Received: 4 April 2018 | Accepted: 7 January 2019

DOI: 10.1111/1365-2745.13152

RESEARCH ARTICLE

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

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] | Rebecca E. Irwin[1,2]

[1]Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina

[2]Rocky Mountain Biological Laboratory, Gothic, Colorado

[3]Biology Department, Luther College, Decorah, Iowa

[4]Department of Biology, University of Maryland, College Park, Maryland

Correspondence
Gabriella L. Pardee
Email: gabriellapardee@gmail.com

Funding information
Garden Club of America, Centennial Pollinator Fellowship; Rocky Mountain Biological Laboratory Barclay Scholarship; Luther College Imagine Fellowship; Colorado Native Plant Society; Southeast Climate Science Center; National Science Foundation, Grant/Award Number: DEB-1354104 and DGE-1143953; Rocky Mountain Biological Laboratory Graduate Research Fellowship

Handling Editor: Akiko Satake

Abstract

1. Changes from historic weather patterns have affected the phenology of many organisms world-wide. 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, and measured subsequent effects on flowering phenology, peak flower density, frost damage, pollinator visitation and reproduction of four subalpine wildflowers. Additionally, we conducted a pollen-supplementation experiment to test whether the plants in our snowmelt and frost treatments were pollen limited for reproduction. The four plants included species flowering in early spring to mid-summer.
3. The phenology of all four species was 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, we recorded fewer pollinators during flowering for the earliest-blooming species in the snow removal plots. 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 unfavourable abiotic 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, and some of the mechanisms involved. 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.

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

© 2019 The Authors. Journal of Ecology
© 2019 British Ecological Society

1 | INTRODUCTION

Global climate change is one of the strongest factors impacting ecosystems today (Parmesan, 2006), with the Earth's climate having warmed by *c*. 0.6°C over the past 100 years (Hansen et al., 2006). Increased temperatures have led to changes in seasonality, precipitation and the occurrence of harsh weather events (Chown & Klok, 2003; Meehl et al., 2000; Westra, Alexander, & Zwiers, 2013). 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 effects on reproduction (Both & Visser, 2001; Cleland et al., 2012; 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, 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; Theobald, Breckheimer, & HilleRisLambers, 2017). 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. For example, several studies have suggested that pollen limitation may occur due to plant-pollinator mismatch under climate change (Forrest, 2015; Hegland, Nielsen, Lázaro, Bjerknes, & Totland, 2009; Memmott, Craze, Waser, & Price, 2007). Because advanced phenology can be associated with both increased instances in which species experience harsh environmental conditions and temporal mismatches, studies are needed to assess experimentally 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 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 of the 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 (Laternser & 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 increased opportunities for pollination 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 et al., 2009; Memmott et al., 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, 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, Inouye, & Irwin, 2016). Studies are needed, however, that experimentally manipulate both snowmelt timing and frost exposure to tease apart the individual versus combined effects of these two factors on species interactions and reproduction.

We addressed the following questions: (a) How do snowmelt timing and frost exposure affect flowering phenology, frost damage, floral abundance and pollinator visitation? and (b) 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 et al., 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.

2 | MATERIALS AND METHODS

2.1 | Study system

Research was conducted at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO, USA (elevation 2,950 m). The RMBL is located in a subalpine region and hosts a diversity of flowering plants, with over 680 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 snowmelt date, 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 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 bumblebees (Forrest, Ogilvie, Gorischek, & Thomson, 2011). *Delphinium* produces deep blue to purple protandrous, zygomorphic, nectar-spurred flowers with 1–14 flowers per stalk that are pollinated primarily by bumblebees 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 responds 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).

2.2 | 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 × 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 shovelling (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, Debinski, Caragea, & Germino, 2017). Plots in the frost treatment were exposed to naturally occurring frost events, while plots in the frost-reduction treatment were covered with frost-resistant tarps (Easy Gardener Products, Waco, TX) the evening before a frost event was forecasted to occur, and tarps were removed the following morning once temperatures reached above 0°C. The experiment was conducted between April and July 2015 and again between May and 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).

