

ABSTRACT

PARDEE, GABRIELLA LYNN. Effects of Climate Change on Plants, Pollinators, and Their Interactions. (Under the direction of Dr. Rebecca Irwin).

Climate change is leading to increased temperatures and extreme weather events worldwide, which can impact species and their interactions. In montane regions, climate change has resulted in earlier spring snowmelt, with plants advancing their phenology to coincide with earlier onset of spring. However, little is known about the consequences of advanced phenology for plant growth and reproduction and the mechanisms involved. For example, plants could become more susceptible to frost damage because they are no longer insulated from freezing temperatures by snowpack. Another possible consequence is changes to their interactions with pollinators, if plant and/or pollinator abundance and species richness are impacted by changes in abiotic conditions, or if plants and pollinators respond differently to altered abiotic conditions. I examined the consequences of advancing spring phenology on plants, pollinators, and their interactions through four main questions: 1) What are the direct and indirect effects of frost on plant growth and reproduction? 2) How do snowmelt timing and frost exposure impact flowering communities, their interactions with pollinators, and plant reproduction? 3) How do snowmelt timing and frost impact networks of plant-pollinator interactions? and 4) Do bees respond differently to climate change based on their life history traits?

I conducted four studies on plants and pollinators in a subalpine region of the Rocky Mountains of Colorado, U.S. For my first chapter, I manipulated frost exposure for three flowering species to examine how frost can directly as well as indirectly affect plants due to changes in visitation rates. Frost had species-specific direct effects on plant survival, growth, and reproduction, as well as indirect effects by altering pollinator visits. For my second chapter, I manipulated snowmelt timing and frost exposure to examine how changes in abiotic conditions

affect flowering communities, focusing on four focal plant species that require insects or other animals for pollination and that vary in their bloom time, from early spring to summer blooming. All species advanced their phenology by 7-14 days in the snow removal plots compared with the natural snowmelt plots, which resulted in different effects on frost damage, pollinator visitation, and seed production depending on species' bloom-time. Early-blooming species were at a disadvantage, but mid-blooming species benefitted from early snowmelt and advanced phenology. For my third chapter, I investigated how changes in flowering communities due to early snowmelt and frost affect plant-pollinator networks. Surprisingly, I found no changes in network structure despite finding differences in flowering and pollinator communities under early snowmelt, suggesting that network structure may be robust to compositional change in plants and pollinators under climate change. For my final chapter, I used data from a long-term bee monitoring study to determine whether life history traits can be used to predict bee responses to climate change. Increased summer temperatures and increased precipitation from the previous year had negative effects on abundance for large bees and eusocial bees, but positive effects on abundance for small bees, solitary bees, and bees that excavated their nests, thus climate change may reshape bee communities based on life history traits.

Taken together, my dissertation provides insight into how plants and pollinators will respond to climate change. Early snowmelt and spring frost will likely reshape flowering communities, and changes in temperatures and precipitation can reshape pollinator communities. This reshaping of plant and pollinator communities has the potential to drive changes in plant-pollinator interactions; yet, plant-pollinator networks seem to be robust against climate change. Thus, ecological interactions in subalpine regions may be resilient to climate change despite compositional changes of plants and pollinators in a warming world.

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Effects of Climate Change on Plants, Pollinators, and Their Interactions

by
Gabriella Lynn Pardee

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APPROVED BY:

Rebecca Irwin
Committee Chair

Robert Dunn

Nicholas Haddad

Steven Frank

DEDICATION

To my favorite bee catching partner, Sean R. Griffin.

And to my parents, John and Mirella Pardee.

Thank you for all your love and support over the last 5 years.

BIOGRAPHY

I was born and raised in Toledo, OH, and am the youngest of 6 children. Growing up, I never dreamed of becoming an ecologist because I didn't even know what an ecologist was. Luckily, during my sophomore year of college at the University of Toledo, I met Dr. Stacy Philpott when I enrolled in her introductory Environmental Science course. When Stacy told the class about the research she conducts on parasitoid-host and predator-prey interactions in coffee farms in Mexico, I thought her work was so fascinating that I asked if I could join her for the summer to help with research. That summer, Stacy taught me everything I needed to know about how to become an ecologist through asking questions, designing and implementing experiments, and publishing my findings in peer-reviewed journals. I am so grateful that I got to work with her during undergrad and definitely would not be where I am today if I had never met her.

After graduating from the University of Toledo in 2011, I worked as a research technician in Dr. Rachael Winfree's lab at Rutgers University for 18 months. I started graduate school at Dartmouth in 2013 and transferred to NC State in 2015 with my advisor, Dr. Becky Irwin.

When I'm not frolicking in meadows looking for bees professionally, I'm frolicking in meadows looking for bees for fun with my partner and dog.

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First, I would like to thank my graduate advisor, Becky Irwin, for being such an awesome mentor and friend over the last 5 years. In addition to being an amazing scientist, she is an incredibly kind person who cares so much about her students. I'm so glad Becky gave me an opportunity to be a part of her lab group. I'd also like to thank my committee members Nick Haddad, Steve Frank, and Rob Dunn.

Second, I'd like to thank my parents, siblings, and second family, the Griffins. They showed their love and support for me during grad school through home cooked meals, vacations, silly bee puns and bee-themed presents, long phone conversations, and comforting hugs.

Third, I'd like to thank all the friends I made during grad school at NC State and at RMBL. I'd especially like to thank Christine Urbanowicz, Jacob Heiling, Gina Calabrese, Andrew Sanders, Sara Giacomini, Jonathan Giacomini, Lauren Carley, Becky Dalton, Rachel Steward, and Lindsey Kemmerling. Thanks for all the dance parties, potlucks, bonfires, and silliness over the last 5 years. I'd also like to thank Dash Donnelly. Even though we only knew you for a short amount of time, you made such a big impact on the Irwin lab and are dearly missed.

Fourth, I'd like to thank my partner, Sean Griffin for being the best field tech, collaborator, and bird/dog dad. Thanks for always being there for me.

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CHAPTER 1: Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wild flowers

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Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers

Gabriella L. Pardee^{1,2} | David W. Inouye^{2,3} | Rebecca E. Irwin^{1,2}

¹Department of Applied Ecology, North Carolina State University, Raleigh, NC, USA

²Rocky Mountain Biological Laboratory, Gothic, CO, USA

³Department of Biology, University of Maryland, College Park, MD, USA

Correspondence

Gabriella L. Pardee, 1000 Eugene Brooks Ave., Raleigh, NC 27695, USA.
Email: glpardee@ncsu.edu

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Abstract

Frost is an important episodic event that damages plant tissues through the formation of ice crystals at or below freezing temperatures. In montane regions, where climate change is expected to cause earlier snow melt but may not change the last frost-free day of the year, plants that bud earlier might be directly impacted by frost through damage to flower buds and reproductive structures. However, the indirect effects of frost mediated through changes in plant–pollinator interactions have rarely been explored. We examined the direct and pollinator-mediated indirect effects of frost on three wildflower species in southwestern Colorado, USA, *Delphinium barbeyi* (Ranunculaceae), *Erigeron speciosus* (Asteraceae), and *Polemonium foliosissimum* (Polemoniaceae), by simulating moderate (−1 to −5°C) frost events in early spring in plants in situ. Subsequently, we measured plant growth, and upon flowering measured flower morphology and phenology. Throughout the flowering season, we monitored pollinator visitation and collected seeds to measure plant reproduction. We found that frost had species-specific direct and indirect effects. Frost had direct effects on two of the three species. Frost significantly reduced flower size, total flowers produced, and seed production of *Erigeron*. Furthermore, frost reduced aboveground plant survival and seed production for *Polemonium*. However, we found no direct effects of frost on *Delphinium*. When we considered the indirect impacts of frost mediated through changes in pollinator visitation, one species, *Erigeron*, incurred indirect, negative effects of frost on plant reproduction through changes in floral traits and pollinator visitation, along with direct effects. Overall, we found that flowering plants exhibited species-specific direct and pollinator-mediated indirect responses to frost, thus suggesting that frost may play an important role in affecting plant communities under climate change.

KEYWORDS

climate change, frost, phenology, plant reproduction, plant–pollinator interactions, pollination services

1 | INTRODUCTION

The abiotic environment plays a critical role in shaping plant survival, growth, and reproduction (Chapin, Bloom, Field, & Waring, 1987; Iler et al., 2013a). Plant survival and performance rely heavily on

temperature, precipitation, and photoperiod (Harte & Shaw, 1995); thus, any change in the abiotic environment can have serious effects not only on plant reproduction but also on plant community composition. One abiotic event that can have particularly detrimental effects on plants is episodic spring frosts. Frost, which we define as

the effects of subfreezing temperatures on living organisms (Inouye, 2000), occurs on clear, dry nights when heat is radiated into the atmosphere faster than it can be replaced from nearby warming sources (Leuning & Cremer, 1988; Sakai & Larcher, 1987), or when a cold air mass below freezing moves into an area. The formation of ice crystals due to frost can damage plant tissue, and in some cases, damage can be severe, resulting in the loss of leaves, flower buds, and ovaries, and even plant death (Ågren, 1988; Sutinen et al., 2001).

Frost has become a paradox in the context of climate change. Theoretically, increasing spring temperatures should lead to a decrease in plant damage from frost events, yet, this may not be the case in some montane regions (Bannister et al., 2005; Inouye, 2008). Many plant species in montane regions use spring snow melt as their primary phenological cue, emerging soon after the ground is bare. Recent changes in climatic conditions are causing thinner snowpack and earlier melting (Inouye, 2000; Wipf, 2010), resulting in earlier spring flowering phenology and the missed benefits of snow insulation during freezing temperatures (Baptist, Flahaut, Streb, & Choler, 2010; Lading, Hacker, Neuner, & Wagner, 2013). For example, in the Rocky Mountains of Colorado, USA, the date of first flowering for 30 plant species has advanced by approx. 3.3 days per decade since the 1970s (CaraDonna, Iler, & Inouye, 2014), but the probability that a spring frost will occur on a particular calendar date may not be changing as rapidly (Inouye, 2000). As a result, plants may be increasingly exposed to episodic frost by growing and flowering at times when nighttime temperatures still drop below freezing (Hanninen, 1991; Inouye, 2000; Baptist et al., 2010; Augspurger, 2013; but see Iler, Høye, Inouye, & Schmidt, 2013b; Walsh et al., 2014). For example, the timing of budbreak in conifers in Northern Ontario has advanced due to increasing spring temperatures; yet, the frequency of nighttime temperatures below freezing has remained stable over the last 90 years (Man, Kayahara, Dang, & Rice, 2009), thus resulting in an increase in needle damage and bud injury due to frost.

Early spring frost may have both direct and indirect effects on plant species, with direct effects studied most commonly (Hufkens, Friedl, Keenan, & Sonnentag, 2012; Lindow, 1983; Miller-Rushing, Høye, Inouye, & Post, 2010; Taschler & Neuner, 2004), especially in agricultural systems (Gu et al., 2008; Zheng, Chapman, Christopher, Frederiks, & Chenu, 2015). Frost can directly damage leaves, flower buds, and ovaries, thus reducing plant growth and reproduction (Burke, Quamme, & Weiser, 1976; Pearce, 2001). For example, in *Helianthella quinqueensis* (Asteraceae), bud mortality due to spring frost ranged from 65%–100% over a 7-year period (Inouye, 2008). Furthermore, frost can indirectly affect plant growth and reproduction via changes in the frequency and magnitude of plant–animal interactions. Previous studies have examined how frost affects animal abundance (Boggs & Inouye, 2012; Ehrlich, Breedlove, Brussard, & Sharp, 1972; Nixon & McClain, 1969; Thomas, Singer, & Boughton, 1996), but few have studied how changes in plant traits due to frost influence plant attractiveness to animals that use the plants for food resources. For example, frost events have the potential to modify plant–pollinator interactions if frost results in flowers

that are less attractive to pollinators due to changes in floral morphology or reductions in floral and pollen abundance, both of which could aggravate pollen limitation of plant reproduction. Previous studies on fruit trees have found that tissue and cell damage due to frost results in smaller flowers and damage to the pistil and style (Rodrigo, 2000). Alternatively, pollinators could help plants recoup lost fitness from frost-damaged flowers if they increase visitation rates and seed set in remaining, undamaged flowers. While the direct effects of episodic frost in montane regions have been studied for several plant species (Bannister et al., 2005), the indirect effects via changes in pollination are relatively unexplored.

We studied the effects of frost events on floral morphology and flowering traits, plant–pollinator interactions, and plant reproduction for three montane plant species. We experimentally manipulated frost to isolate the direct effects of frost on plants and indirect effects mediated through changes in plant–pollinator interactions. Studying how frost directly affects pollinators (e.g., Boggs & Inouye, 2012) was beyond the scope of this study, but can be assessed in future research. We addressed the following questions: (i) How do plant growth, floral morphology, and flowering traits respond to early season frost events? (ii) To what degree do pollinators respond to plants that have been exposed to frost? and (iii) How does frost impact plant reproduction? We predicted that plants exposed to early season frost would have a lower probability of flowering and, for those that did flower, would produce fewer, smaller flowers (Gezon, Inouye, & Irwin, 2016). Assuming that frost affects floral or flowering traits, we also predicted that pollinators would avoid frost-damaged plants, resulting in decreased plant reproduction. Given the potential for climate change to increase the incidence of frost damage (Man et al., 2009), this research provides important insight into how frost impacts plant fitness in natural communities and some of the mechanisms involved.

2 | METHODS

2.1 | Study system and focal species

This study was conducted in the spring and summer of 2014 in montane meadows around the Rocky Mountain Biological Laboratory (RMBL), in Gothic, Colorado, USA (elev. 2950 m). The meadows surrounding the RMBL host a diversity of flowering plants that rely on pollinators, especially bees (Gezon et al., 2016), for successful reproduction. The climate around the RMBL exhibits decadal variation in precipitation (Ault & St. George, 2010). It is predicted that climate change in this region will disrupt this pattern, causing temperatures to increase and precipitation to decrease (McCabe, Betancourt, & Hidalgo, 2007; Stewart, Cayan, & Dettinger, 2005). We have already seen a trend toward an increase in spring temperatures by $0.34 \pm 0.21^\circ\text{C}$ per decade over the last 40 years (Iler et al., 2013b), which has led to earlier snow melt and consequently earlier plant bloom. These changes may result in an increased incidence of plant damage from episodic spring frost events, especially if the last frost-free day of the year is not changing at the same rate (Inouye, 2000).

We studied three focal plant species that flower mid-summer and are long-lived perennials: *Erigeron speciosus* (Asteraceae), *Delphinium barbeyi* (Ranunculaceae), and *Polemonium foliosissimum* (Polemoniaceae). Hereafter, we refer to each species by genus. We chose three mid-summer flowering species because they likely preform their buds (Inouye, Morales, & Dodge, 2002), and their leaves and other tissues have been shown to be more frost sensitive than their earlier season congeners (CaraDonna & Bain, 2016); thus, their floral and flowering traits may be susceptible to early season frost damage. *Erigeron* produces light pink-to-purple, hermaphroditic flowers, with 1–11 flower heads per stem (Robertson, 1999), and on average 15 stems per plant (G.L. Pardee, *per. obs.*). *Erigeron* is weakly self-compatible, and the flowers are visited by a wide range of pollinators, including bees, flies, moths, butterflies, and beetles (Kearns, 1992; Pohl, Van Wyk, & Campbell, 2011; Wilson, Wilson, Loftis, & Griswold, 2010). *Delphinium* produces dark purple, protandrous flowers on racemes that can grow up to 2 m in height (Dodson & Dunmire, 2007). Up to 20 flowering stalks can grow from a single root system (Dodson & Dunmire, 2007), each stalk producing approximately 25 flowers (Elliott & Irwin, 2009). *Delphinium* is weakly self-compatible, and the flowers are primarily pollinated by bumble bees (*Bombus* spp.; Elliott & Irwin, 2009). *Polemonium* has purple, campanulate flowers, and populations are either hermaphroditic or gynodioecious (Brody & Waser, 1995; Campbell, Forster, & Bischoff, 2014). In the population we used for our study, 96% of the plants were hermaphroditic. *Polemonium* produces an average of seven stalks per plant and 28 flowers per stalk (G.L. Pardee & R.E. Irwin, *per. obs.*). *Polemonium* is self-incompatible, and the flowers are pollinated by bees and flies (Campbell et al., 2014). *Polemonium* and *Delphinium* have been shown to be pollen limited for reproduction (see Zimmerman & Pyke, 1988 for *Polemonium*, and Elliott and Irwin, 2009 for *Delphinium*). The buds and flowers of *Erigeron* and *Delphinium* are directly sensitive to the effects of frost (Inouye, 2008), but the indirect effects of frost via changes in pollination are unknown.

2.2 | Experimental design

We selected 60 plants of each species within a meadow (*Erigeron* 38°58'00.0"N, −106°59'23.0"W; *Polemonium* 38°51'25.1"N, −107°03'19.4"W; *Delphinium* 38°57'56.6"N, −106°59'36.5"W) and randomly assigned them to a frost treatment or no-frost treatment ($n = 30$ plants per treatment per species). To simulate frost events, we created frost chambers using foam coolers (43.1 × 36.5 × 30.4 cm) that were separated into two sections (upper and lower) by steel mesh. We created below-freezing temperatures by placing 100 g of dry ice and an ice:salt mixture (400 g: 40 g ratio) in plastic bags in the upper chamber to achieve temperatures between −1° and −5°C, typical episodic freezing temperatures postsnow melt in the spring at the RMBL (D.W. Inouye, unpublished data). Each of the frost chambers contained a temperature logger that was attached to the side near the top of the plant (iButtonLink LLC, Whitewater, WI, USA), that recorded the temperature every 3 min throughout the duration of the frost event. In addition, we used a nearby weather

station that recorded temperature every 5 min to determine the ambient temperature throughout the duration of the frost simulation. We used ANOVAS to compare the cooler and ambient air temperature conditions among the three plant species. All analyses (here and below) were performed in R (version 3.2.4).

For plants in the frost treatment, we placed the frost chambers over the entire plant (all stalks) as soon as buds were visible in mid/late June between 05:30 and 09:30 hours, as this is the time of day when frost events are most likely to occur (Bissonette, 1978). All plants from all three species were <30 cm in height at the time of the frost manipulation, and so the coolers were tall enough to fit over the plants. We performed the frost event one time for each plant to simulate a single, episodic frost event. Each cooler contained one plant, and we used 15 coolers over 2–3 days to frost each individual plant of each species; all three species were frosted over a 10-day span. Plants in the no-frost treatment received a chamber without coolant. Because no natural frost events occurred during the course of the experiment, we did not need to protect the frost or no-frost plants from natural frost. We recorded aboveground inflorescence survival within each treatment, and then used chi-square tests to assess whether frost affected survival. We note that it is unlikely that our treatments affected root survival, and they did not result in soil and root disturbance via frost heaving; instead, our treatments only likely affected aboveground plant parts, and thus our measurements only included aboveground buds and stalks that may have died back following treatment application. Separate analyses (here and below) were performed for each plant species.

2.2.1 | Plant and floral measurements

To determine whether frost affected plant growth, we measured the height of the tallest flowering stalk. The floral traits we measured were specific to each plant species based on their floral morphologies (Fig. S1; Galen, 1989; Ishii & Harder, 2006). Measurements were made using digital calipers to the nearest 0.01 mm on three flowers per plant and then averaged per plant. For *Erigeron*, we measured petal length, disk width, and head width. For *Delphinium*, we measured spur length, width of the flower opening, petal width, and petal length. For *Polemonium*, we measured corolla length, corolla diameter (distance from tip to tip of opposite corolla lobes), petal length, and petal width. We also counted total flowers produced per plant and the number of flowers in bloom every 2–3 days throughout the bloom period to assess whether frost affected flower production and phenology. To test whether frost treatment affected floral and flowering traits for each species, we first used a MANOVA, followed by ANOVAS for each response variable if the MANOVA was significant (Scheiner, 1993).

2.2.2 | Pollinator visitation

Upon flowering, we monitored pollinator visitation to test for indirect effects of frost on plant–pollinator interactions. To observe pollinators, we conducted observations for 1 hour each in the morning

and afternoon every 2–3 days throughout the blooming period. We only recorded visits by insects that contacted the sexual organs of flowers. For each observation period, we haphazardly walked among the focal plants, stopping when we saw a pollinator visiting a flower. Using a voice recorder, we noted the identification of each pollinator on the wing to the lowest taxonomic level possible, duration of each floral visit, and the number of flowers visited on each plant. We followed the visitor until it left the area. After each observation period, we netted for pollinators for 10 min to collect voucher specimens.

Across all floral visitors per plant species, we first ran a generalized linear mixed model using a log-link function and Poisson distribution in the R package *lme4* (Bates, Maechler, Bolker, & Walker, 2015), comparing the total number of flowers visited per plant with frost treatment (frost vs. no-frost) and date of observation as fixed factors, and plant as a random effect. For this analysis, we included plants that were not visited, but were flowering on the day of the pollinator observations. Next, we ran a generalized linear mixed model using a gamma distribution, comparing the effect of frost on the average time spent per flower with frost treatment and date of observation as fixed factors, and plant as a random effect. For this analysis, we excluded all the plants that were not visited, regardless of whether they were flowering that day. We assessed the fit of the model by calculating the dispersion ratio. We then used least squares ratio tests of nested models to measure significance. Finally, we conducted the same analyses but separated the visitors into three different pollinator guilds: flies and Lepidoptera, bumble bees, and other bees to test for differences in visitation patterns among pollinator groups. Our "other bee" group contained bees from the following genera: *Halictus*, *Osmia*, *Megachile*, *Panurginus*, *Pseudopanurgus*, *Andrena*, and *Hylaeus*. Because we found significant effects of the frost treatment on pollinator visitation to *Erigeron* (but not to *Delphinium* or *Polemonium*, see Results), for *Erigeron* we conducted an additional analysis including floral and flowering traits in the model to assess the degree to which differences in pollinator visitation were associated with frost-driven changes in floral traits.

2.2.3 | Plant reproduction

Once the flowers senesced, we measured female plant reproduction by collecting fruits and counting the seeds produced per fruit up to three stalks per plant. We recorded the number of aborted fruits (fruits producing no seeds) and counted the seeds produced in all seed-bearing fruits. Furthermore, for any fruits receiving predispersal seed predation, we recorded the number of fruits partially or fully destroyed and also the number of insects found within the fruits (when they occurred). We estimated female reproduction using the following response variables: total seed set per stalk (number of mature seeds), proportion fruit set (number of seed-bearing fruit/total flowers), and mean seeds per seed-bearing fruit.

We examined the effects of frost treatment on total seed set and proportion fruit set for all of the plants that flowered with plant as a random effect by conducting generalized linear mixed models with a zero-inflated negative binomial distribution in the R package

GLMMADMB (Fournier, Skaug, & Ancheta, 2012; Skaug, Fournier, Nielsen, Magnusson, & Bolker, 2013). This analysis allowed us to assess in general how frost affected plant reproduction. Furthermore, because we found a significant effect of frost on pollinator visitation rate and seed set for *Erigeron* (but not *Polemonium* or *Delphinium*, see Results), we ran an additional analysis on each metric of plant reproduction with frost treatment, the covariates pollinator visitation and time spent per flower, and their interaction using linear models.

3 | RESULTS

3.1 | Frost chamber and ambient air temperature conditions

The average lowest temperature the frost chambers reached was $-3.98 \pm 2.45^\circ\text{C}$ (mean $\pm$ SD) for *Erigeron*, $-2.71 \pm 1.24^\circ\text{C}$ for *Polemonium*, and $-3.14 \pm 1.95^\circ\text{C}$ for *Delphinium*, showing that for all three plant species, temperatures dipped below freezing. Examination of the temperature profiles in the frost chambers document a gradual decline in temperature over approx. 50 min until the chambers were below freezing, and a holding of that below-freezing temperature for at least 60 min., followed by a gradual increase in temperature (Figure 1). During the nights of the frost simulations, the average lowest ambient air temperature was $2.75 \pm 0.74^\circ\text{C}$ (mean $\pm$ SD) for *Erigeron*, $2.14 \pm 1.38^\circ\text{C}$ for *Polemonium*, and $2.13 \pm 1.45^\circ\text{C}$ for *Delphinium* (Figure 1). There were no significant differences in the temperature conditions among the three species within the frost chambers or in the ambient air temperatures ($F < 2.6$, $p > .08$ for both the frost chamber and ambient air temperatures).

