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Title: Community context mediates effects of pollinator loss on seed production

Short Title: Community context in ecosystem function

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1 Abstract

A critical goal for ecologists is understanding how ongoing local and global species losses will affect ecosystem functions and services. Diversity-functioning relationships, which are well characterized in primary producer communities, are much less consistently predictable for ecosystem functions involving two or more trophic levels, particularly in situations where multiple species in one trophic level impact functional outcomes at another trophic level. This is particularly relevant to pollination functioning, given ongoing pollinator declines and the value of understanding pollination functioning for single plant species like crops or threatened plants. We used spatially replicated, controlled single-pollinator-species removal experiments to assess how changes in bumble bee species richness impacted the production of fertilized seeds in a perennial herb — *Delphinium barbeyi* — in the Rocky Mountains of Colorado, USA. To improve predictability, we also assessed how traits and abundances in the plant and bumble bee communities were related to *D. barbeyi* reproductive success. We hypothesized that trait-matching between pollinator proboscis length and *D. barbeyi*'s nectar spurs would produce a greater number of fertilized seeds, while morphological similarity within the floral community would dilute pollination services. We found that the effects of pollinator removal differed depending on the behavioral patterns of pollinators and compositional features of the plant and pollinator communities. While pollinator floral fidelity generally increased *D. barbeyi* seed production, that positive effect was primarily evident when more than half of the *Bombus* community was experimentally removed. Similarly, communities comprising primarily long-tongued bees were most beneficial to *D. barbeyi* seed production in tandem with a strong removal. Finally, we observed contrasting effects of morphological similarity in the plant community, with evidence of both competition and facilitation among plants. These results offer an example of the complex dynamics underlying ecosystem function in multi-trophic systems and demonstrate that community context can impact diversity-functioning relationships between trophic levels.

2 Introduction

Biodiversity positively influences many key biological functions, underscoring concerns about the functional impacts of ongoing local and global extinctions (Hooper et al., 2005; Cardinale et al., 2012). Dozens of experiments have confirmed relationships between biodiversity and ecosystem functioning ("BEF"), particularly in primary producer communities involved in functions like biomass production or carbon fixation, to name just two examples (Tilman et al., 2014). However, the evidence for BEF relationships is much more ambiguous for ecosystem functions that are mediated across two or more trophic levels, such as predation, pollination, and seed dispersal (Sih et al., 1998; Casula et al., 2006; Loy and Brosi, 2022). For example, in multi-predator/single-prey studies, increased predator richness may positively or negatively impact the biomass of a target prey species, depending on other properties of the system in which these interactions take place (Sih et al., 1998; Casula et al., 2006). Thus, there remains a need to understand what factors shape how biodiversity in one trophic level affects the functional responses in a single species at another trophic level.

Plant-pollinator communities represent one system in which the question of BEF patterns across trophic levels is particularly important. Insect pollinators, which facilitate reproduction in a vast majority of flowering plants, are experiencing widespread population declines (Kremen et al., 2002; Potts et al., 2010). Yet BEF studies examining the effects of pollinator losses in these systems have yielded contradictory results (Brosi and Briggs, 2013; Hallett et al., 2017), limiting ecologists' ability to predict how global patterns of pollinator loss will impact ecosystem function. Removal experiments involving a single pollinator species have shown negative impacts on pollination in some systems (Brosi and Briggs, 2013) but not in others (Hallett et al., 2017), indicating that functional relationships in these systems could be mediated by more than simply species richness. Indeed, while numerical metrics of biodiversity like pollinator richness and abundance are likely to play major roles in pollination function (Tilman et al., 2014; Winfree et al., 2015), functional traits within both plant and pollinator communities may also affect the BEF dynamics in these systems (Hooper et al., 2005; McGill et al., 2006). For instance trait-matching (or mismatching) is likely to incentivize some plant-pollinator interactions—like pollinators with long tongues visiting flowers with deep corolla tubes—and limit others (Stang et al., 2009; Garibaldi et al., 2015; Peralta et al., 2020). In this case, successful pollination may rely less on the total abundance of pollinators in a community than on the relative abundance of pollinators morphologically adapted to forage upon these morphologically distinct flowers (Inouye, 1980). Since species richness is likely to interact with several other elements of community composition at both trophic levels involved, it is crucial for future multi-trophic studies of BEF patterns to go beyond investigating species richness *per se* and to instead understand how functional trait distributions in all relevant trophic levels modify the functional effects of biodiversity.

In this study, we examined how plant-pollinator community dynamics influenced the relationship between removals of single bumble bee species (*Bombus* sp.) and pollination services in a target plant species (*Delphinium barbeyi*, Ranunculaceae). We conducted short-term, single-species removal experiments with four of the 11 *Bombus* species present in our study area, precluding identity effects that would arise from consistently removing the same pollinator species. We repeated this experiment over three summers, with true spatial replication across our experimental units. We generally hypothesized that the proportion of specific traits in the plant and pollinator communities, as well as the relative abundance of individuals within each guild, mediated the effects of pollinator removal on plant reproductive function (number of fertilized seeds produced). Specifically, we expected that (i) pollinator community composition that promoted visitation to *D. barbeyi* (high *Bombus* abundance, high proportion of long-tongued bees) would increase the production of viable seeds by this focal plant; (ii) pollinator foraging behavior that promoted conspecific pollen transfer (floral fidelity) would also increase seed production; and (iii) morphological similarity between our focal plant species and other flowers in the community would dilute floral visitation and overall reproduction in our focal plant throughout our experiment via interspecific competition.

3 Methods

3.1 Study system

We conducted this study over three years (2011, 2013, 2014) in subalpine meadows surrounding the Rocky Mountain Biological Laboratory, (38°57.5′N, 106°59.3′W, 2,900 m above sea level), Gunnison National Forest, Colorado, United States. We replicated our experiment over 14 sampling events distributed across 13 unique sites, with sites separated by at least 1km to ensure independence of the pollinator communities (Darvill et al., 2004; Knight et al., 2005). To maintain independence in our data, we sampled each site only once during this study, with the exception of one location that we sampled in both 2011 and 2014. At each site, we established a 20-m by 20-m plot that contained comparable densities of target flowers throughout the study system (Brosi and Briggs, 2013).

