



# Using flower strips to promote green lacewings to control cabbage insect pests

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Received: 15 March 2021 / Revised: 3 August 2021 / Accepted: 4 August 2021  
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## Abstract

Habitat management improves biological control by increasing the abundance and fitness of natural enemies through the provision of floral resources along field edges or between crops. Among the natural enemies reliant on flower resources, green lacewings often stand out due to their abundance, predation capacity and polyphagy. We evaluated the impact of tailored flower strips on the enhancement of natural enemies, especially green lacewings, in three organic cabbage (*Brassica oleracea*) farms in Southern Sweden. Insects were sampled from the flower strips, and cabbage pests and predators were visually recorded in the crop. In a laboratory assay, the pollen feeding preferences of *Chrysoperla carnea* (Stephens, 1836) were evaluated in a dual-choice test. The pollen consumed by the Chrysopidae was extracted from laboratory and field specimens, then quantified and identified. Flower strips were found to attract predators and parasitoids, whose abundance increased as flowers bloomed. Cabbage plants next to the flower strips showed lower pest infestation as compared to cabbage plant control, although no significant differences were observed in the number of predators. Chrysopidae used flower strips as feeding, reproduction and shelter sites and mainly consumed pollen from *Phacelia tanacetifolia* Benth. Under laboratory conditions, *C. carnea* showed a preference for *P. tanacetifolia* and *Coriandrum sativum* L. pollen over *Borago officinalis* L. and *Fagopyrum esculentum* Moench. We show that tailored flower strips could be an efficient tool for enhancing beneficial arthropods and should be considered in integrated pest management for cabbage crops.

**Keywords** Ecological infrastructure · Conservation biological control · Pollen · *Chrysoperla carnea* · Predators · Parasitoids

## Key messages

Communicated by Antonio Biondi .

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- Flower strips attract predators and parasitoids.
- Cabbage plants next to flower strips showed lower pest infestation levels.
- *Chrysoperla carnea sensu* Henry, *Chrysoperla lucasina* and *Chrysopa commata* were found in the tailored flower strips in Southern Sweden.
- Field collected Chrysopidae mainly consumed pollen from the flower strips introduced.
- Chrysopidae show a preference for feeding on *Phacelia tanacetifolia* and *Coriandrum sativum* pollen grains.

## Introduction

Increasing agricultural intensification has had a negative impact on the wider environment, which has led to landscape simplification, agrochemical spillover in soil and water, as well as an increasing loss of biodiversity (Conway 2000; Tilman et al. 2002). Farmers have been experiencing major losses of essential ecosystem services, such as pollination, water and fertility regeneration, nutrient recycling and biological pest control (Steffen et al. 2015). A shift towards a more ecosystem-based approach to agriculture has been proposed (Tittonell 2014). This approach includes habitat management in order to restore functional agrobiodiversity in the agricultural landscape. This involves the introduction of non-crop vegetation with the aim of providing beneficial arthropods with shelter, overwintering sites, alternative prey and food resources (Landis et al. 2000; Gurr et al. 2017). Over the last decades, the use of flower strips in cultivated fields in order to enhance plant diversity has attracted growing interest from researchers, farmers and practitioners (Haaland and Gyllin 2011; Penvern et al. 2019; Aparicio et al. 2021). Annual and perennial flower strips have been shown to increase beneficial arthropod diversity and biological pest control (Fiedler et al. 2008; Tschumi et al. 2016; Cahenzli et al. 2019; Albrecht et al. 2020). However, as several factors may influence the success of this strategy, system-specific evaluations need to be carried out prior to implementation (Tscharntke et al. 2016). Parameters such as time of flowering, synchronization with natural enemies and pest occurrence, nectar and pollen production and competition with the main crop for resources should be carefully investigated before this strategy is implemented. Furthermore, flower strips should not attract or host arthropods potentially harmful to the main crop or favour intraguild predation among beneficial arthropods (Tscharntke et al. 2016).

*Brassica*, an important arable crop in Europe, is attacked by a wide range of herbivores, including insects, nematodes, slugs and birds (Alford et al. 2003). Insects are regarded as the main source of yield losses in Swedish vegetable production, for example, the flies *Delia radicum* (L.) and *Delia floralis* (Fallen) are important pests in cabbage and cauliflower (Nilsson et al. 2016). The most economically important *Brassica* vegetables in Sweden are cauliflower (421 ha; 7359 ton), white cabbage (338 ha; 16362 ton) and broccoli (335 ha; 2941 ton) (SCB 2018). Several key *Brassica* insect pests such as *Plutella xylostella* L., *Pieris rapae* L. and *D. radicum* cause substantial economic damage to *Brassica* crops in Sweden, although in the southern and central part of the country, other insect pests (*Pieris brassicae* L., *Pieris napi* L., *Mamestra brassicae* L., *Autographa gamma* L., *Lacanobia oleracea* L.,

*Agrotis spp.* or *Evergestis forficalis* L.) are frequently responsible for minor damages (Andersson et al. 2013). Furthermore, key *Brassica* insect pests, such as *Psylliodes chrysocephala* (L.), *Ceutorhynchus obstrictus* (Marsham) and *Meligethes aeneus* (Fabricius), have developed resistance to certain widely used pesticides, thus making their control increasingly difficult (Heimbach and Müller 2013). One alternative to the use of pesticides is to create a more favourable environment for locally occurring natural enemies that have the potential to control key pests (Balmer et al. 2014; Nilsson et al. 2016). For instance, *Fagopyrum esculentum* Moench sown as a single species or flower mixture along cabbage borders was found to improve natural lepidopteran pest control, with higher parasitism rates observed on *P. xylostella* and *P. rapae*, as well as increased predation on *M. brassicae*. However, these positive effects were not consistent throughout the studies reported (Lee and Heimpel 2005; Pfiffner et al. 2009; Winkler et al. 2009).

Green lacewings, mainly at the larval stage, prey on soft-bodied insects such as aphids, thrips, mites, moths and lepidopteran eggs (Principi and Canard 1984). In this context, cabbage pests such as *P. brassicae*, *P. xylostella*, *M. brassicae* and *B. brassicae* have been reported as green lacewings preys (Klingen et al. 1996; Reddy et al. 2004; Huang and Enkegaard 2010; Ahmad-Ur-Rahman et al. 2016). Conversely, most adults show palyno-glycophagous behaviour, with a diet based on pollen, nectar and honeydew (Stelzl 1991, 1992; Canard 2001; Villenave et al. 2006; Villa et al. 2017). Furthermore, green lacewings are one of the most studied groups of natural enemies given their important role in crop protection, as well as their presence in field crops (Villenave et al. 2005; Pappas et al. 2011). The *Chrysoperla carnea*-group, which includes some of the most abundant green lacewing species in agroecosystems, is comprised of at least 21 sibling species which are extremely similar in morphology (Price et al. 2015). Three or four species of the *Chrysoperla carnea*-group may even coexist on a single tree (Henry et al. 2013; Alcalá Herrera et al. 2019a). Studies of green lacewings rarely provide a breakdown of the *Chrysoperla carnea*-group into their species assemblages (e.g. Bertrand et al. 2019; Villa et al. 2019), which would give us an essential insight into the ecological differences between the different species. *Chrysoperla carnea* species are opportunistic feeders which are attracted to large patches of flowering plants ( $>20\text{ m}^2$ ) (Villenave et al. 2006; Alcalá Herrera et al. 2020).

