}$ is either the proportion of interactions on species i or the proportion of pollen grains of species i for another pollinator. PS values can range from 0 (no resource niche overlap with bumblebees) to 1 (complete resource niche overlap).

To analyse whether honeybee abundance and wild pollinator abundance and diversity were related to greenhouse distance and bumblebee

abundance, we built GLMMs with honeybee and wild pollinator density and pollinator diversity (Shannon-diversity index) (all x+1) as response variables, and the distance to greenhouses, bumblebee density, floral density and floral richness as explanatory variables. The models were fitted with gamma error distribution and log link function. All explanatory variables were scaled with the 'scale' base function in R. 'Landscape identity' was included in the models as a random factor to account for the non-independence of plots located in the same landscapes. A different analysis was conducted for the abundance of wild bees and non-bees because, in contrast to non-bee species, all bees depend on pollen and nectar throughout their entire life-cycle (Michener, 2007) and are central place foragers which constrain distances to foraging locations (Beutler and Loman, 1951). Thus, wild bees can be more reliant on natural habitats than non-bee insects (Rader et al., 2016). We checked for spatial autocorrelation through model residual maps in the geographical space and no signs of spatial autocorrelation were observed.

3. Results

In total, we recorded 3434 pollinators belonging to the 14 categories defined in Table 1. We observed 101 bumblebee workers (3% of records; 70 individuals during the first round and 31 during the second) in 31 different plots (28 during the first round and 13 during the second). Remarkably, bumblebees were only present at distances ≤500 m from greenhouses. Honeybees were the most abundant pollinator (74% of records) present in all plots. We observed 1675 and 858 individuals during the first and second rounds, respectively. Finally, we observed 128 and 667 wild pollinators during the first and second rounds, respectively. The most abundant wild pollinators during the second round belonged to the following categories: "native bee small size" (331), Coleoptera (146), Diptera (49), Syrphidae (42), "native bee large size" (33) and "native bee medium size" (33).

3.1. Density of bumblebees at increasing distances from greenhouses

The density of commercial bumblebees in natural areas was negatively related to increasing distances from greenhouses (Table 2, Fig. 2). In fact, more than half (52%) of the recorded bumblebees were observed in plots at 30 m from greenhouses, 14% at 100 m, 29% at 200 m and only 5% in plots 500 m away from greenhouses. We did not observe bumblebees at one, five or seven kilometres away from greenhouses. Bumblebee density was not related to floral density or to plant richness (Table 2).

3.2. Parasite prevalence in bumblebees

Of the 209 dissected bumblebee workers, 40.7% of them had parasite spores. We did not detect the presence of the tracheal mite *Locustacarus buchneri*. The prevalence of *Apicystis bombi* was low; only seven bumblebees (3.4% prevalence) contained spores in their fat body. However, spores of *Nosema* and *Crithidia* were found in 27 (12.9% prevalence) and 57 (27.3% prevalence) individuals, respectively. Six bumblebee individuals had spores of two different parasites: five with *Crithidia* and *Nosema* and 1 with *Crithidia* and *A. bombi*.
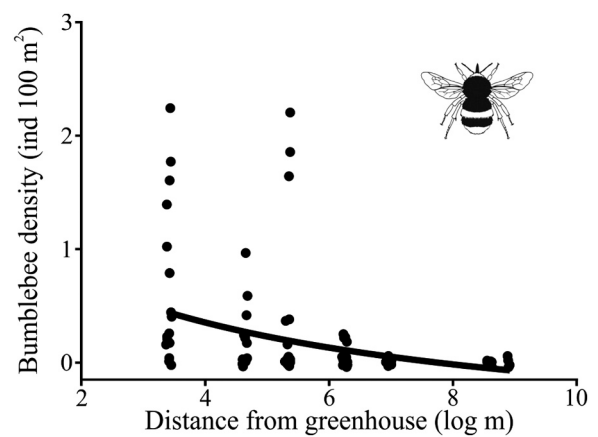

Fig. 2. Commercial bumblebee density in natural areas at increasing distances from vegetable greenhouses. The solid line indicates a significant relationship. Bumblebee silhouette taken from www.divulgare.net.