To estimate reproductive success, we counted the production of fertilized ovules (seed production) by *Delphinium barbeyi* (Ranunculaceae), a common perennial wildflower in the Rocky Mountains that is visited by a wide range of bumble bee (Hymenoptera: Apidae: *Bombus*) pollinators. *D. barbeyi* is notable for its long nectar spur (10 – 18mm, Warnock, 1993), which generally results in a high abundance of visits by pollinators with long proboscises like *Bombus* (Inouye, 1980; Pyke, 1982). Previous work in this system has confirmed that nearly all *Bombus* species in the region, including those with relatively shorter proboscises, visit *D. barbeyi* (Inouye, 1978; Brosi and Briggs, 2013).

3.2 Field data collection

3.2.1 Assessment of seed production

Prior to each sampling event, we selected 30 focal *D. barbeyi* individuals at which we would assess seed production. We randomly assigned half of these individuals to the control period and the other half to the manipulation. Approximately two days prior to the control survey at each site, we covered racemes of immature floral buds on all focal plants with pollinator exclusion bags to prevent pollination outside of the study period. Then at the beginning of each study period, we removed the pollinator exclusion bags on the assigned half of mature *D. barbeyi* flowers. After four hours, during which our focal individuals were exposed to insect pollinators, we re-bagged the flowers and allowed any fertilized ovules to mature without further pollination. We harvested mature fruits from bagged flowers 7–15 days after the study period and counted the developing seeds produced in each fruit to estimate the reproductive function of each flower during its assigned study period.

3.2.2 *Bombus* removal manipulations

Each of our 14 sampling events comprised two 4-hour study periods. First, we established a control period during which we allowed pollinators to forage without intervention. During this control period, we performed baseline surveys of *Bombus* richness and abundance via timed aerial netting within our 20-m by 20-m plot. Two days after each control period, we performed a manipulation in which we non-destructively removed all individuals of the bumble bee species deemed most abundant from these baseline community compositional surveys. To achieve this removal, we performed targeted aerial netting of every individual of the selected species present in our plot (Inouye, 1978). We kept these removed bumble bees alive in vials in a cooler until the end of the study period, at which time we released them (Brosi and Briggs, 2013). Once we had not observed any individuals of the target *Bombus* species in our plot for one hour, we removed pollinator exclusion bags from our focal *D. barbeyi* individuals and commenced the 4-hour study period. During this study period, field personnel patrolled the perimeter of the plot and removed any individuals of the target *Bombus* species that were seen entering the area, and also repeated surveys of the remaining *Bombus* richness and abundance.

3.2.3 Floral morphological similarity

In order to assess the composition of the floral community around our focal *D. barbeyi* individuals for each sampling event, we surveyed the species identity and number of flowers for each plant in bloom along two 1-m by 20-m transects (Brosi and Briggs, 2013). We then calculated the proportion of the floral community that overlapped with *D. barbeyi* on two morphological characteristics — color (blue/violet) and corolla length (10 – 18mm) (Warnock, 1993). We selected these two characteristics because we expected them to influence the rate of bumble bee visitation to *D. barbeyi* compared to other plants at each site.

3.2.4 Pollinator traits and floral fidelity

We observed the foraging patterns of individual bumble bees during each study period in order to assess how our manipulation affected the number and fidelity of visits to *D. barbeyi*. We followed individual bumble bees foraging within our plots until they either reached 100 foraging visits or left the perimeter of the study area. We recorded a foraging event each time a bumble bee interacted with the reproductive organs (anther or stigma) of a flower. We then calculated floral fidelity as a binary trait for each bee, with an individual considered faithful when all of its visits were made to the same floral species.

Because the morphology of *D. barbeyi* allows long-tongued bees to more efficiently obtain its nectar

(Inouye, 1980), we assessed the proportional distribution of this trait in the pollinator communities that we surveyed during each study period. We then calculated the percent of each *Bombus* community that was considered "long-tongued," using accepted expert descriptions (Inouye, 1977; Colla et al., 2011; Koch et al., 2012; Williams et al., 2014).

3.3 Data analysis

We conducted all analyses in the statistical software R (R Core Team, 2021). A fully reproducible R markdown report of our analyses is included in the supplementary material.

3.3.1 Relationship between manipulation and *Bombus* community composition

Our experimental manipulation involved the removal of the most abundant *Bombus* species from each site. We assessed whether this removal significantly altered the total *Bombus* abundance and proportion of long-tongued bees across our study system, since such results could have influenced the effects of our experimental removal. To test for an effect of our manipulation on total *Bombus* abundance, we ran a generalized linear mixed effects model (GLMM) with negative binomial errors using *Bombus* abundance as the response variable, the strength of our removal as a fixed effect, and site and year as a random intercepts. To test for an effect of our manipulation on the distribution of tongue-lengths in our community, we used a similar GLMM, but with the proportion of long-tongued bees as our response variable and binomial errors.

3.3.2 Model selection and averaging

To identify the determinants of seed production in *D. barbeyi*, we used an Akaike information criterion (AIC) model selection framework that tested each combination of fixed effects to identify the best set of variables to describe our observed data. Multi-model inference is an effective tool through which a range of likely ecological processes can be assessed simultaneously (Johnson and Omland, 2004; Symonds and Moussalli, 2011). We used this analytical framework because multiple combinations of community ecological factors could affect our outcome of interest — in this case, the production of fertilized *D. barbeyi* seeds. A traditional hypothesis-testing approach would only determine whether a fixed set of explanatory variables significantly affected our response variable, effectively testing our ability to build an *a priori* prediction of the relevant ecological processes in this system. Rather than testing a specific hypothesis, this model selection approach allowed us to evaluate the relevance of a range of community ecological variables on the production of fertilized ovules and build a more robust understanding of the most important ecological processes governing this outcome.

After detecting overdispersion in our data, we built our global model using a GLMM following a zero-inflated negative binomial distribution using the *glmmTMB* package for R (Magnusson et al., 2017). We included the number of fertilized ovules collected from each *D. barbeyi* carpel in our models as the response variable. Each *D. barbeyi* flower typically contains three carpels, which resulted in an average of three distinct seed counts per flower. Our fixed effects included strength of our experimental manipulation—measured as the proportion of the total pollinator community that we manually removed—and a series of metrics describing community composition and trait values for both the plant and pollinator communities (Table 1). For the plant community, we included the relative abundance of our focal plant at each site (PD) and the proportion of the floral community that shared one of two morphological traits—color (CL) and corolla length (CR)—with *D. barbeyi*. For the pollinator community, we included the total abundance of bumble bees (BA), the proportion of the bumble bees in each community observed engaging in faithful foraging behavior (i.e., visiting only a single plant species; MF), and the relative abundance of long-tongued bumble bees (PL), which we expected to engage in trait-matching with the long corolla spurs of *D. barbeyi*. Since we hypothesized that the experimental *Bombus* removal could have had indirect effects via alteration to the pollinator community, as shown by Inouye (1978), we also examined pairwise interactions between each fixed effect associated with the pollinator community and our manipulation. We included site, *D. barbeyi* plant, and *D. barbeyi* flower as nested random intercepts and year as a separate crossed random intercepts.