While Villenave et al. (2006) have suggested that bordering vegetation constitutes a reservoir habitat and resting place for most Chrysopidae, mixed results have been found about the effect of cover crops and on increased lacewing activity during the cropping season (Smith et al. 1996; Bone et al. 2009). Conversely, other studies have observed that

flowering plants in apple, citrus and olive orchards, as well as sown flower strips adjacent to cotton and cabbage crops, increased lacewing populations and recruitment into crop fields (Fye and Carranza 1972; Burleigh et al. 1973; Ridgway and Murphy 1984; Wyss 1995; Silva et al. 2010; Porcel et al. 2017). The following factors need to be taken into account when managing habitats to increase and maintain naturally occurring Chrysopidae populations (Ridgway and Murphy 1984): availability and suitability of plant species containing flowers with an ultraviolet pattern, open corollas for accessible pollen, and well-exposed nectaries (Villenave et al. 2005; Nave 2016; Van Rijn and Wackers 2016; Hatt et al. 2019); the presence of preimaginal-stage eggs and larvae indicating the suitability of the habitat as a reproduction site (Bianchi et al. 2013); improved nesting sites for a better colonization and healthier predator and parasitoid movement between these habitats adjacent to crop (Nicholls et al. 2001; Wilkinson and Landis 2005); knowledge of local population dynamics of both Chrysopidae and target pest species (Ridgway and Murphy 1984; Landis et al. 2000).

Thus, the overall goal of this study was to determine whether selected flowering plant species arranged in strips can be used to attract and support predators and parasitoids, with particular emphasis on green lacewings. In addition, we estimated the suitability of flower strips to enhance biological pest control of the lepidopterans: *M. brassicae*, *P. xylostella*, *P. brassicae*, *P. rapae* and the aphid *Brevicoryne brassicae* L. by green lacewings in organic *Brassica oleracea* crops. We hypothesized that adult green lacewings would be attracted to and use flower resources such as pollen and nectar and use suitable oviposition sites in adjacent cabbage crops, where their larvae would prey on the most important

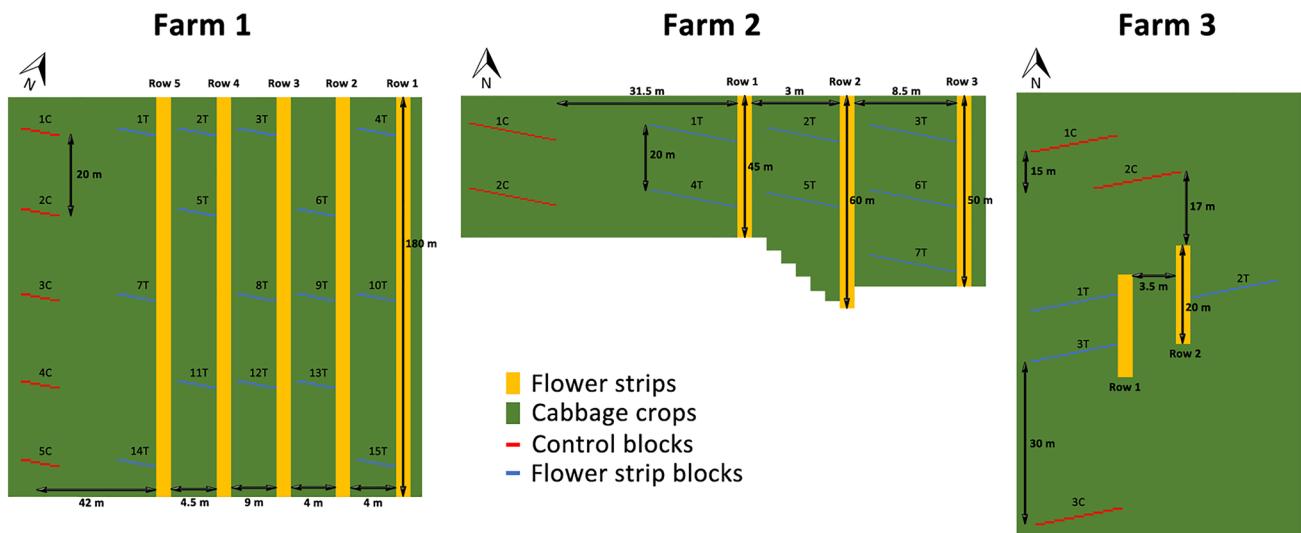
cabbage pests. The specific objectives of this study were: (i) to evaluate the impact of flower strips on pest and predator abundances in Brassica crops, (ii) to identify the arthropod community, especially green lacewings, associated with plant species present in the flower strips, (iii) to identify and quantify the pollen consumed by green lacewing adults collected from the flower strips and (iv) to determinate their pollen feeding preferences in a laboratory assay.

## Material and methods

### Experimental design

The field experiment was conducted between May and September 2017. Three commercial farms were selected in the county of Scania in the south of Sweden (Figure S1), which is dominated by arable crops and meadows. The most common crops are rye, barley, oats, wheat, sugar beet, potatoes and cabbage vegetables. All three farms were organically certified under EU regulations by KRAV Sweden. Each farm had an area planted with cabbage (*B. oleracea*), with three to seven varieties per farm (Table S1). In each farm, standardized 1.80 m wide and between 20 to 180 m long (farm 1: 180 m; farm 2: 45–60 m; and farm 3: 20 m) flower strips (rows) were established between May and June 2017 (Figure 1 and S1) at a distance of 1.5 to 2 m from the crop. The number of rows varied between two and five (Figure 1 and S1). Distances between rows ranged from 3 to 9 m, according to the grower planting diagram (Figure 1 and S1).

In the three farms, the cabbage plants were sown in two treatments: (i) adjacent to flower strips (flower strip blocks)



**Fig. 1** Experimental design in each farm. 1C–5C are the control blocks, and 1T–15T are the flower strip blocks. The location of each farm in Scania, plant species distribution within the flower strips and seed rate per plant species are given in Figure S1

and (ii) with no adjacent flower strips (control blocks) (Figure 1 and S1). A diagonal line of eighteen cabbage plants, randomly selected and marked the first time, was regarded as a block. The number of blocks per farm depended on the cabbage crop surface available by the grower and to maximize differences between treatments. In farm 1, there were 20 blocks (5 control blocks and 15 flower strip blocks), 9 blocks in farm 2 (2 control blocks and 7 flower strip blocks) and 6 blocks in farm 3 (3 control blocks and 3 flower strip blocks) (Figure 1 and S1). Blocks were sampled repeatedly on six to eight occasions in each farm at least once a week from June to September. The minimum distance between blocks and treatments was five and 25 m, respectively. The minimum distance between control blocks and rows was 17 m (Figure 1 and S1).

The flower strips were comprised of six different plant species: *Borago officinalis* L., *Coriandrum sativum* L., *F. esculentum*, *Foeniculum vulgare* Mill., *Helianthus annuus* L. and *Phacelia tanacetifolia* Benth. (Figure S1). These species were chosen on the basis of the following two criteria: (i) floral resources had demonstrated positive effects on the survival, reproduction, development and population dynamics of lacewings (Villenave et al. 2005, 2006; Robinson et al. 2008; Van Rijn 2012; Gonzalez et al. 2016; Tschumi et al. 2016; Villa et al. 2016) and (ii) the locally adapted ecotype plant species are present throughout Sweden and around the world.

The seeds of the plant species produced under organic conditions were supplied by a company specializing in locally adapted ecotypes (Lindbloms Frö, Sweden). Plants were sown using a hand seeder in single species rows (Figure S1). Each flower strip was established coinciding with the planting schedules of the adjacent cabbage row. The presence of other flowering species in the crops was scarce, and weeds in flower strips were manually managed. Flower strips were irrigated at the same time as the cabbage crops. No pesticide treatments, mowing or fertilization was conducted in the flower strips.

Flower bloom was estimated as the percentage of open flowers (scale 0–100%) for each plant species in each row and farm at least once a week from June to September, with a total of between six and nine times per farm. In general, the flower bloom was  $27.72 \pm 3.99\%$  (mean  $\pm$  SE).

