

## ARTICLE

# Removing flowers of a generalist plant changes pollinator visitation, composition, and interaction network structure

Justin A. Bain<sup>1,2,3</sup>  | Rachel G. Dickson<sup>3,4</sup> | Andrea M. Gruver<sup>1,2</sup> | Paul J. CaraDonna<sup>1,2,3</sup> 

<sup>1</sup>Negaunee Institute for Plant Conservation Science and Action, Chicago Botanic Garden, Glencoe, Illinois, USA

<sup>2</sup>Plant Biology and Conservation, Northwestern University, Evanston, Illinois, USA

<sup>3</sup>Rocky Mountain Biological Laboratory, Crested Butte, Colorado, USA

<sup>4</sup>Division of Biological Sciences, University of Montana, Missoula, Montana, USA

**Correspondence**

Justin A. Bain  
Email: [justin.a.bain@outlook.com](mailto:justin.a.bain@outlook.com)

**Funding information**

NSF Research Experience for Undergraduates Program, Grant/Award Number: DBI 1262713; National Science Foundation (NSF) Graduate Research Fellowship Program; Chicago Botanic Garden

**Handling Editor:** Debra P. C. Peters

**Abstract**

Pollination is essential for ecosystem functioning, yet our understanding of the empirical consequences of species loss for plant–pollinator interactions remains limited. It is hypothesized that the loss of abundant and generalized (well-connected) species from a pollination network will have a large effect on the remaining species and their interactions. However, to date, relatively few studies have experimentally removed species from their natural setting to address this hypothesis. We investigated the consequences of losing an abundant, generalist native species from a series of plant–pollinator networks by experimentally removing the flowers of *Helianthella quinquenervis* (Asteraceae) from half of a series of 10 paired plots (15 m diameter) within a subalpine ecosystem. We then asked how the localized loss of this species influenced patterns of pollinator visitation, floral visitor composition, and interaction network structure. The experimental removal of *Helianthella* flowers led to an overall decline in plot-level pollinator visitation rates and shifts in pollinator composition. Species-level responses to floral removal differed between the two other abundant, co-flowering plants in our experiment: *Potentilla pulcherrima* received higher visitation rates, whereas *Eriogon speciosus* visitation rates did not change. Experimental floral removal altered the structural properties of the localized plant–pollinator networks such that they were more specialized, less nested, and less robust to further species loss. Such changes to interaction network structure were consistently driven more by species turnover than by interaction rewiring. Our findings suggest that the local loss of an abundant, well-linked, generalist plant can bring about diverse responses within intact pollination networks, including potential competitive and facilitative effects for individual species, changes to network structure that may render them more sensitive to future change, but also numerous changes to interactions that may also suggest flexibility in response to species loss.

**KEYWORDS**

disturbance, floral resources, networks, pollination, Rocky Mountain Biological Laboratory, species interactions, visitation

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## INTRODUCTION

Plant–pollinator interactions are essential for ecosystem functioning. It is estimated that nearly 90% of flowering plant species depend on animal visitors for some aspect of their own reproduction (Ollerton et al., 2011), and more than 200,000 animal species rely on floral resources for food (Inouye & Ogilvie, 2017). Global environmental change—including climate change, habitat loss, and invasive species—is threatening nearly 40% of vascular plant species with extinction globally (Lughadha et al., 2020), and declines in pollinator populations are becoming increasingly documented (Burkle et al., 2013; Cameron & Sadd, 2020; Potts et al., 2016). Because of their mutualistic relations, the loss or reduction of plants or pollinators can have important consequences for the remaining species, their interactions, and the structure of plant–pollinator networks (e.g., Burkle et al., 2013; Maia et al., 2021; Mathiasson & Rehan, 2020; Memmott et al., 2004).

Rare and more specialized species are hypothesized to be the most susceptible to ecological disturbances (Burkle et al., 2013; Mathiasson & Rehan, 2020). However, more generalized species are also susceptible to ecological disturbances (Inouye, 2008; Renjifo, 1999), although less common, and the loss of generalist species from interaction networks may have disproportionately strong effects on the remaining species and their interactions (Biella et al., 2020; Memmott et al., 2004; Palacio et al., 2016). In particular, generalist plant species are often abundant and well connected within pollination networks (Fort et al., 2016; Ollerton et al., 2007; Waser et al., 1996). Therefore, ecological disturbances that reduce or remove generalist plants from a network may bring about considerable and immediate consequences for the pollinator community. While this prediction is well supported by simulation models that sequentially eliminate species (and therefore all of their interactions) from networks to understand which species may bring about the largest consequences (e.g., Kaiser-Bunbury et al., 2010; Maia et al., 2021; Memmott et al., 2004), it remains less clear how the loss of a well-connected generalist plant may play out in natural ecosystems.

To date, relatively few studies have experimentally removed native plant or pollinator species from intact pollination networks to investigate the consequences of their loss, but the available evidence suggests a variety of responses (plant removals: Goldstein & Zych, 2016; Kaiser-Bunbury et al., 2017; Biella et al., 2019, 2020, and pollinator removals: Brosi & Briggs, 2013; Brosi et al., 2017; but see Lopezaraiza-Mikel et al., 2007; Ferrero et al., 2013 for examples of invasive plant removals). Complete removal of

flowers of generalized plants from a landscape can dramatically reduce visitation rates and redistribute visitors to the remaining plant community (Bartomeus et al., 2008; Biella et al., 2019). If generalist plants facilitate pollinator visitation (Ghazoul, 2006), their loss may reduce visitation to the remaining flowering plant species, with potential consequences for their reproduction (Mitchell et al., 2009). By contrast, if the remaining flowers are less attractive to pollinators, the loss of a generalist plant may cause pollinators to forage elsewhere. Such shifts in pollinator foraging may functionally act as pollinator species loss from localized interaction networks. In concert, these changes may restructure interaction networks such that they become more specialized (e.g., Biella et al., 2020) or more generalized (e.g., Brosi et al., 2017; Goldstein & Zych, 2016)—with possible consequences for their sensitivity to future perturbations.

Here, we investigated the empirical consequences of losing an abundant, generalized flowering plant species from an intact plant–pollinator community in the Colorado Rocky Mountains. We did this by experimentally removing the flowers of the native plant, *Helianthella quinquenervis* (Asteraceae; hereafter *Helianthella*), from half of a series of 10 paired plots (15 m diameter). Our floral removal experiment aims to mimic a natural ecological disturbance that alters the local plant–pollinator landscape in our ecosystem—episodic spring frost events (Inouye, 2000; Pardee et al., 2018). In particular, in high-elevation and high-latitude locations, a consistent pattern of recent climate change is earlier spring snowmelt, which causes plants to begin flowering earlier in the spring (CaraDonna et al., 2014; Iler et al., 2013). This shift in flowering time means that some species are more susceptible to nighttime frost events when the flower buds are developing, including *Helianthella* (which is particularly sensitive to these frost events in our system). As a result, *Helianthella* flower abundance can be dramatically reduced, and in some years, there may be no *Helianthella* flowers in entire meadows due to frost damage (Iler et al., 2019; Inouye, 2008). Using this experimental setup, we asked how the removal of *Helianthella* flowers influenced: (1) community-level pollinator visitation rates, species-level visitation rates to other co-flowering plant species, and the composition of the pollinator community; (2) the structural properties of the plant–pollinator interaction networks, including whether changes in structure were attributed to interaction rewiring or species turnover. Altogether, this work provides an experimental test to improve our empirical understanding of the consequences of losing an abundant, well-linked, generalist plant species within a natural ecosystem.