3.2 | Aboveground inflorescence survival and growth

We found no effect of the frost treatment on aboveground inflorescence survival for *Erigeron* ($\chi^2 = 2.41$, $df = 1$, $p = .12$) or *Delphinium*

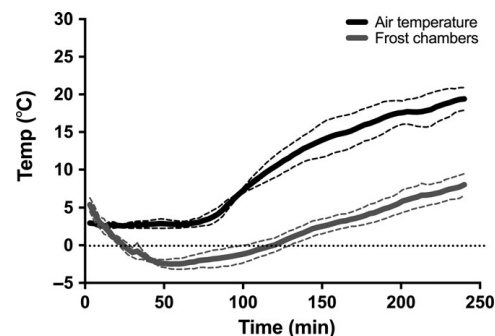


FIGURE 1 Temperature profiles for ambient air temperature (black lines) and frost chambers (gray lines) throughout the duration of the frost simulation for all three species combined. The dotted lines represent the standard deviation

($\chi^2 = 0.07$, $df = 1$, $p = .79$). For both species, survival was 80%–100% across both treatments. However, for *Polemonium*, frost reduced aboveground inflorescence survival by 12% ($\chi^2 = 6.249$, $df = 1$, $p = .01$).

When we examined plant and floral morphology, we found significant differences between the frost and no-frost treatments, but only for *Erigeron* (MANOVA: $F_{4,46} = 6.0$, Wilks' $\lambda = 0.65$, $p < .01$). We found that frost reduced *Erigeron* plant height by 29% ($F_{1,49} = 29.26$, $p < .001$), the size of the flower head by 7.8% ($F_{1,49} = 4.91$, $p = .03$), and the total number of flowers produced per plant by 40% ($F_{1,49} = 6.20$, $p = .01$). In contrast, the frost treatment had no effect on plant height, flower morphology, or flower production for *Delphinium* or *Polemonium* (MANOVA'S: *Delphinium*: $F_{5,25} = 1.92$, Wilks' $\lambda = 0.72$, $p = .12$; *Polemonium*: $F_{4,31} = 0.74$, Wilks' $\lambda = 0.08$, $p = .56$). Finally, we did not find a difference in flowering phenology, measured as the first flowering date and flowering duration, between the frost and no-frost treatments for any of the species ($F < 0.98$, $p > .33$ in all cases).

3.3 | Pollinator visitation

The frost treatment significantly affected pollinator visitation but again, only to *Erigeron* and not to *Delphinium* or *Polemonium*. For *Erigeron*, plants in the frost treatment received 48% fewer visits from pollinators ($\chi^2 = 4.20$, $df = 1$, $p = .04$; Figure 2a) but pollinators spent 33% more time per flower compared to plants in the no-frost treatment ($\chi^2 = 5.91$, $df = 1$, $p = .02$; Figure 2b). Across both treatments, 37% of the floral visits to *Erigeron* were by flies from the Syrphidae and Muscidae families and Lepidoptera, 36% were by bumble bees, and 23% were by other bees. When we conducted analyses by pollinator group, we found that plants in the frost treatment received 66% fewer visits by flies and Lepidoptera ($\chi^2 = 7.17$, $df = 1$, $p = .007$) but not by bumble bees ($\chi^2 = 0.04$, $df = 1$, $p = .83$) or other bees ($\chi^2 = 0.68$, $df = 1$, $p = .41$). Furthermore, we found that flies and Lepidoptera spent, on average, 65% more time on flowers from plants in the frost compared to no-frost treatment (Flies and Lepidoptera: $\chi^2 = 4.95$, $df = 1$, $p = .03$), but bumble and other bees did not vary in the time they spent per flower ($p > .37$ in both cases). When we included floral traits in the statistical models for

Erigeron, we found that frost treatment ($\chi^2 = 4.15$, $df = 1$, $p = .04$) and floral traits were important for affecting pollinator visitation rates. Plants with smaller floral display ($\chi^2 = 379.13$, $df = 1$, $p < .01$; Figure 3a) and smaller flower heads ($\chi^2 = 108.76$, $df = 1$, $p < .01$; Figure 3b) attracted fewer visits from pollinators, with no interaction between frost treatment and either covariate ($p > .06$ in both cases). However, we did not find any association between floral traits and the amount of time visitors spent on each flower ($p > .30$ for each trait measured).

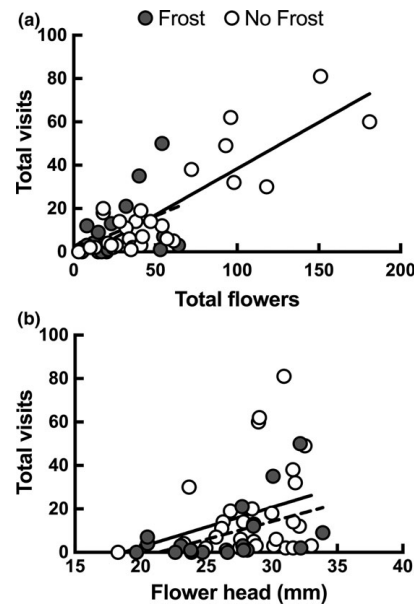


FIGURE 3 For *Erigeron speciosus*, there was a significant positive relationship ($p < .01$ in all cases) between total pollinator visits and (a) floral display and (b) flower head size, with no interaction between frost treatment and either covariate. The gray circles and dashed lines represent the frost treatment and the open circles and solid lines represent the no-frost treatment

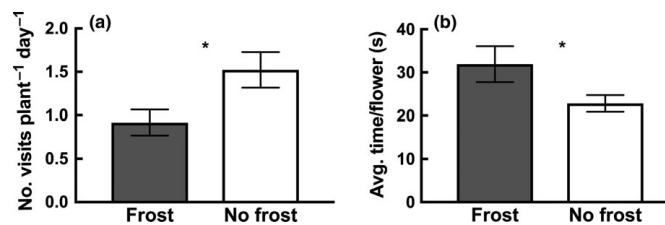


FIGURE 2 Pollinator visitation to *Erigeron speciosus* measured as (a) mean number of pollinator visits per plant per day and (b) mean time spent per flower (in seconds) in the frost (gray) and no-frost (white) treatments. Pollinators visited significantly fewer plants per day in the frost treatment but spent more time on average on flowers in the frost treatment. Bars represent means $\pm$ SE. The asterisks denote statistical significance at $p < .05$

For *Delphinium* and *Polemonium*, we found no differences in any measures of pollinator visitation or by pollinator group composition between the frost and no-frost treatments ($p > .35$ in all cases).

3.4 | Plant reproduction

When we examined the effect of frost on plant reproduction, we found that the frost treatment had significant negative effects on the reproduction of *Erigeron* and *Polemonium*, but not *Delphinium*. For *Erigeron*, frost decreased the proportion of successful fruits by 8.5% ($\chi^2 = 4.28$, $df = 1$, $p = .03$; Figure 4a), the total number of seeds by 27% ($\chi^2 = 4.49$, $df = 1$, $p = .03$; Figure 4b), and the

average number of seeds per fruit by 9% ($\chi^2 = 3.92$, $df = 1$, $p = .04$; Figure 4c). For *Polemonium*, frost did not have an effect on proportion fruit set ($\chi^2 = 0.63$, $df = 1$, $p = .42$; Figure 4a), but frost reduced total seeds produced by 45% ($\chi^2 = 4.39$, $df = 1$, $p = .03$; Figure 4b) and average seeds per fruit by 39% ($\chi^2 = 3.90$, $df = 1$, $p = .05$; Figure 4c). Frost had no effect on *Delphinium* reproduction ($p > .14$ for all response variables).

Because we found significant effects of frost treatment on pollinator visitation rate to *Erigeron*, we ran an additional model that included not only frost treatment but also pollinator visitation rate as a covariate to tease apart the direct effects of frost on plant reproduction vs. the effects mediated through changes in pollinator visitation. We found seed production increased with pollinator visitation ($\chi^2 = 11.04$, $df = 1$, $p = .001$; Figure 5), but the slope of this relationship was steeper for the frost compared to no-frost treatment (frost treatment $\times$ visitation interaction: $\chi^2 = 33.95$, $df = 3$, $p < .01$). This result suggests that while frost has negative effects on plant reproduction, increasing pollinator visitation to plants in the frost treatment helps partially, but not fully, to rescue seed production. However, we did not find any effect of visitation on fruit set or average seeds per fruit ($p > .08$ in both cases).

4 | DISCUSSION

Climate change is resulting in plants being increasingly exposed to episodic spring frost events. Although previous studies have examined the direct effects of frost on tissue damage in flowers and leaves (Taschler & Neuner, 2004), the indirect effects of frost mediated through changes in pollination services have received less attention. Overall, we found species-specific effects of a single frost event on aboveground inflorescence survival, growth, pollinator visitation, and seed set. These direct and indirect effects could have long-term impacts on montane flowering species under climate change as plants may become exposed to more frost events as the snow melt date continues to advance.

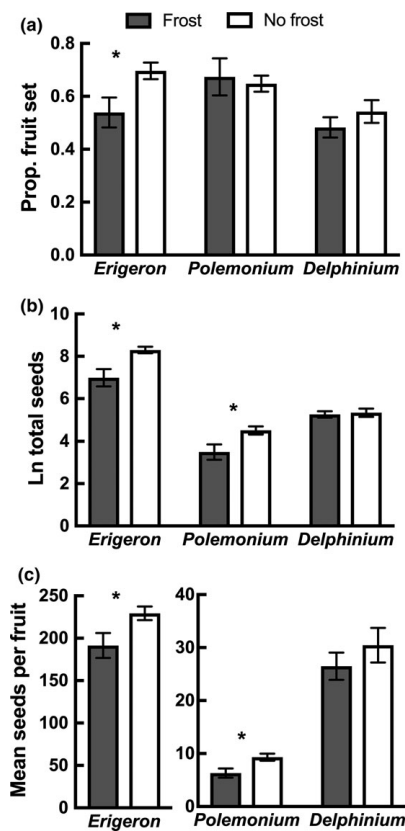


FIGURE 4 (a) Proportion fruit set, (b) total seeds produced, and (c) mean seeds per fruit in frost (gray) and no-frost (white) treatments for *Erigeron speciosus*, *Polemonium foliosissimum*, and *Delphinium barbeyi*. In (c) we report *Erigeron* separate from *Polemonium* and *Delphinium* so that differences in the y-axis scale do not affect the ability to observe patterns in mean seeds produced per fruit per species. Bars are means $\pm$ SE. The asterisks denote statistical significance at $p < .05$

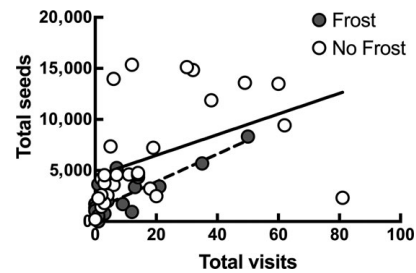


FIGURE 5 For *Erigeron speciosus*, there was a significant positive relationship ($p < .01$) between total seeds produced and pollinator visits. The gray circles and dashed lines represent the frost treatment and the open circles and solid lines represent the no-frost treatment

We found that frost had direct impacts on *Erigeron* and *Polemonium* but not on *Delphinium*. The frost treatment did not affect *Erigeron* aboveground inflorescence survival, but *Erigeron* in the frost treatment produced fewer, smaller flowers than the plants that did not undergo a frost event. For *Polemonium*, frost significantly reduced aboveground inflorescence survival by 12%. Our findings for *Erigeron* extend prior observational field studies and laboratory studies suggesting that this wildflower species is frost sensitive (Boggs & Inouye, 2012; CaraDonna & Bain, 2016; Inouye, 2008), in our case using experimental frost events in wild-growing plants. CaraDonna and Bain (2016) exposed individual leaves and flowers of *Erigeron* to different below-freezing temperatures in the laboratory. They found that 10% of the flowers showed frost damage at temperatures of -3.2°C , and 50% of flowers showed frost damage at -5°C . Between 1973 and 2005, Inouye (2008) reported that the average minimum spring temperature was -4.3°C , with some nights getting as cold as -8°C in the location where this research was conducted. Therefore, by combining these findings with our results, just one spring frost event could greatly reduce the number of *Erigeron* flowers in a summer. Although studies have suggested that *Polemonium* is frost sensitive (Brody, 1997), there are no in-depth studies to our knowledge on the effects of frost on aboveground inflorescence survival and morphology for this species. Our results suggest that *Polemonium* aboveground survival is sensitive to frost, but if plants do flower, floral traits remain unchanged.

It was surprising that we did not find any effects of frost on aboveground inflorescence survival or morphology for *Delphinium*. Previous observational studies on this species that used natural frost events as opposed to experimental frost found that frost was associated with reduced flower bud survival and damaged leaves and flowers (Inouye, 2008; Inouye et al., 2002). Moreover, in the study by CaraDonna and Bain (2016), *Delphinium* was only slightly more frost tolerant than *Erigeron*; thus, we expected to find frost-induced effects on *Delphinium* flower survival and reproduction. One hypothesis explaining the lack of effect is that there could be a period of time after the snow melts when *Delphinium* leaves shelter the small, early forming buds from radiation frost (Inouye et al., 2002). Because we subjected the plants to a single frost event soon after plant emergence, the buds could have been small enough to be protected from frost by the leaves. Or very early buds may not be as sensitive as larger, later bud stages that have been observed to have frost damage. Studies that experimentally frost plants single vs. multiple times during the spring season would help assess the degree to which *Delphinium* leaves protect the plants from frost early in the growing season.

Our study provides evidence of changes in plant–pollinator interactions due to episodic spring frost events. While other studies have alluded to changes in the frequency, behavior, and species of pollinators visiting plants following frost events (CaraDonna & Bain, 2016; Forrest, Inouye, & Thomson, 2010; Inouye, 2000), none have examined differences in pollinator visitation rates between frost-damaged and nonfrost-damaged plants. For one of the species we observed, *Erigeron speciosus*, we found that plants in the frost

treatment experienced reduced pollinator visitation. *Erigeron* was also the only species for which frost affected floral traits. Thus, one parsimonious explanation for this reduction in pollinator visitation to *Erigeron* in the frost treatment is that frost induced changes in floral traits that affected pollinator visitation. For example, we found that frost reduced the number of flowers and flower head size. In other systems, plants with fewer open flowers are often less attractive to pollinators than those with more open flowers (Makino & Sakai, 2007). In a similar vein, reductions in flower size often are associated with reduced pollinator attractiveness and pollinator visitation (Galen & Stanton, 1989). We find similar patterns with *Erigeron* as well, with plants with fewer open flowers and smaller flower heads receiving fewer pollinator visits. In our statistical model when we examined the effects of both frost treatment and floral traits on pollinator visitation, we found effects of all factors, suggesting that frost has additional effects on pollinator visitation beyond that incurred through changes in flower number and size. A more detailed documentation of frost-induced effects on plant and flower size and shape and floral rewards, and how pollinators respond to such changes, may provide additional mechanistic insight. One surprising result for *Erigeron* is that even though we found higher pollinator visitation to flowers in the no-frost treatment, the pollinators spent, on average, more time on the frost-damaged flowers. The mechanisms driving this result are unknown but could be due to frost affecting how hard it is to extract floral rewards or changes in floral rotational symmetry, factors known to affect flower handling time in other systems (Harder, 1983). Another possible explanation could be that the flowers in the frost treatment are less-visited and therefore could have more resources; thus, pollinators would spend more time at the flowers when they did visit. Finally, when we looked across different pollinator groups, we found that flies and Lepidoptera showed a preference for flowers in the no-frost treatment and spent more time on the frost-damaged flowers, but bumble and other bees did not show changes in foraging behavior. Pollinator groups often vary in how responsive they are to changes in flower morphology and floral rewards (Gomez et al., 2008; Kaczorowski, Gardener, & Holtsford, 2005). One reason that may explain these differences is that flies and Lepidoptera primarily collect nectar from flowers (Larson, Kevan, & Inouye, 2001), while bees collect nectar and pollen (Thorp, 2000). Thus, the difference in visitation rates among the groups could be explained if frost-damaged flowers experienced a reduction in nectar but not in pollen, suggesting that flies and Lepidoptera would avoid frost-damaged flowers due to lower resource availability. For additional mechanistic insight, future studies should examine the direct effects of frost on nectar and pollen production, their extraction rates, and subsequent effects on pollinator behavior.

We found evidence for both direct and indirect, pollinator-mediated negative effects of frost on plant reproduction, but the responses were species-specific. For *Polemonium*, we only found evidence for direct, negative effects of frost on plant reproduction, given that frost had no effect on either floral traits or pollinator visitation. For *Erigeron*, however, we found evidence for both direct and

indirect, pollinator-mediated effects of frost. In our statistical model including both frost treatment and pollinator visitation on *Erigeron* seed production, we found effects of both factors, suggesting that not only does visitation affect plant reproduction but frost also has additional direct effects on plant reproduction beyond those incurred through changes in pollination. Surprisingly, we also found a significant interaction between frost treatment and pollinator visitation with increasing pollinator visitation more strongly affecting seed set in plants in the frost compared to no-frost treatment. This result suggests that increasing pollinator visitation may help partially, but not fully, rescue the negative effects of frost on seed production. Why increasing pollinator visitation more strongly benefits seed production in the frost compared to no-frost treatment is unknown, but could be due to resource reallocation within the plant. These results lend support to the importance of the abiotic environment having not only direct effects on plant reproduction but also indirect effects via changes in pollination (Carroll, Pallardy, & Galen, 2001; Potts et al., 2003) and suggest that changes in species interactions may be critically important in assessing the mechanistic response of plants to climate change.

Five caveats are important to consider in the interpretation of our study. First, we only examined the effects of frost on three mid-blooming species, and not on any early blooming species. Plant species that bloom soon after snow melt might be at a greater risk to frost damage because their buds and flowers might undergo several frost events before the later blooming plants even begin to germinate and produce buds. Alternatively, early blooming plants might have a higher tolerance to frost as a means of withstanding harsh, early spring weather conditions (CaraDonna & Bain, 2016). Future experiments should be conducted to examine the direct and indirect effects of frost on early blooming species in the field. Second, we only exposed the plants in our experiments to one moderate frost event. Unless plants are hit with a hard frost ($<-5^{\circ}\text{C}$), they might be able to withstand one or two moderate frost events before any damage to the buds occur (Augsburger, 2009; Thomson, 2010). Studies that have examined the direct effects of frost on plant reproduction have found that frost damage to seed set varies from year-to-year depending on the frequency and severity of frost events (Ågren, 1988; Kudo & Hirao, 2006). Although we only conducted our experiment for 1 year, it gives insight as to how seed set can be affected by just one moderate frost episode. We may expect seed set to be even lower in years with more severe frost events, which could possibly lead to long-term consequences for *Erigeron* and *Polemonium* populations. Third, we did not expose pollinators to frost events; yet, frost likely directly affects pollinator survival (Boggs & Inouye, 2012), phenology, and/or behavior and could have long-term implications for feedbacks between plants and their pollinators. The direct effects of climate change, including frost, on most pollinator groups are understudied and require further observational and experimental work (Forrest, 2014). Fourth, the three plant species we studied are long-lived perennials, and for *Erigeron* and *Polemonium*, it is possible that they may compensate for frost damage in 1 year with higher reproduction in the next, assuming they are not

hit again with frost. Studies that experimentally manipulate frost over multiple seasons and that assess the degree of compensatory responses to changes in plant reproduction will provide additional ecological insights. Fifth, our experiment did not consider that frost might indirectly affect pollination and plant fitness via changes in the aboveground survival and floral display of co-flowering species, which could lead to changes in competition or facilitation for pollinators (Feldman, Morris, & Wilson, 2004; Ghazoul, 2006) if some species are more frost tolerant than others, which is the case at our study site. Understanding the community-level implications of species-specific frost tolerance has important implications for how this episodic event could affect webs of interactions between plants and pollinators.

Declining snowpack and earlier snow melt due to climate change (Mote, Hamlet, Clark, & Lettenmaier, 2005; Solomon et al., 2007; Stewart, 2009) can lead to changes in plant communities. Studies that have examined how plant communities have become reshaped under climate change have primarily focused on abiotic conditions such as snow melt timing, increasing temperatures, and loss of precipitation as indicators (Cleland, Chuine, Menzel, Mooney, & Schwartz, 2007; Hanson & Weltzin, 2000; Henry & Molau, 1997). Given our results, frost may also likely play an important role in shaping flowering communities under climate change (Inouye, 2008). We found that frost has strong species-specific effects on plant reproduction, and effects can be both direct and indirect via changes in floral traits and pollinator visitation. How pollinators will respond to such community-level changes in floral communities and food resources, and how frost will directly affect their survival and health, has important implications for the stability of pollination mutualisms and warrants further investigation.

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ORCID

Gabriella L. Pardee  <http://orcid.org/0000-0003-1575-3024>

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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CHAPTER 2: The individual and combined effects of snowmelt timing and frost exposure on the reproductive success of montane forbs

Gabriella L. Pardee^{1,2*}, Isaac O. Jensen^{2,3}, David W. Inouye^{2,4}, and Rebecca E. Irwin^{1,2}

¹Department of Applied Ecology, North Carolina State University, Raleigh, NC 27607 USA

²Rocky Mountain Biological Laboratory, Gothic, CO 81224 USA

³Biology Department, Luther College, Decorah, IA 52101 USA

⁴Department of Biology, University of Maryland, College Park, MD 20742 USA

*Corresponding author email: gabriellapardee@gmail.com

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ABSTRACT

1. Changes from historic weather patterns have affected the phenology of many organisms worldwide. Altered phenology can introduce organisms to novel abiotic conditions during growth and modify species interactions, both of which could drive changes in reproduction.

2. We explored how climate change can alter plant reproduction using an experiment in which we manipulated the individual and combined effects of snowmelt timing and frost exposure, as well as pollination and measured subsequent effects on phenology, peak flower density, frost damage, pollinator visitation, and reproduction of four subalpine wildflowers. The four plants included species flowering in spring to mid-summer.

3. The phenology of all four species significantly advanced and the bloom duration was longer in the plots from which we removed snow, but with species-specific responses to snow removal and frost exposure in terms of frost damage, flower production, pollinator visitation, and reproduction. The two early-blooming species showed significant signs of frost damage in both early-snowmelt and frost treatments, which negatively impacted reproduction for one of the species. Further, the earliest-blooming species had lower visitation rates in the snow removal plots, which might result in a mismatch with pollinators under advanced phenology. We also found lower fruit and seed set for the early-blooming species in the snow removal treatment, which could be attributed to the plants growing under unfavorable weather conditions. However, the later-blooming species escaped frost damage even in the plots where snow was removed, and experienced increased pollinator visitation and reproduction.

4. *Synthesis*. This study provides insight into how plant communities could become altered due to changes in abiotic conditions. While early-blooming species may be at a disadvantage under climate change, species that bloom later in the season may benefit from early snowmelt, suggesting that climate change has the potential to reshape flowering communities.

INTRODUCTION

Global climate change is one of the strongest factors impacting ecosystems today (Parmesan, 2006), with the Earth's climate having warmed by approx. 0.6° C over the past 100 years (Hansen et al., 2006). The effects of global temperature increase are highly variable, depending on differences in topography, elevation, latitude and regional climate (Chown & Klok, 2003; Meehl et al., 2000; Westra, Alexander, & Zwiers, 2013), differentially impacting seasonality, precipitation, and the occurrence of harsh weather events. These changes in environmental conditions under climate change have been linked to shifts in species abundance and turnover rates, localized species extinctions and colonizations, and latitudinal range shifts for plants and animals in both aquatic and terrestrial ecosystems (Battisti et al., 2005; Gibson-Reinemer, Sheldon, & Rahel, 2015; Walther et al., 2002). These ecological responses are typically preceded by changes in species phenology, which can respond rapidly to annual weather conditions (Badeck et al., 2004). One study documented phenological shifts for 368 of 481 species of plants, invertebrates, and vertebrates under climate change, with the majority showing phenological advancements rather than phenological delays (Parmesan & Yohe, 2003). There is mounting evidence that changes in species phenology are linked to subsequent reductions in reproduction (Both & Visser, 2001; Liu, Reich, Li, & Sun, 2011). What remains unclear, however, are the mechanisms driving changes in species reproduction with altered phenology under climate change.

