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Contrasting occurrence patterns of managed and native bumblebees in natural habitats across a greenhouse landscape gradient

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ABSTRACT

In recent decades, there has been a remarkable expansion of pollinator-dependent crops. An increase in the use of commercial pollinator colonies associated with these crops may promote the spillover of managed pollinators into nearby natural habitats. There, these managed pollinators can exploit floral resources similar to those of wild pollinators, and thus increase competition for local resources. Nonetheless, managed pollinator spillover has been poorly studied and research has focused on only one species, the western honeybee (*Apis mellifera*). In south-western Spain, we investigated the presence, density and exploitation of floral resources by managed (*Bombus terrestris*) and native bumblebees (*B. t. lusitanicus*) in 19 Mediterranean pinewood understories across a landscape gradient of strawberry polytunnel greenhouse cover. Sampling was performed over two consecutive years in two seasons: winter, when strawberries begin flowering and farmers frequently use colonies, and spring, when there is greater availability of floral resources and wild pollinators thrive. In winter, the density of managed bumblebees in pinewoods was higher than that of native bumblebees. The presence of managed and native bumblebees in pinewoods showed contrasting patterns in relation to greenhouse cover in the landscape. The presence of managed bumblebees was positively associated with greenhouse cover, whereas that of native bumblebees was negatively associated with greenhouse cover. Overall, the presence and density of bumblebees did not differ between seasons. The two bumblebee subspecies showed similar wild flowering plant preferences, particularly in winter, when flowering plant species are scarce. We conclude that, although managed bumblebees are placed in greenhouse crops, their pollination role extends beyond these crops. Further studies are needed to assess the pollination function of managed pollinators in crops in order to reduce their spillover into natural habitats and thus, the risks posed to native pollinators.

1. Introduction

Human demand for fruits and seeds has increased over the past few decades (Aizen and Harder, 2009; Gallai et al., 2009). Consequently, the area devoted to flowering crops, many of which require or benefit from animal-mediated pollination (Klein et al., 2007), has disproportionately increased compared to that of non-pollinator dependent crops (Aizen et al., 2008). These expanding agricultural systems offer floral resources that can attract pollinators from nearby natural habitats (Blitzer et al., 2012).

From an economic point of view, the spillover of wild pollinators from natural habitats to crops may not be sufficient to meet the pollination services required. In other words, there may be insufficient pollinators visiting crop flowers to achieve maximum seed and fruit set, thereby affecting the quality and quantity of crop yield (Klein et al., 2007; Ricketts et al., 2008). For instance, the remaining small patches of natural habitat may not support enough pollinators to pollinate large areas of flowering crops (Garibaldi et al., 2011; Holzschuh et al., 2016; Eeraerts et al., 2017). In addition, the bloom period of crops often does not overlap with the flying period of wild pollinators because crops are cultivated to complete their life cycle in a different period than pollinators. Further, greenhouse crops may not be accessible to pollinators.

For these reasons, managed pollinators are frequently used in crops to supplement wild pollinator visitation to flowers (Morse, 1991; Velthuis and van Doorn, 2006; Rucker et al., 2012).

Commercial production of bumblebees began in the late 1980s to replace hand- and mechanical-pollination of tomato crops (van Ravestijn and van der Sande, 1991). This quickly led to the mass production and worldwide transport of these bees to supplement the pollination of about 20 different crops (Velthuis and van Doorn, 2006). Bumblebees are generalist pollinators (Memmott, 1999) and require a diverse pollen diet to thrive (Brunner et al., 2014; Roger et al., 2017). Despite their use in crops, they frequently escape and spillover into adjacent natural habitats to forage (Inari et al., 2005; Murray et al., 2013; Whittington et al., 2004), as these areas typically provide more consistent and diverse floral resources. Managed bumblebees exploit a wide range of wild flowering plant species, increasing the likelihood of competitive interactions with local pollinator species (Ishii et al., 2008; Whittington et al., 2004), hybridisation with congeners (Ings et al., 2005; Kraus et al., 2011), as well as the spread of parasites (Goka et al., 2006).

In addition to the evidence of bumblebees moving from crops to natural habitats, spillover has been shown to vary spatially and temporally. Spillover from crops to natural habitats may depend on crop cover in the surrounding landscape (e.g. Gaines-Day and Gratton, 2016; Klein et al., 2012; Magrach et al., 2017). Large crop areas will require a larger number of commercial colonies to meet pollination demands, eventually spilling over into natural habitats (González-Varo and Vilà, 2017). Furthermore, other factors such as the temporary management of colonies and floral resource availability throughout the year may mediate the magnitude of the spillover, with potential detrimental impacts on local pollinator fauna (González-Varo and Vilà, 2017).