We compared the AIC values from models created from every combination of fixed effects that successfully converged. Since our model selection did not yield a single obvious “best” model, we then averaged the coefficients estimated by all models with $\Delta AIC < 2$ (Grueber et al., 2011; Symonds and Moussalli, 2011; Dormann et al., 2018) to draw general conclusions about the effect plant and pollinator community composition on *D. barbeyi* seed production.

4 Results

4.1 Overview of ***Bombus*** removal experiment

Over three study years and 14 paired control/manipulation survey periods, we counted 6,816 developing *D. barbeyi* seeds (control: 3,016; manipulation: 3,800) from 679 flowers (334; 345) on 439 unique plants (218; 221). Based on relative abundance during each control period, we removed four different species of bumble bees (*B. appositus*, *B. flavifrons*, *B. kirbiellus*, *B. nevadensis*; Table 2). As expected, the removal of the most abundant pollinator species resulted in a significant ($p < 0.001$) reduction in *Bombus* abundance when averaged across all sites (control: 66.7 ± 52.9 individuals; manipulation: 53.4 ± 30.1 individuals). For 11 of the 14 manipulation periods, the bumble bee species removed was a long-tongued species (i.e., all removals except for *B. flavifrons*); however, our manipulation did not have a statistically signifiant effect on the relative abundance of long-tongued bees across communities ($p = 0.76$). Though hummingbirds are common pollinators of *D. barbeyi* (**?**), we observed no hummingbird visits during any of our study periods, likely in response to researcher presence in the plots.

4.2 Model selection and averaging

Model selection yielded 16 models within our threshold range of $\Delta AIC < 2$ (Table 3). Because there was no clear best model from this workflow, we performed model averaging on all 16 models using equal weights for all models (Dormann et al., 2018). This workflow yielded the average model coefficients shown in Table 4. All seven main effects and four of the six interactions were included in one or more models in the best set. The two interaction terms that were dropped were the interaction between the removal and color-similarity (PR:CL) and the interaction between the removal and corolla-length similarity (PR:CR). For the main effects, each was present in at least half (8/16) of the best models, while for the interactions, each was present in at least two of the best models. Two fixed effects, both related to the plant community, were present in every model in the set of best models; these were the proportion of the community with flower colors (CL) and corolla lengths (CR) similar to *D. barbeyi*.

After model averaging, our model predicted seed production values for one manipulation period that were more than three times higher than any other survey period. To check whether some element of this site was abnormal and could have skewed our results, we repeated our model selection and averaging protocol with this site (both control and manipulation surveys) removed. This analysis yielded coefficients that were consistent with the model including all sites and plots that were nearly identical, so we chose to report our findings with all 14 sites included.

4.2.1 Effect of *Bombus* removal

Contrary to our expectations, our model predicted an increase in the production of fertilized *D. barbeyi* seeds in response to our *Bombus* removal manipulation. Additionally, all three metrics describing *Bombus* community composition interacted with the manipulation to influence the production of fertilized ovules, though the effect of that manipulation generally depended on the relative abundance of the *Bombus* species that we removed. For sites at which the removed bumble bee species represented less than half of the overall *Bombus* community, all three variables related to pollinator community composition had modest negative impacts on the production of *D. barbeyi* seeds that were largely similar to the effects of each variable in the control setting. However, for sites at which more than half of the total bumble bee community was removed in our manipulation, we see dramatically different relationships between all three variables and the production of fertilized seeds (Figure 1). In these high-removal settings, overall *Bombus* abundance had a steeply negative effect, while the proportion of long-tongued bees and pollinator floral fidelity both had steeply positive effects (Figures 2, 3).

4.2.2 *Bombus* community composition

In addition to the interactive effects with *Bombus* removal described in the previous paragraph, all three of our pollinator community compositional variables were also identified as important additive predictors of *D. barbeyi* seed production. Pollinator floral fidelity exhibited a positive effect on *D. barbeyi* seed production consistent with previous work in this system (Brosi and Briggs, 2013). However, two other elements of the pollinator community that we also expected to be positively related to *D. barbeyi* reproduction did not show such effects in predictions by our averaged model. The total number of bumble bees in a community and the proportion of those bumble bees that were categorized as “long-tongued” both had negative effects on the production of fertilized seeds in our target plant when assessed as main effects.

4.2.3 Plant community composition

Unsurprisingly, *D. barbeyi* in communities with a higher relative abundance of conspecifics predicted a higher number of fertilized seeds. We also saw an interaction between our experimental manipulation and the relative abundance of *D. barbeyi*, though this interaction was consistent with the overall positive effect of these conspecific flowers in the community. Specifically, sites at which more than half of the *Bombus* community was removed experienced the strongest benefit from high *D. barbeyi* relative abundance, though this variable was also beneficial in the control.

Morphological similarity between *D. barbeyi* and the rest of the floral community had contrasting effects on *D. barbeyi* seed production. *D. barbeyi* yielded more fertilized seeds in communities with a high proportion of flowers with similar corolla lengths, but fewer in communities with a high proportion of similarly colored flowers. This finding partially contradicted our expectation that floral similarity would result in lower reproductive function due to increased heterospecific pollen transfer as pollinators visit different species of flowers with similar morphological traits.

5 Discussion

In order to link ongoing biodiversity losses to likely functional outcomes in a way that reflects real-world dynamics—particularly for ecosystem functions that cross trophic levels—ecologists must integrate BEF theory and community ecology. In this study, we do so by examining how the plant and pollinator communities influenced the production of fertilized seeds in a focal plant species—*D. barbeyi*—following the experimental removal of a single *Bombus* species. Our model offers compelling evidence that the effect of a single species removal on *D. barbeyi* reproduction occurs via changes to the behavior and composition of the pollinator community. Three major sets of results stand out from our analysis. First, we found that some elements of pollinator community composition likely influenced the effect of our single-pollinator-species removals on the production of fertilized *D. barbeyi* seeds. Second, we identified patterns in pollinator foraging behavior that also affected reproductive function in this focal plant species. Finally, we found that the plant community exerted both competitive and facilitative effects on *D. barbeyi* reproduction, likely via access to insect pollinators that can transfer conspecific pollen. These findings demonstrate that the relationship between biodiversity and ecosystem function is not always easy to predict and can be influenced by community dynamics in important and unexpected ways.

