Pollinators, not predators, affect florivore survival in Cirsium arvense (Asteraceae) reveals an experiment based on Structural Causal Modelling

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Pollinators, not predators, affect florivore survival in *Cirsium arvense* (Asteraceae) reveals an experiment based on Structural Causal Modelling

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Abstract

Predators can affect plant fecundity by interfering with plant-pollinator or plant-herbivore interactions. While these indirect effects are usually studied in isolation, new insights can be obtained by studying multiple interactions between predators, pollinators, herbivores, and plants within a single framework. Here we applied this approach to understand the cascading effect of predatory insects on the fecundity of *Cirsium arvense* via their interaction with pollinators and florivores. This study focused on endophagous florivores, specifically on pre-dispersal seed predators. We used an integrated approach of combining simulation modelling with field experimentation. We first designed our experiment and then validated it through a generative simulation model, using sensitivity analysis to assess the expected statistical power of our experimental design. The sensitivity analysis confirmed that our experiment was robust enough to detect weak effects. Despite this, our field experiment did not reveal any cascading effects of predators on plant fecundity. However, we discovered that florivores in this system depend on pollinators to complete their life cycle, highlighting an overlooked link between these interactions. Our study demonstrates the usefulness of simulation models in designing ecological studies and emphasises the need to consider indirect effects in multitrophic systems.

Keywords: Cascading effect, Simulation, Interactions, Pollinators, Florivore, Fecundity

Introduction

A major component of plant fitness is its reproductive success, often measured as plant fecundity, i.e. the average number of viable seeds produced by a plant. It is the net result of the plant's interaction with mutualists and antagonists (Grass et al., 2018). Interactions with mutualists such as pollinators positively impact plant fecundity, whereas antagonistic interactions with herbivores tend to reduce it. The net effect of pollinators and herbivores on plants can be additive (Abdala-Roberts et al., 2009) when the effects from both groups are independent or non-additive (Munguía-Rosas et al., 2015; Herrera, 2000) when the interactions between pollinators and herbivores can modify the direction and strength of the net impact (Barber et al., 2012; Russell-Mercier & Sargent, 2015; Kessler et al., 2011).

Predators can also indirectly affect plant fecundity by altering pollination and/or herbivory. These cascading effects could be positive when predators control herbivores, either demographically or behaviourally (Romero & Vasconcellos-Neto, 2004), or negative if predators dissuade the pollinators from visiting the flowers or shorten visitation duration (Suttle, 2003). The net cascading effect of predators can depend on different factors, ranging from insect composition to insect size and patch size (Schmalhofer, 2001) etc.

The cascading impacts of predators can be quantified using exclusion experiments in the field. Many studies that used exclusion experiments to understand the cascading effect have either focused on plant-pollinator interaction or plant-herbivore interaction. However, studying pairwise interactions in isolation yields only a limited understanding of the ecosystem. In reality, all the interactions occur simultaneously in nature, and therefore, experiments involving multitrophic interactions would furnish a more realistic understanding of the impact of biotic interactions on different trophic levels (Seibold et al., 2018; Abdala-Roberts et al., 2019).

However, designing manipulative experiments for a multitrophic setting could be challenging. This is because an observed pattern could be linked to many processes (O'Sullivan & Perry, 2013), leading to a possibility of missing critical causal linkages in a complex web of interactions (Kimmel et al., 2021). Another challenge could be pseudoreplication, arising due to improper interspersion and randomisation of treatments, as well as the combination of experimental design and statistical model (Hurlbert, 1984). Statistical models that overlook proper causal links in the experiment design risk misinterpreting significant correlations as causal relations (Pearl, 2009). Ignoring causal linkages while designing a study might lead to confounding bias when one variable affects both the predictor and response variables (McElreath, 2020), collider bias when a variable that is affected both by the predictor and response is controlled for (Arif & Massey, 2023), or overcontrol bias when the indirect causal relationship between the predictor and response is ignored (Arif & Massey, 2023; Elwert & Winship, 2014).

The limitations highlighted in the multitrophic study system's experimental design can be addressed using Generative Simulation Modelling under a Structural Causal Modelling (SCM) framework. Having no restriction on sampling locations, generative simulation models can effectively solve the problem of pseudoreplication by creating replicates that are truly independent of each other and can control the biases by selecting statistical models that fit the hypothesis based on causal linkages in each system (Pearl, 2009).

We propose that a more accurate assessment of interactions' direct and indirect impacts on different trophic levels can be made when multitrophic interactions are studied integratively, and the experiments can be more efficiently designed by integrating simulation modelling with field experiments. We applied this framework to understand the net effect of pollinators and florivores and the indirect effect of predators and pollinator-florivore interactions on female plants of *Cirsium arvense*. We address four questions: (1) What is the net effect of predator exclusion on

seed production? (2) Does the net effect of predator exclusion vary spatially? (3) How does predator presence affect the foraging behaviour of pollinators and florivores? (4) Do pollinators and florivores indirectly influence each other?

We hypothesise that plant fecundity will increase with a higher pollinator visitation rate. The pollinator visitation rate will depend on the local pollinator richness and will increase with pollinator richness (Albrecht et al., 2012). Second, the abundance and richness of florivores will determine the seed predation rate. Our third hypothesis posits that plant fecundity will increase with increased pollinator visitation rate in the absence of predators (Romero et al., 2011). Similarly, plant fecundity will decrease when florivore abundance increases without predators (Horvitz & Schemske, 1984; Trager et al., 2010). Therefore, the net cascading impact of predators on plant fecundity will be determined by their relative impact on pollinators and florivores. Our fourth hypothesis is that the abundance of florivores will increase with increased pollination. We created a Directed Acyclic Graph (DAG) (Fig. 1) to represent all the hypotheses visually.

