The carry-over effects of pollen shortage decrease the survival of honeybee colonies in farmlands

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Summary

1. Many studies have reported honeybee colony losses in human-dominated landscapes. While bee floral food resources have been drastically reduced over past decades in human-dominated landscapes, no field study has yet been undertaken to determine whether there is a carry-over effect between seasonal disruption in floral resource availability and high colony losses.

2. We investigated if a decline in the harvest of pollen by honeybees in spring affected managed honeybee colony dynamics (brood size, adult population and honey reserves) and health (*Varroa* mite loads and colony survival) throughout the beekeeping season.

3. A decline in pollen harvest was associated with a direct reduction in brood production, leading to a negative effect on the adult population size later in the season, and lower honey reserves before the onset of winter. Furthermore, the decline in pollen harvest negatively impacted the health of the colony, resulting in higher *Varroa* mite loads and higher seasonal and winter colony losses.

4. Early-warning signs of these carry-over effects were identified, showing that preferential investment in honey reserves instead of brood production early in the season increased the decline in pollen harvest and its associated carry-over effects.

5. *Synthesis and applications.* The results suggest that the decline in pollen harvest may have been overlooked as a cause of pollen shortage and associated bee colony losses. Strategies to avoid such losses in intensive farmland systems include (i) limiting or avoiding honey harvests in spring, (ii) monitoring colonies for early-warning signals of colony failure and (iii) increasing the amount of floral resources available through wise land-use management.

Key-words: agricultural landscapes, *Apis mellifera*, carry-over effects, floral resource scarcity, honeybee colony losses, life-history strategy, pollen, trade-offs, *Varroa* mite

Introduction

Many studies have reported honeybee, *Apis mellifera*, colony losses in winter in human-dominated landscapes (e.g. Potts *et al.* 2010a; Seitz *et al.* 2016). Currently, a third to a half of managed honeybee colonies are lost every winter in Europe (Potts *et al.* 2010a) and North America (Seitz *et al.* 2016), which is a cause for concern for both beekeepers and farmers (Potts *et al.* 2010b). Colony losses in winter appear to be explained by a combination of stress

from parasites, pesticides and a lack of floral resources (Potts *et al.* 2010b; Goulson *et al.* 2015).

In intensive farmland systems, mass-flowering crops result in abundant floral resource pulses over relatively short bloom times, followed by periods of floral resource scarcity. This boom-bust cycle of flowering can affect pollinator population dynamics (Williams, Regetz & Kremen 2012; Holzschuh *et al.* 2016). For example, honeybee individual fitness and health is directly related to a shortage of available floral resources (Fewell & Winston 1992; Mattila & Otis 2006; Alaux *et al.* 2010), but no causal link between floral resource availability and winter colony losses has yet been demonstrated in intensive farmland

systems. Given that there is a 7-month lag between the suspected spring food shortage and winter colony losses (Requier *et al.* 2015), any such causal link must result from a carry-over effect. Carry-over effects are based on some kind of food shortage which exerts detrimental effects at a later life-history stage and affect the fitness or reproductive value of individuals (Harrison *et al.* 2011). Given that insects always have several life-history stages (egg, larvae, pupae and adult), a food shortage at an early life-history stage, e.g. larvae, could result in detrimental carry-over effects on subsequent life-history stages, e.g. adult (Stoks & Cordoba-Aguilar 2012).

Bee floral food resources consist of nectar and pollen. Nectar is used by bees as an energy fuel and is also transformed into honey by dehydration to build food reserves (Winston 1994). Pollen is the main source of protein used to feed the young adults, the queen and the larvae. Unlike nectar, pollen is not stored in large quantities (Keller, Fluri & Imdorf 2005; Brodschneider & Crailsheim 2010) and is used depending on the colony's needs and floral resource availability.

The amount of pollen harvested drives brood production (e.g. Fewell & Winston 1992; Mattila & Otis 2006) and is further regulated by a feedback loop depending on the brood size and the amount of pollen that has already been stored (Fewell & Winston 1992). When there is a decline in pollen harvest, the colony adjusts its foraging activity, giving preferential allocation to foraging for pollen rather than for nectar (Fewell & Winston 1992). However, if the pollen harvest becomes limiting, the colony may then decrease its pollen consumption by reducing the queen's egg-laying rate or even reducing the number of larvae by worker cannibalism, and finally totally stopping any egg-laying activity (Schmickl & Crailsheim 2001). The brood size at a given time, thus, determines the adult population 21 days later, which in turn determines the colony's food intake capacity about 1 month later (the adult foraging age, Winston 1994). This sequence of life-history stages may indicate that a decline in pollen harvest will have a carry-over effect on the colony dynamics of honeybees at a later time period. Although this has been suspected with laboratory work (Mattila & Otis 2006) and theoretically assessed (Horn *et al.* 2015), it has not yet been demonstrated in professional beekeeping operations under field conditions.

The main objective of this study was to assess if the seasonal disruption to pollen availability in farmland systems is likely to affect the colony dynamics of managed honeybees. Intensive cereal farmland systems typically include the cultivation of two mass-flowering crops: rapeseed *Brassica napus* in April and sunflower *Helianthus annuus* in July. Between the mass-flowering period of these two crops, in May–June, the diet of honeybees is restricted to scarce floral resources in semi-natural habitats. At this time, honeybee colonies managed by professional beekeepers tend to harvest less pollen (Requier

et al. 2015). This 2-month spring period coincides with peak brood production; hence, the spring decline in pollen harvest was suspected to result in a food shortage for bees (Requier *et al.* 2015).

