



GfÖ

GfÖ Ecological Society of Germany,
Austria and Switzerland

Basic and Applied Ecology xxx (2017) xxx–xxx

Basic and
Applied Ecology

www.elsevier.com/locate/baae

Managed bumble bees increase flower visitation but not fruit weight in polytunnel strawberry crops

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Received 22 November 2017; received in revised form 16 May 2018; accepted 17 May 2018

Abstract

Animal-mediated pollination is essential for the production and quality of fruits and seeds of many crops consumed by humans. However, crop pollination services might be compromised when wild pollinators are scarce. Managed pollinators are commonly used in crops to supplement such services with the assumption that they will enhance crop yield. However, information on the spatiotemporal pollinator-dependence of crops is still limited. We assessed the contribution of commercial bumble bee colonies compared to the available pollinator community on strawberry ('Fortuna' variety) flower visitation and strawberry quality across a landscape gradient of agricultural intensification (i.e. polytunnel berry crop cover). We used colonies of bumble bees in winter and in spring, i.e. when few and most wild pollinators are in their flight period, respectively. The placement of colonies increased visits of bumble bees to strawberry flowers, especially in winter. The use of bumble bee colonies did not affect flower visitation by other insects, mainly honey bees, hoverflies and other Diptera. Flower visitation by both honey bees and wild insects did not vary between seasons and was unrelated to the landscape gradient of berry crop cover. Strawberries were of the highest quality (i.e. weight) when insect-mediated pollination was allowed, and their quality was positively related to wild flower visitors in winter but not in spring. However, increased visits to strawberry flowers by managed bumble bees and honey bees had no effect on strawberry weight. Our results suggest that the pollination services producing high quality strawberry fruits are provided by the flower visitor community present in the study region without the need to use managed bumble bees.

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Keywords: Agricultural intensification; *Bombus terrestris*; Ecosystem service; *Fragaria x ananassa* ('Fortuna' variety); Fruit quality; Flowering; Non-bee pollinator

Introduction

Around 75% of world food crops require or benefit from animal-mediated pollination to increase the production and quality of fruits and seeds (Klein et al. 2007). During

the last half century, the area devoted to these crops has disproportionately increased compared with non-dependent crops (Aizen, Garibaldi, Cunningham, & Klein 2008). Several factors can affect the presence of pollinators in crops and compromise the service they provide. For instance, the reduction of natural habitats can decrease wild pollinator abundance and richness in agroecosystems (Williams et al., 2010; Winfree, Bartomeus, & Cariveau 2011) because it decreases the availability of nesting sites and flower resources over time. Furthermore, the temporal variability of wild pol-

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linators, which is due to their life-cycle and their activity depending on temperature, creates periods in which their abundance is low (Pisanty, Klein, & Mandelik 2014; Ellis, Feltham, Park, Hanley, & Goulson 2017). If crops bloom outside of the main pollinator flying phenophase period or for a long period, wild pollinators might not fulfill crop pollination services. For these reasons, many farmers do not solely rely on wild pollinators, but rather managed insect pollinators are used to supplement visitation rates on pollinator-dependent crops regardless of the occurrence of wild pollinators.

Honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) are the most common managed pollinators used worldwide. Honey bees have been historically domesticated for honey production and crop pollination (Aizen & Harder 2009; Garibaldi et al. 2013), being present in many crop systems worldwide (Winfree, Williams, Dushoff, & Kremen 2007). In contrast, bumble bees have been domesticated more recently (i.e. last four decades) mainly to pollinate greenhouse tomato crops (Velthuis & van Doorn 2006). Because bumble bees show higher activity when weather conditions are cool and cloudy and require less management effort compared with honey bees, their use has been extended to many other crops, such as berries and apples. Nowadays, over a million colonies are annually commercialized all over the world (Velthuis & van Doorn 2006).

Contrasting results have been found in relation to the use of managed pollinators and crop yield. In fact, their contribution might depend on the spatiotemporal variability of wild pollinators in crops. For instance, when the services provided by managed pollinators are estimated in a scenario where wild pollinators are absent, an overall positive effect is found (Roldán Serrano & Guerra-Sanz 2006; Albano, Salvado, Duarte, Mexia, & Borges 2009). However, in a scenario where wild pollinators are present, this relationship can vary. On the one hand, if wild pollinator populations are relatively small and do not complete the required pollination services, managed pollinators can make a significant contribution to crop yield as seen in blueberry (Isaacs & Kirk 2010), raspberry (Lye, Jennings, Osborne, & Goulson 2011) and sunflower crops (Pisanty et al. 2014). On the other hand, if wild pollinator populations are large and diverse, managed pollinators may drop back to a secondary role and supplement the pollination services, which in many cases does not translate into an increased crop yield (Holzschuh, Dudenhöffer, & Tschamntke 2012; Garibaldi et al. 2013; Mallinger & Gratton 2015). But even negative effects, for example in raspberry drupelet set, have been found when non-native bumble bees and also honey bees visit flowers quite frequently (Sáez, Morales, Ramos, & Aizen 2014).

