



# Experimental species introduction shapes network interactions in a plant-pollinator community

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**Abstract** Invasive species that form mutualistic interactions can perturb resident communities by creating new interactions, or weakening the strength of existing interactions via competition. We hypothesized that introducing a super-generalist plant species to bee-plant networks would lead to (1) increases in the weighted and unweighted average degree, nestedness, and connectance and (2) decreases in the modularity. We constructed visitation networks of bees to five native plant species in experimental research plots, in the presence and absence of an

invasive thistle and while varying thistle abundance and time/duration of introduction. Species introduction did not change the visitation rate of bees to co-occurring native plants, or the interaction structure between the native plant species and bee visitors; seed set of a focal native plant species was also unaffected. We found the number of bee species with which the introduced species interacted (generality) correlated with significant increases in unweighted and weighted average degree, nestedness, and connectance, but not modularity. When comparing the impact of the introduced species either early or late in the season, we found similar relationships between introduced species generality and weighted and unweighted average degree and connectance; there was a significant negative relationship between introduced species generality and modularity early in the season, and a significant positive relationship with nestedness late in the season, suggesting introduction timing within a season may affect some measures differently. Overall, the native community was robust to the introduction of the super-generalist; our experimental test of network theory predictions demonstrates an improving mechanistic understanding of how mutualistic networks respond to ecological perturbations.

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## Introduction

The addition of a novel species to an extant community can be considered as a disturbance that perturbs the structure of existing interactions (Stinson et al. 2006; Ehrenfeld 2010; Walsworth et al. 2013; Campbell et al. 2015). In the context of a mutualistic interaction network, a species introduction may act as a disturbance, for example by competing for interactions or weakening the strength of existing interactions (Aizen et al. 2008; Bartomeus et al. 2008; Russo et al. 2014). These impacts may be species-specific and vary over time (Moragues and Traveset 2005). The impact of a given disturbance varies based on a number of aspects including its timing, duration, and intensity (Shea et al. 2004; Zhang and Shea 2012). Moreover, mutualistic interactions could accelerate and distribute the impacts of disturbances on ecological communities because they link the fate of interacting partners (Bond et al. 1994; Kiers et al. 2010) and existing theory suggests that specialised species with high interaction strengths and a greater mutual dependency will be the most vulnerable to disturbances (Weiner et al. 2014). Thus, it is essential to better understand the impacts of disturbances on mutualistic interactions in the interest of biodiversity conservation (Tylianakis et al. 2010; Kiers et al. 2010).

While many studies show resilience in plant-pollinator interactions (Lopezaraiza-Mikel et al. 2007; Hegland et al. 2009; Kaiser-Bunbury et al. 2010), evidence indicates that these networks will eventually reach a tipping point where they become dramatically degraded and challenging to restore (Forup et al. 2007; Burkle et al. 2013). There is existing theory suggesting the impact of a species introduction is mediated by the generality of the invader; in other words, how many (Morales and Aizen 2005; Russo et al. 2014; Carvalheiro et al. 2014) and which (Campbell et al. 2015) interactions the invader forms in its introduced range determines the magnitude of the change observed in the extant network. Moreover, there is an established relationship between the abundance and impact of invaders (Kaiser-Bunbury et al. 2011; Sofaer et al. 2018). Finally, when viewed as a disturbance, it seems likely that the impact of a novel species' introduction to a mutualistic network will depend not only on the number of interactions it forms, but also on the timing of its introduction and the duration of its activity in the

network (McKinney and Goodell 2011) and may vary over time (Moragues and Traveset 2005) and with flowering phenology (Morente-López et al. 2018).

Though there is much theory on the topic of disturbances and the impacts of invasive species on networks, it is challenging to observe a species invasion in a controlled experimental fashion that could provide evidence of causal interactions. Our main objective, therefore, was to experimentally explore the effect of a species introduction into replicated mutualistic networks, using a published theoretical study to provide a framework and testable hypotheses (Russo et al. 2014). For example, we expect an introduced mutualist that adds interactions without competing for interactions will have positive effects on the network measures of weighted and unweighted degree, connectance, and nestedness, while having a negative effect on modularity (Table 1) (Russo et al. 2016). We designed a field experiment varying the density of a plant invader and the timing/duration of its introduction into a fixed plant community in central Pennsylvania, USA. We used a well-known invasive plant species (*Carduus acanthoides*), previously shown to be a pollinator super-generalist (Russo et al. 2016) because it was likely to have a strong effect on network structure. The experimental unit was a plot comprising a community of five native plants. Five experimental treatments were applied at the plot level and the treatments were replicated six times, yielding 30 research plots, each separated by 100 m or more. The treatments were: (1) control (no introduction), (2) low density of thistles introduced early in the flowering season (early-low), (3) high density of thistles introduced early in the season (early-high), (4) low density of thistles introduced late in the season (late-low), and (5) high density of thistles introduced late in the season (late-high).

Previous research suggested the thistle drove an increase in both bee species richness and abundance without competing for interactions (Russo et al. 2016); thus, we may expect it to add new interactions to the plant-pollinator network without removing extant interactions. In other words, if the introduced thistle behaves in an additive fashion, as in previous studies, we expect that it will not affect the visitation rate of bees to native plant species, the structure of interactions between native plants and their pollinators, or the seed set of a focal native species. By experimentally