5.1 Pollinator community composition

The composition of the bumble bee community impacted *D. barbeyi* reproduction, though these impacts did not follow our initial hypotheses. We had hypothesized that access to pollinators represented an important limitation on *D. barbeyi* reproduction and thus, that a community with more bumble bees would support a higher plant reproductive success. However, our averaged model predicted a *negative* effect of *Bombus* abundance on the production of fertilized *D. barbeyi* seeds. While this negative relationship was consistent across our study, it was steepest in our "high" removal scenario in which 50% or more of the overall bumble bee community was removed (Figure 1). Since we found a significant negative correlation between our manipulation and *Bombus* abundance, we believe that these negative trends are largely reflective of our removal. However, if correlation between these variables was the only driver of this effect, we would expect that our model selection would have simplified the output to include only one of these two variables. In addition, if the effects of our manipulation on bumble bee abundance were the primary driver of the results on seed production, we would expect to see a different pattern of abundance when examining only the data from the control state of each site (solid, dark gray line in Figure 1). Those data, however, are also consistent with a negative relationship of bumble bee abundance on seed production, albeit with a relatively small negative slope. Thus, while we expect that correlation with our manipulation explains a portion of the surprising effect of *Bombus* abundance, we presume that there are other biological processes also underlying this finding that we do not understand.

We also expected that trait-matching between long-tongued bumble bees and *D. barbeyi*'s nectar spurs would make the presence of these bees in a community an important predictor of reproductive function for this focal species. We were again surprised to see that this was not consistently true and that the proportion of long-tongued bees was only strongly beneficial under high-removal scenarios (Figure 2). This disparity in functional importance for long-tongued bees could have some relationship to the ability for shorter-tongued species to obtain nectar from these flowers if reduced visitation events allowed nectar to collect in the spurs (Inouye, 1978). Thus, visitation by non-long-tongued species could depend on the overall reduction in pollinators due to our manipulation, which could reasonably have varied depending on the strength of those removals.

The divergent effects of pollinator community composition in low- and high-removal scenarios are interesting as they suggest that the morphological traits and relative abundance of a pollinator jointly contribute to its functional role in a community. When a numerically dominant pollinator is removed from the community, we see that the functional role that is lost is best filled by long-tongued bees. However, in more even communities where the removed bee species represents less than half of the total community, long-tongued bees do not have such an important functional role. Frameworks for understanding BEF relationships that solely focus on functional traits or numerical abundance may therefore overlook important information about how community dynamics shape interspecific interactions.

5.2 Pollinator foraging behavior

In addition to having access to a pool of abundant and morphologically suitable pollinators, plant reproduction also requires these pollinators to engage in conspecific pollen transfer. Our model emphasizes the importance of pollinator foraging behavior—specifically floral fidelity—in promoting pollen transfer between plants of the same species. Floral fidelity has been previously identified as an important component of plant reproductive success in the context of plant-pollinator interactions (Amaya-Márquez, 2009; Brosi and Briggs, 2013; Brosi, 2016). Our work here is consistent with that past work, with our averaged model predicting a positive effect of site-level floral fidelity. Interestingly, the effect of floral fidelity is only strongly evident in our high-removal scenario (Figure 3). This result suggests that pollinator behaviors that support plant reproduction, like high floral fidelity, could functionally compensate for negative compositional effects, such as the loss of *Bombus* abundance caused by our experimental removal.

At five of our seven “high-removal” sites, *D. barbeyi* produced more fertilized ovules during the manipulation than they did in the control. Interestingly, these included the three sites with the highest seed production during the control period, which indicates that these unexpected trends could have been driven by other site-level variables that promoted the transfer or germination of conspecific pollen. For instance, environmental conditions like soil chemistry or precipitation could have influenced the success of *D. barbeyi* seed fertilization in such a way that overcame the loss of pollinators and floral fidelity at these sites. Other elements of both the plant and pollinator communities also likely influenced these seed production trends, as evidenced by our model selection results. Thus, we take these initial findings as evidence that plant reproductive function cannot be solely determined by pollinator loss or floral fidelity and must be considered in the context of other community-level trends.

5.3 Competition and facilitation in the plant community

Our model contains evidence of both competitive and facilitative interactions within the plant communities that we studied. We expected that morphological similarity within the floral community would promote competition among flowers for insect pollinators that are attracted to those traits. By potentially attracting the same pollinators, we surmised that morphological similarity would increase the likelihood of heterospecific pollen transfer and thus, reduce ovule fertilization in our focal *D. barbeyi* individuals. Indeed, we observed a negative relationship between color similarity and *D. barbeyi* seed production that was consistent with our expectation of interspecific competition within the plant community. While many bees are attracted to blue/violet flowers (Briscoe and Chittka, 2001), they may not discriminate well between flowers of the same color. Pollinators may switch floral partners more frequently in the presence of equally attractive, similarly colored flowers and thus increase the chances for heterospecific pollen transfer throughout this floral community. However, we observed more fertilized *D. barbeyi* seeds at sites with a large proportion of other flowers with long corolla lengths, which contradicted our expectation of intraspecific competition on this axis of morphological similarity. Rather than competing for pollen vectors, plants in this community with similar corolla lengths may facilitate pollination of co-flowering individuals. Complex floral morphology like *D. barbeyi*'s long nectar spur may signal increased quantity and quality of floral rewards (Krishna and Keasar, 2018). On the community level, the prevalence of such a trait across multiple plant species may therefore appear more rewarding to pollinators, resulting in facilitation via the attraction of more pollinators to this community (Ghazoul, 2006; Hegland et al., 2009). Pollinators attracted by this trait might also be better suited to forage upon these flowers and through this form of trait-based environmental filtering, provide more consistent pollen transfer services between conspecific individuals (Messier et al., 2010).

For these traits to truly facilitate plant reproduction, they must not only increase access to pollinators but also increase the overall transfer of conspecific pollen. Because we see a stronger positive effect of corolla-length similarity than we see a negative effect of color (via a larger model coefficient, in variables that are similarly scaled), we suspect that in this plant community facilitation plays a stronger role in plant reproductive function. Specifically, the cost of heterospecific pollen transfer from pollinators switching partners within a pool of similarly colored flowers may be outweighed by the benefits of increased visitation by pollinators that are well suited to transfer pollen between flowers with long corolla tubes (Peter and Johnson, 2008; Wei et al., 2021). Pollinators could also discriminate between similar-shaped flowers along another niche axis, like a flower's nutrient profile or display size, which would increase the likelihood of conspecific pollen transfer. These results also potentially indicate that pollination in this system is more limited by the availability of pollen vectors (pollinators) than of appropriate pollen donors (conspecific plants), which may limit the negative impact of conspecific pollen loss by unfaithful pollinators.