### Pest and predator presence in cabbage crops

All leaves and the stem of the cabbage plants in flower strip and control blocks were closely inspected to count and record the presence and abundance of the lepidopterans *M. brassicae*, *P. xylostella*, *P. brassicae*, *P. rapae* and the aphid species *B. brassicae* L., as well as the predators Chrysopidae, Coccinellidae and Syrphidae.

### Arthropod collection in flower strips

Arthropod collection began on the 15th of July, approximately seven weeks after sowing the flower strips, once the flowers started to bloom, and came to an end on the 1st of September. Suction samples were taken from the flower strips on a weekly basis using a Stihl® SH 85C aspirator (Stihl AG & Co, Waiblingen, Germany). For each sample, the aspirator was moved across the flower strip for 40 seconds covering all plant species. The sampling points within the flower strip were selected randomly, with a minimum distance of four metres between samples. The number of samples per farm ranged from eight to 30 on each sampling occasion. Suction samples were transported under cold conditions and stored in a freezer at  $-20^{\circ}\text{C}$  until identification.

The arthropods collected were counted and identified to family and genus level where possible using standard taxonomic keys (Goulet et al. 1993; Barrientos 2004). We pooled the individuals collected into two groups: predators (Chrysopidae, Coccinellidae, Cantharidae, Anthocoridae, *Deraeocoris* sp. and Nabidae) and parasitoids (Ceraphronidae, Megaspilidae, Aphelinidae, Encyrtidae, Eulophidae, Mymaridae, Pteromalidae, Torymidae, Cynipidae, Figitidae, Braconidae, Ichneumonidae, Platygastridae, Diapriidae and Proctutropidae).

The Chrysopidae larvae were determined under a stereomicroscope to genus level based on larval cephalic chaetotaxy (Monserrat 2016). Chrysopidae adults were sexed and identified to species level as described by Plant (2013) and Aspöck et al. (1980). Apart from *Chrysoperla lucasina* (Lacroix, 1912), cryptic species of the *Chrysoperla carnea*-group were identified to species level, according to the method described by Henry et al. (2002) and Chapman et al. (2006), by measuring the basal dilation of the metatarsal claw and the genital lip and chin of the male abdomen, and the values were compared to those reported by Henry et al. (2002).

### Pollen-foraging preference assay

A laboratory assay was performed in August 2017 to assess the pollen-foraging preferences of the common green lacewing, *Chrysoperla carnea* (Stephens, 1836), for the different plant species used in the field experiment. A colony was established for the trial, with lacewing individuals provided by Sautter & Stepper GmbH (Ammerbuch-Altingen, Germany). *Chrysoperla carnea* adults were fed with a mix of organic multi-flower honey (Ekologisk honung, COOP, Sweden) and pollen (Bipollen, Life, Sweden) (1:1 v/v) and kept in a plastic cage (31 × 21 × 12 cm) which was placed in a climatic chamber under controlled conditions at  $25 \pm 2^{\circ}\text{C}$ , 50–60% humidity and 16:8 hours L:D photoperiod. Larvae were fed *ad libitum* with *Ephestia kuhniella* Zeller eggs (Biotox, Livron-sur-Drôme, France) until pupation. Newly

emerged adults were sexed and starved for 24 h. A female and a male *C. carnea* were placed inside a plastic cage (18 × 18 × 10 cm) with a couple of fresh well-developed flowers with the stems in a 25 ml water cup with all possible plant species pairs combinations. Flowers were obtained from fresh plant material collected daily from the flower strips and kept in water. Adults were allowed to feed on the flowers for 24 h. They were then immediately frozen at -20 °C for gut content analysis. Six replicates of all pairs combinations were run, with a total of 72 *C. carnea* adults assayed (36 females and 36 males).

To analyse pollen consumption, the acetolysis technique was used for protein, lipid and insect tissue digestion, which facilitated accurate pollen identification (Jones 2014). This methodology has been previously used to study gut content in Chrysopidae adults (Medeiros et al. 2010; Andrade et al. 2017; Resende et al. 2017; Villa et al. 2019). Prior to acetolysis, wings, legs and antennae were removed from defrosted adults at room temperature to reduce residues following acetolysis. Each specimen was placed in a 2 ml microcentrifuge tube filled with 96% ethanol, where it was vortex-washed three times to remove external pollen on the cuticle. It was subsequently transferred to another microcentrifuge tube with 0.5 ml of glacial acetic acid to eliminate any residual water from the sample and was then crushed with a micropesle. Then, 0.5 ml of an acetolysis mixture of glacial acetic acid and sulphuric acid (9:1) was added and heated at 100 °C in a dry heater for 8 min. Afterwards, 0.5 ml of glacial acetic acid was added to stop the acetolysis process and centrifuged for 10 min at 10000 rpm, and the supernatant was discarded. The remaining reagents were removed by washing each sample three times with 1 ml of distilled water and then centrifuged for 3 min at 10000 rpm. The supernatant was discarded and the sample was placed in a fume hood at 70 °C for 12 h to evaporate the remaining distilled water.

For microscope slide preparation, 10 µl of 99% glycerine:distilled water (1:1) were added to the microcentrifuge tube, mixed with the sample, placed on the slides and protected by a glass coverslip sealed with nail varnish.

For pollen examination, three traverses were carried out across the coverslip at the centre of each slide under a microscope at 400X for pollen grain counting and 1000X for identification. Pollen grains were identified based on their morphological polar and equatorial axes, shape, aperture and exine ornamentation traits (Punt and Clarke 1980; Valdés et al. 1987; Faegri and Iversen 1989). The results obtained were compared with the surrounding vegetation present in the farm, the reference pollen collection from fresh flowers obtained from the flower strips and slides of Chrysopidae adults feeding on a single plant species. As pollen grain size varies enormously between plant species (Punt and Clarke 1980), we measured pollen consumption in

two different ways: as the number of pollen grains and as the volume of these pollen grains, as described by Buchmann and O'Rourke (1991).

Once the Chrysopidae adults collected in the field were identified to species level, their gut contents were examined following the methodology described above.

## Statistical analysis

We analysed the data using R version 3.6.3 (R Development Core Team 2017) and R Studio version 1.1.456 (RStudio Team 2016), with packages *glmmTMB*, (Brooks et al. 2017), *Matrix* (Bates and Maechler 2019), *lme4* (Bates et al. 2014) and *vegan* (Oksanen et al. 2018). For each model, residuals were examined for model validation using package *DHARMA* (Hartig 2018). We checked fixed factors for significance using Wald test from the *car* package (Fox and Weisberg 2019) and multiple comparisons between levels of the fixed factor were tested using Tukey's test with the packages *lsmeans* and *emmeans* (Lenth 2016, 2020).

To investigate the effect of farm and flower bloom on the number of predators and parasitoids collected in flower strips, suction sampling data were analysed using generalized linear mixed models (GLMMs). In each model, flower strips (row number) and sampling date were established as random effects to account for repeated measures on the same row at different times and population dynamics variation, respectively. Models were corrected for overdispersion by using a negative binomial (NB) distribution or by adding a quasi-Poisson structure (Table S3). We used non-metric multidimensional scaling (NMDS) based on the Bray–Curtis distance to graphically represent the level of association between the different predator and parasitoid groups and the farm and flower bloom for each plant species. For each NMDS, stress was checked using the goodness plot and the Shepard stress test. A permutational multivariate analysis of variance (PERMANOVA) using Bray–Curtis distance and 999 permutations was performed to test whether predator and parasitoid communities differed between farms and were affected by flower bloom of the different plant species.

