Original Articles

Wildflowers-pollinator-crab spider predator food-web as indicator of the agroecosystem biodiversity

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A B S T R A C T

Wildflowers play a crucial role in the functionality of the agroecosystem food chain. In order to investigate the role of biodiversity in the relationships between wildflowers, relative pollinators and their crab spider predators, six wildflower communities, characterized by a different botanical complexity, were sown in a two-year experiment. The selected Mediterranean species flowered between March and September, with a peak during May. The indices of dominance (D) and biodiversity (H') of the wildflower communities confirmed two expected levels of floristic complexity of the oligo-specific plant communities (only three species) in comparison to the poly-specific wildflower communities (a further 20 species for each oligo-specific plant community). The greater floristic biodiversity facilitated a greater pollinator biodiversity (bees, solitary bees, bumblebees, lepidoptera, diptera syrphidae and bombyliidae) but the respective total visitation rate was unchanged. In the case of the lower floristic complexity (oligo-specific plant communities), the dominance of bees increased, confirming their tendency to always choose the same wildflower species to visit. The evaluation of the crab spiders detected on flowers, waiting for pollinators, showed a significant reduction in the case of additional botanical complexity. Indeed, significant linear regressions ($p < 0.05$) showed a direct relationship between the number of crab spiders observed and the dominance indices of the various wildflower communities. The paper also discusses the possible ecological role of crab spiders in the biodiversity sustainability of the various wildflower communities.

1. Introduction

The growing need to protect and/or restore the biodiversity of the agroecosystem has stimulated research on feasible agronomic strategies to increase biological diversity both in terms of flora and fauna (Haaland et al., 2011). The sowing of wildflower strips has thus aroused great interest as these species, which have chromatically evolved to attract pollinators (Trunschke et al., 2021), and are closely linked by the mutualistic relationships with their pollinators.

The increasing erosion of floristic biodiversity is one of the causes of the rarefaction of bees and all other categories of pollinators. Often the scarcity of wildflowers in and outside of cultivated fields is one of the main causes of the rarefaction of pollinators (Twerski et al., 2022). This rarefaction can also occur as a side effect as the mutualistic wildflower-pollinator relationships are closely linked and increasingly hindered by the current climate change (Nicholson and Egan, 2020).

Wildflower strips on cultivated field margins favours biodiversity over time through sustainability criteria (Nicholls and Altieri, 2013). This ecosystem service not only benefits pollinators, but also other useful insects such as pest predators (Mei et al., 2021), thus promoting the agronomic sustainability of eco-friendly cropping systems (Kujawa et al., 2020), and can lead to increased crop productivity (Tschumi et al., 2016).

The level of the ecosystem service produced by wildflower strips is proportional to the diversity of their floristic associations, since each species has a specific mutualistic interaction with pollinators. For example, within the floristic diversity, pollinating butterflies are able to find the species, or those few species, that are suitable for their specialized oviposition and nourishment of the relative caterpillars (Kolkman et al., 2021). Similarly, hoverflies attracted to flowers with corollas that are particularly conformed to their mouthparts (Van Rijn and Wäckers, 2016) are not only useful as pollinators but also as aphidophages during their larval stage (Pekas et al., 2020) thus constituting a further ecosystem service aimed at implementing sustainability in the defense of adjacent crops. Indeed is now widely believed that environmentally-friendly systems need biodiversity to achieve long-term sustainability (Crowder and Jabbour, 2014).

In terms of assessing the level of agroecosystem sustainability, arthropods are a valid indicator of the ecosystem service supported by biodiversity (Hoffmann et al., 2022), especially in terms of spiders (Campbell et al., 2020). Of these, crab spiders (Thomisidae) are crucial as they have evolved to prey on pollinators: camouflaged on flowers or inflorescences the spiders wait for the pollinators, and then capture and feed on them. The abundance of crab spiders highlights the richness of

wildflowers and their pollinators, thus resulting in a valid indicator of the biodiversity of the agroecosystem's flora and fauna. In other words, the abundance of crab spiders indicates the trophic pyramid of the plant-pollinator-predator food-webs.

Crab spiders do not weave webs (Dumke et al., 2018), but camouflage themselves by imitating the flowers' colours and attracting pollinators with deception using chromatic reflections of ultraviolet light (Heiling et al., 2003), thus simulating the natural flower reflectance (Cheng et al., 2006). However, it seems that pollinators are aware of these crab spider strategies, and thus reduce their rate of flower visits (Dukas and Morse, 2003), often stopping on them for shorter amounts of time (Huey and Nieh, 2017). In fact, despite the mimicry and chromatic attraction strategies of crab spiders, pollinators tend to avoid the flowers on which they are able to identify their predators (Reader et al., 2006), especially in the case of solitary bees and syrphid flies (Brechbühl et al., 2010).

The rate of flower visits has been shown to decrease to a third in the presence of crab spiders and that the time spent on the flowers was halved (Romero et al., 2011) with the consequent decrease of the relative pollination.

The present experimentation arises from the hypothesis that the abundance of crab spiders may have an ecological role in maintaining a floristic balance by discouraging the pollination of the most abundant species. Indeed the complex relationship between the trophic levels of plants-pollinators-spiders is not completely clear, especially in terms of biodiversity conservation.

The aims of this work were: i) to clarify the relationship between the biodiversity of wildflowers and that of their respective pollinators and ii) crab spiders and iii) to find a key to understanding the ecological role of crab spiders in the dynamics of floristic equilibrium in wildflower communities.

2. Materials and methods

2.1. Plant material

Floristic investigations carried out in the last decade have identified various wildflower communities in diversified agro-environments in Tuscany (Table 1).

These wildflower communities were shown to be dominated by three species in each of their respective agro-environments. A simultaneous seed collection from different rural environments in Tuscany (Table 2) led to an increased biodiversity of the aforementioned plant communities with twenty additional wildflower species. The criterion for choosing the various wildflower species (listed in Tables 1 and 2) derives from their marked and demonstrated attractiveness (Rollin et al., 2016) for pollinators.

In the summer of 2018 and 2020, seeds of all the cited wildflower species were collected from the relative senescent plants and were transferred to the laboratory, where they were cleaned and stored at room temperature until use.

Table 2

Botanical information (Pignatti, 1982) of the additional wildflower species included in the three aforementioned plant communities.

