Agricultural landscape composition affects the development and life expectancy of colonies of *Bombus impatiens*

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Abstract. Bumble bees are essential, efficient pollinators of numerous crops, and scientists are increasingly concerned about their global decline. Although several potential causes have been put forward, the concurrent modification of landscape structure and pesticide use by agriculture are often pointed out as the main drivers of the decline. Yet, the mechanisms through which these factors cause species to decline remain elusive. Most field studies have been conducted at the individual level, and the few studies that addressed higher levels of organization only covered narrow ecological conditions. We undertook a field experiment in spring 2016 to overcome these limitations by placing four commercial *Bombus impatiens* colonies in each of 20 sites located along a gradient of agricultural intensity in southern Québec, Canada. We hypothesized that landscape habitat composition and configuration, as well as local floral resources, would affect colony development. We expected colonies surrounded by low-intensity and/or flowering crops in heterogeneous landscapes to develop better and live longer, partly linked to more abundant and diversified food resources, than colonies embedded in landscapes dominated by high-intensity crops. Colonies were weighed once a week, and workers were captured to assess pollen load diversity. Final colony weight, queen production, and presence of depredators (*Aphomia sociella*, Lepidoptera: Pyralidae) were also recorded. Landscape habitat composition and configuration were characterized within 1 km of colonies. Local availability of floral resources was assessed within 100 m of colonies every two weeks. As predicted, colony weight and longevity decreased with the proportion of intensive crops and increased with the amount of flowering crops, but queen production and the occurrence of *A. sociella* were not affected by landscape composition. Contrary to our prediction, local availability of floral resources also did not affect colonies. The pollen richness brought back to colonies decreased with the proportion of flowering crops and varied according to the proportion of intensive crops interacting with local floral resources. Our work contributes to generalize the more restrictive conclusion of local-scale, crop-specific studies that intensive crop farming undermines bumble bee colony development and that it may thus play a role in the large-scale population decline of these insects.

Key words: *Aphomia sociella*; Bumble bees; colonies; corn crops; land cover; landscape; margins; neonicotinoids; pollen load; queen.

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INTRODUCTION

Pollination by native pollinators, such as bumble bees and honey bees, is an essential ecosystem service for human societies. In fact, insect pollination represented $175 billion USD worldwide in 2005 (Gallai et al. 2009). Among 87 crops grown around the world, Klein et al. (2007) found that only 28 did not rely on animal pollination. In most countries, honey bees are the prime pollinators of commercial crops. However, bumble bees are increasingly appreciated for their efficiency due to a combination of hard work and an ability to use sonication, that is, the act of extracting pollen by using wing muscles to induce vibration of the flower's anthers (De Luca and Vallejo-Marín 2013). Sonication is particularly efficient for extracting pollen from poricidal anthers found in many crop genera, such as *Cistus*, *Papaver*, *Pedicularis*, *Solanum*, and *Vaccinium* (De Luca and Vallejo-Marín 2013). Furthermore, the generalist diet of bumble bees makes them great pollinators for numerous crops. Indeed, bumble bees may benefit from a diverse pollen diet to thrive as pollen nutritional content varies greatly between plant species (de Groot 1952, Thorp 2000, Tasei and Aupinel 2008). The above characteristics have led bumble bees to be used commercially for pollination purposes since 1987, and their popularity is rising (Velthuis and van Doorn 2006).

Recent studies have however shown that native bumble bee populations are in critical decline worldwide. In the UK, three of the 25 reported species are extinct and eight are experiencing major range decline (Goulson 2015). In Central and Western Europe, similar patterns can be observed. For instance, 13 species became extinct in 11 countries between 1950 and 2000 (Bommarco et al. 2012). Although long-term data are sparse for North America, a similar decline has been observed in many species, including *B. occidentalis*, *B. affinis*, *B. franklini*, *B. terricola*, *B. sonorus*, and *B. pensylvanicus* (Jacobson et al. 2018, Richardson et al. 2018, Strange and Tripodi 2019). A combination of several factors may be responsible, yet to differing degrees, for the observed declines (Potts et al. 2010, Goulson et al. 2018). These comprise climate change (Kerr et al. 2015), diseases (Cameron et al. 2011), pesticide use, and alteration of landscape structure (i.e., habitat composition and configuration), including loss of natural habitats, as well as reduced availabilities of floral resources and nesting sites (Goulson 2015). For instance, recent evidence shows that neonicotinoid pesticides (hereafter referred to as neonics) alter the development (growth, queen production, worker foraging, immunity, and defense) of bumble bee colonies, both in the laboratory and in the field (Gill et al. 2012, Whitehorn et al. 2012, Goulson 2015, Moffat et al. 2015, Sanchez-Bayo et al. 2016, Ellis et al. 2017, Stanley and Raine 2017, Kenna et al. 2019). For example, colonies exposed to various doses of imidacloprid gained up to 12% less weight than control colonies (Whitehorn et al. 2012). Similar conclusions were obtained in the context of raspberry (*Rubus idaeus*) crops treated with thiacloprid, as colonies exposed to the pesticide weighed significantly less than non-treated colonies (Ellis et al. 2017). These studies also found that reproduction was significantly impaired, with a drastic drop in the number of queens produced when colonies were placed on treated sites (Whitehorn et al. 2012, Goulson 2015, Ellis et al. 2017). Neonics have also even been shown to impair crop pollination services provided by bumble bees. In fact, bumble bees exposed to neonics in apple (*Malus* sp.) orchards visited apple flowers at a lower rate and collected less pollen (Stanley and Raine 2017). Furthermore, neonics, when combined with other stressors, are known to reduce immunity in honey bees, and probably in bumble bees as well (Sanchez-Bayo et al. 2016).

Landscape habitat composition and configuration likely play an important role in colony growth and reproduction as they can be linked, for instance, to the availability and diversity of food resources as well as to the level of pesticide exposure. Weight and queen production of bumble bee colonies have however been found to be unrelated to the proportion of natural habitats in the landscape (Williams et al. 2012), or to only be influenced by it in ways that depended upon local floral resources (Spiesman et al. 2017). Moreover, previous studies found that in the presence of floriferous crops, bumble bee colonies gain more weight, produce more workers early in the season, and gather pollen from a greater number of plant species, but failed to detect an improved queen production (Westphal

et al. 2009, Jha et al. 2013). As for landscape configuration, bumble bee colonies were shown to produce larger foragers in landscapes composed of numerous small fields than in simpler landscapes that comprised only a few large fields (Persson and Smith 2011). Furthermore, while simpler landscapes were found to harbor fewer long-tongued bumble bees (generally considered flower specialists) than heterogeneous ones (Persson et al. 2015), weight gain and queen production of colonies of *B. impatiens*, a small-tongued species, were not affected by habitat fragmentation (Herrmann et al. 2017). These results suggest that vulnerability to landscape simplification, such as the one occurring through agricultural intensification (Benton et al. 2003), may depend upon species-specific characteristics linked to their morphology and life history.

At a local scale, most studies have shown that bumble bees thrive when floral resources are highly available. For instance, floral resource abundance has been found to enhance colony growth by affecting positively both the number of workers and males, but had no influence on queen production (Williams et al. 2012). Furthermore, floral abundance and shrub diversity had a positive impact on colony growth rate and fecundity (Adhikari et al. 2019). That said, floral dominance ($1 - J$, where J is Pielou's evenness metric), rather than overall abundance or species richness of flower resources, has stood out as the local factor (i.e., within a ~100 m radius) that best explains colony growth and queen production in agroforested landscapes (Spiesman et al. 2017). Yet, the influence of floral dominance on colony performance has been shown to depend on the amount of natural habitats (i.e., grasslands and forest) within a 2 km radius; while performance increased with floral dominance at high amounts of natural habitats, it decreased at low amounts (Spiesman et al. 2017). These results are further complicated by the fact that the composition in natural habitats changed with their overall cover, which was assumed to be negatively correlated with large-scale floral resource availability (see Spiesman et al. 2017). Hence, local floral dominance could mitigate the negative impact of low floral resource availability at the landscape scale, likely through improved foraging efficiency, providing that the dominant local floral resource is of high enough quality. Furthermore, colonies

with greater access to nectar resources were shown to defend themselves better against predators and kleptoparasites than colonies deprived from nectar resources (Cartar and Dill 1991). Pollen diet is also known to impact on the initiation success of colonies, where colonies with only some pollen type (like *Cistus* sp.) had dramatically reduced numbers of later-stage offspring in their nest (Watrous et al. 2019). Lastly, it has been observed that colonies collect pollen from a wider array of plant species, and may thereby benefit from a more constant and diversified diet, when plant species richness is higher locally (Jha et al. 2013, Vaudo et al. 2018).