In this study, we gauged the decline in pollen harvest and then assessed the possible subsequent effects on colony life-history traits, including colony dynamics (brood size, adult population and honey reserves) and state of health (*Varroa destructor* ectoparasitic mite loads, and finally the seasonal and overwintering colony survival). We tested the following hypotheses:

1. The decline in pollen harvest in spring decreases the current brood size, with a carry-over effect that would lead to a reduction in the adult population after a delay of 1–2 months (a combination of the time taken to rear the larvae and the adult foraging phase, Winston 1994), followed by a subsequent reduction in honey reserves.

2. The decline in pollen harvest decreases the seasonal and overwintering colony survival as an ultimate cost of the carry-over effect. Given that *Varroa* mite load is related to colony dynamics (van der Zee *et al.* 2015), colonies affected by the decline in pollen harvest and associated carry-over effects are more susceptible to increased *Varroa* mite loads, known to weaken the bee colonies, i.e. is on the list of multiple drivers of colony losses (Goulson *et al.* 2015).

3. Differing life-history strategies early in the season (i.e. relative investment in honey reserves or brood production) will mediate the impact of declines in pollen harvest and associated carry-over effects.

4. The availability of floral resources within semi-natural habitats will buffer any declines in pollen harvest.

Materials and methods

STUDY AREA AND MONITORING DESIGN

The study was carried out in the Poitou-Charentes region in central western France (46°23′N, 0°41′W) as part of ECOBEE, a long-term ecological research programme setup to monitor honeybee colonies in a context of real professional beekeeping practices (Odoux *et al.* 2014). The land-use of each field (around 15 000 fields) in the study area (450 km²) was recorded twice a year and was highly heterogeneous in land-use type and level of intensification. This study was carried out over five consecutive years, from 2008 to 2012. The study area was divided into a 3-km grid (see Fig. S1, Supporting Information), i.e. twice the average foraging distance of honeybees for collecting pollen in habitats of this type (Steffan-Dewenter & Kuhn 2003). Each year, 10 of the 50 grid cells were selected at random without replacement, and an apiary was set up in each of these 10 grid cells. Each of the 50 grid cells was, therefore, sampled during 1 year of the 5-year study. The apiaries were located as close as possible to the centre of the grid cell. There were five managed colonies in each apiary. The monitoring design, therefore, included about 250 colonies × year, since surviving colonies from each year were not reallocated among the experimental sites.

INITIAL CONDITIONS OF COLONIES

All the colonies came from an *A. mellifera mellifera* × *caucasica* strain and were managed in accordance with local professional beekeeping practices, without migration, with honey collections, systematic supplemental feeding with syrup when necessary, and systematic treatment against *Varroa* mites (see Odoux *et al.* 2014 for more details). Their health was checked for any visible disease symptoms and queens were 1-year old at the beginning of 2008. Queen cells from our same own strain, i.e. *A. mellifera mellifera* × *caucasica*, were introduced for re-queening colonies. At the end of the beekeeping season, the hives were gathered together in a wintering apiary. After overwintering, colonies entered the monitoring programme again in April. The typical colony structure at the beginning of the monitoring (April) was composed of 18 936 ± 8382 adult individuals (mean ± SD) and 6·4 ± 2·9 kg of honey reserves. Hives were then randomly re-assigned to the new experimental plots with particular attention to minimize the among-apiary variation. New colonies intended to replace lost ones were equally allocated among plots. The beehives were standard 10-frame Dadant-Blatt hives made with microcrystalline-waxed wood.

MONITORING POLLEN HARVESTS

Pollen harvest (g colony^{-1}) was monitored using pollen traps set at the entrance of each colony for 24 h every 8–12 days. Given that a general slowdown in pollen harvest occurs during the 2-month period between rapeseed and sunflower mass flowering (see Requier *et al.* 2015 for more details), we focused on this spring period to gauge the extent of decline in pollen harvest (i.e. from the end of the rapeseed mass flowering, on average at day 132, to the onset of the sunflower mass flowering at day 177). This spring period lasted for about 40–50 days, and therefore covered four or five pollen sampling dates. The spring decline in pollen harvest was then estimated from the apiary level as the relative peak-to-trough decline in the daily pollen harvest (see Data analysis, Fig. 1e).

MONITORING HONEYBEE COLONY DYNAMICS

The colony dynamics were monitored by measuring three core colony traits: the amount of brood in the colony (termed brood size), adult population and honey reserves. Three of the five colonies in each apiary were monitored bimonthly throughout the monitoring period. Complete details may be found in Odoux *et al.* (2014). About 15 sets of measurements were taken each year (Table S1) giving 1636 series of colony traits measured one 257 different colonies × year (three colonies per apiary and the replacement of dead colonies). The brood size was based on the total area (cm^2) covered by brood cells (eggs, larvae and pupae), obtained by measuring the length and width of brood areas on each frame. The worker and drone brood sizes were assessed. The colony size (number of adult individuals) was assessed by weighing each brood frame, the hive brood chamber and the honey super chambers, with and without bees, and then calculating the difference in mass (±10 g). Honey reserves (kg) include the stored honey, nectar and pollen, although the weight of the pollen is negligible compared to the weight of the nectar and honey because pollen is only stored in very small quantities (Brodschneider & Crailsheim 2010).

The honey reserves on each frame were assessed based on the difference between the weight of the frame and the initial (empty) weight measured at the beginning of the season. If a frame also contained brood cells, the brood mass equivalent was subtracted. The space to store the honey reserves was split into two parts in the beehives following professional beekeeping practices, i.e. the hive brood chamber where honey reserves were kept available to bees all year round, and the honey super chambers where honey reserves were harvested by beekeepers (honey harvest). The weight of honey reserves was calculated separately for these two storage spaces. In accordance with local professional beekeeping practices, the honey was harvested twice a year, just after the rapeseed and the sunflower mass-flowering periods (at day 131 ± 8 and 214 ± 6, respectively, see Fig. S2 and Requier *et al.* 2015 for more details).