Huelva (SW Spain) is the second largest strawberry producer in the world after California. In Spain, the strawberry flowering period spans from November to May, and commercial bumblebee colonies are used for pollination services. We investigated the presence, density and exploitation of floral resources by managed (*Bombus terrestris*) and native bumblebees (*B. t. lusitanicus*) in Mediterranean pinewood understories across a gradient of strawberry greenhouse cover, over two consecutive years. We surveyed plant-bumblebee interactions in understorey pinewoods during the two seasons in which managed and native bumblebees overlap: in winter, when farmers use more bumblebee colonies to overcome a shortage in wild pollinators, and in spring, when most wild plants bloom and wild pollinators thrive. Specifically, we addressed the following questions: (1) Are the presence and density of managed bumblebees in pinewoods across the landscape similar to those of native bumblebees? (2) Are the presence and density of managed and native bumblebees associated with greenhouse cover in the landscape? (3) Does their presence and density differ between seasons? (4) Do managed and native bumblebees exploit similar flowering plant taxa?

We expect the presence and density of managed bumblebees in pinewoods to be greater than those of native bumblebees in winter and the reverse in spring. We expect the presence and density of managed bumblebees to be positively associated with greenhouse cover and to be greater in winter than in spring. We expect the reverse to be true for native bumblebees. Finally, we expect both managed and native bumblebees to forage on similar wild flowering plants, as well as on strawberry flowers.

2. Materials and methods

2.1. Study area and bumblebee species

The study was conducted in a 40 km × 20 km area in the Guadalquivir Valley in the province of Huelva (SW Spain) (Fig. 1) in 2014 and 2015. Since 1965, in the province of Huelva there has been an increase in the production of berries, especially strawberries

(*Fragaria × ananassa*). This area has become one of the major producers worldwide (i.e. around 7330 ha producing 308,500 tons of strawberries in 2014–2015; see FYH, 2015). The study area is characterised by a mosaic of intensive strawberry crops under semi-open polytunnel greenhouses and remaining patches of natural habitat. These patches are either shrublands or pinewoods (*Pinus pinea*), which are composed of a rich understorey vegetation (González-Varo et al., 2016). Amongst the most representative entomophilous species are the shrubs *Cistus* spp., *Erica* spp., *Halimium* spp., *Lavandula stoechas*, *Rosmarinus officinalis* and *Ulex australis* and the herbs *Echium* spp., *Leontodon* spp. and *Linaria* spp. The climate is Mediterranean with warm and dry summers and mild winters (mean annual temperature and precipitation are 18.2 °C and 525 mm, respectively; AEMET, 2015).

Strawberry fruit quality benefits from insect-mediated pollination (Klatt et al., 2014; Trillo et al., 2018; Zebrowska, 1998). To secure maximum revenue in south-western Spain and worldwide (Velthuis and van Doorn, 2006), farmers frequently use commercial *B. terrestris* colonies. Here, the strawberry flowering period spans from November to May. Notably, the use of bumblebee colonies is typically more intensive in winter, because wild pollinators are scarce and the weather conditions do not favour their activity (although see Trillo et al., 2018).

Bombus terrestris is native to Europe, North Africa and West and Central Asia, and is represented by 9 subspecies (Estoup et al., 1996; Lecocq et al., 2016; Rasmont et al., 2008). Two subspecies represent the bulk of the bumblebees traded around the world for pollination services. Before the 2000s, the subspecies *terrestris* was the most widely traded, while the subspecies *dalmatinus* has dominated in the last decade (Ings et al., 2009, 2006; Owen et al., 2016). Until recently, it has not been common for countries to use managed bumblebees from their native subspecies (e.g. *B. t. audax* in UK; see Graystock et al., 2016).

In the study region, the managed bumblebees used are not the native bumblebee *B. t.* subsp. *lusitanicus*. Here, bumblebees occur at very low frequency (Magrach et al., 2017), as they are at the limit of their distributional range (Goulson, 2010).