**Table 1** A description of the network measures evaluated, including an ecological interpretation and our expectation of how they will be affected by the thistle introduction

| Network measures      | How we calculate it   | Ecological interpretation   | Hypotheses  |
|-----------------------|---|---|---|
| <i>Average degree</i> |   |   |   |
| Unweighted            | $\frac{h_i + b_i}{h + b}$   | The average number of species every species interacts with. In other words, how many bee species each plant interacts with or floral hosts a bee visits in the network  | We expect the average unweighted degree to increase along with the abundance of the thistle, and when it is present for a longer duration                     |
| Weighted              | $\frac{h_{iw} + b_{iw}}{h + b}$   | The weighted average degree accounts for bee abundance. Thus, the total number of bee visits each plant species receives weights the degree   | Because the thistle is a supergeneralist, we expect it to have an even larger positive effect on the average weighted degree                                  |
| <i>Connectance</i>    |   |   |   |
| Plot-wide             | $\frac{\sum \frac{h_i}{h} + \sum \frac{b_i}{b}}{h + b}$                         | The plot-wide connectance evaluates the number of realised interactions out of the total number of possible interactions, if we account for only bee species detected within a given plot   | If the thistle adds new interactions without altering existing interactions), it may not have an effect on plot-level connectance                             |
| Experiment-wide       | $\frac{\sum \frac{h_i}{h} + \sum \frac{b_i}{b_{tot}}}{h + b_{tot}}$             | The experiment-wide connectance evaluates the number of realised interactions out of the total number of possible interactions, if we account for all bee species present in the landscape that may choose to visit the plant species flowering in each plot. Thus, it is a more inclusive calculation of “possible” interactions | If the thistle attracts new species in from the landscape, relative to the other plant species, it will have a positive effect on experiment-wide connectance |
| Modularity            | We measure modularity using the algorithms proposed by Newman and Girvan (2004) | Modularity describes how tightly the species within compartments interact. Higher modularity values indicate densely interacting subgroups of species, and may destabilize mutualistic networks (Thébault and Fontaine 2010), so it is possible that generalist species might be a stabilizing force if they connect modules      | We expect the thistle to decrease the modularity by interacting diffusely with all modules  |
| Nestedness            | We calculate nestedness using the NODF algorithm (Almeida-Neto et al. 2008)     | Nestedness is a measure of the ways in which specialists interact with subgroups of species that interact with generalists (Fortuna et al. 2010; Campbell et al. 2012)  | Because the thistle is a generalist, we expect the nestedness of the community to increase in response to the introduction (Aizen et al. 2008)                |
| <i>Node measures</i>  |   |   |   |
| Generality            | $h_i$ or $b_i$  | Generality is the number of species with which each plant host or bee visitor interacts   | We expect the thistle generality to increase with the number of thistle plants introduced to the plots  |

**Table 1** continued

| Network measures | How we calculate it  | Ecological interpretation  | Hypotheses   |
|------------------|--|--|--|
| $d'$             | Calculated as a normalized species level specialisation index (see Blüthgen et al. 2006) | $d'$ calculates how strongly a species deviates from a random sampling of interacting partners available and ranges from 0 (no specialisation) to 1 (perfect specialist). This measure takes into account the abundance of the interacting partner | Because we do not expect the thistle to compete with the native plant species, we do not expect their specialisation index to change in response to the introduction |

Where  $h_i$  is the number of interactions of a host plant  $i$ ,  $h_{iw}$  is the weighted interaction frequency of a host plant  $i$ ,  $h$  is the number of host plant species in each plot,  $b_i$  is the unweighted interaction frequency of a bee species  $i$ ,  $b_{iw}$  is the weighted interaction frequency (abundance) of a bee species  $i$ ,  $b$  is the number of bee species in each plot, and  $b_{\text{tot}}$  is the total number of bee species in the landscape

manipulating introduced species density, we test our theoretical predictions of how the impact of the introduced species on network structure is mediated by the number of bee species with which it interacts (Table 1). Though the impact of the timing/duration of the species introduction is less well-established theoretically, we expect that introducing the thistle earlier in the season (and thus increasing the duration of its presence in the network) will increase the magnitude of its impact on these network attributes, without changing their directionality (Memmott and Waser 2002). Finally, as an independent way of testing changes to the function of the community, we test the impact of the introduction treatments on the seed set of a focal native plant species.

## Methods

### Focal invasive species

The plumeless thistle (*Carduus acanthoides*) is native to Eurasia and was first recorded in the United States in 1879 (Desrochers et al. 1988), where it is now widely distributed. It is considered a pest species by land managers, mainly in mown or grazed areas (Allen and Shea 2006), where it can reach high population densities (Tipping 1992). Once established, it is difficult to eradicate (Desrochers et al. 1988; Zhang and Shea 2012) as it reproduces by wind-dispersed seeds (Skarpaas and Shea 2007). It depends on insect pollinators for outcrossing (Yang et al. 2011) and is

highly attractive to resident pollinators, significantly increasing bee abundance and species richness in a previous experimental invasion study (Russo et al. 2016). The previous study addressed the impact of the thistle introduction into a replicated community of 10 flowering ornamental species, but the flower-visiting insects were collected at the plot level. Thus, it was not possible to determine whether there were changes in the insect visitation patterns to specific plant species in the plots in the previous study. Moreover, we did not previously vary the timing or density of the species introduction.

### Experimental design

The background community of plants in all research plots was five native species including four asters (*Helianthus annuus*, *Gaillardia pulchella*, *Rudbeckia hirta*, and *Coreopsis tinctoria*), and one legume (*Chamaecrista fasciculata*). These plants were grown from seed in 30 2 × 2 m plots at the Russell Larson Agricultural Research Center at Rock Springs, Pennsylvania State University, PA, USA. The Russell Larson Agricultural Research Center comprises a heterogeneous mix of crop fields, fragments of semi-natural habitat, and grass fields. The research farm is bordered on the south by Rothrock State Forest and on the other three sides by private farmland and some fragments of deciduous forest (Fig S1). All plots were sown with identical (according to scale precision) seed mass (7.5 g) of each plant species. Plots were seeded once in early April, and once in early May. We thus

used weight of the seed to standardize the density of native species in each plot. Plots were located more than 100 m from one another and along crop field edges to ensure they were separate habitat patches. Though many bee species can fly this distance to forage, it is more than 4000 times the body length of the largest bee found in this region (*Xylocopa virginica*). Moreover, a spatial autocorrelation analysis for this experimental design found no significant effect of plot proximity on the bee community (Russo et al. 2019). We used a blocked design to ensure treatments within a block experienced a relatively similar background environment; all treatments were replicated in six blocks (Fig. S1). We watered during periods of drought (> 5 consecutive days without rain) and removed contaminant plants (species other than the six we deliberately planted) throughout the season.