2.3 | Flowering phenology and frost damage

We recorded the date of complete bare ground within each plot to measure the effectiveness of shovelling 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. If a plot at a sampling date had less than 10 plants in bloom of a particular focal species, we counted the number of plants in bloom and conducted flower counts on all available plants (range 1–10 plants). These data allowed us to calculate the day when plants started blooming (first bloom), the day when the flowers reached the highest abundance (peak bloom) and the day the plants completed blooming (last bloom) within each plot. We also measured visible frost damage by recording the number of plants that showed frost damage (brown or translucent flowers) out of the total plants observed on up to 10 randomly chosen plants per species. From these measurements, we calculated the proportion of plants that experienced frost damage for each plot.

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., 2015) with year, snow treatment (removal vs. natural) and frost treatment (frost vs. frost-reduction) and their interactions as factors and flowering phenology (dates of first, peak, and last flower) and bloom duration, measured as the number of days between the first and last bloom, as the response variables. Separate analyses were performed for each plant species. We note that in 2015, we missed recording the date of last flower and frost damage for *Potentilla*, and so for this species, we could not analyse the date of last flower or bloom duration. 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 used likelihood-ratio tests with type 2 sum of squares test to measure model significance. We assessed the fit of each model by calculating the dispersion ratio. To test how treatments affected the probability of frost damage, we ran linear models using a binomial distribution and log link function 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).

2.4 | Pollinator visitation

Upon flowering, we recorded flower-visitor interactions every other day throughout the blooming period of each focal species. For every observation round, we observed each plot for 10 min 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 conducted 18 observation rounds in 2015 and 12 observation rounds in 2016, resulting in 180 min and 120 min of observation per plot per year respectively. We calculated three metrics of pollinator visitation per focal species per plot: (a) total number of visitors (number of bouts summed across the bloom period), (b) number of flower visits (total flowers visited summed across plants) and (c) average time spent per flower (total time spent at flowers divided by total flower visits). We ran generalized linear models in lme4 on the three 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 floral resources. Additionally, if we saw differences in pollinator visitation and bloom duration between treatments (see Results), then we ran a linear regression to determine whether total number of visitors was correlated with a lengthened growing season. We used a Poisson distribution with the log link function for total number of visitors across all pollinator groups, a negative binomial distribution with the log link function for total number of flower visits, and a gamma distribution with the inverse link function for the average time spent per flower.

2.5 | 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 eight plants per focal species and assigned them to either a pollen-supplementation treatment or open-pollination (not supplemented control) treatment (four 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. For *Mertensia*, we only measured reproduction on plants in the open-pollination treatment.

Once the plants senesced on each of our marked plants per plot, 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.

3 | RESULTS

3.1 | 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. 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). Overall, we found that snow removal significantly advanced flowering phenology for all focal species (Tables S2–S5). 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, except for *Potentilla* ($\chi^2 < 1.47$, $p > 0.22$ in all cases). We outline the species-specific effects of snow and frost manipulation on flowering phenology and frost damage below.