2.2. Sampling design

We selected 19 pinewood plots (50 m × 50 m) within the study area. The 2 km landscape radius surrounding each plot represented a gradient of greenhouse cover ranging from 0% to 64% (mean ± SD = 20.3 ± 19.4%), and a gradient of natural habitat ranging from 10% to 99% (mean ± SD = 47.9 ± 26.6%). These two main land-use types are negatively correlated (Spearman rank correlation: R = -0.50, n = 19, p = 0.03) and thus, we considered greenhouse cover the key factor influencing bumblebee patterns in natural habitats. On average, the remaining land-use types (e.g. denuded areas, urban areas, wetlands and other agricultural fields) ranged from 0.7% to 89% (mean ± SD = 31.6 ± 23.6%).

Plots were located at different distances from the closest greenhouse, ranging from 25 m to 4389 m. The average (± SD) distance between study plots was 4246 ± 946 m (range = 2977–6577 m) and most plots (~70%) were separated by > 4 km, with no overlap in their 2 km radius buffers. However, the overlap for the closest plots was negligible, affecting only 11.5% (range = 4.1–23.5%) of their buffer area (see Fig. 1). Moreover, 2 km is considered to be the maximum foraging range of workers from most bumblebee species (see Osborne et al., 2008), as bumblebees often limit their foraging range at the local scale if floral resources are abundant (Bommarco et al., 2010; Osborne et al., 2008). Thus, we are confident that our study plots meet independence criteria in terms of the individual bumblebees recorded during our surveys. The percentages of greenhouse cover were calculated using ArcGIS (ESRI, 2011) based on the land-cover map of Andalucía from 2011 (Moreira et al., 2011), and updated with Google maps (2013) and field surveys (Table A1).

To ensure similar flowering plant species identity across the landscape gradient, all sampling plots shared at least five species. These
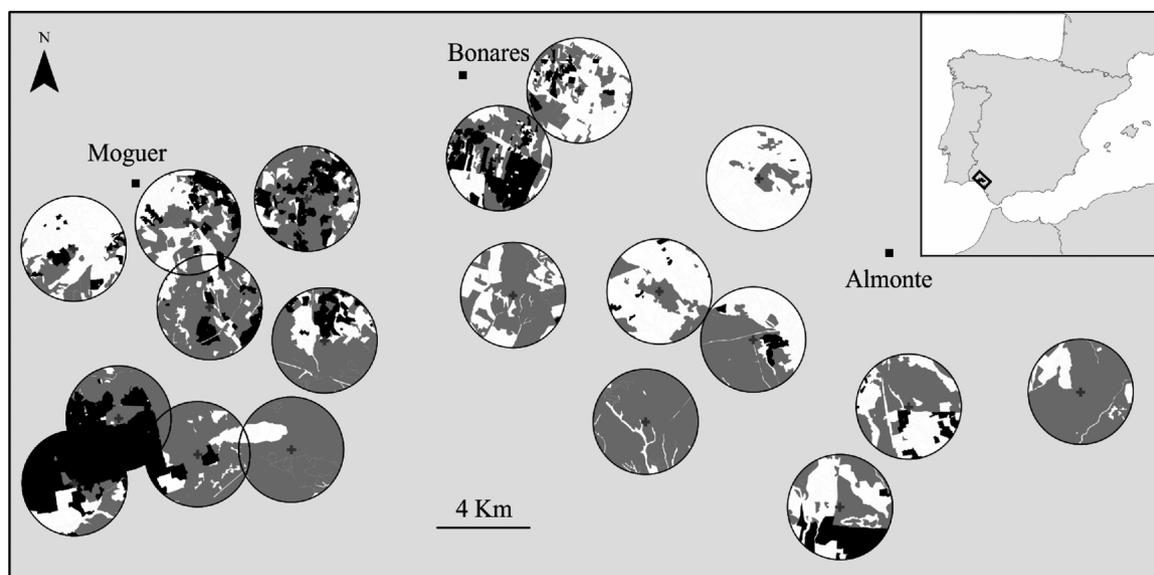
Fig. 1. Study area in the province of Huelva (SW Spain). Circles indicate the 2 km landscape radius surrounding each pinewood plot. Greenhouse (black colour), natural habitat (grey colour) and other land-use types such as agricultural fields, wetlands and urban areas (white colour) represent the main land covers in the study area. Names denote towns (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

plant species were *Halimium calycinum*, *R. officinalis* and *U. australis*, which bloom mainly in winter but also in spring, and *Cistus salvifolius* and *L. stoechas*, which bloom only in spring.