The visual sampling data revealed a low number of pests and predators in cabbage plants. Thus, and to account for zero inflation, we used hurdle generalized linear mixed models (hurdle GLMMs) with a negative binomial (NB) error distribution for parasitoids and Poisson for predators, to investigate the effect of floral attractiveness, farm and treatment (control blocks and flower strip blocks) on the number of pests and predators. In each model, block number was set as random effect to correct for repeated measures over time.

To analyse pollen-foraging preferences, the number and volume of pollen grains from the dual-choice assay were analysed using GLMMs with a NB distribution and linear

mixed effect models (LMMs), respectively. A GLMM and a LMM were run for each pair of plants. Models included plant species and lacewing gender as fixed factors and specimen as random effect due to the paired data recorded on the same combination of plant species. Furthermore, separate NB GLMM and LMM were used for the whole dataset to establish the differences between genders in number and volume of pollen grains consumed. In each of these models, gender was included as fixed factor and specimen and plant species were set as random effects.

To analyse the pollen identified in the gut of the Chrysopidae collected by suction sampling from the field experiment, we used a GLMM with a NB distribution for number of pollen grains and a LMM for the volume of pollen grains. The models included gender, plant species (*B. officinalis*, *C. sativum*, *F. esculentum* and/or *P. tanacetifolia*), Chrysopidae species (*Chrysopa commata* data were excluded due to the low number of adults collected) and flower bloom of each plant species in the specific row where the Chrysopidae adults were collected as fixed effects. The interaction term between plant species and flower bloom was included to explore the effect of bloom at different times on pollen

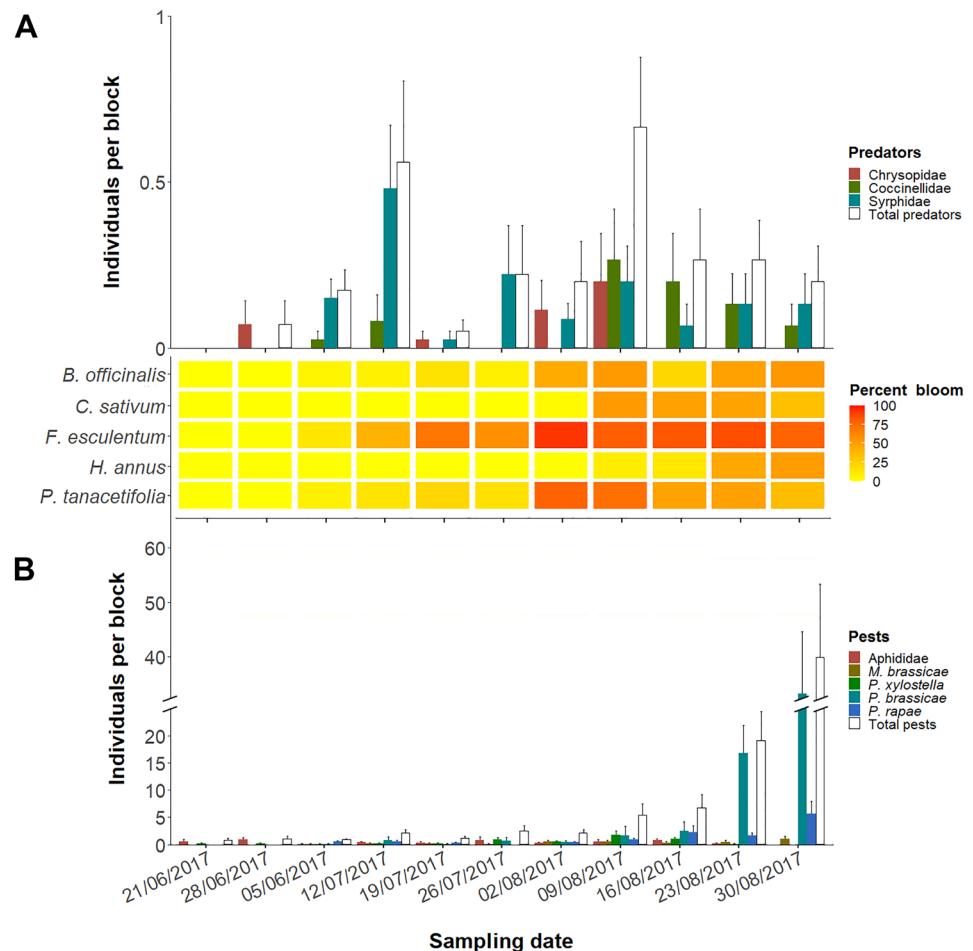
utilization by lacewings. Both models included farm as random effect.

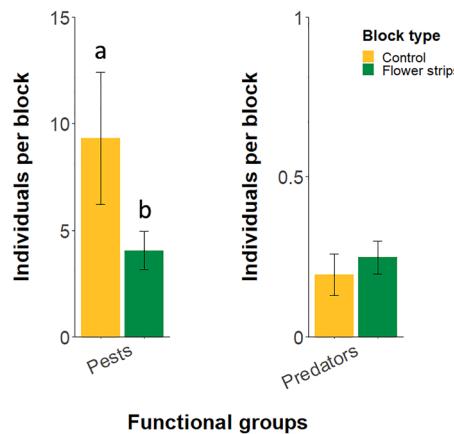
## Results

### Impact of flower strips on pests and predators in cabbage crops

The number of pests and predators recorded by visual inspection in cabbage plants throughout the season was, respectively,  $5.68 \pm 1.15$  (mean  $\pm$  SE) and  $0.23 \pm 0.04$  individuals per block (Fig. 2 and Table S2). The abundance of cabbage pests remained low during almost the whole sampling period, with a sharp increase observed at the end of August (Fig. 2b). Of the five pest species found in this study, *P. brassicae* and *P. rapae* were the most abundant (Fig. 2b and Table S2). We observed a drastic reduction in pest abundance in flower strip blocks ( $4.06 \pm 0.89$ ) compared with control blocks ( $9.32 \pm 3.11$ ) (Hurdle model,  $\chi^2 = 9.6$ , d.f. = 1,  $P < 0.01$ ) (Fig 3 and Table S3), farm (Hurdle model,  $\chi^2 = 15.1$ , d.f. = 2,  $P < 0.001$ ) and flower bloom variables (Hurdle model,  $\chi^2 = 6.4$ , d.f. = 1,  $P = 0.012$ ) (Table S3).

**Fig. 2** Number of **a** predators and **b** pests (mean  $\pm$  SE) recorded on each sampling date by visual inspection of the cabbage plant blocks. The heatmap shows the bloom percentage for each plant species in the flower strips





**Fig. 3** Number of predators and pests (mean  $\pm$  SE) recorded in blocks situated in the vicinity of flower strips and in the control blocks through visual inspection of cabbage plants. Different letters indicate statistically significant differences between treatments (Wald test,  $P < 0.05$ )

Predators showed two peaks of abundance in the middle of July and at the beginning of August (Fig. 2a). The most common predatory taxa found were Coccinellidae, Syrphidae and Chrysopidae (Table S2). The abundance of predators