Methods

Model plant species

We used *Cirsium arvense*, a functionally dioecious ruderal plant species native to Europe (Tiley, 2010), as our model species. It starts flowering in late spring and continues throughout the summer. Each plant is either functionally male or female, but morphologically hermaphrodite (Heimann & Cussans, 1996). This species is highly resilient to abiotic stressors, allowing it to thrive in disturbed environments (Guggisberg et al., 2012; Tiley, 2010) and has fast vegetative propagation assisted by a widely spread deep-rooting system (Tiley, 2010), which helps in rapid and easy local colonization (Heimann & Cussans, 1996). However, *Cirsium arvense* depends on sexual reproduction via pollinators to colonise new sites outside the population (Heimann &

Cussans, 1996). A large amount of nectar and emission of volatile compounds indicate that *Cirsium arvense* invests significantly in attracting pollinators and other insects. (Theis 2006, Tiley, 2010). Established populations of *Cirsium arvense* form large, mostly female-biased patches (Van Drunen & Dorken, 2012), with male plants at the edges. Studies on populations of *Cirsium arvense* show that both, the distance between female and male plants (Drunen & Dorken, 2012; Tiley, 2010), and the sex ratio within the population (Heimann & Cussans, 1996) can affect plant fecundity (but see Drunen & Dorken, 2012 for no effect).

Experimental design

For this study, we combined simulation modelling with field experimentation. First, we developed a simulation model using an SCM framework, followed by a sensitivity analysis to test the power of our experimental design. Then, we used the verified design to collect data on pollinator visitation rate and florivore abundance in the field. In this section, we describe the experimental design applied in the field.

We designed a blocked experiment with four treatments: control, predator exclusion, pollinator exclusion, and predator model in four populations of *Cirsium arvense*, located at sites in the vicinity of České Budějovice, Czechia. The experiment was set up in June 2022, approximately one week before the flowering season, to ensure that the flowers were not pollinated before the experiment. In each site, 12 blocks of four plants close to each other were chosen to apply four treatments. The first plant in each block served as the control (T1, Fig. 2a). Pollinators were excluded from the second plant using organza bags that covered three or more flowerheads to evaluate the dependency of *Cirsium arvense* on sexual reproduction (T2, Fig. 2a). Pollinator access through the mesh was avoided by surrounding the branch in a wire structure, preventing the flowerheads from touching the exclusion bags (Fig. S1b). The third plant was used to test the cascading effect of predators by excluding them using adhesive traps on the plant stem. The sit-

and-wait predators were removed manually using a brush (T3, Fig. 2a). For the last treatment, we placed an artificial predator model, a plastic spider 1.5 cm x 1 cm painted green, on or near the flowerheads to test the impact of predator presence on pollinator and florivore behaviour (Fig. S1a). This was done in addition to removing real predators (i.e., ants, spiders, and other predatory arthropods) in the same way as T3 (T4, Fig. 2a). The treatments were randomly assigned to the plants within each block by randomly picking a pre-made treatment tag for each plant. There were 48 blocks with 196 plant individuals in the four sites. We ensured the effective removal of predators from the second and fourth treatments by manually clearing the plants and grasses in the surroundings of our experiment. The manual clearance of the surroundings was repeated periodically throughout the experiment period. Before starting observations, we also measured the distance between the closest male individual to the centroid of each block. At the end of the flowering season, just before the seed dispersal, we collected mature flowerheads for florivore sampling and plant fecundity measurement.

Two sites were established in abandoned patches of agricultural land, surrounded by agricultural fields (Site 1: 48°58'31.8"N 14°26'40.5"E, Site 2: 48°59'29.9"N 14°25'52.9" E). The remaining two populations were located at the edge of fields that are regularly used for agricultural purposes (Site 3: 48°58'40.7"N 14°26'15.4"E, Site 4: 48°58'32.4"N 14°24'47.5" E). All the populations were female-biased, with male plants at the edge of the populations. In Site 3 and Site 4, the female plants were spread along the edge of the agricultural fields (Fig. S2a), and the male plants were located towards the extremes of the edges (Fig. 2b). Ants and spiders were ubiquitous in this landscape and were the main predators of arthropods in these habitats (Sanders & van Veen, 2011).

Pollinators, florivores, and plant fecundity

Plant-pollinator interactions were recorded by observing pollinator activity in control, predator exclusion, and predator model treatments. We visually observed plant-pollinator interactions for 15 minutes in each plant. We recorded the number of visited flowerheads per plant for each pollinator and the number of open flowerheads per plant at the observation time. The description of the pollinators was noted, and videos and pictures were recorded in the field to help with identification. We observed the pollinators from 7 AM to 1 PM, considering that pollinators from different groups were most active in this time window. The blocks and sites for observation were selected randomly during the observation period, with a target of collecting 10 hours of observation at each site. The observation continued till the end of July 2022 (Table S1), after which the flowerheads started to mature. We used photos and videos from the field to identify the pollinators into species or morphospecies.

Based on the power analysis in the simulation model (see Results), we collected three mature flowerheads per treatment from all blocks to understand the effect of the treatments on plant-florivore interactions. We collected 609 flowerheads for all four sites (Table S2) and counted the number of florivores per flowerhead, followed by storing them in a 70% ethanol solution for identification up to species or morphospecies.