Additional wildflower species	Botanic family	Life form[1]	Environment of seed collection	Chorology
Adonis annua L.	Ranunculaceae	T	Winter cereals	Eur-Medit.
Althea cannabina L.	Malvaceae	H	Field margins	S-Europ.
Anchusa hybrida Ten.	Boraginaceae	H	Arid grasslands	Steno-Medit.
Anthemis mixta L.	Asteraceae	T	Arid grasslands	Steno-Medit.
Campanula rapunculus L.	Campanulaceae	H	Grasslands	Eur-Asiat.
Cirsium arvense (L.) Scop.	Asteraceae	T	Winter cereals	Eur-Asiat.
Glebionis coronaria (L.) Spach	Asteraceae	T	Pastures	Steno-Medit.
Lavatera punctata All.	Malvaceae	T	Arid grasslands	Steno-Medit.
Linum bienne Mill.	Linaceae	H	Grasslands	Eur-Medit.
Lycnis flos cuculi L.	Cariophyllaceae	H	Grasslands	Eur-Siber.
Malva sylvestris L.	Malvaceae	H	Field margins	Eur-Asiat.
Matricaria chamomilla L.	Asteraceae	T	Winter cereals	Sub-Cosmop.
Orlaya grandiflora (L.) Hoffm.	Apiaceae	G	Rocky soils	Cent. Europ.
Ranunculus arvensis L.	Ranunculaceae	T	Field margins	Eur-Asiat.
Salvia verbenaca L.	Lamiaceae	H	Arid grasslands	Eur-Medit
Saponaria officinalis L.	Cariophyllaceae	H	Road borders	Eur-Asiat.
Scabiosa columbaria L.	Dipsacaceae	H	Road borders	Eur-Asiat.
Silene conica L.	Cariophyllaceae	T	Arid grasslands	Eur-Asiat.
Silene gallica L.	Cariophyllaceae	T	Arid grasslands	Eur-Medit
Trifolium incarnatum L.	Fabaceae	T	Pastures	Eur-Medit.

1 T = Therophyte, H = Hemicryptophyte, G = Geophyte.

2.2. Agronomic environment

The trials were carried out in the experimental fields of the Department of Agriculture, Food and Environment of Pisa University (43°40′39′′N, 10°19′46′′E) in a sandy-soil (sand 70 %; lime 18 %; clay 12 %; pH 7.5; organic matter 1.8 %). The fields surrounding this experimental site were cultivated with non-entomogamous crops such as wheat and maize. The wildflower plots were placed in such a way that they could have the role of "ecological corridor" between the agricultural land and the surrounding Mediterranean Chaparral located a few hundred meters away.

Table 1

Botanical information (Pignatti, 1982) of the plant species found into the three different agro-environments and studied as "dominant" wildflower communities.

Wildflower Community	Agro-environment[1]	Locality	Species	Botanic family	Life Form[2]	Chorology
1	Emmer wheat fields	Corfino(LU) 44°19′ N 10°38′E	*Agrostemma githago* L.	Cariophyllaceae	T	Eur-Siber.
			Centaurea cyanus L.	Asteraceae	T	Steno-Medit.
			Papaver rhoeas L.	Papaveraceae	T	Eur-Medit.
2	Forage crops fields	S. Piero (PI) 43°68′ N 10°34′E	*Anthemis arvensis* L.	Asteraceae	T	Eur-Medit.
			Consolida regalis Gray	Ranunculaceae	T	Eur-Medit.
			Nigella damascena L.	Ranunculaceae	T	Steno-Medit.
3	Pastures	Santa Luce (PI) 43°47′N 10°56′ E	*Echium vulgare* L.	Boraginaceae	H	Eur-Medit.
			Glebionis segetum (L.)Fourr.	Asteraceae	T	Eur-Medit.
			Verbascum blattaria L.	Scrophulariaceae	H	Eur-Asiat.

1 Agronomic environment where the three plant communities were observed and where the relative seeds were collected.
2 T = Therophyte, H = Hemicryptophyte.

In order to minimize the presence of unwanted weeds, the soil was prepared in a stale seedbed during autumn–winter in order to limit the majority of the weed seed bank (Benvenuti et al., 2021). The climate of this agro-environment is typically Mediterranean with annual minimum and maximum daily air temperatures that are vary greatly throughout the year (means of experimental periods: February 2/10 °C, March 5/15 °C, April 7/18 °C May 12/22 °C, June 16/27 °C, July 19/30 °C, August 18/30 °C, September 15/26 °C, min/max respectively). The rainfall in this area during the experimental period (February-September 2019 and 2021) was about 460 mm and 510 mm during 2019 and 2021, respectively, mainly distributed in the spring.

2.3. Wildflower field sowing

All wildflower communities were sown in plots (3 × 5 m) in the first ten days of February of 2019 and 2021. The sowing was also carried out in 2020 but the collection was affected by the Covid-19 pandemic and consequently was not used.

The sowings of the three wildflower communities (shown in Table 1, with the abbreviations 1, 2 and 3) were carried out alone (only three "dominant" species) in a balanced mixture between each species (one third respectively). In the remaining plant communities (hereafter 1+, 2 + and 3 +), the seed quantity per square meter was halved and replaced by further wildflower biodiversity.

This additional biodiversity was achieved by replacing the missing half dose of seeds of the above-cited plant communities with a balanced mixture of twenty other wildflower species (hereafter "additional biodiversity") shown in Table 2. The seed dose for all plant communities was standardized to an expected density of about 300 plants m^{-2}.

In order to obtain the aforementioned balanced plant density within each wildflower community, the seed dose was calculated according to previous experiments (Bretzel et al., 2012). This calculation enables the dose of each species, of the desired plant density, to be obtained in the directly proportional way to their 1,000 seed weight and inversely to their germinability. After seeding, light rolling was applied in order to facilitate contact with the soil in order to provide a uniform seed germination and seedling emergence.

Furthermore was checked the absence of spatial autocorrelation between wildflower plots and pollinator visits using Moran's I test.

All the experiments entailed the use of a randomized complete block design for both years (2019 and 2021) and were conducted with three replicates per experiment with a total of 24 plots (six different wildflower communities (three with only three species, and three with an additional biodiversity of a further 20 species) × 4 replicates).

2.4. Verification of the expected botanical structure in emerging wildflower communities

In mid-March, one month after sowing the six plant communities, their current botanical structure was checked. In each plot, a metal rectangle (20 × 30 cm) was randomly placed in order to identify and count the seedlings of the various species sown. In each plot, 10 counts were carried out for a total of 240 (10 counts × 6 plant communities × 4 replicates). The data were processed as absolute density (plants m^{-2}) and then transformed into relative density (%) in order to calculate (see after) the biodiversity and dominance indexes of the various plant communities.