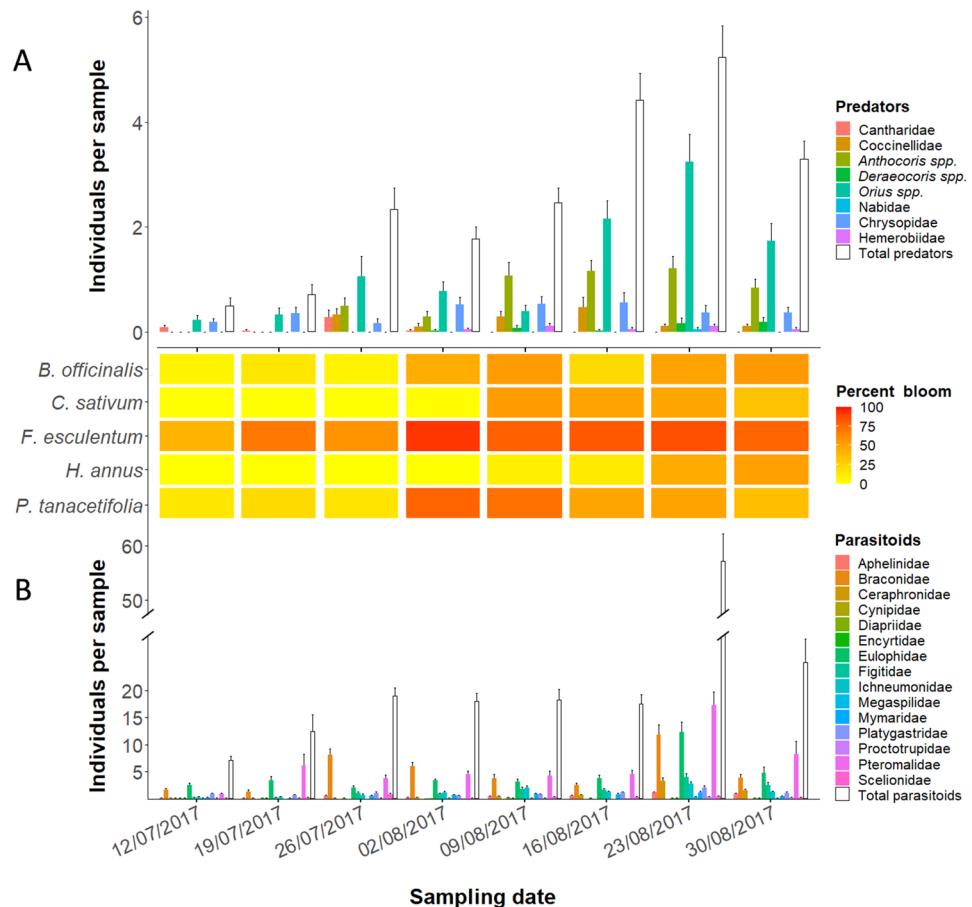
in the crop was not influenced by any of the three variables tested (Hurdle model,  $P > 0.050$ ; Table S3 and Fig. 3).

### Arthropod collection in flower strips

A total of 38,081 arthropods were collected from the flower strips. Diptera were the most abundant, accounting for 50.2% of the total, followed by Thysanoptera (13.81%) and Coleoptera (5.42%) (Table S4).

In terms of functional groups, parasitoids represented 17% of the total arthropods collected, with 6,349 individuals, with the families Pteromalidae (4.8%), Braconidae (3.61%) and Eulophidae (3.51%) being the most abundant. Predators, accounting for 2.59% of the total, belonged to the groups *Orius sp.* (0.95%), Syrphidae (0.64%), *Anthocoris sp.* (0.46%), Neuroptera (0.33%); Coccinellidae (0.12%), *Deraeocoris sp.* (0.05%), Cantharidae (0.03%) and Nabidae (0.01%) (Table S4). The number of predators and parasitoids gradually increased over the sampling period from the beginning of July to the highest peak of abundance at the end of August (Fig. 4a, b). The abundance of both these natural enemies also rose significantly with increasing percentages of flower bloom in the flower strips (predators GLMM,  $\chi^2 = 3.9$ , d.f. = 1,  $P = 0.047$ ; parasitoids GLMM,  $\chi^2 = 14.2$ ,

**Fig. 4** Number of **a** predators and **b** parasitoids (mean  $\pm$  SE) on each sampling date collected by suction sampling from the flower strips. The heatmap shows the percent bloom of each plant species in the flower strips



d.f. = 1,  $P < 0.001$ ) (Fig. 5 and Table S3). Predatory insect community analysis showed significant differences between farms (PERMANOVA,  $R^2 = 0.546$ , Pseudo-F = 16.2, d.f. = 2,  $P < 0.001$ ), but no flower bloom effect (PERMANOVA,  $R^2 = 0.040$ , Pseudo-F = 2.5, d.f. = 2,  $P = 0.076$ ) on community composition (Fig. 6a and Table S5). Cantharidae was associated with *P. tanacetifolia* bloom, while Coccinellidae, *Orius sp.*, *Anthocoris spp.* and *Deraeocoris spp.* were associated with *B. officinalis*, *F. esculentum*, *C. sativum* and *H. annus* bloom (Fig. 6a and Table S5). The parasitoid community was significantly affected by both farm (PERMANOVA,  $R^2 = 0.494$ , Pseudo-F = 17.1, d.f. = 2,  $P < 0.001$ ) and flower bloom (PERMANOVA,  $R^2 = 0.102$ , Pseudo-F = 7.1, d.f. = 2,  $P < 0.01$ ) (Fig. 6b and Table S5). Encyrtidae were associated with *C. sativum* and *H. annus* bloom, while Megaspilidae and Torymidae were associated with *B. officinalis* and *F. esculentum* bloom. The other parasitoid families were associated with *P. tanacetifolia* bloom (Fig. 6b).

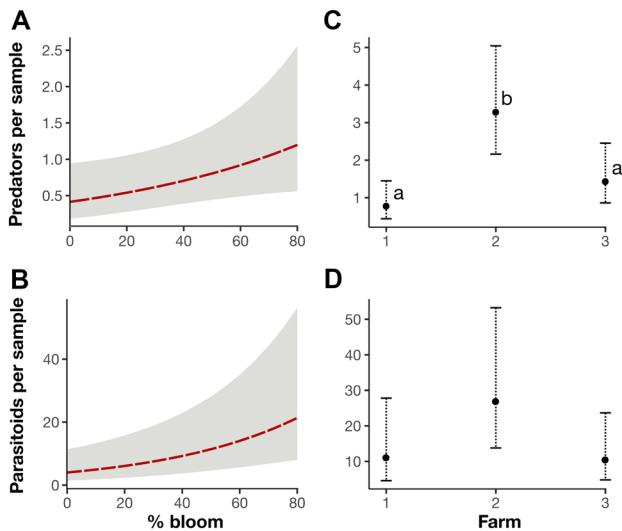
### Pollen consumption by Chrysopidae

*Chrysoperla carnea* individuals tested in the dual-choice laboratory assay consumed significantly more pollen from the paired plant species *P. tanacetifolia* and *C. sativum* as compared to *F. esculentum* and *B. officinalis*, with no differences observed between either paired plant species (Fig. 7a and Table S3). The overall number of pollen grains

consumed was significantly higher for females as compared to male specimens (Fig. 7c and Table S3). The volume of pollen consumed by lacewings yielded the same results for plant species and gender as for the number of pollen grains (Fig. 7b and d and Table S3).

A total of 54 adults (35 females and 19 males) and 59 larvae from the Chrysopidae family were captured in the flower strips. 48.2% (19 females and 7 males) were identified as *Chrysoperla carnea* (Stephens, 1836) *sensu* Henry, 42.6% (14 females and 9 males) as *C. lucasina* and 9.3% (2 females and 3 males) as *Chrysopa commata* Kis & Újhelyi, 1965. 71.2% (42 individuals) of the 59 larvae collected were identified at genus level as *Chrysoperla sp.* Steinmann, 1964 and 28.8% (17 individuals) as *Chrysopa sp.* Leach 1815.