3.1.1 | Claytonia

Dates of first bloom, peak bloom and last bloom were significantly advanced ($\chi^2_1 > 39.64$, $p < 0.001$ for all dates; Figure 1a and Table S2), and bloom duration was significantly longer by 7.93 ± 6.93 days ($\chi^2_1 = 49.49$, $p < 0.001$; Figure S1) in the snow removal relative to the natural snowmelt plots. We also found a significant difference in first, peak and last dates of bloom between 2015 and 2016, where phenology was shifted earlier in snow removal and natural snowmelt plots, and the bloom duration was longer in 2015 compared to 2016, due to an earlier arrival of spring in 2015 ($\chi^2 > 12.84$, $p < 0.001$ in all cases; Figure 1a). For peak flower density, we found that the snow removal plots had 39% fewer flowers than the natural snowmelt plots ($\chi^2_1 = 10.38$, $p = 0.001$), and that peak flower density was higher in 2015 than 2016 ($\chi^2_1 = 11.05$, $p = 0.001$). When we looked at frost damage, we found that plants in the frost treatment exhibited 16.7% more frost damage (0.23 ± 0.03; mean ± *SE*) than plants in the frost-reduction treatment (0.07 ± 0.02; $F_{1,72} = 18.59$, $p < 0.001$; Figure 2a). Moreover, the proportion of plants that exhibited frost damage was 17.7% higher in the snow removal treatment (0.24 ± 0.03) than the natural snowmelt treatment (0.06 ± 0.02; $F_{1,72} = 22.46$, $p < 0.001$), and there was a significant interaction between the snow and frost treatments ($F_{1,72} = 13.57$, $p < 0.001$; Figure 2a), with the magnitude of frost damage having a larger effect on plants in the snow removal plots. Additionally, we did not find a difference in frost damage between years ($F_{1,72} = 2.73$, $p = 0.102$).

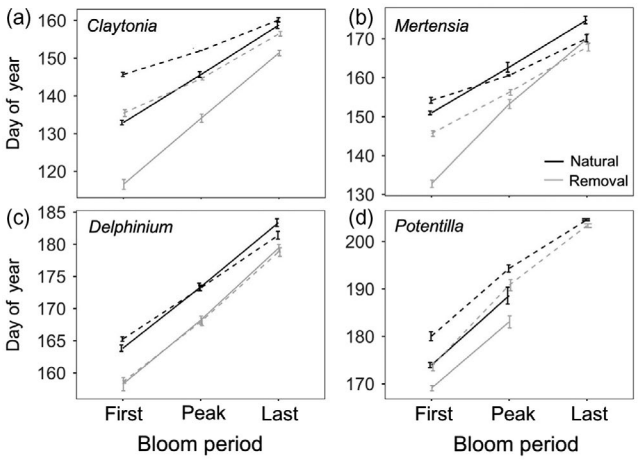


FIGURE 1 Flowering phenology in the snow removal (grey lines) and natural snowmelt (black lines) treatments for (a) *Claytonia lanceolata*, (b) *Mertensia fusiformis*, (c) *Delphinium nuttallianum* and (d) *Potentilla pulcherrima* (missing date of last flower in 2016 for *Potentilla*). Note the difference 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 *SE*

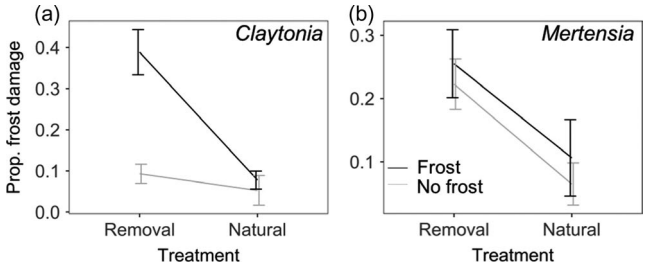


FIGURE 2 Proportion of plants experiencing frost damage in the frost (black line) and frost-reduction (grey line) treatments and in the snow removal and natural snowmelt plots for (a) *Claytonia* and (b) *Mertensia*. Note the difference in scale on the y-axis of the two panels. For *Claytonia*, there 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 *SE*