2.5. Flowering dynamics

For each of the six wildflower communities (1, 2, 3, 1+, 2 + and 3 +), ten plants of each species were marked with paper labels to evaluate the flowering dynamics. Data were expressed as flowering period (beginning and end) throughout the experimental period. Data were collected weekly for each wildflower species.

2.6. Evaluation of flower visitors

Evaluations regarding pollinator biodiversity were carried out during the full flowering period of May because during this period, all species reached the flowering phenological-stage (generally as a peak of maximum flowering). For each date (two days per week, for a total of eight days per year), each evaluation was carried out at the following times: morning (from 10:00 to 12:00) and early afternoon (from 14:00 to 16:00). Data were collected by placing a 1 m^2 plastic frame along of each experimental plot. Pollinators landing on the flowers inside this area (with evident feeding of pollen and/or nectar) were observed, counted and classified into the following pollinator categories: honey bees, solitary bees, bumblebees, flies (hoverflies, bee flies), and butterflies.

Pollinator species that could not be reliably identified in the field were collected using a sweep net and identified in the laboratory. Observations lasted five minutes (for each of the aforementioned morning and afternoon evaluations) for each of the 24 plots. From these data, the percentages of visits of the various pollinator categories (bees, solitary bees, bumblebees, flies (hoverflies, bee flies), and butterflies) were calculated. The number of total pollinator visits for each wildflower community was also calculated as pollinator visits m^{-2}h^{-1}.

2.7. Quantification of crab spiders

Since the presence of crab spiders is typically less frequent than pollinators, the surveys of these arthropods were carried out along the entire 50 cm edge of each plot for a total of 7 m^2 of surface of each sampled sub-plot. This sampling method was chosen in order not to damage the vegetation. The low mobility of crab spiders (long periods on the flowers waiting to capture the pollinators) also entailed quantifying them in terms of the number of crab spiders observed per sampling-day (altogether eight days during the two experimental years) per unit of surface area. In other words, the outermost areas of each plot (delimited by a colored ribbon placed at the height of one meter using sticks fixed to the ground) were observed extremely accurate in one single attempt The number of observed individuals per day per square meter was recorded.

The samplings of crab spiders camouflaged on the flowers waiting to capture the pollinators were carried out on the same dates selected for the evaluation of pollinator dynamics eight per year carried out in May 2019 and 2021). The sunniest hours of the day (12:00 to 14:00) were selected for logistical reasons (chronological distribution of both pollinator and crab spider evaluations within the sampling day).

2.8. Calculation of dominance and diversity indexes of wildflowers and pollinator communities

Data of the six wildflower communities and the related pollinator groups were used to calculate the Shannon diversity index (H') as follows:

$$H' = - \sum_{i=1}^{k} p_i \log p_i$$

where k is the number of wildflower species or pollinator groups, and p_i is the fraction of individuals belonging to the ith each wildflower community or pollinator group.

In addition, Simpson's index of dominance (D) of wildflowers or pollinators was calculated, as below:

$$D = \sum (n_i/N)^2$$

where n_i is the number of individuals of a specific wildflower community or pollinator group (bees, solitary bees, bumblebees, flies (hoverflies, bee flies), and butterflies), and N is the total number of plants or pollinators.

2.9. Statistical analyses

All the experiments used a randomized complete block design for both 2019 and 2021, and were all conducted with four replicates. Data were pooled over the two experimental years because there was no significant interaction (means analysed by one-way ANOVA, p < 0.05) between 2019 and 2021. After the homogeneity test of variance and arcsine transformation of these data expressed as a percentage to normalize their distribution (Steel & Torrie 1980), all data were subjected to a two-way ANOVA (with two factors: a) species composition (three levels), and b) species diversity (two levels: basic and enriched) using Duncan's Multiple Range test (p < 0.05 and/or p < 0.01) for mean separation (least-significant difference, LSD) among all treatments.

In addition, linear regressions between the Shannon diversity index (H') or Simpson dominance index (D) of wildflower communities and the number of crab spiders detected were calculated. For each statistical analysis, CoHort (Minneapolis, MN) was used.

3. Results

3.1. Performance of wildflower communities

The three oligo-specific floristic communities obtained by sowing only three wildflower species, showed a much higher level of dominance
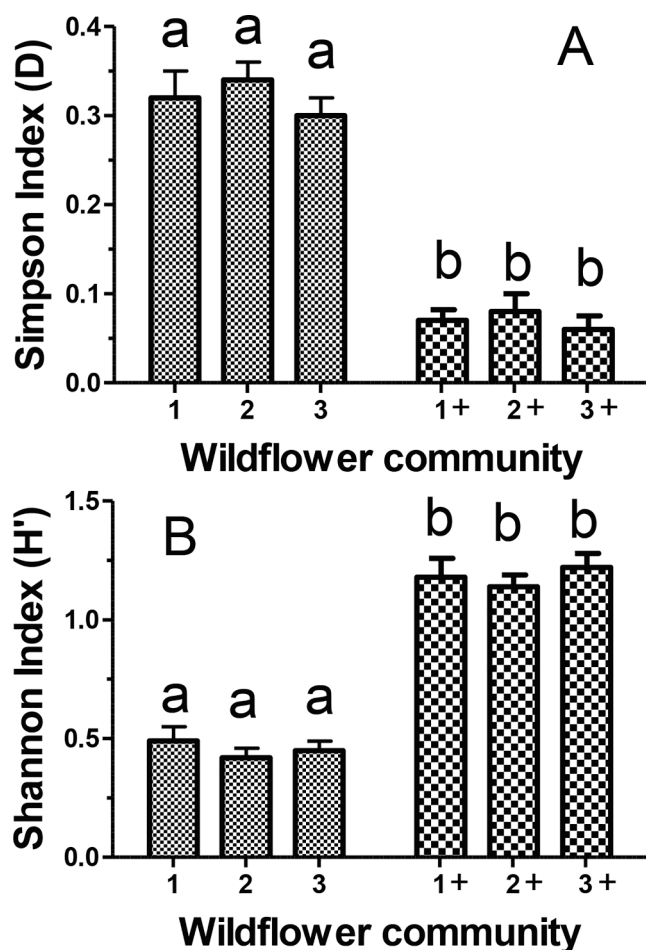

Fig. 1. Indexes of dominance (Simpson index, D, A) and biodiversity (Shannon index, H') of oligo-specific (1, 2 and 3, see Table 1) or poli-specific (1+, 2 + and 3+, where "+" means the further wildflower species shown in Table 2) plant communities. Means with the same letter did not differ at P < 0.05 according to the Duncan's Multiple Range Test. Vertical bars indicate the ± standard error of the means.