Despite the contribution of numerous recent studies that have investigated the potential causes of decline in bumble bee populations, there are still significant knowledge gaps to fill. In particular, most studies involving neonics have been performed in the laboratory (Cresswell et al. 2012, Laycock and Cresswell 2013) and those conducted in the field have only examined potential exposure to these pesticides over a small window of ecological conditions (Whitehorn et al. 2012, Goulson 2015, Rundlöf et al. 2015). While these studies have focused mostly on sub-individual and individual effects of pesticides, the consequences of pesticide exposure on higher levels of organization such as colonies and populations have been less documented, as pointed out by Godfray et al. (2014) and Lundin et al. (2015), but see Stanley and Raine (2017) as well as Wu-Smart and Spivak 2018) for examples of impacts on higher levels of organization. Moreover, landscape habitat composition and configuration, together with local floral resources, could lessen or amplify the effect of acute threats, such as neonics, to bumble bees (Henry et al. 2014, Park et al. 2015). In this study, we aimed to quantify experimentally the effects of landscape habitat composition and configuration, including local floral resource availability, on bumble bee colony development. For this, we placed a cluster of four commercial bumble bee colonies in each of 20 sites located along a gradient of agricultural intensity in southern Québec, Canada. Key to our design was the large variation in the amount of row crops, namely maize and soybeans, treated with neonics and herbicides along this gradient of intensity. We hypothesized that an increasing proportion of intensive

crops in the landscape would impair colony development by reducing weight gain, longevity, queen production, and the diversity of pollen collected by workers, as well as by increasing susceptibility to depredation by nest parasites. We also hypothesized that a landscape composed of a higher cover of flowering crops would dampen or even override the magnitude of these negative effects. Lastly, we expected that increased local diversity of floral resources would be beneficial for colony development.

METHODOLOGY

Model species

The Common Eastern Bumble bee (*Bombus impatiens* Cresson) is the only native species in eastern Canada that is currently commercialized. In nature, the entire life cycle of a colony lasts one year, starting with the fertilized hibernating queen in spring, and ending with the new male and queen offspring in autumn. The hibernating queen exits its hibernaculum in spring to find a suitable place to nest, often in an abandoned mammal burrow. The queen gathers resources from flowers, such as pollen and nectar, and starts laying eggs. After the first cohort of workers has been reared, the latter take charge of food gathering and brood rearing while the queen continues to lay eggs. At the end of the colony's life, the queen starts rearing males and future queens. These newborn queens will be fertilized and will then hibernate before starting their own hive in the next spring (Goulson 2010). Upon delivery, commercial hives contain enough workers (~150) to ensure efficient pollination services when crops, such as cranberries, apples, and blueberries, bloom (Velthuis and van Doorn 2006). Each colony also has an initial nectar supply to ensure its survival in the first few days after its field deployment, especially if the environment does not provide enough food.

Study area

The study was conducted in southern Québec, which is characterized by an east–west gradient of agricultural intensification. Its eastern part is mostly composed of small-scale, dairy farms (hayfields and pastures), and its western part increasingly hosts large-scale, homogeneous cropping farms that concentrate on maize,

soybean, wheat, and other cereals (Jobin et al. 2005). Forest cover follows a similar gradient, with large expanses of continuous forest separated by roads and punctured by fields in the east, and increasingly numerous, small, and isolated forest patches toward the west (Bélanger and Grenier 2002). Between 2011 and 2018, a large proportion of maize, soybean, and other cereals grown in southern Québec stemmed from seeds coated with neonics resulting in a general, and sometimes severe contamination of surface waters by these pesticides (along with numerous others, especially herbicides, such as glyphosate and atrazine) in the western part of the study area (Giroux 2019, Montiel-León et al. 2019). A total of 20 bumble bee quads (QUAD POLLINATION, Koppert Canada), each hosting 4 colonies, were installed at the field margin of 20 different farms (1 quad/farm) distributed along the above gradients on 3 May 2016 (Fig. 1). Before installation, all quads were opened to ensure that they had sufficient nectar reserve and to certify the absence of *Aphomia sociella*. Two agroenvironmental clubs helped identify 40 local growers willing to participate in the study and whose farms reflected the range of agricultural landscape habitat compositions (i.e., relative amounts of extensive, flowering, and intensive crops within a 1 km radius) found in the study area. Farms were then randomly selected with the constraint that they had to be separated from one another by a distance of at least 5 km (nearest-neighbor distance: 69 ± 37 km, mean ± SD).

Quad monitoring

Quads were monitored once a week (during the day and at night) until all of the colonies they contained were dead (max. 14 weeks). Each quad was weighed (±0.01 kg) using a handheld scale (Jennings/JScale UltraSport V2-30; J-SPORT^{TM} 30kg, JJSCALE, Bath, UK). Weighing occurred at night to ensure that most workers had returned to the hives and to limit disturbance. We attempted to capture one worker per colony per week (from week 4 to week 10) during daily visits for a maximum of eight workers per colony. Sampled workers were stored for ~5 weeks at −20°C until further processing. A hive was deemed dead when no entry or exit was observed during a 15-min observation period. Dead colonies were left in the field until all
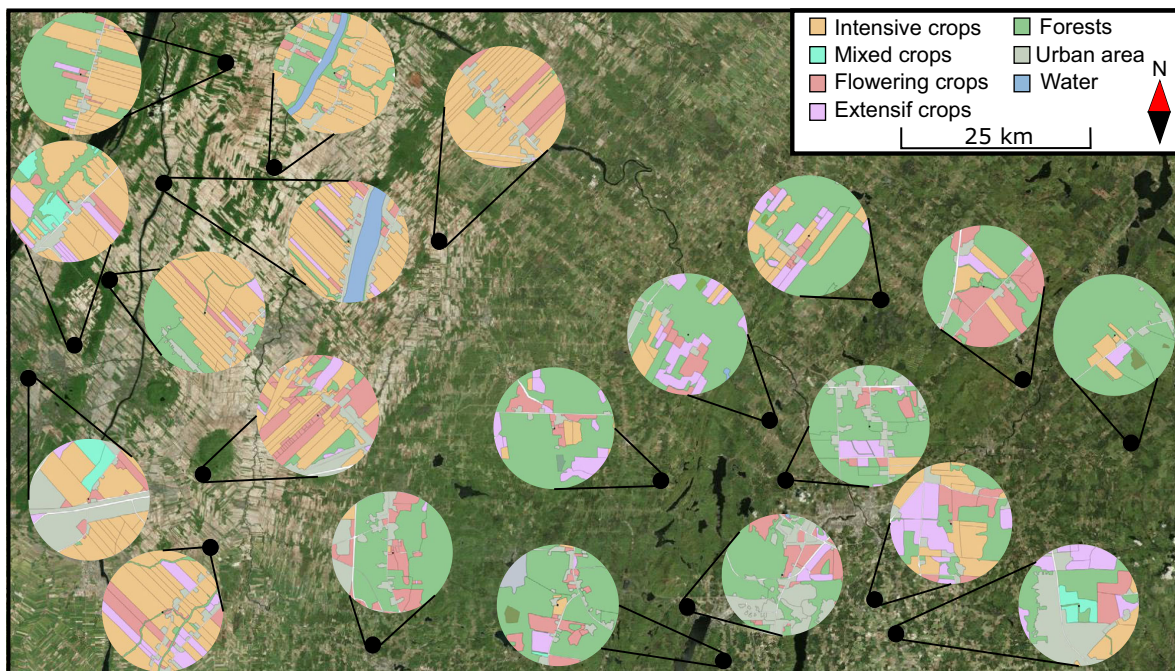
Fig. 1. Habitat composition within 500 m of the 20 clusters (quads) of experimental *Bombus impatiens* colonies monitored in 2016 in southern Québec, Canada.

four colonies of a given quad were dead. Dead quads were then stored at −4°C for ~2 weeks before their necropsy. Each nest was weighed (±0.01 g) individually (METTLER TOLEDO; model ML 1502E, Mississauga, Canada). Queen cells were counted, and the presence of depredators (e.g., *Aphomia sociella*) was noted.