We measured the fecundity per flowerhead to estimate the net effect of pollinators, florivores, and the cascading effect of predators. For this, we counted the number of fertilised seeds, the number of unfertilised seeds, and the number of seeds damaged by florivores in each of the three flowerheads collected from every plant.

Generative Simulation Model

We used DAG to create generative simulation models. Creating a generative simulation model is a two-step process: i) conceptualising the problem (conceptual model), and then ii) defining the conceptual model in mathematical form (mathematical model). We tested the resulting generative simulation model using statistical methods (statistical model). The difference between the prediction from the statistical model and the known value from the generative simulation model informs about the model's (hypothesis) ability to infer causality. The lesser the deviation between the estimated value from the statistical model and the known value from the generative simulation model, the better the model predicts causal linkages (Arif & MacNeil, 2023). Further, sensitivity analysis on generative models can inform us about the power of tests ahead of experimenting in the field.

We created a generative model fitting the hypotheses described in the DAG (Fig. 1) and the experimental setup described in the experimental design section (Fig. 2b). The parameters in the generative model were used to calculate the probability of fertilisation for each flower. The variables related to experimental design were the number of sites, the number of blocks per site, treatments, the distance from male plants, and the number of open flowerheads. We created two variables, site pollinator effect and site florivore effect, to simulate the variation in pollinator richness and florivore richness between sites. Similarly, we created a treatment effect variable to account for the relative effect of experimental manipulation compared to control (Eq. 1).

To simplify the model, we assumed that the positive effect of predator exclusion on pollinators would be greater than the negative effect of predator exclusion on the florivores. The probability of fertilisation was calculated for each of these flowers as an additive effect of the site pollinator effect, site florivore effect, total treatment effect, distance from male plants, variation between plant individuals, and variation between flowerheads within a plant individual, all on a logit scale

(Eq. 1). This probability of fertilisation was employed on every flower in each flowerhead to determine the total number of viable seeds per flowerhead (Eq 2).

$$logit(p) = t_p + t_f + s_p + s_f + \mathrm{d} + r_i + r_f \qquad \text{Eq (1)}$$

$$N\ binomial(p, flw) \qquad \text{Eq (2)}$$

$$r_i\ normal(0,0.5) \qquad \text{Eq (3)}$$

$$r_{if}\ normal(0,0.5) \qquad \text{Eq (4)}$$

In Eq. (1), s_p is the site pollinator effect, s_f is the site florivore effect, *tp* is the treatment effect on pollinators, t_f is the treatment effect on florivores, *d* is the distance from male plants, r_i is the variation between plant individuals, and r_f is the variation between flowerheads within a plant individual. The total site effect was a combination of s_p and s_f. Variables s_p and s_f were generated at the site level; therefore, plants from the same site were assigned the same values for s_p and s_f in the simulations. The total treatment effect ($t_p + t_f$) was calculated assuming that the effect of predator exclusion treatment on florivores is 20 % of pollinators. Distance from male plants, *d,* was generated at the block level; hence, plants from the same block were equidistant from the closest male plant. Random variation between plant individuals (r_i) and variation between flowerheads (r_f) was generated from a normal distribution with a mean of 0 and a standard deviation of 0.5 (Eq (3) & Eq (4)). In Eq (2), *N* represents the total number of viable seeds per flowerhead, which is a binomial function of the probability of fertilisation (*p*) and the number of flowers per flowerhead (*flw*).

Sensitivity analysis

We used sensitivity analysis to understand how the proportion of fertilised seeds responds to changes in different predictor variables. We defined four test cases (Table 1) by varying the effect of one single predictor variable at a time while keeping other effects constant (Local

sensitivity analysis). For example, in the first case, we varied the strength of the effect of predator exclusion on seed fertilisation. For this, we varied the treatment effect of predator exclusion from 0 to 2 on a logit scale while other variables were fixed at 0. The next two test cases were used to test the effect of variation in site pollinator (or site florivore effect) and predator model effect on seed fertilisation. The last test case aimed to estimate the power of our experiment by varying the sampling effort. We have used one set of parameter values from each case to illustrate the causal model and analysis for this manuscript (see results)

STATISTICAL ANALYSIS

Site richness & plant fecundity estimation

We estimated the richness of pollinators and florivores in each site from the data obtained from pollinator observations in the field and florivore samples reared in the lab. We used the abundance-based Chao1 index (Chao & Shen, 2003 as implemented in the *vegan* R package (Oksanen et al., 2024) instead of observed richness to account for possible sampling incompleteness. We calculated the pollinator visitation rate per hour per flowerhead and used it to understand the effect of treatments on pollinator behaviour.

We calculated the proportion of fertilised seeds, the proportion of unfertilised seeds, and the proportion of predated seeds for each flowerhead. The effect of the treatments on plant fecundity was determined by comparing the proportion of fertilised seeds between treatments. Similarly, the proportion of predated seeds per flowerhead in each treatment signified the effect of treatments on florivore survival.