We counted a total of 112,319 pollen grains from the 54 Chrysopidae adult guts, six of which contained no pollen. Despite feeding on all plant species present in the flower strips, the most consumed pollen was *P. tanacetifolia* in terms of quantity and total volume, as well as in the sampling period (Fig. 8a, Fig. 9 and Table S3). A small percentage (5.61%) of the pollen consumed corresponded to other plant taxa such as the families Asteraceae (1.24%), Brassicaceae (3.32%), Cariophyllaceae (0.59%), Pinaceae (0.001%), Poaceae (0.18%), Polygonaceae (0.03%) and Rosaceae (0.19%). No statistical differences were detected between Chrysopidae species (Fig 8b and Table S3). In addition, Chrysopidae females were observed to consume more pollen than males (Fig 8c and Table S3).

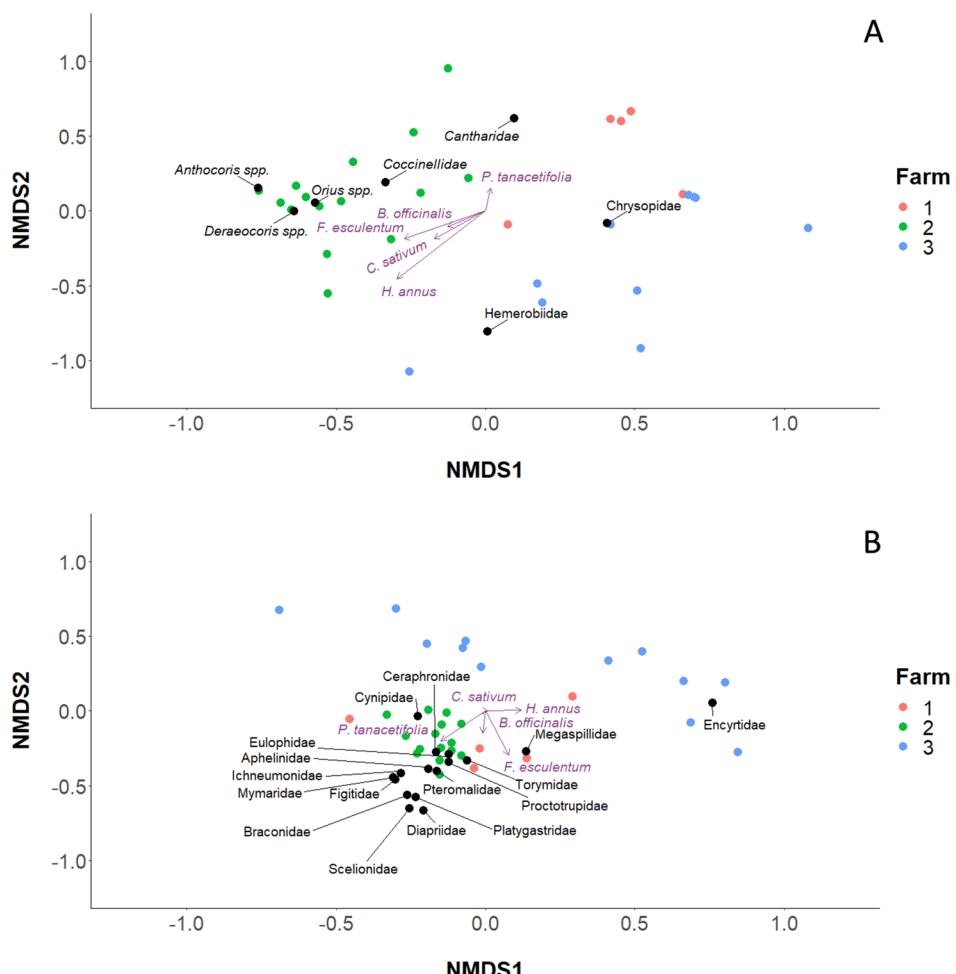


**Fig. 5** Predicted GLMM values ( $\pm 95\%$  CI) for the relationship between mean percentage of flower bloom in the flower strips and **a** predators ( $\chi^2 = 3.9$ , d.f. = 1,  $P = 0.047$ ) and **b** parasitoids ( $\chi^2 = 14.2$ , d.f. = 1,  $P < 0.001$ ) per suction sample, and **c** predators ( $\chi^2 = 17.9$ , d.f. = 2,  $P < 0.001$ ) and **d** parasitoids ( $\chi^2 = 4.8$ , d.f. = 2,  $P = 0.092$ ) per sample in the different farms. Different letters indicate statistically significant differences between farms (Tukey's test,  $P < 0.05$ )

### Discussion

The use of tailored flower strips as a conservation biological control strategy has been gaining traction in recent years, both as a research topic and in practical application terms (Gurr et al. 2017). This type of habitat manipulation strategy benefits from a cropping system-specific design and laboratory research, and requires multiple-year field testing showing pest control to achieve its full potential, to prevent failure and to improve adoption. In this study, we show that the implementation of tailored flower strips increases biodiversity and enhances natural enemy populations in cabbage crops. Through visual inspection, we demonstrated that flower strips in their vicinity have a positive impact on pest reduction in cabbage plants as compared to those in control blocks although, due to space constraints, we were forced to limit the amount of control blocks in each farm. Arguably, with a higher replication differences between treatments would have been more marked; however, we cannot discard a reduction in the pest pressure variability covered by our control plots that could have affected the results obtained. Furthermore, we cannot totally discard a certain interference of the flower strips on control blocks

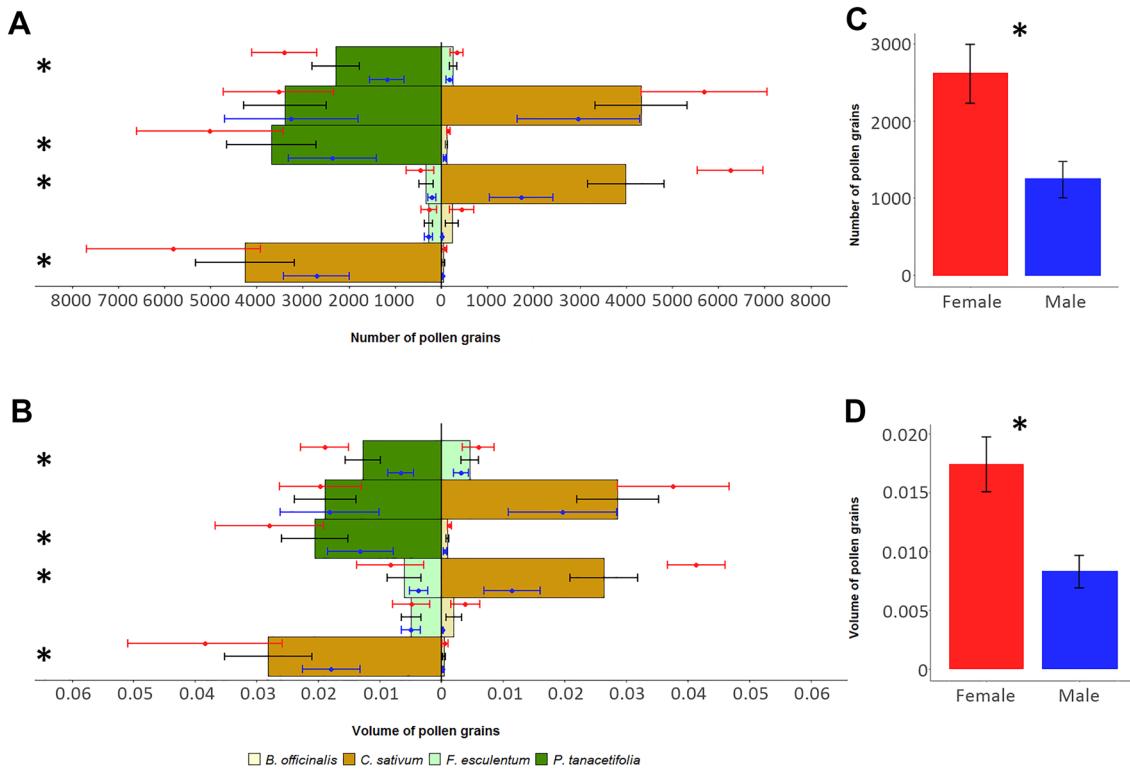
**Fig. 6** Non-metric multidimensional scaling (NMDS) biplot representing the community composition of **a** predators (stress = 0.156) and **b** parasitoids (stress = 0.143) collected by suction sampling from the flower strips. Coloured dots indicate different farms, and black dots indicate the position of predator and parasitoid groups in the multivariate space. Plant species in relation to the predators and parasitoids are represented by orchid-coloured arrows. Farms differed in relation to both predator and parasitoid communities (PERMANOVA, Pseudo- $F$  = 16.2,  $P < 0.001$ ; Pseudo- $F$  = 17.1,  $P < 0.001$ ; respectively). The parasitoid community composition was affected by the bloom rate of the plant species in the flower strips (PERMANOVA, Pseudo- $F$  = 7.1,  $P < 0.01$ )