MONITORING THE STATE OF HEALTH OF THE COLONY

The *Varroa* mite load was assessed using a sticky board placed at the bottom of the hive brood chamber. Dead *Varroa* mites on the sticky board were counted every 8–12 days. The *Varroa* mite load was assessed for one colony per apiary (except in 2012). Given the high inter-annual variations typically observed in *Varroa* infestation levels, the *Varroa* mite load values in each colony were normalized to an annual mean of zero. In accordance with the local professional beekeeping practices, an anti-*Varroa* mite treatment, i.e. inside-hive evaporator of one active agent, was applied systematically to each colony every year, late in the season (late-August, see Fig. S3 for more details). Analyses were restricted to the natural *Varroa* mite dynamics, excluding the post-treatment period, and were staggered from May to August (305 measurements collected on 40 different colonies × year).

The criteria for the seasonal survival and the overwintering survival of the colony were whether the colony remained operational for beekeeping activity during the season or after winter arousal, respectively. This excluded colonies that collapsed or were depopulated, as well as weak or orphan colonies that would be unable to recover without human intervention (e.g. queen replacement, colony merging). The colony survival state was reassessed before winter, so that colonies that successfully recovered after human intervention during the season could be included *de novo* in overwintering survival statistics.

EARLY-WARNING SIGNS OF CRITICAL FOOD SHORTAGES

Managed beehives are composed of a main brood chamber that contains both brood and honey reserves and super chambers that only contain the honey to be harvested by beekeepers. Queen excluders prevent the queen from laying eggs in super chambers. Therefore, the amount of honey reserves actually stored by bees in the brood chamber is partly constrained by the space dedicated to brood. Colonies may allocate more space to honey reserves in the hive brood chamber by reducing the brood size (both workers and drones). To quantify the in-hive space allocation strategy, colonies were qualified as *reserve maximizers* or *brood maximizers* in comparison with the average pattern using the relationship between honey reserve and total brood area (see *data analysis*, n = 1636), and using the residuals as a proxy for the strategy. The sexual reproductive effort of the colony was also assessed using the relationship between worker (female) brood and drone
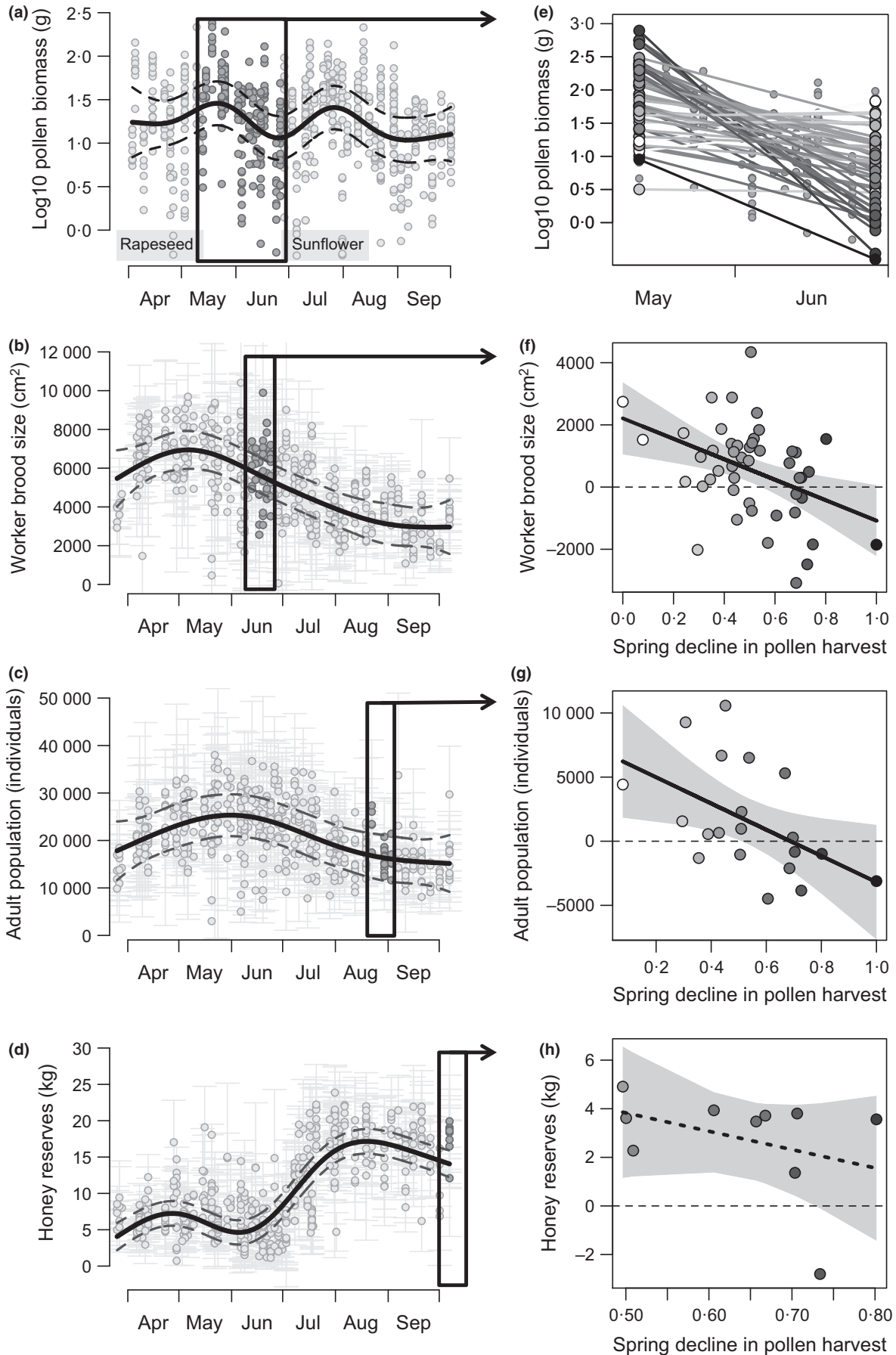
Fig. 1. Seasonal patterns of honeybee colony dynamics in intensive farmland habitats showing (a) pollen harvests (redrawn from Requier *et al.* 2015 from the same study area, $n = 780$), (b) worker brood size, (c) adult population and (d) honey reserves ($n = 1636$). Each dot represents a measurement of colony dynamics, thick lines show generalized additive mixed models (GAMM) predictions and dashed lines show the 95% CI. For clarity, measured values are shown as means ± SE per apiary. Dark grey dots within the time windows represent the subset of samples selected for evaluating the carry-over effect of the decline in pollen harvest. Light grey shading delineates the rapeseed and sunflower mass-flowering periods. (e) The decline in pollen harvest was estimated at apiary level ($n = 50$) as the relative peak-to-trough decline in daily pollen harvests between the rapeseed and sunflower blooming periods (in May–June). Smaller points show observed pollen harvests, while higher points show the Linear models (LM) predictions of peak-to-trough declines in pollen harvest. The predicted values subsequently were graduated from 0 (no decline in pollen harvests) to 1 (high decline in pollen harvests) and were also depicted by a colour gradient from white to black, respectively. The carry-over effects of pollen decline are shown for (f) worker brood size in June ($n = 48$), (g) adult population in August ($n = 20$) and (h) honey reserves in October ($n = 10$). The GAMM predictions are used for seasonal adjustment, and horizontal dashed lines indicate zero effect. Thick lines show the linear model predictions with shaded areas indicating the 95% CI. These lines are dashed if they are non-significant.