5.4 Future Directions

As our experiments were conducted at the scale of individual days, we are limited in our ability to draw conclusions about the long-term effects of species loss on ecosystem function. Though our short-term approach had several notable advantages, particularly in ensuring nearly equivalent floral resources between control and manipulation states, facilitating non-destructive bumble bee removals, and allowing us to conduct multiple true replicates over each season, our conclusions could be strengthened by future single-species removal experiments conducted at longer timescales. We also focused our experiment on a single target plant species, which limits our ability to extend our conclusions to other flowering plants in this or other communities. Though the limitations of time and effort likely preclude the inclusion of every flowering plant at a site in such a study, an expanded experiment that examines the effect of pollinator removal on a subset of plants known to compete for pollinator visitation could provide valuable insight into how community context impacts BEF relationships in plant-pollinator communities.

6 Conclusion

Biodiversity loss is eroding the economic, social, and biological functions of ecosystems on a global scale (Cardinale et al., 2012; Oliver et al., 2015,?). Many important ecosystem functions and services are driven by relationships in which species richness at one trophic level impacts functional outcomes in other trophic levels. However, previous work on such systems has yielded inconsistent diversity-functioning relationships. We show here that in a study system comprising a single plant species visited by multiple pollinator species, single pollinator species removals (with the identity of that removed species changing among replicates) have negative functional impacts on seed production in our focal plant via the effect of this removal on the plant and pollinator communities. This work underscores the need to understand how community context interacts with diversity to mediate biodiversity-ecosystem functioning relationships.

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8 Conflict of Interest Statement

The authors declare no conflict of interest.

References

Amaya-Márquez, M. (2009, December). Floral constancy in bees: a revision of theories and a comparison with other pollinators. *Revista Colombiana de Entomología 35*(2), 206–216.

Briscoe, A. D. and L. Chittka (2001). The evolution of color vision in insects. *Annual Review of Entomology 46*(1), 471–510.

Brosi, B. J. (2016). Pollinator specialization: from the individual to the community. *New Phytologist 210*(4), 1190–1194.

Brosi, B. J. and H. M. Briggs (2013, August). Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proceedings of the National Academy of Sciences 110*(32), 13044–13048.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, et al. (2012). Biodiversity loss and its impact on humanity. *Nature 486*(7401), 59–67.

Casula, P., A. Wilby, and M. B. Thomas (2006). Understanding biodiversity effects on prey in multi-enemy systems. *Ecology Letters 9*(9), 995–1004.

Colla, S., L. Richardson, and P. H. Williams (2011). *Bumble bees of the eastern United States.* USDA Forest Service and the Pollinator Partnership.

Darvill, B., M. E. Knight, and D. Goulson (2004). Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos 107*(3), 471–478.

Dormann, C. F., J. M. Calabrese, G. Guillera-Arroita, E. Matechou, V. Bahn, K. Bartoń, C. M. Beale, et al. (2018). Model averaging in ecology: a review of Bayesian, information-theoretic, and tactical approaches for predictive inference. *Ecological Monographs 88*(4), 485–504.

Garibaldi, L. A., I. Bartomeus, R. Bommarco, A. M. Klein, S. A. Cunningham, M. A. Aizen, V. Boreux, et al. (2015). Trait matching of flower visitors and crops predicts fruit set better than trait diversity. *Journal of Applied Ecology 52*(6), 1436–1444.

Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. *Journal of Ecology 94*(2), 295–304.

Grueber, C. E., S. Nakagawa, R. J. Laws, and I. G. Jamieson (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology 24*(4), 699–711.

Hallett, A. C., R. J. Mitchell, E. R. Chamberlain, and J. D. Karron (2017, May). Pollination success following loss of a frequent pollinator: the role of compensatory visitation by other effective pollinators. *AoB PLANTS 9*(3), plx020.

Hegland, S. J., J.-A. Grytnes, and Ø. Totland (2009, July). The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecological Research 24*(4), 929–936.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, et al. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs 75*(1), 3–35.

Inouye, D. (1977). Species structure of bumblebee communities in North America and Europe. In *The Role of Arthropods in Forest Ecosystems*, pp. 35–40. Springer Berlin Heidelberg.

Inouye, D. W. (1978, July). Resource partitioning in bumblebees: Experimental studies of foraging behavior resource partitioning in bumblebees: Experimental studies of foraging behavior. *Ecology 59*(4), 672–678.

Inouye, D. W. (1980). The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. *Oecologia 45*(2), 197–201.

Johnson, J. B. and K. S. Omland (2004, February). Model selection in ecology and evolution. *Trends in Ecology & Evolution 19*(2), 101–108.

Knight, M. E., A. P. Martin, S. Bishop, J. L. Osborne, R. J. Hale, R. A. Sanderson, and D. Goulson (2005). An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. *Molecular Ecology 14*(6), 1811–1820.

Koch, J., J. Strange, and P. H. Williams (2012). *Bumble bees of the western United States.* USDA Forest Service and the Pollinator Partnership.

Kremen, C., N. M. Williams, and R. W. Thorp (2002, December). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences 99*(26), 16812–16816.

Krishna, S. and T. Keasar (2018, June). Morphological complexity as a floral signal: from perception by insect pollinators to co-evolutionary implications. *International Journal of Molecular Sciences 19*(6), 1681.

Loy, X. and B. J. Brosi (2022). The effects of pollinator diversity on pollination function. *Ecology 103*(4), e3631.

Magnusson, A., H. Skaug, A. Nielsen, C. Berg, K. Kristensen, M. Maechler, K. van Bentham, B. Bolker, M. Brooks, and M. M. Brooks (2017). Package ‘glmmtmb’. *R Package Version 0.2. 0*.

McGill, B., B. Enquist, E. Weiher, and M. Westoby (2006, April). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution 21*(4), 178–185.

Messier, J., B. J. McGill, and M. J. Lechowicz (2010, May). How do traits vary across ecological scales? A case for trait-based ecology: How do traits vary across ecological scales? *Ecology Letters 13*(7), 838–848.

