nature
ecology & evolution

Articles
DOI: 10.1038/s41559-017-0249-9

Honeybee spillover reshuffles pollinator diets and affects plant reproductive success

Ainhoa Magrach[1,2]*, Juan P. González-Varo[1,3], Mathilde Boiffier[1], Montserrat Vilà[1] and Ignasi Bartomeus[1]

During the past decades, managed honeybee stocks have increased globally. Managed honeybees are typically used within mass-flowering crops and often spill over to adjacent natural habitats after crop blooming. Here, we uniquely show the simultaneous impacts that honeybee spillover has on wild plant and animal communities in flower-rich woodlands through changes in plant–pollinator network structure that translate into a detrimental effect on the reproductive success of a dominant wild plant. Honeybee spillover leads to a re-assembly of plant–pollinator interactions through increased competition with other pollinator species. Moreover, honeybee preferential foraging for the most abundant plant species decreases its seed set, driven by high honeybee visitation that affects plant pollen tube growth. Our study therefore calls for an adequate understanding of the trade-offs between providing pollination services to crops and the effects that managed pollinators might have on wild plants and pollinators.

Cropland has increased by ~25% in the past half century, particularly involving crops that depend to some extent on pollinators[1]. This has been accompanied by a ~45% increase in stocks of the main crop pollinator, the honeybee (Apis mellifera L.)[2]. At present, honeybees are the single most important crop pollinator globally[3,4], particularly used within mass-flowering crops[5] (that is, highly productive crops that flower intensively for brief periods). In this context, and after crop flowers have disappeared, honeybees often spill over towards adjacent natural habitats in search for floral resources[6–8]. Although such an increase in the abundance of this generalist pollinator could be beneficial for some wild plants, there is occasionally a saturating relationship between pollinator visitation rate and fruit set, with a threshold above which more visits do not render greater success or could even lead to a decrease in fruit set. This could happen if, for instance, excess pollen prevented pollen tube development[9].

In addition to the effects on wild plant reproductive success, sudden increases in honeybee abundances might also affect other pollinator species through direct competition for floral resources (pollen and nectar)[7,10–14]. This competition is particularly evident within simplified agricultural landscapes where diverse floral resources are limited to natural or semi-natural habitats[15]. Competition for resources at these sites can have implications for the reproductive success of many pollinator species[11]. Indeed, although the honeybee is perceived as a generalist species due to its ability to feed on many floral resources[16], on the local scale it tends to focus on the flowers of the most abundant species[17,18]. This, coupled with their unique ability to communicate the location of flower-rich areas to the rest of their colony[19], makes honeybees highly efficient pollen and nectar foragers, able to outcompete other pollinator species, which could be forced to shift their diets towards less profitable or scarce resources[7,10]. Such changes in pollinator diets would not only have consequences for pollinator population dynamics, but also feedback into plant reproductive success (see ref. [20]).

Recent research in southern Spain has shown a strong spillover of managed honeybees from orange groves (a very relevant mass-flowering crop occupying >150,000 ha[21]) to adjacent woodlands following orange blooming[8]. This finding is particularly significant in the case of Spain, which is also one of the honey-producing countries that has seen their honeybee stocks rise steeply[22], more than tripling since the 1960s[23]. This study system provides the perfect situation to test honeybee spillover effects on wild pollinator species, associated plant–pollinator networks and, as a consequence, on wild plant reproductive success. To this end, we surveyed landscapes with high and low cover of orange groves during and after orange blooming to specifically investigate how mass-flowering crop cover and the associated honeybee spillover that follows crop flowering affected wild plant and animal communities (Fig. 1). In particular, our study aims to evaluate whether honeybee spillover (1) resulted in an increased competition with other pollinator species for floral resources, (2) led to a re-assembly of plant–pollinator interaction networks through changes in the identity of plant species visited by pollinators, and (3) affected wild plant reproductive success. We find that increased honeybee abundance in woodlands surrounded by high grove cover increases apparent competition between honeybees and wild pollinators, and modifies plant–pollinator network structure by monopolizing visits to the most abundant plant species and by shifting wild pollinator diets. These persistent honeybee visits to the most abundant plant species finally result in a decreased seed set for these species (Fig. 1c).

Results

In the sampled woodlands, we recorded 9,958 pollinator visits (Supplementary Table 1; 67% of which were honeybees, 30% solitary bees, 3% hoverflies and 0.3% bumblebees) involving 240 species (or morphospecies) of pollinator and 59 species of plant (Supplementary Table 2). Besides the honeybee, the most abundant pollinator was the solitary bee Flavipanurgus venustus E., identified in 855 visits (9%). The most visited plants in the community were Cistus crispus L. (4,330 visits, 70% by honeybees and 20% by F. venustus), Lavandula stoechas Lam. (2,126 visits, 79% by

---

[1]Estación Biológica de Doñana (EBD-CSIC), Avda. Américo Vespucio 26, Isla de la Cartuja, 41092 Sevilla, Spain. [2]Basque Centre for Climate Change-BC3, Edif. Sede 1, 1°, Parque Tecnológico UPV, Barrio Sarriena s/n, 48940 Leioa, Spain. [3]Conservation Science Group, Department of Zoology, University of Cambridge, The David Attenborough Building, Pembroke Street, Cambridge CB2 3QZ, UK. *e-mail: ainhoamagrach@hotmail.com