In addition, managed pollinators can also spillover into adjacent natural areas (Ishii, Kadoya, Kikuchi, Suda, & Washitani 2008; González-Varo & Vilà 2017). There, they can compete with native pollinators for floral and nesting resources (Inoue, Yokoyama, & Washitani 2008; Ishii et al. 2008), as well as drive parasite spread into native pollinator populations (Colla, Otterstatter, Gegear, & Thomson 2006;

Fürst, McMahon, Osborne, Paxton, & Brown 2014). Furthermore, managed pollinators can disrupt plant-pollinator networks and impact the reproductive success of wild plants (Magrach, González-Varo, Boiffier, Vilà, & Bartomeus 2017).

Huelva province in SW Spain is the second largest producer of strawberries (*Fragaria x ananassa*) in the world (~300,000 t per year; Freshuelva 2016). Farmers typically grow strawberries under semi-open polytunnels for a long period; the flowering period spans from November to May. Many farmers rely on managed pollinators (honey bees and/or bumble bees) to aid crop pollination, because studies have shown that strawberry fruit quality is enhanced when insect-pollinated (e.g. Klatt et al. 2014). In general, honey bee hives are used throughout the entire crop flowering period, while bumble bee colonies are mainly used in winter when it is cold. However, in this region wild pollinators are diverse and abundant in the remaining natural habitats, especially in spring (Magrach et al. 2017).

Our goal in this study was to determine whether strawberry quality increases with the use of commercial bumble bee colonies, and the role of the native strawberry flower visitor community. For this purpose, we placed colonies of *Bombus terrestris* in 12 strawberry plots along a landscape gradient of polytunnel berry crop cover. We surveyed strawberry flower visitors when colonies were both absent and present, and evaluated the pollination services provided during winter (early-January to mid-February), a period when major revenues might be compromised by the scarcity of wild pollinators, and in spring (early-March to mid-April), a period when most wild plants bloom and pollinators are very active in adjacent natural habitats. We addressed the following questions: (a) Does the use of bumble bee colonies affect flower visitation rates in strawberries? (b) Are strawberry flower visitors affected by seasonal differences along a landscape gradient? (c) To what extent does strawberry quality rely on insect-mediated pollination? and (d) Does the use of bumble bee colonies and/or the increase of flower visitors enhance strawberry quality?

Materials and methods

Study system

The study was conducted in the Guadalquivir valley, province of Huelva (SW Spain), in 2016. The climate is typically Mediterranean with hot and dry summers and mild winters. Mean annual temperature and precipitation are 18.2 °C and 525 mm, respectively (AEMET 2016). In the study region, berry crops are quite widespread, especially strawberries (~75% of the area devoted to berry crops; Freshuelva 2016), forming an intermingled mosaic with woodland patches composed of a rich entomophilous understorey that mostly blooms in spring.

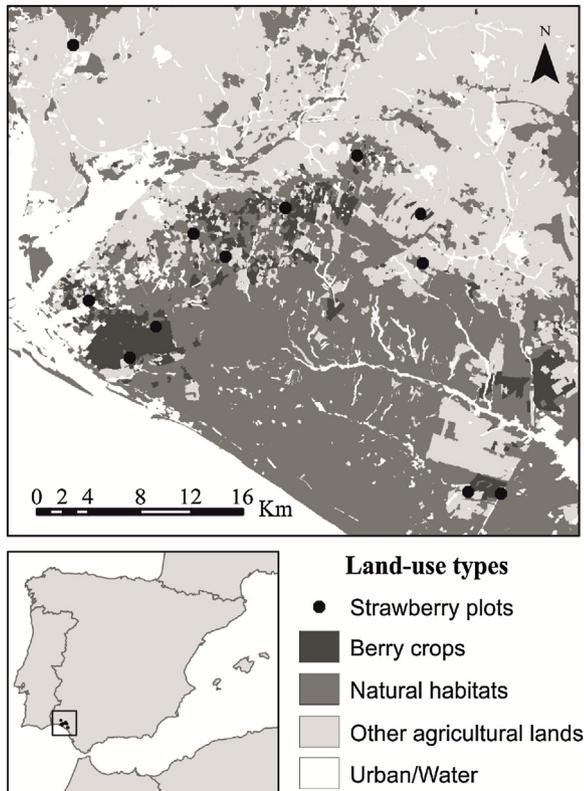


Fig. 1. Geographical distribution of the 12 study strawberry plots in the province of Huelva (SW Spain). Latitude $37^{\circ}23'24.19''$ N to $37^{\circ}19'14.65''$ N and longitude $6^{\circ}52'34.16''$ W to $6^{\circ}31'13.31''$.