Worker pollen load

The pollen load carried by sampled workers was removed into a 50-mL Falcon tube using a needle and repetitive 70% ethanol rinses (max 40 mL) under a microscope (40×). Tubes were then centrifuged at 3,000 rpm for 12 min to isolate the pollen. The supernatant was removed, and the bottom (containing pollen and alcohol) was left to dry for three to five days. We then added 0.5 or 0.1 mL of Tween solution (0.5%) to the tube, depending on whether we could detect the pollen load by eye (0.5 mL) or not (0.1 mL). One slide per sample was then mounted using 20 µL of the pollen–Tween solution. Fuchsin-stained glycerin gelatin (Louveaux et al. 1970) was used to color the slide and facilitate examination of pollen features. Lastly, a haphazard sample of 150 pollen grains per slide were identified to the lowest

taxonomic level possible with a microscope (1000×) by a melissopalynology expert, namely Mélissa Girard (Girard et al. 2012, Girard 2014).

Landscape characterization and local floral resources

We determined landscape structure within a 1 km radius around each bumble bee quad by characterizing all polygons (e.g., fields, roads, forests) we could delineate on orthophotographs (1:15,000) during a field visit in August. Data were then compiled in QGIS (QGIS Development Team 2017). Landscape habitat composition was determined based on the proportion of land cover of the following habitat classes: forest, water, urban area, intensive crops, extensive crops, and melliferous flower crops. Habitats were grouped according to their physical or vegetational similarity, as well as to their typical levels of food/nesting resource availability and pesticide inputs (Table 1). Note that extensive and flowering crops were not mutually exclusive classes but were never included in the same statistical model. We characterized landscape configuration based on total edge length. Total edge length was determined independently of habitat

Table 1. Habitat composition within 500 m of the 20 clusters (quads) of experimental *Bombus impatiens* colonies.

Categories	Description	Land cover range (proportion)
Forest	Forests, hedgerows, riparian borders, plantations	0.00–0.65
Water	Lakes, rivers, streams	0.00–0.30
Urban area	Houses, parking lots, buildings, roads	0.01–0.35
Intensive (INT)	Maize, soybean, apple, wheat, oat	0.00–0.76
Extensive (EXT)	Garlic, hay, alfalfa, clover, buckwheat, strawberries, blueberries, sunflowers, gardens, pastures, organic vines, melon, cantaloupe, uncultivated fields	0.04–0.48
Flowering (FLO)	Garlic, alfalfa, clover, buckwheat, strawberries, blueberries, sunflowers, gardens, organic vines, melon, cantaloupe, uncultivated field	0.02–0.28

classes because the ecotones between all of the classes we considered typically harbor strips of flower-rich fallow habitats used by bumble bees for both nesting and foraging (Goulson 2010). Lastly, we recorded on each visit all the plant species in bloom within 100 m from the quad by walking thoroughly over the entire area. This allowed us to estimate the weekly species richness of flower-bearing plants available to bumble bees.

Statistical analyses

Three quads (12 colonies) had to be removed from all analyses since they were intensely pilfered by honey bees during the experiment. Using the 17 remaining quads, we first determined the spatial scale (500 vs. 1000 m) at which response variables were most influenced by landscape structure variables. Based on the Akaike information criterion (AIC; Burnham and Anderson 2002), all models performed similarly or better (averaged $\Delta AIC_c = 0.18$; Appendix S1: Table S1) when landscape structure was characterized within 500 m of colonies; the 1000-m scale was thus disregarded in all subsequent analyses. We unfortunately had to exclude total edge length from all analyses because it often prevented model convergence when included in models that already contained the proportion of intensive crops. The fact that

total edge length was collinear with the proportion of intensive crops in our experimental sites ($r = 0.64$ at 500 m) implies that some of the putative effects of intensive crops may be attributable to the amount ecotones in the landscape. For all models, except the maximum weight models, local floral richness represented the number of species found at a site within the first 10 weeks of the experiment (all quads were then still alive). For the maximum weight models, local floral richness represented the number of species found at a site within the first six weeks of the experiment (most of the quads reached their maximum weight on week 6).

Due to our hierarchical design (repeated measures on quads or measures on colonies nested within quads), we modeled certain response variables using mixed models where quad ID was included as a random factor. For each response variable, several models were contrasted based on AIC or its second-order value (AIC_c; Burnham and Anderson 2002). Except for generalized additive mixed models (GAMMs; Wood 2017), we report model-averaged effect sizes (Appendix S1: Table S2) and predictions, along with their unconditional confidence intervals, based on Akaike weights (w_i) following Burnham and Anderson (2002). Model selection and multimodel inferences were performed with the AICcmodavg package (v. 2.2-2; Mazerolle 2019) in R (v. 3.5.1; R Core Team 2018). Explanatory variables were standardized (zero mean and unit variance) for all analyses except GAMMs.

We addressed the development of colonies through quad weight in two ways. We first modeled the growth curves of quads with GAMMs and then modeled the maximum weight reached by quads with linear models. We only considered weight measures of the first 12 weeks of the experiments as many colonies died or were deemed heavily colonized by *Aphomia sociella* past this time point (see *Results*). Specifically, growth curves were fitted with GAMMs which included a single common tensor product smoother for the effect of week number and habitat amount on weight, plus group-level smoothers of the same wiggliness for within-quad week by habitat amount effects (i.e., Model 2 in Pedersen et al. 2019). Two different GAMMs assuming a normal error distribution and an identity link function were fitted by maximum likelihood.

While the effect of week was modulated by the proportion of intensive crops in the first one, it was modulated by the proportion of flowering crops in the second. These two models allowed us to partly contrast the effect of crops heavily treated with pesticides from that of crops offering foraging opportunities (intensive vs. flowering crops: $r = 0.25$). We could not fit more complex models due to the limited number of quads. Both GAMMs were fitted with the mgcv package (v. 1.8-23; Wood 2017) in R. The fit and appropriateness of the model parameters (e.g., basis dimensions) were verified with the gam.-check function (Wood 2011, 2017). Regarding the maximum weight reached by quads, three linear regressions (1, 4, and 5; Table 2) were compared, as for all the following models, based on second-order AIC (AIC$_c$; Burnham and Anderson 2002). While model 4 assessed the influence of landscape habitat composition on maximum weight through the proportion of intensive and flowering crops, model 5 determined if flower species richness contributed to the weight gain of colonies once landscape composition was taken into account (Table 2). Flower species richness was here defined as the mean richness across weeks 0–6 as 14 out of the 17 quads had reached their maximum weight by week 6. Again, the number of quads limited the complexity of the models we could fit.

We assessed the influence of landscape habitat composition and local floral resources on colony survival using mixed Cox regression models (Therneau and Grambsch 2000) fitted with the

coxme package in R (Therneau 2015). In addition to the three models for maximum weight (1, 4, and 5), we considered two additional models that included a two-way interaction between habitat variables (6) as well as two-way interactions between habitat and floral resource variables (7; Table 2). We considered the same five models (1 and 4–7; Table 2) when modeling the number of queens produced by a given colony using generalized linear mixed models (GLMMs) assuming a negative binomial error structure and log link function. These GLMMs were fitted with the glmmTMB package (v. 0.2.3; Brooks et al. 2017) in R.

We also used GLMMs to model the nest weight of colonies (once dead) and their likelihood of being depredated by *Aphomia sociella*. While GLMMs assumed a normal error distribution and used an identity link function for nest weight, the GLMMs for depredation probability assumed a binomial error distribution and used a logit link function. Candidate models for nest weight were the same as for nest survival and the number of queens but also considered whether the colony had suffered from depredation by *A. sociella* as this can lead to weight loss (3; Table 3). Regarding depredation probability, candidate models were the same as for maximum quad weight except that (1) the proportion of flowering crops was replaced by that of extensive crops to reflect the generalist habitat use of *A. sociella*, and (2) they also included the maximum weight reached by the focal colony's quad because larger colonies may be more attractive to *A. sociella* (1, 2, 8, and 9;

Table 2. Candidate models considered in model selection and multimodel inference procedures based on the second-order Akaike information criterion (AIC$_c$) for response variables related to colony growth and fitness.