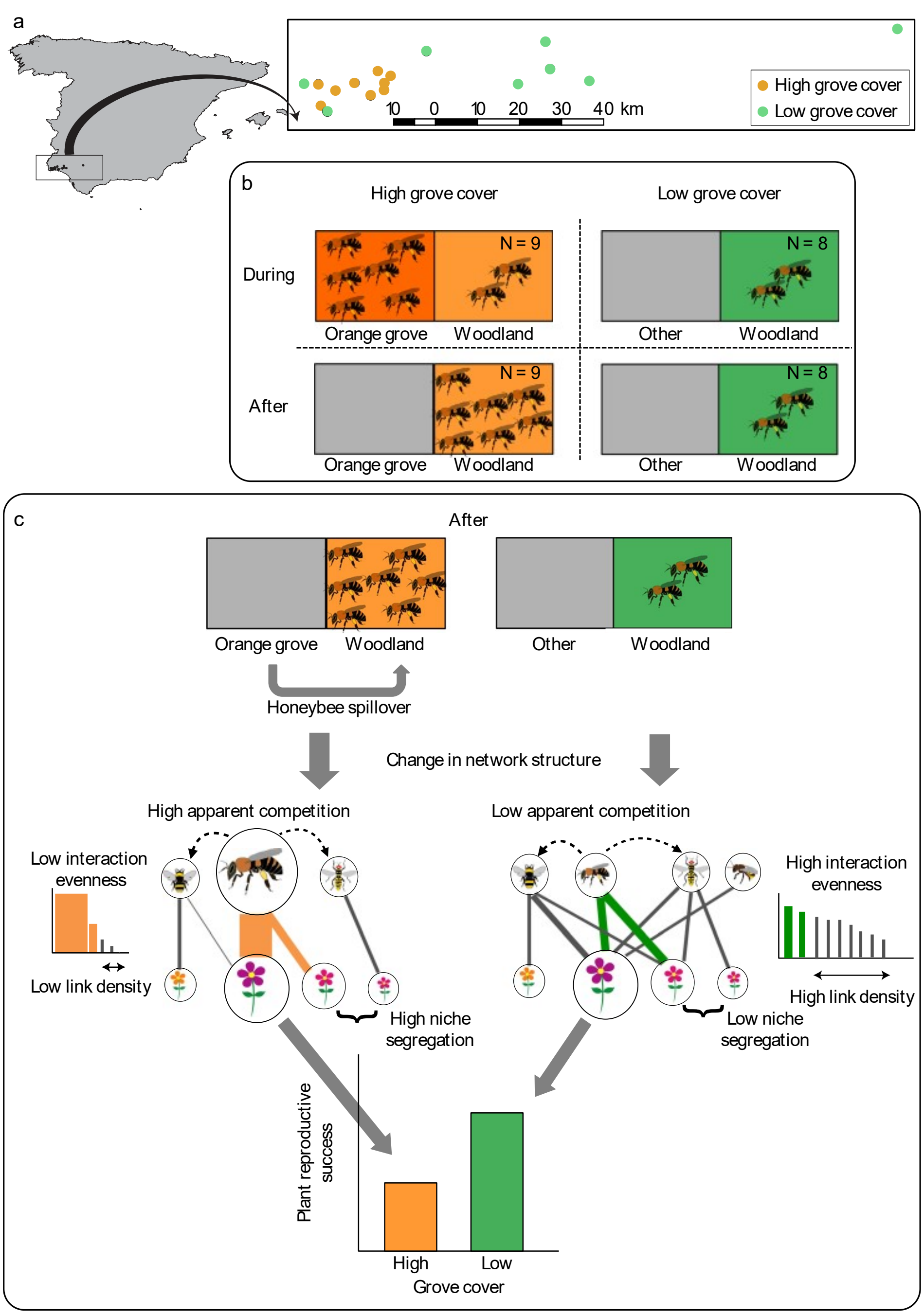

Fig. 1 | Description of study area and experimental design. a, Location of the study area in southwest Spain. Inset shows distribution of woodlands surrounded by high (orange) and low (green) orange grove cover in a 1 km radius. b, Study design showing eight (nine in the case of high grove cover) woodlands sampled within each landscape type (high and low grove cover) during orange flowering and resampled after orange blooming. c, Schematic representation of the effects of honeybee spillover after orange blooming on interaction network structure and plant reproductive success. Within networks, node size denotes plant and animal abundance. Increasing honeybee abundances in woodlands surrounded by high grove cover are expected to shift network structure by visiting the most abundant plant species and leading to a change in the diet of other pollinators, with final negative consequences for the reproductive success of these common plant species.

honeybees) and Cistus salvifolius L. (791 visits, 66% by honeybees), which combined received 80% of all honeybee visits.

After orange blooming, honeybee abundance in woodlands greatly increased in areas surrounded by high grove cover[8]. This honeybee spillover resulted in a clear dominance of honeybees within these woodlands, which were recorded in 72.93 ± 10% of the interactions for this period. In contrast, honeybees were involved in 38.66 ± 15% of the interactions recorded in woodlands surrounded by low grove covers during the same period (Supplementary Table 3). At the network level, this implied a decrease in interaction evenness after honeybee spillover (Supplementary Fig. 1a; Table 1, 'Interaction evenness'), as the frequency of interactions between honeybees and common plant species was an order of magnitude greater than those involving other pollinator species.

As a consequence of honeybee dominance, apparent competition between honeybees and each of the other pollinator species

Table 1 | Estimates ± s.e.m. of spatial and temporal variables affecting network metrics at the network and pollinator group levels included in the average models (for all models with ΔAIC values < 6).

		Estimates ± s.e.m.
Network level	Interaction evenness	
	(Intercept)	0.41 ± 0.02
	Period	0.04 ± 0.02
	Landscape type	0.05 ± 0.02
	Honeybee abundance	−0.06 ± 0.01
	Pollinator species diversity	0.04 ± 0.02
	Period × Pollinator species diversity	−0.03 ± 0.02
	Landscape type × Honeybee abundance	−0.06 ± 0.03
	Landscape type × Pollinator species diversity	−0.04 ± 0.02
	H_2'	
	(Intercept)	97.28 ± 80.15
	Period	−0.05 ± 0.04
	Landscape type	−0.13 ± 0.05
	Year	−0.06 ± 0.03
	Honeybee abundance	−0.03 ± 0.02
	Landscape type × Pollinator species diversity	−0.19 ± 0.06
Group level	Apparent competition	
	(Intercept)	0.62 ± 0.02
	Period	0.04 ± 0.03
	Landscape type	0.31 ± 0.17
	Honeybee abundance	0.07 ± 0.02
	Year	−0.05 ± 0.02
	Flower cover	0.00 ± 0.00
	Period × Landscape type	−0.42 ± 0.17
	Period × Honeybee abundance	0.08 ± 0.04
	Landscape type × Honeybee abundance	0.58 ± 0.19
	Period × Landscape type × Honeybee abundance	−0.56 ± 0.19
	Functional complementarity	
	(Intercept)	−6,042 ± 2,013
	Period	−32.55 ± 14.10
	Landscape type	−3.78 ± 14.56
	Honeybee abundance	176.43 ± 7.28
	Pollinator species diversity	−43.26 ± 11.34
	Year	30.14 ± 10.00
	Period × Pollinator species diversity	42.08 ± 11.47
	Period × Honeybee abundance	−36.61 ± 15.58
	Landscape type × Pollinator species diversity	43.62 ± 10.50
	Landscape type × Honeybee abundance	−41.50 ± 20.03
	d′	
	(Intercept)	0.26 ± 0.01
	Period	−0.01 ± 0.01
	Landscape type	−0.01 ± 0.02
	Honeybee abundance	−0.01 ± 0.01
	Pollinator species diversity	−0.00 ± 0.00
	Flower cover	−0.00 ± 0.00
	Year	−0.01 ± 0.01
	Period × Honeybee abundance	−0.00 ± 0.01
	Landscape type × Period	−0.00 ± 0.00
	Landscape type × Honeybee abundance	−0.00 ± 0.01

Fixed factors included were period (during or after), landscape type (low or high orange grove cover) and year (2011 or 2012). In all cases, 'during' and 'low' were used as the reference categories for the variables period and landscape type. Honeybee abundance was included as a covariate. Bold numbers indicate significant variables. Results for apparent competition (Müller's index) are those from models including the subset of data for which Müller's index > 0 analysed using a Gaussian error structure.

increased (Table 1, 'Apparent competition', Fig. 2a; Supplementary Table 4). Increased competition was particularly evident in woodlands surrounded by high grove cover (Fig. 2a), yet interestingly, there were signs of competition between honeybees and other pollinator species in woodlands surrounded by low grove cover, indicating that competition starts at relatively low honeybee abundances (Fig. 2a).