Statistical Model & Causal Effect

We used mixed-effects models (GLMM) with a binomial distribution and logit link function to estimate the odds of fertilisation for each flower within a flowerhead, for all treatments in all the

sites, using the package *lme4* (Bates, 2014). We applied the same statistical method to both the generative model and, subsequently, to data from our experiment. Treatment and site were used as fixed effects to understand the effect of treatments and spatial variation in insect richness on plant fecundity. The fixed effect of distance from male plants determined the impact of pollen sources on plant fecundity. Our model used blocks, individual plants, and individual flowerheads as random factors (Eq. 5). We did a post hoc pairwise comparison of treatments using the *emmeans* package (Rusell, 2023). To assess model accuracy, the estimated parameter values from the statistical analysis of the generated data were compared with known parameter values used in the generative models, following Table 1.

$$logit(p) = S + T + D + (1 \vee block) + (1 \vee individualplant) + (1 \vee flowerhead) \quad \text{Eq(5)}$$

In Eq (5), the total site effect is represented by *S*, the total treatment effect is *T,* and the distance from male plants is *D*. As explained in the previous section, the total site effect *(S)* corresponds to the combined effect of s_p + s_f, total treatment effect *(T)* corresponds to the value of t_p + t_f, and distance from male plants *(D)* is same as *d* in Eq (1).

We also tested whether site differences were better explained by pollinator or florivore richness instead of the site as a categorical variable for the empirical data. Additionally, we tested the effect of pollinator visitation rates in different treatments and sites. GLMM with all possible combinations of the fixed predictors were compared, and the most parsimonious model was selected using the corrected Akaike Information Criterion (AICc) (Burnham & Anderson, 2004). Simulation modelling and statistical analysis were done using R software for statistical computing (R Core Team, 2022).

RESULTS

Simulation Model - Causal Effect Estimates

In our first test case, we recovered the simulated effects for predator exclusion (simulated: 1.6, measured (mean ± s.e.): 1.638 ± 0.03) and the other parameters (control, site effects, and distance from male plants all close to 0). This result indicates that our statistical model can correctly estimate the effects of all the variables in this situation. Our statistical model fits our assumption of causal linkages between predators and net plant fecundity. For the second case in which we varied site pollinator effects, Sites A, B, and C were set to 0, 1, and -1, respectively, the estimated values were -0.005 ± 0.03, 1.03 ± 0.04, and -0.987 ± 0.04. This result again indicated that our statistical model can accurately estimate the known effects of spatial variation along with the treatment effect on plant fecundity. For the third test case, where the known effect of predator exclusion and predator model was 0.8 and -0.8, we recovered the estimated effect equal to 0.809 ± 0.03 and -0.841 ± 0.03. Therefore, we again verified for the third case that our statistical model can correctly estimate the causal effect of multiple treatments on plant fecundity.

Sensitivity Analysis

We analysed the sensitivity of the proportion of fertilised seeds per flowerhead to the variation in treatment effect for test case 1 (Table 1). The results show that the proportion of fertilised seeds per flowerhead increased with an increase in the treatment effect of predator exclusion. In other words, the odds of seed fertilisation will increase with the increase in the treatment effect of predator exclusion (Fig. 3A). The differences between treatments became statistically significant with $p < 0.05$ from a treatment effect of 0.2. For test case 2, testing the impact of variation in the site pollinator effect. The results in Fig. 3B show that the likelihood of detecting an effect of site

variation increases with increasing site variability and is detectable with a $p < 0.05$ from the site pollinator effect of Site B, and Site C equal to 0.2 and -0.2, relative Site A, fixed at 0.

We analysed the sensitivity of the proportion of fertilised seeds per flowerhead to the variation in treatment effect for the predator model in test case 3 (Table 1). The proportion of fertilised seeds per flowerhead increased with the increase in the treatment effect of the predator model (Fig. 3C). The odds of fertilisation started lower than all other treatments (0 to 0.8), became like the control (1 to 1.2), then intermediate between control and predator exclusion (1.4 to 1.6), finally becoming like predator exclusion (1.8 to 2.0). In test case 4, we tested the impact of sampling effort on detecting the effect of the predator exclusion treatment. Our results showed that the odds of detecting an effect of treatment become significant after a sample size equal to three (Fig. 3D).

Field Experiment

Pollinator visitation and florivore abundance

The observed pollinator richness of sites A, B, C, and D was 12, 15, 14, and 15, respectively. The Chao1 index for these sites was 13, 22, 15, and 17.5. This indicates sufficient sampling to estimate the richness in the different sites, allowing us to use local pollinator richness as a predictor in our models. Overall, the highest number of visits were from families Apidae, followed by Syrphidae and Sarcophagidae. Other visitors were from orders Coleoptera, Diptera, Lepidoptera, and Hymenoptera. There was variation in visitation rate between sites, however, the overall pattern remained consistent. Pollinators from Apidae were most frequent in all the sites, except Site D, where plants received more visits from Syrphidae (Fig. S3A-S3C). In our experiment, the pollinator visitation rate was slightly negatively affected by distance from male plants. However, the effect was driven by a few individuals who were much further away from males. These very leveraged

observations had a high potential to bias the observed values, as no relationship is observed when these individuals are removed from the analysis.

The florivores belonged mainly to the families of Cecidomyiidae, Syrphidae, Platygastaridae, and Tephritidae. Insects from the Cecidomyiidae group were the most abundant in all sites and treatments (Fig. S4).

Treatment effects on pollinator visitation and florivore abundance

Predator exclusion did not affect the pollinator visitation rate in our experiment (Table S3). We showed that predator exclusion and predator model treatments do not differ from the control (Fig. 4A), and there was no spatial variation in this pattern (Fig. S5). Similarly, predator exclusion and predator model did not affect the florivore abundance; however, we noticed a significant reduction in florivore abundance when the pollinators were excluded from the plants (Fig. 4B). This pattern was repeated in all the sites (Fig. S6). These results are supported by our model selection, with the model including only treatment as the best performing (Table S4), and multiple pairwise comparisons showing that only pollinator exclusion was different from the other treatments.