The treatments included: control (no thistle), two early treatments (high and low density of thistle individuals), and two late treatments (high and low number of thistle individuals). Because the thistles are typically biennial, we planted a mix of nursery (3 out of 4) and wild grown (1 out of 4) thistles, all of which over-wintered outdoors to ensure vernalisation. We planted 4 thistles (3 nursery grown and 1 wild grown) at low density plots and 8 thistles (6 nursery grown and 2 wild grown) at high density plots. For the early treatment, we planted the thistles before sampling began in July. At this stage, the thistles were bolting but not yet flowering and none of the remaining species were flowering. The thistle was the first species to bloom in July, followed by *C. tinctoria*, *C. fasciculata*, *H. annuus*, and *R. hirta*, in order. *G. pulchella* began flowering last, in August (Fig. S2). The thistle individuals for the late treatment were planted while already flowering on 1 August.

Beginning in July, we checked each plot every week for flowering and removed any contaminant plants. On each sampling date throughout the flowering season (July and August), each plant species flowering in the plot was observed for five minutes and all insect visitors contacting the reproductive parts of the inflorescences were collected by insect vacuum. There were a total of 30 sample dates from 11 July through 2 September. We spent a total of 58.3 h (700 5-min samples) sampling insects during the course of the field season. Sampling was evenly divided between morning (9 am–12 pm) and afternoon (12 pm–4 pm) for each plot to ensure we accounted

for variation in visitation rates during different times of the day (Vaudo et al. 2014). Additional sampling demonstrated little bee activity outside of these sampling hours because cool, wet mornings precluded most early bee activity.

For the purposes of this study, we focus on the bee visitors to the different plant species, because they have been shown to respond strongly to the presence of the thistle (Russo et al. 2016). The vacuum collections show the number of bees visiting the inflorescences of a given plant species over a 5-min sampling period, for the duration of its flowering season. At each sampling date, we also recorded the number of inflorescences of every species. Sampling only occurred on plants in flower and, as species and plots began flowering at different times, this resulted in variable sampling effort among the different species. We thus used the number of individual inflorescences multiplied by the average size of the floral unit (capitula for the asters and individual blossoms for the legume) to calculate the total size of the floral display for each sample. We include the size of the total floral display rather than the number of individual inflorescences because the species vary in the size of individual inflorescence. Some species produce a large number of small inflorescences, while some species produce a small number of large inflorescences (Table S1). Both the number of inflorescences and the size of each inflorescence effectively influence floral visibility to pollinators (DeBarros 2010). We measured the average size of the inflorescences by randomly selecting 10–20 individual blossoms at random for each species and measuring the diameter with a ruler, and then taking the average of the diameters (Table S1).

In order to passively sample the background community of bees present in the landscape, we conducted bowl trap surveys by placing blue, yellow, and white plastic bowls with soapy water (Droege et al. 2010) at each plot each week of the flowering season (13 total bowl surveys). The bowls were left out for 24 h during each survey. These bowl surveys allowed us to look at background bee diversity, including species present in the landscape that did not visit our plant species. Collecting these non-interacting bees allows calculation of network connectance (i.e. the number of realized interactions that occur given the number of “possible” interactions); non-interacting bee species contribute to the

denominator (Table S2). Thus, we use this background diversity to calculate experiment-wide connectance: the number of realized plant-bee interactions identified via vacuum divided by the number of possible interactions ( $N \times M$  where  $N$  = plant species in flower and  $M$  = bee species collected via vacuum and/or bowl collection) (Russo and Shea 2016). In contrast, plot-wide connectance: the number of realized plant-bee interactions identified via vacuum divided by the number of possible interactions ( $N \times M$  where  $N$  = plant species in flower and  $M$  = bee species collected via vacuum only) (Table 1). We identified 98% of the collected bee specimens to species with help from Samuel Droege, a bee taxonomist with the United States Geological Survey (USGS).

### Data analyses

We first compared the visitation rate (number of bee visitors per unit floral area per 5-min sample) among the different plant species, and then tested whether the presence of the thistle changed the visitation rate of bees to the native plant species. To determine the relationship between the visitation rate for each plant species (response) and the presence/absence of the thistle (fixed effect), we used linear mixed effects models with the `lmer` function in the package `lme4` in R (Bates et al. 2014), with experimental block as the random effect and Gaussian distributions.

The experiment was designed to have high and low intensity introduction treatments, where the high intensity treatment had twice as many individual thistles with the expectation that they would produce twice as many inflorescences, and attract a greater number of insect visitors (Carvalho et al. 2014). However, our early high intensity treatment did not produce significantly more inflorescences than the low intensity treatment (Fig. S3A). Thus, we used a model selection process with the `anova` function in R to determine whether the five original categorical treatments or the continuous number of thistle inflorescences with the timing treatment (early or late) as a categorical effect was the best model for our analyses of the impact of the introduced species on the bee abundance and species richness at the plot level, with block as a random effect. We report the best model as the model with the lowest AIC value.

Next, for each plot, we pooled all the samples from across the season for each plant species, including all

bees collected on flowers at all time points. Then we constructed bipartite networks of plant and bee species nodes with links between them weighted by the abundance of bee visitors on inflorescences. From these networks, we calculated the following measures: average degree (weighted and unweighted), connectance (plot-wide and experiment-wide), nestedness (NODF, Almeida-Neto et al. 2008), and modularity (Newman and Girvan 2004). We examined these measures because they describe the basic structure of the visitation networks, and are commonly reported in many different plant-pollinator studies (Table 1 for full descriptions). Moreover, we have well-established conceptual explanations of the ways in which these measures relate to structure (Newman and Girvan 2004; Blüthgen et al. 2006; Almeida-Neto et al. 2008; Fortuna et al. 2010; Weiner et al. 2014) and predictions of how they respond to perturbations (Lopezariza-Mikel et al. 2007; Vilà et al. 2009; Kaiser-Bunbury et al. 2011; Russo et al. 2014; Campbell et al. 2015). We used Pearson correlation coefficients to test the plot-level relationships between (1) thistle inflorescences and bee abundance, (2) the number of bee species visiting the thistle and average weighted and (3) unweighted degree of the plot networks, and (4) the number of bee species visiting the thistle and the number of thistle inflorescences.