Throughout the study area, we selected 12 strawberry semi-open polytunnel plots across a landscape gradient of agricultural intensification and natural habitat cover (Fig. 1). The distance between selected strawberry plots ranged from 2532 to 17,406 m (5060 ± 1192 m, mean \pm SE, hereafter). These distances are larger than the reported values of maximum foraging distances of most pollinators (Gathmann & Tschamtkke 2002; Osborne et al. 2008), assuring independence of study plots. Agricultural intensification was measured as the percentage of berry crop cover in circular areas around each plot. Natural habitats, in contrast, consisted of the sum of grasslands, pinewoods and shrubland areas. We considered the cover of berry crops, rather than other agricultural lands such as olive trees and vineyards, a good proxy of agricultural intensification because these are the most extensive and most disturbed in the area in terms of labour and inputs. Multiple scales with radii of 500, 1000, 1500 and 2000 m were used to select the best scale predictor. For instance, at a scale with 2-km buffer radius, berry crop cover in the landscape ranged from 0.1 to 64% ($24.0 \pm 5.9\%$), while natural habitat cover ranged from 6 to 53% ($33.4 \pm 4.4\%$). We used the ArcGIS (ESRI 2011) programme based on the land cover map of Andalucía from 2011 (Moreira et al. 2011) to calculate the percentage of each land-use type.

Within each strawberry plot we selected an area c. $25 \text{ m} \times 70 \text{ m}$ (experimental plot), which consisted of five semi-open polytunnels. Each tunnel was 5 m wide, 70 m long and had five to six parallel 0.5 m wide ridges. Each ridge had two rows of strawberry plants and was elevated half a metre aboveground (see Supplementary Appendix A: Fig. A1). Experimental plots met three criteria. First, the strawberry variety was 'Fortuna', because it is one of the most cultivated in this region and therefore important in the economic sector. Second, plots were adjacent to natural habitats. Third, there were no commercial bumble bee and honey bee colonies within 300 m of the experimental plots. This distance was the maximum for which we could ensure the absence of managed bee colonies without compromising farmers' demands.

Supplementation of *B. terrestris* colonies

We used 48 bumble bee colonies from Koppert Biological Systems, which were kept with a syrup solution ad-libitum in a plastic box covered by cardboard. Each colony included the founding queen and approximately 100 workers. From 7 to 26 January 2016 (i.e. 20 days of treatment; round one), we placed two colonies in six (i.e. half) randomly assigned selected strawberry plots. One colony was positioned in the middle of the first tunnel (i.e. tunnel one) and the other in the middle of the last tunnel (i.e. tunnel five) in each plot. After this period, we removed the colonies and placed new colonies in the remaining six plots for the following 20 days (round two). We hereafter refer to round one and round two as the 'winter' season. Starting on 3 March 2016, we repeated the procedure to cover the period when wild pollinators thrive in natural habitats. We thus refer to rounds three and four, both also 20 days in length, as the 'spring' season. In sum, each plot had a consecutive absent/present colony treatment or vice-versa in winter and in spring.

Flower visitor censuses

Five days after the start of each round (i.e. after the placement of bumble bee colonies) we conducted two flower visitor censuses on two days per plot at different times: one day in the morning and another day in the afternoon. The same sampling procedure was also conducted in the six plots without colonies. Sampling was performed on sunny and calm days with minimum interior temperatures of 16°C in winter and 20°C in spring. Plots were selected at random on those days. For each census, we walked 200 m within tunnels two to four for 60 min. We recorded and identified managed bumble bees and honey bees visiting strawberry flowers along the four middle ridges of each tunnel, comprising a total area of $2 \text{ m} \times 200 \text{ m}$ per census. For wild flower visitors, we reduced the observed area and sampled the two central ridges of each tunnel, comprising a total area of $1 \text{ m} \times 200 \text{ m}$ per census. The difference in the survey area was justified given that managed

flower visitors (i.e. bumble bees and honey bees) are highly active and larger than most wild flower visitors (i.e. Diptera), and therefore more easily detected.