We conducted bumblebee censuses in 2014 and 2015 during two seasons: winter (early-January to early-February) and spring (late-March to late-April). We sampled each plot on four random days per season and year, two in the morning (09:30–13:00) and two in the afternoon (13:00–17:00), by walking 5–8 parallel transects (on average 7.91 transects per plot, 50 m × 5 m each) for 15 min, selecting a different transect each sampling time. Along each transect, we recorded the number of bumblebees of each subspecies foraging on flowers, and noted the plant taxa. Bumblebees were visually identified by colouration patterns according to Rasmont et al. (2008) and Ornosa and Ortiz-Sánchez (2004). Native bumblebees are characterised by reddish hair, especially on the scutellum and legs, while managed subspecies (hereafter, 'managed bumblebees') have black hair. In total, we conducted 2348 transect walks on 78 days accounting for a total of 587 sampling hours. Sampling was conducted on sunny and non-windy days with temperatures above 11 °C.

Prior to this study, we conducted pilot sampling from November 2013 to May 2014 to test the proposed protocol and explore the distribution of native and managed bumblebee populations across the landscape (Appendix: Pilot sampling, Table A1 and Fig. A1).

To quantify floral resource availability for pollinators, in each plot and on each census day, we walked five randomly placed 50 m parallel transects. We counted and identified receptive flowers within 40 cm × 40 cm quadrants placed every two meters. Therefore, the number of flowers was counted in 130 quadrants per plot and day. Overall, nine flowering plant taxa were recorded in winter (2.49 ± 0.13 plants per plot, mean ± SE, hereafter), and 29 flowering plant taxa in spring (6.39 ± 0.34 plants per plot). The average floral density (flowers m²) was 10.18 ± 3.18 in winter, and 30.87 ± 6.14 in spring (see Table A2 for more details).

2.3. Pollen loads

In addition to recorded plant-pollinator interactions and to amplify the resolution of floral resource exploitation, we captured both managed and native bumblebees during the censuses to identify their pollen loads. Bumblebees were captured while foraging using aerial nets and were frozen in individual clean vials for later identification of pollen

loads. Sample sizes were small, particularly for native bumblebees, due to their low abundance and our intention to avoid disrupting their populations (see results).

The body of each bumblebee was rubbed with a cube (0.3 cm × 0.3 cm) of fuchsin jelly (Beattie, 1971). The cube was then mounted on a slide to identify pollen grains under a microscope. For each slide, we counted and identified all pollen grains in 20 fields at ×200 magnification. For the identification of pollen grains, we used a reference pollen collection prepared during the sampling period. Because some plant taxa cannot be identified to the species level via the microscope, some pollen was assigned to the genus level (Table A3).

For the plant-bumblebee interactions recorded, we identified pollen loads in a total of 33 (0 in 2014 and 33 in 2015) managed bumblebees in winter and 23 (10 in 2014 and 13 in 2015) in spring. We also collected a total of 4 (0 in 2014 and 4 in 2015) native bumblebees in winter and 12 (8 in 2014 and 4 in 2015) in spring. Overall, we counted 28,039 and 37,840 pollen grains from the bodies of bumblebees. They belonged to 10 different plant taxa in winter and 20 plant taxa in spring, respectively. We identified 76.2% of the pollen to the species level (Table A3).

2.4. Statistical analyses

Bumblebee presence and density data were pooled over the two sampling years because there were no significant differences between years for any season (Wilcoxon signed-rank paired tests, all p > 0.05).

Differences in presence and plot density (bumblebees per 100 m²) between managed and native bumblebees in each season were analysed by Wilcoxon signed-rank paired tests. Plot density differences between seasons were also compared by Wilcoxon signed-rank paired tests. Because of small sample sizes (see results), we could not perform more complex analyses on bumblebee density.

The relationship between presence/absence of managed and native bumblebees with greenhouse cover, season (winter/spring) and their interaction were analysed using generalised linear mixed models (GLMM). Models were fitted with binomial error structures. The site was included as a random factor to account for re-sampling plots in winter and in spring. Models were evaluated based on the minimum value given by the second order Akaike Information Criterion (AICc; Burnham et al., 2011) and the model with the lowest AICc score was considered 'the best model' (see Table A4). Autocorrelation was

checked using the variograms of the residuals.