This increased competition between honeybees and the rest of the pollinator species had consequences for the network of interactions linking wild plants and pollinators. First, the disproportionate increase in honeybee abundance in woodlands surrounded by high grove cover after orange blooming led to a shift in the identity of plants that pollinators interacted with, that is, to a re-assembly or turnover of interactions. This shift occurred despite the fact that the plants they interacted with before were still flowering after orange blooming (Table 2, Fig. 2b; 16.30 ± 6.44 pollinator and 6.77 ± 2.06 plant species were present at both periods).

Second, these diet shifts as a consequence of increased competition were not random. This was supported by an increase in functional complementarity among bee species, a measure describing niche segregation and a further indication of competition[13] (Supplementary Fig. 2; Table 1, 'Functional complementarity'). Specifically, functional complementarity was four times larger in woodlands that received large numbers of honeybees after orange blooming, suggesting that pollinator diet overlap decreased as a consequence of honeybee spillover.

At the network level, this increase in functional complementarity after orange blooming implied a decrease in pollinator niche breadth (that is, the number of interactions per pollinator species or link density; Supplementary Fig. 1b, Supplementary Table 5a). In particular, while link density during orange blooming was very similar in both types of woodland (2.63 ± 0.55 and 2.78 ± 0.50 in woodlands surrounded by high and low grove cover, respectively), it showed a decrease in woodlands surrounded by high grove cover after blooming (1.97 ± 0.37) compared with that in woodlands surrounded by low grove cover, which remained rather constant regardless of the time period (2.71 ± 0.57). These patterns were driven by honeybee abundance, as calculations of link density for the subset of only wild pollinator species (excluding honeybees) confirm this decrease in link density, which was particularly pronounced in woodlands surrounded by high grove cover (Supplementary Fig. 1c, Supplementary Table 5b).

The decrease in niche breadth is also supported by our calculations of complementary specialization (H_2'), a measure of the degree of niche divergence among species, which shows that species become more selective as honeybee numbers increase (Supplementary Fig. 1d; Table 1, 'H_2''). However, this pattern was not found at the species level (Table 1, 'd''), probably because species-level specialization (d') is rather sensitive to low sample sizes, which may be preventing us from detecting any patterns here[24].

Honeybee spillover further had an effect on pollination function, reducing the number of seeds per fruit in two of the most visited plant species, *C. crispus* and *C. salvifolius*, particularly for the former. In the case of *C. salvifolius*, we further found a reduced seed set in woodlands surrounded by high grove cover (Table 3, Fig. 3a). This was partly explained by the fact that individuals in these woodlands suffered higher levels of pollen limitation than those in woodlands surrounded by low grove cover (Supplementary Table 6, Fig. 3b; with an individual considered to be pollen-limited when

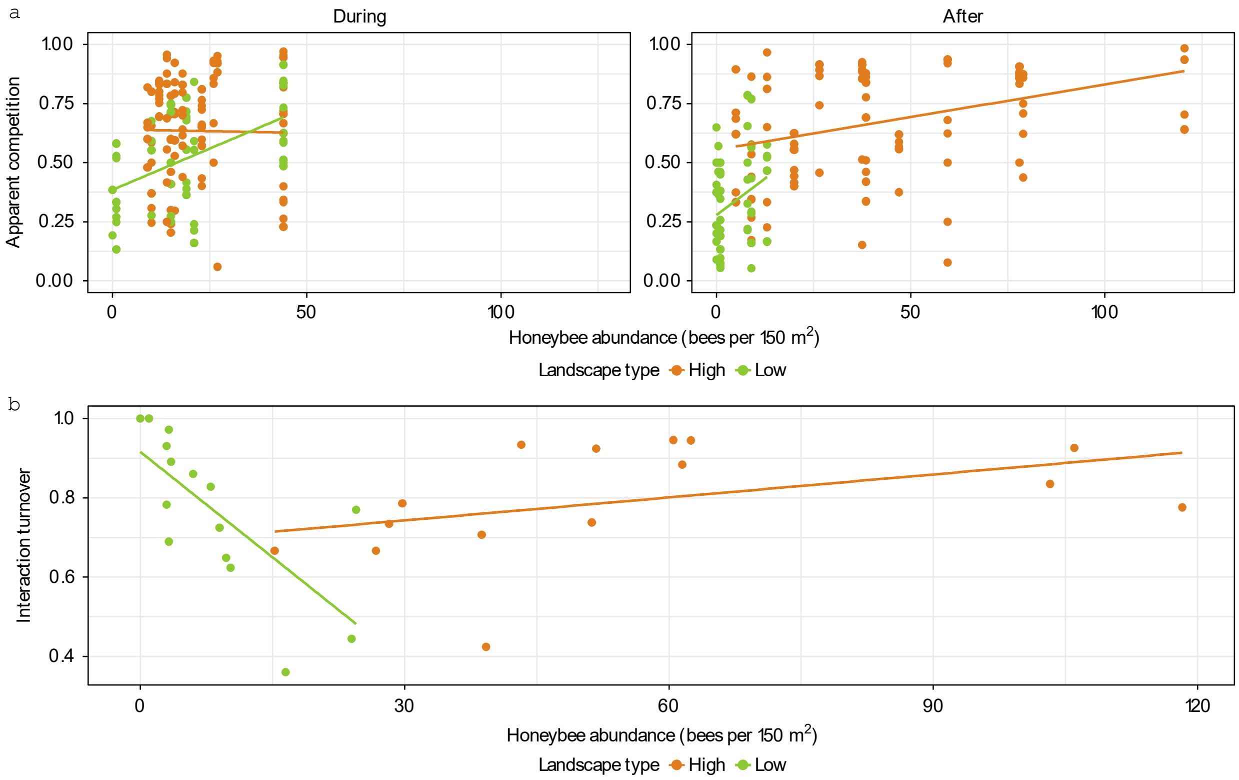

Fig. 2 | Effect of honeybee abundance on apparent competition between honeybees and other pollinator species, and interaction turnover. a, Scatter plots showing the effect of honeybee abundance (bees per $150\,m^2$) in woodlands surrounded by high (orange) and low (green) orange grove cover on apparent competition between honeybees and each of the other pollinator species during and after orange blooming. Data represent the subset of pairwise calculations between honeybees and other pollinators that showed values > 0. N = 687. b, Scatter plot showing the effect of the difference in honeybee abundance (bees per $150\,m^2$) during and after orange blooming in woodlands surrounded by high (orange) and low (green) orange grove cover on interaction turnover for pollinator and plant species that are consistently present at both study periods. N = 30.

Table 2 | Estimates ± s.e.m. of variables affecting interaction evenness included in the averaged models (for all models with ΔAICc values < 6).

Interaction evenness	Estimate ± s.e.m.
(Intercept)	0.61 ± 0.08
Landscape type (low)	0.25 ± 0.08
Honeybee abundance after	0.00 ± 0.00
Year	0.12 ± 0.04
Flower cover after	0.01 ± 0.01
Landscape type (low) × Honeybee abundance after	− 0.02 ± 0.00

Fixed factors included were landscape type (low or high orange grove cover) and year (2011 or 2012). Honeybee abundance after orange blooming was included as a covariate. Bold numbers indicate significant variables.