Phenological change can affect reproduction in at least two ways. First, alterations in phenology can change the environmental conditions species experience while growing (Wheeler, Hoye, Schmidt, Jens-Christian, & Forchhammer, 2015). For example, even though climate change is advancing leaf-out and flowering time (CaraDonna, Iler, & Inouye, 2014; Fitter &

Fitter, 2002), the last frost-free day of the year may not be advancing as quickly as phenology is advancing (Augspurger, 2013; Badeck et al., 2004; Inouye, 2000). Thus, species may find themselves experiencing cooler temperatures in the spring than they are adapted for, as well as more extreme weather events, including temperatures below freezing (Inouye, 2000; Inouye, 2008). Second, changes in phenology can alter species co-occurrence patterns with subsequent effects on species interactions (Blois, Zarnetske, Fitzpatrick, & Finnegan, 2013; Elzinga et al., 2007). Because climate change can have differential effects on species phenologies, especially those at different trophic levels, there is the potential for temporal mismatches between species that have historically co-occurred. In cases where those interactions negatively affect fitness, species reproduction may benefit from phenological mismatch (Saino et al., 2009). However, in cases where mismatch results in loss of mutualists or associations with alternate mutualists (Miller-Rushing, Høye, Inouye, & Post, 2010; Petanidou et al., 2014; Rafferty, CaraDonna, & Bronstein, 2015), species reproduction may decline with phenological change. Because advanced phenology can be associated with both increased instances in which species experience harsh environmental conditions and temporal mismatches, studies are needed that experimentally assess the relative importance of these factors individually and in combination to understand the mechanisms driving phenologically-induced changes in reproduction.

Within trophic levels, species have shown strong variability in how they are responding to a changing climate. For example, flowering plant phenology has advanced more rapidly in early-flowering species compared to species that flower later in the season (CaraDonna et al., 2014; Dunne, Harte, & Taylor, 2003). Similarly, only 8 out of 19 butterfly species advanced their phenology in the Mediterranean Basin, due to differences in life history traits (Stefanescu, Penuelas, & Filella, 2003). This variability in phenological response within trophic levels can

reshape ecological communities. For instance, a study on 100 European bird species showed that the species that advanced their spring migration timing showed stable or increased population size under climate change whereas species that did not advance their spring phenology declined (Moller, Rubolini, & Lehikoinen, 2008). Thus, it is important to examine species-specific responses to climate change to understand shifts in species interactions and community dynamics in response to earlier onset of spring.

In this study, we manipulated snowmelt timing, exposure to extreme low-temperature conditions (frost), and pollination to assess their effects on plant reproduction. We focused on flowering plants in montane environments because montane, alpine, and polar regions are especially vulnerable to a changing environment due to the strong link between phenology and the timing of snowmelt (Inouye & Wielgolaski, 2013; Stinson, 2004). Snow cover is rapidly responding to current climate through decreased snowfall and earlier melting (Latenser & Schneebeli, 2003; Wipf, 2010), thus leading to phenological advancement for many montane plant species (Miller-Rushing & Inouye, 2009). While there may be benefits to earlier bloom time, such as a longer growing and flowering season and potentially reduced competition for pollinators for early-flowering species, both of which could increase plant growth and reproduction, there may also be costs. These costs may include temporal mismatches with mutualist pollinators (Forrest, 2014; Hegland, Nielsen, Lázaro, Bjerknes, & Totland, 2009; Memmott, Craze, Waser, & Price, 2007), as well as a higher risk of being exposed to spring frost due to early snowmelt, which can damage plant tissue through the formation of ice crystals at or below freezing temperatures (Inouye, 2000; Inouye, 2008; Sakai & Larcher, 1987). Prior research that experimentally manipulated snowmelt timing and pollination found that earlier snowmelt advanced phenology, resulting in enhanced pollination services to a spring-flowering

plant, but only in years without spring frosts (Gezon et al. 2016). Studies are needed, however, that experimentally manipulate both snowmelt timing and frost exposure to tease apart the individual vs. combined effects of these two factors on species interactions and reproduction.

We addressed the following questions: 1) How do snowmelt timing and frost exposure affect flowering phenology, frost damage, floral abundance, and pollinator visitation? and 2) To what degree do snowmelt timing and frost exposure affect plant reproduction and pollen limitation? We predicted that species that bloom earlier in the season would show a stronger phenological response to earlier snowmelt than the species that bloom later in the season (Gezon, Inouye, & Irwin, 2016), and that advanced phenology would cause all focal species to be exposed to more frost events. We also predicted that snow removal would result in a phenological mismatch between flowers and their pollinators, but only for species that bloom early in the season as these plants might flower before pollinator emergence. Further, we predicted that phenological mismatch would result in reduced seed set in plants, assuming pollinator limitation of plant reproduction. This study provides much needed empirical insight into how climate change can alter plant-pollinator interactions with potential cascading effects on community composition within montane ecosystems.

METHODS

Study System

Research was conducted at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO, USA (elevation 2950 m). The RMBL is located in a sub-alpine region and hosts a diversity of flowering plants, with over 120 non-graminoid plants species, many of which rely on pollinators for successful reproduction. Over the last 40 years at the RMBL, there is a trend

towards increasing spring temperatures (Iler, Høye, Inouye, & Schmidt, 2013) and earlier spring snowmelt, with an average advancement of 3.5 days per decade (CaraDonna et al., 2014). However, the probability that a spring frost event will occur may not be changing as rapidly (Inouye, 2000). Snowmelt timing will likely continue to advance under climate change, which can lead to increased incidence of frost damage as plants miss the benefits of snow insulation in early spring (Badeck et al., 2004; Inouye, 2008). Inouye (2008) found that plants are being exposed to more frequent mid-June frost events, and that frost damage is correlated with the date of bare ground, where early snowmelt years result in more plant damage due to frost.

We selected four focal plant species that commonly grow in meadows around the RMBL and flower sequentially during the spring and early/mid-summer (listed in their phenological bloom order): *Claytonia lanceolata* (Portulacaceae), *Mertensia fusiformis* (Boraginaceae), *Delphinium nuttallianum* (Ranunculaceae), and *Potentilla pulcherrima* (Rosaceae) (hereafter referred to by genus). All of these species require pollinators for the transfer of pollen from anthers to stigmas and range from weakly self-compatible to self-incompatible (Bosch & Waser, 1999; Forrest & Miller-Rushing, 2010; Stinson, 2004). *Claytonia* is the first species to flower each spring and produces 3-15 white-to-pink actinomorphic flowers per inflorescence that are primarily pollinated by solitary *Lasioglossum* and *Andrena* bees (Gezon et al., 2016). *Mertensia* flowers shortly after *Claytonia* and each plant produces numerous campanulate, blue-purple, hermaphroditic flowers per stalk (Forrest & Thomson, 2010) that are pollinated by solitary *Osmia* and *Andrena* bees as well as eusocial bumble bees (Forrest, Ogilvie, Gorishek, & Thomson, 2011). *Delphinium* produces deep blue to purple protandrous, zygomorphic, nectar-spurred flowers with 1-14 flowers per stalk that are pollinated primarily by bumble bees and hummingbirds (Bosch & Waser, 1999). *Potentilla* is a mid-summer flowering species that

produces up to 50 yellow flowers per plant (Stinson, 2004) that are visited by both solitary bees and flies. The phenology of all four species respond strongly to snowmelt timing, resulting in phenological advancement with earlier snowmelt (CaraDonna et al., 2014; Gezon et al., 2016). *Claytonia*, *Delphinium*, and *Mertensia* have high frost tolerance, but years with hard frost events result in lower flowering and reproduction for these species (CaraDonna & Bain, 2016; Forrest et al., 2011).

Experimental setup

We used a two-way factorial design in which we manipulated snowpack (removal/natural) crossed by frost events (yes/no) with ten 2 x 2 m plots per treatment for a total of 40 plots. All plots were located across two 100 m transects and spaced 3 m apart (38°58'00.0" N, -106°59'23.0" W); treatments were randomly assigned to plots using a random number generator. We removed snow from each of the snow removal plots when the natural snowpack melted to approx. 1 m in depth, but left approx. 3 cm of snow in the plots as to not disturb the soil while shoveling (Petraglia, Tomaselli, Chiari, & Carbognani, 2014). The plots in the natural snowmelt treatment were allowed to melt out naturally. Snow removal has been shown to be an effective way to induce early flowering in plants (Gezon et al., 2016; Wipf, 2010). Although snow removal could affect soil moisture, few studies have documented any significant differences in soil moisture between snow removal and natural snowmelt treatments (Wipf 2010, but see Sherwood et al. 2017). Plots in the frost treatment were exposed to naturally occurring frost events, while plots in the no-frost treatment were covered with frost resistant tarps (Easy Gardener Products, Waco, TX, USA) the evening before a frost event was forecasted to occur, and they were removed the following morning once temperatures reached above 0°C. The

experiment was conducted between April-July 2015 and again between May-August 2016 using a different set of plots that were located 10 m north of the 2015 plots. Our experiments focused on manipulating snowmelt timing and frost experienced by plants in plots to determine how sensitive plants are to changes in these abiotic conditions. Although manipulating pollinator phenology was beyond the scope of this study, future studies should assess how the pollinator community responds to changes in snowmelt timing and frost exposure (Boggs & Inouye, 2012).

Flowering phenology and frost damage

We recorded the date of complete bare ground within each plot to measure the effectiveness of shoveling at advancing snowmelt timing. Every 2-3 days throughout plant bloom we recorded the phenology of each focal plant species by counting the total number of plants in each plot and the number of flowers per plant for up to 10 plants per species that were randomly selected each sample date. We also measured visible frost damage by recording the proportion of plants that showed frost damage (brown or translucent flowers) on up to 10 plants per species.

To test for effects of snow manipulation and frost exposure on flowering phenology, we used generalized linear models using a Poisson distribution in the lme4 package (Bates et al. 2009) with year, snow treatment (removal vs. natural) and frost treatment (frost vs. no-frost) and their interactions as factors, and flowering phenology (date of first, peak, and last flower) and bloom duration as the response variables. Separate analyses were performed for each plant species. To assess how year and treatments affected peak flower density (highest observed flower density per plot), we used a generalized linear model using a quasipoisson distribution in the lme4 package to account for over-dispersion in the Poisson distribution. We assessed the fit

of each model by calculating the dispersion ratio. We then used least squares ratio tests of nested models to measure significance. To test how treatments affected the probability of frost damage, we ran linear models comparing proportion frost damage with year, snowmelt treatment, frost treatment, and their interactions as factors. All statistical analyses (here and below) were performed in R 3.3.2 (R Core Team, 2016).

Pollinator visitation rates

Upon flowering, we recorded flower-visitor interactions every other day throughout the blooming period of each focal species. We observed each plot for 10 mins using hand-held digital recorders, noting the duration of each floral visit, and the number of flowers visited on each plant. We only recorded flower-visitor interactions in which the visitor contacted the sexual organs of flowers, suggesting that they were indeed pollinating. We calculated four metrics of pollinator visitation per focal species per plot: (1) total number of visitors (number of bouts summed across the bloom period), (2) number of plant visits (total number of plants visited summed across visitors), (3) number of flower visits (total flowers summed across plants), and (4) average time spent per flower (total flower visits divided by total time spent at flowers summed across visits). We ran generalized linear models in lme4 on the four metrics of pollinator visitation with year, snow treatment, frost treatment, and their interactions as factors. Further, for focal species that showed a difference in flower production between treatments (see Results), we ran an additional analysis in which we divided the total visitors by the number of available flowers per sample date on a per plot basis to determine whether differences in visitation were associated with available resources. We used a Poisson distribution for total

visitors across all pollinator groups, a negative binomial distribution for total plants and flowers visited, and a gamma distribution for the average time spent per flower.

Pollen limitation and plant reproduction

In 2016, to determine the interactive effects of early snowmelt and frost on pollen limitation and plant reproduction, we conducted a pollen supplementation experiment on all of our focal species except for *Mertensia*. Within each plot, we randomly selected 8 plants per focal species and assigned them to either a pollen-supplementation treatment or open-pollination (not supplemented control) treatment (4 plants per treatment per plot). For the pollen-supplementation treatment, we applied pollen that was collected from plants within the same study population growing 5-10 m away to the stigmas of open flowers. For the open-pollination treatment, no pollen was added, but we handled flowers to control for flower handling (Cahill, Castelli, & Casper, 2002). Flowers in the pollen-supplementation and open-pollination treatments were both exposed to natural pollinator visitation.

Once the plants senesced, we measured female plant reproduction by collecting fruits and counting the seeds produced per fruit. Female reproduction was estimated as proportion fruit set (successful fruits/total flowers), mean seeds per successful fruit, and total seeds per plant. We then ran linear models with proportion fruit set and mean seeds per fruit as response variables with snow treatment, frost treatment, and their interactions and pollen treatment (supplemented/not supplemented) nested within snow and frost treatments as factors. We used a similar generalized linear model with a quasipoisson distribution for total seeds produced as the response variable.

RESULTS

Flowering phenology and frost damage

The date of bare ground was 15 days earlier in the snow removal plots than the natural snowmelt plots in 2015 and 12 days earlier in 2016. We found that snow removal significantly advanced flowering phenology for all focal species (Table 1). Phenology advanced by almost two weeks in the snow removal treatments for *Claytonia* ($\chi^2_1 = 119.97$, $p < 0.001$; Fig. 1a) and *Mertensia* ($\chi^2_1 = 139.93$, $p < 0.001$; Fig. 1b) and by almost one week for *Delphinium* ($\chi^2_1 = 58.88$, $p < 0.001$; Fig. 1c) and *Potentilla* ($\chi^2_1 = 56.75$, $p < 0.001$; Fig. 1d). Further, bloom duration (Fig. 2) was significantly longer by 3-10 days on average in the snow removal relative to control plots for *Claytonia* (7.93 ± 6.93 days longer; $\chi^2_1 = 49.48$, $p < 0.001$), *Mertensia* (10 ± 7.09 days longer; $\chi^2_1 = 77.82$, $p < 0.001$), *Delphinium* (2.89 ± 3.07 days longer; $\chi^2_1 = 8.98$, $p = 0.002$), and *Potentilla* (5.23 ± 4.29 days longer; $\chi^2_1 = 9.51$, $p = 0.002$). We also found a significant difference in phenology between 2015 and 2016 for all species ($\chi^2 > 28.90$, $p < 0.001$ in all cases; Fig. 1), except for *Delphinium* where there was no difference in first and peak bloom between years ($\chi^2_1 < 1.24$, $p > 0.21$; Fig. 1c). For all other species, phenology was shifted earlier in snow removal and control plots in 2015 compared to 2016 due to an earlier arrival of spring in 2016. For peak flower density, we found that the snow removal plots had 39% fewer *Claytonia* flowers than the natural snowmelt plots ($\chi^2_1 = 9.70$, $p = 0.001$), but did not find a difference in peak flower density for *Mertensia* ($\chi^2_1 = 0.60$, $p = 0.43$), *Delphinium* ($\chi^2_1 = 0.01$, $p = 0.90$) or *Potentilla* ($\chi^2_1 = 0.29$, $p = 0.58$).

Once the snow melted, the frost plots experienced 25 nights below 0° C in 2015 and 18 nights below 0° C in 2016 (Table S1). For *Claytonia*, we found that plants in the frost treatment exhibited 16.7% more frost damage than plants in the no-frost treatment ($F_{1,74} = 18.18$, $p < 0.001$;

Fig. 3a). Moreover, the proportion of *Claytonia* that exhibited frost damage was 24.2% higher in the snow removal treatment than the natural snowmelt treatment ($F_{1,74} = 23.53$, $p < 0.001$), and there was a significant interaction between the snow and frost treatments ($F_{1,74} = 13.13$, $p < 0.001$; Fig. 3a), with the proportion frost damage dependent on the snow removal treatment. For *Mertensia*, we also found higher rates of frost damage in the snow removal than the natural snowmelt treatments ($F_{1,33} = 17.59$, $p < 0.001$; Fig. 3b), but we did not find any difference in frost damage between the treatments for *Delphinium* or *Potentilla* ($F < 0.35$, $p > 0.58$ in all cases). Despite finding significant visual differences in frost damage between treatments for *Claytonia* and *Mertensia*, we did not find any significant effects of the frost treatment or interactions between the frost and snow removal treatments on estimates of flowering phenology or peak flower density for any of the focal species ($\chi^2 < 1.47$, $p > 0.22$ in all cases, Table 1).

Pollinator visitation rates

Across all four plant species, we observed 6,656 flower-visitor interactions. For *Claytonia*, we found that plants in the snow removal treatment experienced 78.71% fewer visitors than in the natural snowmelt treatment ($\chi^2_1 = 7.38$, $p < 0.001$; Fig. 4a) and that pollinators (mostly *Lasioglossum* and *Andrena* bees) spent on average 51.42% less time on the flowers in the snow removal relative to natural snowmelt treatments ($\chi^2_1 = 4.29$, $p = 0.03$; Fig. 4b). However, when we looked at the number of *Claytonia* plants and flowers visited by pollinators, we found no difference in visitation rates between snow removal and control plots ($\chi^2_1 = 0.66$, $p = 0.41$), but we did find that visitation rates increased by 48.3% in the frost compared to no-frost treatment ($\chi^2_1 = 5.04$, $p = 0.02$; Fig. 4c). Because we found a difference in flower production between the snow removal and natural snowmelt treatments for *Claytonia*, we analyzed per-plant

visitation rates and did not find a difference in visitation rates between snow treatments on a per-plant basis ($\chi^2_1 = 2.35$, $p = 0.13$). There were no significant interactions between snow removal and frost treatments for metrics of pollinator visitation ($\chi^2_1 < 0.60$, $p > 0.40$ in all cases). Further, we did not find any effect of year on visitation rates ($\chi^2_1 < 0.26$, $p > 0.60$ in all cases).

We found the opposite pattern of pollinator visitation for *Potentilla* (Table 1), with plants in the snow removal treatment receiving 21.19% more visitors (mostly *Lasioglossum* and *Panurginus* bees and flies in the Muscidae and Tachinidae families) than plants in the natural snowmelt treatment ($\chi^2_1 = 5.60$, $p = 0.012$; Fig. 4d), and pollinators visited 1.3 times more plants and flowers in the snow removal than control treatment ($\chi^2_1 = 7.35$, $p = 0.006$ and $\chi^2_1 = 8.25$, $p = 0.004$, respectively; Fig. 4d). However, we did find that that pollinators spent 11.48% more time on average on *Potentilla* flowers in the natural snowmelt treatment relative to snow removal treatment ($\chi^2_1 = 6.66$, $p = 0.009$; Fig. 4f). Further, for *Potentilla*, the frost treatment had no effect on patterns of pollinator visitation, and there were no significant interactions between the snow removal and frost treatments ($\chi^2_1 < 0.65$, $p > 0.41$ in all cases). We also found an effect of year on all aspects of visitation for *Potentilla* ($\chi^2_1 > 71.49$, $p < 0.001$ in all cases), with 2016 having lower metrics of visitation than 2015, but there was no interaction effect of year with snowmelt or frost treatment ($\chi^2_1 < 1.36$, $p > 0.24$ in all cases). When we looked at the relationship between bloom duration and visitation rates, we found that a longer bloom period increased visitation rates ($F_{1,37} = 5.14$, $p = 0.02$).

We found very few differences in pollinator visitation to *Mertensia* and *Delphinium* among treatments (Table 1). For both species, we did not find any effect of snow removal or frost treatments or their interaction on total visitors, total plants visited, total flowers visited, or average time spent per flower ($\chi^2_1 < 0.53$, $p > 0.46$ in all cases). The only significant effect we

found was for *Delphinium*, with pollinators spending on average 36.87% more time at the flowers in the no-frost treatment than the flowers in the frost treatment ($\chi^2_1 = 3.71$, $p = 0.05$). Further, we found an effect of year on all aspects of visitation for *Mertensia* ($\chi^2_1 > 17.64$, $p < 0.001$ in all cases), with 2016 having higher metrics of visitation than 2015, but there was no interaction effect of year with snowmelt or frost treatment ($\chi^2_1 < 0.75$, $p > 0.13$ in all cases).

Pollen limitation and plant reproduction

The one common pattern across all species is that none were pollen-limited for plant reproduction in 2016 ($p > 0.35$ in all cases). Thus, any differences in plant reproduction among treatments likely cannot be attributed to differences in pollinator visitation.

Despite the lack of pollen limitation, we did find some significant effects of the snow removal treatment on plant reproduction, but the results were species-specific (Table 1). For *Claytonia*, we found a significant reduction in proportion fruit set by 22.83% ($F_{1,69} = 31.37$, $p < 0.01$; Fig. 5a) and total seeds produced by 46.04% ($\chi^2_1 = 5.46$, $p = 0.01$; Fig. 5b) in the snow removal treatment compared to the natural snowmelt treatment, with no difference in average seeds produced per fruit ($F_{1,66} = 0.12$, $p = 0.72$; Fig. 5c). For *Delphinium* and *Potentilla*, we found opposite patterns. Snow removal resulted in 31.6% higher seeds produced in *Delphinium* ($\chi^2_1 = 1.95$, $p = 0.05$; Fig. 5d), but no effect on fruit set or average seeds produced (Figs. 5e and 5f). Further, while snow removal did not affect fruit set or total seeds produced in *Potentilla* (Figs. 5g and 5h), we did find that plants in the snow removal treatment produced 20.8% higher average seeds per fruit ($F_{1,71} = 5.58$, $p = 0.01$; Fig. 5i) than plants in the natural snowmelt treatment. Snow removal had no effect on plant reproduction in *Mertensia* ($p > 0.34$ in all cases).

Despite visible frost damage in both *Claytonia* and *Mertensia*, the only effect of frost treatment that we observed was for *Mertensia* (Table 1), with a non-significant trend towards 7.23% lower fruit set in the frost compared to no-frost treatment ($F_{1,36} = 3.30$, $p = 0.07$). We also found a trend in the interaction between snow removal and frost treatments on proportion seed set for *Mertensia* ($F_{1,36} = 3.31$, $p = 0.07$), suggesting that snowmelt timing moderates the degree to which plants are damaged by frost with reproductive consequences. No other effects of frost as a main effect or interaction were observed for any species or metric of plant reproduction ($p > 0.30$ in all cases).

DISCUSSION

Using a snow removal experiment, we successfully advanced the phenology of all four plant species through early snowmelt, and then examined subsequent effects on plant-pollinator interactions and reproduction. A growing number of studies have manipulated snowmelt timing to understand the consequences of climate change on flowering communities, but many of them have used artificial warming as a way to simulate early snowmelt (Sanders-Demott & Templer, 2017; Wipf & Rixen, 2010) whereas we only manipulated snowmelt timing, but not the ambient temperature. In arctic and montane systems climate change can lead to thinner snowpack, but not necessarily an increase in spring temperatures (Wipf & Rixen, 2010). Thus, our experimental manipulations allowed us to examine how harsher growing conditions, such as cooler spring temperatures and frost events, can affect patterns of flowering, pollination, and plant reproduction.