To quantify similarity in the exploitation of floral resources between managed and native bumblebees, we separately analysed the observed plant-bumblebee interactions and body pollen loads. We pooled the data for each season during the two sampling years due to the small sample size per plot. Data were analysed with the proportional similarity index (PS; Hurlbert, 1978). PS was calculated as: $PS = \Sigma_i$ min $(p_{i,m}, p_{i,n})$, where $p_{i,m}$ is either the proportion of interactions on plant taxa i or the proportion of pollen grains of plant taxa i for managed bumblebees and $p_{i,n}$ is either the proportion of interactions on plant taxa i or the proportion of pollen grains of plant taxa i for native bumblebees. PS values can range from 0 (no niche overlap between bumblebees) to 1 (complete niche overlap).

Furthermore, the number of bumblebees carrying strawberry pollen and the percentage of strawberry pollen grains were compared between managed and native bumblebees, and between seasons using Chi square tests.

All statistical analyses were run with R (v. 3.1.2; R Core Team, 2014). We used the R packages *bipartite* (Dormann et al., 2009), *lme4* (Bates et al., 2014) and *MuMIn* (Barton, 2009).

3. Results

3.1. Bumblebee presence and density in pinewoods

We found managed bumblebees in 12 (8 in 2014 and 10 in 2015) pinewood plots in winter and in 9 (6 in 2014 and 7 in 2015) pinewood plots in spring. Native bumblebees were present in 7 (7 in 2014 and 3 in 2015) pinewood plots in winter and in 7 (4 in 2014 and 4 in 2015) pinewood plots in spring. There were no significant differences between the presence of managed and native bumblebees in pinewoods in either winter (W = 10, P = 0.11) or spring (W = 13.5, P = 0.53).

The presence of managed bumblebees in pinewood plots was positively related to greenhouse cover in the landscape, whereas that of native bumblebees was negatively related, although both patterns were significant at the 0.10 significance level (managed bumblebees: z = 1.823, P = 0.068 and native bumblebees: z = -1.728, P = 0.084; Fig. 2a and 2b). Season had no significant effect on the presence of bumblebees, as this variable was not selected in the models with the lowest AICc (i.e. only the variable greenhouse cover remained in the models, see Table A4).

Over the two sampling years, we recorded a total of 73 (17 in 2014 and 56 in 2015) managed bumblebees in winter and 37 (12 in 2014 and 25 in 2015) in spring. Native bumblebees were even less abundant with a total of 15 (10 in 2014 and 5 in 2015) bumblebees in winter and only 13 (7 in 2014 and 6 in 2015) in spring. In winter, the density of managed bumblebees in the plots was significantly higher than that of

native bumblebees (0.48 ± 0.17 bumblebees per 100 m², mean ± SE, hereafter, and 0.10 ± 0.04 bumblebees per 100 m², respectively; W = 16, P = 0.04), but similar in spring (0.26 ± 0.11 bumblebees per 100 m² and 0.09 ± 0.03 bumblebees per 100 m², respectively; W = 15, P = 0.22). Further, managed and native bumblebee density did not differ significantly between seasons (W = 58, P = 0.14 and W = 34, P = 0.96, respectively).

3.2. Floral resource use

In winter, *R. officinalis* (79.8%) and *U. australis* (17.8%) were the most common flowering plants of the 9 plant taxa recorded in the pinewoods (Table A2). These plant species were the most visited by both managed (~88%) and native bumblebees (100%), and were represented by the most abundant pollen types recorded on bumblebee bodies (overall, ~56% of managed bumblebee pollen loads and ~97% of native bumblebee pollen loads). Overall, managed bumblebees were observed on a total of 5 plant taxa while their pollen loads included pollen from a total of 10 plant taxa (half of them were not present in our study plots). Native bumblebees were observed on a total of 2 plant taxa and their pollen loads contained pollen from 3 plant taxa (one of them was not present in our plots) (Fig. 3 and Table A3).

In spring, *L. stoechas* (46.1%) and *Erica* spp. (20.8%) were the most common flowering plants of the 29 plant taxa recorded in the pinewoods (Table A2). However, there was great variability in plant-bumblebee interactions and bumblebee pollen loads. Most managed bumblebees were observed visiting *L. stoechas* (43.2%) and *H. calycinum* (24.3%), whereas native bumblebees continued to visit *R. officinalis* (61.5%) and *L. stoechas* (38.5%) as well. Managed bumblebees mainly carried pollen from *Cytisus grandiflorus* (25.1%) and *Linaria* spp. (33.0), while native bumblebees carried pollen from *Cerinthe gymnandra* (21.7%), *C. grandiflorus* (35.8%) and *U. australis* (24.9%) (Fig. 3 and Table A3). Managed bumblebees were observed visiting a total of 9 plant taxa, while their pollen loads showed a total of 16 plant taxa, most of which were absent or in low abundance in our plots. Native bumblebees were observed visiting a total of 2 plant taxa and their pollen loads contained a total of 9 plant taxa, most of them absent or in low abundance in our plots (Table A2 and Table A3).

