

density through competition with native plant that can impact the plant community composition, flowers production and visibility (Fenesi et al. 2015), and (ii) during the flowering of *S. canadensis*, when the invader additionally provides insects with important floral resources that can lead to a modification of insect community composition and behaviour (Sun et al. 2013).

We expected *visitation density* to be determined by three ecological drivers of interest: (1) the focal environmental gradient decomposed into the density of *S. canadensis* (*Invader density*) and the surface occupied by its flowers (*Invader flowers area*), (2) the availability of native floral resources (*Native flowers area*), and (3) the local specificities of each meadow in terms of soil, and surrounding landscape configuration (*meadow ID*). In this regard, meadow ID is treated as a fixed effect and not a random effect in this model. In addition, *visitation density* could be influenced by other ecological factors linked to sampling conditions, which can blur the effect of the drivers of interest: (4) phenological variations within (*hour*) and across (*date*) days for plants and insects, and (5) meteorological conditions during observation (*temperature, wind, cloud*).

1. Phenology and meteorology

To quantify the relative importance of phenology and meteorological conditions compared to the ecological drivers of interest on *visitation density*, we used boosted regression trees algorithms (BRT; Elith et al. 2008) A BRT is a recognized form of machine learning technique that assembles (via boosting) multiple small regression trees into a powerful predictive model, and has the advantages of allowing flexible response shapes and interactions among many predictors without losing too many degrees of freedom due to internal regularization. The models were built to predict pollinator *visitation density* from nine explanatory variables including four ecological drivers of interest: *invader density, invader flowers area, native flowers area, meadow ID*, and five sampling condition variables: *day, hour, temperature, wind* and *cloud*. We performed a BRT for nine measures of *visitation density* (all visits, visits per insect type and per plant families) before and during *S. canadensis* flowering. The results of these 18 models are the “reference regressions” for

all subsequent analyses as they correspond to the most accurate analysis possible with our dataset. For each of these models, we estimated their global goodness-of-fit (model R^2) and the relative contribution of each variable (hereafter contribution).

2. Frequency of flowers area measurement

In order to check if the frequency of floral area measurement affected the precision of the analyses, we downgraded our data by redefining the *native flowers area* of each plot as its value measured at mid-period (respectively late June and early august). We obtained a dataframe that correspond to the one we would have had if we had measured the flower area only once per period.

We expected the number of visits received per flowers area to be highly sensitive to the patch attractiveness (estimated by *native flowers area*). However, quantifying this area at each sampling event is time consuming and doing it once per period significantly fastens protocols. Within each period (before and during *S. canadensis* flowering), we redefined The response variable, *visitation density* was also modified as it is measured per unit of *native flower area*. We then performed the 18 regressions on this new dataset and compared the R^2 and the contribution of the ecological drivers of interest obtained with those of the reference regressions.

3. Influence of observed but non-captured visits

The number of insects we observed but escaped may varied among observers and may be linked to *visitation density* as the more insects there are in the observed area, the more difficult it is to catch them all. To quantify this potential bias on the results we excluded the non captured visits from our dataset and ran the 18 regressions on this new dataset. Then we compared the model R^2 and contribution of the ecological drivers of interest obtained with these of the reference regressions.

All statistical analyses were conducted in R v.3.6.3 (R Core Team 2020) with the packages `gbm` to run the models and `dismo` to determine the number of trees to include in the models (Brandon Greenwell et al. 2020; Robert J. Hijmans et al. 2020).