Model	Model sets	Max quad weight			Colony survival			Queen production			Nest weight		
		K	ΔAIC_c	w_i	K	ΔAIC_c	w_i	K	ΔAIC_c	w_i	K	ΔAIC_c	w_i
1	~null (intercept only)	2	4.99	0.06	1	218.50	0.00	**3**	**0**	**0.74**	...	...	...
2	~quad mass	...	...	...	...	...	...	...	...	...	...	...	...
3	~depredation	...	...	...	...	...	...	...	...	...	4	6.31	0.02
4	~INT + FLO	**4**	**0**	**0.75**	3	56.12	0.00	5	3.29	0.14	6	2.51	0.16
5	~INT + FLO + LOC	5	2.70	0.19	4	12.75	0.00	6	4.99	0.06	**7**	**0**	**0.58**
6	~INT + FLO + INT:FLO	...	...	...	**5**	**0**	**1.00**	7	6.11	0.03	8	2.23	0.19
7	~INT + FLO + LOC + INT: FLO + INT:LOC + FLO:LOC	...	...	...	7	15.00	0.00	9	7.17	0.02	10	5.07	0.05
8	~INT + EXT	...	...	...	...	...	...	...	...	...	...	...	...
9	~INT + EXT + INT:EXT	...	...	...	...	...	...	...	...	...	...	...	...

Notes: K, number of model parameters; w_i, Akaike weight; INT, proportion of intensive crops; FLO, proportion of flowering crops; EXT, proportion of extensive crops; and LOC, local floral resources (species richness).
Bold values are associated with the best model in the candidate models for the given response variable.

Table 3. Candidate models considered in model selection and multimodel inference procedures based on the second-order Akaike information criterion (AIC_c) for response variables related to colony defense and foraging behavior.

Model	Model sets	Depredation			Pollen		
		K	ΔAIC_c	w_i	K	ΔAIC_c	W_i
1	~null (intercept only)	**2**	**0**	**0.72**	**2**	**0**	**0.56**
2	~quad mass	3	2.18	0.24	. . .	. . .	. . .
3	~depredation	. . .	. . .	. . .	. . .	. . .	. . .
4	~INT + FLO	. . .	. . .	. . .	4	0.74	0.39
5	~INT + FLO + LOC	. . .	. . .	. . .	5	4.80	0.05
6	~INT + FLO + INT:FLO	. . .	. . .	. . .	6	9.24	0.01
7	~INT + FLO + LOC + INT:FLO + INT:LOC + FLO:LOC	. . .	. . .	. . .	8	15.02	0.00
8	~INT + EXT	5	6.70	0.03	. . .	. . .	. . .
9	~INT + EXT + INT:EXT	6	9.09	0.01	. . .	. . .	. . .

Notes: K, number of model parameters; w_i, Akaike weight; INT, proportion of intensive crops; FLO, proportion of flowering crops; EXT, proportion of extensive crops; and LOC, local floral resources (species richness).

Bold values are associated with the best model in the candidate models for the given response variable.

Table 3). These GLMMs were also fitted with the glmmTMB package in R.

We conducted analyses on pollen loads at the quad level instead of the colony because we were not able to collect similar numbers of workers across colonies on weekly quad visits. Furthermore, we decided to investigate possible pollen selection strategies at the colony level instead of at the worker level, which is to be treated in a future companion article. We hence aggregated all pollen load data for a given quad to determine the plant species visited by the workers from that quad. We modeled pollen species richness with GLMs assuming a negative binomial error distribution and a log link function and included the number of workers sampled per quad (mean $\pm$ SD = 14.1 $\pm$ 3.4, range = 6–18) as an offset to control for sampling effort with the glm.nb function of the MASS package (v.7.3-49; Venables and Ripley 2002)in R. Candidate models were the same as for survival, number of queens, and nest weight of a colony (1, 4–7; Table 2). We also ran a redundancy analysis (RDA) to assess how the proportions of intensive and flowering crops, as well as local floral species richness, influenced the species composition of pollen loads. We opted to apply the RDA to a presence–absence species matrix (following Hellinger transformation; Legendre and Gallagher 2001) because we did not estimate pollen load abundance. We fitted the RDA using the rda function of the vegan package (Oksanen et al. 2018) in R and assessed the significance of the marginal effect of each explanatory variable with permutation tests via the anova.cca function using 9999 permutations. Means are reported along with their standard deviation unless specified otherwise.

RESULTS

Quad weight

Quad weight showed little variation at the onset of the experiment (mean $\pm$ SD = 5.38 $\pm$ 0.06 kg), and no strong association was found with either the proportion of intensive ($r = -0.10$) or flowering ($r = 0.17$) crops. Initial quad weight was however negatively correlated with local flower species richness averaged over the first 10 weeks of the experiment ($r = -0.49$), the period during which most colonies were alive (Fig. 2). All quads followed the same pattern: They lost weight during their first week and then gained weight up to a maximum, generally between weeks 5 and 7, before they started losing weight again. GAMMs showed that this pattern was strongly influenced by landscape habitat composition, yet more tightly by the proportion of flowering crops ($R^2 = 0.93$) than by the proportion of intensive crops ($R^2 = 0.89$; $\Delta AIC = 72.9$; Table 4). Quads gained weight at a higher rate and over a longer time period in landscapes composed of less intensive crops or more flowering crops (Fig. 3) This resulted in a maximum weight that
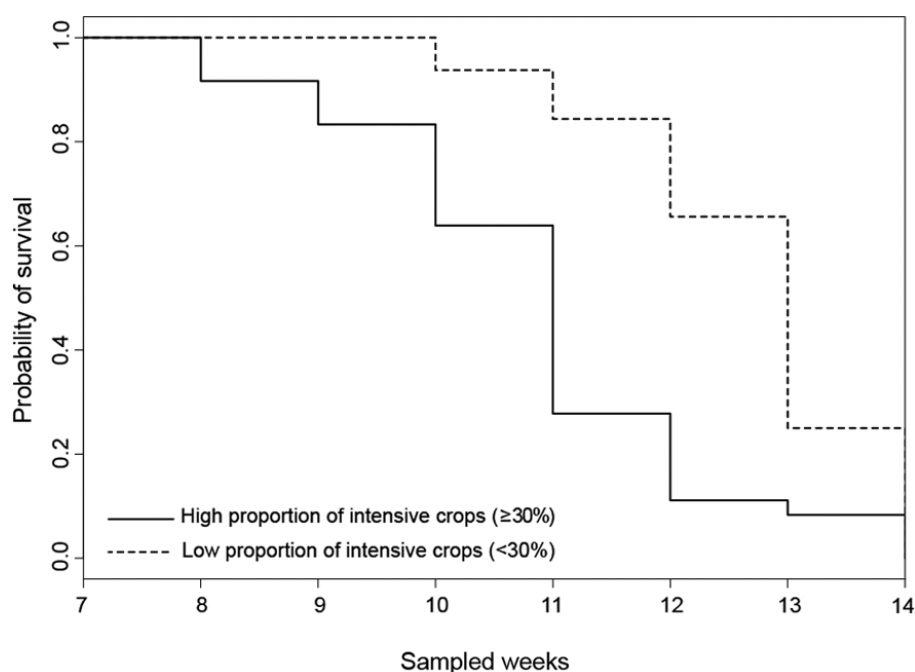
Fig. 2. Influence of intensive crop cover (500 m radius) on the survival rate of 68 experimental *Bombus impatiens* colonies clustered into 17 agricultural sites monitored in 2016 in southern Québec, Canada.

decreased with the proportion of intensive crops and increased with the proportion of flowering crops (Table 2; Figs. 4 and 5). No effect of local flower species richness on maximum quad

Table 4. Outputs of the two generalized additive mixed models (fitted with mgcv in R) used to quantify the (univariate) influence of intensive and flowering crop covers (500 m radius) on the temporal weight profiles of 17 clusters composed of four experimental colonies (quads) of *Bombus impatiens* monitored in 2016 in southern Québec, Canada.

Model	Tensor product smooth terms	Edf	F	Approximate P value	R^2 (scale)
1	te(week, FLO)	45.84	26.73	<0.001	0.93 (0.097)
	te(week, FLO, quad_ID)	29.27	13.19	<0.001	
2	te(week, INT)	33.56	14.99	<0.001	0.89 (0.138)
	te(week, INT, quad_ID)	29.70	19.23	<0.001	

Note: Edf, estimated degrees of freedom for model terms.

weight was detected (Table 2; Fig. 5). Lastly, although quads lost weight more rapidly in landscapes composed of less intensive crops or more flowering crops, they nevertheless ended their life at a greater weight (Fig 3).