As with honey bees, we assumed that all bumble bees observed were managed bumble bees. In fact, in this region, bumble bees are rare (Magrath et al. 2017). Most wild flower visitors recorded were identified as hoverflies and other Diptera. Specimens are deposited at the EBD-CSIC. For analyses, flower visitors were assigned to one of the three following groups: bumble bees, honey bees and wild insects.

To quantify the availability of strawberry flowers, we surveyed two 50 m transects in tunnels two to four each census day. Along each transect we counted receptive flowers within 40 cm × 40 cm quadrates placed every five metres. Flower density was two times larger in spring than in winter but variability among plots was very low (16.97 ± 1.83 flowers/m² in winter and 32.18 ± 1.34 flowers/m² in spring). Therefore, we decided not to include flower density in the statistical analyses as a factor influencing spatial differences within a season.

Contribution of flower visitors to strawberry quality

To quantify the dependence of strawberry crops on insect-mediated pollination, and specifically to compare the contribution of managed bumble bees and other flower visitors to strawberry yield, we tagged 30 virgin primary flowers from different plants in each experimental plot. In each plot, virgin flowers were selected five to six days after the start of each round throughout tunnel three. Virgin flowers were identified as those not yet open and therefore not visited by insects before treatment. Each flower was randomly assigned to one of the following two treatments: net-bagged or open. For the net-bagged treatment, nylon tulle bags (size: 10 cm × 10 cm; openings: 1 mm × 1 mm) were used to cover virgin flower buds. Thus, the net-bagged treatment allowed for self- and wind-pollination, while the open treatment additionally permitted insect pollination. It should be noted that the treatments were only applied to primary flowers as they produce fruits with the largest commercial value. After fruit set, i.e. approximately 10 days after treatment, we bagged all fruits to avoid hand picking. Fruits were harvested and weighed in the field when ripe. We used weight and degree of deformation as measures of fruit quality (Klatt et al. 2014). Degree of deformation was estimated based on European marketing criteria (European Commission 2011). All fruits showed extremely low deformation rates and overall 99.3% of them were classified into class I (good quality). Thus, differences in the degree of deformation between treatments were not compared.

Statistical analyses

For each season we calculated strawberry flower visitation by bumble bees, honey bees and wild insects as the average

number of recorded interactions per census and round. To deal with differences in surveyed area for each flower visitor group we standardized the data for an area of 100 m².

First, we evaluated whether the use of commercial bumble bee colonies increased bumble bee visits to strawberry flowers. For this purpose, we built a generalized linear mixed model (GLMM; gamma error distribution and log link function) with bumble bee flower visitation as the response variable, and colony treatment (absent/present), season (winter/spring) and their interaction as fixed factors. A contrast matrix was built for post hoc comparison when significant interactions were found. Prior to this, we ascertained that there were no significant differences (Wilcoxon signed-rank test: $p = 0.10$ in winter and $p = 0.06$ in spring) in the placement of bumble bee colonies at different rounds in winter (round one vs. round two) and in spring (round three vs. round four).

Second, we evaluated whether the use of commercial bumble bee colonies affected flower visitation by honey bees and wild insects using paired Wilcoxon signed-rank tests. Because colony treatment (absent/present) had no effect on flower visitation by honey bees and wild insects (see results), data from colony supplementation treatments were pooled. Then, we tested whether flower visitation by honey bees and wild insects varied between seasons and/or was related to the landscape gradient. For this purpose, we built GLMMs (gamma error distribution and log link function) with flower visitation by honey bees and wild insects as the response variables, and season (winter/spring), land use-type (berry crop or natural habitat cover) and their interaction as fixed factors. Multiple scales for each land use-type were tested in separate models. Models were selected based on the minimum value given by the second order Akaike Information Criterion (AICc; Burnham, Anderson, & Huyvaert 2011) and compared with null models (models without explanatory variables).

Third, we assessed the degree of strawberry dependence on insect-mediated pollination using linear mixed models (LMMs) with a Gaussian error distribution. In the models, the log transformed ($\log x + 1$) strawberry weight was used as a response variable, and colony treatment (absent/present), season (winter/spring), and pollination treatment (net bagged/open flowers) were included as fixed factors. In the model, we also included the interaction between colony treatment and season.

Finally, we evaluated the direct contribution of each flower visitor group (bumble bees, honey bees and wild insects) and also total flower visitors to strawberry fruit weight in winter and in spring, using separate LMMs for each season. The difference in the weight of fruits from open flowers and net-bagged flowers was used as the response variable. The indirect contribution of the landscape to strawberry weight was not compared as it had no effect on flower visitors (see results).