(Simpson index significant for P < 0.05, Fig. 1A) compared to the poly-specific communities in which a further 20 species were included. Consequently, the latter poly-specific floristic communities highlighted a much lower level of biodiversity (also significant for P < 0.05, Fig. 1B). The sowing of suitable seed mixes (see Materials and methods) led to a decidedly balanced seedling emergence (total plant density of about 300 plants m^{-2}, data not shown) so that within each floristic community (oligo-specific and poly-specific), no significant differences (P < 0.05) were detected for either of the above mentioned indexes.

All 29 wildflower species (nine of the oligo-specific plant communities plus 20 of the additional biodiversity) reached the phenological stage of flowering between March and September (Table 3).

However, during May, all the species tested showed full flowering and thus this was the most suitable period to monitor the pollinator activity within the various plant communities.

3.2. Pollinator abundance and biodiversity

The number of pollinator visits was significantly different (P < 0.05) in the three oligo-specific wildflower communities consisting of only three species (Table 4).

The most visited plant community was the one composed of *Centaurea cyanus*, *Agrostemma githago* and *Papaver rhoeas*, while the least visited (in any case well over 100 visits m^{-2}h^{-1}) was *Echium vulgare*, *Glebionis segetum* and *Verbascum blattaria*. Intermediate values were shown by the floristic association constituted by *Anthemis arvensis*, *Consolida regalis* and *Nigella damascena* (132.5 visits m^{-2}h^{-1}).

Replacing half of the species with the inclusion of 20 additional species did not significantly change (P < 0.05) the visit rate shown by the oligo-specific wildflower communities. The increase in floristic biodiversity often led to a diversification in the category of pollinators monitored on flowers. The increase in the biodiversity of wildflowers tends to decrease, almost always significantly (P < 0.05), the percentage of flower visits carried out by bees and/or bumblebees, but in our case this led to an increase in diptera, both syrphidae and bombyliidae and overall in solitary bees. Finally, the increase in floristic diversity led to a small and non-converging change in the percentage of flower visits carried out by lepidoptera.

The fact that floristic diversity influences the diversity of pollinators was confirmed by the linear regressions (significant at P < 0.05) obtained using the dominance and biodiversity indexes of both wildflowers and pollinator communities. In fact these linear regressions showed that with the higher wildflower dominance in the three oligo-specific plant communities, the relative pollinator biodiversity decreased (Fig. 2A). Consequently, as the floristic biodiversity increased, the dominance of the relative pollinators decreased (Fig. 2B).

3.3. Number of crab spiders and connection with the wildflower biodiversity

The highest number of spider crabs (Table 5) was found in oligo-specific wildflower community 1 (0.31 individuals m^{-2} day^{-1}) in which the highest rate of pollinator visits was detected (see Table 4).

On the other hand, the lowest number of spider crabs was observed in poly-specific community 2 (0.11 individuals m^{-2} day^{-1}) in which the lowest rate of visits by pollinators was found. In each of the three wildflower communities, the increase in floristic diversity (insertion of additional 20 species) resulted in a significant reduction (P < 0.01 in 1 and P 0.05 in 2 and 3) in the number of crab spiders. Some of these observations of spider crabs camouflaged on the flowers, for example the araneid family of thomisidae (belonging to genus *Thomisus*, *Misumena* and *Synema*), are shown in Fig. 3. Sometimes they were observed in an unequivocal phase of pollinator capture (Fig. 3F).

To test the hypothesis that the abundance of pollinators may be related to the number of crab spiders, linear regressions were performed between these two parameters. However, this regression did not show a

Table 3
Calendar of flowering of the tested wildflowers during the experimental periods.

Wildflower Species	Flowering period (months of the year)											
	J	F	M	A	M	J	J	A	S	O	N	D
Adonis annua L.			●	●	●							
Agrostemma githago L.			●	●	●	●						
Althea cannabina L.				●	●	●	●	●				
Anchusa hybrida Ten.				●	●	●						
Anthemis arvensis L.				●	●	●						
Anthemis mixta L.				●	●	●	●					
Campanula rapunculus L.				●	●	●						
Centaurea cyanus L.				●	●	●						
Cirsium arvense (L.) Scop.			●	●	●	●						
Consolida regalis Gray				●	●	●	●	●				
Echium vulgare L.				●	●	●	●					
Glebionis coronaria (L.) Spach				●	●	●	●					
Glebionis segetum (L.)Fourr.			●	●	●	●						
Lavatera punctata All.				●	●	●	●					
Linum bienne Mill.			●	●	●							
Lycnis flos cuculi L.			●	●	●	●						
Malva sylvestris L.				●	●	●	●	●	●			
Matricaria chamomilla L.			●	●	●	●						
Nigella damascena L.				●	●	●						
Orlaya grandiflora (L.) Hoffm.			●	●	●							
Papaver rhoeas L.			●	●	●	●						
Ranunculus arvensis L.			●	●	●							
Salvia verbenaca L.				●	●	●						
Saponaria officinalis L.				●	●	●	●					
Scabiosa columbaria L.				●	●	●	●	●				
Silene conica L.			●	●	●	●						
Silene gallica L.			●	●	●	●						
Trifolium incarnatum L.			●	●	●							
Verbascum blattaria L.				●	●	●	●					

Table 4
Flower visits percentages by different pollinator groups into the several wildflower communities and relative total visitation rate. Values are followed by ± standard error of the means. Means with the same letter did not differ within rows at P < 0.05 according Duncan's Multiple Range test.

Wildflower community	Pollinator groups						Total visitation rate[1]
	Bees	Solitary bees	Bumblebees	Diptera syrphidae	Dipera bombyliidae	Lepidoptera	
	Visits (% of the total)						
1	33.4 ± 1.4 a	28,7 ± 2,1b	17.2 ± 1.8c	5.7 ± 0.3 d	3.4 ± 0.2 e	11.6 ± 1.5 cd	153,4 ± 22.6 a
1 + ADDITIONAL BIODIVERSITY	7.5 ± 1.3 d	38,8 ± 1,6 a	15.5 ± 1.5c	12.8 ± 1.2 cd	6.6 ± 0.3 d	18.8 ± 1.7c	149.7 ± 11.2 a
2	38.4 ± 2.5 a	25.2 ± 3.1b	14.8 ± 1.2c	3.9 ± 1.2 e	2.2 ± 0.1 e	15.5 ± 0.2c	132.5 ± 13.1b
2 + ADDITIONAL BIODIVERSITY	5.3 ± 2.2 d	44.5 ± 2.2 a	11.3 ± 1.1 cd	15.6 ± 1.4c	3.1 ± 0.5 e	20.2 ± 1.3 bc	140.7 ± 15.8b
3	35.0 ± 2.1 a	23.4 ± 3.1b	24.2 ± 2.0b	12.2 ± 1.1 cd	1.2 ± 0.2 e	4.1 ± 0.5 e	117.4 ± 15.3c
1 + ADDITIONAL BIODIVERSITY	26.2 ± 1.4b	38.9 ± 2.5 a	14.5 ± 1.6c	18.4 ± 2.0c	9.5 ± 0.6 d	12.5 ± 1.1 cd	123.0 ± 10.7c

[1] Flower visitors m^{-2}h^{-1}.

close relationship between them (Fig. 4), highlighting a lack of statistical significance (at P < 0.05) and a decidedly low R^2 value.