3.3. Floral resources use

In total, we observed pollinator interactions with 50 plant species and identified pollen loads from 40 plant taxa (Table A.4). During the first sampling round, bumblebees visited 11 species and carried pollen from 12, while honeybees visited 26 (nine shared with bumblebees) and carried pollen from 16 (seven shared with bumblebees) plant species. Bumblebees focused most of their visits (69% of recorded visits and 76% of pollen) on a single species, *Echium creticum*. In contrast, honeybee visits and pollen carried were less specialised (e.g., ~20% *Thymus hyemalis*, ~20% *Asphodelus tenuifolius* and ~20% *E. creticum*). Therefore, honeybees had broader diets than bumblebees (Table 3). The proportional similarity index (PD) between bumblebees and honeybees showed values of 0.38 and 0.32 for the observed interactions with plants and for the carried pollen, respectively.

During the second sampling round, on the basis of observed plant-pollinator interactions, the patterns were similar. Bumblebees had lower diet breadth compared with honeybees but also with the rest of

Table 3
Pollinator diet breadth values per round based on floral visits and body pollen loads.

Round	Data	Pollinator	Diet breadth
First			
	Floral observations	Bumblebee	2.10
		Honeybee	7.08
	Pollen loads	Bumblebee	1.64
		Honeybee	8.12
Second			
	Floral observations	Bumblebee	3.14
		Honeybee	4.52
		Syrphidae	12.30
		Bee large	6.77
		Diptera	6.09
		Bee medium	4.36
		Coleoptera	4.35
		Bee small	2.54
	Pollen loads	Bumblebee	1.48
		Honeybee	7.01
		Rhodanthidium sticticum (Megachilidae)	2.82
		Anthophora leucophaea (Apidae)	2.18
		Eucera notata (Apidae)	2.05
		Eucera elongatula (Apidae)	2.03
		Anthophora hispanica (Apidae)	1.05

Table 2
Results of the GLMM obtained for bumblebees. Significant p-values are in bold.

Model	Terms	Estimate	SE	Statistic	p value
Bumblebee density	Intercept	0.18	0.06	3.16	**0.00**
	Distance	−0.10	0.04	−2.40	**0.02**
	Floral density	0.04	0.03	1.36	0.17
	Plant richness	0.00	0.04	−0.10	0.92

the most abundant pollinator groups (Table 3). This was in part due to bumblebees again mainly visiting *E. creticum* (55% of recorded visits) but also seven other plant species. Honeybees visited 22 species (six shared with bumblebees), while the group "native bee large size" visited 13 species (four shared with bumblebees) (Fig. 3a). Bumblebee diet largely coincided with that of honeybees (PS=0.42) and with the "native bee large size" group (PS=0.42). The diet of bumblebees overlapped to a lesser extent with the other most abundant pollinator groups (Syrphidae: PS=0.29; "native bee medium size": PS=0.21; Diptera: PS=0.14; and there was almost no overlap with "native bee small size": PS=0.04; Coleoptera: PS=0.03) (Fig. 3a).

During the second round, on the basis of pollen loads, the honeybee was the only species that showed high values of diet breadth (Table 3). Bumblebees carried pollen from 25 species but 82% of the total pollen