(male) brood as a surrogate of the brood sex-ratio allocation strategy (Page & Metcalf 1984). This determined whether colonies were *worker maximizers* or *drone maximizers* in comparison to the average pattern.

It was expected that the decline in pollen harvest would be buffered by the extent of attractive foraging habitats available around the colony. Given that no data were available on the abundance of weeds and their spatial distribution in our study area, the study focused on the two other main foraging resources used by bees during the food shortage period, i.e. permanent grasslands and forest (including hedgerows and forest edges, see Requier *et al.* 2015 for more details). These foraging habitats were summed in successive buffers every 500 m from 500 to 3000 m, i.e. twice the average foraging distance of honeybees for harvesting pollen in intensive farmland systems (Steffan-Dewenter & Kuhn 2003).

DATA ANALYSIS

All statistical analyses were performed using the R Project for Statistical Computing version 2.11.1 (R Development Core Team, 2009). To make our statistical methods easy to understand and to stimulate further studies in the field, we provide full R software codes and examples in Appendix S1.

Detrending the seasonal patterns

The typical seasonal dynamics of the three core colony traits were modelled as a function of the day of year (see Fig. S1 and Requier *et al.* 2015 for detailed methods of inter-annual adjustment), using generalized additive mixed models (GAMMs) and a Gaussian error structure (*gamm* function in the R mgcv package, Odoux *et al.* 2014). GAMMs are modelling techniques that allow temporal spline fitting while taking account of repeated measurements on statistical units in a nested design (colonies nested within apiaries and within years). The detrended data obtained by subtracting the best-fit line of the GAMM, rather than raw colony dynamics data, was used for subsequent carry-over analyses. This additional step was necessary to control for the variations arising from seasonal effects and isolate the effects of the decline in pollen harvest per se. Unless otherwise stated, colony dynamics data refer to detrended data.

Estimating the decline in pollen harvest

The decline in pollen harvest was quantified as the relative peak-to-trough decline in the log-transformed daily pollen harvests.

We focused on the spring period between rapeseed and sunflower mass flowering (i.e. from day 132, the end of the rapeseed mass flowering, to day 177, the start of the sunflower mass flowering; see Requier *et al.* 2015 for more details). This period covered four or five pollen sampling dates for each apiary. Linear models (LMs, *lm* function in the R stats package) were then used to estimate the overall decline in the pollen harvest for each apiary and to predict the expected pollen harvest at the peak date (day 132) and trough date (day 177). The relative peak-to-trough decline was rescaled to range from 0 (no decline in pollen harvest from the day 132–177) to 1 (high decline in pollen intake). The decline in pollen harvest was then compared to subsequent colony dynamics and state of health parameters, also at apiary level (colony traits averaged per apiary) so that we could compute all explanatory (environmental) and response (colony) variables in line at the same level, while controlling for the usually high inter-colony variations in a more conservative way.

Identifying carry-over effects

The carry-over effects of the decline in pollen harvest on subsequent colony dynamics were determined by testing correlations with ever-increasing time-lags. Colony dynamics data were binned into 2-week periods, starting from the beginning of the pollen decline period ($n = 10$–48 per period, Table S1). The statistical link between the decline in pollen harvest and subsequent colony dynamics (the three core colony traits and the *Varroa* mite load) was assessed for each period using LMs (see Table S1 for complete results). The seasonal and overwinter colony survival rates were calculated once a year and analysed using binomial generalized linear models (GLM) with a logit link function (*glm* function in the R nlme package).

Disentangling direct vs. indirect effects

As a series of carry-over effects of the spring decline in pollen harvest were detected across the whole season and possibly affected colony survival, it appeared critical to determine which variables were likely to be responsible for triggering the excess mortality. Binomial GLMs based on a subset of the core colony traits (and with the sample size of hives being kept constant throughout the season, $n = 20$) involved in the carry-over effects were used to compare their respective contributions to survival. All the possible combinations of one or more variables were evaluated. The candidate models were ranked according to the Akaike information criterion (AIC) to find the best compromise between fit and complexity (Tables S2 and S3).