Table 3 | Estimates ± s.e.m. of variables included in the final models evaluating the effect of landscape type and pollination supplementation on seed set for the two most abundant wild plants.

	Estimate ± s.e.m.
Cistus salvifolius	
(Intercept)	72.0 ± 29.76
Landscape type	− 0.17 ± 0.05
Honeybee abundance	− 0.01 ± 0.00
Year	− 0.36 ± 0.01
Landscape type × Honeybee abundance	0.01 ± 0.00
Cistus crispus	
(Intercept)	76.4 ± 41.5
Landscape type	− 0.03 ± 0.05
Honeybee abundance	− 0.00 ± 0.00
Year	− 0.38 ± 0.02
Landscape type × Honeybee abundance	0.00 ± 0.00

Fixed factors were landscape type (low or high orange crop cover) and year. In all cases, ‘low’ and ‘supplemented’ were used as the reference categories for the variables landscape type and treatment. Honeybee abundance was included as a covariate. Bold numbers indicate significant variables.

the number of seeds produced by flowers manually supplemented with pollen from other individuals was larger than in non-manipulated open flowers). This observed pollen limitation in woodlands surrounded by high grove cover was probably related to the lower abundance of wild pollinators at those sites during the period in which C. salvifolius flowered (coinciding with orange blooming, mean ± s.d.: 11.80 ± 6.95 and 15.80 ± 8.36 pollinator individuals, in woodlands surrounded by high and low grove cover respectively, $z = 5.35$, $P < 0.001$).

Conversely, the decrease in seed set observed in C. crispus following honeybee spillover (Table 3, Fig. 3c) was not a consequence of differences in pollen limitation, which did not differ between woodland types (Supplementary Table 6; Fig. 3d), or lack of wild pollinators, as there was a positive relationship between honeybee abundance and that of C. crispus's main wild pollinator, F. venustus ($z = 2.42$, $P < 0.05$). Rather, for this species, reduced seed set was probably driven by honeybee visitation rates, given that there was a negative relationship between the number of honeybee visits and the number of pollen tubes produced (Supplementary Table 7; Fig. 3e). There was no effect of honeybee abundance or any of the variables measured on fruit set, suggesting that plants are not compensating seed loss with increased fruit production (Supplementary Table 8). Spatial autocorrelation analyses of model residuals confirm that any spatial autocorrelation that could be present within our raw variables is eliminated with our analyses (Supplementary Figs. 3–7).

A structural equation model for C. crispus led to roughly similar results (Supplementary Fig. 8). Honeybees had a direct negative effect as well as significant indirect effects on seed set for C. crispus via changes on plant–pollinator networks (that is, decreases and increases in interaction evenness and complementary specialization, respectively). The effect of complementary specialization was, however, somewhat surprising as the increase in complementary specialization as a result of honeybee spillover had a positive effect on seed set despite stronger direct negative effects of honeybees.

Discussion

Our study shows the simultaneous effects of honeybee spillover from a mass-flowering crop on pollinator diet preferences, plant–pollinator network structure and plant reproductive success. The twofold increase in honeybee abundances in woodlands surrounded by high orange grove cover after blooming compared with their abundance during blooming[8] resulted in a dominance of plant–pollinator interactions involving honeybees. Honeybees outcompeted other pollinator species in feeding on the most abundant resources, shifting and narrowing the diet of other pollinator species towards less abundant resources (for example, 3–5 pollinator species visited either Cistus species after blooming in areas surrounded by high grove cover and high honeybee abundances, while 7–12 pollinator species visited these two species at the same period in areas with low grove cover and few honeybees). A similar shift in pollinator diets in response to increased honeybee abundances has also been observed in bumblebee species in California. This study found that bumblebees avoided the plant species most frequently visited by honeybees in years where honeybees were most abundant, and as a consequence suffered strong declines in their populations[10]. In our study, the shift in pollinator diets became evident in the re-assembly of interactions between plant and pollinator species occurring in both periods in response to honeybee spillover.

Further, our results confirm that, as opposed to other less-dominant pollinators, honeybees tend to preferentially visit the most abundant or rewarding resource within an area[18,25]. In our study system, particularly C. crispus and to a lesser extend C. salvifolius. However, as shown for other species[26], honeybees are not the most efficient pollinators for these two species, as seed set for C. crispus in particular decreases in response to honeybee spillover. In the case of C. salvifolius, whose flowering does not coincide with the peak of honeybee spillover but rather coincides with orange blooming, we find greater levels of pollen limitation in woodlands in intensely managed landscapes. This increased pollen limitation is probably a consequence of the lower visitation rates to C. salvifolius in general, but particularly so in woodlands surrounded by high grove covers. However, we found a positive effect of honeybee abundance on seed set in woodlands surrounded by low grove cover. In this case, more honeybees meant more pollination for a species that receives few visits by wild pollinators. Although C. salvifolius shares its flowering period with oranges, which represent a much more abundant resource, wild bee visitation rates to orange flowers in the study area were extremely low (<4%[27]). Recent research on the effect of other mass-flowering crops on plant–pollinator interaction networks in natural areas suggests the effect of crop flowering is negligible[28], and thus we can be confident that the patterns observed are not driven by the attraction of wild pollinators towards orange groves during blooming.

Conversely, in the case of C. crispus the decrease in seed set in response to honeybee spillover was most probably related to the high honeybee visitation rates suffered by this species, and not a consequence of pollen limitation or reduced visitation rates. Indeed, in the case of C. crispus we found a decrease in seed set in areas that hosted greater numbers of honeybees but also greater numbers of the plant's main wild pollinator, F. venustus[29]. Moreover, we found