For GLMMs and LMMs, 'plot' was included as a random factor to account for re-sampled plots between rounds and/or seasons. The statistics yielding both F- and p-values were

Table 1. The effects of colony treatment (absent/present) and season (winter/spring) on strawberry flower visitation by bumble bees.

Contrast	Estimate	SE	Z	P-value
Winter: colony present × absent	0.111	0.022	5.139	<0.001
Spring: colony present × absent	0.053	0.022	2.433	0.050
Colony absent: winter × spring	0.023	0.022	1.070	0.652
Colony present: winter × spring	0.082	0.022	3.776	<0.001

calculated using Satterthwaite's approximations to determine denominator degrees of freedom. Moran's I index was used to check for spatial autocorrelation of each flower visitor group, and no signal was found ($I < 0.4$). All statistical analyses were computed in R (v.3.1.3, R Core Team 2014) using packages *lmerTest* (Kuznetsova, Brockhoff, & Christensen 2013), *lme4* (Bates, Maechler, Bolker, & Walker 2014), *multcomp* (Hothorn, Bretz, Westfall, & Hothorn 2013), *MuMIn* (Barton 2009) and *Ncf* (Bjørnstad 2013).

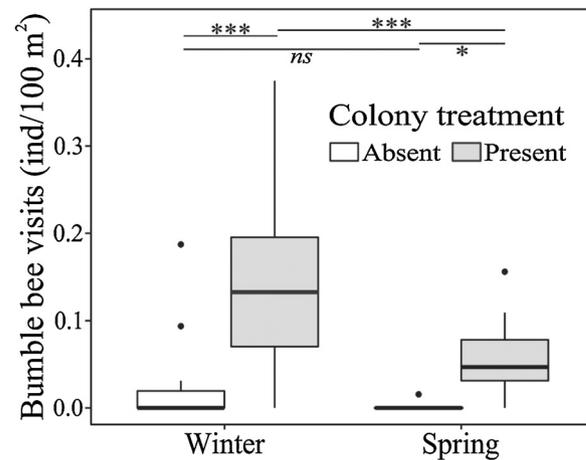
Results

Strawberry flower visitors

Overall, we recorded 790 and 1092 strawberry flower visitors belonging to 20 and 27 species (or morphospecies) in winter and in spring, respectively. Despite the apparent high richness of insect taxa visiting strawberry flowers, five taxa accounted for 94.5% of all records (see Supplementary Appendix A: Table A1). These included managed bumble bees (*B. terrestris*) and honey bees (*A. mellifera*), and wild insects such as *Eupeodes corollae* (47.4% of total recorded wild insects), Diptera sp1 (morphotype 1; 9.3%) and *Episyrphus balteatus* (7.9%). Overall, the total number of visits was not significantly different between winter and spring (mean \pm se = 0.47 ± 0.08 ind/100 m² in winter and 0.70 ± 0.13 ind/100 m² in spring; paired Wilcoxon signed-rank tests: $n = 12$, $p = 0.09$).

When commercial bumble bee colonies were absent, strawberry flower visitation by bumble bees was very low and did not differ significantly between winter and spring (Table 1, Fig. 2). Most of the records (~78%) derive from two strawberry plots in winter with the highest area of berry crops in the landscape. However, the placement of colonies significantly increased strawberry flower visitation by bumble bees (Table 1, Fig. 2), being higher in winter than in spring (Table 1, Fig. 2).

Flower visitation by both honey bees and wild insects was not affected by colony treatment in any season (paired Wilcoxon signed-rank tests: $n = 12$, $p > 0.05$). Honey bees

**Fig. 2.** Box-plot showing strawberry flower visitation by bumble bees (visits per 100 m²) when commercial bumble bee colonies were absent and present in winter and in spring. Significance levels: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; $ns = p > 0.05$.

and wild insects were recorded visiting strawberry flowers in all plots throughout the sampling dates. Overall, honey bees were the most abundant flower visitors. Flower visitation by honey bees was 0.36 ± 0.07 ind/100 m² in winter and 0.56 ± 0.11 ind/100 m² in spring, while flower visitation by wild insects was 0.07 ± 0.01 ind/100 m² in winter and 0.12 ± 0.05 ind/100 m² in spring. There were no significant effects of season (winter/spring) and land-use type (berry crop or natural habitat cover) on either honey bee or wild insect flower visitation at any of the scales examined, as null models showed the lowest AICc values.