## MATERIALS AND METHODS

### Study site and study species

Our floral resource removal experiment was conducted in an intact subalpine meadow primarily composed of perennial herbs and bunchgrasses near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (38°57.50 N, 106°59.30 W, 2900 m above sea level). The growing season in this subalpine ecosystem is brief, lasting approximately 3–5 months (CaraDonna et al., 2014). The plant and pollinator communities contain relatively high numbers of generalist species and almost exclusively native plants and pollinators (CaraDonna et al., 2017; CaraDonna & Waser, 2020). The European honey bee (*Apis mellifera*) is absent near the RMBL. Because non-native honey bees can compete with and influence the behavior of native foraging pollinators (e.g., Cane & Tepedino, 2017; Thomson, 2016), our study

provides a unique opportunity to explore the effects of an experimentally induced flower removal within an intact plant–pollinator community without the confounding effects of the presence of invasive honey bees.

Among the 15 co-flowering wildflower species at our study site during midsummer, three are most dominant: (1) *H. quinquenervis* (Asteraceae), (2) *Potentilla pulcherrima* (Rosaceae; hereafter *Potentilla*), and (3) *Erigeron speciosus* (Asteraceae; hereafter *Erigeron*) (Figure 1). Together, these three species comprise 91.5% of flowers in our plots during the period of study (Appendix S1: Figure S1). *Helianthella* is self-incompatible with relatively large, yellow composite inflorescences (capitulum) that are up to 10 cm in diameter, and plants have flower stalks that are 50–150 cm tall (Weber, 1952); it provides moderate to high nectar rewards (mean: nectar sugar concentration = 0.661 mg sugar/ $\mu$ l; nectar volume per flower head = 0.218  $\mu$ l; sugar per flower head = 0.158 mg); *Helianthella* is particularly sensitive to spring



**FIGURE 1** Photographs illustrating (a) the study site at the Rocky Mountain Biological Laboratory in Gothic, CO, USA, and (b–d) the three most abundant co-flowering plant species. (a) An example of the abundance of *Helianthella quinquenervis*, the focal flowering plant species whose capitula were removed from experimental plots at our study site. (b) *Helianthella* being visited by a common bumble bee species (*Bombus flavifrons*). (c) *Potentilla pulcherrima* flowers being visited by a common fly species. (d) *Erigeron speciosus* being visited by a common bumble bee species.

frost events (Inouye, 2008). *Potentilla* is self-compatible with small, yellow flowers up to 1 cm in diameter and stalks 30–80 cm tall (Aitken, 2004); it provides low to moderate nectar rewards (mean: nectar sugar concentration = 0.379 mg sugar/ $\mu$ l; nectar volume per flower = 0.160  $\mu$ l; and sugar per flower = 0.068 mg). *Erigeron* is largely self-incompatible (P. J. CaraDonna, unpublished data) with moderate-sized composite inflorescences measuring 3–5 cm diameter, composed of yellow disk florets and lavender ray florets, with stems 15–80 cm tall; it provides moderate nectar rewards (mean: nectar sugar concentration = 0.471 mg sugar/ $\mu$ l; nectar volume per flower head = 0.227  $\mu$ l; and sugar per flower head = 0.071 mg). Unlike *Helianthella*, both *Potentilla* and *Erigeron* are not particularly sensitive to spring frost events (CaraDonna & Bain, 2016; CaraDonna & Bain, unpublished data). All three species are visited by a diverse, generalized suite of pollinators (e.g., CaraDonna & Waser, 2020), including bees, butterflies, moths, flies, and beetles.

## Flower removal experiment

We removed *Helianthella* flowers from a series of large plots at our study site on 5 July 2017 (Figure 1; Appendix S1: Figure S2). The specific arrangement of our study plots is based on a previously established *Helianthella* flower removal experiment, which was set up to address distinctly different questions (e.g., floral albedo; Iler et al., 2021). The experiment used a paired plot design: 10 *Helianthella* flower removal plots and 10 control plots (*Helianthella* flowers left intact). Each circular plot measured 15 m in diameter (Appendix S1: Figure S2). Plots were paired based on their spatial proximity within the meadow, whereby the edge of each experimental pair was, on average, 3 m apart. The flower removal treatment was assigned randomly within each plot pair. We cut all *Helianthella* capitula within floral removal plots, while they were in the flower bud stage, approximately 1 week before the start of *Helianthella* flowering. Any flower heads missed during the initial removal were subsequently removed when they began to bloom. We controlled for any trampling effects that may have occurred during the removal treatment application by replicating a similar trampling effect in all control plots.

## Plant–pollinator interaction observations

We focused our plant–pollinator interaction observations across the peak flowering period of *Helianthella* over 3 weeks (10–28 July 2017). Although the entire flowering period of *Helianthella* is somewhat longer than our

interaction observations, our sampling period effectively captures pre-peak (Week 1), peak (Week 2), and post-peak flowering (Week 3). The same two researchers monitored plant–pollinator interactions in control and removal plots throughout the entire experiment (J.A.B. and R.G.D.). We observed each plot twice per observation day: One observation session occurred during the morning (8:30 AM–12:00 PM) and the other during the afternoon (1:00–5:00 PM). During an observation session, we observed each plot for 15 min; researchers would slowly move throughout the entire plot, scanning all open flowers a few meters at a time and recording all floral visits by pollinators (following CaraDonna et al., 2017). We randomized the order of plot observations before each daily observation session, and researchers swapped treatments after each observation period. We performed all plant–pollinator interaction observations during weather favorable for pollinator activity when the temperatures were above 15°C and when it was not raining or excessively windy (<16 kmh) (Vicens & Bosch, 2000). We observed all flowering plant species present during observation sessions to minimize sampling bias attributed to floral abundance. Each plot received six observations per week, resulting in 90 min (1.5 h) of total observation time per plot per week. Across all plots and treatments, this resulted in 30 h per week and 90 h of total observation time across the 3-week study period. This sampling level has been shown to characterize the plant–pollinator interactions in this subalpine system effectively (e.g., Burkle & Irwin, 2009; CaraDonna et al., 2017).