The similarity index in exploited floral resources between managed and native bumblebees was, overall, larger for plant-bumblebee interactions than for pollen loads, and also in winter compared to spring. In fact, in winter, the overlap was almost complete for observed plant-pollinator interactions with PS = 0.83, while for pollen loads, it was PS = 0.56. In spring, the overlap for interactions was PS = 0.41 and for pollen loads it was PS = 0.28.

Finally, we found strawberry pollen only on the bodies of managed bumblebees. In winter, both the number of bumblebees carrying
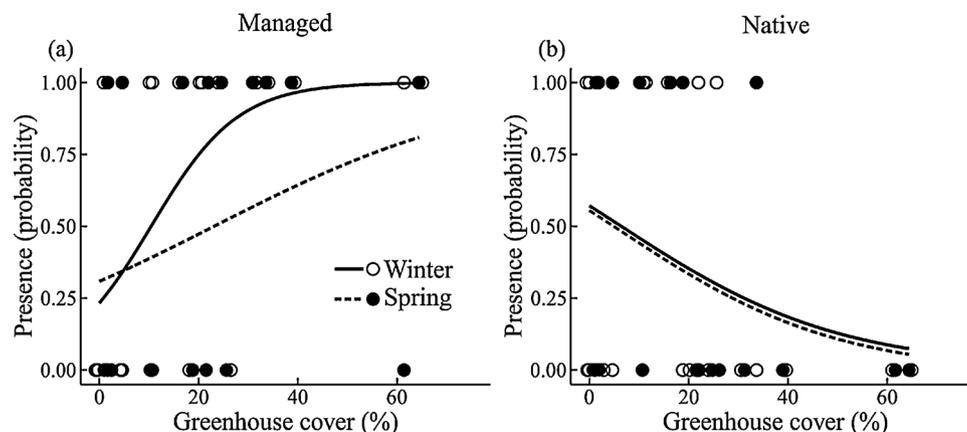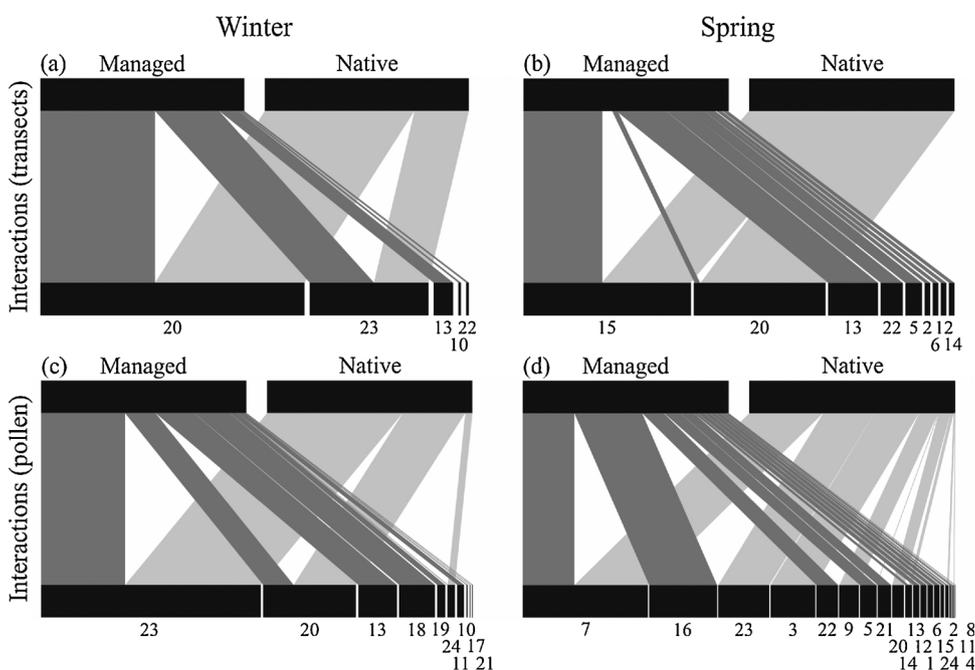
Fig. 2. Patterns of the occurrence of (a) managed (*Bombus terrestris*) and (b) native bumblebees (*B. t. lusitanicus*) in pinewood plots across a gradient of greenhouse cover in a 2 km landscape radius in winter and in spring.