Colony survival

Colony survival, and thus longevity, followed patterns congruent with those found for quad weight. Survival decreased with the proportion of intensive crops and increased with the proportion of flowering crops, but was not affected by local flower species richness (Table 2; Fig. 5). For instance, colonies surrounded by <30% intensive crops lived on average two weeks longer than those surrounded by more than 30% intensive crops (Fig. 2). No evidence of interaction between landscape habitat variables or between the latter and local flower species richness was found (Table 2; Fig. 5).

Queen production

Despite the fact that the number of queens produced by colonies was highly variable (mean ± SD = 10.6 ± 6.06), within-quad
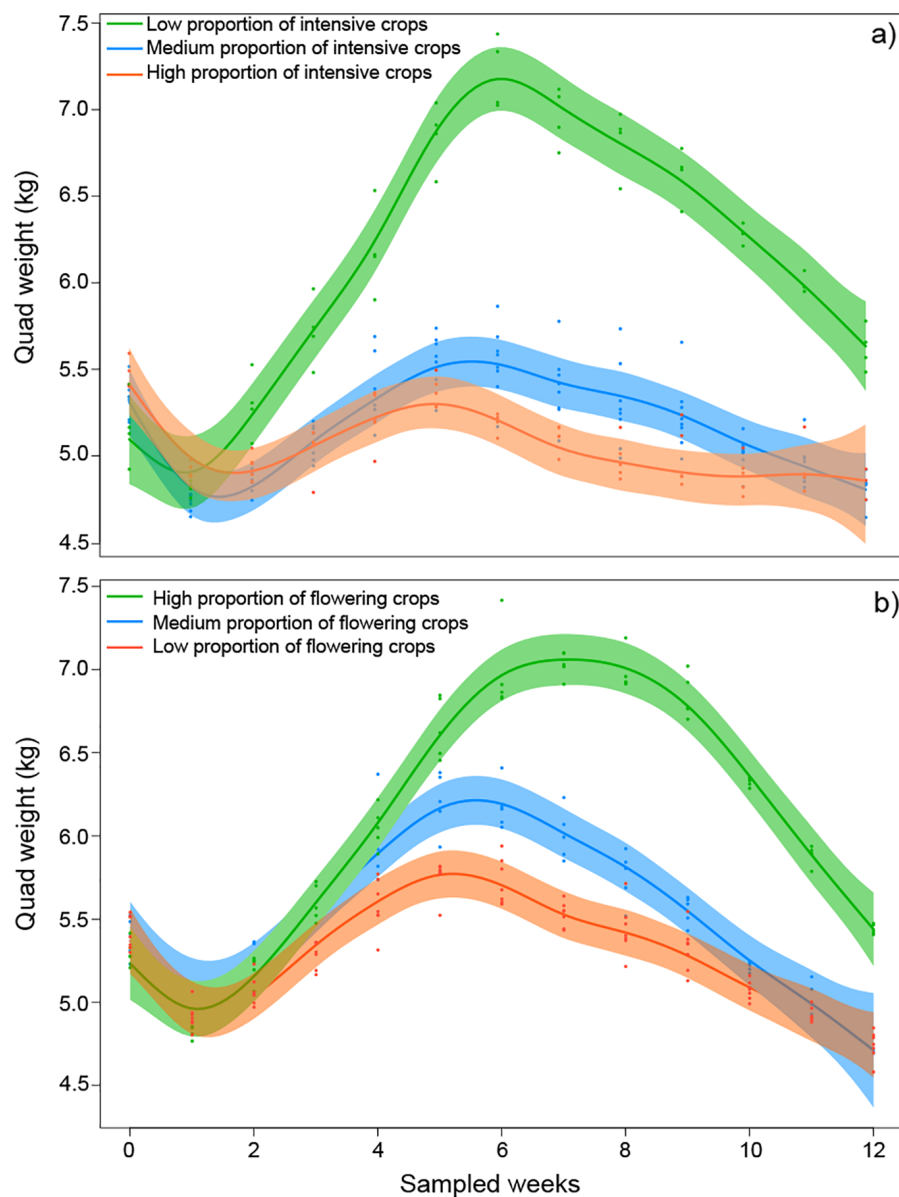
Fig. 3. Influence of intensive (a) and flowering (b) crop covers (500 m radius) on the temporal weight profiles of 17 clusters composed of four experimental colonies (quads) of *Bombus impatiens* monitored in 2016 in southern Québec, Canada. Profiles were estimated with generalized additive mixed models (Table 4). (a) Green, blue, and red lines represent low (0–30%), medium (31–67%), and high proportion (>68%) of intensive crops, respectively. (b) The red, blue, and green lines represent low (0–5%), medium (6–17%), and high proportion (>18%) of flowering crops, respectively. Data points depict partial residuals, and model predictions are shown with 95% confidence intervals.

variability was such that we found no clear evidence that landscape habitat composition or local flower species richness influenced this fitness component (Table 2; Fig. 5).

Nest weight

Nest weight of dead colonies was influenced by both landscape habitat composition and local flower species richness, but no interaction within
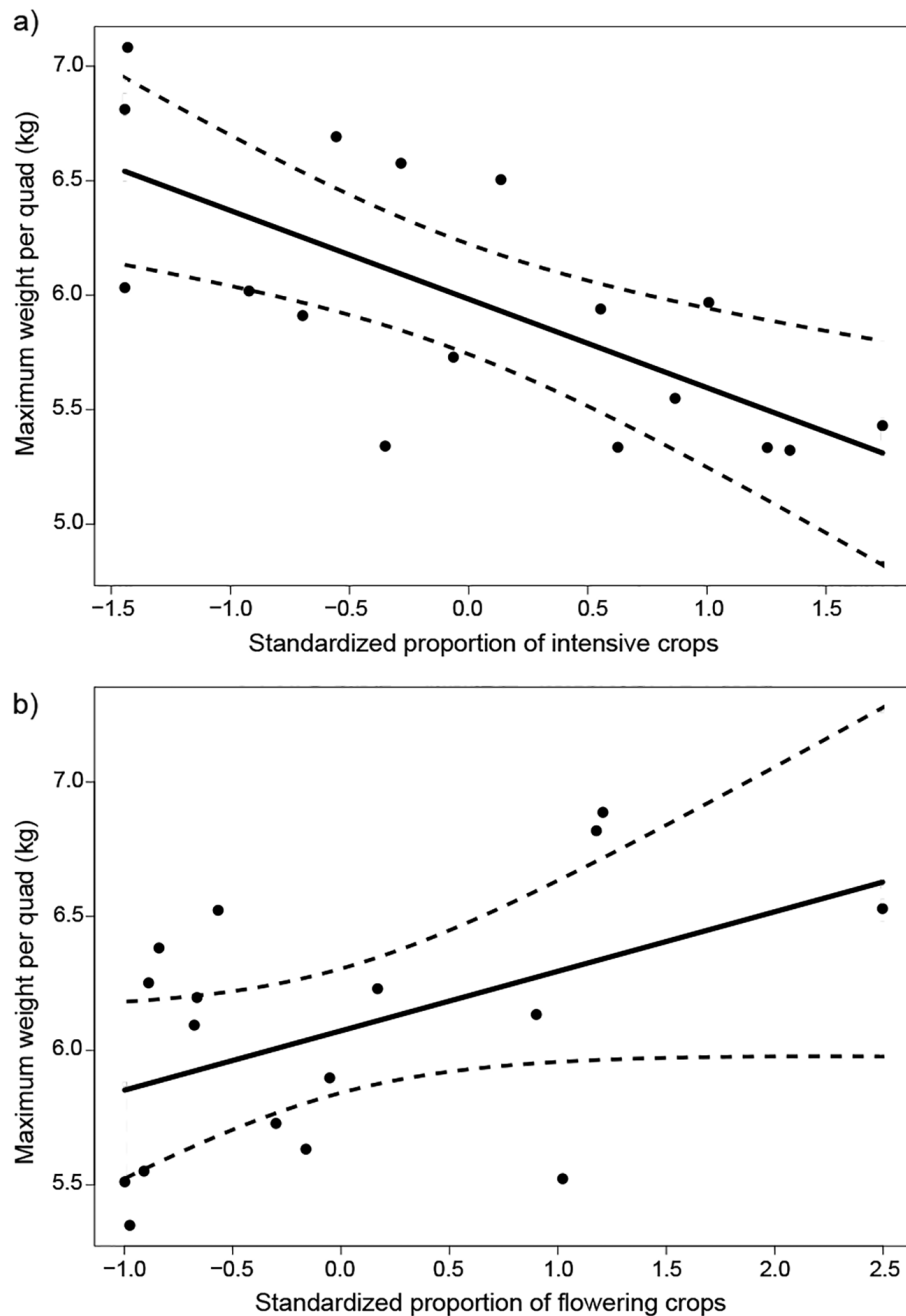
Fig. 4. Influence of intensive (a) and flowering (b) crop covers (500 m radius) on the maximum weight attained by the 17 clusters composed of four experimental colonies (quads) of *Bombus impatiens* monitored in 2016 in southern Québec, Canada. Data points depict standardized values, and model-averaged predictions under average conditions are shown with 95% unconditional confidence intervals.