Strawberry weight

A total of 1296 fruits were weighed (144 fruits were excluded from analyses because damage and/or fungus was visible). Strawberries from bagged flowers were, overall, 15% smaller than fruits developed from open flowers ($F_{1,1281.3} = 104.37$, $p < 0.001$) (Fig. 3). Strawberries were also smaller in spring than in winter ($F_{1,1282.8} = 153.78$, $p < 0.001$) (Fig. 3) because flowers become smaller throughout the growing season (see Supplementary Appendix A: Fig. A2). Colony treatment had no effect on strawberry weight in any season ($F_{1,1281.7} = 2.28$, $p = 0.131$).

In winter, the difference in strawberry weight between net-bagged and open flowers was not related to flower visitation by bumble bees (Fig. 4A) or honey bees (Fig. 4B), but rather by wild insects (Fig. 4C) (Table 2). However, there was no significant relationship with total visits of pollinators in any season (Table 2, Fig. 4D).

Discussion

The use of commercial bumble bee colonies in 'Fortuna' variety strawberry crops increased bumble bee strawberry

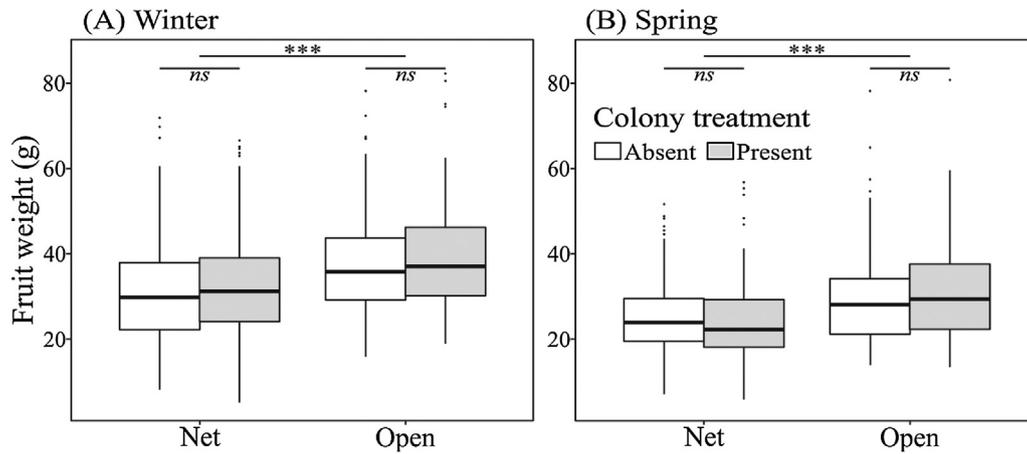


Fig. 3. Box-plots showing strawberry weight of net-bagged and open-pollinated flowers when commercial bumble bee colonies were absent and present in winter (A) and in spring (B). Box-plots show medians and, 25th and 75th percentiles. The whiskers indicate the 5th and 95th percentiles, while black dots denote outliers. Significance levels: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; $ns = p > 0.05$.