Results

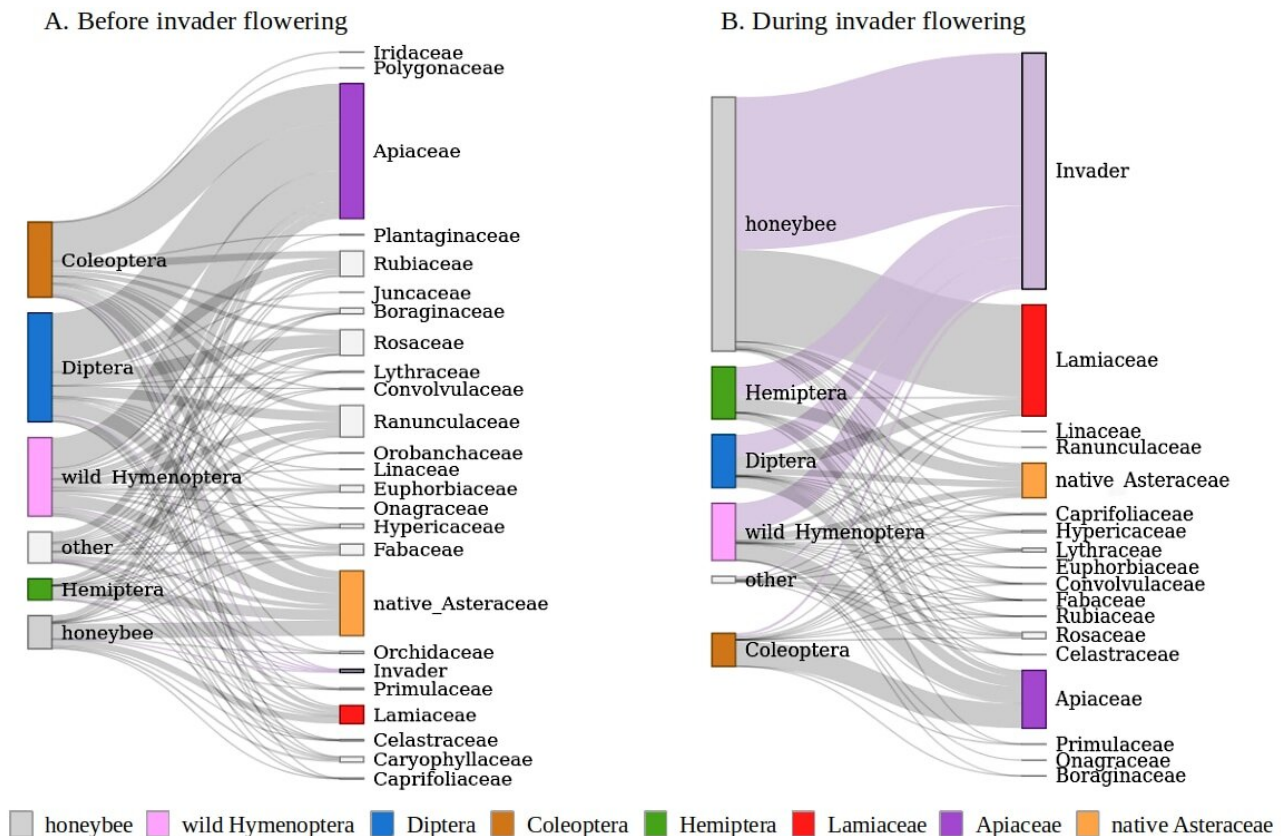


Figure 1. Representation of the relative number of insects captured per flowers before (A) and during (B) invader flowering. Insect species are grouped by taxonomic order (and separating honeybees), and plants species by families (with an extra group for the invasive species). The size of the boxes represents the number of visits given or received by the group.

Overall, we recorded 11,132 insect visits on the 69 native plant species flowering during our study and 6,214 visits on the invader (*S. canadensis*), distributed across 720 transects (60 transects visited 10-13 times each). Apart from visits on the invader (not included in the analyses), most

insect visits (79%) were made to three native plant families: Apiaceae (29%), Lamiaceae (28%), and Asteraceae other than *S. canadensis* (23%). Each plant family attracted different types of insects: Lamiaceae were mostly visited by honeybees (80% of the visits on Lamiaceae), Apiaceae by Coleoptera (35% of the visits on Apiaceae), wild Hymenoptera (25%), and Diptera (17%), and native Asteraceae were visited evenly by all insects groups. Each insect group showed specific preferences: honeybees (28% of all visits) mainly visited Lamiaceae (81% of honeybees visits), Coleoptera (17% of all visits) primarily visited *Apiaceae* (59% of Coleoptera visits), while Diptera (25% of all visits) and wild Hymenoptera (22% of all visits) visited more evenly the different plant families (Figure 1).