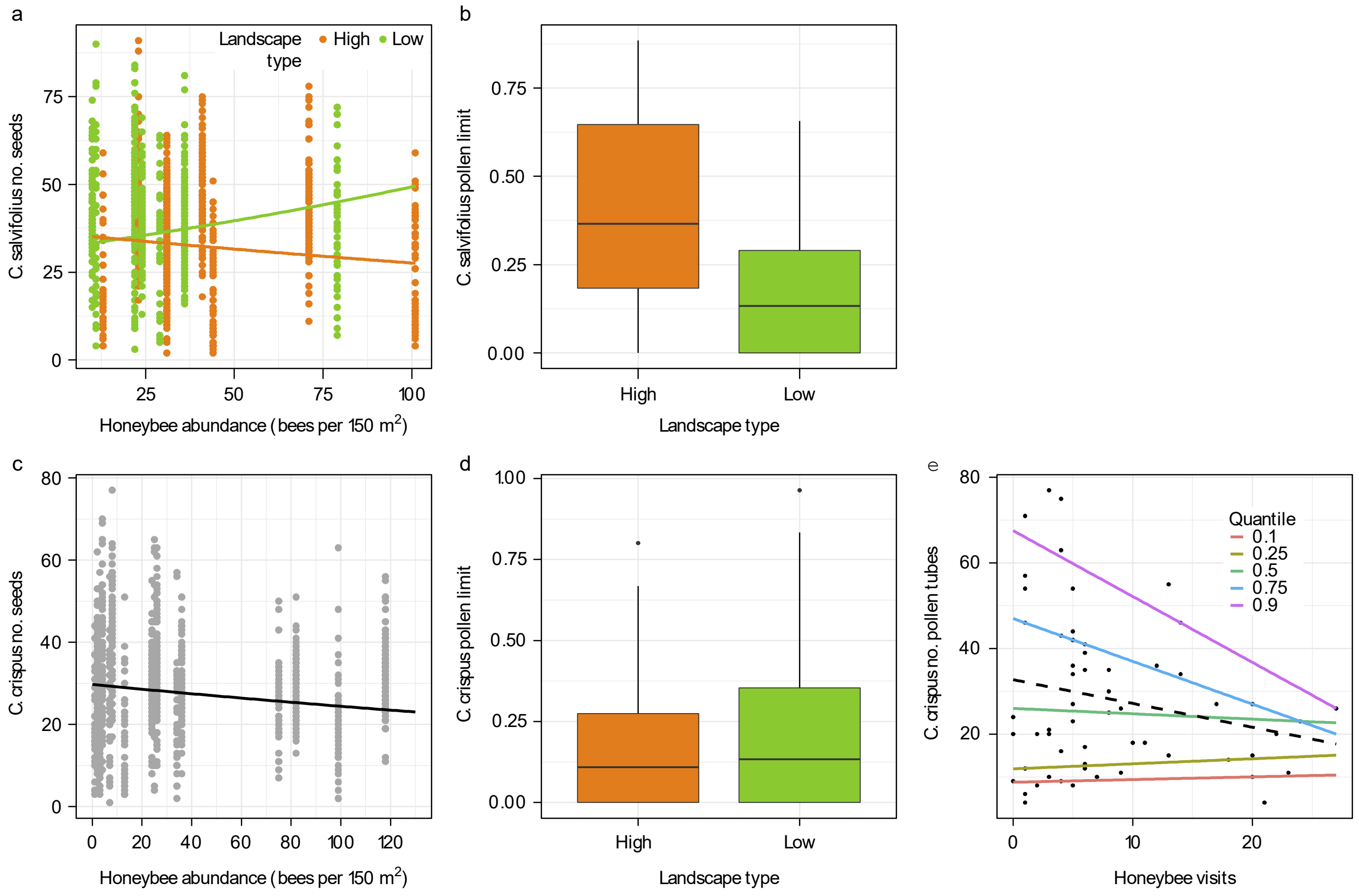

Fig. 3 | Effect of honeybee abundance on seeds and pollen limitation for the two most abundant plant species. a, Scatter plot showing the effect of honeybee abundance (bees per 150 m²) on number of seeds per fruit for each of ten mother C. salvifolius plants in woodlands surrounded by high (orange) and low (green) orange grove cover, N = 1,192. **b**, Box plot showing the effect of landscape type on pollen limitation for C. salvifolius, N = 1,638. **c**, Scatter plot showing the effect of honeybee abundance on number of seeds per fruit for C. crispus, N = 1,093. **d**, Box plot showing the effect of landscape type on pollen limitation for C. crispus, N = 2,190. **e**, Relationship between honeybee visits per individual flower on C. crispus pollen tube number. Different coloured lines indicate different fits using quantile regression given that visual inspection suggested a non-constant variance, N = 63. In the box plots whiskers extend to extreme points < 1.5 times the length of the box.

no difference in pollen limitation across sites, which suggests that it is not a lack of pollen deposition that affects reproductive output in this species, but rather something occurring after the pollen is delivered, as even the hand-pollinated flowers showed a reduced seed set after receiving several honeybee visits. More importantly, we found a significant reduction in pollen tube numbers with increasing honeybee visits. This might indicate that honeybee foraging behaviour somehow affected pollen deposition. Several mutually non-exclusive reasons could explain this result. Most species in the Cistaceae family are self-incompatible[30] and honeybees could, for instance, increase the amount of pollen from other species (heterospecific)[31] or from the same plant (geitonogamy) deposited in the stigmas, which could reduce seed set. But honeybees could also feed on some of the pollen already deposited[32] or somehow damage the style when landing on the flower, given their larger body sizes compared with other wild pollinators[9]. Indeed, when F. venustus, the species' main pollinator under natural conditions, lands on a C. crispus flower, it does so sideways in the outer part of the anthers; it slowly walks around this outer part feeding on pollen and eventually gently touches the stigma (see Fig. 2c–e in ref. [29]). In contrast, honeybees, which are larger than F. venustus, tend to land directly on top of the flower's stigma to collect pollen quickly, potentially damaging the style[9]. Further, the foraging patterns of both species of bee are completely different. While F. venustus visits flowers in an apparently random way, honeybees tend to systematically visit flowers in sequence within individual plants and therefore much of the pollen transfer occurs between flowers of the same plant or between neighbouring individuals (A.M., personal observations), limiting cross-pollination and potentially increasing inbreeding depression[33], particularly given self-incompatibility in the family[30]. The large abundances of honeybees observed in some of our sites resulted in many flowers receiving multiple honeybee visits (>50 visits over the course of a flower's lifespan of one day), while the number of visits performed by F. venustus was much lower (<5 in one day; A.M., personal observations). This excess of pollen grains from closely related individuals deposited in the stigma might be the most plausible reason for the negative effect we found of honeybee visits on pollen tube growth for C. crispus, as well as that of honeybee abundance on seed set.

Finally, of special relevance is our finding that a greater functional complementarity had a negative effect on seed set. Our result contrasts a previous microcosms study that found a positive effect of increasing functional complementarity within the pollinator guild on seed production[34]. However, this particular study was based on evenly distributed experimental communities, a pattern that is not common in nature, where communities tend to present a ubiquitous dominance pattern[35]. In our study, we found that the dominant honeybees visit two common plant species, representing the most abundant floral resources. Honeybees were the most competitive species, yet not the most efficient pollinators[26] and hence reproductive success for highly visited plant species decreased. Our study thus suggests that the positive effect of functional complementarity on pollinator function depends on the dominance/evenness of the community as well as the efficiency of the dominant species. It is worth noting, however, that our study has concentrated on the reproductive output of rather abundant and ubiquitous plant species within the study area, yet the effects on rare plant species remain to be studied.

In summary, managed honeybees are needed for the production of many entomophilous crops, as the great increase in the cover of pollinator-dependent crops in the past decades cannot rely solely on the services provided by wild pollinators[2]. However, an excess of honeybees and their spillover towards adjacent natural habitats might change plant–pollinator networks[36] and jeopardize the reproduction of some wild plants (as opposed to that suggested by ref. [37]). It is thus of paramount importance that we first understand the densities of honeybees needed to ensure a successful crop pollination[9] and the threshold at which these honeybees might impact the reproductive success of wild plant species[10,12], as well as that of other pollinators in the community[11].