Russo et al. (2014) specifically predicted that the impact of novel mutualists would be mediated by the number of species they interact with (generality, Table 1) in their introduced range. We manipulated the number of introduced thistles with the intent to manipulate the number of bee species they would attract and determine whether we could recapture the expected relationship between thistle generality and other network measures. In other words, we manipulated the number of plants, and therefore the number of inflorescences, which correlated with the number of bee species visiting the thistle in each plot (Fig. S3C). Because our goal was to manipulate introduced species generality, we test the relationship between thistle generality and network measures in the main text, and describe the relationship between the number of inflorescences and network measures in the supplemental materials (Fig. S4). We used linear mixed-effects models to test the fixed effect of thistle generality (# of bee species the thistle interacts with in each plot) on the plot-level network measures of average (1) weighted and (2) unweighted degree, (3)



plot-wide and (4) experiment-wide connectance, (5) nestedness, and (6) modularity. For these models, we used a Gaussian distribution and block was again used as a random effect.

To further test the impact of the introduced thistle on the interactions between the bee visitors and the native plant species, we compared these same measures to just the native plants in the invaded plots (calculating the network measures with only visits to the native plant species). We then compared these to the network measures in the control (never invaded) plots with the same models outlined above. Finally, we compared the effect of the presence or absence of the thistle on the mean degree and  $d'$  of each native plant species, using a Welch test for unequal variance. We used  $d'$  as a way of testing whether the introduction of the thistle altered the specialization of the native plant species because  $d'$  measures how strongly the species deviates from a random sampling of available partners (Blüthgen et al. 2006). Thus, a more specialized plant species will have a greater  $d'$  value (Table 1).

To evaluate whether the timing of the thistle's presence had an impact on the network structure, we separated the data into early and late periods of the summer, where the "early" data were collected before the late treatment was applied only, and the "late" data were collected after the late treatment was applied only. The purpose of this analysis was to determine whether there are important seasonal differences in the effect of the thistle on the interactions between the plants and their insect visitors.

We used the dredge function in the R package MuMIn (Barton 2018) to identify the best model (defined here as having the lowest AICc value) for the relationship between the experimental treatment and the response of the different measured network properties (average weighted and unweighted degree, experiment and plot-wide connectance, nestedness, and modularity), using block as a random effect and comparing the fixed effects of the treatment design, time of year, number of thistle inflorescences, and thistle generality. Then we tested the relationship between each network property and the thistle generality separately in the early and late season.

To further test the impact of the non-native thistle on the pollination services the native species received, we selected one focal native species on which we collected flower heads and counted the number of pollinated seeds. For this purpose, we chose

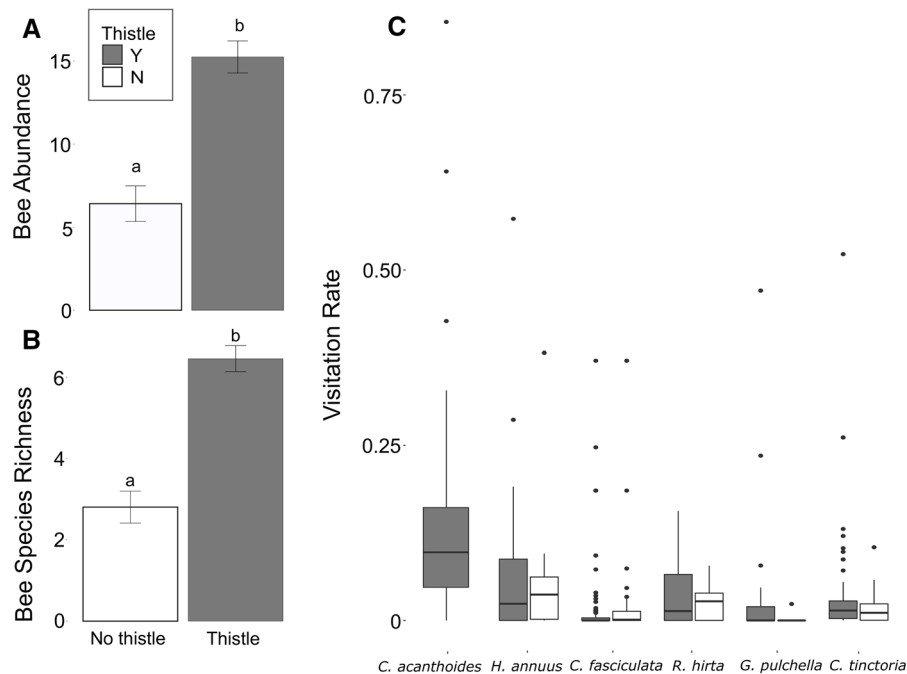
*Helianthus annuus* as our focal native plant species because the flower heads could be easily covered in bags when they finished flowering to limit seed loss. We then harvested all the flower heads at the end of the season and (a) counted the total number of bagged (flowering completed) and unbagged (still flowering) inflorescences, (b) measured the head diameter from five randomly selected heads for each plot, and (c) counted the number of viable (full) and nonviable (flat or hollow) seeds from these randomly selected heads. Where there were five or fewer inflorescences produced in a plot, we counted seeds on all the heads collected.

We only placed bags over flower heads after they finished flowering in order to avoid interfering with open pollination. This resulted in the collection of some unbagged flower heads because the experiment ended before they finished flowering. To analyse these data, we performed model selection by comparing the AIC values of the models with the number of thistle inflorescences, treatments, or the presence/absence of the thistle (all models had a Gaussian distribution and experimental block was the random effect). We report the model with the lowest AIC as the "best" model.

## Results

Overall, we vacuum-collected 3749 bee specimens, representing 69 species visiting the six plant species during our timed collections. The bowl traps collected an additional 495 bee specimens of 43 species (adding 10 new species to the total species richness) (Table S2). Thus, the denominator for experiment-wide connectance was 79 bee species multiplied by the total number of flowering plant species. Treatment plots (plots with thistles) did not have a significantly larger number of plant species flowering overall because not all plant species flowered in each plot (Fig. S5).