Detecting early-warning signs in life-history strategies

To quantify the in-hive space allocation strategy, colonies were assessed as *reserve maximizers* or *brood maximizers* using the residuals of the smoothed spline relationship between honey reserves and total brood area [using generalized additive models (GAM, *gam* function in the R mgcv package) with a Gaussian error structure, because the relationships were usually non-monotonic in time]. Positive residual values indicate a *reserve maximizer* strategy, whereas negative residual values indicate a *brood maximizer* strategy. Similarly, the brood sex-ratio allocation strategy was assessed using the residuals of the smoothed relationship between worker (female) brood and drone (male) brood (using a GAM). Positive residual values indicate a *worker maximizer* strategy, and negative residual values indicate a *drone maximizer* strategy. The residuals were then averaged at apiary level for each sampling date. The sampling dates for the month before to the month after the pollen decline period were selected to determine whether poorly adapted life-history strategies (i.e. life-history strategies leading to reduced fitness) might have increased the decline in pollen harvest and associated carry-over effects (Table S1). The correlation between preferential allocation strategies (residuals) and the decline in pollen harvest was determined using an LM. LM regression was also used to test for a possible buffer effect of the abundance of attractive foraging habitats on the decline in pollen harvest. These models were computed for each landscape buffer distance (every 500 m, from 500 to 3000 m) and the best candidate models identified by AIC were selected (Table S4).

Results

SEASONAL VARIATIONS IN BEE COLONY DYNAMICS

Pollen harvests in intensive farmland systems were highly seasonal and nonlinear (temporal variations tested using GAMM, $F_{6,773} = 7.33$, $P < 0.001$), with a general slowdown in pollen harvest during the 2-month period between the mass flowering of rapeseed and sunflower (i.e. the pollen decline period, Fig. 1a). Each of the three core bee colony traits (i.e. brood size, adult population and honey reserves, monitored bimonthly) also showed seasonal patterns, with significant temporal variations (Fig. 1b–d). The worker brood size peaked early in spring (early May), at the end of rapeseed mass flowering and before the pollen decline period ($F_{4,1631} = 189.1$, $P < 0.001$; Fig. 1b). The worker brood size then decreased gradually over the season. The adult population peaked about 1 month after the peak in brood size, coinciding with the pollen decline period ($F_{4,1631}=77.22$, $P < 0.001$; Fig. 1c). Honey reserves (i.e. the honey in brood chamber only, available to bees all year round) showed a bimodal pattern ($F_{5,1630} = 238.0$, $P < 0.001$) with a first small peak during the rapeseed mass-flowering period and a larger peak during the sunflower mass-flowering period (Fig. 1d).

CARRY-OVER EFFECT OF THE DECLINE IN POLLEN HARVEST ON BEE COLONY DYNAMICS

As expected, the decline in pollen harvest varied between apiaries, as shown by the variable peak-to-trough decline in pollen harvest (Fig. 1e). No relationship was detected between the initial condition of colonies (i.e. worker brood size, adult population, honey reserves in the beginning of April) and the decline in pollen harvest (respectively, $F_{1,37} = 0.12$, $P = 0.74$; $F_{1,37} = 0.36$, $P = 0.55$; $F_{1,37} = 2.67$, $P = 0.11$), confirming that the decline in pollen harvest was not an artefact of our experimental design. At the end of the pollen decline period, when brood size decreased sharply (late June), brood size was negatively correlated with the decline in pollen harvest (LM, $F_{1,46} = 9.42$, $P < 0.01$; Fig. 1f), indicating that the pollen decline had a direct effect on bee reproductive effort, i.e. with no time-lag. Two months later, this reduction in reproductive effort, the adult population size was negatively correlated with the spring decline in pollen harvest ($F_{1,18} = 5.98$, $P = 0.03$; Fig. 1g) as a significant carry-over effect. We also found indications that the decline in pollen harvest negatively predicted the honey reserve stores 1 month after the adult population slowdown, although non-significant ($F_{1,8} = 1.27$, $P = 0.29$; Fig. 1h). This additional carry-over effect on honey reserves should be confirmed by future studies since fewer data were collected later in the season in our study ($n = 10$, Table S1).

THE DECLINE IN POLLEN HARVEST WEAKENS THE STATE OF HEALTH OF THE BEE COLONY

With a 4-month carry-over effect, the *Varroa* mite load in the beehive was positively correlated with the decline in pollen harvest (LM, $F_{1,13} = 9.85$, $P = 0.01$; Figs 2a and S3). No significant correlation was found in other periods (Table S1). As final carry-over effects, the seasonal and overwintering survival of the colony was negatively predicted by the decline in pollen harvest (binomial GLMs, $F_{1,48} = 7.23$, $P = 0.01$ and $F_{1,48} = 4.17$, $P = 0.04$, respectively; Fig. 2b,c). Colony survival was fairly high (90 ± 12% and 60 ± 27% colony seasonal and overwintering survival, respectively) when the levels of decline in pollen harvest were at their lowest, but both seasonal and overwintering colony survival were halved when there was a severe pollen decline.