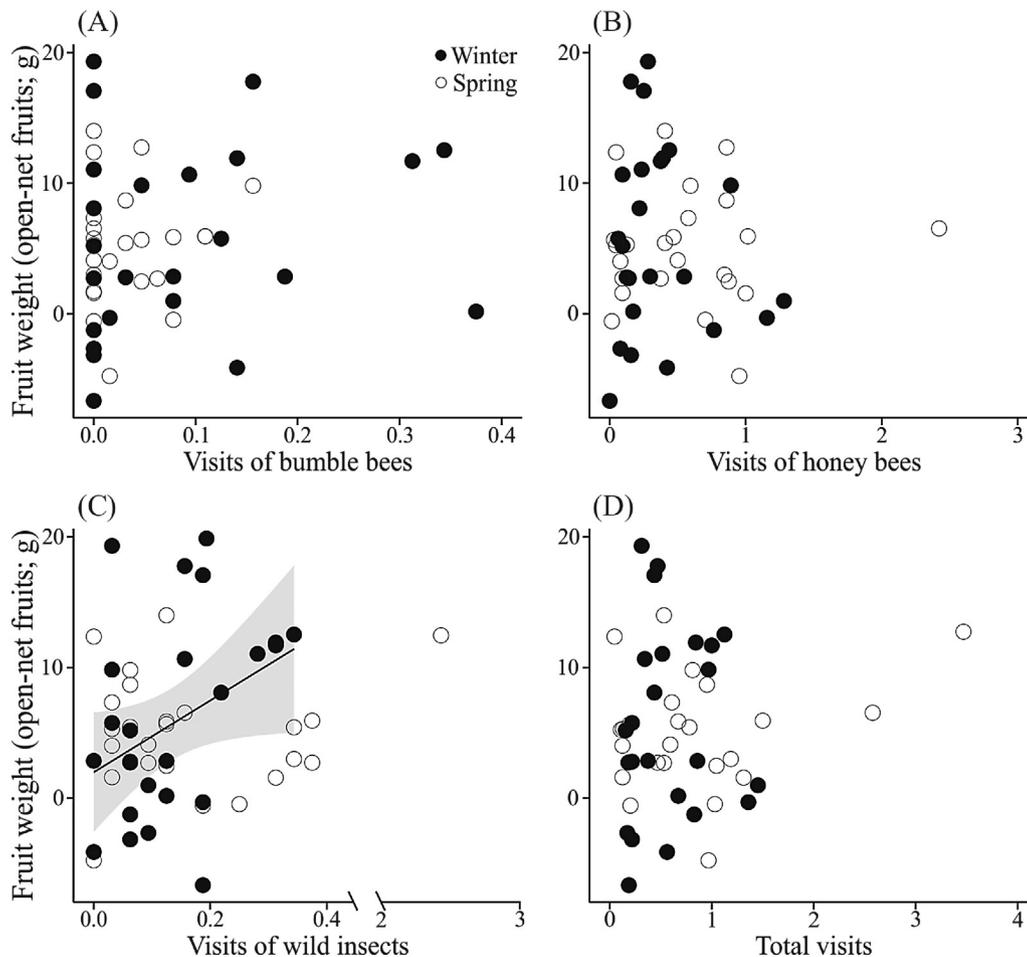


Fig. 4. Relationships between the difference of strawberry fruit weight (open – net fruits) and flower visitation (n/100 m²) by bumble bees (A), honey bees (B), wild insects (C) and total insects (D) in winter (black dots) and in spring (open dots). The line indicates a significant relationship and the shaded area indicates the 95% confidence interval.

flower visitation, especially in winter. This increase did not affect flower visitation by other insects, composed mainly of honey bees, hoverflies and other Diptera. Surprisingly,

strawberry weight, which was higher when insect pollination was allowed, did not increase when bumble bee colonies were used. Moreover, strawberry weight was not related to

Table 2. The effects of flower visitation by bumble bees, honey bees and wild insects and also total insects on strawberry weight in winter and in spring. Satterthwaite's approximations were performed for statistic results.

Models	Flower visitor group	Df	F	P-value
Winter				
<i>Model1</i>				
	Bumble bee	1, 14.9	0.21	0.656
	Honey bee	1, 18.3	0.25	0.621
	Wild insects	1, 19.9	5.01	0.037
<i>Model2</i>				
	Total visitors	1, 19.4	0.02	0.899
Spring				
<i>Model3</i>				
	Bumble bee	1, 13.5	0.35	0.565
	Honey bee	1, 20.0	0.02	0.891
	Wild insects	1, 19.9	2.48	0.131
<i>Model4</i>				
	Total visitors	1, 20.7	1.66	0.212

increases in the number of flower visits by bumble bees and honey bees in any season. However, in winter, strawberry weight increased when wild flower visitors proliferated, although such an effect did not occur in spring.

Strawberry flower visitors

Flower visitation by bumble bees was higher in winter than in spring. Seasonal differences are probably due to a spillover of bumble bees from crops to adjacent habitats in spring, a period when most wild plants bloom (Whittington, Winston, Tucker, & Parachnowitsch 2004). However, the flower visitor community present in strawberry crops was neither affected by the use of commercial bumble bee colonies in winter nor in spring. Lye et al. (2011) also reported no competition when bumble bee colonies were used in raspberry crops in Scotland. It is likely that the massive and continuous bloom offered by these crops provides sufficient floral resources for all flower visitors.

Even when we used commercial bumble bee colonies and did not observe honey bee hives in the area, honey bees were the most common flower visitors, as also reported by other authors (Chagnon, Gingras, & De Oliveira 1993; Bartomeus et al. 2014). Honey bee hives harbour a high number of individuals (~60 K individuals; Seeley & Morse 1976), approximately 50 times that of bumble bee colonies. Honey bees fly long distances (~1.5 km; Steffan-Dewenter & Kuhn 2003) and thus could have come from other areas. We noticed that flower visitation by honey bees was quite similar among strawberry plots. In our study region, most honey bee hives are maintained over the crop flowering period and it is likely that the number of honey bee hives is related to berry crop cover in the landscape (González-Varo & Vilà 2017). Thus, although in spring honey bees are attracted to forage in natural habitats, because more attractive resources are available (Free

1993), the growth of honey bee hive populations from winter to spring could maintain similar numbers of individuals foraging in strawberry crops between seasons.