The visitation rate (bees per unit floral area per five minute sample) did differ significantly between the different plant species, with the thistle having approximately twice the visitation rate of the native species on average, demonstrating that the thistle is more attractive to floral visitors than the native confamilial species or the native legume used in this study (Fig. 1c). Thus, plots with thistles present had a significantly higher bee abundance and species



**Fig. 1** Bar plots showing the mean abundance (**a**) and species richness (**b**) of bees in the plots in the presence (grey) and absence (white) of the invasive thistle, with standard error bars. Letters above the bars represent significant ( $P < 0.05$ ) differences in the mean values. Box and whisker plots of the visitation

rate (number of bee visits per unit floral area per five minute sample) to the rest of the plant species (**c**) in the presence (grey) and absence (white) of the thistle. The introduced species, *C. acanthoides*, is on the far left of the visitation rate plot

richness on average than plots without thistles (Fig. 1a, b). The presence of the thistle did not significantly change the visitation rate for any of the native plant species (Fig. 1c, Table S3), similar to other studies of the impact of introduced species on visitation rates (e.g. Montero-Castaño and Vilà 2016).

The number of thistle inflorescences (range 0–811, mean: 265.4, standard error: 46.4) correlated with bee abundance at the plot level ( $df = 28$ , Pearson correlation coefficient = 0.80,  $P \ll 0.001$ ). The number of bee species with which the thistle interacted (generality) correlated with the weighted ( $df = 28$ ,  $r = 0.83$ ,  $P \ll 0.001$ , Fig. S3B) and unweighted ( $df = 28$ ,  $r = 0.75$ ,  $P \ll 0.001$ ) average degree of the plot networks. The number of thistle inflorescences each plot produced also correlated with number of visits the thistle received in each plot ( $df = 28$ ,  $r = 0.91$ ,  $P \ll 0.001$ , Fig. S3C).

For bee species richness, the model with the lowest AIC value involved the original categorical treatments (rather than the number of thistle inflorescences or the timing of the introduction), while for bee abundance, the model with the lowest AIC value included the

continuous number of thistle inflorescences (Table S4). For bee species richness, there was a significant positive effect of the early-high and early-low treatments on bee species richness and abundance (Table S5). There was a significant relationship between the number of thistle inflorescences and bee abundance (Table S5).

The generality of the thistle (number of bee species it interacts with), was a significant predictor of the average unweighted and weighted degree, nestedness, and experiment-wide connectance of the networks. However, there was no relationship between the thistle generality and the modularity or plot-wide connectance (Table 2, Fig. 2).

There is no significant difference in the mean degree or  $d'$  of the native plants in the presence or absence of the thistle (Table S6, Fig. S6). When we focus only on interactions between native plants and the resident pollinators, there is no significant effect of the treatment on any of the network measures evaluated here (Fig. 3, Table S7).

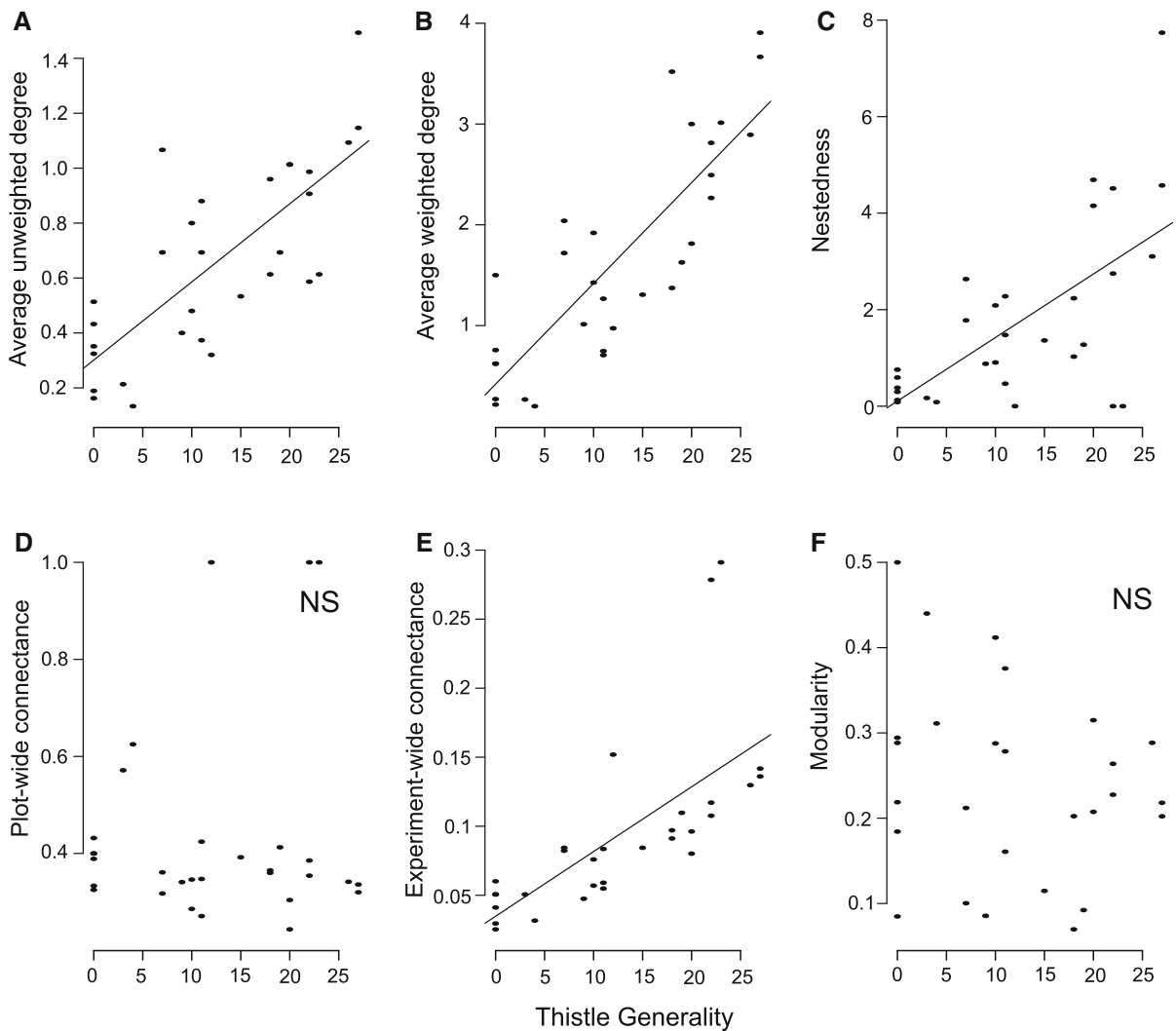
When looking at the role of the timing of the introduction, for the relationship between the