Seasonal survival was best explained by the decline in pollen harvest (AIC) with 46% probability that the decline in pollen harvest was the most likely candidate, against 24·9–13·2% for all other candidates (Table S2) indicating that the pollen decline had a direct effect on colony seasonal survival. Overwintering survival was best explained by the adult population size in August (AIC probability of 51%, against 20·8–0·3% for all other candidates, Table S3), indicating that the pollen decline had an
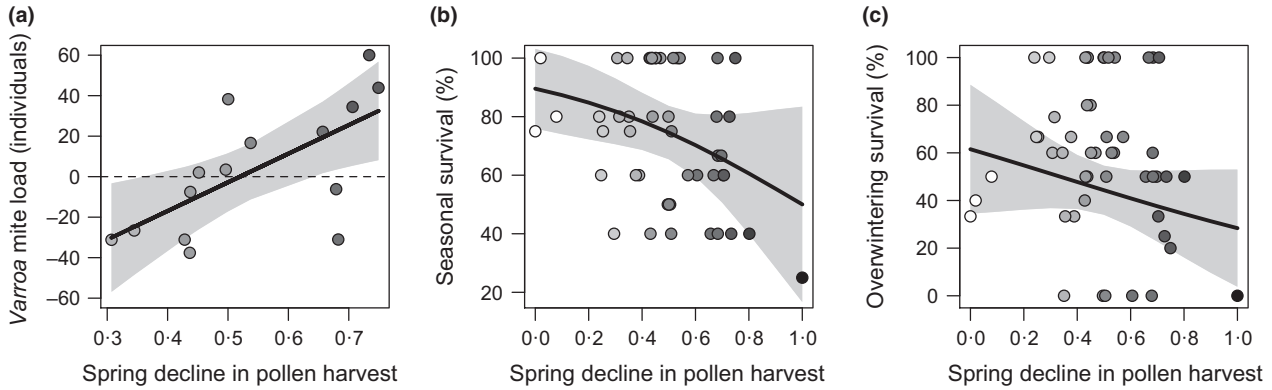
Fig. 2. State of health of the colony with (a) the *Varroa* mite load in September, (b) colony seasonal survival (March–November) and (c) overwintering survival (November to March of the next year). Each dot represents the measurement for one apiary (*n* = 15, *n* = 50 and *n* = 50, respectively) and colour gradient shows the decline in pollen harvest (see Fig. 1). The horizontal dashed line indicates zero effect on *Varroa* mite load, and the thick lines show the binomial generalized linear models (GLM) predictions with shaded areas indicating the 95% CI.

indirect effect on colony overwintering survival in intensive farmland systems.

EARLY-WARNING SIGNS OF CARRY-OVER EFFECTS

In honeybee colonies managed by professional beekeeping practices, there was evidence that brood size was not independent of honey reserves (GAM, $F_{3,1632}$ = 163, $P < 0.001$; Fig. 3a), indicating that some colonies may be viewed as *reserve maximizer* or *brood maximizer*. A colony may also allocate energy to the breeding of more workers or use a dispersal strategy that favours increased male (drone) production. We found evidence of non-independent variations in the brood sex-ratio allocation strategy
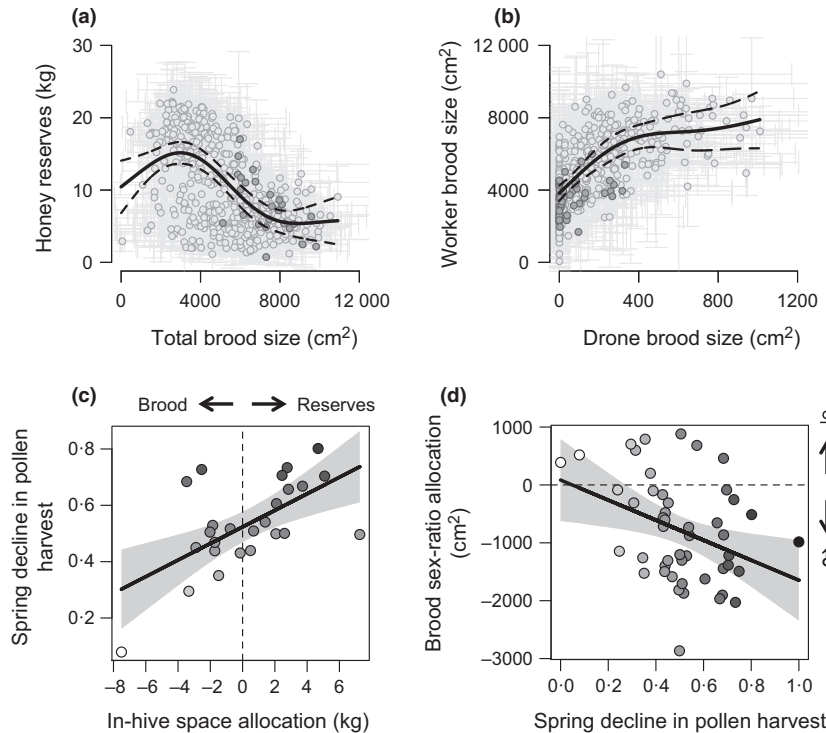
Fig. 3. The colony life-history strategies of managed honeybee differentiate between (a) *reserve maximizers* vs. *brood maximizers* in an in-hive space allocation strategy and (b) *worker maximizers* (female) vs. *drone maximizers* (male) in a brood sex-ratio allocation strategy. Each dot represents a measurement of colony dynamics (*n* = 1636), the thick lines show generalized additive models (GAM) predictions and the dashed lines show the 95% CI. For clarity, the measurements are shown as mean ± SE per apiary. Dark grey dots represent the subset of samples selected for determining early-warning signs of carry-over effects, 1 month before the spring pollen decline (two graphs on the left) and 1 month after (two graphs on the right). (c) The significant effect of the colony preferential in-hive space allocation strategy is shown with respect to the decline in pollen harvest (April, *n* = 25), and (d) the effect of the decline in pollen harvest on the colony preferential brood sex-ratio allocation strategy (July, *n* = 48). The colour gradient shows the decline in pollen harvest (Fig. 1). Vertical and horizontal dashed lines indicate zero effect, and thick lines show the linear model predictions with shaded areas indicating the 95% CI.

of colonies ($F_{3,1632} = 178·3$, $P < 0·001$; Fig. 3b), with a differentiation between *worker (female) maximizers* and *drone (male) maximizers*.