We recorded an interaction when an insect contacted the reproductive parts of an open flower. We recorded the plant species visited for each visitation event and the floral visitor's identity (hereafter referred to as pollinator). We identified pollinators to species or the lowest taxonomic resolution possible in the field. When species-level identification was not possible, specimens were identified as unique morphospecies (i.e., pollinators were first identified to the lowest taxonomic level and then given a functional name in place of a species name). We did not destructively sample pollinators to avoid influencing plant–pollinator interactions in our plots and the following observation periods. All plants were identified to species. We quantified floral abundance during each week of the experiment by counting the number of open flowers for all plant species present in a 2 × 2 m square plot located within the center of each larger, circular plot.

## Pollinator visitation and composition

We quantified species-level visitation rates by dividing the total number of visits to each plant species (per plot

and observation session within each study week) by the number of flowers recorded for each species (per plot in each week). To quantify community-level pollinator visitation rates, we then calculated the mean visitation rate across all species present (per plot per observation session and week). In a few cases, a visitation event was observed for a plant, but no open flowers were recorded for this species within the census plot; in these cases, to avoid recording this visitation event as a zero, we divided the number of visits by one flower.

We compared the composition of floral visitors between control and removal plots across all weeks combined using nonmetric multidimensional scaling ordination and permutational multivariate analysis of variance. Ordinations were created using square-root-transformed visitation data with Bray–Curtis dissimilarity distances (Oksanen et al., 2019).

## Plant–pollinator interaction network structure

We constructed a series of plant–pollinator interaction networks by aggregating interaction data within each week across each plot and each treatment (one interaction matrix per plot per week). These plot-level networks can be seen as replicates of localized interaction networks whose relative structures can be compared across treatments and weeks. To investigate how the removal of *Helianthella* flowers from our experimental plots influenced the structure of plant–pollinator interaction networks, we compared six measures of network structure between the control and removal treatments: connectance, links per species, network-level specialization, nestedness, network robustness, and plant and pollinator niche overlap. Together, these metrics help reveal whether the relative structure of these interaction networks became more or less specialized or generalized and whether they are more or less sensitive to future change. *Connectance* describes a fundamental component of network complexity and is calculated as the proportion of observed links out of all possible links (values range from 0 to 1). *Network-level specialization* ( $H_2'$ ) is a frequency-based metric that characterizes the level of interaction specialization within a bipartite network; it indicates how much niche partitioning there is across all interactions within the entire pollination network (Blüthgen et al., 2006). Values of  $H_2'$  range from 0 to 1, with higher values indicating greater specialization and therefore less interaction niche overlap among plants and pollinators within the network. *Weighted nestedness* based on overlap and decreasing fill ( $wNODF$ ) (Almeida-Neto et al., 2008), describes the extent to which specialist species (those with few links)

interact with generalist species (those with many links) (Bascompte et al., 2003), and takes into account interaction frequencies; values range from 0 to 100, where 100 indicates a perfectly nested network.

*Plant and pollinator niche overlap* measures the amount of interaction specialization separately for plants and pollinators within the network; values range from 0 to 1, where 1 indicates perfect niche overlap (greater generalization) among species. *Network robustness* quantifies a network's sensitivity to the simulated extinction of plants or pollinators (or both; Memmott et al., 2004; Burgos et al., 2007); robustness values range from 0 to 1, where 0 indicates that all species become secondarily extinct after removing the first species (zero robustness and high sensitivity) and 1 indicates that no species become secondarily extinct (maximum robustness and low sensitivity). We calculated network robustness separately for plants and pollinators by simulating the random loss of species from the opposite group.

Finally, we quantified the amount of interaction turnover (i.e., changes in the composition of interactions) between each pair of control and removal networks. Total interaction turnover between a pair of networks can be attributed to two additive components: species turnover (interactions gained or lost because a species is gained or lost) and interaction rewiring (interactions are reassembled because of changes in who is interacting with whom among species that are present) (Poisot et al., 2012). In the context of our experiment, species turnover represents interactions that are lost (or gained) because a species is absent (or present) between a pair of control and removal networks. Interaction turnover values range from 0 to 1, where 0 indicates no differences in the composition of interactions between networks, and 1 indicates complete interaction turnover (no similarity in composition).

## Data analysis

We analyzed the effect of experimental treatment (flower removal vs. control) on all of our response variables using linear mixed-effects models. In our statistical models, we included the phenological period of *Helianthella* (pre-peak, peak, and post-peak) as a categorical interaction term to determine whether any treatment effects varied with flowering time. We also included plot pair (categorical) and sampling date (continuous) as random effects to account for the spatial proximity of the study plots and repeated sampling of study plots within each phenological stage. All models were checked to ensure they met the assumption of normality and heteroscedasticity. Values were log-transformed for our visitation rate analyses to

improve normality and heteroscedasticity. We first assessed the significance of treatment effects in our statistical models with a type III ANOVA (i.e., to test whether there was a significant treatment  $\times$  phenology interaction); if the interaction term was not statistically significant, we then used a type II ANOVA to assess the significance of the main effects (a type II ANOVA assumes no interaction term and is statistically more powerful for assessing main effects; Langsrud, 2003).  $p$ -Values were generated from our models using Satterthwaite's method for denominator df and  $F$ -statistic. When relevant, we describe  $p$ -values using the language of evidence following (Muff et al., 2022), where approximate boundaries indicate the strength of evidence, e.g.,  $p > 0.1$  indicates no evidence,  $p < 0.1$  indicates weak evidence,  $p < 0.05$  indicates moderate evidence,  $p < 0.01$  indicates strong evidence, and  $p < 0.001$  indicates very strong evidence.

To understand whether the patterns we observed between treatments resulted from additive or synergistic effects of the experimental removal of *Helianthella* flowers, we repeated each analysis described above with the omission of all interactions with *Helianthella* from our control plot data before treatment comparison. We refer to these as “*Helianthella* interaction omission” analyses. If treatment effects vanish when we omit visits to *Helianthella* from the control plot data, this would suggest that the effect is additive (i.e., driven mainly by the loss of *Helianthella* flowers); by contrast, differences between treatments would suggest that the effect is synergistic (i.e., the removal of *Helianthella* flowers has consequences beyond the loss of its flowers). *Helianthella* omission analyses were otherwise identical to those described above.

All data analyses were performed using R version 4.0.4 (R Core Team, 2021). Linear mixed-effects models were performed using the *lme4* package (Bates et al., 2014); statistical significance of models was assessed using the *lmerTest* package (Kuznetsova et al., 2017); all network metrics and interaction turnover were calculated in the *bipartite* package (v. 2.15) (Dormann et al., 2008; Fründ, 2021; Poisot et al., 2012).