**Table 2** Results of linear mixed-effects models run to determine impact of increasing thistle generality on network measures

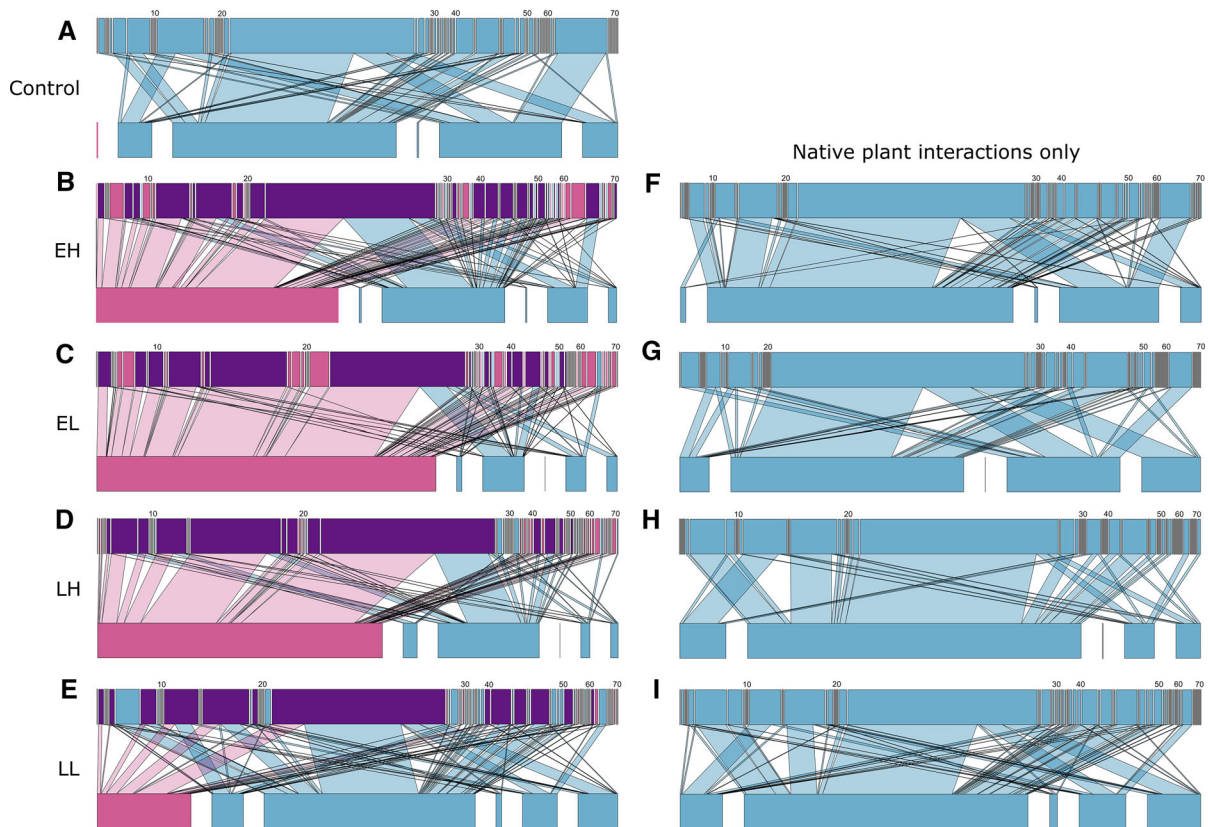
| Network measure             | Estimate | SE    | t value | P value        | Marginal R <sup>2</sup> |
|-----------------------------|----------|-------|---------|----------------|-------------------------|
| Unweighted degree           | 0.03     | 0.005 | 6.07    | ≪ <b>0.001</b> | 0.56                    |
| Weighted degree             | 0.1      | 0.01  | 8.26    | ≪ <b>0.001</b> | 0.69                    |
| Nestedness (NODF)           | 0.13     | 0.02  | 4.45    | ≪ <b>0.001</b> | 0.41                    |
| Modularity                  | − 0.003  | 0.002 | − 1.08  | 0.28           | 0.04                    |
| Plot-wide connectance       | 0.002    | 0.004 | 0.54    | 0.59           | 0.01                    |
| Experiment-wide connectance | 0.005    | 0.001 | 5.02    | ≪ <b>0.001</b> | 0.46                    |

For all models, thistle generality is the fixed effect and experimental block is used as a random effect. Significant effects are highlighted in bold



**Fig. 2** Relationships between thistle generality (number of bee species the thistle interacts with) and average unweighted (a) and weighted degree (b), nestedness (c), plot-wide

connectance (d), experiment-wide connectance (e), and modularity (f). Lines indicate a significant relationship and “NS” denotes no significant relationship