One month before the pollen decline period, the colony *reserve maximizer* strategy positively predicted the decline in pollen harvest, while the *brood maximizer* strategy mitigated it (LM, $F_{1,37} = 13·52$, $P < 0·01$; Fig. 3c). The colony *reserve maximizer* strategy was targeted by beekeepers during the period of rapeseed mass flowering for honey harvest (i.e. the honey harvested by beekeepers in honey super chambers). However, the rapeseed honey harvest (i.e. just before the pollen decline period) accentuated the decline in pollen harvest ($F_{1,48} = 4·7$, $P = 0·04$; Fig. S2). Nevertheless, the quantity of rapeseed honey harvest was not correlated with the reduced brood activity in June ($F_{1,48} = 0·85$, $P = 0·36$), as well as the winter colony survival (binomial GLMs, $F_{1,48} = 0·62$, $P = 0·43$). One month after the pollen decline period, however, there was no sign of any carry-over effect on sunflower honey harvest ($F_{1,48} = 0·14$, $P = 0·71$; Fig. S2). Nonetheless, the decline in pollen harvest negatively predicted the brood sex-ratio allocation strategy in the colonies (LM, $F_{1,46} = 7·02$, $P = 0·01$; Fig. 3d).

The extent of permanent grasslands around the apiary (within a 1,000 m radius) tended to reduce the decline in pollen harvest, although non-significant ($F_{1,48} = 3·36$, $P = 0·07$, Fig. S4 and Table S4), as did the extent of forest cover (including hedgerows and forest edges, $F_{1,48} = 2·67$, $P = 0·10$, Fig. S4 and Table S4). Those trends should be confirmed by future studies since the majority of apiaries were surrounding by a low range of grassland and forest abundances in our study (Fig. S4).

Discussion

As the brood size at a given time determines the subsequent size of adult population, there was, as expected, a strong carry-over effect of the spring pollen decline on the adult population (August). As workers may be allocated either to larval rearing (in-hive task) or foraging (Winston 1994), colonies that suffered a severe decline in pollen harvest had lower honey reserves later in September, because of the decrease in the adult forager population. The combination of these two factors (smaller adult population and lower honey reserves) halved the state of health of the colony (Fig. 4). The spring pollen decline, thus, directly triggered seasonal colony losses and indirectly triggered winter colony losses. The size of the adult population in August was the best indicator of the risk of winter colony loss, whereas this colony trait was itself constrained by spring pollen decline. Consequently, the spring decline in pollen harvest should be considered a pollen shortage and as one of the drivers of honeybee colony losses in intensive cereal farmland systems.

Our study confirms that resource acquisition and allocation strategy are critical components driving the life history of colonies; hence, carry-over effects need greater research attention to facilitate our understanding of honeybee declines. While theoretical bee colony models can predict such carry-over effects (Horn *et al.* 2015), field assessments are very challenging and resulted in several limitations. As our study was conducted at the apiary scale, we averaged colony traits as a simple, conservative, approach to control for inter-individual variability. This means that we may have reduced the ability of our analyses to detect some secondary, more subtle, links in the causal chain leading to reduction of colony survival. To facilitate more detailed investigations in these fields (e.g. inter-individual variations), we have provided full R software codes and examples in Appendix S1 to assess such carry-over effects on honeybee colony dynamics.

While our results showed that the *Varroa* mite load on colonies at the end of the season (September) was greater in areas with a severe spring pollen shortage (Fig. 4), it was not clear whether *Varroa* mite pressure resulted directly from pollen shortage in spring or rather from the smaller adult population later in August, or as an artefact of the correlation between pollen shortage and brood size. This question, thus, requires greater attention with more robust data sets (only 40 colonies with consistent *Varroa* estimates here). Nevertheless, this empirical result confirms that *Varroa* mites play a leading role in causing the death of honeybee colonies in winter (van der Zee *et al.* 2015). Pollen shortage may also encourage additional opportunistic parasites and pathogens (Alaux *et al.* 2010) and increase the sensitivity of bees to pesticides (di Prisco *et al.* 2013). These stressors may be especially important in environments where pollen quality is also low (e.g. Alaux *et al.* 2010).

This study also suggests that certain current beekeeping practices in intensive farmland systems should be reviewed. While beekeepers promoted the *reserve maximizer* colony strategy (i.e. preferential allocation of workers taskforce to honey storage instead of brood production) to improve honey production, the setup of such practices just before the pollen shortage period accentuated the severity of pollen shortage (Fig. 4). Therefore, by maximizing honey production in spring, beekeeping management triggered detrimental carry-over effects after pollen shortage periods. Incidentally, these colonies produced less honey during the sunflower blooming period, and their overwintering survival probability was halved. These results, therefore, cast doubt on the efficacy of current beekeeping practices in such intensive farming systems. Furthermore, current beekeeping practices include artificial supplementary feeding (i.e. feeding bees with sugar syrup in May and June). This practice may not compensate for a deficiency in brood production and hence spring (after rapeseed flowering) may not be a suitable time to harvest honey. Avoiding honey harvesting in spring may in fact improve honeybee survival and increase honey production during the sunflower mass-flowering period.