Unexpectedly, flower visitation by wild insects did not vary between seasons and it was unrelated to natural habitat cover. Although there is a large amount of evidence reporting that land-use intensification negatively affects wild pollinators, mostly bees (Isaacs & Kirk 2010; Klein et al. 2012; Holzschuh et al. 2016), in our study the community of observed flower visitors was composed of hoverflies and other Diptera. Pollinators show variable sensitivity to landscape composition depending on their life history. Non-bee insects can be less reliant on natural areas than bees (Rader et al. 2016); for instance, hoverflies and other Diptera are able to exploit resources from habitat types highly altered (Winfree et al. 2011; Raymond et al. 2014). This, together with the mild winter weather conditions in the study region, could favour wild flower visitor populations to be sustained throughout the strawberry flowering period regardless of changes in landscape characteristics.

Strawberry weight

As expected, strawberry crops produced heavier fruits when insect-pollinated (Klatt et al. 2014), but the measures that we recorded as proxies for fruit quality (shape and weight) did not increase with the use of bumble bee colonies nor with an increase in visitation frequency of managed bumble bees and honey bees. There are several studies which have found that managed pollinators promote crop yield, but they are less valuable than wild pollinators (Holzschuh et al. 2012; Garibaldi et al. 2013). This is widely related to flower damage when pollinators are at saturation levels or, as is likely in our system (although we lacked these measures), to the transfer of low-quality or incompatible pollen (Morris, Vazquez, & Chacoff 2010; Aizen et al. 2014; Sáez et al. 2014). Honey bees were the most frequently recorded flower visitor. Thus, it is likely that honey bees provided the pollination services to produce larger fruits when insect-pollinated, but increases in flower visitation frequency did not produce an added effect (Garibaldi et al. 2013; Mallinger & Gratton 2015). A similar process could have occurred when colonies of bumble bees were present or even, as their recorded visits to strawberry flowers were low, bumble bee effects on strawberry weight may have been diluted. Importantly, when the number of wild non-bee flower visitors increased, they provided supplementation of the pollination services by increasing the weight of fruits. But this positive effect was only significant in winter. A possible explanation for such seasonal differences could be related to the time spent foraging by wild flower visitors. It is likely that in spring, when weather conditions are optimal, the visitation rates of each individual are greater, providing the maximum pollination service across the landscape.

Conclusions

Our study indicates that bumble bee colony supplementation in the most cultivated strawberry variety, ‘Fortuna’, in Huelva (SW Spain) does not yield an increase in fruit weight. We found that the community of flower visitors present, composed of honey bees, hoverflies and other Diptera, provides the necessary pollination service to this strawberry variety. It is important to take into account that most flower visitors were honey bees which probably play an important role in the pollination function, and that bumble bees are not common pollinators in this Mediterranean region. Moreover, the strawberry plots in which we conducted our experiments were located adjacent to natural habitats where the presence of wild flower visitors could be greater than at larger distances within the crop. In any case, our findings emphasize the need to consider the spatiotemporal variability of managed and wild pollinators in pollinator-dependent crops. The unnecessary use of managed pollinators reduces farmers’ profits. It is very important, therefore, that crop type, fruit variety and region are considered before the widespread use of commercial bumble bee colonies.

Acknowledgements

We thank D. Ragel for fieldwork help, A. Vujic for identifying the Syrphidae, P.J. Macaluso for the English revision, K. Hövemeyer and two anonymous reviewers for their comments on a previous version of the manuscript. We thank the farmers who generously let to conduct our experiments in their fields. This research was supported by the Spanish Ministry of Economy and Competitiveness project FLORMAS (‘Influence of mass flowering crops on pollinator biodiversity’, project no CGL2012-33801) and by the Biodiversa-FACCE project ECODEAL (‘Enhancing biodiversity-based ecosystem services to crops through optimized densities of green infrastructure in agricultural landscapes’ <http://www.cec.lu.se/ecodeal>, project no PCIN-2014-048). AT was supported by a Severo-Ochoa predoctoral fellowship (SVP-2013-067592) and by the Super-B COST Action (FA1307:18100). JMH was supported by the Spanish Ministry of Education and Science through a postdoctoral fellowship ‘Juan de la Cierva’ (FPDI-2013-16335), and by the Portuguese national funding agency for science, research and technology (FCT) through the contract IF/00001/2015 and the project PTDC/AAG-REC/6480/2014.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.baae.2018.05.008>.

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Please cite this article in press as: Trillo, A., et al. Managed bumble bees increase flower visitation but not fruit weight in polytunnel strawberry crops. *Basic and Applied Ecology* (2017), <https://doi.org/10.1016/j.baae.2018.05.008>