Treatment effects on plant fecundity and seed predation

The proportion of fertilised flowers per flowerhead did not vary between control, predator exclusion and predator model treatments. This indicates that predators do not affect female plant fecundity in *Cirsium arvense*. However, the proportion of fertilised seeds significantly dropped when pollinators were excluded from plants (Fig. 4C). This shows that *Cirsum arvense* depends on pollinators for sexual reproduction. This result aligns with our first hypothesis that plant fecundity will increase with increasing pollinator visitation rate.

The proportion of predated seeds remained unaffected by predator exclusion or predator model treatments (Fig. 4D). This indicates that florivore survival was not affected by predators in this

system. The proportion of predated seeds in pollinator exclusion treatment was higher than in the control plants (Fig. 4D); however, the number of florivores that emerged from flowerheads in the pollinator exclusion plants was lower than in the control group (Fig. 4B). This result contradicts our second hypothesis, which proposed that seed predation will increase with the number of florivores. The pattern was consistent in all four sites, suggesting no spatial variation in this pattern. This again indicates that the fecundity of *Cirsium arvense* is more dependent on pollinators than on the florivores, and endophagous florivores in *Cirsium arvense* depend on pollinators to complete their life cycle.

Discussion

In this study, we combined simulation models with field experimentation to study the cascading effect of predatory insects on pollination, florivory (seed-predation), and plant fecundity in *Cirsium arvense*. Our results from the three test cases (Fig. 3A-3D) showed that our model is adequate for detecting very small effects of the treatments. We used this inference to support our results from the actual field experiment and observed no cascading effect of predators on plant fecundity. We also did not find any effect of predators on plant-pollinator or plant-florivore interactions in our field experiment. In contrast, we found that florivores are highly dependent on pollination in this system.

For the generative model, we simulated datasets that fit our causal assumption, as described in Fig. 1. Our approach is in line with some other studies on causal inference (Runge, 2023; Arif & MacNeil, 2023), and our results show that our models can estimate the causal effects accurately. Additionally, the sensitivity analysis showed that our design can detect weak cascading effects. We consider this approach a step beyond selecting the best-fitting model out of all possible predictor combinations, as that might choose a model with variable combinations that do not reflect the hypothesised causal relationships in the system. In other words, to infer causality, models

should be based on causal assumptions backed by the biological knowledge of the system (Arif & MacNeil, 2023). Our sensitivity analysis on sample size also allowed us to fine-tune the necessary sampling effort. In our case, we chose a sample size of three flowerheads per individual based on the output of the simulations. This approach is useful in planning the experiment, especially because sampling is often constrained by logistics and finances and, in our case, requires a high effort in seed sorting and counting.

Our field experiment shows that the proportion of fertilised seeds significantly reduces when the pollinators are experimentally removed, as expected for a dioecious species (Fig. 4A). This indicates that pollinator-mediated sexual reproduction could be a strategy to maintain genetic variability within and outside the population in *Cirsium arvense* (Tiley, 2010). This could be especially important in *Cirsium arvense* because it engages extensively in vegetative propagation, and the importance of sexual reproduction has been downplayed in this species (Heimann & Cussans, 1996). However, we have shown that pollination is essential for seed production and, thus, long-distance dispersal. This finding is important, especially in the context of partially self-compatible plant species, because it shows that, despite the ability of self-fertilisation, plants rely on pollinators for reproduction. Other studies on self-compatible plant species also show a positive effect of pollinator richness on plant fecundity (Klein et al., 2003).

Our data suggests that the fecundity of *Cirsium arvense* is driven by pollinators and not florivores (Fig. 4B & 4D). Apidae was the family with the highest visitation rates, followed by Syrphidae in all four sites (Fig. S3A-C). This implies that visitation by generalist species has a high impact on plant fecundity, and our inference is in line with some other studies (Ollerton et al. 2024).

We observed that pollinator visitation rates for treatments were not significantly different in any of the sites, and the pollinator richness of sites was not very different. Consequently, in this experiment, we could not verify if the pollinator richness from our experiment drove the pollinator

visitation rates and plant reproductive success. However, various studies have demonstrated a positive correlation between pollinator richness and plant reproductive success (Klein et al., 2003; Albrecht et al., 2012).

In our experiment, we did not find any effect of predators on pollinator visitation rate. This could be because pollinators from some families, especially the large-sized pollinators, show weaker responses to the predator presence (Romero et al., 2011). Another possible reason could be that the predation risk of ants and sit-and-wait predators might not be very significant in the pollinator groups present in the experimental sites (Romero et al., 2011). Consequently, as predators show no effect on pollinator visitation (Fig. 4A), we also saw no cascading effect of predators on plant fecundity (Fig. 4C). The same applies to florivores and seed predation: we saw no increase in florivore abundance when predators were removed, in other words, no cascading effect on seed predation. Some other studies show variation in the cascading effect of predators on herbivores (Trager et al., 2010). In addition to this, our results show no evidence of differences in the effect of predators on pollinators vs florivores.