or between these two components was found (Tables 2 and 3; Fig. 6). While nest weight showed a decreasing trend with local flower species richness, it clearly increased with the proportion of flowering crops (Table 2; Figs. 5 and 6). These effects were substantial as, for example, colonies in
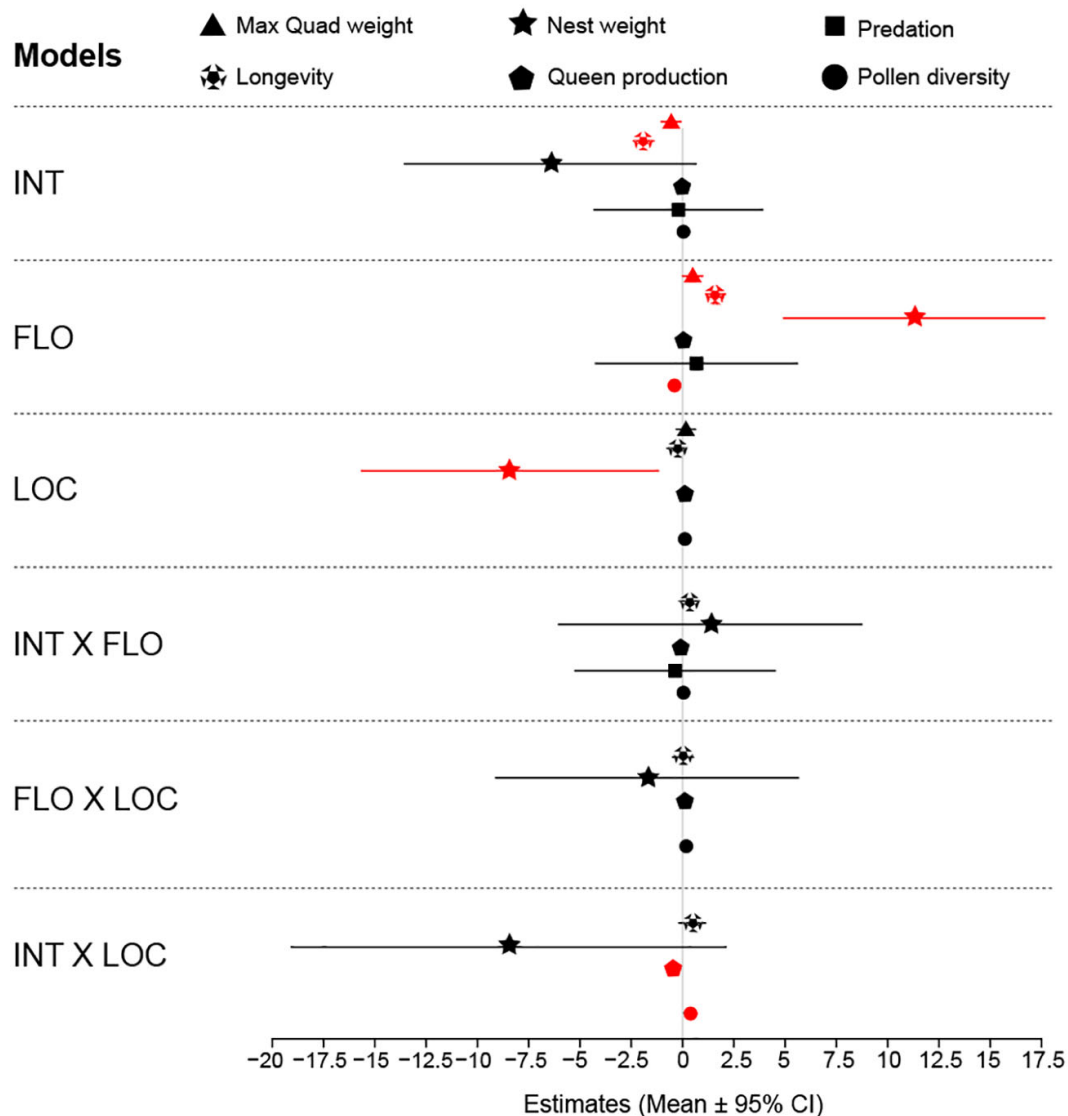
Fig. 5. Forest plot of model-averaged coefficients and their 95% unconditional confidence intervals estimated by multimodel inference following model selection (see Table 2). Model coefficients were computed with standardized explanatory variables (zero mean and unit variance). Red symbols indicate confidence intervals that do not contain zero. Abbreviations are INT, proportion of intensive crops; FLO, proportion of flowering crops; and LOC, local floral species richness.

a landscape with 25% flowering crops would weigh 35% more than those in a landscape without flowering crops if other landscape variables were fixed at their mean value (Fig. 6a). Conversely, colonies locally surrounded by 15 flower species would weigh 30% less than colonies surrounded by only five species (Fig. 6b); local flower species richness averaged 8.10 ± 2.57 (mean ± SD).

Depredation probability

No infestation by *Aphomia sociella* was detected at the beginning of the experiment, and yet more than one-third (36.8%) of the 68 colonies were subjected to depredation by this moth. Level of infestation varied only slightly between colonies of a same quad. Depredated colonies were distributed among 8 of the 17 quads, of which 6 had
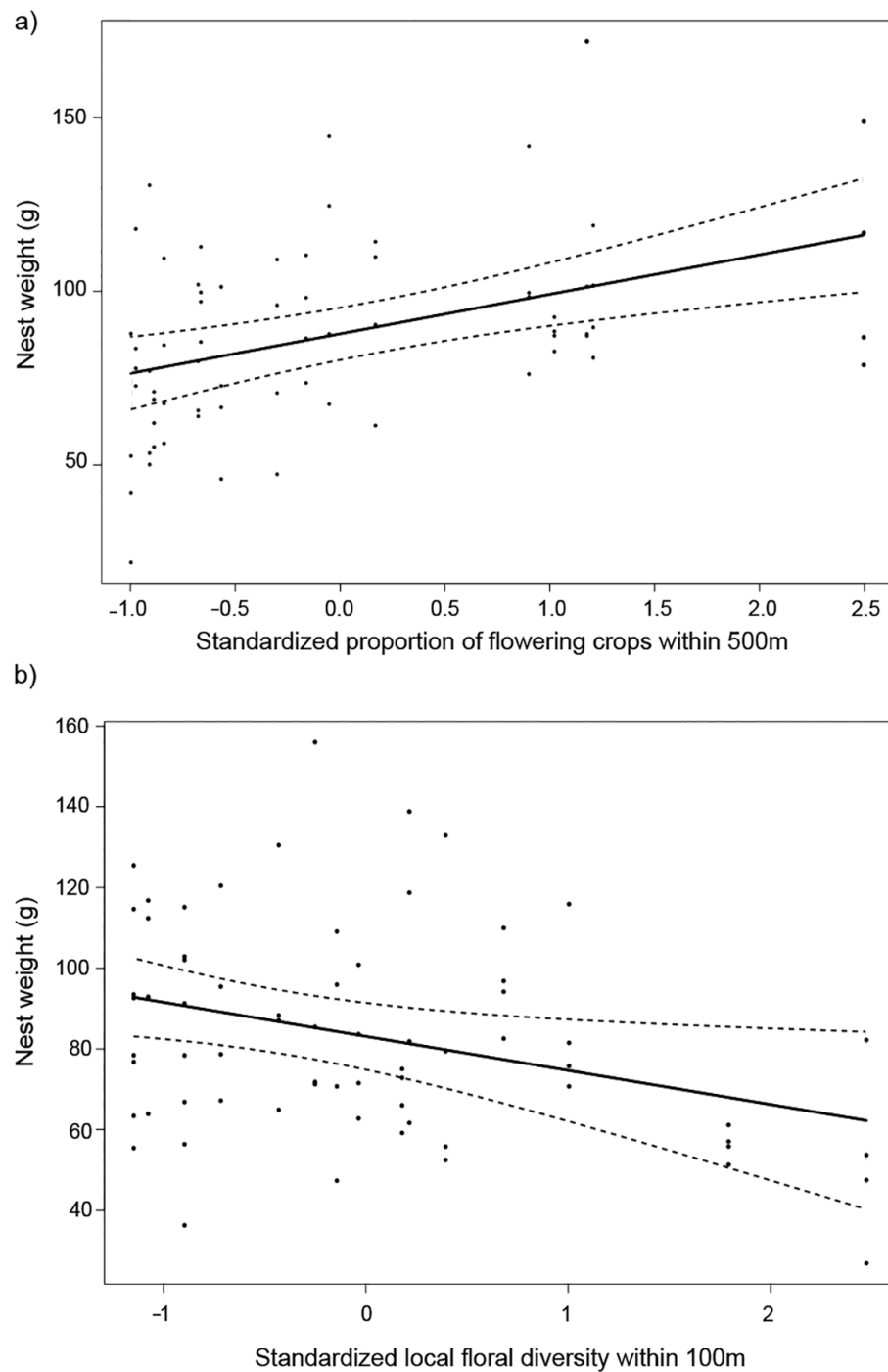
Fig. 6. Influence of flowering crop cover (500 m radius) (a) and local flower species richness 100 m radius) (b) on the nest weight at death of 68 experimental *Bombus impatiens* colonies clustered into 17 agricultural sites monitored in 2016 in southern Québec, Canada. Data points depict standardized values, and model-averaged predictions under average conditions are shown with 95% unconditional confidence intervals.