1. Phenology and meteorology

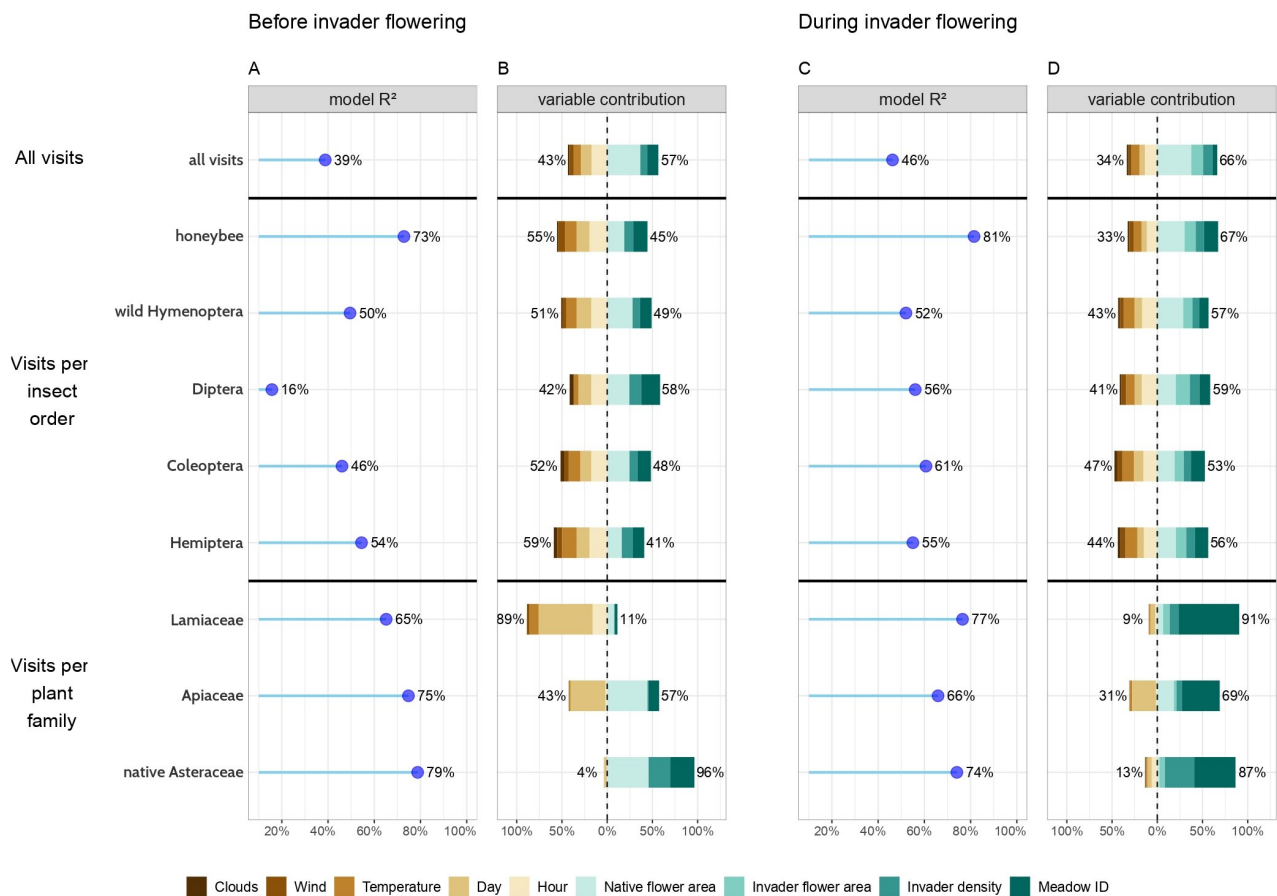


Figure 2. Model performance and variable contribution for the 18 reference models. Model performance is quantified by its proportion of variance explained: R² (A & C), and variable