Oliver, T. H., M. S. Heard, N. J. B. Isaac, D. B. Roy, D. Procter, F. Eigenbrod, R. Freckleton, et al. (2015, November). Biodiversity and resilience of ecosystem functions. *Trends in Ecology & Evolution 30*(11), 673–684.

Oliver, T. H., N. J. B. Isaac, T. A. August, B. A. Woodcock, D. B. Roy, and J. M. Bullock (2015, December). Declining resilience of ecosystem functions under biodiversity loss. *Nature Communications 6*(1), 10122.

Peralta, G., D. P. Vázquez, N. P. Chacoff, S. B. Lomáscolo, G. L. W. Perry, and J. M. Tylianakis (2020). Trait matching and phenological overlap increase the spatio-temporal stability and functionality of plant–pollinator interactions. *Ecology Letters 23*(7), 1107–1116.

Peter, C. I. and S. D. Johnson (2008). Mimics and magnets: the importance of color and ecological facilitation in floral deception. *Ecology 89*(6), 1583–1595.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin (2010, June). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution 25*(6), 345–353.

Pyke, G. H. (1982). Local geographic distributions of bumblebees near Crested Butte, Colorado: competition and community structure. *Ecology 63*(2), 555–573.

R Core Team (2021). *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing.

Sih, A., G. Englund, and D. Wooster (1998, September). Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution 13*(9), 350–355.

Stang, M., P. G. L. Klinkhamer, N. M. Waser, I. Stang, and E. van der Meijden (2009, June). Size-specific interaction patterns and size matching in a plant–pollinator interaction web. *Annals of Botany 103*(9), 1459–1469.

Symonds, M. R. E. and A. Moussalli (2011, January). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology 65*(1), 13–21.

Tilman, D., F. Isbell, and J. M. Cowles (2014, November). Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics 45*(1), 471–493.

Warnock, M. J. (1993). *Flora of North America: Volume 3: Magnoliophyta: Magnoliidae and Hamamelidae*, Chapter *Delphinium barbeyi*. Oxford University Press on Demand.

Wei, N., R. L. Kaczorowski, G. Arceo-Gómez, E. M. O'Neill, R. A. Hayes, and T.-L. Ashman (2021, September). Pollinators contribute to the maintenance of flowering plant diversity. *Nature 597*(7878), 688–692.

Williams, P. H., R. W. Thorp, L. L. Richardson, and S. R. Colla (2014). *Bumble Bees of North America: an Identification Guide.* Princeton, NJ: Princeton University Press.

Winfree, R., J. W. Fox, N. M. Williams, J. R. Reilly, and D. P. Cariveau (2015). Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecology Letters 18*(7), 626–635.

Category	Variable Code	Variable description
Experimental	PR	Proportion of the *Bombus* community removed by our manipulation
Pollinator	BA	*Bombus* abundance, scaled to center at 3
Pollinator	PL	Proportion of the *Bombus* community with a long tongue
Pollinator	MF	Proportion of the pollinator community exhibiting floral fidelity during foraging surveys
Plant	PD	Relative abundance of *D. barbeyi* flowers
Plant	CL	Proportion of the floral community with the same color as *D. barbeyi* flowers (blue/violet)
Plant	CR	Proportion of the floral community with a mean corolla length that overlaps with the range for *D. barbeyi* nectar spurs (10 – 18mm)
Interaction	PR:BA	Interaction between the experimental removal and *Bombus* abundance
Interaction	PR:PL	Interaction between the experimental removal and the proportion of long-tongued bees in the community
Interaction	PR:MF	Interaction between the experimental removal and floral fidelity
Interaction	PR:PD	Interaction between the experimental removal and the relative abundance of *D. barbeyi*
Interaction	PR:CL	Interaction between the experimental removal and the proportion of the community with color similarity
Interaction	PR:CR	Interaction between the experimental removal and the proportion of the community with corolla-length similarity

Table 1: Description of fixed effects included in the model selection framework.

Site	Year	PD	PR	Species Removed	Tongue
Brush Creek Fork	2011	0.57	0.52	Bombus appositus	long
Deer Creek Gate 2	2011	0.14	0.36	Bombus nevadensis	long
Gothic Road Grate.2011	2011	0.17	0.46	Bombus appositus	long
Gothic Town	2011	0.44	0.30	Bombus appositus	long
Teocali View	2011	0.19	0.38	Bombus nevadensis	long
Back of Baldy Bend	2013	0.04	0.50	Bombus flavifrons	medium
Emerald Lake 2	2013	0.07	0.49	Bombus kirbiellus	long
Gothic Road 2	2013	0.09	0.60	Bombus appositus	long
Judd Falls TH 2	2013	0.19	0.48	Bombus flavifrons	medium
Past Rustlers	2013	0.17	0.43	Bombus flavifrons	medium
Brush Creek Sign	2014	0.48	0.66	Bombus appositus	long
Cold Springs Ranch	2014	0.71	0.62	Bombus appositus	long
Emerald Lake	2014	0.16	0.53	Bombus kirbiellus	long
Gothic Road Grate.2014	2014	0.23	0.52	Bombus appositus	long

Table 2: Summary of *Bombus* removal and *D. barbeyi* relative abundance by site. Tongue categories were long (4 species), medium (5 species), and short (2 species).

ID	AIC	delta	PR	BA	PL	MF	PD	CL	CR	PR:BA	PR:PL	PR:MF	PR:PD
1	6317.69	0.00		-0.29	-1.1		0.18	-0.45	1.46			3.72	
2	6317.99	0.30	3.39	-0.12	-1.52	3.44	0.51	-0.63	1.78	-3.77			
3	6318.00	0.31		-0.17	-1.64	2.58	0.58	-0.67	1.70				
4	6318.27	0.58	3.19			2.89		-0.49	1.58	-3.95			
5	6318.55	0.86	5.73	0.06	-3.01	3.65	1.96	-0.83	1.99	-4.9			-4.79
6	6318.71	1.02		-0.16	-1.8	2.77	0.57	-0.49	1.73		4.18		
7	6318.73	1.04		-0.3	-1.19		0.13	-0.27	1.47		4.06		
8	6318.88	1.19		-0.16	-2.45		1.46	-0.67	1.62			5.84	-3.99
9	6319.14	1.45	3.51		-1.49	3.38		-1.01	1.80	-3.94			
10	6319.28	1.59	3.35	-0.14	-1.38	3.2		-0.83	1.77	-3.79			
11	6319.44	1.75				2.1		-0.61	1.50	-3.62			
12	6319.50	1.81	3.07	-0.31	-0.69		-0.04	-0.37	1.46	-4.11			
13	6319.53	1.84		-0.19	-1.48	2.33		-0.90	1.68				
14	6319.54	1.85	2.97	-0.22		2.63		-0.25	1.55	-3.72			
15	6319.66	1.97	3.53		-1.62	3.59	0.53	-0.78	1.81	-3.9			
16	6319.68	1.99		-0.07	-2.74	2.32	1.64	-0.84	1.80				-3.42

Table 3: Model selection output for models with $\Delta AIC < 2$. Variable names as in Table 1.