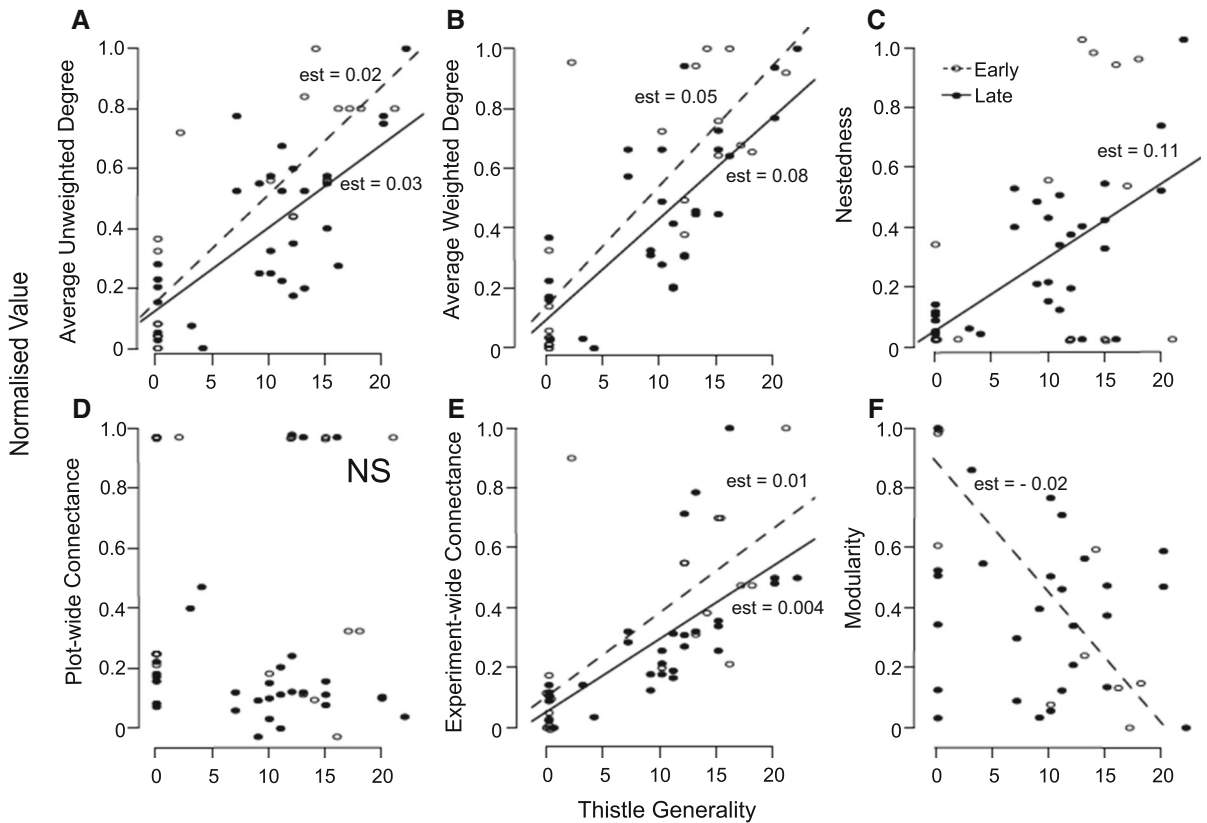
**Fig. 3** Plant-bee network diagrams of the treatments with the thistle (a–e) and the same treatment plots with only interactions with the native plants (f–i). The experimental treatments are early high thistle density (EH), early low thistle density (EL), late high thistle density (LH), late low thistle density (LL), and control (Control). The boxes represent species and the lines between them represent observed interactions. The width of the boxes and the lines are normalised proportions of the total

number of interactions in each network. The thistle and its interactions are black and the native plants and their interactions are white. Pollinators exclusively visiting the thistle are coloured in black and pollinators exclusively visiting the native plants are coloured in white. Pollinators that visit both native species and the thistle are coloured in grey. A colour version of the figure is available online

experimental treatment and the response of the different measured network properties (average weighted and unweighted degree, experiment and plot-wide connectance, nestedness, and modularity), the model with the lowest AICc had thistle generality as the fixed effect and block as the random effect. Including time of year as a fixed effect or an interaction term did not significantly improve the model fit. We found a significant positive relationship between thistle generality and average unweighted and weighted degree and experiment-wide connectance both early and late in the season. In addition, there was a significant positive relationship between thistle generality and nestedness late in the season and a significant negative relationship between thistle

generality and modularity early in the season (Fig. 4). The early season section of the network had a higher mean plot-wide connectance than late in the season or over the entire season (early:  $0.72 \pm 0.06$  se, late:  $0.44 \pm 0.04$  se, entire:  $0.43 \pm 0.04$  se).

*H. annuus* produced inflorescences in 18 of the 30 plots, with an average of 43.57 inflorescences per plot (range 1–407). We counted 1307 seeds of 88 flower heads, 35 of which were bagged (finished flowering before the end of the experiment) and 53 of which were unbagged (still in flower when harvested at the end of the experiment). There was a significant difference in the head diameter, number of good seeds, and number of bad seeds between bagged and



**Fig. 4** Relationship between thistle generality and the normalised network properties [average unweighted (a) and weighted (b) degree, nestedness (c), plot-wide connectance (d), experiment-wide connectance (e), and modularity (f)] measured in just the early portion of the summer (open circles,

dashed lines) or just the late portion of the summer (filled circles, solid lines). Lines indicate significant relationships, and the estimate of the fixed effect of thistle generality is provided for each line. “NS” indicates that there was no significant relationship

unbagged flower heads, so we analysed them separately (Table S8).

For bagged *H. annuus* flower heads, the model with the lowest AIC for number of viable seeds and diameter had the number of thistle inflorescences as the fixed effect, while for the number of nonviable seeds the model with the lowest AIC had the presence/absence of the thistle as the fixed effect. For unbagged *H. annuus* flower heads, the model with the lowest AIC for number of viable and nonviable seeds had the number of thistle inflorescences as the fixed effect, while for the diameter, the model with the lowest AIC had the presence/absence of the thistle as the fixed effect. Nonetheless, there was no significant effect of the fixed effects on any of the measured traits (number of viable or nonviable seeds or diameter) of *H. annuus* (Table S9).

**Discussion**

The introduction of a novel mutualist into an existing network of plant-pollinator interactions can act as a disturbance to network structure and its impact may be mediated by multiple aspects of the disturbance, including intensity, timing, and duration (Shea et al. 2004). Formally, we hypothesize that the introduction of a mutualist that adds new interactions, without removing existing interactions will increase the network measures of weighted and unweighted average degree, connectance, and nestedness, while having a negative effect on modularity (Russo et al. 2014). Here we used a field experiment to determine whether we could alter the magnitude of impact on network structure by manipulating the density and timing/duration of an invasive species introduction. We tested the density of the introduced species because other

studies have shown a relationship between impact and high levels of introduced species density (Kaiser-Bunbury et al. 2011) and timing/duration because the effects of an introduced species on plant-pollinator interactions can vary over time (Moragues and Traveset 2005).