## RESULTS

We observed 9489 pollinator visits to flowers representing 15 plant species and 60 pollinator species. Across all plots, treatments, and observations, visits to *Helianthella*, *Potentilla*, and *Erigeron* comprised 91% of observed visitation events. In the control plots, there were a total of 5479 visitation events across 15 plant species and 49 pollinator species; *Helianthella* received 2122 visits (39%) from 32 pollinator species, *Potentilla* received 1530 visits (28%) from 35 pollinator species,

and *Erigeron* received 1452 visits (27%) from 26 pollinator species. In the *Helianthella* floral removal plots, there were 4010 visitation events across 11 plant species and 48 pollinator species; here, *Potentilla* received 1824 visits (45%) from 35 pollinator species, and *Erigeron* received 1703 visits (42%) from 29 pollinator species. *Potentilla* received visits from 43 pollinator species across all plots and treatments, and *Erigeron* received visits from 35 pollinator species.

*How does experimental floral removal influence pollinator visitation rates and composition?* Removing *Helianthella* flowers reduced pollinator visitation rates at the community level. On average, community-level visitation rates were 47.3% lower in removal plots compared to control plots (Table 1; Figure 2a). When we examined these treatment effects when all interactions to *Helianthella* were omitted (i.e., *Helianthella* interaction omission analysis), we found mixed results, indicating the presence of both synergistic and additive effects (Table 1; Appendix S1: Figure S3). We found moderate evidence of a treatment  $\times$  phenology interaction, whereby there is a reduction in visitation rates in Week 1, and then no effect of treatment on visitation in subsequent weeks. This suggests that overall reductions in community-level visitation patterns in response to the experimental removal of *Helianthella* flowers (Figure 2a) result from synergistic effects in Week 1 and additive effects in Weeks 2 and 3.

Overall, the composition of pollinators differed between control and removal treatments ( $F_{1,58} = 4.46$ ;  $p = 0.001$ ; Appendix S1: Figure S4). Just 10 species contributed over 60% of the compositional differences between the treatments. Six of the top 10 species that contributed most to compositional differences consisted of bumble bees. Other species that contributed most to compositional differences include *Halictus virgatellus* (solitary bee), *Rhamphomyia* spp. (dance flies), *Speyeria atlantis* (butterfly), and *Miridae* spp. (leaf bugs) (Appendix S1: Table S1). Our *Helianthella* omission analysis found no difference in pollinator composition between the treatments ( $F_{1,58} = 1.52$ ,  $p = 0.11$ ; Appendix S1: Figure S5), suggesting that the treatment effect was additive and due to the loss of *Helianthella* flowers.

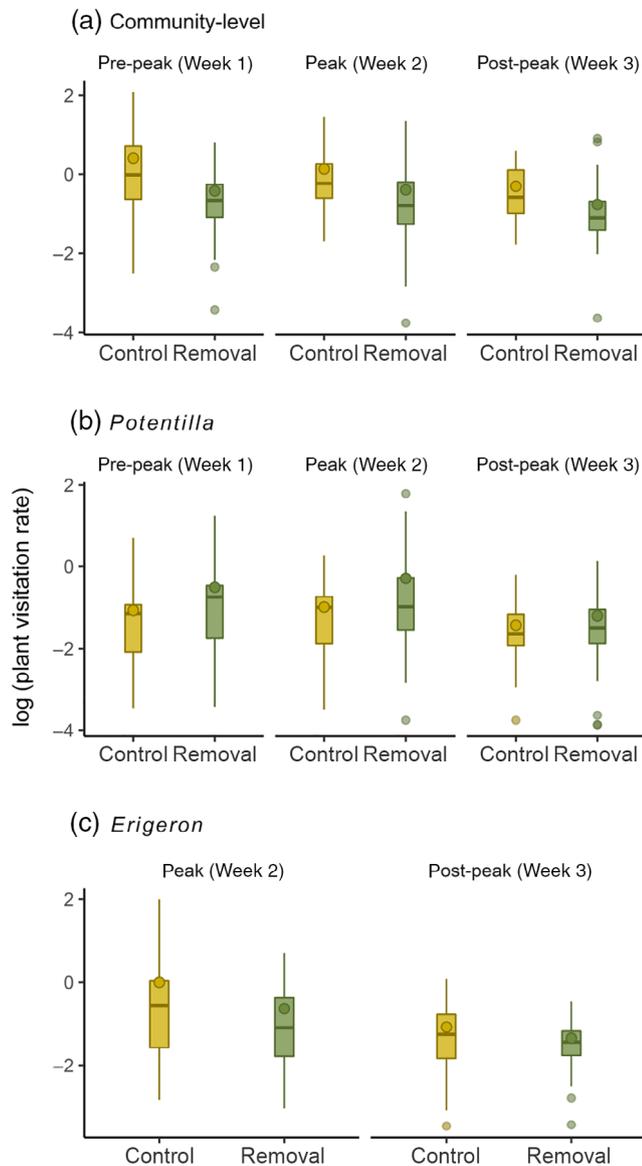
*How does experimental flower removal influence species-level visitation rates to other co-flowering plants?* Removing *Helianthella* flowers resulted in species-specific effects on pollinator visitation rates to the other two abundant co-flowering plant species in the landscape. Visitation rates to *Potentilla* were 43% higher on average in floral removal plots compared to control plots (Table 1; Figure 2b). By contrast, visitation rates to *Erigeron* were 39.1% lower on average in floral removal plots compared to control plots (Table 1; Figure 2c).

*How does experimental floral removal influence plant-pollinator network structure?* We found consistent

**TABLE 1** Results of the effects of experimental floral removal of an abundant generalist floral resource, *Helianthella quinquenervis*, on pollinator visitation and network structure from linear mixed-effects models.