recorded belonged to *E. creticum*. Anecdotally, only eight bumblebee individuals carried pollen from tomato crop flowers, the target crop for which most of them are deployed. Bumblebee pollen loads largely overlapped with the rest of the captured bees (Fig. 3b). Specifically, bumblebees shared all of their plant visits with *Anthophora hispanica* (PS=0.84), 83% with *Eucera notata* (PS=0.63), 75% with *E. elongatula* (PS=0.60), 70% with *Rhodanthidium sticticum* (PS=0.64), 59% with *A. mellifera* (PS=0.36) and 50% with *A. leucophaea* (PS=0.62) (Fig. 3b). Note that except for *A. mellifera*, all these species belong to the native bee extra-large, large and medium size groups.
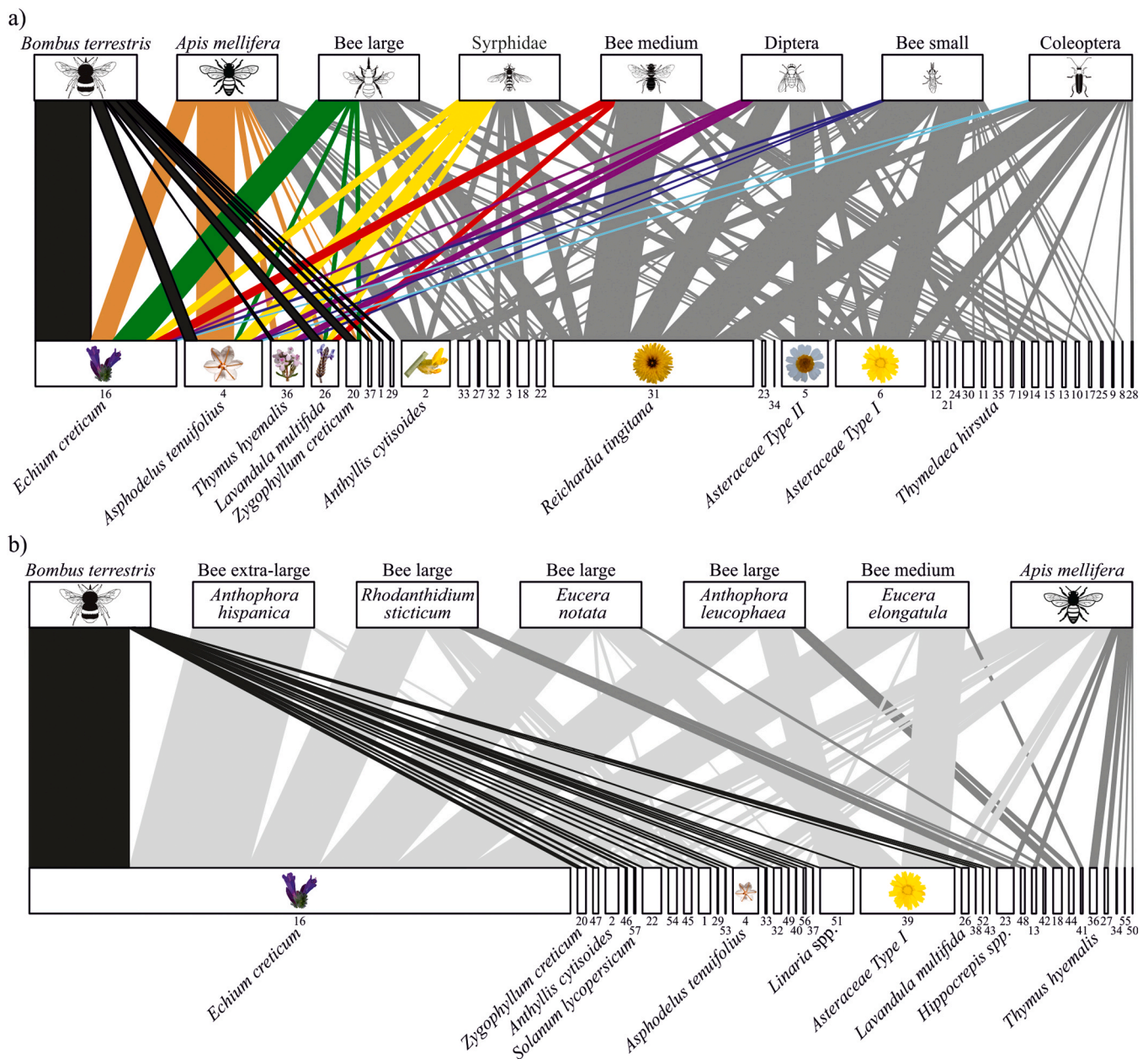

Fig. 3. Illustration of plant-pollinator (a) and pollen load (b) interaction networks in the second sampling round of the study. As sample sizes varied among pollinators and captured bee species, interaction networks were proportionally homogenised (i.e., interaction bar widths sum the unity for each pollinator and bee species). The widths of the links are proportional to the number of interactions observed or pollen grains counted on pollinators. Black lines indicate bumblebee plant interactions. Dark grey links indicate non-shared plant and pollen interactions between bumblebees and the rest of the pollinators. For more details of plant taxa names, here referred to by numbers, see Tables A.2 and A.4. Insect silhouettes (except the honeybee) taken from www.divulgare.net.