3.1.2 | Mertensia

Dates of first bloom, peak bloom and last bloom were significantly advanced ($\chi^2_1 > 15.13$, $p < 0.001$ for all dates; Figure 1b and Table S3), and bloom duration was significantly longer by 10 ± 7.09 days ($\chi^2_1 = 78.14$, $p < 0.001$; Figure S1) in the in the snow removal relative to natural snowmelt plots. We also found a significant difference in first and last dates of bloom between 2015 and 2016, where phenology was shifted earlier in snow removal and natural snowmelt plots and bloom duration was longer in 2015 compared to 2016 ($\chi^2 > 7.84$, $p < 0.005$ in all cases; Figure 1b). Further, we did not find a difference in peak flower density between the snow removal and natural snowmelt plots or between years ($\chi^2_1 > 2.83$, $p > 0.09$). Finally, when we looked at frost damage, we found 21% higher rates of frost damage in the snow removal (0.25 ± 0.03) than the natural snowmelt treatments (0.04 ± 0.01; $F_{1,33} = 21.73$, $p < 0.001$; Figure 2b) and higher frost damage in 2015 than 2016 ($F_{1,33} = 6.05$, $p = 0.013$).

3.1.3 | Delphinium

Dates of first bloom, peak bloom and last bloom were significantly advanced ($\chi^2_1 > 31.29$, $p < 0.001$ for all dates; Figure 1c and Table S4), and bloom duration was significantly longer by 2.89 ± 3.07 days ($\chi^2_1 = 8.89$, $p = 0.002$; Figure S1) in the snow removal treatments compared to the natural snowmelt treatment. When we looked at year effects, we did not find a difference between first and peak bloom between years ($\chi^2_1 < 1.46$, $p > 0.22$; Figure 1c), but we did find that bloom duration was longer in 2015 than 2016 ($\chi^2_1 = 6.64$, $p = 0.009$). Further, though we found higher peak flower density in

the snow removal and natural snowmelt treatments in 2015 than 2016 ($\chi^2_1 = 7.69$, $p = 0.005$), we did not find a difference in peak flower density between the snow treatments ($\chi^2_1 = 0.008$, $p = 0.92$). Finally, there was no difference in frost damage between the snow and frost treatments (Table S4).

3.1.4 | Potentilla

Dates of first bloom and peak bloom were significantly advanced in the snow removal treatment in 2015 and 2016 ($\chi^2_1 > 29.43$, $p < 0.001$; Figure 1d and Table S5), and date of peak bloom was earlier in the frost treatment than the frost-reduction treatment ($\chi^2_1 = 8.39$, $p = 0.003$), but the date of last bloom in 2016 was not different between the snow treatments ($\chi^2_1 = 3.01$, $p = 0.08$). Further, the 2016 bloom duration was significantly longer by 5.23 ± 4.29 ($\chi^2_1 = 9.56$, $p = 0.001$; Figure S1) in the snow removal than the natural snowmelt treatment. We found that the first and peak flower dates were earlier in 2015 than 2016 ($\chi^2_1 > 50.05$, $p < 0.001$ for both dates), but we did not compare date of last flower or bloom duration between 2015 and 2016 due to missing values in 2015. Further, we did not find a difference in peak flower density between the snowmelt treatments ($\chi^2_1 = 0.39$, $p = 0.52$), but found higher peak flower density in 2015 than 2016 ($\chi^2_1 = 232.50$, $p < 0.001$). Finally, there was no difference in frost damage in 2016 between the snow and frost treatments (Table S5).

3.2 | Pollinator visitation

Across all four plant species, we observed 6,656 flower–visitor interactions. We found species-specific differences, which we outline below, in visitation rates due snow and frost manipulation.

3.2.1 | Claytonia

We found that plants in the snow removal treatment experienced 78.71% fewer visitors than in the natural snowmelt treatment ($\chi^2_1 = 8.31$, $p = 0.003$; Figure 3a), but when we looked at the number of flowers visited by pollinators, we found no difference in flower visits between snow removal and natural snowmelt plots ($\chi^2_1 = 0.42$, $p = 0.51$; Figure 3b). Moreover, we found that the pollinators spent on average 51.42% more time on the flowers in the snow removal relative to natural snowmelt treatments ($\chi^2_1 = 6.15$, $p = 0.01$; Figure 3c). When we looked at pollinator visitation between the frost and frost-reduction treatments, we found that flower visits increased by 48.3% in the frost compared to frost-reduction treatment ($\chi^2_1 = 3.79$, $p = 0.05$). Because we found a difference in flower production between the snow removal and natural snowmelt treatments, we analysed per-flower visitation and did not find a difference in visitation between snow treatments on a per-flower basis ($\chi^2_1 = 2.35$, $p = 0.13$). Although we did find independent effects of both the snow and frost treatments on pollinator visitation, we did not find any difference in the interaction between snow and frost treatments for any metrics of pollinator visitation ($\chi^2_1 < 0.31$, $p > 0.57$ in all cases). Finally, we did not find any effect of year on metrics of pollinator visitation ($\chi^2_1 < 0.78$, $p > 0.37$ in all cases, Table S2).