at least three colonies that were depredated (mean ± SD = 3.1 ± 1.1, n = 8). Yet, none of the predictors that we considered influenced the likelihood that a colony is depredated by *A. sociella* (Table 3; Fig. 5).

Pollen load diversity and composition

A total of 247 workers were captured during the course of this study for an average of 14.5 ± 2.8 workers by site. These workers visited 141 species or genus of flowers including 21 morphotypes and 29 unknown pollen grains across study sites (quads). Pollen grain richness varied between 21 and 55 between sites and averaged 33.2 ± 8.8 per site. Landscape composition as well as local floral richness did not clearly influence the richness of pollen bought back to the colonies (Table 3; Fig. 5). Indeed, pollen grain richness only tended to decrease with the amount of intensive cultures when the local floral species richness was high and stayed constant when the local floral richness was low. In contrast, the RDA showed that the species composition of pollen loads varied with landscape habitat composition ($F_{3,13}$ = 1.21; P = 0.02; Fig 7). The proportion of variance explained by the three constrained axes was 9.1%, 7.5%, and 5.3%, for a total of 21.8%. While the proportion of both intensive ($F_{1,15}$ = 1.43; P = 0.01) and flowering ($F_{1,15}$ = 1.22; P = 0.11) crops modulated the composition of pollen loads, local floral richness had no bearing on this variable ($F_{4,12}$ = 0.95; P = 0.59).

Discussion

Our objective was to measure experimentally the influence of agricultural intensification through its effects on landscape habitat composition and configuration, including local floral resource availability, on the development of *Bombus impatiens* colonies. As expected, colonies exposed to an increasing proportion of intensive row crops showed reduced weight gains and longevity. Greater exposure to flowering crops had the opposite effect. However, we found no effect of intensive crops on queen production, diversity of pollen collected by workers, nor on depredation probability by nest parasites; flowering crops only had a positive effect on nest weight. Lastly, we expected that increased local

diversity of floral resources would be beneficial for colony development but only found a negative effect on nest weight. Overall, our results bring support to the accumulating number of studies showing negative effects of intensive agricultural practices on bumble bee populations and contribute evidence as to the mechanism leading to these declines.

Landscape habitat composition

The weight of bumble bee colonies was affected by landscape habitat composition during their entire growth as well as during their last development stages. These results are consistent with those of numerous previous studies, which found that colony growth was enhanced by the proportion of either flowering crops (Westphal et al. 2009) or natural habitats within 2–3 km from the colonies (Williams et al. 2012, Crone and Williams 2016, Milano et al. 2019). Flowering crops, here within 500 meters, likely provided bumble bee workers with an extensive supply of pollen and nectar at a smaller travel cost, resulting in heavier colonies (Figs. 3 and 4). This contention is also supported by the positive influence of flowering crops on nest weight (Fig. 6). However, some experiments found that some flowering crops, and particularly orchards, can have marginally negative on colony growth (Proesmans et al. 2019). Flowering crops in our study area were mostly composed of alfalfa and clover, cultures which provide a relatively stable food supply to bumble bees (Goulson and Darvill 2004) and typically require much less pesticides than the intensive crops grown therein (Table 1). The negative influence of intensive crops on colony growth that we observed (Figs. 3 and 4) was thus likely related to their lower value as food sources and to a greater exposure to pesticides. Indeed, maize, soybean, wheat, and apple crops in our study area are associated to high inputs of pesticides, including several neonicotinoid insecticides and herbicides, which are not only found in the surrounding water (Giroux 2019, Montiel-León et al. 2019) but also in flying insects (Haroune et al. 2015). These compounds, and notoriously neonicotinoids, are known to have the capacity to impair the neural system of workers and thereby reduce their ability to provision food to the colony (Mommaerts et al. 2010, Siviter et al. 2018, Kenna et al. 2019).
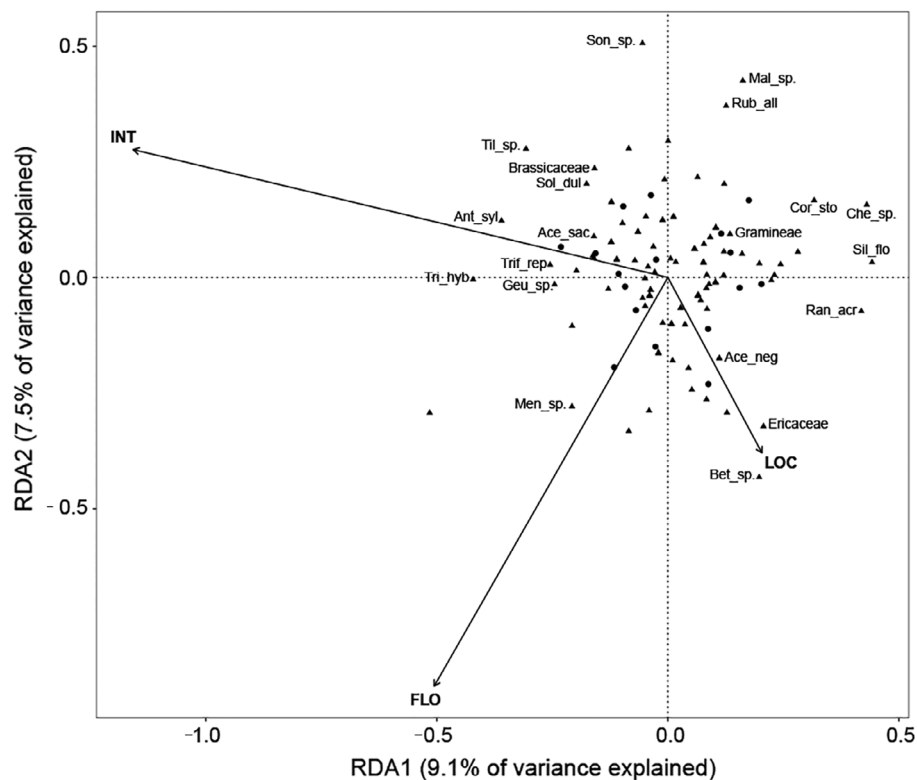
Fig. 7. Influence of landscape habitat composition and local floral resources on the species composition of pollen loads collected by the workers of 68 experimental *Bombus impatiens* colonies clustered into 17 agricultural sites monitored in 2016 in southern Québec, Canada. Arrows of the RDA biplot depict the effects of explanatory variables. INT and FLO stand for the proportion of intensive and flowering crops (500 m radius), respectively. LOC corresponds to local floral species richness (100 m radius). Circles and triangles depict sites and pollen species, respectively. Ace_neg, *Acer negundo*; Ace_sac, *Acer saccharinum*; Ant_syl, *Anthriscus sylvestris*; Bet_sp., *Betula* sp.; Che_sp., *Chenopodium* sp.; Cor_sto, *Cornus stolonifera*; Geu_sp, *Geum* sp.; Mal_sp, *Malus* sp.; Men_sp., *Mentha* sp.; Ran_acr, *Ranunculus acris*; Rub_all, *Rubus allegheniensis*; Sil_flo, *Silene flos-cuculi*; Sol_dul, *Solanum dulcamara*; Son_sp., *Sonchus* sp.; Til_sp., *Tilia* sp.; Tri_hyb, *Trifolium hybridum*; Tri_re, *Trifolium repens*.