This experiment was an empirical test of established theory on how interaction networks equilibrate in response to species additions (Russo et al. 2014; Carneiro et al. 2014); indeed, we found that the magnitude of the thistle's effect on the network measures of average degree (weighted and unweighted), connectance (experiment-wide), and nestedness was mediated by increasing plot-level thistle generality. Because this relationship was linear, this suggests a constant per-capita (per-inflorescence here) effect of the impact of increasing the thistle abundance (Sofaer et al. 2018), rather than an abrupt response to the addition of a novel species. Moreover, for the average unweighted and weighted degree and experiment-wide connectance, there was no effect of the timing in the introduction on the magnitude, or the slope of the observed relationship, suggesting the number of interactions formed by the introduced species plays a bigger role than the timing of its introduction for these measures. For bee species richness and abundance across the entire season, we found significant effects only when the introduction was early in the season, but because we have shown that the impact of the thistle is mediated by the number of interactions it forms, it seems likely that the duration of its introduction played a greater role than the timing of its introduction in this effect. In other words, when the thistle is introduced early in the season, it has more opportunities to interact with bees over time (Memmott and Waser 2002, but see McKinney and Goodell 2011).

On the other hand, there did seem to be timing-specific effects on nestedness and modularity. The significant relationship between nestedness and thistle generality found late in the season and across the entire season was not seen in the isolated early section of the summer. Because nestedness is related to the ways in which specialists and generalists share interaction partners, this may relate to the phenology of particular groups of species with more or less specialised interactions. For example, *Halictus ligatus*, which visited every plant species, was present across the entire season, while *Hylaeus affinis/modestus*, which

visited only one plant species, was present only late in the season.

Similarly, timing seemed to matter when we looked at the effect of the introduction on modularity. When looking across the whole season, we did not see the predicted negative effect of introduced species generality on modularity, but we did see a significant negative effect of the thistle generality on modularity when looking only at the first part of the season. Plant-pollinator networks tend to exhibit a positive correlation between nestedness and modularity, mediated by the connectance (Fortuna et al. 2010); it is possible we would have seen an effect on modularity at higher levels of connectance. The early season section of the network did have higher mean plot-wide connectance than late in the season or over the entire season. An alternative explanation is that phenology may affect modularity; indeed, previous work has shown phenology was a strong predictor of modularity in networks (Morente-López et al. 2018).

Plot-wide connectance was not significantly affected by any of our treatments across the season or in either section of the year. In this measure of connectance, we did not account for the total possible insect visitors in the landscape, rather we only used the species recorded visiting the flowers in the plot. When we account for all possible bee species (experiment-wide connectance includes both bowl and vacuum collected species across all plots), we found a positive relationship with thistle generality because plots where thistles were present had a higher abundance and species richness of bees. Although it is possible for population sizes of social bees to increase in the same season (Williams et al. 2012), the populations of solitary bees will likely not increase in the same year in response to a novel resource (Crone 2013) and because there is no significant change to the visitation rate of bees on native plants, this suggests the thistle, as an attractive host plant, is drawing a greater number and species richness of bees in from the greater landscape.

Based on our previous work with this invasive thistle species, we expected that the thistle would act as an additive mutualist by interacting with resident pollinators without changing their interactions with native plant species (Russo et al. 2016). In this study, we were able to more thoroughly examine impacts on the visitation to the other plant species, and to compare the network structure of invaded plots to uninvaded

controls. From this comparison, it seems the structure of the interactions between the resident pollinators and native plants has not changed, suggesting changes to network structure are due to the added interactions between the thistle and resident pollinators. The introduction of the thistle early in the season increased both the abundance and the species richness of bees without significantly changing the visitation rates, degree, or  $d'$  of the other plant species in the invaded plots. This absence of a significant effect on the visitation rates to other plant species has also been found in other studies (e.g. Montero-Castaño and Vilà 2016). Moreover, we found no change in the pollination services (i.e. the proportion of pollinated seeds) delivered to a focal native plant species, *H. annuus*, in agreement with previous work showing no effect of non-native species on native plant seed set (Moragues and Traveset 2005).

With experimental manipulations of community structure, it is difficult to work with large numbers of species, yet many network properties are sensitive to changes in network size (Dormann et al. 2009). The size of the plant-pollinator community evaluated in this study was relatively small (mean network size:  $21.5 \pm 1.6$  species), making some of the network measures (e.g. connectance) less reliable, and may interfere with our ability to detect changes in modularity (Dormann et al. 2009). However, our experimental treatments were replicated in a blocked design, allowing us to compare variation within and between treatments. These analyses allowed us to see significant differences in network measures, even at relatively small network sizes.

This study further progresses our understanding of structure in mutualistic networks, as we were able to directly manipulate network properties consistent with theoretical expectations by experimentally introducing a supergeneralist plant species. Further work will need to be done on disentangling timing from duration, and also on other aspects of disturbance. For example, it would be interesting to explore how the spatial extent of a novel mutualist may affect the structure of networks across its introduced range, given many invasive species are spatially patchy (Allen and Shea 2006). More work is also needed to better understand the theoretical underpinnings of how changing timing and duration may alter network properties, for example, by experimentally altering the synchronicity of the flowering (McKinney and

Goodell 2011). In addition, it would be interesting to further explore the empirical impact of species introductions on modularity and nestedness in plant-pollinator networks and the way these measures may be affected by the phenology of specialist and generalist bee species. For example, if climate change and land-use change affect specialists differentially (Cariveau and Winfree 2015), when generalist novel species are introduced, there could be significant implications for the community and, accordingly, also for the measures used for community characterization.

We designed this study with a fair amount of background knowledge about the system [e.g. the attractiveness of the thistle to resident pollinators, (Russo et al. 2016)] and with theoretical expectations about how it might respond to the introduction of a supergeneralist (Russo et al. 2014). Nonetheless, it is encouraging that we were able to validate our theoretical predictions in an experimental field study, and that the network structure responded in a way that agreed with most of our hypotheses and a previous experiment. This suggests that we could make and test similar predictions about introduced species in other systems, where there is some knowledge of the species' behaviour. Moreover, it demonstrates that it may be possible for an introduced mutualist to add new interactions to an existing network without significantly modifying the interactions between the original species. As in other studies, some attributes of the network structure remained robust to species introductions (Vilà et al. 2009). This study suggests we are gaining a better mechanistic understanding of how networks of mutualistic interactions form, and how they respond to disturbance, with significant implications for our understanding of the impacts that different types of introduced species may have on communities.

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