Our study highlights the important role that the abiotic environment has on flowering communities. The early-blooming species performed worse with snow removal; thus, the

advantage of a longer flowering period was outweighed by potential costs on development and reproduction due to cooler growing temperatures (Wipf, 2010). These results are consistent with other studies that have found that early-blooming species in montane regions have lower flowering density and seed set under early snowmelt conditions (Kudo, Nishikawa, Kasagi, & Kosuge, 2004; Lambert, Miller-Rushing, & Inouye, 2010; Thomson, 2010), and one study in particular found that early blooming *Claytonia* plants were smaller in size compared to *Claytonia* in a natural snowmelt treatment (Gezon et al., 2016). However, when snow removal is accompanied with experimental warming, these early-blooming species can perform better than controls under advanced phenology (Price & Waser, 1998). While there are trends towards increased spring temperatures after snowmelt, if the snow melts out too early, then the spring growing season is accompanied by cooler temperatures in arctic and montane regions (Lambert et al., 2010; Walker et al., 1999; Wipf, 2010). It is interesting that we found the opposite pattern for the later-blooming plant species, where they either remained unaffected, or performed better, under advanced phenology. This is consistent with other studies that did not find any immediate effects of snowmelt timing on plant production in the tundra, including mid-season flowering species (Hollister, Webber, & Bay, 2005; Livensperger et al., 2016). One important caveat, however, is that it may take multiple early snowmelt seasons for any ecological effects of advanced phenology to accumulate (Livensperger et al., 2016). Indeed, several long-term studies found that it took several years before a difference in flower production due to advanced phenology was observed, possibly due to slow growth and recruitment rates (Henry & Molau, 1997; Hollister et al., 2005; Wahren, Walker, & Bret-Harte, 2005). Long-term demographic studies would provide valuable insight to fully assess the effects of snowmelt timing on flowering for key vital rates.

We found similar patterns for pollinator visitation where the early-blooming species, *Claytonia*, received fewer visits from pollinators in the snow removal treatments, but the later-blooming species were either unaffected or positively affected by advanced phenology. Further, pollinators visited more flowers in the frost treatments than the no-frost treatments for *Claytonia*, but pollinators spent more time on the flowers in the no-frost treatment than the frost treatment for *Delphinium* (Table 1). Our results for *Claytonia* could be attributed to abiotic conditions directly altering flowering and floral morphology (Gezon et al., 2016), which could in turn indirectly alter the plant's attractiveness to pollinators. Alternatively, abiotic factors influence bee foraging activity (Polatto, Chaud-Netto, & Alves-Junior, 2014); thus, early-blooming species in the snow removal treatment might have been growing under unfavorable weather conditions for pollinators, or their peak flowering may not have coincided with their pollinators' emergence and peak activity. This interpretation suggests that a mismatch can occur between early-blooming species and their pollinators under climate change. However, Gezon et al. (2016) found that in some years, *Claytonia* received more visits from pollinators in the snow removal treatment; thus, future studies that examine the environmental variables that drive the emergence time of *Claytonia* pollinators could help explain these differences in visitation rates among years with snow removal.

Considering other patterns of pollinator visitation we observed (Table 1), our findings of higher pollinator visitation rates in the snow removal treatment for *Potentilla* coincide with previous research that has found that advanced phenology in mid- and late-blooming plants increases pollinator visitation rates in Wisconsin prairies (Rafferty & Ives, 2011). Since we found a positive relationship between bloom duration and number of plants visited, a longer bloom period in the snow removal treatment likely provided more opportunities for pollination.

Finally, the differences we observed in pollinator visitation between the frost and no-frost treatment could indicate that frost damaged floral rewards, and thus pollinators did not spend much time trying to extract resources (for *Delphinium*), or that frost reduced floral rewards and thus pollinators had to visit more flowers (for *Claytonia*) in order to collect enough pollen and nectar rewards (Pardee, Inouye, & Irwin, 2017).

Our trends in pollinator visitation rates coincided with our trends in seed set, where the treatment in which the plants that received more visits was also the treatment with higher seed production. However, we found no effect of pollen supplementation on plant reproduction for any of the species; thus, any effects of the snow removal and frost treatments on plant reproduction were likely due to direct effects rather than indirect effects mediated through pollinator visitation. These results contrast with a previous study that found *Claytonia* to be more pollen limited with snow removal, even in years with higher pollinator visitation rates, compared to ambient snowmelt (Gezon et al., 2016). The presence and strength of pollen limitation can vary interannually (Dudash & Fenster, 1997; Holm, 1994); thus, insight would be gained from experiments that measure pollen limitation and plant reproduction over multiple years to determine in more detail the effect of snowmelt timing and the biotic and abiotic factors that may be driving those variable effects.

At least two non-mutually exclusive hypotheses could explain increased seed production of *Delphinium* and *Potentilla* in the snow removal plots. First, these species might not be as affected by cooler temperatures during spring plant growth. Second, *Potentilla* flowers throughout July, which is a month of high precipitation in the Rocky Mountains (Adams & Comrie, 1997). However, July 2016 had low precipitation, thus the plants in the natural

snowmelt treatment might have experienced drought-like conditions during growth which might have resulted in lower seed set in the natural snowmelt treatments.

We found higher frost damage for *Claytonia* between the frost and no-frost treatments, and these differences were greater between the snow removal and control treatments. We also found higher frost damage in the snow removal treatment for *Mertensia*. However, frost damage did not translate into lower flower production for either species. One reason may be that the nighttime temperatures during the natural frost events in our study were usually between -1 and -4 °C, and early blooming species have been shown to be able to withstand these mild frost events (CaraDonna & Bain, 2016). However, Gezon et al. (2016) did find that frost negatively impacted *Claytonia* seed set in 2012, a year where the snow melted out particularly early and temperatures reached -8°C once plants had emerged, and Forrest and Thomson (2010) found that two hard frost events in May 2007 severely damaged *Mertensia* flowers. Thus, although we detected frost damage, the frost events might not have been severe enough to affect flower production. Finally, we did not find any significant frost damage to *Delphinium* or *Potentilla*, which is not surprising given that *Delphinium* is highly frost tolerant (CaraDonna & Bain, 2016), and *Potentilla* bloomed after the date of last frost, even in the snow removal treatment. Several studies have documented a direct link between frost and flower production for mid- to late-blooming species (Augspurger, 2009; Inouye, 2008; Pardee et al., 2017). Thus, the differences in results suggest strong interannual variation in how plants respond to frost events under early snowmelt conditions and the magnitude of the frost events occurring.

We did not find any effect of the frost treatment on seed set for *Claytonia*, *Delphinium*, or *Potentilla*, but we did find negative effects of the frost treatment on seed set for *Mertensia*. This suggests that while *Mertensia* might be able to grow under cooler temperatures, its reproduction

may directly suffer with freezing temperatures during the night. Also, since we found higher rates of frost damage in the frost-treatment, but no difference in floral production or visitation rates between the frost and no-frost treatments for *Mertensia*, our results indicate that frost-damaged flowers will fail to reproduce even if they receive sufficient visits from pollinators (Jacquemart, 1997). Therefore, advanced phenology due to early snowmelt could put *Mertensia* at greater risk for frost damage and reduced reproduction under climate change, especially if the number of annual frost events within subalpine regions remains constant or increases (Forrest et al., 2011; Forrest & Thomson, 2010). Our results show that frost may play an important role in shaping flowering communities, but more studies should be conducted that manipulate both the occurrence and temperature of frost events to determine the thresholds that control frost tolerance in early-blooming species.

Given our findings, early snowmelt timing and advanced phenology negatively impact early-blooming species likely through exposure to harsher growing conditions with the potential for mismatches with pollinators. However, species that flower later in the season benefit from advanced phenology through increased seed set. These differences in plant reproduction between early- and late-blooming species could reshape flowering communities as plant phenology continues to advance under climate change, but more long-term studies are needed to assess lasting demographic consequences of climate change due to changes in both abiotic and biotic conditions.

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AUTHOR CONTRIBUTIONS

GLP, REI, and DWI conceived and designed the study. GLP and IOJ conducted the fieldwork. GLP performed the analysis and wrote the paper. All authors provided edits to prior drafts of the manuscript.

DATA ACCESSIBILITY

The data used in this study will be archived at the Dryad Digital Repository and be made publicly available.

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Table 2-1: Summary of the effect of the snow removal treatment and frost treatment on phenology, pollinator visitation, and seed set for each flowering species. The + denotes a positive effect in comparison to the natural snowmelt and no-frost treatments and a – denotes a negative effect. The 0 represents non-significant differences. The table only includes significant effects for either the snow treatment, frost treatment, or their interaction effect. Any metrics not included for the species means that there was no effect across the treatments.

Plant Species	Snow Removal	Frost Treatment	Snow* Frost
<i>Claytonia lanceolata</i>			
Phenology	+	0	0
bloom duration	+	0	0
flower production	-	0	0
Frost damage	+	+	+
Visitation			
total visitors	-	0	0
total flowers	0	+	0
avg. time spent per flower	-	0	0
Seed set			
prop. Fruit set	-	0	0
total seeds	-	0	0
<i>Mertensia fusiformis</i>			
Phenology	+	0	0
bloom duration	+	0	0
Seed set			
prop. Fruit set	0	-	+
<i>Delphinium nuttallianum</i>			
Phenology	+	0	0
bloom duration	+	0	0
Visitation			
avg. time spent per flower	0	-	0
Seed set			
total seeds	+	0	0
<i>Potentilla pulcherrima</i>			
Phenology	+	0	0
bloom duration	+	0	0
Visitation			
total visitors	+	0	0
total flowers	+	0	0
avg. time spent per flower	-	0	0
Seed set			
avg. seeds per fruit	+	0	0

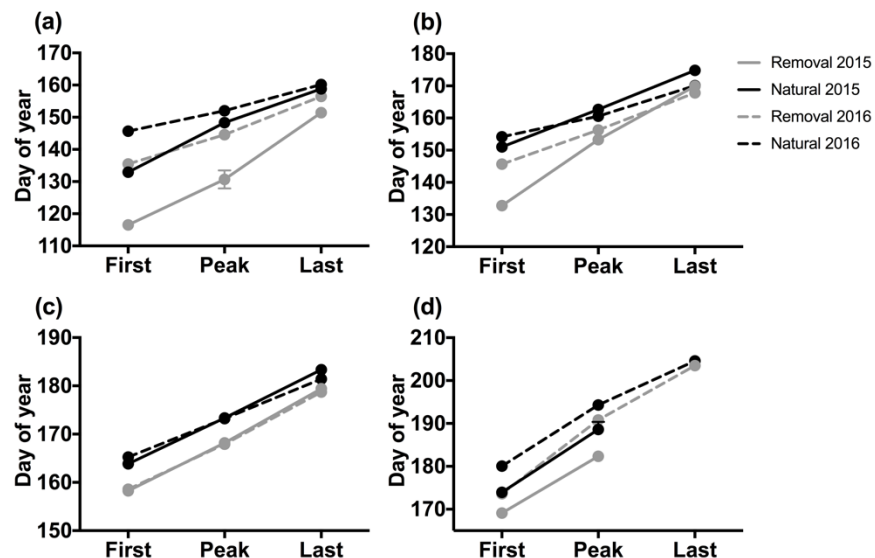


Figure 2-1: Flowering phenology in the snow removal (grey lines) and natural snowmelt (black lines) treatments for a) *Claytonia 43anceolate*, b) *Mertensia fusiformis*, c) *Delphinium nuttallianum*, and d) *Potentilla pulcherrima* (though we are missing date of last flower in 2016 for *Potentilla*). Note the difference in scale on the Y-axis of each panel. The solid lines show the phenology for 2015 and the dotted lines for 2016. Phenology was significantly advanced in the snow removal treatment for all focal species ($p < 0.05$ in all cases). Points are means and bars are standard error.

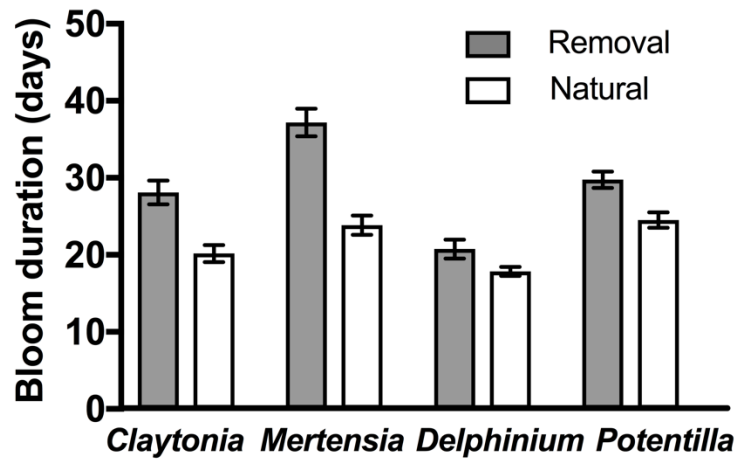


Figure 2-2: Bloom duration for four focal plant species in the snow removal (grey bars) and natural snowmelt (white bars) treatments. Bloom duration was significantly longer in the snow removal treatment for all species ($p < 0.05$ in all cases). Bars are means $\pm$ standard error.

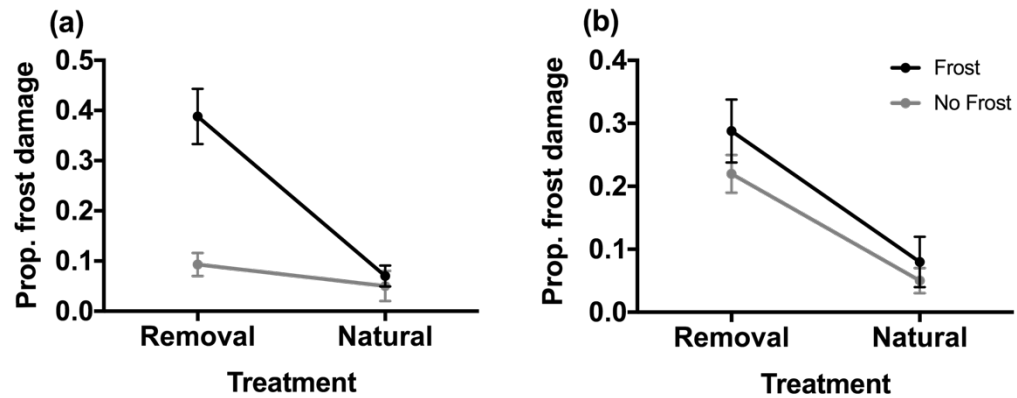


Figure 2-3: Proportion frost damage to flowers in the frost (black line) and no-frost (grey line) treatments and in the snow removal and natural snowmelt plots for a) *Claytonia* and b) *Mertensia*. Note the difference in scale on the y-axis of the two panels. For *Claytonia*, there was a higher proportion of frost damage in the snow removal and frost treatments as well as a significant interaction between the two treatments. For *Mertensia*, we found higher frost damage in the snow removal treatment but no significant interaction. Points are means and bars denote standard error.

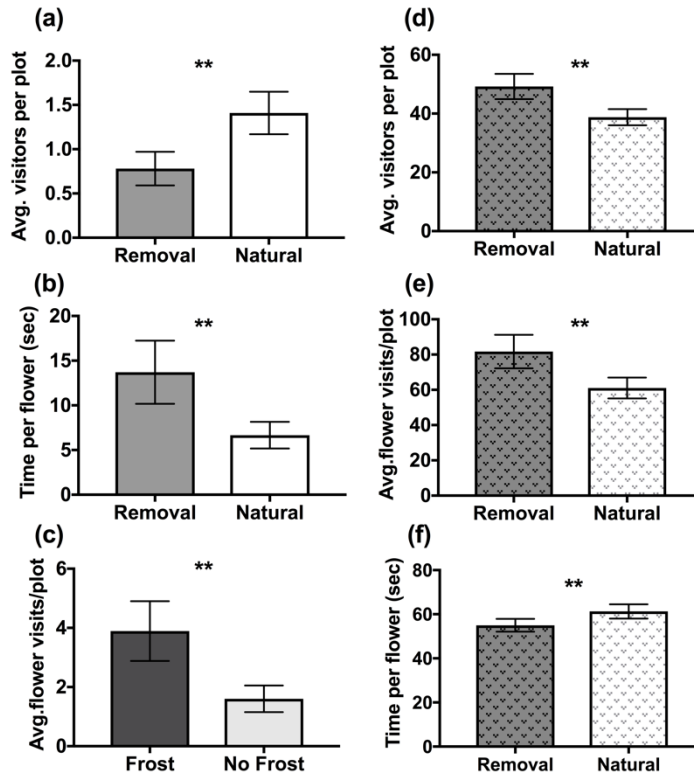


Figure 2-4: Patterns of flower-visitor interactions for *Claytonia* (solid bars) and *Potentilla* (dotted bars) between the snow removal and natural snowmelt treatments, except for panel c which shows differences between the frost and no-frost treatment. Panels a and d show average visitors per plot, panels b and e show the average time spent per flower, and panels c and f show the average number of flowers visited per visitor. Asterisks denote significance at $p < 0.05$. Bars are means $\pm$ standard error.

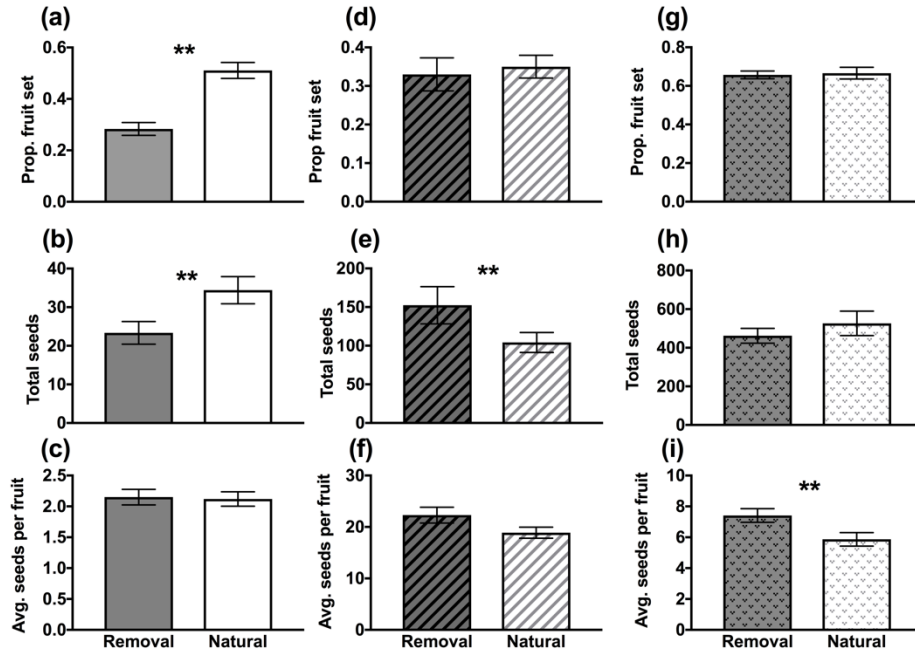


Figure 2-5: Mean fruit set and seed set for *Claytonia* (solid bars), *Delphinium* (striped bars), and *Potentilla* (dotted bars) between the snow removal (dark bars) and natural snowmelt (light bars) treatments. Panels a, d, and g show the proportion of successful fruits, panels b, e, and h show the total seeds produced, and panels c, f, and i show the average seeds produced per fruit. Asterisks denote significance at $p < 0.05$, and bars are means $\pm$ standard error.

CHAPTER 3: Snowmelt timing and frost shift flowering communities in montane regions without disrupting plant-pollinator networks

Gabriella L. Pardee^{1,2*}, David W. Inouye^{2,3}, and Rebecca E. Irwin^{1,2}

¹Department of Applied Ecology, North Carolina State University, Raleigh, NC 27607 USA

²Rocky Mountain Biological Laboratory, Gothic, CO 81224 USA

³Department of Biology, University of Maryland, College Park, MD 207442 USA

*Corresponding author email: gabriellapardee@gmail.com

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ABSTRACT

Changes in abiotic conditions under climate change can have profound effects on species richness, abundance, and community composition. Such effects could alter the network structure of interacting organisms within a community. Under climate change, we could see losses in interactions between organisms due to one organism responding differently to changes in abiotic conditions than its interacting partner. Alternatively, we could find that networks are robust to climate change through network rewiring where interactions are reassembled to avoid losses in ecosystem function. Thus, we conducted a snow manipulation experiment (snow removal vs natural snowmelt) crossed with a frost component (frost vs no-frost) in a montane meadow to examine how species composition and plant-pollinator networks respond to changes in abiotic conditions. We found that flowering communities were heavily influenced by snow and frost,

while patterns of the pollinator visitation community were only different between the snow treatment for one year of the study. We did find that eusocial and solitary bees visited proportionally more flowers in the frost treatment than the no-frost treatment, but the opposite was true for syrphid flies. However, we did not find a difference in network specialization, connectance, or nestedness between the snowmelt and frost treatments. Our findings show that high generalization among plants and pollinators might make networks robust to climate change in subalpine communities, despite finding differences in composition and visitation rates among groups. Thus, plant-pollinator interactions might remain stable under climate induced changes to montane ecosystems.

INTRODUCTION

Climate change is having profound effects on the phenology, abundance, and biodiversity of plants and animals worldwide (Walther et al. 2002, Parmesan and Yohe 2003, Van der Putten et al. 2010). For example, climate change is resulting in the advancement of organisms' phenologies at unprecedented rates (Visser and Both 2005, Cleland et al. 2007, Wolkovich et al. 2012). Additionally, changes in temperature and other abiotic factors associated with climate change are affecting the survival and abundance of populations, with subsequent effects on metrics of biodiversity (Bellard et al. 2012). However, studies are also finding that not all species are responding to a changing climate, or if they are, they may be responding at different rates (Parmesan and Yohe 2003, Stefanescu et al. 2003), which can result in the reshaping of ecological communities and co-occurrence patterns of historically interacting species. That species interactions can be modified by the abiotic environment is not surprising. Classic studies have shown that abiotic conditions, such as temperature and moisture, can strongly affect predator-prey, plant-herbivore, and plant-pollinator interactions (Møller et al. 2008, Gilman et al. 2011). What remains unresolved, however, is how individual variation in species abundance and phenology and changes in biodiversity scale to affect community structure, and in particular, the structure of networks of species interactions. The goal of this study was to assess how a changing climate affects networks of interactions between plants and pollinators.

Over 87% of all flowering plant species on Earth are dependent on pollinators for successful reproduction (Ollerton et al. 2011), and in return pollinators receive food and nesting resources. Climate change can have implications for ecosystem services by disrupting this interaction through several ways (Memmott et al. 2007, Hegland et al. 2009). First, climate change can lead to temporal mismatching, where plants and pollinators are no longer active at

the same time (Rafferty et al. 2015). Second, climate change can alter plant and/or pollinator abundance and species richness, which can in turn affect visitation rates and diet breadth. Third, changes in abiotic conditions can affect rates of visitation. For example, small bees have been shown to increase foraging activity under increased temperatures while eusocial bumble bees decrease their activity level (Rader et al. 2013). Any of these scenarios can result in community level shifts in plant-pollinator interactions and loss in pollination services.

While many studies have explored the consequences of climate change on pairwise plant-pollinator interactions and pollinator diversity (Rafferty and Ives 2011, Iler et al. 2013c, Kudo and Ida 2013, Gezon et al. 2016), evaluating these interactions at the community level using networks can provide greater insight into how communities respond to environmental variation rather than just biodiversity metrics or pairwise interactions (Morris 2010, Urbanowicz et al. 2017). For example, networks can tell us whether increase temperatures result in lower connectance, specialization, or loss in interactions. As higher connectance helps with community stability (Heleno et al. 2012), a decline in connectance can lead to losses in pollination opportunities for flowers or food resources for pollinators. A study conducted on a plant-pollinator network in a temperate forest understory found interaction losses for 50% of bees over 120 years, and one reasoning behind this result was a phenological mismatch (Burkle et al. 2013). Further, a network study conducted across a climate gradient in the Mediterranean Basin found that temperature and precipitation were correlated with modularity and specialization, two metrics that are associated with phenology (Petanidou et al. 2018). Network studies can also tell us whether plant-pollinator interactions are robust to climate change through network rewiring where pollinators can switch to different flowering species (CaraDonna et al. 2017) if they become mismatched with their preferred host plant. Thus, network rewiring could prevent

population declines for both interacting species under climate change. Previous studies have used natural elevational or latitudinal gradients to measure network responses to increased temperature in temperate and tropical regions (Cannone 2006, Dalsgaard et al. 2013, Benadi et al. 2014, Petanidou et al. 2018). While these gradients have provided insights into simultaneous effects of multiple abiotic factors, they miss some basic effects of climate change, in particular the advancement of phenological events relative to historic patterns and extreme events, that may have large effects on webs of interactions and warrant investigation.