Methods

Study site. The study was carried out in the provinces of Huelva and Sevilla, southern Spain. The region is characterized by a Mediterranean climate featuring warm, dry summers and cold and humid winters, with a mean annual precipitation of 525 mm. The area presents numerous flowering crops (such as oranges and different berries) as well as fragmented pine (Pinus pinea) and Mediterranean oak (Quercus ilex subsp. ballota and Q. suber) woodlands with a diverse understory of flowering species[29].

Pollinator and flower survey. We selected 17 semi-natural woodlands. Nine of these sites were located in areas with a high relative cover of orange groves for the region (ranging from 28 to 57% of total cover within a 1 km radius from the study site edge) while eight sites were located in areas with low crop cover (0–8% orange grove cover). We selected a 1 km radius to account for the majority of foraging flights of the pollinators considered[38]. All woodlands were surveyed four times per year for two consecutive years (2011–2012, except for one site that could not be surveyed in 2012 and was replaced by a new site); the first two surveys overlapped with orange flowering (March–April), while the last two surveys were carried out immediately after flowering had ceased (April–May). We recorded all flower-visiting bees (Hymenoptera: Apiformes) as well as hoverflies (Diptera: Syrphidae) along two randomly selected 150 m long × 1 m wide transects for 30 min (spanning 15 min per transect), as well as the plant species they were visiting. We collected all pollinators that could not be readily identified in the field and did so in the laboratory with the aid of taxonomist experts. We collected most rare pollinator species but did not collect honeybees as they can be readily identified in the field. We do not believe this to be a caveat in our study, given the disproportionate dominance of honeybees in woodlands surrounded by high grove covers. Capturing a subset of them at those sites would not have made much of a difference. All voucher specimens are deposited at EBD-CSIC (Estación Biológica de Doñana—Consejo Superior de Investigaciones Científicas). At all sites we estimated flower cover as the sum of:

$$\frac{\text{number of flower units} \times \text{mean flower unit size}}{\text{transect area}}$$

for every species in the transect surveyed.

Reproductive success in wild plants. For the two most abundant and visited plant species in the area, Cistus crispus L. and Cistus salvifolius L. (see Results section), we recorded seed set (that is, the number of seeds produced per fruit) in 2011 and 2012 for ten fruits per plant from ten different plants in each site (N = 1,400 fruits per year and species). During 2012, we also recorded fruit set for ten plant individuals in each site. C. salvifolius flowers during the period of orange blooming, while C. crispus flowers immediately after. Both Cistus species are shrubby species with few flowers arranged in cymes. Flowers are actinomorphic with five petals, white in C. salvifolius and purple in C. crispus.

During 2012, to evaluate the level of pollen limitation for both species, ten flowers per plant from ten plants were bagged before flower opening, supplemented with cross-pollen and then left uncovered. Pollen limitation was quantified as $1 - P_o / P_s$, where P_o is the number of seeds in open-pollinated flowers and P_s is the number of seeds in plants that received supplemental cross-pollen. We gave the index a lower bound of 0, as negative values would indicate greater fertility in open flowers, probably a consequence of experimental or statistical errors (occurring in N = 6 flowers and N = 8 flowers for C. crispus and C. salvifolius, respectively).

For C. crispus, and to test the effect of increasing honeybee visitation levels on reproductive output, we collected flowers with increasing numbers of visits by honeybees. To this end, during five days we bagged closed flowers early in the morning. We then uncovered one flower each time and recorded all honeybee visits to the focal flower. Flowers were collected after a fixed number of visits were attained. Flowers (N = 63) were collected on the same day and watered for 24 h to allow pollen tubes to grow, after which the style was kept in ethanol. Styles were then fixed with NaOH for 20 min at 65 °C and stained with aniline blue for another 20 min. Styles were mounted in a slide glass and pollen tubes were then observed and counted under a fluorescence microscope.

Test for sampling completeness. We evaluated sampling completeness for both the pollinator community and the plant–pollinator links using Chao 1 asymptotic species richness estimators. We first estimated the richness of pollinator species and plant–pollinator links accumulated as sampling effort increased up to 100% sampling coverage using package iNEXT[39]. Extrapolation of species and link diversity estimates showed that our survey was able to capture 58–90% of the pollinator species richness as well as 56–64% of the plant–pollinator link richness (Supplementary Fig. 9).

Effects of honeybees on plant–pollinator interaction networks. To evaluate whether orange flowering affected plant–pollinator interactions in adjacent woodlands, we constructed weighted interaction networks for each woodland, combining data for the two transects and surveys per period. In total we built 64 (16 sites × 2 periods × 2 years) quantitative networks where links represented the frequency of plant–pollinator visits.

We selected a set of network metrics at the network and at the pollinator level relevant for our hypotheses. At the network level, we calculated link density as the weighted number of interactions per species. We calculated this metric both for the full networks as well as for networks where only wild species of pollinators were considered, that is, honeybees were excluded from this analysis. We also calculated interaction evenness (IE), a measure of niche breadth or the uniformity in the distribution of species of interactions as per ref. [40]:

$$\mathrm{IE} = \frac{\sum p_i \log_2(p_i)}{\log_2 N}$$

where p_i is the proportion of the total number of plant–pollinator interactions represented by interaction i and N is the total number of interactions. Finally, we calculated complementary specialization (H_2'), a measure of interaction exclusiveness, which measures the deviation of interaction frequencies from a completely generalized network ($H_2' = 0$) to a completely specialized one ($H_2' = 1$). We selected these three metrics because the first informs of the diversity of interactions while the other two reveal the relative distribution of interactions.

At the pollinator level (which includes all floral visitors) we calculated functional complementarity, apparent competition and species-level specialization (d′). We selected these three metrics because they provide complementary non-redundant information on the effect of honeybees on other pollinator species. The first represents a measure of niche segregation between all species, the second provides a specific understanding of the potential for honeybees to interfere with the diet of other pollinator species, while the third provides information on the level of selectivity for each pollinator species. Functional complementarity is a community-level measure of the overlap in ecological niches between pollinator species. It is measured as the total branch length of a dendrogram representing functional diversity, where functional diversity is calculated as the similarity in interactions among different pollinator species[41]. Apparent competition measures the potential for honeybees to influence other pollinator species via their shared plants using Müller's index[42]. This metric evaluates potential competition between two species in one trophic level via the partners they share in another trophic level (for example, between two pollinators via their shared plants). We calculated Müller's index as:

$$d_{ij} = \sum_k \left(\frac{\alpha_{ik}}{\sum_l \alpha_{il}} \times \frac{\alpha_{jk}}{\sum_m \alpha_{mk}} \right)$$

where α_{ik} represents the number of interactions between the target pollinator i and a plant k, while l represents the total number of plant species visited by that pollinator species. Similarly, α_{jk} represents the number of interactions between the same plant k with pollinator j (with m representing the total number of pollinator species with which plant k interacts). This metric takes values ranging from 0 (pollinators share no plants) to 1, with greater values indicating greater potential for a pollinator species to influence another one via shared resources. We calculated this metric for each site for the subset of pairs involving honeybees and each of the other pollinator species in the pollinator community. d′ was calculated using the Kullback–Leibler distance as a measure of partner diversity[43]. All analyses were performed using package bipartite in the R environment[41].