due to the high mobility of the natural enemies attracted to the flower strips, such as lacewings, and the distances established in our experimental design. Our findings are in line with other studies of biocontrol with regard to *P. xylostella*, *P. rapae* and *M. brassicae* in the presence of flowering plants (Zhao et al. 1992; Lee and Heimpel 2005; Pfiffner et al. 2009; Winkler et al. 2009). For instance, *B. officinalis*, *C. sativum*, *F. vulgare* and *P. tanacetifolia* have previously been reported to be effective attractants of natural enemies such as Coccinellidae, Syrphidae, Chrysopidae and certain parasitoids (Colley and Luna 2000; Morris and Li 2000; Verkerk 2001; Boller et al. 2004; Sievwright et al. 2006; Alcalá Herrera et al. 2019b). Surprisingly, the large presence of predators and parasitoids on the flower strips did not increase their populations on adjacent cabbage plants. Cabbage pest populations were low in the study area at the time of the study according to the growers and our own observations. This may explain why predators preferred to remain in the flower strips, where more resources were available. Furthermore, we recorded other generalist predators in the flower strips, such as Araneae, Anthocoridae, Carabidae and Dermaptera (Cahenzli et al. 2019) which may contribute to

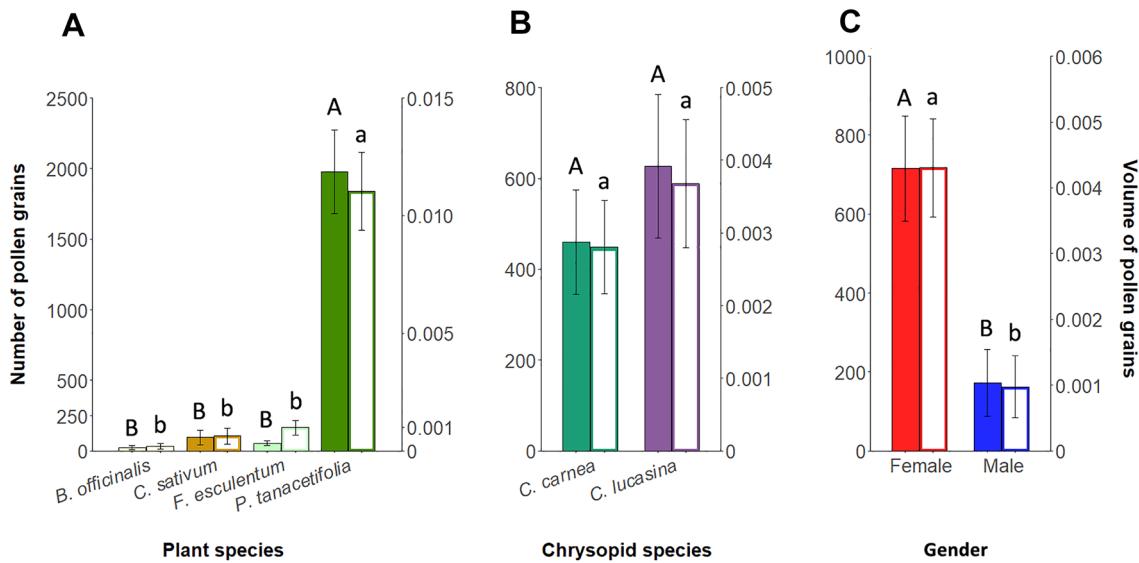
the reduction in pest populations in the vicinity of the flower strips, although these generalist predators were not recorded on the cabbage plants in our study.

It has been well established that an increase in landscape heterogeneity in terms of composition and configuration promotes natural enemies on agricultural land (Fahrig et al. 2011; Martin et al. 2019; Serée et al. 2020). We found that farm location had a major impact on predatory communities. For instance, the higher density of surrounding trees in farm 2 may have improved the abundance of predators collected compared to farms 1 and 3. Previous studies have reported that predators such as Chrysopidae, Coccinellidae and Syrphidae are attracted to flowering plants (Bertolaccini et al. 2011; Hatt et al. 2017), which is consistent with the predator community that we identified from suction samples. We show that parasitoid abundance was closely correlated with flower bloom, indicating a high attraction to plant resources in the cropping system of a large number of families within this group. This finding corroborates a simulation model developed by Bianchi and Wäckers (2008), which suggests that parasitoid aggregation in flower strips is due to feeding on floral nectar. Furthermore, Irvin et al. (2006) have shown



**Fig. 7** **a** Number and **b** volume ( $\text{mm}^3$ ) of pollen grains (mean  $\pm$  black SE) consumed by all *C. carnea* tested in each dual-choice combination. **c** Number and **d** volume of pollen grains consumed by gender (mean  $\pm$  black SE) for the whole trial. The red dots and error bars (mean  $\pm$  SE) in **a** and **b** represent pollen consumption by females and

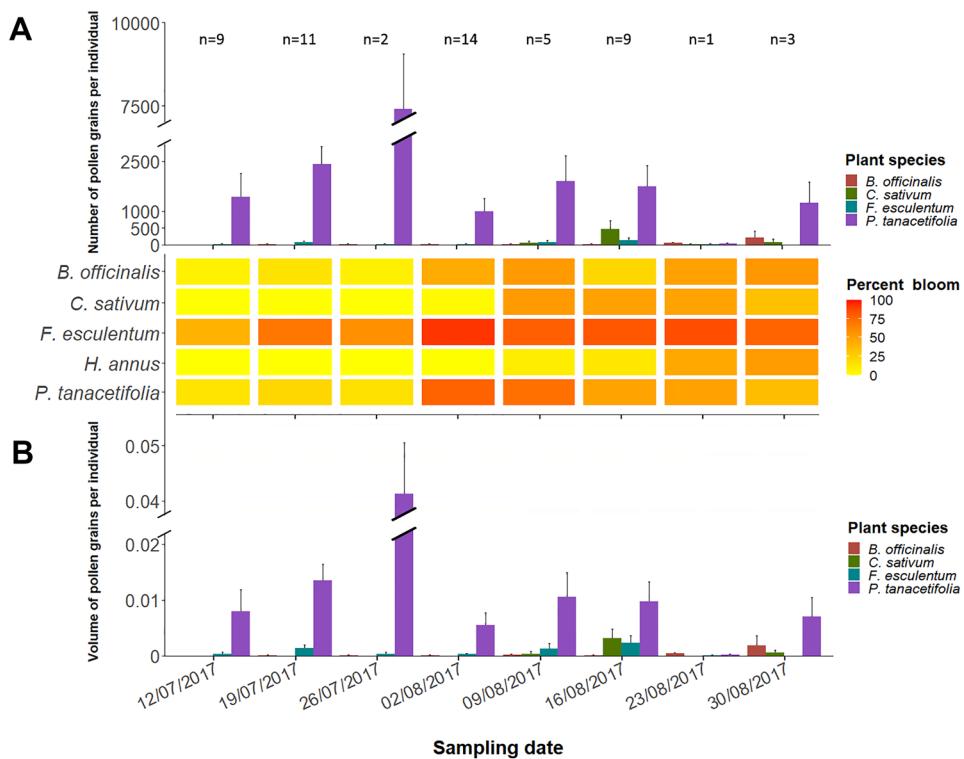
the blue dots and error bars (mean  $\pm$  SE) represent pollen consumption by males for each combination. Significant differences in number and volume of pollen grains consumed between paired plants and between genders are indicated with an asterisk (GLMM, Wald test,  $P < 0.05$ )