Plants in montane and arctic regions have been advancing their phenology over the last 40 years due to decreased snowpack and earlier melting (Miller-Rushing and Inouye 2009, Iler et al. 2013a, CaraDonna et al. 2014). Advanced phenology in plants could alter their interactions with pollinators, in two non-mutually exclusive ways. First, advanced phenology in plants could introduce them to novel pollinator communities if pollinators respond differently to snowmelt timing in terms of their phenology. Second, advanced phenology could expose plants to novel abiotic conditions while growing, which could alter species richness and abundance. For example, advanced phenology under early snowmelt can put plants at a higher risk of being exposed to extreme events, such as spring frost, which can damage plant tissue through the formation of ice crystals at or below freezing temperatures (Sakai and Larcher 1987, Inouye 2008). This could alter plant-pollinator networks if frost reduces flower abundance or if pollinators switch to species that are more frost tolerant while foraging. Thus, when examining the effects of early snowmelt on plant-pollinator networks, it is important to incorporate other harsh weather events that can result from early bloom. While previous studies have looked at pairwise interactions of snowmelt timing and frost exposure (Boggs and Inouye 2012, Gezon et

al. 2016, Pardee et al. 2017), none have examined these effects across networks of plant-pollinator interactions.

We manipulated snowmelt timing and frost exposure in a montane flowering community to address the following questions: 1) How do snowmelt timing and frost exposure affect flowering composition, and does advanced flowering phenology result in differences in the visitation patterns of pollinator communities? 2) Do the proportion of flowers visited by the main pollinator groups change in response to snowmelt timing and frost exposure? and 3) How do plant-pollinator networks respond to snowmelt timing and frost exposure? We predict that advanced phenology could alter community composition due to species-specific responses to snowmelt timing and frost exposure. Because we are not manipulating pollinator phenology, we predict that advanced phenology in plants due to early snowmelt will alter the pollinator communities that visit the plants. Further, we predict that pollinators will respond differently to differences in the plant communities, depending on their biology (fly vs bee) and life history traits (eusocial bee vs solitary bee), thus we will see shifts in visitation rates for each of these groups under advanced flowering phenology and frost exposure. Finally, we predict that we will observe shifts in network specialization, connectance, and nestedness due to snowmelt timing and frost exposure altering community composition and plant-pollinator interactions. Alternatively, we could find no change in network structure among the treatments if networks are robust to phenological change via network rewiring.

METHODS

Study System

Fieldwork was conducted at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO, USA (elevation 2950 m). The RMBL is located in a sub-alpine region, and over the last 40 years in this region, there has been a trend towards increasing spring temperatures (Iler et al. 2013b) and earlier spring snowmelt, which has consequently led to earlier plant bloom (CaraDonna et al. 2014). These changes may result in an increased incidence of plant damage from episodic spring frost events, especially if the last frost-free day of the year is not changing at the same rate (Inouye 2000).

We conducted our study in a dry meadow, which is the dominant habitat type around the RMBL (Langenheim 1962). These dry meadows contain at least 40 flowering plant species (Burkle and Irwin 2009) that are pollinated by an assemblage of bees, hummingbirds, and flies (Forrest 2010). The flowering species that typify these communities are *Claytonia lanceolata*, *Delphinium nuttallianum*, and *Potentilla pulcherrima*, and the main pollinators in these meadows are bumble bees, syrphid flies, and solitary bees, from the Halictidae, Andrenidae, and Megachilidae families. Studies in this region have found that networks experience high turnover from week-to-week as well from year-to-year, and that pollinators are flexible in their plant interactions, suggesting strong evidence for network rewiring (Burkle and Irwin 2009, CaraDonna et al. 2017).

Field methods

We conducted a factorial design manipulating snowmelt timing (snow removal vs. natural snowmelt) and frost exposure (frost vs. no frost), as in Pardee et al. (in review and

Chapter 2). We used 40 2 x 2 m plots along two 100 m transects, each plot spaced 3 m apart. We manipulated snowmelt timing in 20 plots by manually removing snow once the snowpack melted down to 1 m (snow removal treatment), and left the remaining twenty plots to melt out naturally (natural snowmelt treatment) (akin to Gezon et al. 2016). We crossed the snowmelt treatment with a frost treatment, where plots were either exposed to naturally occurring frost events (frost treatment), or plots were protected from natural frost events (no-frost treatment) using a frost protectant cover (Easy Gardener Products, Waco, TX, USA). The experiment was conducted between April-July 2015 and again between May-August 2016 using a different set of plots that were located 10 m north of the 2015 plots. Our prior research in these plots documented that date of bare ground occurred 15 days earlier in 2015 and 12 days earlier in 2016 in the snow removal treatment relative to natural snowmelt treatment (Pardee et al. in review and *Chapter 2*).

Moreover, the frost plots experienced 25 natural frost events in 2015 and 18 frost events in 2016, resulting in visible flower damage (brown or translucent flowers suggesting cell damage) to the earliest blooming species (Pardee et al. in review and *Chapter 2*). Although we only manipulated the flowering community, we could see results similar to what we found in this study under climate change if either pollinators respond to different abiotic cues, or to the same cues (i.e. snowmelt timing), but at different rates, both of which could alter plant-pollinator interactions.

Flower phenology. Every 2-3 days throughout the flowering season, we recorded flowering community composition and phenology within each plot by recording the number of plants with open flowers for each species in bloom. Additionally, we counted the number of flowers per plant for up to 10 plants per species. We then estimated the total flowers in bloom per plot for each species by multiplying the average number of flowers per plant by the total number of plants in bloom.

Plant-pollinator interactions. We recorded plant-pollinator interactions every other day throughout the flowering season. During each observation period, we observed each plot for 10 mins using hand-held digital recorders, noting every time a visitor entered the plot, the plant species visited, and the number of flowers visited on each plant per species. We identified each visitor on the wing to a pre-assigned morphogroup within genus for solitary bees and syrphid flies and family for non-syrphid flies, butterflies, wasps, and moths. Bumble bees were identified to species based on pile color markings (Williams et al. 2014). We only recorded flower-visitor interactions in which the visitor contacted the sexual organs of flowers, suggesting that they were pollinating. We attempted to collect each visitor as it left the plot to ensure accurate identification. Further, we netted for floral visitors for 10 min after every hour sampled to collect voucher specimens. Specimens were pinned and identified the taxonomic level listed above.

Statistical analyses

All statistical analyses were conducted using R 3.3.2 (R Core Team, 2016).

Flower phenology. In addition to the four species analyzed in Pardee et al. (in review and Chapter 2), we examined phenology for seven species that were well represented in our plots across both years: *Androsace septentrionalis* (Primulaceae), *Collomia linearis* (Polemoniaceae), *Erigeron speciosus* (Asteraceae), *Lathyrus lanszwertii* (Fabaceae), *Taraxacum officinale* (Asteraceae), *Vicia americana* (Fabaceae), and *Viola praemorsa* (Violaceae). To test for effects of snow manipulation and frost exposure on flowering phenology, we used generalized linear models using a Poisson distribution in the lme4 package (Bates et al. 2009) with year, snow treatment (removal vs. natural) and frost treatment (frost vs. no-frost) and their interactions as factors, and flowering phenology (date of first, peak, and last flower) as the response variable.

Community composition. We used multivariate methods in the R package ‘vegan’ (Oksanen et al. 2015) to compare between-treatment differences in community composition of flowering species and pollinator morphogroups within each plot. We calculated dissimilarities between all plots using the Bray-Curtis metric, which compares sites using the identity and relative abundance of species (Griffin et al. 2017). We first used non-metric multidimensional scaling plots (NMDS) to visually compare species similarity of sampled plots. Next, we tested significance of visually distinguishable groups from the NMDS plots with a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function with SS type III to test for differences in community composition between snow treatments (removal vs natural), frost treatments (frost vs no-frost), and their interaction (Anderson 2001). We used 999 permutations to assess significance.

Proportion flower visits. To gain better insight into how pollinators respond to advanced phenology and frost exposure in plants, we examined the proportion of flower visits within each plot by the four most common pollinator groups: bumble bees, solitary bees, syrphid flies, and flies that belong to Anthomyiidae, Muscidae, and Tachinidae families (hereafter, referred to as “other flies”). Next, we ran generalized linear models with a quasibinomial distribution to account for underdispersion with proportion of flower visits per pollinator group as the response variables and snow treatment, frost treatment, their interactions, and year as the fixed effects.

Plant-pollinator networks. We constructed plant-pollinator interaction networks to examine how communities respond to changes in abiotic conditions using the bipartite package (Dormann et al. 2009). Networks were represented as two-dimensional matrices where the columns represented plant species and the rows represented pollinator morphogroups. We

constructed one quantitative network per plot for each year for a total of 80 networks, in which cells were the frequency of observed visits for each plant-pollinator interaction.

We used three commonly used metrics that describe network structure: network specialization, connectance, and relative nestedness (Blüthgen 2010). All three metrics were calculated using the `networklevel` function. Network specialization (H_2') describes the degree of specialization between plants and pollinators while incorporating abundances into the network (Blüthgen et al. 2006). Connectance is the number of observed links divided by the total possible links between the plants and pollinators within each network (Jordano 1987). We weighted connectance, which takes link weights (visitation rates) into account, to capture the shape of the visitation distribution (Altena et al. 2016). Nestedness, which measures ecosystem structure, is a common pattern in plant-pollinator networks where specialists tend to interact with generalists (Staniczenko et al. 2013), has a value of 0 to 100 where 0 represents perfect nestedness and 100 represents complete randomization (Dormann et al. 2009). We weighted nestedness by visitation rates using the NODF function (Ulrich et al. 2009). As nestedness can be influenced by matrix size and number of interactions we standardized nestedness prior to comparing networks (Strona and Fattorini 2014). We did this by first randomly generating networks using the `vaznull` function in the 'Bipartite' package, iterating each null model 1000 times (Vazquez and Aizen 2003). Next, we calculated relative nestedness with the equation, $(x - \mu) / \mu$, where x represents the observed nestedness value and μ is the mean nestedness of the randomized matrices (Olesen et al. 2007). The null models were used to determine whether the observed models were more or less nested than by chance with the resulting p-value denoting a significant difference between the observed and randomized networks. We tested the effects of snowmelt timing and frost on network metrics by running generalized linear models with the quasibinomial distribution using

H2', connectance, and relative nestedness as the response variables and year, snowmelt timing, frost exposure, and their interactions as fixed effects.

Combining networks across the growing season could dilute the effects of frost and snowmelt timing on plant-pollinator interactions since blooming plant species are more susceptible to changes in abiotic conditions than species that flower later in the season (Lambert et al. 2010a, Wipf 2010). Thus, we conducted an additional analysis where we examined changes in network metrics for 5 early blooming species (listed their phenological bloom order):

Claytonia lanceolata, *Mertensia fusiformis*, *Delphinium nuttallianum*, *Androsace septentrionalis*, *Ranunculus inamoenus*, and *Viola praemorsa*. Though we did not have sufficient data to run 80 individual networks like we described in the above paragraph, we instead combined each of the 10 plots per treatment into one network and compared each of the constructed networks against a null model to determine whether our observed values were significantly different from our null values for nestedness, connectance, and specialization. For nestedness and specialization we ran null models using the *vaznull* function and for connectance we ran null models using Patefield's algorithm (Blüthgen et al. 2008). All models were iterated 1000 times.

RESULTS

Overall, we conducted 206 hours of observations, which allowed us to observe a total of 7,870 unique pairwise plant-pollinator interactions from 26 flowering species and 57 pollinator morphogroups. We also recorded an additional 17 species in the plots during our phenology counts that were not visited by pollinators during our observations. The most common pollinators were small houseflies belonging to the *Muscidae* family (48.77% of flower visits), solitary *Lasioglossum* spp. bees (13.83%), and eusocial *Bombus bifarius* bumble bee (6.01%),

and the most abundant flowering plant species were *Potentilla pulcherrima*, *Androsace septentrionalis*, and *Claytonia lanceolata* (Table S1).

Flowering phenology

Date of first bloom and peak bloom significantly advanced in the snow removal treatment compared to the natural snowmelt treatment for six of our seven species (table 1). Further, we found that date of last bloom was significantly earlier in the snow removal treatment compared with the natural snowmelt treatment for three of the seven species. Finally, there was a significant difference in year where plants bloomed earlier in 2015 than 2016. When we looked at the effects of frost on phenology, we found that plants reached date of peak bloom and date of last bloom earlier in the frost treatments than the no-frost treatments for *Lathyrus lanszwertii* ($\chi^2_1 = 4.70, p = 0.03$, and $\chi^2_1 = 9.65, p = 0.001$, respectively), but did not find any other effects of frost on flowering phenology ($\chi^2_1 < 2.87, p > 0.09$, for all species). Finally, we did not find any interaction effect between the snow and frost species on phenology for any of our species ($\chi^2_1 < 2.87, p > 0.08$, for all species).

Community composition

Our PERMANOVA analysis showed that snowmelt timing and frost exposure had a significant effect on the composition of flowering communities in 2015 ($R^2 = 0.06, F_{1,36} = 2.57, p = 0.04$, and $R^2 = 0.05, F_{1,36} = 2.53, p = 0.05$, respectively), but there was no significant interaction effect ($R^2 = 0.01, F_{1,36} = 0.48, p = 0.75$). However, though visual analysis of the NMDS plots for 2015 showed that points in the different treatments were somewhat differentiated (stress = 0.12, and stress = 0.15, respectively; figs. 1a & b), the ellipses overlapped showing lack of independence in clustering. In 2016, we found that community composition was

significantly impacted by snowmelt timing ($R^2 = 0.05$, $F_{1,36} = 2.19$, $p = 0.05$), but not by frost exposure ($R^2 = 0.01$, $F_{1,36} = 0.45$, $p = 0.87$) nor by an interaction between snow and frost ($R^2 = 0.05$, $F_{1,36} = 2.15$, $p = 0.08$). Visual analysis of the NMDS plots for 2016 showed that points in the snow removal and natural snowmelt treatments were somewhat differentiated, but the ellipses overlapped showing lack of independence in clustering (stress = 0.17; fig 1c). The NMDS plot for frost exposure showed complete overlap between the plots in the frost and the plots in the no-frost treatment (stress = 0.16; fig. 1d).

For pollinator community composition, we found an effect of the snowmelt timing on pollinator communities in 2015 ($R^2 = 0.05$, $F_{1,36} = 1.99$, $p = 0.02$). However, we did not see any significant effect of frost exposure nor an interaction between snow and frost on pollinator communities ($R^2 < 0.02$, $p > 0.59$, all cases). We did not see any difference in pollinator community composition between treatments in 2016 ($R^2 < 0.02$, $F < .80$, $p > 0.32$, for all cases, fig 2b). Visual analysis of the NMDS plots for 2015 showed that plots in the snow removal and natural snowmelt treatments were clustered apart, but the ellipses showed slight overlap indicating lack of independence in clustering (stress = 0.13; fig. 2a). NMDS plots for 2016 showed almost complete overlap between plots in snow removal and natural snowmelt treatments (stress = 0.25; fig. 2b).

Plant-pollinator interactions

When we looked at the proportion of flowers visited by the most common pollinator groups, we found differences in their responses to the snow removal and frost treatments. For the snow removal treatment, we found a non-significant trend towards higher proportion visits compared to the natural snowmelt plots for solitary bees ($\chi^2_1 = 2.77$, $p = 0.09$, Fig 3a), but did

not find any other effect of snow removal on flower visits by the other three pollinator groups ($\chi^2_1 < 0.561, p > 0.45$, for all groups). For the frost treatment, we found that solitary bees had significantly higher proportion of visits in the frost treatment compared to the no-frost treatment ($\chi^2_1 = 5.48, p = 0.01$, fig. 4a), but that the syrphids had higher proportion of flower visits in the no-frost treatment than the frost treatment ($\chi^2_1 = 5.33, p = 0.02$, 3b). Bumble bees and other flies show no effect of visitation between the frost and no-frost treatment ($\chi^2_1 < 0.44, p > 0.37$ for both groups; figs 3c & d). For the interaction between snow and frost, we found a trend where bumble bees visited more flowers in the removal treatments with frost vs without frost ($\chi^2_1 = 3.38, p = 0.06$, fig 3c). Finally, we found a significant effect of year for all pollinator groups ($\chi^2_1 > 10.88, p < 0.001$), except bumble bees ($\chi^2_1 = 1.33, p = 0.24$).

Network structure

Although we ran statistical analyses using our 80 networks, we combined each of our plots by treatment type to create one large network per treatment to visually display the networks across treatments (fig. 4). Network specialization across the 80 plots ranged from 0.14-0.96 in 2015 and 0.18-1.0 in 2016. Connectance ranged from 0.11-0.27 in 2015, and 0.06-0.33 in 2016, and nestedness ranged from 0-43.33 in 2015 and 0-40.44 in 2016. When we compared these metrics across treatments, we did not find any difference in network structure between the snow removal treatments, frost treatments, or their interactions for either year ($\chi^2_1 < 2.76, p > 0.09$ in all cases). When we compared the observed nestedness with the nestedness of the randomized matrices, we found that 40% of the networks in the snow removal and no-frost treatment, 60% of the networks in the snow removal and frost treatment, 55% of the networks in the natural

snowmelt and no-frost treatment, and 45% of the networks in the natural snowmelt and frost treatment were significantly more nested than by chance ($p < 0.05$ in all cases).

When we examined networks for just the 5 early blooming species, we found that for both years, all networks were significantly different from the null models for nestedness, connectance, and specialization (table 2). For nestedness, the observed networks had significantly lower nestedness values than the randomized nestedness for both years. The observed networks also had significantly higher specialization than the null models for both years (table 2). For connectance, the observed networks had higher connectance than the null models in 2015, but lower connectance than the null models in 2016.

DISCUSSION

Climate change can disrupt plant-pollinator interactions, leading to a decrease in survival and reproduction for both partners (Memmott et al. 2007, Hegland et al. 2009). By manipulating the date of bare ground and frost exposure, we were able to examine how plant communities respond to changes in abiotic conditions, and whether those changes led to differences in the visitation patterns of pollinator communities and networks of interactions between plants and pollinators. While many studies have explored the effects of advanced or delayed snowmelt on flowering communities (Wipf 2010, Semenchuk et al. 2016, Sherwood et al. 2017), few have looked at whether these shifts have led to differences in the pollinator communities visiting entire plant assemblages. When we examined pollinator communities, we found differences in composition between the snow removal and natural snowmelt treatments for one year of the study (2015), suggesting that advancing snowmelt timing by just two weeks results in plants being visited by a different suite of pollinators than under natural snowmelt conditions.

Surprisingly, however, these changes in the plant communities and assemblages of pollinators visiting those plants did not result in changes in network structure, suggesting that pollinators in this region have broad dietary breadths and can maintain visitation rates through network rewiring if they become mismatched with their preferred host.

We found that snowmelt timing and naturally occurring frost events have profound effects on flowering communities. These results are not surprising considering that plants respond strongly to changes in annual weather patterns in terms of their phenology, growth, and flower production (Badeck et al. 2004, Forrest et al. 2010). Six out of the seven plant species in our study bloomed earlier due to snow removal and date of peak bloom and last bloom was significantly earlier for two of the species in the frost treatment compared to the no-frost treatment. As a result of early snowmelt and frost exposure, we found differences in community composition. Given the projected weather patterns in montane regions under climate change (McCabe et al. 2007, Overpeck and Udall 2010), it is likely that we will see reshaping of flowering communities due to earlier snowmelt and exposure to extreme weather events.

Not only did we detect differences in the flowering communities with manipulations, but we also detected differences in the communities of pollinators visiting those plants. For bumble bees, there was a strong trend towards an interaction effect between the snow removal and frost treatments where there were proportionally more flowers visited in the frost treatment compared to the no-frost treatment for the snow removal treatment. We saw the same trend with solitary bees where there were proportionally more flowers visited in the frost treatment than the no-frost treatment. However, the opposite trend was seen with syrphids visiting proportionally higher flowers in the no-frost treatment than the frost treatment. These differences are similar to another study that has shown that bees and flies respond differently to frost-damaged flowers (Pardee et

al. 2017). One reason that may explain these different patterns of visitation is that flies primarily collect nectar (Larson et al. 2001), while bees collect nectar and pollen (Thorp 2000). Therefore, the difference in visitation rates among the groups could be explained if frost-damaged flowers experienced a reduction in nectar but not in pollen, suggesting that flies may avoid frost-damaged flowers due to lower nectar availability. This hypothesis assumes that bees are more limited by pollen than nectar on foraging bouts, which can occur (Francis et al. 2016). Thus, studies that match measures of floral rewards with pollinator visitation patterns as a function of snow removal and frost damage will yield additional ecological insights.

Given changes in flowering communities and patterns of pollinator visitation under snow removal and frost, we expected to find sweeping changes in plant-pollinator network structure. Instead, we found no difference in network specialization, connectance, or nestedness among treatments for our plant-pollinator networks. This is surprising given that previous studies have shown that plant-pollinator networks are influenced by environmental variables, such as temperature and precipitation (Olesen and Jordano 2002, Petanidou et al. 2018, but see Hoiss et al. 2015). Moreover, one snow removal study in the Alps had contrasting network results to our study. Hoiss et al. (2015) found higher network specialization in the snow removal treatment compared to the natural snowmelt treatment, but the study did not examine nestedness or connectance. There are at least two relevant hypotheses that could explain differences among this study and prior research. First, many of the prior studies that showed effects of environmental variation on network structure were conducted in temperate and tropical regions where networks are highly specialized (Olesen and Jordano 2002). However, montane regions (where this study was conducted) as well as alpine and arctic regions experience shortened growing seasons and harsh weather conditions and thus might contain more generalized pollinators that can readily

alter their foraging activity under changing weather patterns (Ramos-Jiliberto et al. 2010, Miller-Struttmann and Galen 2014). Indeed, when we looked across pollinator groups, we found that that 88.7% of the pollinators that visited flowers within our plots were generalist Dipterans and bees. These results are similar to Urbanowicz et al. (2017) who also found no effect of climate variation (temperature and wind exposure) on Dipteran dominated networks in Greenland. Further, many flowers species in montane and arctic regions are highly generalized and are visited by a wide array of pollinators (Elberling and Olesen 1999, Olesen and Jordano 2002, Tiusanen et al. 2016, Urbanowicz et al. 2017); thus, we might not have seen a difference due to high plant generalization as well. Second, comparing our results from a montane region to Hoiss et al. (2015) in the alps, an explanation for the difference in results could be that snowmelt was advanced by ~28 days in the snow removal plots in Hoiss et al. (2015), but we advanced snowmelt timing by only 15 days. Thus, our snow removal treatment might not have been strong enough to detect differences in network specialization. Nonetheless, our snow removal treatment mimics natural variation in snowmelt timing and phenological responses of plants when compared to a long-term flowering phenology dataset (Gezon et al. 2016). Third, we constructed networks across the entire growing season, which could have diluted the effects of frost and snowmelt timing on plant-pollinator interactions. However, when we compared our networks for the five earliest blooming species with null models, we found that our networks had significantly lower rates of nestedness and higher rates of specialization for both years compared to our null models across all treatments. Further, we found that in 2015 connectance was significantly higher than the null models for all treatments, but in 2016 connectance was significantly lower than the null models for all treatments. This indicates that plant-pollinator networks for the early flowering species also have high stability and generalization.