3.4. Influence of distance to greenhouses and bumblebee presence on pollinators

The density of honeybees did not change with distance to greenhouses (Table 4, Fig. 4a) nor was it related to bumblebee density (Table 4). However, it was positively related to floral density, although not to plant richness (Table 4). For wild pollinators, neither wild bee nor non-bee densities changed with distance to greenhouses (Table 4, Fig. 4b and c) or with bumblebee density, floral density and plant richness (Table 4). In contrast, overall wild pollinator diversity was positively related to floral density and plant richness but not related to the distance to greenhouses and bumblebee density (Table 4). We further analysed if there was any relationship between bumblebee density and honeybee, wild bee and non-bee densities as well as between bumblebee density and wild pollinator diversity only in the plots where bumblebees were present. We did not observe any significant changes (see Table A.5 for more details). Moreover, we plotted the relationships between bumblebee densities and the densities of the most sensitive taxonomic groups on the basis of the diet overlap results (i.e., native bee extra-large, large and medium size groups). The low sample size of each of these groups prevents a full statistical analysis, but no signs of negative relationships were shown except for the group "native bee large size" (see Fig. A.1). However, due to data limitations, we cannot suggest a potential negative effect of bumblebees on large native bees.

4. Discussion

We found most commercial bumblebees (*Bombus terrestris*) foraging over distances less than 200 m from greenhouses. Moreover, in line with previous studies (Graystock et al., 2013; Murray et al., 2013; Trillo et al., 2019a), more than a third of these bumblebees had trypanosomatids (27%), microsporidians (13%) and/or neogregarines (3%) parasites. The diet of bumblebees overlapped to some degree with some pollinator taxa, but especially with honeybees and large bees. Nevertheless, pollinator abundance and diversity were related neither to distance to greenhouses nor to bumblebee abundance.

4.1. Bumblebee density at increasing distances from greenhouses

In this region, vegetables are grown in sealed greenhouses. However, we observed commercial bumblebee individuals foraging adjacent to greenhouses. Similar observations of spillover have been reported in other countries where bumblebee colonies are deployed in part because greenhouses are not airtight (Murray et al., 2013; Whittington et al., 2004). However, bumblebee densities in natural areas decreased markedly at increasing distances from greenhouses with most bumblebees (95%) foraging within a radius of less than 200 m from them. Foraging flight distances of bumblebees, as central-place foraging insects, are constrained by the location of their colony (Beutler and Loman, 1951). In general, mean foraging distances of *B. terrestris* workers have been reported to be about 200–500 m (Osborne et al., 1999; Redhead et al., 2016) and 2.5 km as a maximum distance (Redhead et al., 2016). Small differences in bumblebee flight distances between study systems may be related to foraging availability at the local and landscape scales (Heinrich, 1979; Redhead et al., 2016). Our study provides empirical data with a good degree of resolution and replication confirming previous findings on the distance at which commercial bumblebees spill over from greenhouses. Furthermore, our results suggest that commercial bumblebees have not yet become naturalised in the study area due to the lack of bumblebees at distances far from greenhouses. It is likely that physiological barriers prevent their naturalisation, similar to that of native bumblebees which are also not present.

4.2. Parasites in commercial bumblebees

Of the bumblebees captured foraging, 41% carried parasites. Specifically, bumblebees carried the three commonest parasites: *Apicystis bombi*, *Crithidia* and *Nosema*. These parasites have been frequently reported in commercially produced colonies worldwide with similar high prevalence levels (Graystock et al., 2013; Murray et al., 2013; Trillo et al., 2019a). Worryingly, the three commonest bumblebee parasites not only affect commercial bumblebee health and colony success (Graystock et al., 2016; Rutrecht and Brown, 2009; Shykoff and Schmid-Hempel, 1991), but also can spread to other species. There is robust evidence about the spread of parasites from commercial bumblebees to native congeners (Durrer and Schmid-Hempel, 1994; Goka et al., 2006; Graystock et al., 2015). This fact has been linked to the decline of several species worldwide (Meeus et al., 2011). In our study region, native bumblebees are not present in the lowlands where greenhouses and commercial bumblebees are, and thus disease transmission to native bumblebees is unlikely to occur. However, the rapid

Table 4
Results of the GLMMs obtained for the different pollinators. Significant p-values are in bold.