Fig. 3. Illustration of plant-bumblebee (a, b) and body pollen loads (c, d) interaction networks in winter and in spring. As sample sizes varied between managed (*Bombus terrestris*) and native bumblebees (*B. t. lusitanicus*) and between seasons, interaction networks were proportionally homogenized (i.e. interaction bar widths sum the unity for each bumblebee group). The width of the links is proportional to the number of interactions observed or pollen grains counted for managed and native bumblebees. The numbers represent the following plant taxa: (1) *Acacia* spp., (2) *Centaurea* spp., (3) *Cerinthe gymnandra*, (4) *Cistus cryspus*, (5) *Cistus ladanifer*, (6) *Cistus salviifolius*, (7) *Citisus grandiflorus*, (8) *Citrus* spp., (9) *Equium* spp., (10) *Erica* spp., (11) *Fragaria* × *ananassa*, (12) *Genista triacanthos*, (13) *Halimium calycinum*, (14) *Halimium halimifolium*, (15) *Lavandula stoechas*, (16) *Linaria* spp., (17) Brassicaceae, (18) *Eucalyptus* spp., (19) *Prunus* spp., (20) *Rosmarinus officinalis*, (21) *Rubus idaeus*, (22) *Stauracanthus genistoides*, (23) *Ulex australis* and (24) *Vaccinium corymbosum*. See Table A3 for more details.

strawberry pollen and the percentage of strawberry pollen grains were higher than in spring (49% of individuals in winter and 13% in spring, $\chi^2 = 6.10$, $P = 0.01$; 3.4% of strawberry pollen in winter and 0.3% in spring, $\chi^2 = 592.8$, $P < 0.001$) (Table A3).

4. Discussion

Managed bumblebees used in polytunnel greenhouse crops also foraged in nearby pinewoods. The density of managed bumblebees in pinewoods was greater than that of native bumblebees in winter but similar in spring. The presence of managed bumblebees in pinewoods was positively related to crop cover in the landscape. In contrast, the presence of native bumblebees in pinewoods was negatively related to crop cover in the landscape. Remarkably, managed bumblebees foraged on a wide range of flowering plant species, many of which were shared with native bumblebees, especially in winter, when flowering plant species were scarce.

The occurrence of managed and native bumblebees in pinewoods showed opposite patterns in relation to greenhouse cover in the landscape. As expected, managed bumblebees were positively associated with greenhouse cover. An increase in polytunnel greenhouse cover at the landscape scale may be linked to greater use of commercial bumblebee colonies in the landscape (e.g. Gaines-Day and Gratton, 2016; Klein et al., 2012). A greater abundance of colonies may subsequently result in a higher probability of occurrence of managed bumblebees in nearby small pinewoods, which become isolated within a large matrix of greenhouses. However, the relationship we found was not very strong. One explanation for this is that the presence of greenhouses in the landscape associated with colony use could drive the presence of managed bumblebees in pinewoods, regardless of greenhouse cover. Another possible and complementary explanation for this lack of a strong pattern might be that bumblebees have the potential to fly long distances when foraging (Cresswell et al., 2000) and thus, they were present in many of our selected agricultural landscapes.

In contrast to managed bumblebees, native bumblebees were negatively related to greenhouse cover in the landscape. This pattern was also likely the result of a decrease in natural habitat cover in the landscape, as both habitat types were negatively correlated, as mentioned previously. Several causes may explain this pattern. Overall, bumblebees prefer to forage in natural habitats rather than in crops

(Collado et al., 2018). In this study, we did not find any strawberry pollen on native bumblebee bodies, although this result does not directly indicate that native bumblebees do not forage in strawberry crops, as they were collected in pinewoods surrounded by greenhouses. However, a parallel study that recorded strawberry flower visitors has shown the absence of native bumblebees foraging in greenhouses (Trillo et al., 2018). Therefore, an increase in greenhouse cover linked to a decrease in natural habitats in the landscape could reduce the availability of floral resources for bumblebees. In parallel, greenhouse crops are unsuitable nesting sites due to frequent disturbances (e.g. Holzschuh et al., 2007). Other causes, such as the intensive use of pesticides in strawberry crops and their probable spread into adjacent natural habitats (Botías et al., 2016), could be responsible for the contrasting pattern found between the presence of native bumblebees in pinewoods and crop cover in the landscape. All of these possibilities should be tested, and our results should be interpreted with caution since the relationship that was found was not very strong.