For both plant and pollinator responses and network metrics, one consistent pattern we observed was interannual variation in responses or responses to the treatments in the two years of study. One reason why we might not have seen differences in pollinator communities for both years of the study is differences in the natural environmental conditions of the two years. In meadows surrounding the RMBL, the snow usually melts out in mid- to late May (Inouye 2008). However, 2015 was a particularly early snowmelt year, so the date of bare ground for plots in both snow removal and natural snowmelt treatments was late April, and early May, respectively. In 2016, the date of bare ground in the snow removal and natural snowmelt plots was two weeks later than in 2015. Thus, these results suggest strong interannual differences in pollinator communities, depending on when the snow melts out. We could see similar patterns for natural conditions under projected climate change scenarios if both flowers and bees respond to the same abiotic cues, but at different rates, which could expose plants and/or pollinators to novel interactions. While this could negatively impact early blooming flowering species, or pollinators that specialize on a few species (Kudo et al. 2004, Lambert et al. 2010b, Kudo and Ida 2013), some species that flower later in the season might experience increased visitation or visits from more effective pollinators (Rafferty and Ives 2011), which could positively impact reproduction. However, studies that manipulate abiotic conditions on plants and pollinators simultaneously are needed to fully understand the fate of plant-pollinator mutualisms under climate change. In a similar vein, we also observed a significant difference in network metrics between years. We found lower specialization, higher relative nestedness and higher connectance in 2015 for all treatments than compared to 2016. This is consistent with several studies that found high variation from year to year in plant-pollinator networks (Alarcon et al. 2008, Chacoff et al. 2018,

Cirtwill et al. 2018). Thus, the differences between years could be contributed to high interannual variation, depending on snowmelt timing.

There are several caveats to our study that should be addressed. First, we only manipulated the flowering communities and not the pollinator communities. While this allowed us to examine a scenario in which the plants responded stronger to changes in abiotic conditions than pollinators, future studies are needed that manipulate only pollinators or pollinator and plants simultaneously to fully understand how networks might respond to disruption in mutualistic partnerships. Second, the plots were located within non-manipulated flowering communities and so while plots will provide insight into what could happen with earlier snowmelt and frost exposure, they are still embedded in a scenario in which pollinators have choices of plants not in those treatments. Thus, long-term network studies that harness interannual variation in snowmelt timing and frost exposure may yield insights that cannot be gleaned from smaller scale plots. Third, we only measured visitation rates and did not pair our study with a pollen-transport study to determine the extent to which the insects visiting the flowers were providing pollination services. Previous studies that have incorporated either pollen-transport networks or networks that measure pollinator effectiveness with visitation networks have found strong differences among the networks (King et al. 2013, Ballantyne et al. 2015). Thus, future studies that examine the effects of climate change on plant-pollinator networks should incorporate a component that measures pollination services since the environment under which a plant grows can alter floral rewards (Stang et al. 2006, Mu et al. 2015).

In conclusion, despite detecting differences in community composition and proportion flowers visited per pollinator group in response to snowmelt timing and frost exposure, we did

not find any difference in network structure. Thus, it is likely that the high generalization results in no response in the networks to environmental variation. Therefore, the robustness of networks in this region indicate that pollination mutualisms might remain stable despite climate induced changes to the environment.

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Table 3-1: The number of days in which the plants bloomed earlier, $\pm$ the standard deviation, in the snow removal treatment compared with the natural snowmelt treatment for seven species that were well represented in plots across both years. Bold text indicates that the plants bloomed significantly earlier in the snow removal treatment than the natural snow treatment at $p < 0.05$. For *Erigeron speciosus*, we only had measurements for the date of first bloom and peak bloom.

Flowering species	First bloom (days)	Peak bloom (days)	Last bloom (days)
<i>Androsace septentrionalis</i>	13.76 $\pm$ 1.96	6.13 $\pm$ 1.51	0.04 $\pm$ 3.39
<i>Collomia linearis</i>	2.95 $\pm$.96	2.38 $\pm$ 1.13	0.63 $\pm$ 1.00
<i>Erigeron speciosus</i>	5.04 $\pm$ 1.46	0.02 $\pm$ 0.30	--
<i>Lathyrus lanszwertii</i>	2.62 $\pm$ 1.15	3.02 $\pm$ 1.33	5.16 $\pm$ 2.30
<i>Taraxacum officinale</i>	8.74 $\pm$ 1.13	6.28 $\pm$ 0.74	2.52 $\pm$ 1.46
<i>Vicia americana</i>	0.45 $\pm$ 1.41	0.05 $\pm$ 1.49	0.35 $\pm$ 1.07
<i>Viola praemorsa</i>	7.12 $\pm$ 1.05	7.29 $\pm$ 0.64	5.7 $\pm$ 0.74

Table 3-2: Comparison of the observed vs. null models for nestedness, connectance, and specialization for the networks comprised of the 5 earliest blooming species: *Claytonia lanceolata*, *Mertensia fusiformis*, *Delphinium nuttallianum*, *Androsace septentrionalis*, *Ranunculus inamoenus*, and *Viola praemorsa*. A significant p-value indicates that the value produced from the null model was significantly different from the observed value.

Treatment	Nestedness	Null nestedness	P-value
2015			
Removal_Frost	28.91	46.93	0.001
Removal_NoFrost	21.7	43.11	0.001
Natural_Frost	18.77	41.17	<0.0001
Natural_NoFrost	28.91	46.93	0.001
2016			
Removal_Frost	13.36	27.74	<0.0001
Removal_NoFrost	15.61	23.20	0.03
Natural_Frost	17.32	26.69	0.04
Natural_NoFrost	13.01	26.07	.003
	Connectance	Null connectance	P-value
2015			
Removal_Frost	0.26	0.14	<0.0001
Removal_NoFrost	0.20	0.16	<0.0001
Natural_Frost	0.22	0.11	<0.0001
Natural_NoFrost	0.24	0.15	<0.0001
2016			
Removal_Frost	0.10	0.18	<0.0001
Removal_NoFrost	0.11	0.20	<0.0001
Natural_Frost	0.13	0.25	<0.0001
Natural_NoFrost	0.11	0.20	<0.0001
	Specialization	Null specialization	P-value
2015			
Removal_Frost	0.62	0.19	0.004
Removal_NoFrost	0.46	0.22	0.003
Natural_Frost	0.64	0.21	<0.0001
Natural_NoFrost	0.62	0.19	<0.0001
2016			
Removal_Frost	0.58	0.26	0.003
Removal_NoFrost	0.67	0.39	0.005
Natural_Frost	0.69	0.39	0.01
Natural_NoFrost	0.74	0.19	<0.0001

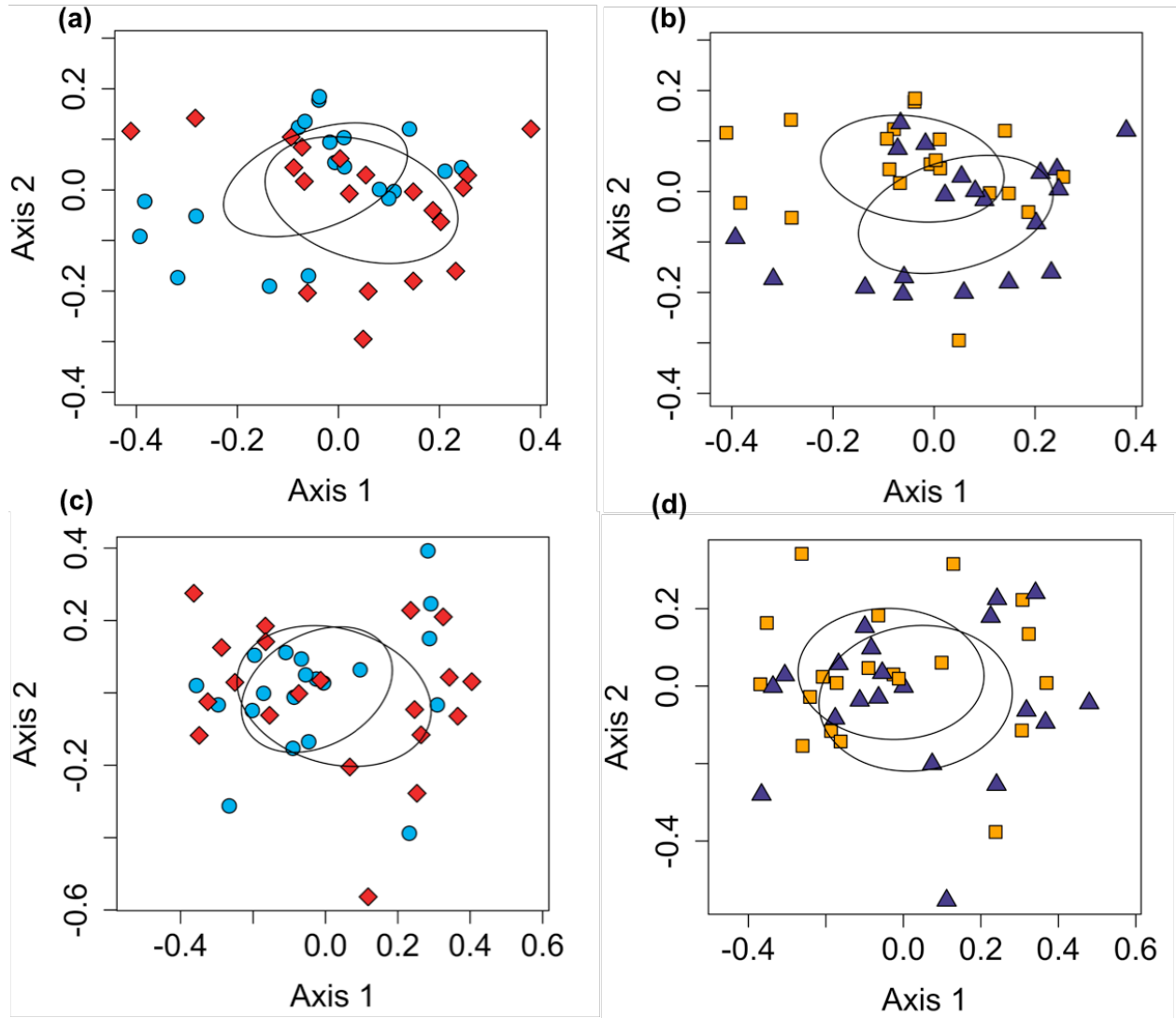


Figure 3-1: NMDS graphs of the flowering community in 2015 (top) and 2016 (bottom). Graphs a and b show the community composition between the snow removal (red diamonds) and the natural snowmelt (blue circles) treatments, and graphs b and d show the community composition between the frost (yellow squares) and no-frost (purple triangles) treatments.

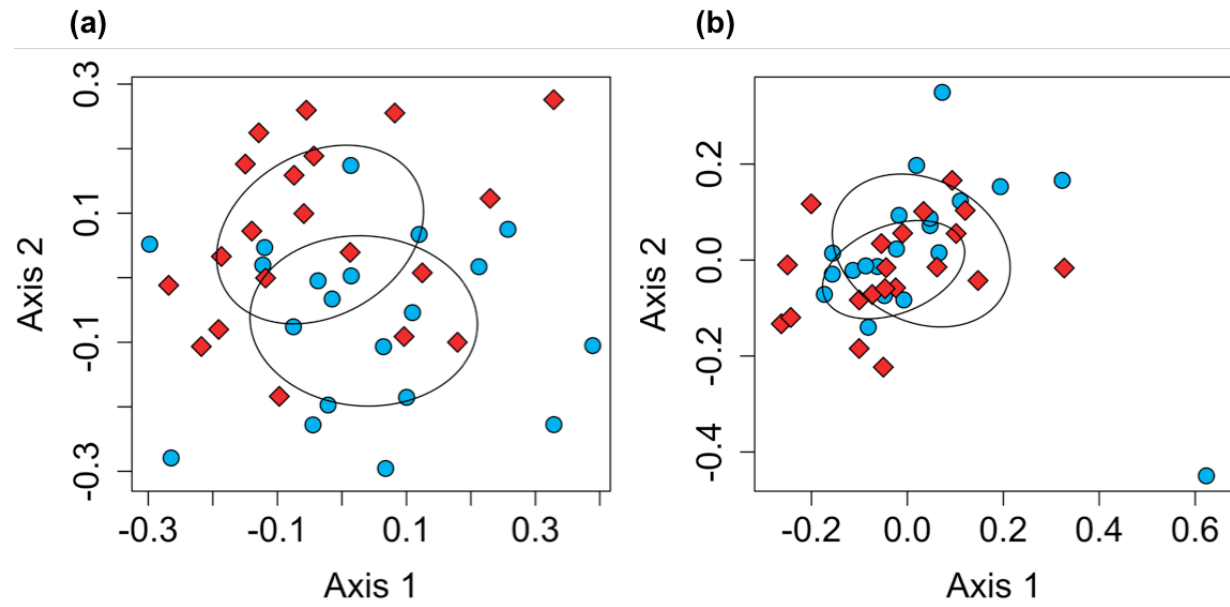


Figure 3-2: NMDS graphs of the pollinator community in a) 2015 and b) 2016 between the snow removal (red diamonds) and the natural snowmelt (blue circles) treatments.

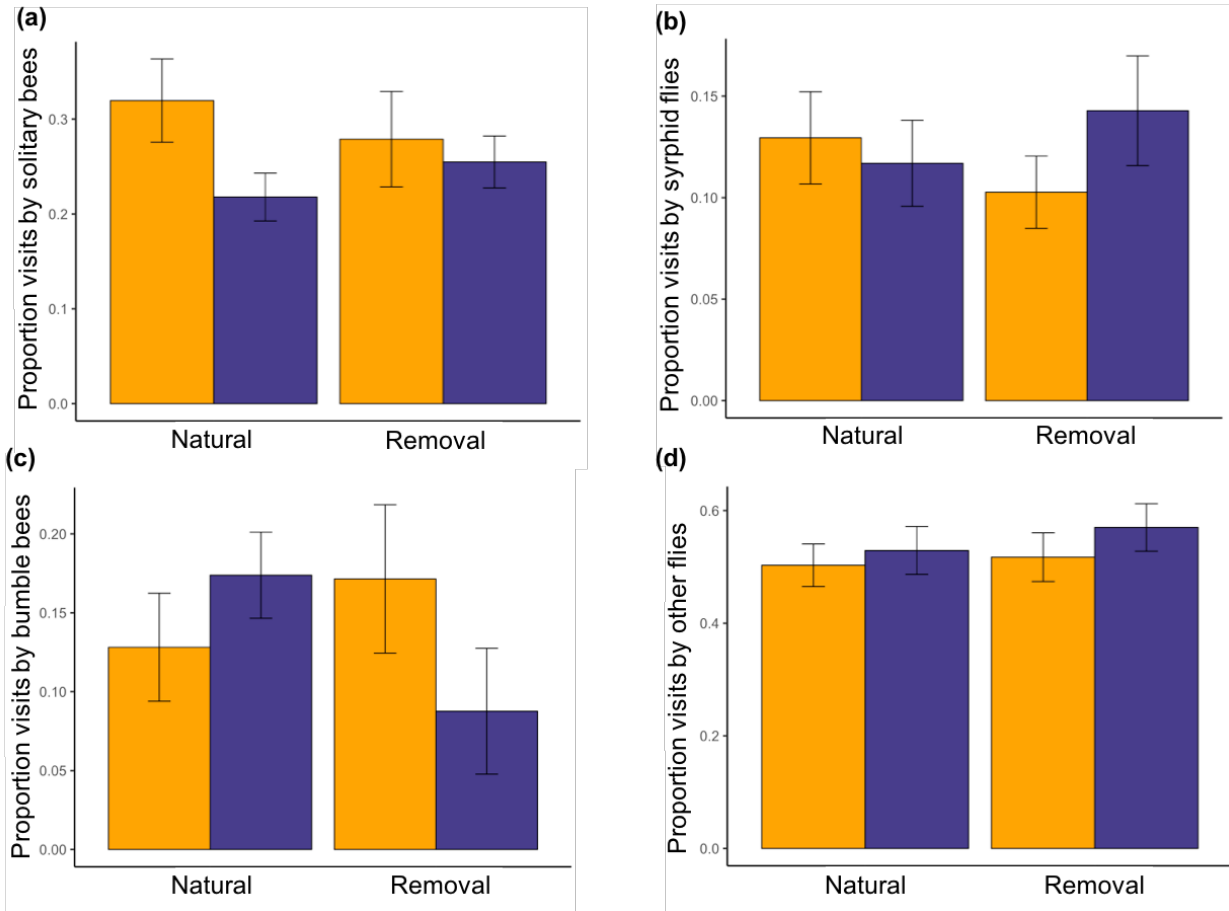


Figure 3-3: Proportion of flowers visited by the main pollinator groups: (a) solitary bees, (b) syrphid flies, (c) bumble bees, and (d) other flies between the natural snowmelt and snow removal treatments. The orange bars denote the frost treatments and the purple bars denote the no frost treatment. Solitary bees visited significantly higher proportion of flowers in the frost treatment and syrphid flies visited significantly higher proportion of flowers in the no-frost treatment ($p < 0.05$). There was no difference in treatment for the proportion of flowers visited by other flies ($p > 0.05$). Bars are means $\pm$ standard error.

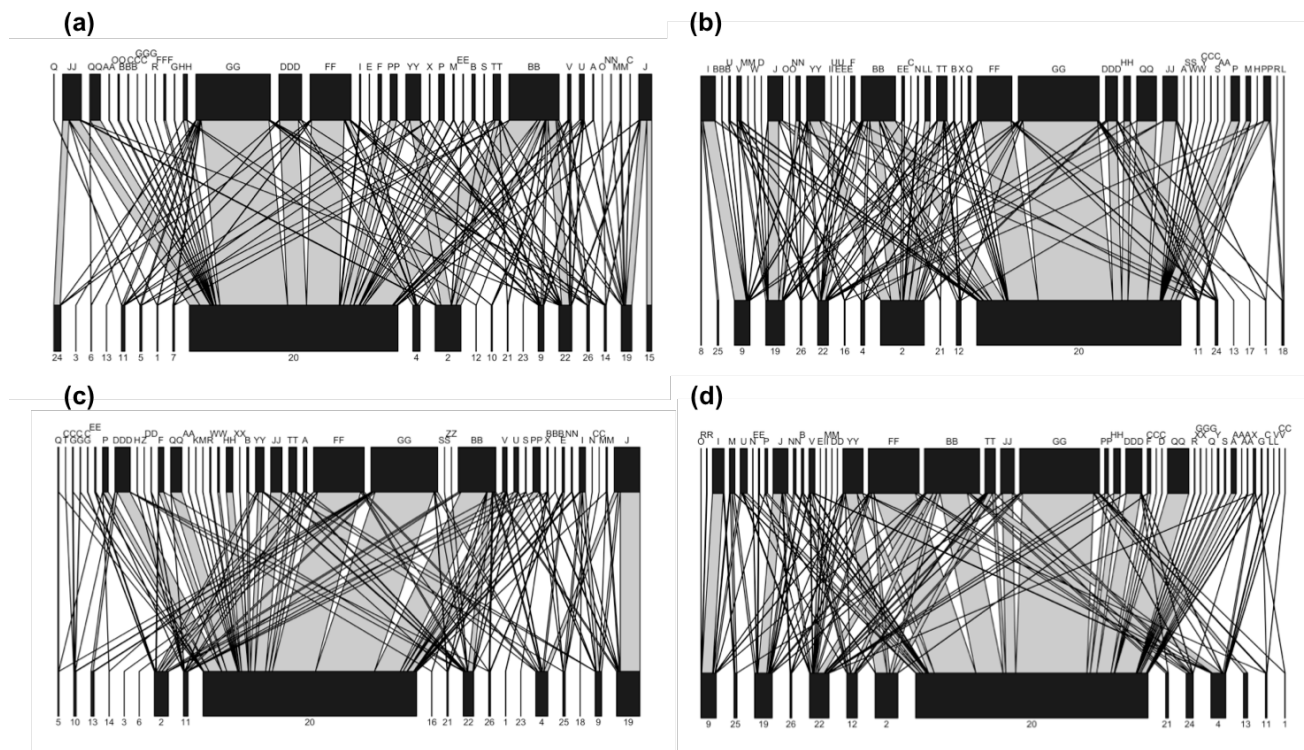


Figure 3-4: Combined networks for two years of observations for a) snow removal and no-frost treatment, b) natural snowmelt and no-frost treatment, c) snow removal and frost treatment, and d) natural snowmelt and frost treatment. The bottom of the networks represent the plant species (indicated as numbers), and the top of the network are pollinator morphogroups (represented by letters). The strength of the interaction is represented by the line width, where the thicker lines denote higher interactions. See appendix 2 for the plant species and pollinator morphogroup list.

CHAPTER 4: Life history traits in wild bees predict responses to climate change

Gabriella L. Pardee^{1,2*}, Sean R. Griffin³, Michael Stemkovski^{1,2}, Tina Harrison⁴, David W. Inouye^{2,5}, and Rebecca E. Irwin^{1,2}

¹Department of Applied Ecology, North Carolina State University, Raleigh, NC 27607 USA

²Rocky Mountain Biological Laboratory, Gothic, CO 81224 USA

³Department of Integrative Biology, Michigan State University, East Lansing, MI 48824 USA

⁴Department of Entomology, University of California Davis, Davis, CA 95616 USA

⁵Department of Biology, University of Maryland, College Park, MD 207442 USA

*Corresponding author email: gabriellapardee@gmail.com

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ABSTRACT

Bees are important contributors to pollination services and ecosystem functioning, yet are declining worldwide. Climate change is one likely driver of bee decline, but we do not fully understand its effects due to the lack of long-term studies that track bee responses to changes in abiotic conditions over time. Moreover, because bees are highly variable in their life history traits, examining bee responses across this life history variation may allow us to predict which bees may be most susceptible to a changing climate. We monitored the abundance of 96 bee species for 8 consecutive years in a subalpine region of the Rocky Mountains and then used fourth corner analysis to examine how bees with varying life history traits and overall communities responded to variation in snowpack, temperature, and precipitation. We described

bee life history based on their nesting behavior, body size, phenology, lecticity, and sociality. We found that body size, sociality, and nesting habits (renters vs excavators) were strongly associated with temperature and precipitation. Specifically, increased summer temperatures and increased precipitation from the previous year had negative effects on abundance for large bees and eusocial bees, but positive effects on abundance for small bees, solitary bees, and excavators. Further, we found shifts in community composition under years with the highest temperatures and lowest precipitation from the previous year. Taken together, our results suggest that climate change may reshape pollinator communities, with warmer, drier years favoring smaller-bodied and solitary species over larger-bodied and eusocial species.

INTRODUCTION

With the Earth warming at unprecedented rates, we are seeing worldwide changes in species distributions, abundance, and richness (1–3). However, these responses are highly variable among species within taxa, where some species are performing better under climate change while other species are in decline (4). Thus, it is difficult to make general predictions about how communities will be affected under a changing climate. However, recent studies suggest that life history traits that differ between increasing and decreasing species might better predict how biodiversity is impacted by climate change. This trait-based approach allows us to look for consistent responses within groups that share the same life history traits (5–7). Further, as responses to abiotic factors vary among species with different life history traits respond differently to abiotic factors, understanding which traits are strongly affected by climate change can allow greater insight into how ecosystems might change with loss of important contributors to ecosystem function, such as pollinators (8,9).

Bees are highly diverse in their life history traits, such as sociality, dietary breadth, body size, and nesting behavior, and a growing number of studies have begun using these traits to predict bee responses to environmental change (10–14). As life history traits relate to foraging period, flight distance, and diet breadth, differences in bee communities through life history traits could lead to changes in flower preference, foraging behavior, and pollination efficiency (15). Changes in pollination services can have cascading effects on plant-pollinator interactions, plant reproductive success, and even plant communities (16–18). While many studies have examined the effects of climate change on pollination services, most have done so from either the plant's perspective (19, 20) or by examining plant-pollinator interactions (21–23). The published studies that directly examine the effects of increased temperatures from the bee's perspective primarily

focus on eusocial bumble bees (24, 18, 25), and few studies exist about how the remaining ~20,000 species of bees, the majority of which are solitary, are responding to a changing climate (26, 27). This lack of data is startling given that the abiotic environment plays a pivotal role on bee physiology and foraging activity (28). Since increased functional diversity helps maximize pollination services and acts as a buffer against human disturbance (17), it is important to explore which species are most vulnerable to climate change and which species might benefit.