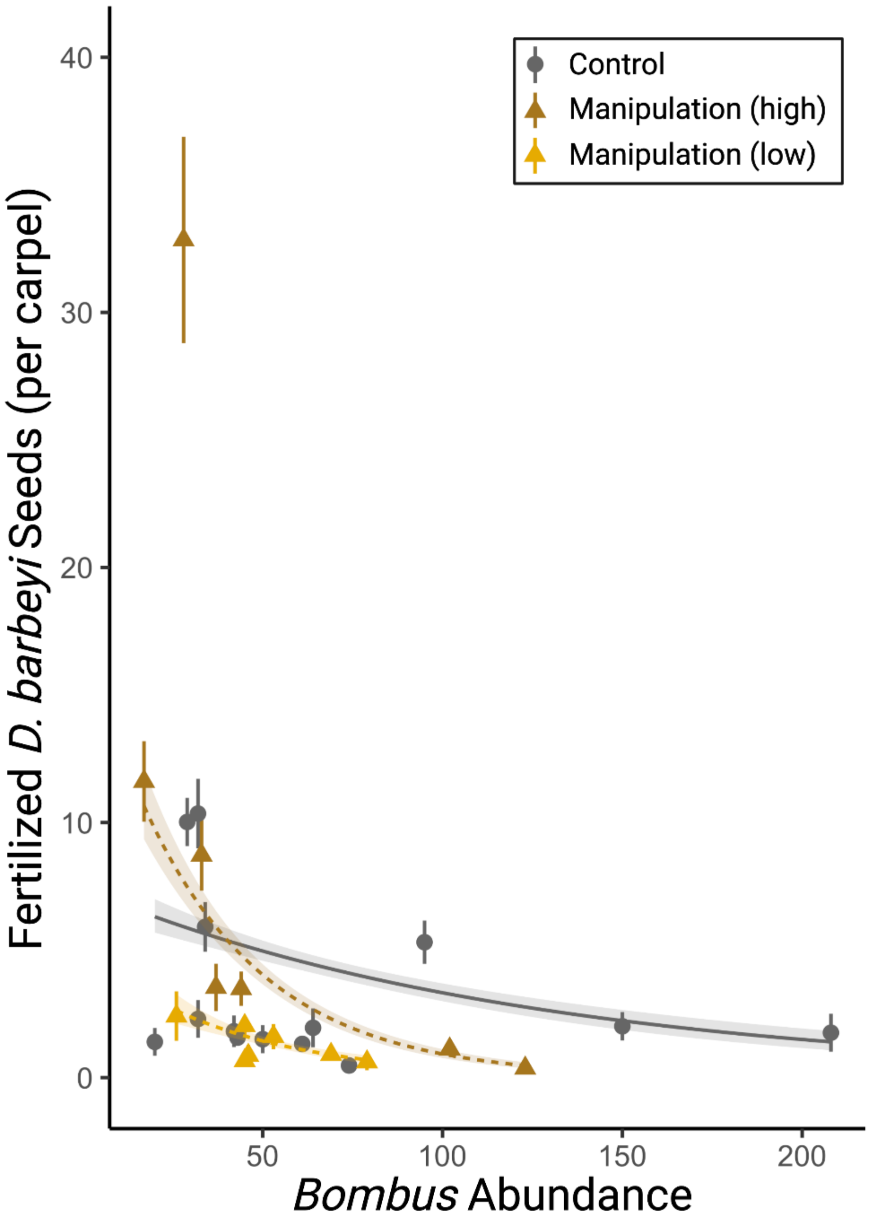
Variable	Estimate	Std.Error	Adjusted.SE	z-value	p-value
Intercept	-0.98	1.86	1.86	0.53	0.60
PR	1.74	2.01	2.01	0.87	0.39
BA	-0.13	0.28	0.28	0.49	0.63
PL	-1.34	1.37	1.37	0.98	0.33
MF	2.12	1.88	1.88	1.13	0.26
PD	0.45	1.00	1.00	0.44	0.66
CL	-0.61	1.14	1.14	0.54	0.59
CR	1.66	0.53	0.53	3.13	0.00
PR:BA	-3.86	0.98	0.98	3.94	0.00
PR:PL	0.53	1.43	1.43	0.37	0.71
PR:MF	1.63	2.24	2.24	0.73	0.47
PR:PD	-0.66	1.86	1.86	0.35	0.73

Table 4: Summary of model averaged from 16 $\Delta AIC < 2$ models. Variable names as in Table 1.

Figure 1: Effect of total *Bombus* abundance predicted by our averaged model. Points represent the mean for each survey period and bars represent 95% confidence intervals. Manipulation is plotted in the “high” bin if half or more of the overall *Bombus* community was removed and in the “low” bin if less than half was removed.

Figure 2: Effect of the proportion of the *Bombus* community categorized as “long-tongued,” per expert descriptions. Points represent the mean for each survey period and bars represent 95% confidence intervals. Manipulation is plotted in the “high” bin if half or more of the overall *Bombus* community was removed and in the “low” bin if less than half was removed.

Figure 3: Effect of pollinator floral fidelity, averaged across all *Bombus* observed during the represented study period. Points represent the mean for each survey period and bars represent 95% confidence intervals. Manipulation is plotted in the “high” bin if half or more of the overall *Bombus* community was removed and in the “low” bin if less than half was removed.