relative contribution distinguishes between the ecological variables of interest (B & D, green gradient) vs. the variables linked to protocol choices (B & D, brown gradient).

The goodness of fit (R^2) of the 18 models ranged from 39% to 81%, except for the visits of Diptera before invader flowering (16%; Figure 2A,C). The ecological variables of interest contributed more to the model (53%-91%) than sampling date, hour and meteorological variables during invader flowering, but we found the reverse before invader flowering for Lamiaceae and insect groups other than Diptera. The contribution of the variables was similar across taxonomic groups and periods when considering all visits or visits per insect group, but varied a lot across plant families within and between periods (Figure 2B,D). *Native flowers area* was the variable that better explained visitation density in all models (19-46% of the explained variance), except for Lamiaceae, Hemiptera and honeybees before invader flowering, and plant families during invader flowering. *Meadow ID* was the main predictor of visits per plant family during invader flowering (42-67% of contribution) and contributed up to 27% in other models. The *invader density* contributed well in models for native Asteraceae (24%-32%; the group of the invader) but only up to 13% in other models. *Invader flowers area* contributed less than 15% in all models.

The sampling time (*day* and *hour*) largely contributed to most models (17%-41%), except for native Asteraceae and Lamiaceae during invader flowering (3% to 10%). *Temperature* had a moderate contribution to most models (8%-16%; except for Diptera before invader flowering and plant family models). *Wind* and *cloud* contributed to less than 8% in all models (Table S2). However, those three weather variables altogether significantly contributed to most models (14%-25% except for Diptera before invader flowering and plant family models).