In contrast, the use of the dominance and biodiversity indexes showed (Fig. 5) significant (P < 0.05) relationships between these two parameters with the observed crab spiders. In particular a significant (P < 0.05, R^2 = 0.75) regression was found that was directly related with the wildflower dominance (Fig. 5A) and number of crab spiders, and this last parameter was found inversely related with wildflower biodiversity Fig. 5B).

4. Discussion

4.1. Biodiversity and flowering dynamics of wildflower communities

The goal of creating two diversified levels of floristic biodiversity was fully achieved (Fig. 1). In fact, the three wildflower communities, consisting of only three species (oligo-specific plant communities), showed decidedly higher dominance indexes (D) compared to the poly-specific plant communities, in which half the plant density (about 300 m^{-2} plants) was replaced by an additional twenty species. The objective of creating two groups of wildflower communities in the field characterized by highly diversified biodiversity levels was thus reached and confirmed by the respective Shannon indices (H'). This floristic balance might, in fact, not have been achieved given the diversified germination ecology of the various wildflower species, especially in terms of seed dormancy (Baskin and Baskin, 2004). We believe our success was due to previous experience regarding the sowing and agronomic management of similar wildflower strips (Benvenuti and Bretzel, 2017).

As expected, the flowering dynamics were not synchronized in the various species, some occurring earlier (e.g. thirteen species began to bloom in March such as *Agrostemma githago*, *Glebionis segetum*, *Papaver rhoeas*, etc.) and others later (some species were still in bloom in August, e.g. *Consolida regalis*, *Scabiosa columbaria* and *Malva sylvestris*). However, during May all the species reached the phenological stage of full flowering as typically happens in the most common wildflowers in Mediterranean environments (Potts et al., 2006). May was thus the most suitable month to carry out surveys of both pollinators and their related predators.
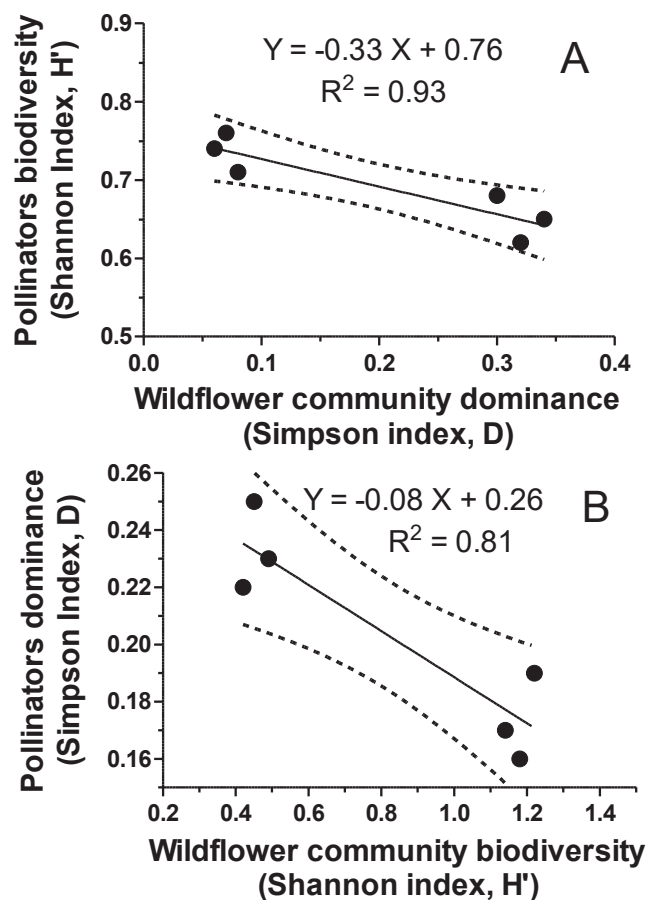
Fig. 2. Linear regression between (p > 0.05) the calculated indexes of dominance (Simpson index, D) of the studied wildflower communities and the biodiversity (Shannon index, H') of the pollinator categories (A) and *vice-versa* (B). The equations (significant at P < 0.05), the relative R^2 and the 95 % of confidence levels are reported.

Table 5
Means of the crab spiders observed into the several wildflower communities during the experimental periods. Values are followed by ± standard error. The pairs of wildflower communities (with or without additional biodiversity) followed by one or two asterisks shows a significant difference for P < 0.05 or 0.001 respectively (df = 38, t = 1.028, p = 0.032) according to the Duncan's Multiple Range test. Asterisks indicate the significance levels (** = p ≤ 0.01; * = p ≤ 0.05).

Wildflower community	Mean observed spider crabs (number m^{-2} day^{-1})	Statistical Significance
1	0.31 ± 0.3	**
1 + Additional biodiversity	0.16 ± 0.2	
2	0.27 ± 0.2	*
2 + Additional biodiversity	0.15 ± 0.1	
3	0.22 ± 0.2	*
3 + Additional biodiversity	0.11 ± 0.1	

4.2. Number of pollinator visits and biodiversity

The greater diversity of wildflowers did not lead to a higher number of visits, but rather to a diversification of the pollinator categories (Table 4). The fact that the increase in floristic diversity decreased the rate of visits by bees is probably due to the habitual nature (Waser, 1986) of these pollinators, who prefer to visit flowers of the same species

daily. A similar trend was also shown by bumblebees, which also seem to select the most abundant species (Gegear and Laverty, 2005) as also occurred in the wildflower communities without additional biodiversity.

In contrast with this generalized decrease in pollinators characterized by constancy with an increase in floristic biodiversity, diptera syrphidae showed an increase in visitation rates, generally due to their scarce or absent pollination specialization with certain wildflower species. In fact these generalist pollinators are less susceptible to rarefaction in those fragmented habitats (Jauker et al., 2019) where there are fewer species to visit.