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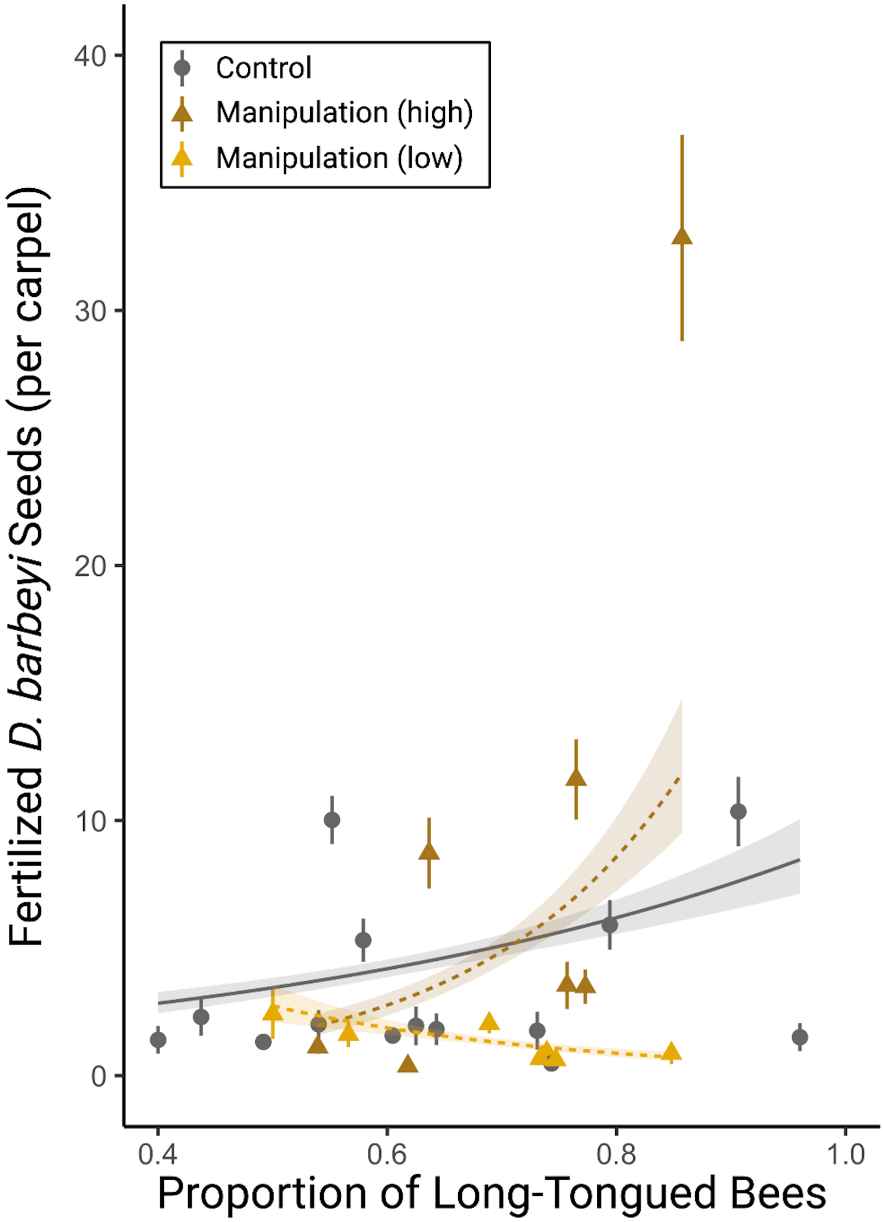

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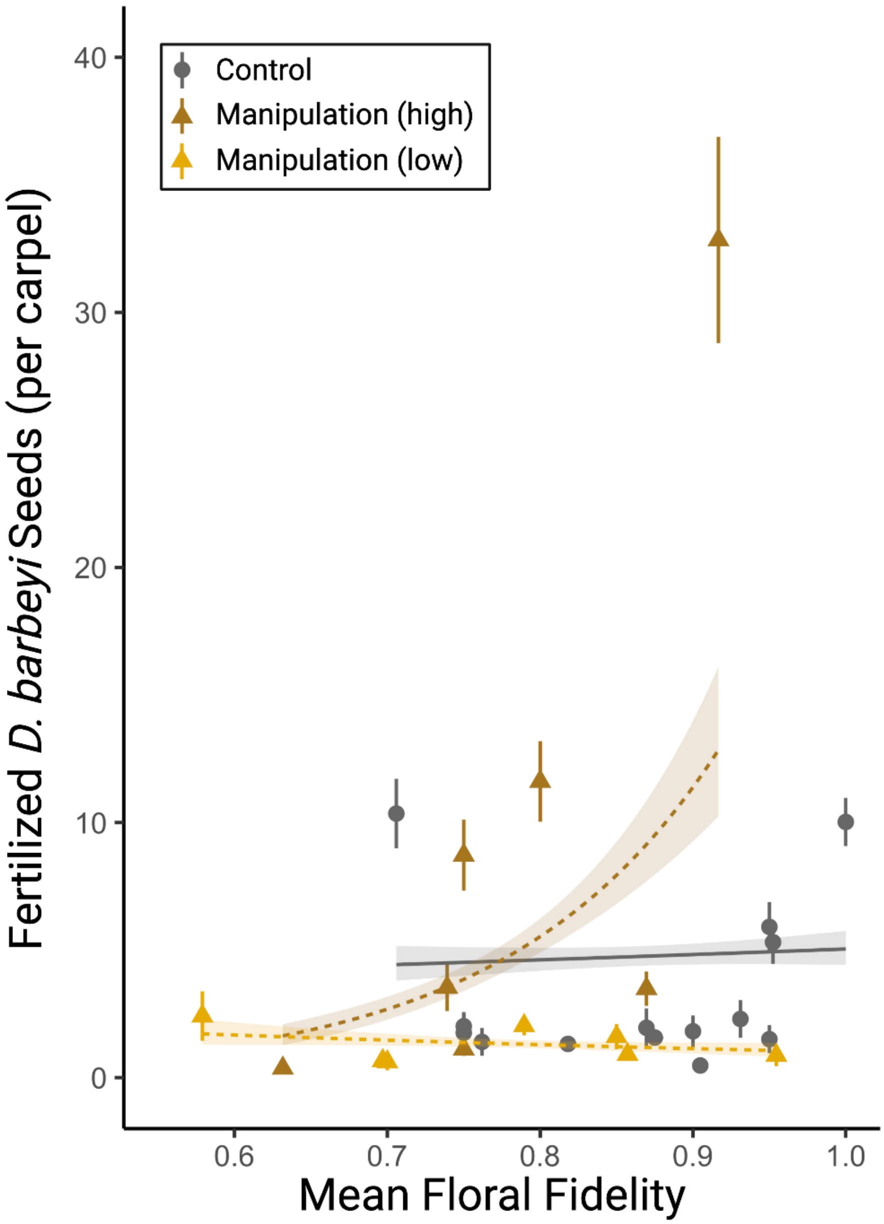

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