2. Frequency of floral area measurement

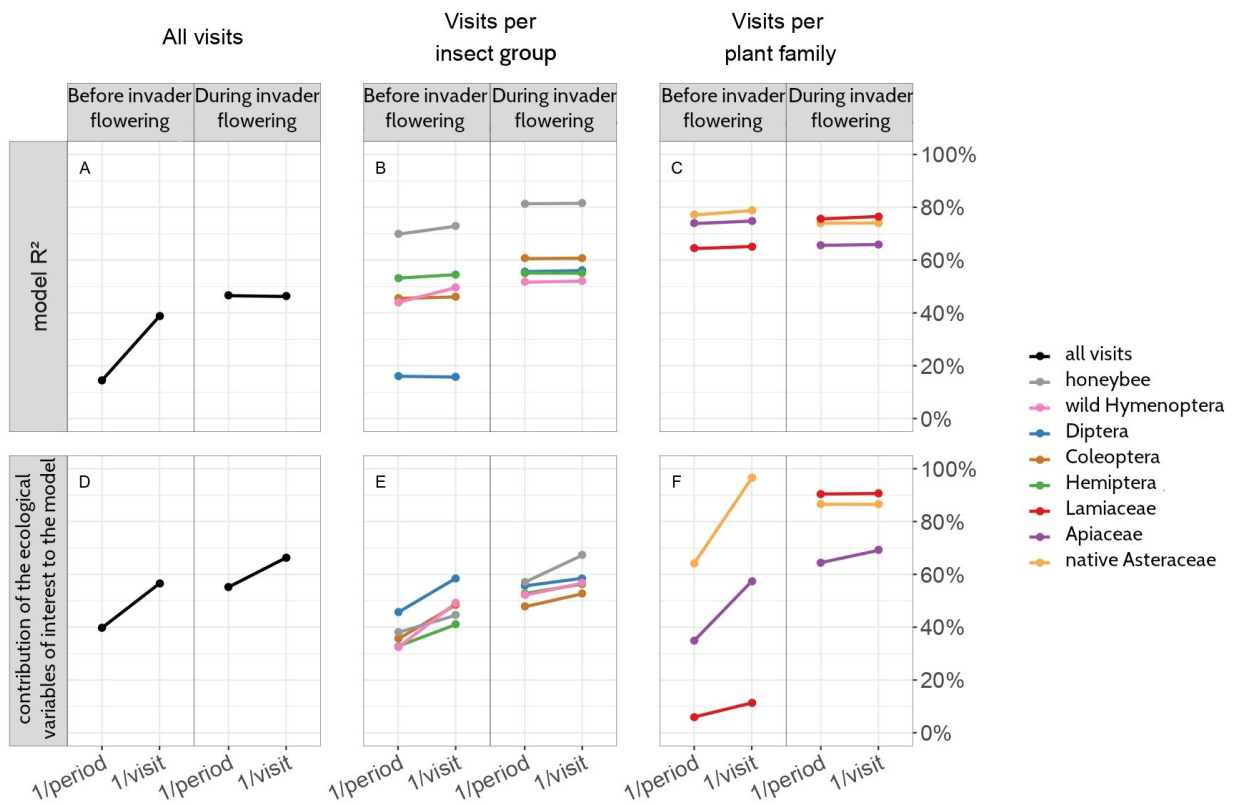


Figure 3. Impact of the frequency of flower area measurement (once per period or once per sampling event) on the model performance (R^2 ; A-C), and on the contribution of the ecological variables of interest (D-F), before and during invader flowering for all groups (A & D), per insect group (B & E), and per plant families (C & F).

The frequency at which flowers area was measured only marginally affected the models in terms of overall performance: reducing this frequency from once per visit to once per flowering period led to a maximum of 5% decrease in R^2 , except when considering all groups before invader flowering (24% decrease in R^2) (Figure 3). However, the total contribution of the ecological variables of interest (*native flowers area, invader flowers area, invader density and meadow ID*) was largely reduced before invader flowering (5% to 32% decrease) and during invader flowering (0 to 11% decrease).