Our experiment shows a surprising result: a strong impact of pollinators on florivores. We found that the proportion of predated seeds was higher in the pollinator exclusion treatment (Fig. 4D), whereas the florivore abundance was the lowest in this treatment (Fig. 4B). To better understand this seemingly contradictory result, we can consider the life cycle of a florivore along the anthesis of the plant. First, florivores lay their eggs in flower buds. The larvae then grow inside the flowerheads, feeding on flower tissues and seeds, and they emerge in the adult stage. In the absence of pollination, these florivores feed on unfertilised seeds and thus have a very low probability of completing their lifecycle due to undernourishment. Conversely, the higher the proportion of fertilised seeds, the more likely florivores are to be able to complete their life cycle. Even though the importance of pollination to seed predators is described in other systems (Strauss & Irwin, 2004), here we quantify this indirect interaction experimentally, showing that even for

florivore species that do not feed exclusively on fertilised seeds, pollination can still have a very high impact. This highlights the importance of considering multiple factors and interactions when assessing plant fitness, as this indirect, context-dependent pollinator-florivore interaction can have a major effect on plant fecundity. (Adler et al., 2001).

Conclusion

This study emphasises the potential benefits of integrating generative simulation models and sensitivity analysis more extensively into experiment planning and sampling design. Drawing robust causal inferences from both observational and manipulative experiments is challenging, mainly because causal analysis ideally requires a closed system (O'Sullivan & Perry, 2013). In the context of ecological research, achieving genuinely controlled environments is often not feasible. Additionally, there are valid concerns about whether the conditions established in an artificially controlled setting accurately represent the complexities of natural environments.

Generative simulation models offer a promising avenue to address these inherent difficulties within ecological studies. We propose that these models can function as powerful tools for developing more effective and informative experiments. By utilising techniques such as sensitivity analysis, researchers can systematically evaluate how the outcomes of these models change in response to variations in their input parameters. This understanding can provide valuable insights for field experiments, such as determining the level of precision required when measuring different parameters to reliably detect a meaningful effect. Furthermore, sensitivity analysis can offer guidance on the necessary sampling effort to confidently identify the impact of a specific cause or treatment under investigation.

Results from our field experiment des not show a cascading effect of predators on the fecundity of *Cirsium arvense* or plant-insect interactions. Some other studies with different model plant species show a significant effect of predators on plant fecundity and plant-pollinator interactions.

This kind of experiment can be further expanded to study plant communities using an interaction network approach. This approach would be useful to decipher why cascading effects are prominent in certain systems but absent in others.

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Declarations

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The data generated during this study are deposited in Zenodo for public accessibility.

Code availability

The R code used to analyse the data is deposited in Zenodo for public accessibility. DOI: 10.5281/zenodo.15364152

Authors' contributions

Yogita Karpate and Leonardo Ré Jorge were involved in designing the field experiment, designing the simulation model, securing funding, conducting field observations, statistical analysis, and manuscript writing. Katerina Sam contributed to designing the field experiment and securing funding. Petr Blažek supported in designing the field experiment. Jan Kollross helped in laboratory analysis. All authors contributed in manuscript writing.

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Table 1: Parameter values used for local sensitivity analysis for four test cases. Each column represents one evaluation, and each row is for one parameter. Each column has all values fixed except the variable under assessment. The values in square brackets show the range of values.

Parameters	Predator exclusion	Site pollinator	Predator model	Sampling effort
Treatment effect	0	0	0	0
Treatment effect	[0,2]	0	1	0.2
Treatment effect	NA	NA	[-1,1]	NA
Site Pollinator effect	0	[0,1, -1]	0	0
Site florivore effect	0	0	0	0
Sampling effort	3	3	3	[1,10]

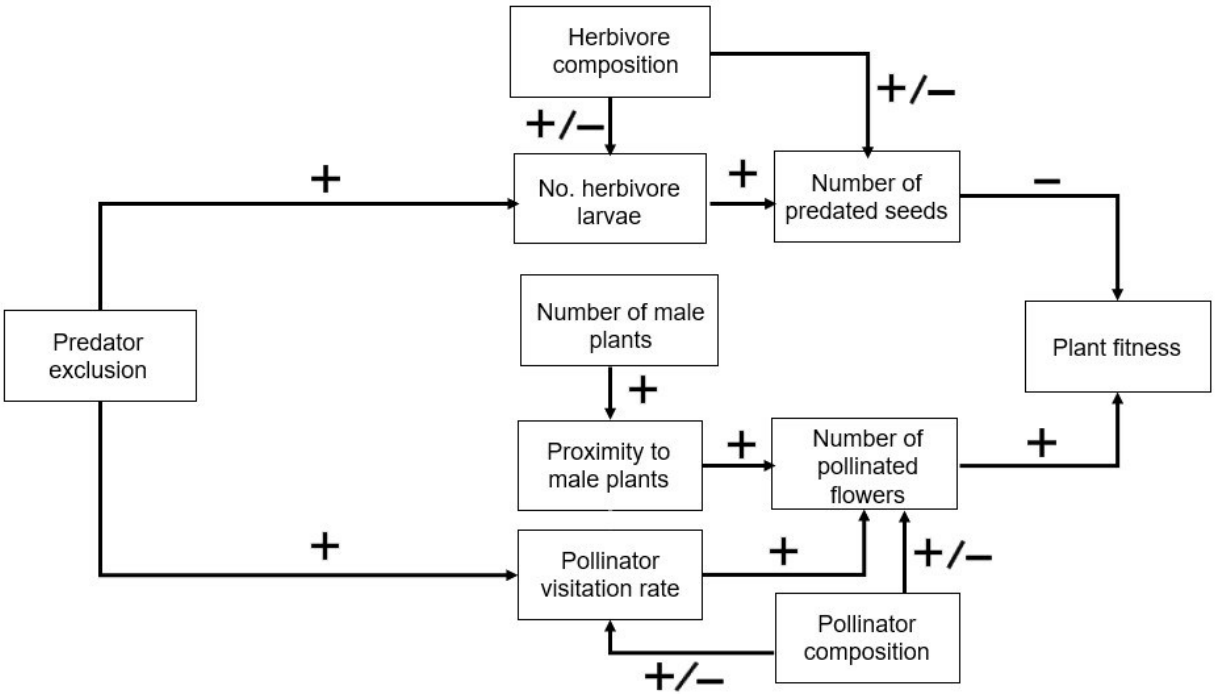
Figure 1: Directed Acyclic Graph (DAG) of hypothesised processes driving plant fecundity in *Cirsium arvense*. Arrows represent causal relationships, and the plus and minus signs indicate whether the relationship is positive (+), negative (-) or uncertain (+/-).