In fact, there is considerable laboratory and field evidence that pesticides can negatively impact the growth of bumble bee colonies (Whitehorn et al. 2012, Goulson 2015, Baron et al. 2017, Stanley and Raine 2017), yet no studies had so far clearly shown a link between colony weight and the proportion of intensive crops in the surrounding landscape.

As for weight, landscape composition greatly affected colony longevity. Despite the scarcity of data on the relationship between colony and queen or worker longevities, it is reasonable to assume that they are positively correlated. Besides, many factors are known to decrease bumble bee longevity, such as high foraging workloads (O'Donnell et al. 2000), diseases (Fauser-Misslin et al. 2014), small colony size (Blacher et al. 2017), or pesticide intoxication (Fauser-Misslin et al. 2014, Fauser et al. 2017, Wu-Smart and Spivak 2018). Hence, workers from colonies established in landscapes dominated by intensive agriculture where food is likely scarcer and contaminated by pesticides would be expected to have a shortened lifespan, leading in turn to a reduced colony longevity, just as we found (Fig. 2). This rationale could be reversed and used to explain why we also observed that colonies placed in landscapes harboring more flowering crops lived longer. These causal links nevertheless remain speculative and cannot

benefit from the evidence gathered by past studies on the effect of landscape structure or pesticide exposition on bumble bee colonies as researchers typically froze colonies prior to their natural death (Goulson et al. 2002, Whitehorn et al. 2012, Cutler and Scott-Dupree 2014, Spiesman et al. 2017, but see Samuelson et al. 2018). Our work definitely stresses the importance of monitoring the development of bumble bee colonies throughout their entire lifespan under field conditions so that the causal determinants of survival related to agriculture are uncovered.

Unexpectedly, bumble bee queen production was not affected by landscape habitat composition. Indeed, we expected that the availability of flowering resources and exposition to pesticides would affect colony size and longevity, and thereby the ability of colonies to invest in reproduction. Yet, the proportion of neither intensive nor flowering crops did affect the number of queens that were produced. Similar results were obtained in other studies that found landscape effects on colony growth but not on gyne production (Westphal et al. 2009, Williams et al. 2012, Crone and Williams 2016). One possible explanation for that result may simply be that colonies will prioritize queen production and allocate the resources accordingly. Hence, colony development would be negatively impacted, but no effect on the number of queens would be detected. However, while the number of queens produced by colonies did not vary, the size of the queens may have been affected. This possibility warrants further investigation as the size of queens is positively correlated with their chances of winter survival (Owen 1988), and possibly with their capacity to establish a colony the following spring, and their fecundity (Carvell et al. 2017). The downside of not collecting colonies before their natural death is that we could not verify this possibility nor that of a greater number and size of males in more extensively farmed landscapes (Westphal et al. 2009).

Colonies placed in different landscapes were subjected to the same probability of depredation by *A. sociella.* Similar results have been found in regard to depredation by other invertebrates, such as *Vitula edmandsii* (Lepidoptera: Pyralidae), with infestation levels uncorrelated with landscape composition (Williams et al. 2012). The infestation rate found in this study was,

however, greater than that found within native bumble bee colonies (36% this study vs. 26% in Goulson et al. 2017). It is difficult to interpret such a difference because several confounding factors could be at play, including the artificial setup (i.e., quads) in which our colonies were established or the semi-wild strain(s) of *B. impatiens* that we used. Another important potential confounding factor that we could not take into account was the density of bumble bee nests surrounding our experimental sites, which may be positively correlated with *A. sociella* density and thus to infestation rate by this nest parasite (Goulson et al. 2002). How the different ecological components related to agricultural intensification affect bumble bee and *A. sociella* densities, as well as the ease of nest colonization and destruction by this nest parasite should definitely be addressed. Indeed, *A. sociella* is an important pest to bumble bee nests, since the genus is their main host. It eats the entire nest, including wax cells, food reserves, and larvae, and an infestation by *A. sociella* larvae can totally destroy a mature bumble bee nest in only 40 d (Pouvreau 1988, Goulson et al. 2018).

Landscape habitat composition had no effect on the diversity of pollen brought back to the nest by workers. This was surprising given that workers, and even colonies, will specialize on a few plants under optimal conditions, namely an abundance of a sufficiently nutritious flowering plant and low competition for this resource (Kunin and Iwasa 1996, Fontaine et al. 2008, Vaudo et al. 2016). Again, most of the flowering crops in our experimental sites were Fabaceae (*Trifolium* spp. and *Medicago* spp.), which are highly nutritious for pollinators (Goulson and Darvill 2004). In our study area, those crops were allowed to bloom before harvest, thus representing an important food source for workers. Yet, we found no evidence of such specialization within our data, even in flower-rich landscapes. That may be explained by the fact that we aggregated data among the quad, hence hiding the possible flower constancy exhibited by workers. We also had a small sample size, representing just a few workers per week per site, which limited our statistical power. Nevertheless, both the proportion of flowering and intensive crops predicted the composition of the pollen collected by colonies (Fig. 7). As we mentioned above, the

intensive crops found in our study area are associated with substantial uses of pesticides. For instance, the dominant intensive crops consisted in maize and soybean which were then mainly sown as seeds coated with neonicotinoids (Giroux 2019, Montiel-León et al. 2019), a class of pesticides known to affect the foraging ability of workers (Feltham et al. 2014). Moreover, it has been found that bumble bees intoxicated by neonicotinoid pesticides tended to forage on different flower species than unexposed bumble bees (Stanley and Raine 2016). Altered foraging behavior may thus have played a role as to why we did not find a difference in pollen load diversity but a difference in the pollen composition brought back to the nest in more intensively cultivated landscapes.

Local floral resources

Contrary to our predictions, local floral resources estimated through species richness within 100 m from colonies had no consistent bearing on their development as it only influenced, and negatively, the weight of dead nests (Fig. 6). This is surprising given the large body of evidence that shows the positive importance of floral resources for bumble bee colonies (Crone and Williams 2016, Herrmann et al. 2007, 2017, Williams et al. 2012, Spiesman et al. 2017). The lack of consistent results may however stem from the fact that there is no consensus as to how we should estimate floral resource (pollen and nectar) availability and profitability for pollinators under field conditions (Szigeti et al. 2016). For instance, Spiesman et al. (2017) found that floral dominance, rather than floral abundance or richness, was the best predictor of bumble bee colony growth. Yet Adhikari et al. (2019) found that flower density and shrub richness were positively correlated with colony growth and reproduction. Hence, we may have ended with other results had we chosen another index of floral resource availability. Such an outcome definitely implies that some research efforts should be devoted at designing integrative methods and indices to estimate floral resource availability at various spatial scales.

Conclusion

Our experiment provides strong evidence that landscape structure has observable effects on the development of bumble bee colonies. Notably, the relative amount of intensive and flowering crops was the best predictor of colony growth and survival but did not cascade down to queen production. We however have to acknowledge that 20 sites represent a small sample size and that future studies should increase the number of colonies to monitor. Nonetheless, although studies showing that pesticides, such as neonicotinoids, impair colonies are accumulating, our field study is among the first to attest that intensive crop farming undermines colony development. Also, the significant magnitude of the impacts we observed with commercial *B. impatiens* colonies suggests that current intensive agricultural practices may be even more negative to other bumble bee species and the essential ecological services they provide. *Bombus impatiens* remains common and abundant in Eastern North America and possesses generalist functional traits that make it competitive in the landscape (Persson et al. 2015). Already endangered bumble bees, which tend to be more specialist with respect to their food sources or nesting habitat (Goulson and Darvill 2004), may thus prove more responsive to landscape factors and management. Further studies should try to assess such questions with wild bumble bees.

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