3. Influence of observed but non-captured visits

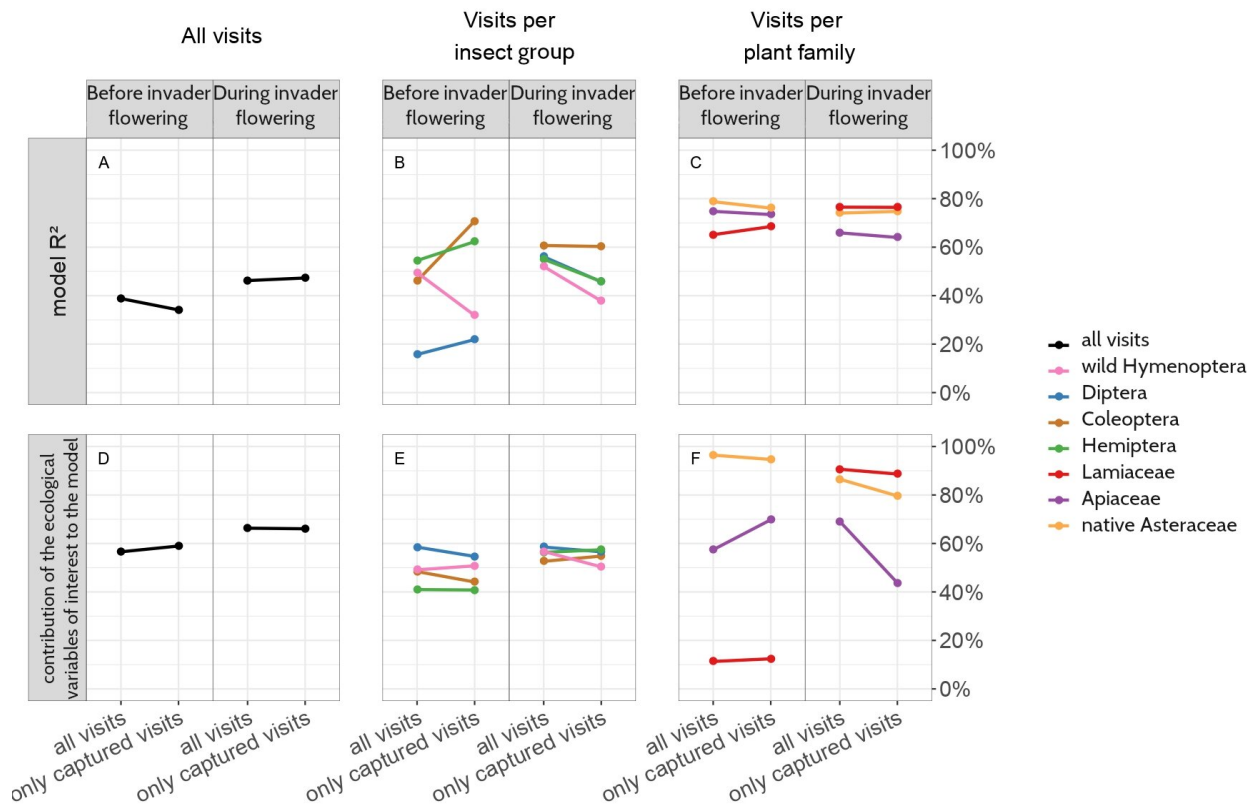


Figure 4. Impact of ignoring observed but non-captured visits on model performance (R^2 ; A-C), and on the accumulated contribution of the ecological variables of interest (D-F). The results are presented before and during invader flowering for all groups (A & D), per insect group (B & E), and per plant families (C & F).

Accounting for observed but non captured visits in the analysis had only marginal effects when considering all species together or honeybees (Figure 4A,D). However, in analyses specific to insect orders, accounting for all visits highly influenced model performance, with various effects across groups but it generally decreased model fit before invader flowering and increased it during invader flowering (by 5% to 24%; Figure 4B). Analyses specific to plant families showed that accounting for all visits only marginally influenced both model performance (less than 3% decrease in R^2) and contribution of the ecological variables of interest, except when considering Apiaceae

(12% decrease in contribution before invader flowering and 25% increase during invader flowering; Figure 4C,F).

Discussion

Our results show that in general, the visitation density was highly influenced by: the ecological variables of interest (mainly native and invader flowers areas), phenology and meteorological variables. The contribution of these different variables were consistent across insect groups, but varied a lot across plant groups. We additionally showed that, depending on the focal taxonomic group, models could be largely improved by the use of high quality measures of patch attractiveness (i.e., the frequency of floral area measurement) and the inclusion of escaped insects in the dataset.

1. Phenology and meteorology need to be accounted for in protocols

With the complete dataset (all captured and non-captured interactions, flower area measured at each sampling event), pollinator visitation density was well predicted by all predictors both before and during invader flowering. The ecological variables of interest, especially the *native flowers area* in the transect, explained most of insect visitation density. This variable is a component of the patch attractiveness (Conner and Rush 1996; Lázaro and Totland 2010) and thus expected to influence insect visits (Templ et al. 2019). Apart from the ecological variables of interest, sampling conditions in terms of phenology (both *day* and *hour*) and meteorological conditions (especially *temperature*) did also strongly influence *visitation density* before and during the invader flowering.