To better understand the effects of climate on bee communities and to assess the degree to which a trait-based approach can predict the response of bees to changing abiotic conditions, we conducted an 8-year study in the subalpine region of the Rocky Mountains, USA. We collected bees every two weeks throughout the flowering season to first ask whether bee abundance responded differently to changes in climate based on seven life history traits. Second, we asked how changes in the abiotic environment affected bee community composition in years with differing temperatures, precipitation, and snowpack. One of the most pressing issues with climate change is assessing how it will affect ecosystem services providers, such as bees. Our results suggest that climate change may reshape bee community composition based on life-history traits, with potential ramifications for the pollination services these bees provide.

METHODS

Study system and site selection

This study was conducted in meadows surrounding the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (elevation 2950 m). The RMBL is located in a sub-alpine region and hosts over 139 species of bees and at least 120 flowering species, many of which rely on bees for successful reproduction (29, 30). This area is particularly vulnerable to

climate change as spring temperatures have increased by about 1.6°C over the last 40 years (31), and the snow is melting out at a rate of 3.5 days earlier per decade (30). Despite the long-term trends of increased temperature and earlier snowmelt, during the 8 years of data collection for this study (2009-2016), the spring and summer weather patterns were highly variable, with some seasons experiencing early snowmelt and dry summers and other years experiencing late snowmelt and heavy precipitation (Table S1). This interannual variation allowed us to examine how bees respond to changes in weather patterns. Previous research on flowering communities in montane regions has shown that phenology and abundance of flowers show species-specific responses to increased temperature and early snowmelt, resulting in a reshaping of the flowering community (30, 32, 33), but we do not know if and how bees will respond.

We used nine sampling sites from which to collect bees. The nine sites were grouped into three blocks, each block containing three sites (Table S2). Sites within each block were between 200 m and 1 km apart, and blocks were separated from each other by a distance of at least 1 km. Each block consisted of three different habitat types: dry meadow, *Salix* spp.-dominated wet meadow, and *Veratrum tenuipetalum*-dominated wet meadow, representing three dominant habitat types in the region (29, 34).

Bee dataset

Sites were sampled for bees every two weeks throughout the flowering season using the methods outlined in Gezon et al. (2015). Briefly, for each sampling period we placed bowls filled with soapy water in alternating colors of florescent blue, florescent yellow, and white every 3 m along two fixed 30 m transects between approx. 0900 and 1700 on clear, sunny days. We placed all of the specimens into vials with 70% ethanol to later dry, pin and label. As the bee bowls

usually catch small to medium sized bees (29), we used a non-lethal method of collecting larger-bodied bumble bees by netting for 2 hours at each site (1 hour each in the morning and afternoon each day that bowls were out at a site). The bumble bees were identified on the wing using identification guides (35), marked with non-toxic paint to avoid re-capturing, and then released back into the site. We calculated yearly bee abundance in each year by taking the weighted arithmetic mean of bees caught per hour of sampling (i.e., total bees of a species/total hours of sampling). Because of inconsistency in small bee netting ability, we only considered bees (except for *Bombus* spp.) which were caught in pan traps. For *Bombus* species, we only considered bees caught by netting because pan traps are poor at capturing this genus (36). For pinned bees, we identified them to species using taxonomic keys, our collection, and taxonomists (i.e., *Lasioglossum*: J. Gibbs; *Melissodes*: K Wright; *Megachile*: J. Neff; *Anthophora*: M. Orr; Remaining genera: J. Ascher). In exception to this, we treated individuals of the genera *Sphecodes*, *Nomada*, and *Triepeolus* as belonging to one species per genera because there are no recent revisions for these genera. Due to difficulties in identification, we excluded species of the genera *Andrena*, *Hylaeus*, and *Osmia*. All specimens are housed at North Carolina State University in Raleigh, NC and the RMBL in Gothic, CO.

Bee species traits

We described the life-history of each bee species we sampled based on seven traits: lecticity, nesting (location, substrate, and habit), phenology, sociality, and body size. These traits are good descriptions of a bee's life history and have low pairwise correlations (37). For lecticity, we determined whether a species was polylectic or oligolectic based on whether bees collected pollen from flowers in multiple plant families or from flowers in just one family,

respectively (38). We described nesting based on three factors: nest location (above or below ground), nest substrate (wood, stem, soil, cavity, rock) and nesting habit (bees that seek out cavities to nest in were classified as “renters” while bees that create nesting holes in wood, stems, or soil were classified as “excavators”). We estimated phenology as a continuous variable by determining the average number of days past date of bare ground that an individual of each species was first recorded in pan traps for non-bumble bees and collected with nets for bumble bee queens across all years. Sociality included three traits: eusocial, solitary, and parasitic. The parasitic bees were included in the analysis for sociality and nesting behavior of their host (29), but were excluded from the lecty analysis as they do not collect pollen (39). Although some sweat bees can be semi-social at lower elevations (40), our sampling sites were high enough in elevation that these bees likely do not exhibit social traits (41, 42); thus, we categorized all sweat bees as solitary. We estimated bee body size by measuring the intertegular distance, which is the distance across a bee’s thorax between the base of the wings (43) for up to 10 specimens per species using the software program ImageJ (National Institutes of Health, USA).

Environmental variables

We compiled data on 40 environmental variables to describe the climate at the RMBL; environmental variables were based on knowledge of the site as well as the literature (30, 34, 44, 45). These variables included growing degree days, mean annual temperature, mean snowpack in April, mean annual precipitation, and mean summer temperature. For mean summer temperature and precipitation values, we defined summer months as May-September. We included the current year's and the previous year's estimates for each variable as some species could show a lag effect to changes in the environment. As our sites were not paired with weather stations and

were located on varied terrain in terms of elevation, slope, and aspect, we used interpolation models to estimate the local climate variables. To do this, we constructed linear mixed effects models using historical data from 29 weather stations in Gunnison and Pitkin Counties, Colorado. These 29 weather stations were the closest weather stations to our field sites. Models to predict the variables of interest incorporated slope, aspect, and elevation as fixed effects, and year and weather station as random effects. For model selection, we compared AICc scores of variations of the models in which the effects were iteratively removed. For all models besides growing degree days and mean annual temperature (where including aspect did not improve model fit), we found that including all available effects produced the best fits. We trained the models on data from 1978 to 2016, and predicted variables for the years of this study. From this analysis, we selected five variables that were not strongly correlated with one another ($r < 0.40$ in all cases) and have been shown to affect bee foraging behavior, physiology, and colony buildup in other studies (16, 26). These 5 variables were: mean April snowpack, current and previous year's mean summer temperature, and current and previous year's mean annual precipitation. All analyses were done in R version 3.4.0 here and below (R Core Team 2016).

Statistical analyses

How does climate variation affect bee abundance as a function of life history traits?

We used fourth-corner analysis in the R package *ade4* (Dray and Dufour 2007) to test for differences in trait compositions across environmental variables. The fourth-corner analysis calculates the correlation between species' traits and the average environmental conditions of blocks occupied by each species, weighting by species' abundances (47). Then, we used a null model to investigate how the environment affected life history traits by testing the significance of

the trait-by-environment correlations through randomization across block per year. Because differences in bee responses could be attributed to similarities in phylogeny, we ran a second null model permutation test in which we constrained species randomizations so that trait values were more likely to be exchanged between more closely related species using the methods outlined in Harrison et al. (2017). We used 9,999 randomizations for each of our permutations, and only interpreted correlations between traits and environment if they were significant in both null model analyses.

How do changes in the abiotic environment affect bee community composition?

To further explain how bee communities shift with varying environmental variables, we used the multivariate methods in the R package ‘vegan’ (48) to examine how the bee community responds to changes in environmental conditions. We used the calculated dissimilarities between all three blocks per year using the abundance-based Morisita-Horn index, which is highly sensitive to dominant species and not as affected by uncommon species or singletons (49). We first used non-metric multidimensional scaling plots (NMDS) to visually compare species similarity of sampled blocks. We plotted each community per block per year and colored the points based on four categories to create a gradient from years with low precipitation, temperature and April snowpack to years with the highest precipitation, temperature, and snowfall to see whether years with similar environmental conditions are more closely clustered than years with different environmental conditions. Next, we tested the significance of visually distinguishable groups from the NMDS plots with a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function with type III SS and holding block constant

to test for differences in community composition against each environmental variable (50). We used 999 permutations to assess significance.

RESULTS

How does climate variation affect bee abundance as a function of life history traits?

Overall, we collected 19,999 individuals comprised of five families, 28 genera, and 96 species. We were able to collect life history traits for all 96 species (Appendix 1). Our fourth corner analysis showed that three of our seven traits were significantly correlated with the environment: body size, sociality, nesting habit (Fig. 1). We did not find any effect of the environment on phenology, lecticity, nesting location (above or below ground), or nesting substrate (Table S2). Further, the two environmental variables that had significant effects on life history traits were the current year's temperature and the previous year's precipitation.

Body size response to environmental variables. We found that the small bees (ITD < 2.0 mm; 51 species; Fig. 1) responded positively to increased temperatures ($R^2 = 0.12$, $p = 0.04$; Fig. 2a), while the medium bees (ITD 2.01-3.00 mm; 20 species) did not respond to increased temperatures ($R^2 = -0.01$, $p = 0.44$; Fig. 2a), and large bees (ITD >3.01 mm; 25 species) negatively responded to increased temperature ($R^2 = -0.11$, $p = 0.06$; Fig. 2a). Further, we found that small bees increased with increased precipitation from the previous year ($R^2 = 0.32$, $p = 0.001$; Fig 2b), but the medium and large bees exhibited a non-significant decline with increased precipitation from the previous year ($R^2 = -0.05$, $p = 0.14$, and $R^2 = -0.01$, $p = 0.527$, respectively; Fig. 2b).

Sociality response to environmental variables. We found that sociality responded to current summer temperature ($Pseudo-F = 15.54$, $p = 0.02$; Fig. 1), and the previous year's annual

precipitation ($Pseudo-F = 20.48$, $p = 0.01$; Fig. 1). When we looked at response trends, we found that the eusocial bees (14 species) responded negatively to increasing summer temperature ($R^2 = -0.19$, $p = 0.03$) and to the previous year's annual precipitation ($R^2 = 0.18$, $p = 0.008$), while solitary bees (70 species) responded positively to increasing summer temperature ($R^2 = 0.18$, $p = 0.05$) and the previous year's precipitation ($R^2 = 0.20$, $p = 0.01$). The parasitic bees (12 species) did not show any response to environmental conditions ($R^2 < 0.03$, $p > 0.15$).

Nesting habit response to environmental variables. We found that bees respond differently to mean summer temperature and previous year's precipitation based on whether they rent out cavities to nest in or excavate tunnels into soil, wood, or stems (temperature: $Pseudo-F = 26.82$, $p = 0.038$; previous precipitation: $Pseudo-F = 37.97$, $p = 0.009$). The bees that excavate nests (54 species) positively responded to temperature ($R^2 = 0.18$, $p = 0.03$; Fig 1), while the renters (30 species) showed no response ($R^2 = 0.086$, $p = 0.76$). The same pattern was shown for precipitation where the excavators responded positively to increase in previous year's precipitation ($R^2 = 0.19$, $p = 0.01$), where the renters showed no response ($R^2 = -0.01$, $p = 0.29$).

How do changes in the abiotic environment affect bee community composition?

We found community composition shifts due to mean summer temperature ($F_{1,64} = 6.20$, $R^2 = 0.057$, $p = 0.006$; Fig. 3a) and the previous year's precipitation ($F_{1,64} = 12.70$, $R^2 = 0.121$, $p = 0.001$; Fig. 3b). In the NMDS plot, the years with the highest summer temperature (13.0-13.6 °C) were clustered away from the years with low (11.06-11.56 °C), medium (11.70-12.25 °C), and high summer temperatures (12.34-12.86 °C). Further, the NMDS plot for the previous year's precipitation showed that the years with the lowest precipitation (< 610.00 cm) were clustered away from the years with medium (610-706 cm), high (713-812 cm), and highest precipitation

(819-919 cm). We also found a non-significant trend in differences in bee composition due to April snowpack ($F_{1,64} = 6.20$, $R^2 = 0.022$, $p = 0.09$), but did not find any effect of the previous year's mean summer temperature or the current year's annual precipitation on bee communities ($F < 0.805$, $p > 0.139$, in both cases).

DISCUSSION

With growing concerns over bee declines under climate change, it is imperative that we monitor responses to variation in temperature and precipitation. However, the lack of long-term studies on entire assemblages of bees makes it difficult to fully examine the extent of bee pollinator losses under shifting environmental conditions. Specifically, we are lacking information on whether all bees will respond in the same way or whether we will find some winners and some losers with a changing climate. Sampling over 8 consecutive years allowed us to evaluate the effects of variable temperature and precipitation on bee abundance as a function of life history traits and community composition. Our results show that the fourth corner analysis is a powerful tool because it allows one to determine whether a given environment will select for species with certain traits. The fourth corner analysis combined with our community composition analysis provided insight into how bee with particular life history traits might decline under increased temperature and decreased precipitation, which might increase, and how climate change will reshape bee communities.

The trait-specific responses allowed us to predict which species might be winners and losers under increased temperatures. For example, given that bumble bees make up the eusocial bee category and are the biggest bees in our study system, we might see declines in this group with increased temperature. This is consistent with several other studies that have found bumble

bee declines with increased temperatures (16, 50), possibly due to having lower thermal tolerance to increased temperature than smaller sized bees (51). Larger bees also tend to forage earlier in the day when temperatures are cooler (16); thus, elevated summer temperatures can reduce foraging activity for these bees. Further, while we saw overall positive associations between solitary bees and temperature, some of the larger solitary bees, such as *Megachile*, *Eucera*, and *Anthophora* could decline with increasing temperature. In contrast, our analyses suggest that small, solitary bees that excavate their own nests, such as *Lasioglossum*, *Colletes*, and *Agapostemon*, will increase under climate change. Indeed, many species within these genera were shown to be stable or increasing in abundance in a study that examined bee responses to climate change over a 140-year period in the Northeastern U.S. (5).

We also found both trait responses and overall community shifts with previous year's precipitation. The groups that declined with increased precipitation were eusocial bees, while the small and solitary bees increased with increased precipitation in the previous year. Further, large and medium sized bees remained stable under increased precipitation from the previous year. The community composition for the year following a year with low precipitation was clustered away from years with medium, high, and highest precipitation suggesting that drought has the potential to reshape bee communities. This result may have been indirectly driven by floral resource availability, as previous studies in the same region have found that floral abundance is highly correlated with precipitation, which indirectly affects bee abundance (34, 52). A reduction in flowers during years with low precipitation could result in bee declines for the following year due to reduced brood production, but these results only hold for eusocial bumble bees. These results are in contrast with a previous study that did not find a lag effect in response to precipitation for three commonly occurring bumble bee species in our study system (34). These

differences could be due to how precipitation rates were calculated between their study and ours. We only included rainfall for our precipitation measurements while their measurements on precipitation included both rainfall and snowfall. These differences could also mean that lag effects from decreased precipitation are caused by other changes in biotic conditions, such as competition, that was beyond the scope of our study (53).

We did not find any correlation between nesting behavior, phenology, or lecticity and environmental variables. Our results for phenology and lecty are unexpected given that these traits have been shown to be sensitive to climate change both for bees (54) as well as other invertebrates and plants (55–57) Our findings could be attributed to our sampling methods, as we only sampled every two weeks and therefore could not obtain complete phenological measures for our species. Further, pan traps have been shown to miss the rarer species and specialist pollinators (14); thus, we might not have an accurate measure of the species abundance and richness for this group. Nesting behavior has been shown to be influenced by amount of impervious surface, proximity to forests, and floral abundance (37, 58, 59); thus, other site characteristics could have a greater influence on nesting behavior than the environmental variables we considered in this study.

Based on our findings we will likely see shifts in plant-pollinator interactions and plant fitness due to changes in bee communities under climate change. For example, as body size is correlated with flight distance (60), if the larger bees decline under increased temperature then we could see a decline in long distance pollen transfer (61), which can reduce plant fitness and increase population genetic structure. Further, though we might see declines in bumble bees under increased temperatures, the smaller, solitary bees might be able to act as a buffer against pollination loss (13, 17). However, bumble bees have been shown to have higher pollination

efficiency than smaller bodied bees (62), thus loss of these pollinators could have strong effects not only on plant fitness but also patterns on natural selection in floral traits. Finally, since bees showed a lag effect in precipitation, consistently low precipitation over multiple years could have long-term effects on bee communities. As previous studies have shown that climate change is reshaping plant communities (32, 33), and our study shows that climate change is also reshaping pollinator communities, research is now needed that assesses how the webs of interactions will change over time and the consequences for ecosystem functioning in a changing climate.

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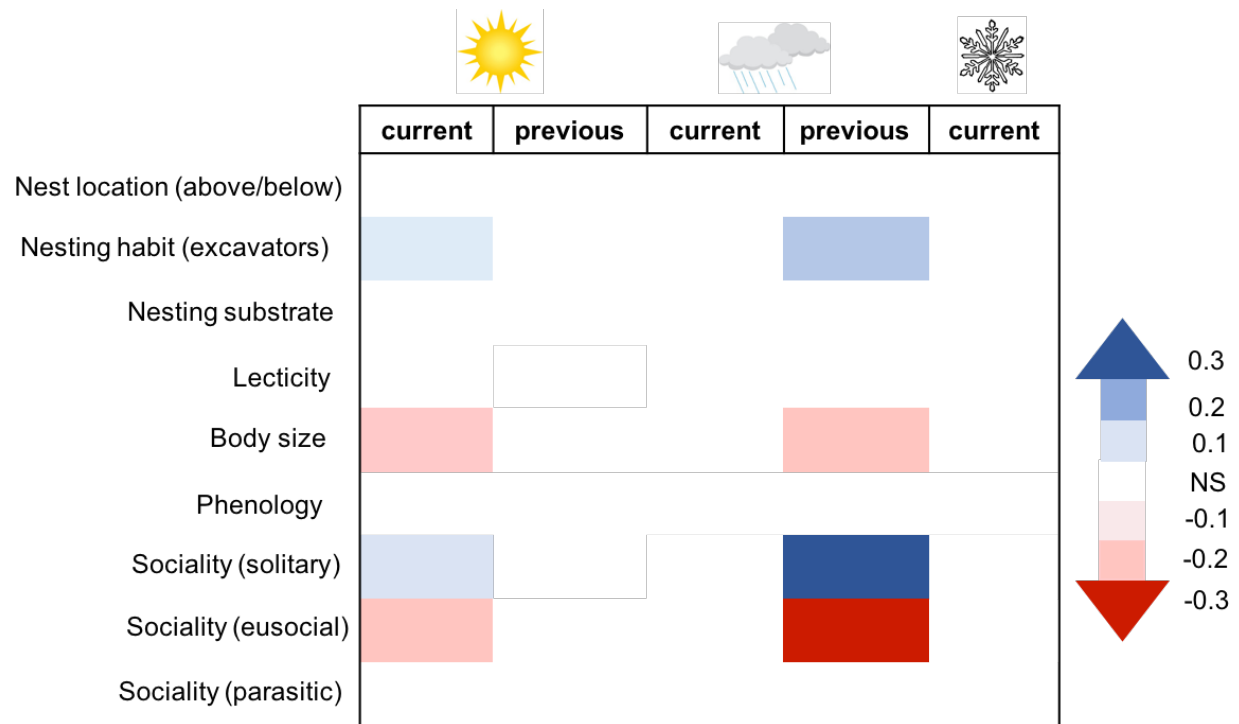


Figure 4-1: Effects of current and previous year's summer temperature and annual precipitation on bee abundance based on life history traits. Red denotes a negative effect, blue denotes a positive effect, and white denotes a non-significant effect. The darker colors represent a strong correlation while the lighter colors represent a weaker correlation.

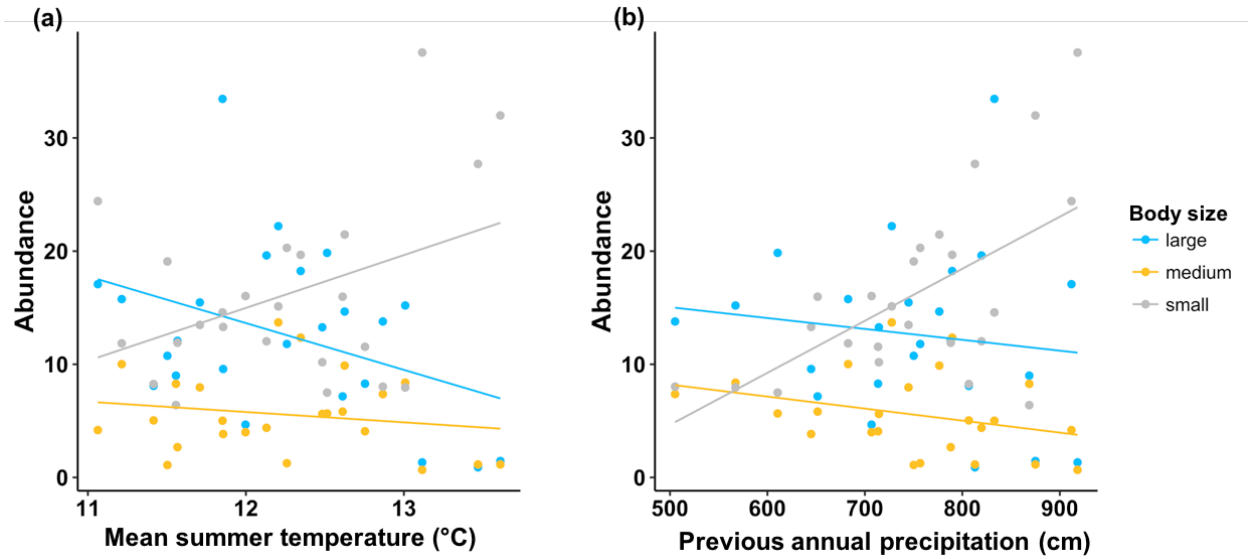


Figure 4-2: Effects of **a)** current year's mean summer temperature and **b)** previous year's annual precipitation on the abundance (bees per hour) of small bees (ITD < 2mm), medium bees (ITD 2.01-3.00 mm), and large bees (ITD > 3.01 mm). The small bees significantly increased with increased temperature from the current year ($p = 0.04$) and increased precipitation from the previous year ($p = 0.001$), while the large bees declined with increased temperature from the current year ($p = 0.06$). We did not find any effect of temperature and precipitation on medium size bees or precipitation on large bees ($p > 0.14$ for all cases).