| Model	Terms	Estimate	SE	Statistic	p value
Honeybee density	Intercept	1.87	0.12	15.92	**0.00**
	Distance	−0.12	0.09	−1.33	0.19
	Bumblebee density	0.05	0.06	0.72	0.48
	Floral density	0.29	0.06	5.02	**0.00**
	Plant richness	−0.07	0.07	−0.90	0.37
Wild bee density	Intercept	0.79	0.17	4.76	**0.00**
	Distance	0.06	0.11	0.49	0.62
	Bumblebee density	−0.05	0.08	−0.72	0.47
	Floral density	−0.06	0.08	−0.75	0.45
	Plant richness	0.10	0.10	1.04	0.30
Wild non-bee density	Intercept	0.75	0.13	5.81	**0.00**
	Distance	0.12	0.09	1.33	0.18
	Bumblebee density	−0.04	0.07	−0.61	0.54
	Floral density	0.04	0.07	0.52	0.60
	Plant richness	0.03	0.09	0.34	0.74
Wild pollinator diversity	Intercept	0.52	0.05	10.25	**0.00**
	Distance	0.04	0.04	0.94	0.35
	Bumblebee density	0.03	0.03	0.79	0.43
	Floral density	0.08	0.03	2.49	**0.01**
	Plant richness	0.10	0.04	2.65	**0.01**
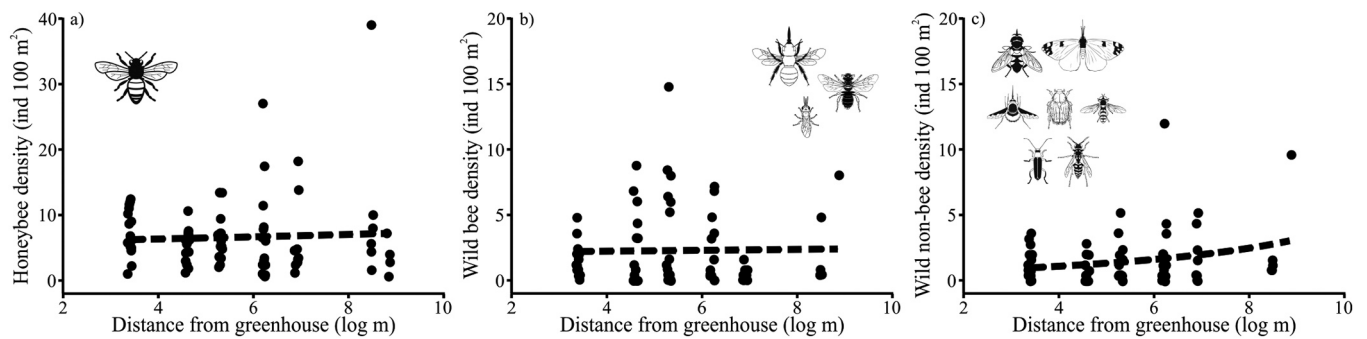
Fig. 4. Managed honeybee (a), wild bee (b) and non-bee (c) densities in natural areas at increasing distances from vegetable greenhouses. The dashed lines indicate non-significant relationships. Insect silhouettes (except the honeybee) taken from www.divulgare.net.

agricultural expansion in the last few decades towards highlands could lead to a higher likelihood of interaction between commercial and native bumblebees through shared visited flowers. Parasite spread from bumblebees to other bee genera may also occur. For instance, it has been reported that honeybees (*Apis mellifera*) could be infected by the parasites *A. bombi* (Graystock et al., 2013) and *N. bombi* (Vavilova et al., 2017). Moreover, infections by the parasites *A. bombi* and *C. bombi* have also been detected in bees of the genera *Osmia* and *Megachile* (Figueroa et al., 2020; Ngor et al., 2020). Nevertheless, there are also other studies in the literature that have found no evidence of parasite transmission from bumblebees to wild bees (e.g., Fernández et al. (2020), Whitehorn et al. (2013)). In any case, companies should agree to produce parasite-free colonies to avoid posing risks to bee health.

4.3. Diet breadth and resource niche overlap

As expected, bumblebees visited and carried the pollen of a large diversity of plant taxa. Bumblebees are frequent pollinators of many crops and wild plants worldwide (Goulson, 2010). They possess ample flower handling skills (Dafni and Shmida, 1996) and the health and success of their colonies depend to some degree on the quality of their diets (Brunner et al., 2014; Tasei and Aupinel, 2008). Surprisingly, in our study area, bumblebees were highly attracted to *Echium creticum*. As a result, bumblebees showed low diet breadth compared with honeybees and in general with most pollinators. Nevertheless, bumblebee diet partially overlapped with the pollinator community. There was substantial variation in diet overlap between bumblebees and other pollinators, being higher with honeybees and large wild bees. This variability is expected as wild pollinators structure its foraging preferences along a trait matching axes, with larger pollinators visiting larger flowers (Naghiloo et al., 2021). As Goulson (2003) mentioned and other researchers have subsequently found (Matsumura et al., 2004; Trillo et al., 2019b), it seems reasonable to find high potential for competition between bumblebees and other bees with a similar ecological niche. However, high floral resource overlap does not always imply competition if, for instance, floral resources are not a limiting factor (Herbertsson et al., 2016).