Interaction turnover. We assessed how the composition of interactions between plants and pollinators varied through space and time by means of β-diversity measures across sites during and after crop flowering[44]. We focused on comparing the turnover in interaction composition before and after honeybee spillover (that is, during and after orange blooming) involving species of plants and pollinators that were present during both periods (betaOS measure in ref. [44], which refers to rewiring in species interactions). To this end, we used a quantitative measure of β-diversity based on the Ruzicka distance coefficient, which is analogous to its qualitative counterpart based on the Colwell and Coddington measure[45]. This metric takes values of 0 when interaction composition is exactly the same and values of 1 when interaction composition is completely different. Code to calculate this is available at github (https://github.com/ibartomeus/betalink/tree/new_features).

Data analyses. First, we evaluated how landscape type, period and honeybee abundance affected both the network-level (interaction evenness and complementary specialization) and the pollinator-level metrics (functional complementarity and apparent competition) by fitting generalized linear mixed models (GLMMs) where we included the following variables: landscape type, period and year as fixed factors; honeybee abundance, flower cover and pollinator species diversity (averaged across both transects, to account for differences in diversity across sites) as covariates; and the three-way interactions between landscape type, period and pollinator species diversity, and that of landscape type, period and honeybee abundance. For apparent competition and given the large number of zeroes found in our dataset, we proceeded in a two-step approach (as in previous studies[46]). In a first step, we analysed the probability of honeybees influencing other pollinators by converting Müller's index into a binomial variable (taking a value of 0 if a pollinator species shared no resources with honeybees and a value of 1 otherwise) and fitting a GLMM with a binomial structure that included period, landscape type, year, flower cover and honeybee abundance as well as the three-way interactions between period, landscape type and honeybee abundance (Supplementary Table 7). In every analysis, we included site as a random effect to account for the non-independence of repeated sampling across time (two periods and years) for all sites except two that were sampled only once. We ran all combinations of models using the 'dredge' function in the MuMIn package[47] and selected the best model based on the lowest Akaike information criteria values corrected for small sample sizes (AICc). If more than one plausible model existed (that is, when ΔAICc < 6 for more than one model[48]), we computed average estimates for each variable across all models in which each variable was retained. In a second step, we used the subset of data for which Müller's index $>$ 0 and analysed them using the same model configuration and a Gaussian error structure.

Second, we assessed whether landscape type, year, flower cover and honeybee abundance after orange blooming influenced interaction turnover, looking just at interaction rewiring between species present at both periods. We also included in the analysis the two-way interaction between landscape type and each of the covariates. For flower cover we used the average value after orange blooming for plant species present during both periods.

Third, we evaluated how seed and fruit set for C. salvifolius and C. crispus changed across sites by fitting a GLMM, which included landscape type, honeybee abundance and their interaction as well as year (only in the case of seed set). For both species we included plant nested within site as a random effect to account for non-independence of flowers treatments within the same plant at each site in the case of seed set and only site in the case of fruit set. In addition, we evaluated whether pollen limitation changed across sites by fitting a GLMM including landscape type, honeybee abundance and their interaction. We also tested how honeybee visitation rates to C. crispus affected pollen tubes. In this case, given that visual inspection suggested a non-constant variance, we used quantile regression[49] to fit a model that included honeybee visits as a covariate using the quantreg package in the R environment[41]. This approach does not allow for the inclusion of random effects, which in this case were not necessary as we had one measure per plant individual.

We evaluated whether there were potential spatial autocorrelation biases in our measured variables by means of Mantel correlograms at different distance lags for the raw variables as well as for the residuals of the models they were included in.

Finally, to summarize our main results we fitted a structural equation model using the piecewiseSEM package in R[50]. Each response variable was fitted to a GLMM where site and plant nested within site were included as random effects. Model fit was evaluated by means of Fisher's C statistic compared with a chi-squared distribution. We evaluated how landscape type and year affected honeybee and wild bee abundance as well as pollinator species diversity directly. These response variables were then included in models to evaluate their effects on network metrics. Given correlations between some of the network metrics considered, we reduced our analyses to two main network metrics: interaction evenness and complementary specialization. Network metrics and pollinator variables were then included in the model for C. crispus seed set. All paths were retained in the final model.

Data availability. The data that support the findings of this study have been deposited in Figshare (https://doi.org/10.6084/m9.figshare.5035709.v1).

Received: 18 January 2017; Accepted: 22 June 2017;

References

1. Aizen, M. A., Garibaldi, L. A., Cunningham, S. A. & Klein, A. M. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18, 1572–1575 (2008).
2. Aizen, M. A. & Harder, L. D. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. 19, 915–918 (2009).
3. Kleijn, D. et al. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6, 7414 (2015).
4. Kennedy, C. M. et al. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol. Lett. 16, 584–599 (2013).
5. Rader, R. et al. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. J. Appl. Ecol. 46, 1080–1087 (2009).
6. Blitzer, E. J. et al. Spillover of functionally important organisms between managed and natural habitats. Agric. Ecosyst. Environ. 146, 34–43 (2012).
7. Montero-Castaño, A. & Vilà, M. Influence of the honeybee and trait similarity on the effect of a non-native plant on pollination and network rewiring. Funct. Ecol. 31, 142–152 (2017).
8. González-Varo, J. P. & Vilà, M. Spillover of managed honeybees from mass-flowering crops into natural habitats. Biol. Conserv. 212, 376–382 (2017).
9. Sáez, A., Morales, C. L., Ramos, L. Y. & Aizen, M. A. Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. J. Appl. Ecol. 51, 1603–1612 (2014).
10. Thomson, D. M. Local bumble bee decline linked to recovery of honey bees, drought effects on floral resources. Ecol. Lett. 19, 1247–1255 (2016).
11. Cane, J. H. & Tepedino, V. J. Gauging the effect of honey bee pollen collection on native bee communities. Conserv. Lett. 10, 205–210 (2016).
12. Torné-Noguera, A., Rodrigo, A., Osorio, S. & Bosch, J. Collateral effects of beekeeping: impacts on pollen–nectar resources and wild bee communities. Basic Appl. Ecol. 17, 199–209 (2016).
13. Brosi, B. J. & Briggs, H. M. Single pollinator species losses reduce floral fidelity and plant reproductive function. Proc. Natl Acad. Sci. USA 110, 13044–13048 (2013).
14. Geslin, B. et al. Massively introduced managed species and their consequences for plant–pollinator interactions. Adv. Ecol. Res. 57, 147–199 (2017).
15. Herbertsson, L., Lindström, S. A. M., Rundlöf, M., Bommarco, R. & Smith, H. G. Competition between managed honeybees and wild bumblebees depends on landscape context. Basic Appl. Ecol. 17, 609–616 (2016).
16. Coffey, M. F. & Breen, J. Seasonal variation in pollen and nectar sources of honey bees in Ireland. J. Apic. Res. 36, 63–76 (1997).
17. Gross, C. L. The effect of introduced honeybees on native bee visitation and fruit-set in Dillwynia juniperina (Fabaceae) in a fragmented ecosystem. Biol. Conserv. 102, 89–95 (2001).
18. Grüter, C., Moore, H., Firmin, N., Helanterä, H. & Ratnieks, F. L. W. Flower constancy in honey bee workers (Apis mellifera) depends on ecologically realistic rewards. J. Exp. Biol. 214, 1397–1402 (2011).
19. von Frisch, K. The Dance Language and Orientation of Bees (Harvard Univ. Press, Cambridge, 1965).
20. Vanbergen, A. J., Woodcock, B. A., Heard, M. S. & Chapman, D. S. Network size, structure and mutualism dependence affect the propensity for plant–pollinator extinction cascades. Funct. Ecol. 31, 1285–1293 (2017).
21. FAOSTAT: Statistical Databases and Data-Sets (FAO, Rome, 2014).
22. Aizen, M. A. & Harder, L. D. Geographic variation in the growth of domesticated honey bee stocks: disease or economics? Commun. Integr. Biol. 2, 464–466 (2009).
23. FAOSTAT Database on Agriculture (FAO, Rome, 2014).
24. Fründ, J., McCann, K. S. & Williams, N. M. Sampling bias is a challenge for quantifying specialization and network structure: lessons from a quantitative niche model. Oikos 125, 502–513 (2015).
25. de Menezes Pedro, S. R. & de Camargo, J. M. F. Interactions on floral resources between the Africanized honey bee Apis mellifera L and the native bee community (Hymenoptera: Apoidea) in a natural "cerrado" ecosystem in southeast Brazil. Apidologie 22, 397–415 (1991).
26. Garibaldi, L. A. et al. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339, 1608–1611 (2013).
27. Holzschuh, A. et al. Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. Ecol. Lett. 19, 1228–1236 (2016).
28. Magrach, A. et al. Plant–pollinator networks in semi-natural grasslands are resistant to the loss of pollinators during blooming of mass-flowering crops. Ecography https://doi.org/10.1111/ecog.02847 (2017).