The results differed across insect groups and plant families. Visitation density was better explained for smaller taxonomic units (i.e., single insect order or plant family), than when considering all visits, except for Diptera. This result supports the expectation that insect foraging behavior is generally more similar within than across orders (Romero et al. 2011). Additionally, the relative

contribution of phenological and meteorological variables remained high but differed widely across taxonomic groups. Compared to the global models, the contribution of sampling time and meteorological variables increased for most insect orders, but decreased for most plant families (except for Lamiaceae before invader flowering). This result can be explained by the strong influence of sampling time and meteorological conditions on insect foraging behaviours (compared to plants), and the large range of pollinators interacting with a given plant family. This result highlights the fact that both sampling time (*hour* and *day*) and meteorological conditions (especially *temperature*) can be important to consider when designing the transect protocol, particularly so when investigating insect visits from different insect orders as they can respond differently to the sampling time and meteorological conditions. To do so, transects should be repeated multiple times during the sampling season, at different times of the day, with a large range of meteorological conditions (within suitable conditions for insects to fly). We found only marginal effects of *wind* and *cloud*, but this may be due to the relatively moderate ranges of conditions experienced during the sampling (e.g. up to 70% cloud coverage, and 13 km/h wind).

2. Floral areas should be frequently assessed

Estimating the amount of floral resources per plant family before a sampling event is crucial because it informs on both the density of insect visits per flower unit, and the attractiveness of the site for prospecting pollinators. It is however a tedious task and it is thus relevant to ask whether reducing the number of assessments of this resource affects the quality of this information. Here we showed that reducing the number of floral surveys from once per sampling event to once per sampling period can affect both the performance of the models (up to 22% reduction of R^2 for global models), and the relative contribution of the ecological variables of interest. We observed that the contribution of these ecological predictors decreases (greatest effect for native Asteraceae and Lamiaceae before invader flowering), while the goodness of fit of the model remains unchanged. This suggests that the influence of sampling conditions can increase and bias our

understanding of the visitation density drivers. The attractiveness of specific flower types is known to be influenced by the surrounding floral resources (Morales & Traveset 2009) that vary a lot across time (mostly before invader flowering). This result brings to attention the importance of floral surveys, and most importantly, their frequency during the sampling period.

3. Non-captured visits mostly influence analyses at insect order level

It is impossible to reach a 100% catch score when catching insects on flowers. Many insects have escape strategies that make them hard to capture: falling like stones, flying away, digging themselves into the flower, etc. But whether ignoring these non-captured visits strongly biases the results remained largely unexplored. Here we show, in the context of our case study, that ignoring non-captured visits has a very moderate effect when considering either the full set of species, or when analysing visitation density at the plant family level. However, when analysing visitation density for specific insect orders, uncaught insects can influence model performance up to 20% (positively or negatively, depending on the insect group and period). This result is consistent with the fact that escape strategies can be shared by species belonging to the same insect order. It is reassuring that for all visits the influence of non-captured visits remains marginal. This result should be transferred to other case studies with caution, as inevitably the proportion of failed captures will differ across: (i) observers depending on their ability to anticipate the escape strategies, and (ii) across studies depending on the insect species pools, abundance and escape strategies.

Conclusion

When studying the impact of protocols on pollination networks characterisation, most studies focus on sampling intensity and transect design. Our results highlight the need for (i) covering large ranges of temporal and meteorological conditions for each site, and (ii) repeating the assessment of patch attractiveness for pollinators (a major covariate, usually derived from once-off vegetation

surveys). Moreover, we show that (iii) for analyses of visitation density across insect groups, failed insect captures should not be neglected. We hope that this research, on three key choices and how they can influence our understanding of plant-pollinator interactions, will open the path for further investigations on how protocol choices influence our understanding of ecological processes, in other ecosystems and sampling approaches.

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Author contributions

MCG, LG and FM conceived the study. All authors contributed to the field work. JR created the database, MCG performed the statistical analyses, and MG helped with the figures. MCG wrote the first draft of the manuscript, which was significantly improved by all authors.

Data availability statement

Raw data and derived data supporting the findings of this study are available from the corresponding author MCG on request.

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Figures titles

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