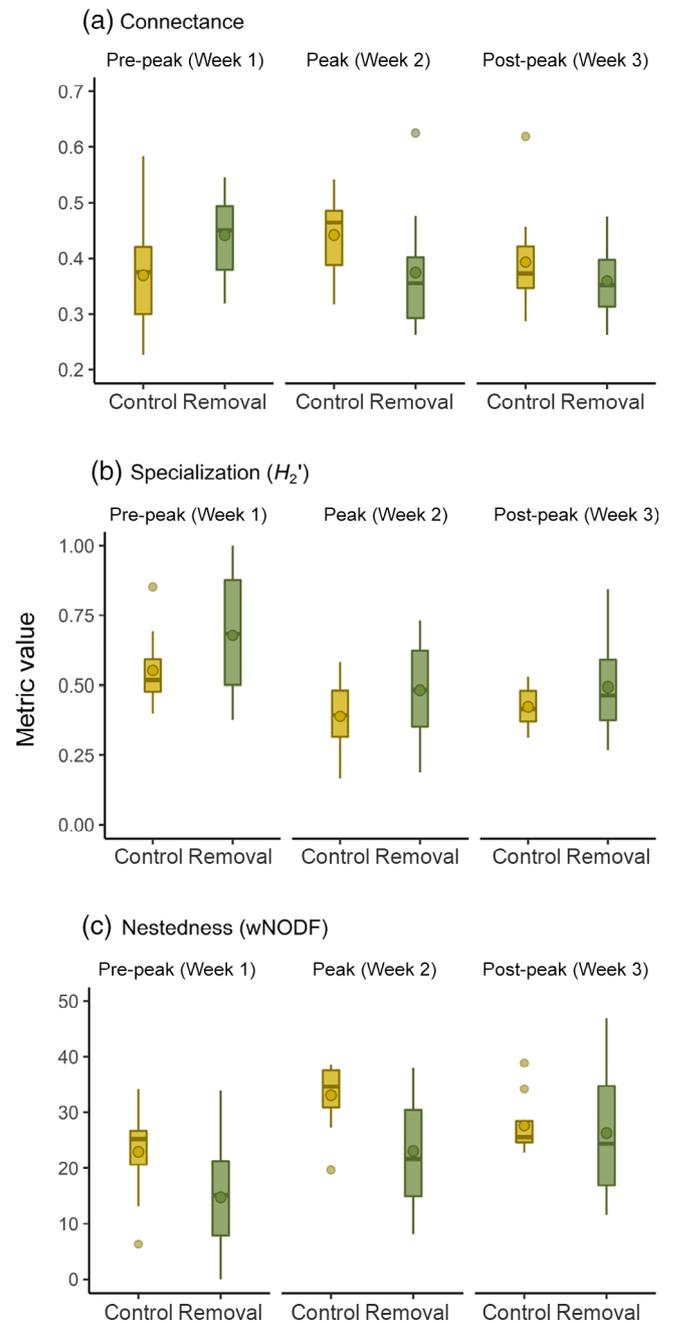
Model	Model fixed effects	F	df	p
Pollinator visitation rates				
Community-level	Treatment	16.009	1; 9	0.003
	Phenology	3.105	2; 11	0.086
	Treatment × phenology	0.135	2; 221	0.874
Community-level ( <i>Helianthella</i> interaction omission)	Treatment	8.653	1; 58	0.005
	Week	1.952	2; 24	0.164
	Treatment × phenology	4.582	2; 214	0.011
<i>Potentilla pulcherrima</i>	Treatment	6.125	1; 9	0.036
	Week	2.727	2; 11	0.110
	Treatment × phenology	1.219	1; 205	0.298
<i>Erigeron speciosus</i>	Treatment	4.413	1; 9	0.066
	Phenology	2.098	1; 7	0.191
	Treatment × phenology	1.301	1; 113	0.256
Interaction network metrics				
Connectance	Treatment	3.215	1; 41	0.08
	Phenology	1.689	2; 36	0.199
	Treatment × phenology	3.280	2; 36	0.049
Specialization ( $H_2'$ )	Treatment	5.277	1; 9	0.047
	Phenology	7.944	2; 36	0.001
	Treatment × phenology	0.172	2; 36	0.843
Nestedness (wNODF)	Treatment	8.379	1; 9	0.017
	Phenology	6.734	2; 36	0.003
	Treatment × phenology	1.387	2; 36	0.263
Niche overlap (pollinators)	Treatment	13.98	1; 9	0.005
	Week	9.083	2; 36	<0.001
	Treatment × week	1.299	2; 36	0.285
Niche overlap (plants)	Treatment	19.18	1; 9	0.002
	Phenology	7.746	2; 36	0.002
	Treatment × phenology	2.525	2; 36	0.094
Robustness (pollinators)	Treatment	11.51	1; 9	0.008
	Phenology	2.749	2; 36	0.077
	Treatment × phenology	2.62	2; 36	0.087
Robustness (plants)	Treatment	8.287	1; 9	0.018
	Phenology	6.797	2; 36	0.003
	Treatment × phenology	2.680	2; 36	0.082
Interaction turnover	Species turnover versus interaction rewiring	31.294	1; 45	<0.001
	Phenology	0.845	2; 45	0.436
	Turnover component × phenology	4.747	2; 45	0.013

*Note:* In all models, “plot pair” is included as a random effect. Statistical significance for main effects was assessed via type II ANOVA when there was not a significant interaction term in the model. *p*-values were generated from the mixed-effects models using Satterthwaite’s method for denominator df and *F*-statistic. “Community-level *Helianthella* interaction omission” indicates analysis where all visits to *Helianthella* are omitted from control plot data before treatment comparison (see *Materials and methods* for details).



**FIGURE 2** Plots comparing differences in pollinator visitation rates between the control plots and treatment plots where *Helianthella quinquenervis* capitula were removed: (a) the community-level, (b) visitation rates to *Potentilla pulcherrima*, and (c) visitation rates to *Erigeron speciosus*. All dots inside the boxplots represent means. The community-level and *Potentilla* models show strong ( $p < 0.01$ ) and moderate ( $p < 0.05$ ) evidence of treatment effects respectively, whereas the *Erigeron* model shows weak evidence of a treatment effect ( $p < 0.1$ ). There was no evidence of week effects or treatment  $\times$  week interactions. Statistical details for all models are included in Table 1.

evidence that the removal of *Helianthella* flowers altered the local structure of plant–pollinator interaction networks. There was moderate evidence that network specialization and nestedness responded to the removal of *Helianthella*, but only weak evidence that network connectance responded to the removal of *Helianthella* (Table 1; Figure 3). Such changes were consistent with an



**FIGURE 3** Plots comparing differences in network metric values between the control plots and treatment plots where *Helianthella quinquenervis* capitula were removed: (a) connectance, (b) specialization ( $H_2'$ ), and (c) nestedness (weighted NODF). All dots inside the boxplots represent means. The connectance model finds weak evidence of a treatment effect ( $p < 0.1$ ) and moderate evidence ( $p < 0.05$ ) of a treatment  $\times$  week interaction, whereas the specialization and nestedness models find moderate evidence ( $p < 0.05$ ) of a treatment effect and strong evidence of a week effect ( $p < 0.01$ ). Statistical details for all models are included in Table 1.