29. González-Varo, J. P., Ortiz-Sánchez, F. J. & Vilà, M. Total bee dependence on one flower species despite available congeners of similar floral shape. PLoS ONE 11, e0163122 (2016).
30. Bosch, J. Floral biology and pollinators of three co-occurring Cistus species (Cistaceae). Bot. J. Linn. Soc. 109, 39–55 (1992).
31. Morales, C. L. & Traveset, A. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. Crit. Rev. Plant Sci. 27, 221–238 (2008).
32. Morris, W. Mutualism denied? Nectar-robbing bumble bees do not reduce female or male success of bluebells. Ecology 77, 1451–1462 (1996).
33. González-Varo, J. P., Albaladejo, R. G., Aparicio, A. & Arroyo, J. Linking genetic diversity, mating patterns and progeny performance in fragmented populations of a Mediterranean shrub. J. Appl. Ecol. 47, 1242–1252 (2010).
34. Fründ, J., Dormann, C. F., Holzschuh, A. & Tscharntke, T. Bee diversity effects on pollination depend on functional complementarity and niche shifts. Ecology 94, 2042–2054 (2013).
35. McGill, B. J. et al. Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. Ecol. Lett. 10, 995–1015 (2007).
36. Watts, S., Dormann, C. F., Martín González, A. M. & Ollerton, J. The influence of floral traits on specialization and modularity of plant–pollinator networks in a biodiversity hotspot in the Peruvian Andes. Ann. Bot. 118, 415–429 (2016).
37. Aebi, A. et al. Back to the future: Apis versus non-Apis pollination—a response to Ollerton et al. Trends Ecol. Evol. 27, 142–143 (2012).
38. Danner, N., Molitor, A. M., Schiele, S., Härtel, S. & Steffan-Dewenter, I. Season and landscape composition affect pollen foraging distances and habitat use of honey bees. Ecol. Appl. 26, 1920–1929 (2016).
39. Hsieh, T. C., Ma, K. H. & Chao, A. iNEXT: Iterpolation and Extrapolation for Species Diversity. R package v. 2.0.8. (R Foundation for Statistical Computing, Vienna, 2016).
40. Tylianakis, J. M., Tscharntke, T. & Lewis, O. T. Habitat modification alters the structure of tropical host–parasitoid food webs. Nature 445, 202–205 (2007).
41. Dormann, C. F., Frund, J., Bluthgen, N. & Gruber, B. Indices, graphs and null models: analyzing bipartite ecological networks. Open Ecol. J. 2, 7–24 (2009).
42. Müller, C. B., Adriaanse, I. C. T., Belshaw, R. & Godfray, H. C. J. The structure of an aphid–parasitoid community. J. Anim. Ecol. 68, 346–370 (1999).
43. Blüthgen, N., Menzel, F. & Blüthgen, N. Measuring specialization in species interaction networks. BMC Ecol. 6, 9 (2006).
44. Poisot, T., Canard, E., Mouillot, D., Mouquet, N. & Gravel, D. The dissimilarity of species interaction networks. Ecol. Lett. 15, 1353–1361 (2012).
45. Legendre, P. Interpreting the replacement and richness difference components of beta diversity. Glob. Ecol. Biogeogr. 23, 1324–1334 (2014).
46. Carvalheiro, L. G. et al. The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. Ecol. Lett. 17, 1389–1399 (2014).
47. Bartoń, K. MuMIn: Multi-Model Inference. R package v. 1.9.13 (R Foundation for Statistical Computing, Vienna, 2013); http://CRAN.R-project.org/package=MuMIn
48. Burnham, K. P., Anderson, D. R. & Huyvaert, K. P. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behav. Ecol. Sociobiol. 65, 23–35 (2011).
49. Cade, B. S. & Noon, B. R. A gentle introduction to quantile regression for ecologists. Front. Ecol. Env. 1, 412–420 (2003).
50. Lefcheck, J. S. piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. Methods Ecol. Evol. 7, 573–579 (2016).

Acknowledgements

This project was partially funded by the EU FP7 STEP project 'Status and trends of European pollinators' (244 090; http://www.STEP-project.net) and Biodiversa-FACCE project 'Enhancing biodiversity-based ecosystem services to crops through optimized densities of green infrastructure in agricultural landscapes' (PCIN-2014-048, http://www.cec.lu.se/ecodeal), the Spanish Ministry of Economy and Competitiveness FLORMAS (CGL2012-33801) and the Severo Ochoa program (SEV-2012-0262). A.M. acknowledges funding from the Juan de la Cierva Incorporación program (IJCI-2014-22558). We thank R. Gómez, A. Carrillo-Gavilán, C. Molina and D. Ragel for their assistance in fieldwork. We thank J. M. Ortiz and A. Vujic for identifying most bee and hoverfly specimens, respectively.

Author contributions

J.P.G.V. and M.V. conceived the experimental design; J.P.G.V., M.B. and A.M. collected field data; A.M. led data analysis and drafted the first version of the manuscript; I.B. participated in data analyses and helped draft the manuscript. All authors commented on manuscript drafts and gave final approval for publication.

Competing interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available for this paper at doi:10.1038/s41559-017-0249-9.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to A.M.