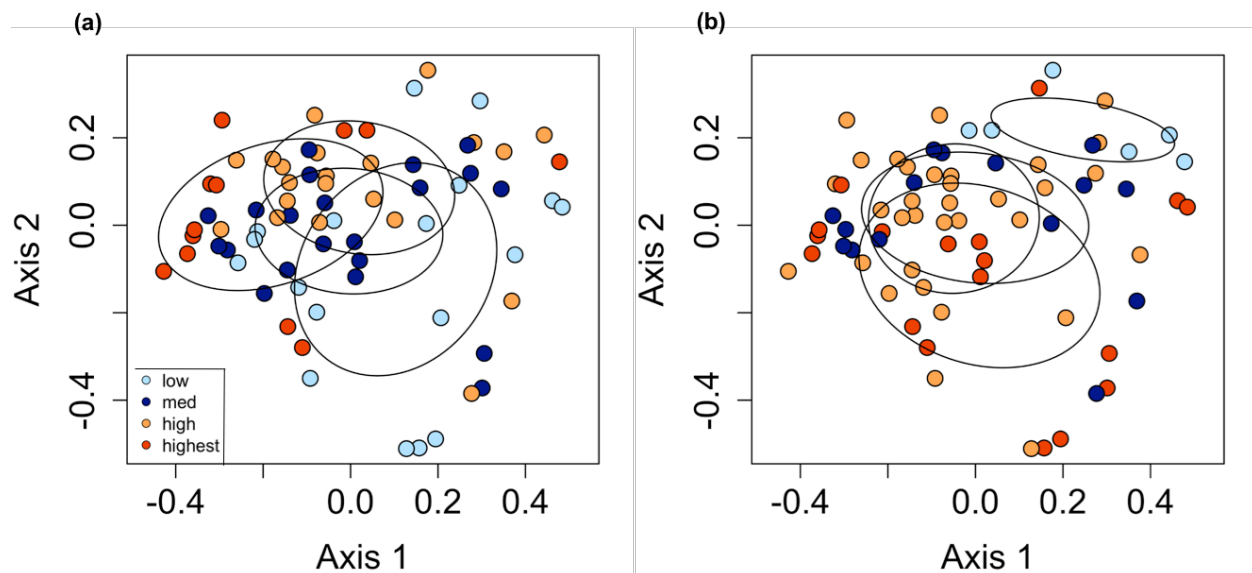


Figure 4-3: Nonmetric multidimensional scaling ordination plots for bee communities with ordinations based on Morisita-Horn index for **a)** current year's mean summer temperature, and **b)** the previous year's annual precipitation. Light blue and dark blue represent years with lower rainfall and temperatures and orange and red represent years with higher precipitation and rainfall, respectively.

APPENDICES

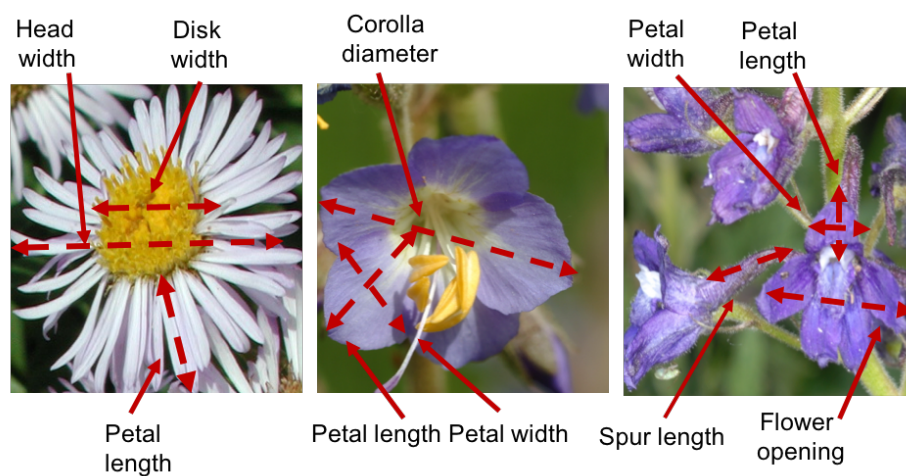


Figure S1: Descriptions of the plant measurements for *Erigeron speciosus* (left), *Polemonium foliosissimum* (center) and *Delphinium barbeyi* (right).

Table S2-1: The night time temperatures (°C) that plants were exposed to in the frost-treatment. The plants in the frost-treatment were exposed to 25 nights below freezing in 2015 and 18 nights below freezing in 2016.

Date	Year	Low temp.
29-Apr	2015	-3.4
30-Apr	2015	-3.6
1-May	2015	-1.8
2-May	2015	-1.8
3-May	2015	-1.5
7-May	2015	-1.7
8-May	2015	-2
9-May	2015	-5.5
10-May	2015	-3.9
11-May	2015	-5.3
12-May	2015	-3.7
13-May	2015	-0.4
14-May	2015	-1.5
15-May	2015	-3.4
16-May	2015	-3.8
17-May	2015	-1.6
18-May	2015	-2
19-May	2015	-0.5
20-May	2015	-1.6
21-May	2015	-2.3
22-May	2015	-0.1
23-May	2015	-1.4
24-May	2015	-4.1
25-May	2015	-1.2
26-May	2015	-0.3
13-May	2016	-3.2
14-May	2016	-3.1
15-May	2016	-0.9
16-May	2016	-1.9
17-May	2016	-2.3
18-May	2016	-1.1
19-May	2016	-0.2
20-May	2016	-0.4
23-May	2016	-3
24-May	2016	-3.9
25-May	2016	-2.2
26-May	2016	-3.7
27-May	2016	-1.9
28-May	2016	-3.6
29-May	2016	-2.6
30-May	2016	-1.4
31-May	2016	-2.3
1-Jun	2016	-1.1

Table S3-1: Plant species and pollinator morphogroup list for all species present in the two-year study at the Rocky Mountain Biological Laboratory, Gothic, CO, USA. Interaction degree indicates the number of interaction links a particular plant or pollinator had with other pollinators or plants across both years of the study. The number on network corresponds to the number (plant) of letter (pollinator) on the interaction networks from figure 4

Species	Family	Number on network	Interaction degree
Plant species			
<i>Achillea millefolium</i>	Asteraceae	1	15
<i>Androsace septentrionalis</i>	Primulaceae	2	649
<i>Boechera stricta</i>	Brassicaceae	3	2
<i>Claytonia lanceolata</i>	Montiaceae	4	237
<i>Collomia linearis</i>	Polominaceae	5	21
<i>Crepis intermedia</i>	Asteraceae	6	4
<i>Cymopterus lemmonii</i>	Apiaceae	7	10
<i>Delphinium barbeyi</i>	Ranunculaceae	8	3
<i>Delphinium nuttallianum</i>	Ranunculaceae	9	256
<i>Erigeron elatior</i>	Asteraceae	10	25
<i>Erigeron speciosus</i>	Asteraceae	11	84
<i>Fragaria virginiana</i>	Rosaceae	12	81
<i>Galium boreale</i>	Rubiaceae	13	47
<i>Helianthella quinquinervis</i>	Asteraceae	14	15
<i>Hydrophyllum capitatum</i>	Hydrophyllaceae	15	31
<i>Hymenoxys hoopesii</i>	Asteraceae	16	9
<i>Lathyrus lanszwertii</i>	Fabaceae	17	1
<i>Mertensia ciliata</i>	Boraginaceae	18	14
<i>Mertensia fusiformis</i>	Boraginaceae	19	451
<i>Potentilla pulcherrima</i>	Rosaceae	20	5366
<i>Ranunculus inamoenus</i>	Ranunculaceae	21	33
<i>Taraxacum officinale</i>	Asteraceae	22	325
<i>Tragopogon pratensis</i>	Asteraceae	23	1
<i>Valeriana capitata</i>	Valerianaceae	24	94
<i>Vicia americana</i>	Fabaceae	25	42
<i>Viola praemorsa</i>	Violaceae	26	50
Pollinator groups			
<i>Andrena</i> sp1	Andrenidae	A	59
<i>Andrena</i> sp2	Andrenidae	B	55
<i>Andrena</i> sp3	Andrenidae	C	11
<i>Andrena</i> sp4	Andrenidae	D	3
<i>Andrena</i> sp5	Andrenidae	E	21
Anthomyiidae group1	Anthomyiidae	F	110
Anthomyiidae group2	Anthomyiidae	G	8
Asilidae group1	Asilidae	H	3
<i>Bombus appositus</i>	Apidae	I	200

Table S3-1 (continued).

<i>Bombus bifarius</i>	Apidae	J	450
<i>Bombus californicus</i>	Apidae	K	2
<i>Bombus fervidus</i>	Apidae	L	2
<i>Bombus flavifrons</i>	Apidae	M	66
<i>Bombus insularis</i>	Apidae	N	10
<i>Bombus rufocinctus</i>	Apidae	O	2
<i>Bombus sylvicola</i>	Apidae	P	145
Bombyliidae group1	Bombyliidae	Q	25
<i>Chrysotoxum</i> sp1	Syrphidae	R	15
<i>Eupeodes</i> sp1	Syrphidae	S	22
<i>Euphydras</i> sp	Syrphidae	T	1
<i>Halictus rubicundus</i>	Halictidae	U	113
<i>Halictus tri/vir</i>	Halictidae	V	112
<i>Hemaris</i> sp	Sphingidae	W	2
Hesperiidae group1	Hesperiidae	X	31
<i>Hoplitis fulgida flugida</i>	Megachilidae	Y	9
<i>Hylaeus</i> sp	Colletidae	Z	1
Ichneumonidae group1	Ichneumonidae	AA	10
<i>Lasioglossum</i> sp	Halictidae	BB	1085
Lycaenidae group1	Lycaenidae	CC	2
<i>Megachile</i> sp	Megachilidae	DD	5
<i>Melangyna</i> sp1	Syrphidae	EE	36
Muscidae group1	Muscidae	FF	1116
Muscidae group2	Muscidae	GG	1871
Muscidae group3	Muscidae	HH	150
Muscidae group4	Muscidae	II	4
<i>Myolepta</i> sp1	Syrphidae	JJ	360
<i>Nomada</i> sp	Apidae	KK	33
<i>Osmia</i> sp1	Megachilidae	LL	24
<i>Osmia</i> sp2	Megachilidae	MM	52
<i>Osmia</i> sp4	Megachilidae	NN	11
<i>Osmia</i> sp5	Megachilidae	OO	163
<i>Panurginus</i> sp	Andrenidae	PP	378
Papilionidae group1	Papilionidae	QQ	3
<i>Platycheirus</i> sp1	Syrphidae	RR	6
<i>Sphaerophoria</i> sp1	Syrphidae	SS	223
<i>Sphecodes</i> sp	Halictidae	TT	1
<i>Sphex</i> sp1	Sphecidae	UU	2
<i>Symphyta</i> group1	unknown	VV	16
Syrphidae group2	Syrphidae	WW	6
Syrphidae group3	Syrphidae	XX	368
Syrphidae group5	Syrphidae	YY	2
<i>Syrphus</i> sp1	Syrphidae	ZZ	12

Table S3-1 (continued).

Tabanidae group1	Tabanidae	AAA	13
Tachinidae group1	Tachinidae	BBB	415
Tephritidae group1	Tephritidae	CCC	1
Unknown fly group1	Unknown	DDD	12
<i>Volucella</i> sp	Syrphidae	EEE	8

Table S4-1: Average summer temperature, annual precipitation, and April snow depth for the three blocks over the last 8 years.

Environmental variable	2009	2010	2011	2012	2013	2014	2015	2016
Temperature (C)	11.34	12.42	12.54	13.39	12.79	11.78	12.13	11.49
Precipitation (cm)	770.12	707.26	868.68	561.00	700.58	783.32	738.55	309.92
April Snow depth (cm)	923.79	785.16	1178.25	218.02	726.23	972.39	433.43	767.37

Table S4-2: GPS locations of each of the 3 sites within each block. Sites within each block were separated by 100 m and each block was at least 2 m apart.

Block	GPS location
Block A	
<i>Site 1</i>	38°57'22.7"N 106°59'18.1"W
<i>Site 2</i>	38°57'19.8"N 106°59'18.5"W
<i>Site 3</i>	38°57'43.8"N 106°59'13.0"W
Block B	
<i>Site 1</i>	38°57'43.9"N 106°59'41.1"W
<i>Site 2</i>	38°57'50.3"N 106°59'42.6"W
<i>Site 3</i>	38°57'41.0"N 106°59'26.9"W
Block D	
<i>Site 1</i>	38°58'14.2"N 106°57'57.6"W
<i>Site 2</i>	38°58'13.3"N 106°57'56.7"W
<i>Site 3</i>	38°58'16.1"N 106°57'54.5"W

Table S4-3: Life history traits that were not correlated with our five environmental variables.

Life history trait	Environmental variable	Pseudo-F	P value
Nesting substrate	Current summer temp.	1.97	0.75
	Previous year's summer temp.	0.57	0.87
	Current precip.	0.73	0.60
	Previous year's precip.	0.63	0.86
	April snowpack	1.21	0.64
Nest location (above/below)	Current summer temp.	2.96	0.499
	Previous year's summer temp.	0.65	0.67
	Current precip.	0.71	0.47
	Previous year's precip.	0.34	0.78
	April snowpack	1.66	0.40
Lecty	Current summer temp.	4.58	0.48
	Previous year's summer temp.	3.55	0.38
	Current precip.	1.66	0.32
	Previous year's precip.	7.17	0.25
	April snowpack	1.84	0.28
Phenology	Current summer temp.	3.63	0.60
	Previous year's summer temp.	0.21	0.94
	Current precip.	0.06	0.93
	Previous year's precip.	0.84	0.85
	April snowpack	5.00	0.09

Table S4-4. Species, morphospecies, and life history trait designations used in analyses. Categorizations were based on information compiled from published literature (Mitchell 1960, Hurd Jr 1979, Cane et al. 2007, Michener 2007) and from a list of species traits compiled by L. Polonsio. Phenology was measured as the average number of days bees emerge after the date of bare ground. Nesting substrate includes nest parasites, which indicate the nesting substrate of the host. IT span refers to intertegular distance measured using imageJ for up to 10 females from each morphospecies, using only workers for *Bombus* species. The category “lecty” refers to pollen specialists (oligolectic) versus pollen generalist (polylectic). “Sociality” was broken into three categories: ‘eusocial’, ‘solitary’, and ‘parasitic’, with ‘eusocial’ containing only non-parasitic *Bombus* spp. Species that could potentially be eusocial based on their phylogenetic placement such as *Halictus* spp. and *Lasioglossum* (*Dialictus*) spp. were considered ‘solitary’ because eusociality is unlikely in high-elevation habitats with short growing seasons (Kocher et al. 2014). Parasitic bees were excluded from analyses of diet and nesting habit (excavators vs. renters).

Morphospecies	Phenology (days)	Nest location	Nesting substrate	Nesting habit	ITD (mm)	Lecty	Sociality
Andrenidae							
<i>Calliopsis coloradensis</i>	113	below	soil	Excavator	1.68	Oligolectic	Solitary
<i>Calliopsis teucarii</i>	72	below	soil	Excavator	1.41	Oligolectic	Solitary
<i>Panurginus cressoniellus</i>	46.25	below	soil	Excavator	1.3	Oligolectic	Solitary
<i>Panurginus ineptus</i>	37.5	below	soil	Excavator	1.35	Oligolectic	Solitary
<i>Pseudopanurgus bakeri</i>	57.25	below	soil	Excavator	1.01	Polylectic	Solitary
<i>Pseudopanurgus didirupa</i>	59.37	below	soil	Excavator	1.3	Oligolectic	Solitary
<i>Pseudopanurgus</i> sp.	77.33	below	soil	Excavator	1.155	Oligolectic	Solitary
Apidae							
<i>Anthophora terminalis</i>	53.33	above	wood	Excavator	3.17	Polylectic	Solitary
<i>Anthophora ursina</i>	20	below	soil	Excavator	3.6	Polylectic	Solitary
<i>Bombus appositus</i>	31.37	below	soil	Renter	3.48	Polylectic	Eusocial
<i>Bombus balteatus</i>	44	below	soil	Renter	2.89	Polylectic	Eusocial
<i>Bombus bifarius</i>	25.62	below	soil	Renter	3.48	Polylectic	Eusocial
<i>Bombus centralis</i>	52	below	soil	Renter	3.45	Polylectic	Eusocial
<i>Bombus fervidus</i>	29.71	below	soil	Renter	3.48	Polylectic	Eusocial
<i>Bombus flavifrons</i>	25.37	below	soil	Renter	2.89	Polylectic	Eusocial
<i>Bombus frigidus</i>	47.83	below	soil	Renter	3.67	Polylectic	Eusocial
<i>Bombus huntii</i>	55	below	soil	NA	3.22	Polylectic	Eusocial
<i>Bombus insularis</i>	42.5	below	soil	NA	4.45	NA	Parasite
<i>Bombus mixtus</i>	72	below	soil	Renter	3.04	Polylectic	Eusocial

Table S4-4 (continued).

<i>Bombus nevadensis</i>	44.33	below	soil	Renter	3.78	Polylectic	Eusocial
<i>Bombus occidentalis</i>	57.2	below	soil	Renter	3.42	Polylectic	Eusocial
<i>Bombus rufocinctus</i>	45.5	below	soil	Renter	3.48	Polylectic	Eusocial
<i>Bombus sylvicola</i>	41	below	soil	Renter	3.18	Polylectic	Eusocial
<i>Ceratina neomexicana</i>	21	above	wood	Excavator	1.37	Polylectic	Solitary
<i>Diadasia diminuta</i>	41	below	soil	Excavator	2.43	Oligolectic	Solitary
<i>Epeolus americanus</i>	49	below	soil	NA	1.43	NA	Parasite
<i>Epeolus compactus</i>	77	below	soil	NA	1.87	NA	Parasite
<i>Epeolus</i> sp.	77	below	soil	NA	2.18	NA	Parasite
<i>Eucera fulvitaris</i>	20	below	soil	Excavator	3.41	Oligolectic	Solitary
<i>Holcopasites</i> sp.	92	below	soil	NA	1.14	NA	Parasite
<i>Melissodes confusus</i>	89.75	below	soil	Excavator	3.01	Oligolectic	Solitary
<i>Melissodes coreopsis</i>	108	below	soil	Excavator	2.09	Oligolectic	Solitary
<i>Melissodes grindeliae</i>	114	below	soil	Excavator	2.77	Polylectic	Solitary
<i>Melissodes hymenoxidis</i>	105	below	soil	Excavator	3.11	Oligolectic	Solitary
<i>Melissodes tristis</i>	106.5	below	soil	Excavator	3.07	Oligolectic	Solitary
<i>Triepeolus</i> sp.	53.5	below	soil	NA	2.4	NA	Parasite
Colletidae							
<i>Colletes consors</i>	36.5	below	soil	Excavator	2.19	Polylectic	Solitary
<i>Colletes hyalinus</i>	64	below	soil	Excavator	2.19	Polylectic	Solitary
<i>Colletes kincaidii</i>	74	below	soil	Excavator	1.97	Polylectic	Solitary
<i>Colletes nigrifrons</i>	44.6	below	soil	Excavator	2.04	Polylectic	Solitary
<i>Colletes paniscus</i>	43.5	below	soil	Excavator	1.64	Oligolectic	Solitary
<i>Hylaeus annulatus</i>	60.28	above	cavity	Renter	1.28	Polylectic	Solitary
Halictidae							
<i>Agapostemon texanus</i>	35	below	soil	Excavator	2	Polylectic	Solitary
<i>Dufourea fimbriata</i>	84	below	soil	Excavator	1.18	Oligolectic	Solitary
<i>Dufourea harveyi</i>	57.75	below	soil	Excavator	1.18	Oligolectic	Solitary
<i>Dufourea maura</i>	58.66	below	soil	Excavator	1.73	Oligolectic	Solitary
<i>Dufourea</i> sp.	82.33	below	soil	Excavator	1.05	Oligolectic	Solitary
<i>Halictus confusus</i>	58	below	soil	Excavator	1.32	Polylectic	Solitary

Table S4-4 (continued).

<i>Halictus rubicundus</i>	23	below	soil	Excavator	1.87	Polylectic	Solitary
<i>Halictus tripartitus</i>	10	below	soil	Excavator	1.24	Polylectic	Solitary
<i>Halictus virgatellus</i>	16.87	below	soil	Excavator	1.44	Polylectic	Solitary
<i>Lasioglossum abundipunctum</i>	26.85	below	soil	Excavator	1.01	Polylectic	Solitary
<i>Lasioglossum ebmerellum</i>	41	below	soil	Excavator	1.35	Polylectic	Solitary
<i>Lasioglossum ephialtum</i>	77	below	soil	Excavator	1.01	Polylectic	Solitary
<i>Lasioglossum granosum</i>	8	below	soil	Excavator	1.59	Polylectic	Solitary
<i>Lasioglossum inconditum</i>	16.62	below	soil	Excavator	1.25	Polylectic	Solitary
<i>Lasioglossum kneri</i>	103.5	below	soil	Excavator	1.2	Polylectic	Solitary
<i>Lasioglossum nigrum</i>	16.62	below	soil	Excavator	1.28	Polylectic	Solitary
<i>Lasioglossum obnubilum</i>	32.4	below	soil	Excavator	0.89	Polylectic	Solitary
<i>Lasioglossum occidentale</i>	113	below	soil	Excavator	0.96	Polylectic	Solitary
<i>Lasioglossum pacatum</i>	20	below	soil	Excavator	1.12	Polylectic	Solitary
<i>Lasioglossum pavoninum</i>	23.57	below	soil	Excavator	1.06	Polylectic	Solitary
<i>Lasioglossum prasinogaster</i>	42.5	below	soil	Excavator	1.14	Polylectic	Solitary
<i>Lasioglossum regis</i>	50	below	soil	Excavator	1.18	Polylectic	Solitary
<i>Lasioglossum ruidosense</i>	16.75	below	soil	Excavator	1.05	Polylectic	Solitary
<i>Lasioglossum sandhousiellum</i>	58.33	below	soil	Excavator	1.08	Polylectic	Solitary
<i>Lasioglossum sedi</i>	16.62	below	soil	Excavator	0.99	Polylectic	Solitary
<i>Lasioglossum semicaeruleum</i>	74.8	below	soil	Excavator	1.03	Polylectic	Solitary
<i>Lasioglossum tenax</i>	18.62	below	soil	Excavator	1.06	Polylectic	Solitary
<i>Lasioglossum trizonatum</i>	36.16	below	soil	Excavator	2.3	Polylectic	Solitary
<i>Sphecodes</i> sp.	24.12	below	soil	NA	0.95	NA	Parasite
Megachilidae							
<i>Anthidium</i> sp.	65.5	above	cavity	Renter	3.03	Polylectic	Solitary
<i>Ashmeadiella</i> sp.	43	above	cavity	renter	1.48	Polylectic	Solitary
<i>Atoposmia abjecta</i>	56	above	cavity	Excavator	1.88	Oligolectic	Solitary

Table S4-4 (continued).

<i>Atoposmia elongata</i>	70	above	cavity	Excavator	1.31	Oligolectic	Solitary
<i>Coelioxys moesta</i>	84	above	cavity	NA	2.27	NA	Parasite
<i>Coelioxys sodalis</i>	45	above	cavity	NA	2.51	NA	Parasite
<i>Dianthidium heterulkei</i>	75.5	above	cavity	Renter	2.08	Polylectic	Solitary
<i>Hoplitis albifrons</i>	44.5	above	stems	Excavator	2.43	Polylectic	Solitary
<i>Hoplitis fulgida</i>	36.83	above	stems	Renter	2.04	Polylectic	Solitary
<i>Hoplitis fulgida fulgida</i>	50	above	stems	Renter	2.04	Polylectic	Solitary
<i>Hoplitis robusta</i>	42.5	above	stems	Renter	1.39	Polylectic	Solitary
<i>Megachile frigida</i>	78	above	cavity	Renter	3.64	Polylectic	Solitary
<i>Megachile gemula</i>	41.5	below	soil	Renter	3.62	Polylectic	Solitary
<i>Megachile inermis</i>	64.66	above	rocks	Renter	4.43	Polylectic	Solitary
<i>Megachile latimanus</i>	80	above	cavity	Renter	5.25	Polylectic	Solitary
<i>Megachile melanophaea</i>	55.16	below	soil	Renter	3.26	Polylectic	Solitary
<i>Megachile montivaga</i>	58.2	above	cavity	Renter	2.61	Polylectic	Solitary
<i>Megachile perihirta</i>	70.33	below	cavity	Excavator	3.53	Polylectic	Solitary
<i>Megachile pugnata</i>	60.66	above	cavity	Renter	3.1	Oligolectic	Solitary
<i>Megachile relativa</i>	59.5	above	cavity	Renter	2.47	Polylectic	Solitary
<i>Megachile subexilis</i>	54	above	cavity	Renter	2.5	Polylectic	Solitary
<i>Nomada</i> sp.	13	below	soil	NA	1.63	NA	Parasite
<i>Stelis foederalis</i>	76	above	cavity	NA	1.745	NA	Parasite
<i>Stelis</i> sp.	41	above	cavity	NA	1.78	NA	Parasite
<i>Stelis submarginata</i>	77	above	cavity	NA	1.71	NA	Parasite