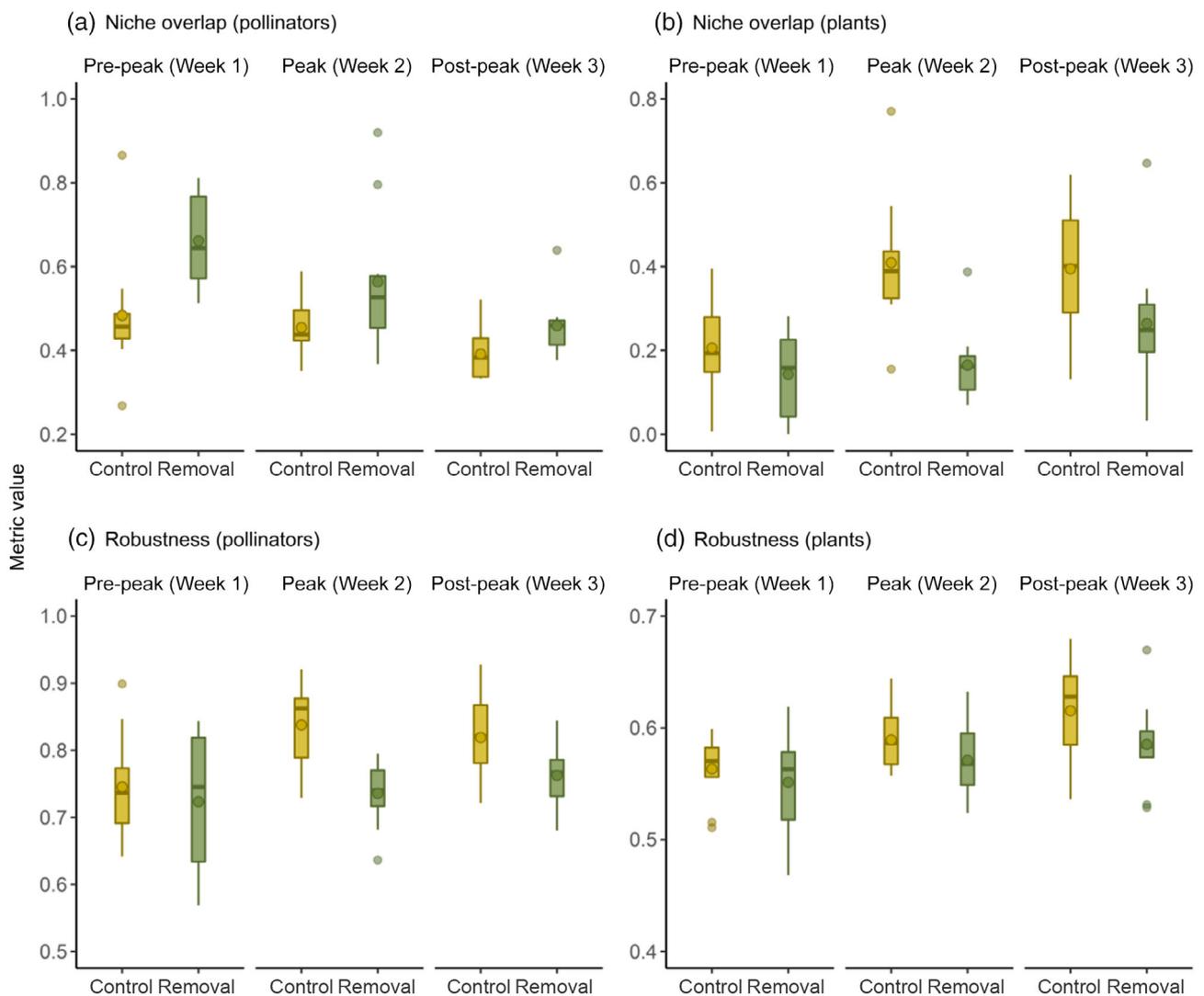
overall reduction in interaction generalization. Average network connectance was 2.5% lower overall in the removal networks compared to the control networks

(Table 1; Figure 3). Average network specialization ( $H_2'$ ) was 17.4% higher in the removal networks compared to the control networks (Table 1; Figure 3). Nestedness (weighted  $NODF$ ) was 23.3% lower in the removal networks compared to control networks (Table 1; Figure 2b). Only connectance showed evidence of a treatment  $\times$  phenology interaction, indicating that for specialization and nestedness, the treatment effects remained consistent with temporal variation in network structure (Table 1).

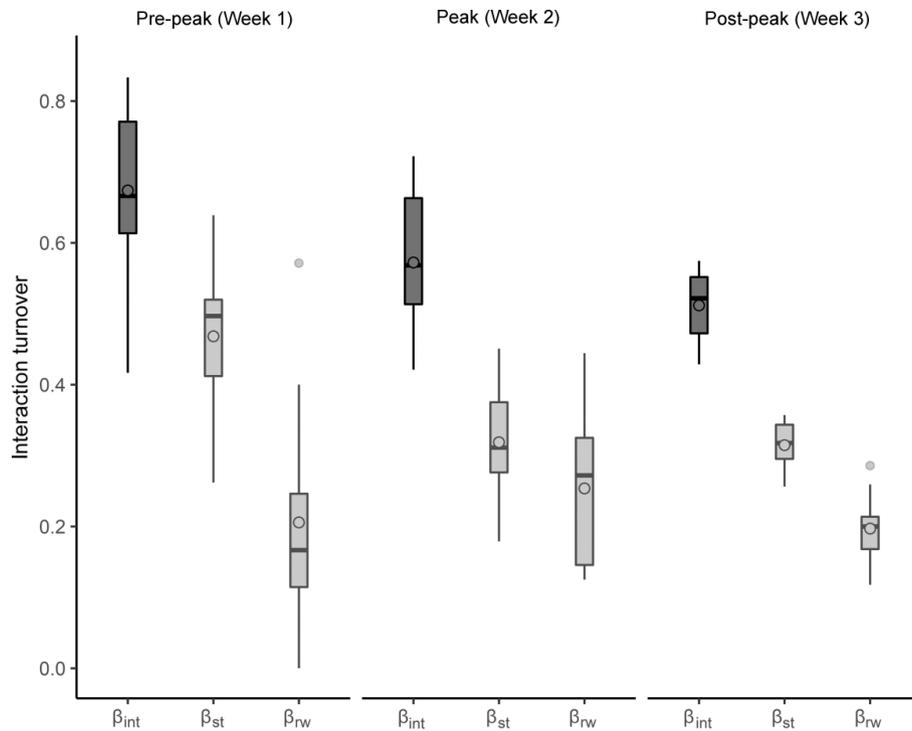
When comparing group-level metrics for both plants and pollinators, we find consistent evidence that both groups responded to the removal of *Helianthella* flowers. Average pollinator interaction niche overlap was 21.1% higher in removal networks than control networks; by contrast, plant interaction niche

overlap was 43.3% lower in removal networks than control networks (Figure 4). We found strong evidence that niche overlap for both groups varied across weeks but no evidence of a treatment  $\times$  phenology interaction for plants or pollinators (Table 1). Network robustness was lower in the floral removal treatment compared to the control networks. Robustness to plant extinctions was 3.5% lower, whereas robustness to pollinator extinctions was 8% lower (Figure 4). We found strong evidence that plant robustness varied over time but only weak evidence that pollinator robustness varied over time. We found weak evidence of a treatment  $\times$  phenology interaction for both plants and pollinators (Table 1).