Figure 2: Description of treatments (a) and experimental design (b). The first treatment (T1) is control, the second treatment (T2) is pollinator exclusion, the third treatment (T3) is predator exclusion, and the last treatment (T4) is predator model addition on a plant without natural predators. The treatments were applied to four closely located female plants that form one block, as described in (b). Male plants surrounded the patch of female plants. The straight line depicts the distance of blocks from the closest male individuals.

Figure 3: Effect of predator exclusion (blue) on the proportion of fertilised seeds as compared to control (pink) (A). Effect of site pollinator variation on the proportion of fertilised seeds in predator exclusion (blue) and control plants (pink) (B). Effect of predator model addition (green) on the proportion of fertilised seeds as compared to predator exclusion (blue) and control (pink) (C). Effect of sampling effort on detecting the difference between treatments (D). Figures (A), (B), (C) & (D) represent test cases 1, 2, 3, & 4 as detailed in Table 1.

Figure 4: (A) Pollinator visitation rate for all treatments. Each point represents one plant (B). Florivore abundance for all treatments. Each point represents one flowerhead. The proportion of fertilised (C) and predated (D) seeds for all treatments. Each point in both plots represents one flowerhead.

697 Figure 1



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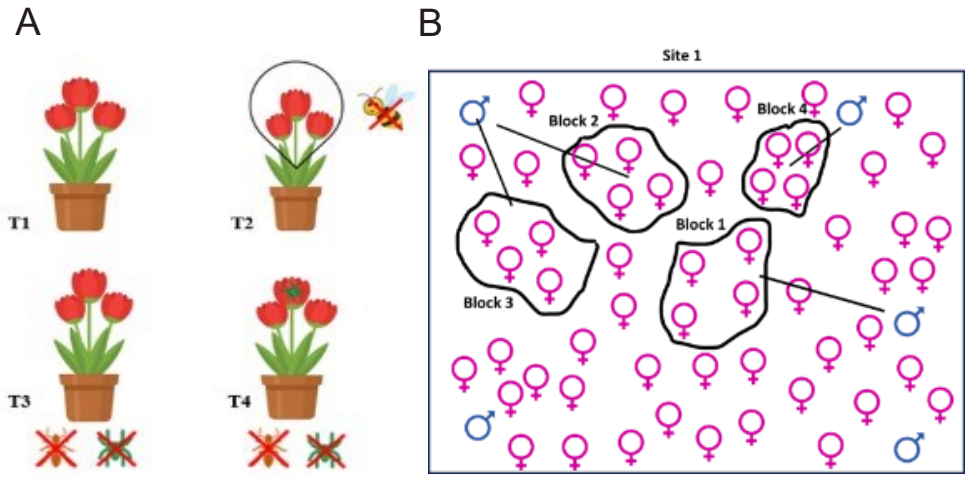