In contrast, although being the least frequent pollinator category, diptera bombyliidae showed an increase with the increase in floristic biodiversity. This is probably due to the fact that they show specialization links with some species with a particular calyx shape, inserted with the additional biodiversity (such as *Anchusa hybrida*, *Salvia verbenaca*, *Silene gallica*, etc.) suitable for being visited by pollinators with small and elongated mouthparts.

Similarly, the increase in lepidoptera, following the increase in floristic diversity, can also be explained by the introduction of additional species with flowers characterized by an elongated calyx (for example *Lycnis flos cuculi*, *Saponaria officinalis*, *Silene conica*, etc.) which can be more easily visited by the mouthparts of this category of pollinators.

Even the volatile compounds emitted by the flowers (Benvenuti et al., 2020) also play a role as they facilitate their identification by certain pollinators according to the respective category (bees, bumblebees, hoverflies, butterflies etc.).

The hypothesis that the floristic dominance of certain wildflower species leads to a reduction in pollinator biodiversity was validated by the linear regression (significant for p < 0.05) shown in Fig. 2A. In fact wildflower-insect pollinator networks are effective bio-indicators of ecological sustainability in agriculture since they constitutes an important ecosystem service for the survival of biodiversity (Bretagnolle and Gaba, 2015). Consequently, when some pollinator species are dominant in the agroecosystem, it means that the floristic biodiversity is scarce as shown in Fig. 2B. In other words, a high pollinator biodiversity needs a high floristic biodiversity.

4.3. Number of crab spiders and their relationship with floristic biodiversity

As expected, the high number of visits by pollinators attracted the presence of crab spiders who lay in wait camouflaged with the same flower colours on which they were positioned (Fig. 3). However, the hypothesis that the number of crab spiders may be strictly related with the number of pollinator visits was not validated. In fact, the linear regression between these two parameters showed only a slight increase in crab spiders with the increase in the flower visit rate (Fig. 4). This does not contradict previous experiments that showed an increase in crab spiders in wildflowers strips (Schmidt-Entling and Döbeli, 2009) or field margins (Middleton et al., 2021). Instead, it highlights that some floristic communities have different effects depending on the species and the relative botanical complexity. The fact that the additional biodiversity of the three different wildflower communities led to a reduction in the number of crab spiders (Table 5) appears to be linked to a sort of specialization of predators in their ability to camouflage themselves with the different flower colors. In other words, crab spiders seem to prefer environments where some wildflower species dominate in order to maintain their scarce detectability by moving onto neighbouring flowers of the same species.

This was supported by linear regressions between the number of crab spiders and wildflower dominance (Fig. 5A) and biodiversity (Fig. 5B). The presence of wildflowers is thus essential for the presence of crab spiders, however their abundance derives from the dominance of a few plant species. This can be explained in the preferences of crab spiders in camouflaging themselves on the flowers of the most abundant species. This does not mean that the floristic diversity is an enemy of the
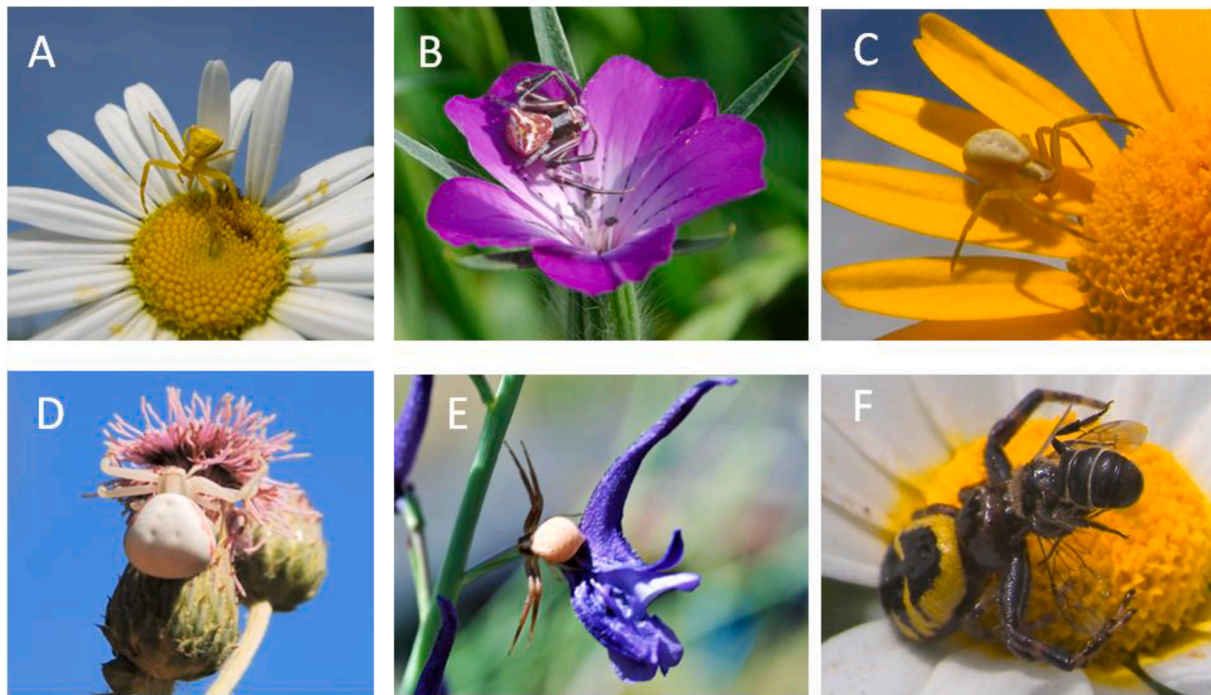
Fig. 3. Observed crab spiders (A and B *Thomisus onustus,* C, D *and E Misumena vatia, F Synema globosum*) waiting to catch pollinators on flower (inflorescence in the case of asteraceae botanic family) of *Anthemis arvensis* (1), *Agrostemma githago* (2), *Glebionis segetum* (3), *Cirsium arvense* (4) and *Consolida regalis* (5) and an evident success in catching a solitary bee observed on *Anthemis mixta* inflorescence (6).
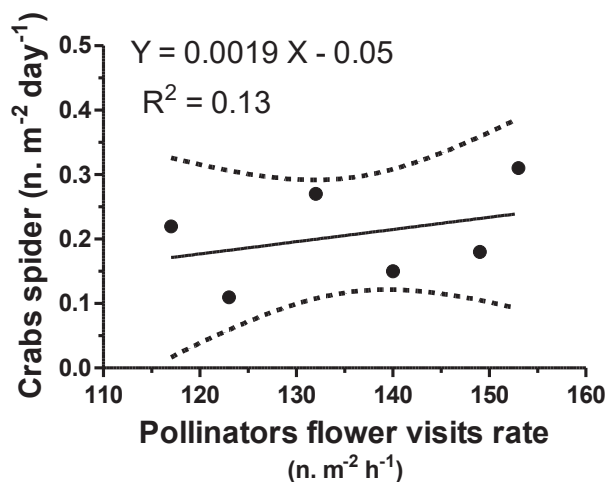

Fig. 4. Linear regression between the pollinator flower visits rate (individuals $m^{-2}h^{-2}$ day^{-1}) in the six wildflower communities (1, 2, 3, 1+, 2 + and 3 +) and the observed crap spiders (individuals m^{-2} day^{-1}). The equation (not significant at $P < 0.05$), the relative R^2 and the 95 % confidence level are reported.

sustainability of predators but rather that predators play an ecological role by maintaining the floristic biodiversity.