When we excluded *Helianthella* interactions from the control dataset, only connectance and plant niche overlap



**FIGURE 4** Plots comparing differences in group-level network metric values (i.e., plants and pollinators separately) between the control plots and treatment plots where *Helianthella quinquenervis* capitula were removed for (a) niche overlap (pollinators), (b) niche overlap (plants), (c) robustness (pollinators), and (d) robustness (plants). All dots inside the boxplots represent means. All models show moderate ( $p < 0.05$ ) or strong evidence ( $p < 0.01$ ) of a treatment effect; weak ( $p < 0.1$ ) to very strong ( $p < 0.001$ ) evidence of a week effect for all metrics; and no more than weak evidence of a treatment  $\times$  week interaction for all metrics. Statistical details for all models are included in Table 1.



**FIGURE 5** Plots showing interaction turnover between the control plots and treatment plots where *Helianthella quinquenervis* capitula were removed, where the dark gray boxplot represents interaction turnover ( $\beta_{\text{int}}$ ), and the light gray boxplots represent species turnover ( $\beta_{\text{st}}$ ) and interaction rewiring ( $\beta_{\text{rw}}$ ) respectively. All dots inside the boxplots represent means. The model shows very strong evidence ( $p < 0.001$ ) of a difference between species turnover and interaction rewiring, no evidence of a week effect ( $p > 0.1$ ), and moderate evidence of a treatment  $\times$  week interaction ( $p < 0.05$ ). Statistical details of the model are included in Table 1.

showed moderate evidence of a treatment response (Appendix S1: Table S2 and Figure S6). This pattern indicates that treatment effects on network structure were mostly additive due to the loss of *Helianthella* flowers.

Finally, total interaction turnover between control and removal treatments ( $\beta_{\text{int}}$ ) was high throughout the experiment (mean  $\beta_{\text{int}} = 0.59$ ) (Figure 5). The effect of species turnover ( $\beta_{\text{st}}$ ) on total interaction turnover was greater than the effect of interaction rewiring ( $\beta_{\text{rw}}$ ) (mean  $\beta_{\text{st}} = 0.37$  and mean  $\beta_{\text{rw}} = 0.22$ ) (Figure 5). We found no evidence that interaction turnover varied over time, but there was moderate evidence of a treatment  $\times$  phenology interaction (Table 1).

## DISCUSSION

We experimentally removed *Helianthella* flowers from localized, plant–pollinator networks in a series of large, replicate plots within a subalpine ecosystem. Our experiment aimed to mimic the effects of a natural ecological disturbance—damaging late-spring frost events—that are becoming more frequent in our study system and have clear and dramatic consequences for *Helianthella* (Iler et al., 2019; Inouye, 2008). Given the scale of our

experiment, our results represent behavioral changes from pollinators in response to the localized loss of an important floral resource (i.e., from large patches), rather than the result of more extensive extirpation of this species (i.e., from the meadow or entire ecosystem), providing empirical evidence for understanding the consequences of a natural disturbance leading to species loss from interaction networks. The findings we present here suggest that losing an abundant, generalist plant from localized pollination networks yields numerous changes in interactions among species, including reduced pollinator visitation rates and altered species composition; potential changes in competition and facilitation among individual plant species; increases in network specialization; and reductions in network robustness. Our results are consistent with findings from invasive species removal studies, indicating that generalist plants have similar effects on the neighboring plant and pollinator communities regardless of their native or non-native status (Bartomeus et al., 2008; Daniels & Arceo-Gómez, 2020; Ferrero et al., 2013).

Experimental removal of *Helianthella* flowers resulted in an immediate reduction in pollinator visitation rates. The observed lower visitation rates in the removal plots appear to result from synergistic effects prior to

*Helianthella* peak flowering (Week 1) and additive effects during peak flowering and after (Weeks 2 and 3). These patterns may partly be explained by the difference in floral density and abundance of the control and removal plots. Dense and more abundant patches of flowers are generally more attractive to pollinators because they reduce travel time between flowers and increase the foraging efficiency and visitation rates within a patch (Ebeling et al., 2008; Pyke, 1984; Sih & Baltus, 1987). Evidence points to pollinator visitation frequency as a strong determinant of plant reproductive output (Vázquez et al., 2005). Increased visitation can change the quality of pollination by increasing conspecific pollen deposition, but may also increase heterospecific pollen or self-pollination rates (Karron et al., 1995). Therefore, the reduction in the visitation rates we observed in the *Helianthella* floral removal plots suggests that the loss of an abundant and generalized flowering plant can potentially alter the quantity and quality of pollination services provided by pollinators.

At the species level, our study showed that the experimental removal of *Helianthella* flowers had contrasting effects on visitation rates for the two other abundant co-flowering plant species. *Potentilla* received higher visitation rates in the absence of *Helianthella* flowers, indicating that it may compete with *Helianthella* for pollinators. A few factors may help to explain these potentially competitive interactions. First, *Helianthella* flowers produce much higher nectar quantities than *Potentilla* (0.158 mg sugar per flower head compared to 0.068 mg sugar per flower), making it an overall higher quality resource to foraging pollinators. Additionally, despite distinct differences in their floral morphology (Figure 1b,c), both *Helianthella* and *Potentilla* exhibit bright yellow floral displays, which may broadly attract similar assemblages of pollinators, but *Helianthella* flower stalks are much taller than *Potentilla*. Therefore, some pollinators may overlook *Potentilla* when *Helianthella* is present because *Helianthella* flowers are larger, more visible, easily accessible, and provide more floral resources.

In contrast to *Potentilla*, the effect of removing *Helianthella* flowers on visitation rates to *Erigeron* indicated evidence for a facilitative relationship. The similarity in floral morphology and visual cues between *Helianthella* and *Erigeron* (Figure 1b,d) may promote facilitation among plants for pollinators via similar handling strategies and search images (Callaway, 1995; Johnson et al., 2003; Moeller, 2004; Thompson, 2001). *Helianthella* may also promote facilitation through a magnet species effect whereby visitors spill over to other nearby, similar, and otherwise less-visited flowers, like *Erigeron* (Ghazoul, 2006; Laverty, 1992; Moeller, 2004). Two traits typically associated with magnet species are large floral

displays and the production of large amounts of nectar (Johnson et al., 2003; Molina-Montenegro et al., 2008), both of which *Helianthella* exhibits. Finally, the peak flowering time of *Helianthella* occurs before the peak flowering period of *Erigeron* at our study site (Weeks 2 and 3 of our study, respectively). As a result, pollinators initially attracted to *Helianthella* may switch to later blooming *Erigeron* flowers when *Helianthella* floral abundance declines (Waser & Real, 1979). The existence of potentially facilitative interactions between generalists like *Helianthella* and other co-flowering species like *Erigeron* may help buffer plant and pollinator communities against species extinctions by increasing interaction overlap among pollinators (Verdú & Valiente-Banuet, 2008). Consequently, the loss (or reduction) of a generalist like *Helianthella*—and therefore the loss of any facilitative effects it provides—may make plant and pollinator communities more vulnerable to future species losses.