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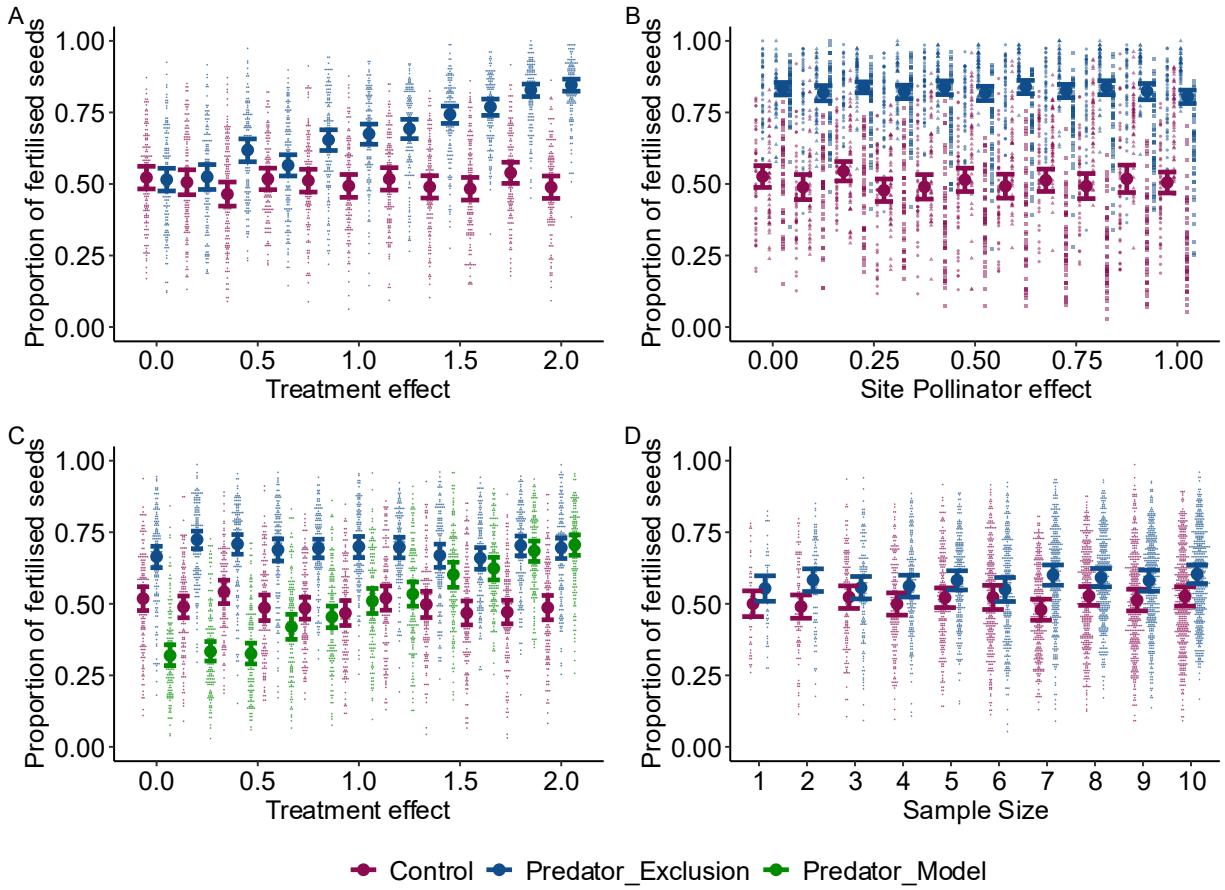

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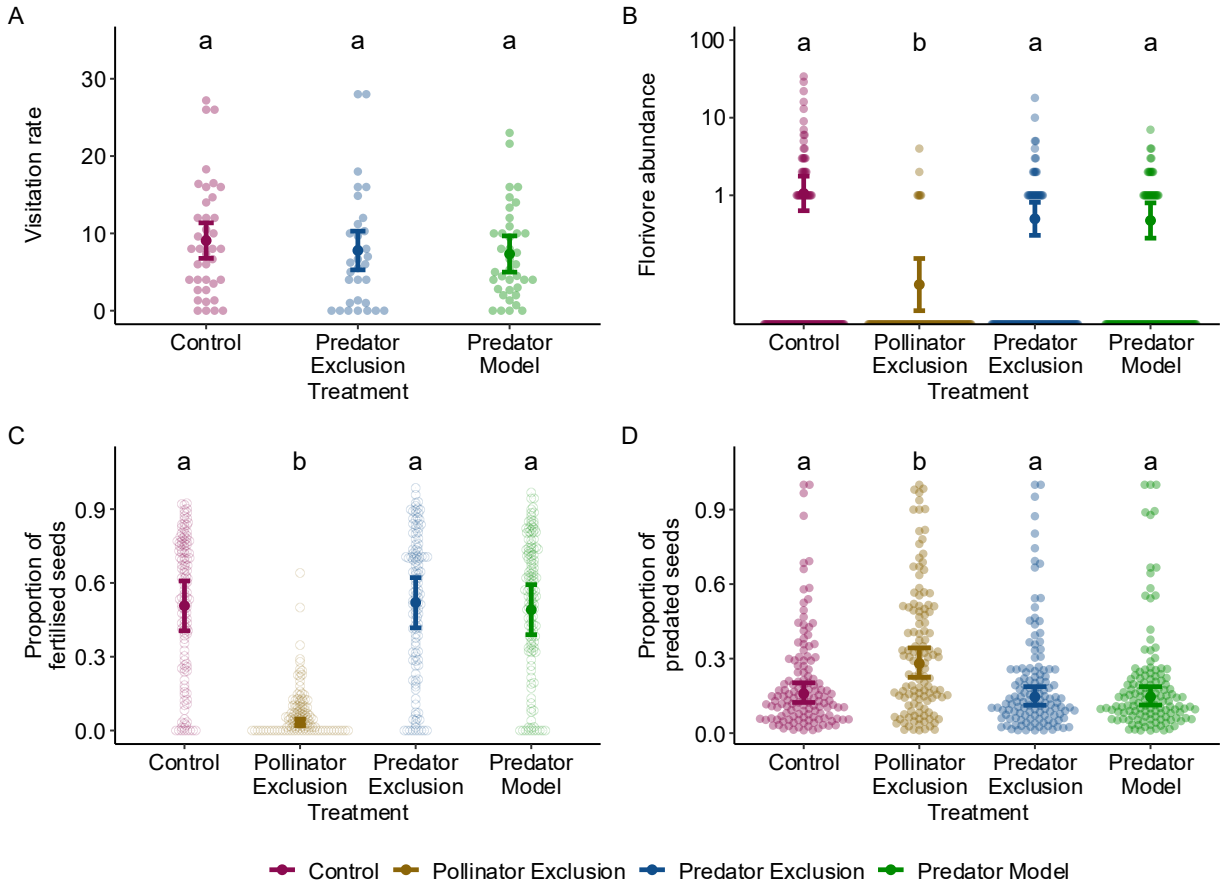

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