Crab spiders appear to hinder the dominance dynamics of one or a few prevalent species since they discourage the rate of visits by pollinators thus implying a reduced seed-set. Indeed the reduced seed-set, defined as inbreeding depression, which is typical of wildflowers not visited by pollinators (Suttle, 2003), could over time hinder the dynamics of the wildflower species that prevail in a given environment. In fact the presence of crab spiders on the flowers has sometimes even shown to halve the seed-set of some insect-pollinated species (Gonçalves-Souza et al., 2008). This also occurs in experimental flowers grown in isolation from pollinators in several wildflower species

(Benvenuti and Mazzoncini, 2021) as a consequence of inbreeding depression (Hedrick and Kalinowski, 2000). This suggests that crab spiders may play a ecological role in the dynamics of the long-time floristic equilibrium of several wildflower communities.

5. Conclusions

Sown wildflower strips play a crucial role in implementing biodiversity and related food-web in the agroecosystem. The biodiversity of such wildflower communities acts not so much to quantitatively increase the dynamics of visits to flowers, but rather, to allow further biodiversity in terms of pollinator complexity. However, the abundance of crab spiders appears to be linked not so much to the overall quantity of wildflowers but, rather, to the dominance of some species. In fact, the increase in crab spiders, in the case of the dominance of a few wildflower species, tends to reduce the rate of flower visits to the dominant species, thus reducing the seed-set level, due to inbreeding depression, with a consequent reduction in the annual rate of seed rain. The biodiversity level of a given agroecosystem in which crab spiders are also present appears to influence the dynamic equilibrium of the plant-consumer-predator trophic pyramid.

Future indices aimed at evaluating the environmental health of the various agroecosystems could include not only floristic biodiversity and related pollinators but also that of arthropods, such as crab spiders, specialized in pollinator predation.

CRediT authorship contribution statement

Stefano Benvenuti: Conceptualization, Investigation, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
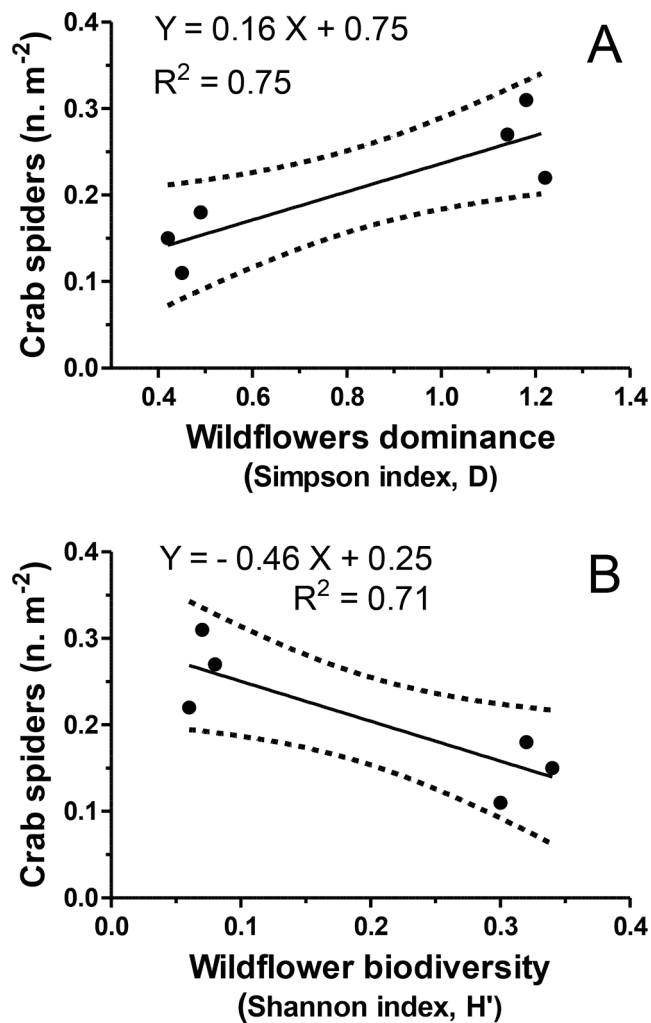
Fig. 5. Linear regression between (P > 0.05) the calculated Simpson indexes of dominance (D) of the several plant communities (A) or their Shannon indexes (H') of biodiversity (B) and the quantity of the observed crab spider quantity. The equations (significant at P < 0.05), the relative R^2 and the 95 % confidence level are reported.

Data availability

No data was used for the research described in the article.

References

Baskin, C.C., Baskin, J.M., 2004. Germinating seeds of wildflowers, an ecological perspective. HortTechnology 14, 467–473.

Benvenuti, S., Bretzel, F., 2017. Agro-biodiversity restoration using wildflowers: What is the appropriate weed management for their long-term sustainability? Ecol. Eng. 102, 519–526.

Benvenuti, S., Mazzoncini, M., Cioni, P.L., Flamini, G., 2020. Wildflower-pollinator interactions: Which phytochemicals are involved? Basic Appl. Ecol. 45, 62–75.

Benvenuti, S., Mazzoncini, M., 2021. Entomogamy in wildflowers: What level of pollinator biodiversity is required? Acta Oecol. 111, 103737.

Benvenuti, S., Selvi, M., Mercati, S., Cardinali, G., Mercati, V., Mazzoncini, M., 2021. Stale seedbed preparation for sustainable weed seed bank management in organic cropping systems. Sci. Hortic. 289, 110453.

Brechbühl, R., Kropf, C., Bacher, S., 2010. Impact of flower-dwelling crab spiders on plant-pollinator mutualisms. Basic Appl. Ecol. 11, 76–82.

Bretagnolle, V., Gaba, S., 2015. Weeds for bees? A review. Agron. Sustainable Dev. 35, 891–909.