The shift in the floral visitor community in response to the localized experimental removal of *Helianthella* flowers was largely driven by abundant bumble bee species, which generally avoided the floral removal plots and instead foraged within control plots containing *Helianthella* flowers (Appendix S1: Table S1). Most of the pollinators in our study system have foraging ranges larger than the scale of our experimental floral removal. If *Helianthella* flowers are a preferred resource, then bumble bees and other pollinators can fly to anywhere in the meadow where these flowers are present. This pattern is also consistent with our interaction turnover results, whereby the observed differences in interactions between control and removal networks were primarily driven by changes in species turnover, indicating the movement of pollinators between control and removal treatments. These localized changes in pollinator composition could bring about reproductive consequences for the remaining plant species. For example, if bumble bees, which are the most effective pollinators in our system, preferentially forage in patches of the meadow with *Helianthella* flowers, then the remaining patches of flowers without *Helianthella* may experience a decline in reproduction if the remaining floral visitors are less effective pollinators (sensu Brosi & Briggs, 2013).

The changes we observed in pollinator visitation rates and species composition in response to the removal of *Helianthella* flowers gave rise to the altered structural properties of the plant–pollinator interaction networks. Other experiments that have removed generalist plant species have found conflicting results: Biella et al. (2020) observed increased network specialization, whereas others have observed increased generalization (Goldstein & Zych, 2016; Kaiser-Bunbury et al., 2017). We find that experimental floral removal gave rise to more specialized

and less nested networks. Such variation in network structure (i.e., the composition of interactions) was due primarily to the effect of species turnover; in other words, the total turnover of interactions between control and removal networks was driven more by interactions lost or gained due to species turnover than it was by interaction rewiring among the remaining species. Overall, the relative differences in network structure between floral removal and control networks remained even with significant week-to-week variation (which is characteristic of our study system: CaraDonna et al., 2017; CaraDonna & Waser, 2020).

More specialized and less nested networks are hypothesized to be less stable for mutualistic networks than more generalized and nested networks (Thébault & Fontaine, 2010; but see Allesina & Tang, 2012). Consistent with this hypothesis, when we simulated the random loss of plants or pollinators from control and removal networks, we observed lower robustness in the floral removal networks, indicating that the removal of *Helianthella* flowers at the scale of our experimental plots made the networks more fragile. Robustness decreased approximately twice as much for pollinators (in response to simulated plant extinctions) as it did for plants (in response to simulated pollinator extinction), suggesting that the pollinator community may be somewhat more vulnerable to the loss of generalist flowers like *Helianthella* than the plant community to the loss of generalist pollinators. This pattern makes sense given that *Helianthella* is an important food source for many pollinators, but most plant species in this system are visited by multiple pollinators (CaraDonna & Waser, 2020). Together, these network properties provide evidence that reducing or removing abundant generalist floral resources, even at a localized plot level, may render networks more susceptible to future disturbances.

Although interaction networks became more specialized in response to experimental floral removal, we observed that interaction niche overlap was lower among plants and higher among pollinators when we examined the plants and pollinators as groups separately. In other words, plants became more specialized in response to the local removal of *Helianthella* flowers, whereas pollinators became more generalized. Two factors likely influenced the observed reduction in plant interaction niche overlap: generalist pollinators moving from *Helianthella* removal plots to control plots with *Helianthella* flowers and less potential pollinator facilitation received by plants in the presence of *Helianthella*. Likewise, the greater pollinator interaction niche overlap may also be explained, at least partly, by the reduction of abundant generalist bumble bees in floral removal plots without *Helianthella* flowers. For example, the absence of a highly abundant and generalized

pollinator may relax competition among the remaining pollinators, thereby allowing their interaction niches to expand, as was found when dominant bumble bee species were experimentally removed from subalpine meadows (Brosi et al., 2017; Brosi & Briggs, 2013). These patterns together suggest variable and flexible responses among plants and pollinator species, which may provide some resilience to the loss (or reduction) of *Helianthella* despite the overall network structure appearing to be at least somewhat more sensitive to further species loss.

Our findings suggest that the removal of an abundant, well-linked, generalist plant from a pollination network can bring about a variety of responses, including potential increases and decreases in competition and facilitation for co-flowering plant species, reductions in network generalization, and robustness to species loss, but also numerous changes in interactions among species suggesting some level of flexibility in response to disturbance. By removing *Helianthella* flowers from local plant–pollinator networks, our experiment takes an important step toward understanding how natural ecological disturbances may affect plant–pollinator interactions on a fine temporal and spatial scale. As the frequency of plant and pollinator declines continues to increase, understanding the consequences of losing abundant and well-connected plant species is a critical component of conserving the most at-risk species and their interactions.

#### AUTHOR CONTRIBUTIONS

Paul J. CaraDonna conceived the project; Justin A. Bain, Rachel G. Dickson, and Paul J. CaraDonna designed the project and conducted the experiment; Justin A. Bain, Rachel G. Dickson, and Paul J. CaraDonna collected the data; all authors analyzed the data and interpreted results. Justin A. Bain wrote the initial draft of the manuscript, and all authors contributed to revisions.

#### ACKNOWLEDGMENTS

We thank A. M. Iler and K. Mooney for providing access to the study plots; the Rocky Mountain Biological Laboratory for providing access to facilities; K. Mooney's laboratory group, and S. Walwema, for assistance with *Helianthella* removal in the field; R. Perenyi for assistance with flower counts; G. Kirschke for collecting floral nectar data; and all anonymous reviewers and the Iler + CaraDonna Lab whose comments greatly improved our manuscript. This work was supported by the Chicago Botanic Garden (Paul J. CaraDonna, Andrea M. Gruver, and Justin A. Bain), the National Science Foundation (NSF) Graduate Research Fellowship Program under Grant No. DGE-1842165 (to Justin A. Bain), and the NSF Research Experience for Undergraduates Program under Grant No. DBI 1262713 (to Rachel G. Dickson).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All novel scripts and analysis files (Bain et al., 2022) are available from the Open Science Framework (OSF): <https://doi.org/10.17605/OSF.IO/EHU73>.

## ORCID

Justin A. Bain  <https://orcid.org/0000-0002-6738-4665>

Paul J. CaraDonna  <https://orcid.org/0000-0003-3517-9090>

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**How to cite this article:** Bain, Justin A., Rachel G. Dickson, Andrea M. Gruver, and Paul J. CaraDonna. 2022. "Removing Flowers of a Generalist Plant Changes Pollinator Visitation, Composition, and Interaction Network Structure." *Ecosphere* 13(7): e4154. <https://doi.org/10.1002/ecs2.4154>