Fig. 4. Summarized conceptual framework of the carry-over effect of spring pollen shortage on winter colony survival in intensive farmlands (note that the overall cascading links from pollen shortage in spring, to bee colony dynamics, health and survival were theoretically supported by Horn *et al.* 2015). Beekeeping recommendation may be derived from this framework to promote friendly bee-management and monitor early-warning signals of colony failure. Future studies should pay particular attention to carry-over and indirect effects in order to better understand the mechanism of bee colony failure and the respective contributions of the multiple suspected stressors.
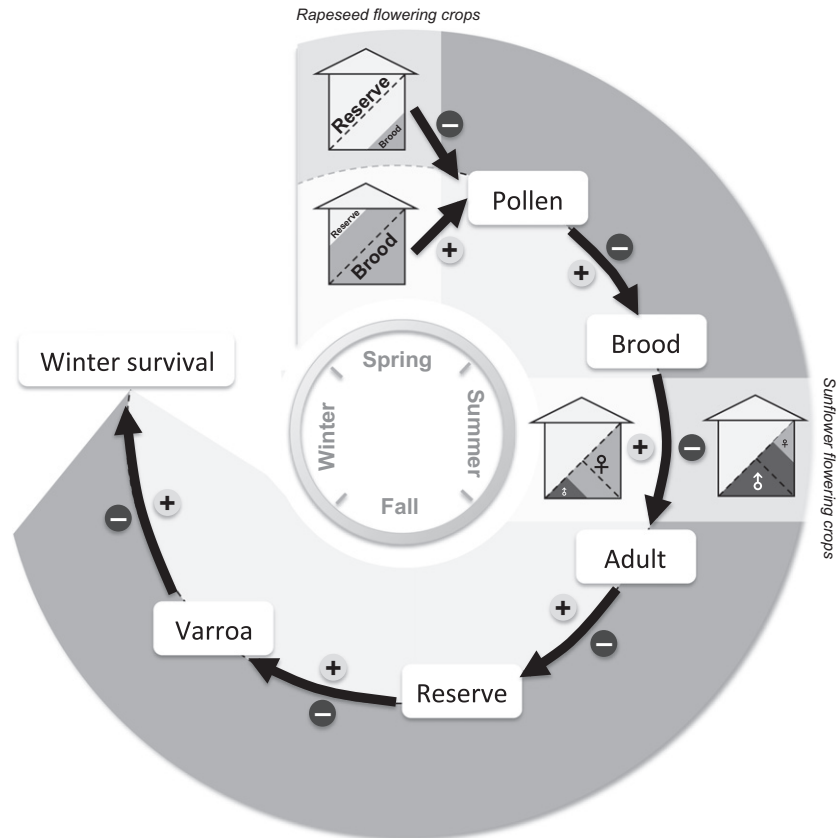
Honeybees are well adapted to their environment, allowing for future adverse or favourable environmental conditions (Perry & Barron 2013). Although female workers were allocated to brood production in the spring, brood production was diverted to drones in July in colonies that had suffered severe pollen shortage (Fig. 4). Given that drones ensure the genetic dispersal in the colony, a survival mechanism probably adopted by colonies in response to pollen shortage, the brood allocation to drones in July should be considered an early-warning sign of colony failure. Monitoring such colony traits could facilitate beekeepers to track of detrimental carry-over effects and anticipate the risk of colony failure.

Many studies have shown the importance of the surrounding landscape composition on honeybee life history (e.g. Requier *et al.* 2015; Sponsler & Johnson 2015). The expected buffering effect of pollen shortage by nearby semi-natural habitats could not be formally established here, possibly due to the limited amounts of grasslands and forests actually covered by our study (Fig. S4). Nonetheless, weeds account for a substantial part of the honeybee diet in this environment (Requier *et al.* 2015). Species such as the poppy *Papaver rhoeas* can provide up to 60% of pollen brought back to the hive in the pollen shortage period (Requier *et al.* 2015). Restoring semi-natural habitats and improving weed richness by reducing the application of herbicides are both part of Agri-Environmental Schemes (AES). Such strategies are also consistent with the conservation of non-*Apis* bees (solitary bees and bumblebees, Bretagnolle & Gaba 2015), since the effectiveness of AES for solitary bees and bumblebees has been already demonstrated (e.g. Carvell *et al.* 2007).

Authors' contributions

F.R. and V.B. conceived the ideas and designed methodology; F.R. and J.-F.O. collected the data; F.R., M.H. and V.B. analysed the data; all authors led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data on bee colony dynamics and health used in this study are available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.70j22 (Requier *et al.* 2016). The R-scripts are available in Appendix S1 – R codes to compute carry-over effects in bee colony dynamics.

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Computing carry-over effects in bee colony dynamics with R.

Fig. S1. The study area, the LTER 'Zone Atelier Plaine et Val de Sèvre', France, set up a honey bee colony monitoring program in intensive farmland habitats in 2008 (ECOBEE).

Fig. S2. The honey harvested by beekeepers during the mass-flowering period of rapeseed (just before pollen shortage period at day 131 ± 8) affects the decline in pollen harvest ($F_{1,48} = 47$, $P = 004$, $n = 50$).

Fig. S3. Seasonal pattern of the natural *Varroa* mite load in honey bee colonies in intensive farmland habitats (France).

Fig. S4. The availability of attractive foraging habitats around the apiary (radius of 1000 m) may mitigate the decline in pollen harvest.

Table S1. Carry-over effects of the spring decline in pollen harvest on subsequent (i) colony dynamics, (ii) state of health and (iii) early warning signs.

Table S2. Model selection by AIC to disentangling the direct vs. indirect (through the worker brood size in June) effects of the decline in pollen harvest on colony seasonal survival.

Table S3. Model selection by AIC to disentangling the direct vs. indirect (through the worker brood size in June, and adult population in August) effects of the decline in pollen harvest on colony overwintering survival.

Table S4. Model selection by AIC for the effect of the extent of attractive foraging habitats on the decline in pollen harvest.