Bretzel, F., Malorgio, F., Paoletti, L., Pezzarossa, B., 2012. Response of sowed, flowering herbaceous communities suitable for anthropic Mediterranean areas under different mowing regimes. Landscape Urban Plann. 107, 80–88.

Campbell, J.W., Milne, M., Dinh, B.T., Daniels, J.C., Ellis, J.D., 2020. Spider (Araneae) abundance and species richness comparison between native wildflower plantings

and fallow controls in intensively managed agricultural areas. Arthropod-Plant Interact. 14, 263–274.

Cheng, K., Heiling, A.M., Herberstein, M.E., 2006. Picking the right spot: crab spiders position themselves on flowers to maximize prey attraction. Behaviour 143, 957–968.

Crowder, D.W., Jabbour, R., 2014. Relationships between biodiversity and biological control in agroecosystems: current status and future challenges. Biol. Control 75, 8–17.

Dukas, R., Morse, D.H., 2003. Crab spiders affect flower visitation by bees. Oikos 101, 157–163.

Dumke, M., Herberstein, M.E., Schneider, J.M., 2018. Advantages of social foraging in crab spiders: groups capture more and larger prey despite the absence of a web. Ethology 124, 695–705.

Gegear, R.J., Laverty, T.M., 2005. Flower constancy in bumblebees: a test of the trait variability hypothesis. Anim. Behav. 69, 939–949.

Gonçalves-Souza, T., Omena, P.M., Souza, J.C., Romero, G.Q., 2008. Trait-mediated effects on flowers: artificial spiders deceive pollinators and decrease plant fitness. Ecology 89, 2407–2413.

Haaland, C., Naisbit, R.E., Bersier, L.F., 2011. Sown wildflower strips for insect conservation: a review. Insect Conserv. Diversity 4, 60–80.

Hedrick, P.W., Kalinowski, S.T., 2000. Inbreeding depression in conservation biology. Annu. Rev. Ecol. Syst. 31, 139–162.

Heiling, A.M., Herberstein, M.E., Chittka, L., 2003. Crab-spiders manipulate flower signals. Nature 421, 334-334.

Hoffmann, H., Peter, F., Donath, T.W., Diekötter, T., 2022. Landscape-and time-dependent benefits of wildflower areas to ground-dwelling arthropods. Basic Appl. Ecol. 59 (2022), 44–58.

Huey, S., Nieh, J.C., 2017. Foraging at a safe distance: crab spider effects on pollinators. Ecol. Entomol. 42, 469–476.

Jauker, F., Jauker, B., Grass, I., Steffan-Dewenter, I., Wolters, V., 2019. Partitioning wild bee and hoverfly contributions to plant–pollinator network structure in fragmented habitats. Ecology 100, e02569.

Kolkman, A., Dopagne, C., Piqueray, J., 2021. Sown wildflower strips offer promising long term results for butterfly conservation. J. Insect Conserv. 1–14.

Kujawa, K., Bernacki, Z., Kowalska, J., Kujawa, A., Oleszczuk, M., Sienkiewicz, P., Sobczyk, D., 2020. Annual wildflower strips as a tool for enhancing functional biodiversity in rye fields in an organic Cultivation System. Agronomy 10, 1696.

Mei, Z., de Groot, G.A., Kleijn, D., Dimmers, W., Van Gils, S., Lammertsma, D., Ruudvan, K., Scheper, J., 2021. Flower availability drives effects of wildflower strips on ground-dwelling natural enemies and crop yield. Agric. Ecosyst. Environ. 319, 107570.

Middleton, E.G., MacRae, I.V., Philips, C.R., 2021. Floral plantings in large-scale commercial agroecosystems support both pollinators and arthropod predators. Insects 12, 91.

Nicholls, C.I., Altieri, M.A., 2013. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agr. Sustain. Dev. 33, 257–274.

Nicholson, C.C., Egan, P.A., 2020. Natural hazard threats to pollinators and pollination. Glob. Change Biol. 26, 380–391.

Pekas, A., De Craecker, I., Boonen, S., Wäckers, F.L., Moerkens, R., 2020. One stone; two birds: concurrent pest control and pollination services provided by aphidophagous hoverflies. Biol. Control 149, 104328.

Pignatti, S., 1982. Flora d'Italia Vol. 1, 790.

Potts, S.G., Petanidou, T., Roberts, S., O'Toole, C., Hulbert, A., Willmer, P., 2006. Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape. Biol. Conserv. 129, 519–529.

Reader, T., Higginson, A.D., Barnard, C.J., Gilbert, F.S., 2006. The effects of predation risk from crab spiders on bee foraging behavior. Behav. Ecol. 17, 933–939.

Rollin, O., Benelli, G., Benvenuti, S., Decourtye, A., Wratten, S.D., Canale, A., Desneux, N., 2016. Weed-insect pollinator networks as bio-indicators of ecological sustainability in agriculture. A review. Agron. Sustainable Dev. 36, 1–22.

Romero, G.Q., Antiqueira, P.A., Koricheva, J., 2011. A meta-analysis of predation risk effects on pollinator behaviour. PLoS ONE 6, e20689.

Schmidt-Entling, M.H., Döbeli, J., 2009. Sown wildflower areas to enhance spiders in arable fields. Agric. Ecosyst. Environ. 133, 19–22.

Steel, R.G., Torrie, D., 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill Inc., Toronto, Canada.

Suttle, K.B., 2003. Pollinators as mediators of top-down effects on plants. Ecol. Lett. 6, 688–694.

Trunschke, J., Lunau, K., Pyke, G.H., Ren, Z.X., Wang, H., 2021. Flower color evolution and the evidence of pollinator-mediated selection. Front. Plant Sci. 12, 617851.

Tschumi, M., Albrecht, M., Bärtschi, C., Collatz, J., Entling, M.H., Jacot, K., 2016. Perennial, species-rich wildflower strips enhance pest control and crop yield. Agric. Ecosyst. Environ. 220, 97–103.

Twerski, A., Albrecht, H., Fründ, J., Moosner, M., Fischer, C., 2022. Effects of rare arable plants on flower-visiting wild bees in agricultural fields. Agric. Ecosyst. Environ. 323, 107685.

Van Rijn, P.C., Wäckers, F.L., 2016. Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control. J. Appl. Ecol. 53, 925–933.

Waser, N.M., 1986. Flower constancy: definition, cause, and measurement. Am. Nat. 127, 593–603.

Further reading

Welti, E.A., Putnam, S., Joern, A., 2016. Crab spiders (T homisidae) attract insect flower-visitors without UV signalling. Ecol. Entomol. 41, 611–617.