4.4. Influence of bumblebees and agricultural expansion on pollinators

There was no significant relationship in pollinator abundance and diversity with respect to bumblebee abundance in natural areas. Despite the fact that our study was performed in a region with the largest concentration of greenhouses in the world, bumblebee abundance was relatively low even in areas close to greenhouses. The abundance of commercial bees seems to play an important role in the degree of competition with native pollinators, along with floral resource limitation (Herbertsson et al., 2016). For instance, honeybee hives harbour a large number of individuals (20–50 K individuals, Jean-Prost and Médori, 1994), approximately 100 times that of bumblebee colonies.

Hordes of honeybees impair wild pollinators all over the world through increased competition for food resources (Angelella et al., 2021; Magrach et al., 2017). In contrast, there is no evidence of competition between commercial bumblebees and native pollinators for food resources in Europe (reviewed by Chandler et al. (2019)). However, some signs of competition have been found in regions where exotic *B. terrestris* has become established in the wild and is invasive and abundant, such as in Japan (Ishii et al., 2008). Thus, we cannot discard similar facts in Europe. Note that we identified pollinators into taxonomic groups instead of at a species level, and hence our results regarding diversity should be interpreted with caution. Our study was not designed to see the effects on specific or rare species, but community wide effects.

Honeybees were by far the most abundant pollinators, with roughly constant densities regardless of the distance to greenhouses or the abundance of bumblebees. In the study area, honeybee hives are placed in natural areas for honey production. In fact, honeybees are currently the most frequent pollinators in crops (Garibaldi et al., 2013) and in natural habitats worldwide (Hung et al., 2018). Therefore, the number of honeybees is closely linked to human decisions at the landscape scale rather than exclusively to agriculture (Trillo et al., 2018). However, at the local scale, there was a positive relationship between honeybee and floral densities. A preference of honeybees for flower-rich patches and their behaviour in communicating the location of such resources within the hive could explain such a pattern, as seen in other studies (González-Varo and Vilà, 2017).

Neither wild pollinator density nor diversity were related to distance from greenhouses, despite the fact that there is large amount of evidence that has found a negative association between the abundance of some pollinator populations and agricultural expansion in the landscape (e.g., Holzschuh et al. (2016)). We propose two explanations for the lack of this relationship in our study. On one hand, most wild bees were small and thus had small foraging ranges (Gathmann and Tscharntke, 2002). Whether or not floral, and also nest, resources are available at the local scale may regulate how wild bees respond regardless of landscape composition (González-Robles et al., 2020; Klein et al., 2007). On the other hand, non-wild bees can be less reliant on natural areas than wild bees due to different needs throughout their life-cycle (Rader et al., 2016). For instance, larvae of some Diptera species can feed on crop aphids or on decaying organic matter in aquatic systems. These resources are closely linked to disturbed habitats (Winfree et al., 2011).

5. Conclusions

Even though the use of commercial bumblebees in greenhouse crops provides a huge benefit to farmers (Velthuis and van Doorn, 2006), there is also evidence showing little or no positive effects (e.g., Trillo et al. (2018)). Moreover, commercial bumblebees may threaten native pollinators for a number of reasons (e.g., Bartomeus et al. (2020); Ishii et al. (2008); Meeus et al. (2011)). Although in our study we did not record a high spillover into adjacent natural habitats or significant impacts on

native pollinator populations, we need to be cautious. The fact that bumblebees are scarce and restricted to the vicinity of greenhouses implies that wild pollinator densities are not significantly impacted. However, more subtle effects related to pathogen transmission or direct competition with specific species should not be discarded. Note that wild bee densities are not high and the long term presence of managed honeybees may also mask such relationships. We propose some actions to be considered to preserve the health of the pollinator community: prevent commercial bumblebees from escaping greenhouses, accurately control their health when they are raised by companies and optimise their use in terms of the number of colonies per hectare of crop.

CRediT authorship contribution statement

AT, IB and MV designed the study. AT performed the field and lab work. FJOS identified the bees and JB supervised pollen identification. AT prepared and analysed the data and IB and MV advised him. AT wrote the first draft of the paper and all co-authors critically contributed to subsequent drafts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2021.107604.

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