Despite the fact that we performed intensive and extensive sampling over two years (i.e. 2348 transect walks on 78 days for a total of 587 h), the density of bumblebees was too low to assess variation across the greenhouse gradient. Bumblebees are rare in this region; for instance, Magrach et al. (2017) showed that bumblebees accounted for only 0.3% of the total visits recorded in an exhaustive plant-pollination network analysis. Surprisingly, in the pilot sampling performed the year before this study, we found similar numbers of bumblebees but with much less sampling effort than in the present study (i.e. 74 h compared to 587 h). Despite methodological differences between the two samplings (i.e. an active search for bumblebees in the pilot sampling), we believe that the low densities observed here probably arose as a result of the strong attraction of bumblebees to plant taxa such as *Acacia* spp. and *C. gymnandra* (Trillo, personal observation). Remarkably, these taxa were absent or in low abundance in the study plots but well represented in the pollen loads (e.g. *C. gymnandra*, *C. grandiflorus* and *Eucalyptus* spp.). Plants from these taxa were isolated and in most cases located in human-modified areas, such as roads and their surroundings, close to our plots.

Unexpectedly, the presence and density of both managed and native bumblebees in pinewoods did not differ between winter and spring. However, this shared pattern is due to the result of different processes operating in the two bumblebee groups. Because commercial

bumblebee colonies placed in winter are active for a short period of time, there are farmers that also use new colonies in spring, regardless of crop flower visitation by wild insects (Trillo, personal observation). The use of bumblebees throughout the strawberry flowering period leads to a constant spillover of managed bumblebees from crops into natural habitats in both winter and spring. Even if the use of colonies is higher in winter than in spring, a greater number of bumblebees may spillover into natural areas in spring, prompted by the attraction of a high diversity and abundance of wild flowering plant species (Trillo et al., 2018; Whittington et al., 2004). Indeed, bumblebees collected less strawberry pollen in spring than in winter. Native bumblebees are adapted to begin their life-cycle when temperatures are mild, extending from spring to summer in temperate regions (Goulson, 2010). In contrast, in our lowland Mediterranean study region, temperatures are extreme (up to 44 °C; AEMET, 2015) from late-spring to late-summer. Mild most of the winter coinciding with the bloom of several abundant flowering plant species (e.g. *R. officinalis* and *U. australis*) that are highly attractive to bumblebees.

Bumblebees are generalist pollinators (Memmott, 1999) that require diverse pollen sources to thrive (Brunner et al., 2014; Roger et al., 2017). In pinewoods, managed bumblebees exploited a wide range of flowering plant species, as did native bumblebees. Both bumblebee groups showed similar floral resource exploitation, especially in winter, when flowering plant species tend to be scarce. These comparable patterns were expected as they are morphologically similar subspecies (Rasmont et al., 2008). Their capacity to exploit most flower types, such as short- and long-tubed flowers (Inouye, 1980), probably led to a decrease in floral resource overlap in spring, when most flowering plants bloom.

5. Conclusions

This study provides correlational evidence that managed bumblebees spillover from pollinator-dependent crops into natural habitats. We found that increasing coverage of these crops at the landscape scale increased the probability of presence of managed bumblebees, which forage on a wide range of plant taxa, and in contrast, decreased that of native bumblebees in natural habitats.

The use of managed pollinators for pollination services has become a common practice worldwide (Aizen and Harder, 2009; Velthuis and van Doorn, 2006). However, it is important to highlight that, on the one hand, their pollination function in crops varies greatly for each particular system (Lye et al., 2011; Petersen et al., 2013; Trillo et al., 2018) and depends on the community of crop flower visitors (e.g. Garibaldi et al., 2011). On the other hand, a range of managed pollinators forage in natural habitats where they can interfere with wild pollinators in multiple ecological processes, such as resource competition, hybridisation and parasitism (reviewer by Geslin et al., 2017). Research on the impacts of agricultural intensification and the introduction of managed pollinators on the native pollinator community is of great importance as the area of pollinator-dependent crops is increasing worldwide (Aizen et al., 2008; Essl et al., 2018; González-Varo et al., 2013). Further studies are needed to optimize the use of managed pollinators in crops in order to reduce or prevent their presence in agricultural landscapes.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2018.11.018.

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