**Fig. 8** Number (left axes) and volume ( $\text{mm}^3$ ) (right axes) of pollen grains (mean  $\pm$  SE) consumed by the Chrysopidae collected from the flower strips by **a** plant species, **b** Chrysopidae species and **c** gender. Solid bars represent the number of pollen grains and hollow bars rep-

resent the volume of pollen grains. Significant differences are indicated by upper-case letters for the number of pollen grains and lower-case letters for the volume of pollen grains (GLMM, Turkey's tests,  $P < 0.05$ )

**Fig. 9** **a** Number and **b** volume of pollen grains ( $\text{mm}^3$ ) (mean  $\pm$  SE) consumed by the Chrysopidae collected from the flower strips in each sampling date. The heatmap represents the bloom percentage for each plant species in the flower strips



that the longevity of parasitoids fed on *F. esculentum* flowers was enhanced as compared to those fed on *P. tanacetifolia*. While predator abundance also correlated with flower bloom, predatory insect community composition was unaffected by the presence of flowers.

The Chrysopidae species assemblage in flower strips was composed of three species, *C. carnea* *sensu* Henry, *C. lucasina* and *C. commata*. This is the first record in Sweden of *C. commata* and *C. lucasina* to the best of our knowledge. Both *Chrysoperla carnea* *sensu* Henry and *C. lucasina*, which have a palyno-glycophagous diet, are frequently found in crops associated with herbaceous vegetation substrates (Canard 2001). In our study, Chrysopidae appeared to use the pollen resources provided by the flower strips, as evidenced by the ingested pollen, which belonged to the plant species sown in the flower strips. Furthermore, the number of pollen grains was correlated with the blooming period of their flowers. These results are in line with the findings of Bertrand et al. (2019), who observed that the majority of pollen consumed by *C. carnea* came from non-agricultural adjacent plants, even in crop-dominated landscapes, shifting from woody plant species in spring to herbaceous plants in the summer. In contrast, Villa et al. (2019) found an opposite trend, with *C. carnea* feeding mainly from olive pollen, rather than non-agricultural plants during the whole year. *Chrysoperla carnea* and *C. lucasina* are known to be opportunistic feeders that consume pollen according to flowering plant availability by blooming period

and abundance (Villenave et al. 2006; Alcalá Herrera et al. 2020). This apparent lack of specificity in feeding habits may underlie the contrasting results obtained in gut analysis of lacewings collected from different crops and adjacent vegetation. *Chrysoperla carnea* *sensu* Henry is considered a more polyphagous feeder than *C. lucasina*. *Chrysoperla lucasina* may prefer field crops and herbaceous plants, while *C. carnea* *sensu* Henry feeds on pollen from trees and herbaceous plants (Villenave et al. 2005). However, we found that *C. carnea* *sensu* Henry and *C. lucasina* collected in the field fed mainly on *P. tanacetifolia* pollen with no differences observed between either Chrysopidae species. Furthermore, under laboratory conditions, we determined that *C. carnea* prefers to consume pollen from *P. tanacetifolia* and *C. sativum*, with no differences being observed between either plant species. The tailored flower strips also attracted *C. commata*, another Chrysopidae species. Despite being predators, gut content analysis of *C. commata* adults also revealed pollen consumption, which is explained by the omnivorous feeding behaviour of *Chrysopa spp.* (Stelzl 1991, 1992; Canard 2001). We also observed that Chrysopidae females consumed a larger number of pollen grains as compared to males. This finding, which has been reported by other authors, is explained by the additional nutrients required by females for reproduction (Villenave et al. 2005; Alcalá Herrera et al. 2020). Flower strips were also found to be used as reproduction and shelter sites by *Chrysopa sp.* and *Chrysoperla spp.*, as evidenced by the larval stages

identified from the suction samples. This is in line with previous studies which have reported preimaginal-stage larvae and/or eggs on non-agricultural grass plants and flowering plants (McEwen and Ruiz 1994; Alcalá Herrera et al. 2019b).

## Conclusion

Our study shows the capacity of tailored flower strips to attract and support natural enemies, particularly green lacewings, in the cabbage agroecosystem. *Chrysoperla carnea sensu* Henry and *C. lucasina* were the most frequently collected Chrysopidae species in the flower strips. The identification of *P. tanacetifolia*, *B. officinalis*, *F. esculentum* and *C. sativum* pollen in the guts of Chrysopidae demonstrates that green lacewings feed from the plant species selected. Furthermore, our laboratory assay, which confirmed the feeding behaviour observed in the field, showed that *P. tanacetifolia* and *C. sativum* pollens were preferred by *C. carnea sensu* Henry and *C. lucasina* with no significant differences observed between either species. Finally, the presence of Chrysopidae larvae on flower strips proves that green lacewings adults use these sites for reproduction and shelter. Thus, in order to enhance conservation biological control in cabbage crops, we recommend using *P. tanacetifolia*, *C. sativum* and *F. esculentum* in flower strips to attract and support natural enemy populations, specifically those of Chrysopidae. However, further research is required to link pest reduction with natural enemy presence and explore the optimal flower strip designs in relation to crop surface. Long-term field studies are required to establish the full potential of our tailored flower strips on biological control services in cabbage and their interaction with local landscape configuration.

## Author contribution statement

BC acquired the funding and performed in supervision. BC and MT administered the project. BC, NA and RAH took part in conceptualization. BC and RAH selected the methodology and carried out the investigation. MP and RAH carried out formal analysis and was involved in visualization. RAH wrote the original draft. BC, MP, MT, NA and RAH took part in writing, reviewing and editing. All the authors revised and approved the manuscript.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10340-021-01419-7>.

**Acknowledgments** We wish to thank Elisabeth Örgen and Christina Winter (Jordbruksverket) not only for helping to make the project possible, but also for their invaluable assistance and comments, as well as Matthieu Laurent for field and laboratory assistance. We owe special thanks to the local farmers, Biskopshagens Odlingar, Mossagårdens Ekologiska Odling and Gårdsbutik, Kåsebergaodlingen Skåne, for granting us the use of their properties. We would also like to thank Oskar Hansson, Anna-Mia Björkholm (Hushållningssällskapet), Teun Dekker and Mattias Jonsson (SLU) for their advice and assistance, as well as Anne Birgitte Nielsen (Lund University) and Victor Monserrat, (Complutense University of Madrid) for resolving pollen and lacewing identification issues. Mercedes Campos and Javier Palenzuela (EEZ-CSIC) assisted with the production of pollen images and Michael O’Shea proofread the manuscript.

**Funding** Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. The study was financed by SLU Ekoforsk at the Swedish University of Agricultural Sciences (Project number 2016.4.1-742-4).

**Data availability** The data are available at <https://digital.csic.es/handle/10261/248177> with <https://digital.csic.es/handle/10261/248177>.

## Declaration

**Conflict of interest** The authors declare no conflict or competing of interest.

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