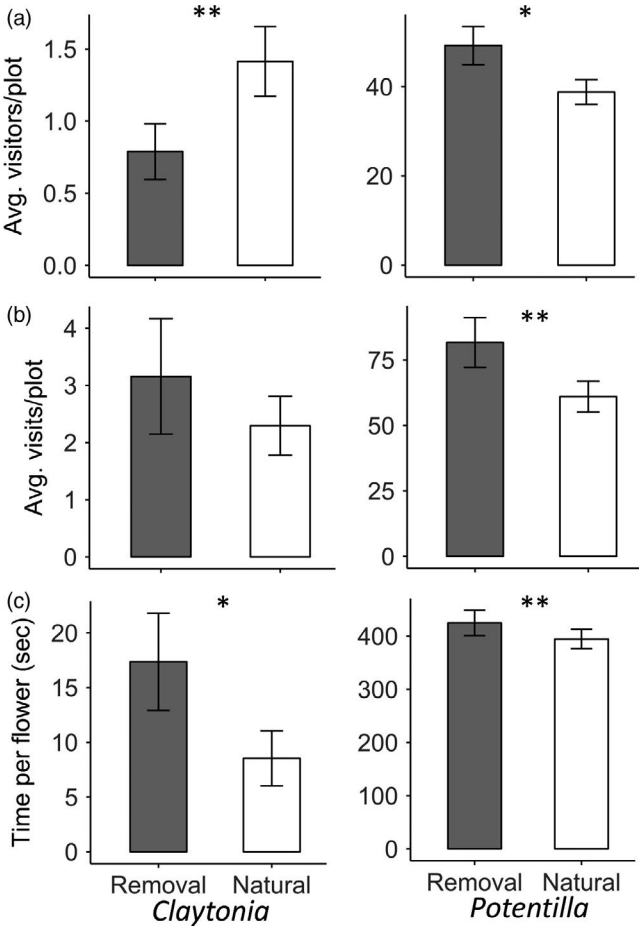


FIGURE 3 Patterns of flower–visitor interactions for *Claytonia* (left panel) and *Potentilla* (right panel) between the snow removal (dark bars) and natural snowmelt (light bars) treatments. Panels in row (a) show the average number of visitors per plot, (b) the average number of flowers visited per plot and (c) the average time spent per flower per plot. Asterisks denote significance at $^{**}p < 0.001$ and $^{*}p < 0.05$. Bars are means + *SE*

3.2.2 | Mertensia

We did not find any effect of snow or frost treatment or their interaction on any metrics of pollinator visitation ($\chi^2_1 < 2.60$, $p > 0.10$ in all cases, Table S3). However, when we looked at pollinator visitation among the snow and frost treatments across years, we observed more visitors and more flowers visited in both the snow removal and natural snowmelt treatments in 2016 than 2015 ($\chi^2_1 > 20.35$, $p < 0.001$ in all cases), and that pollinators spent 70.51% less time on flowers in the frost treatment in 2016 than 2015 ($\chi^2_1 = 4.34$, $p = 0.03$).

3.2.3 | Delphinium

We did not find any effect of snow removal on any metrics of pollinator visitation ($\chi^2_1 < 1.14$, $p > 0.28$ in all cases). Though we did not

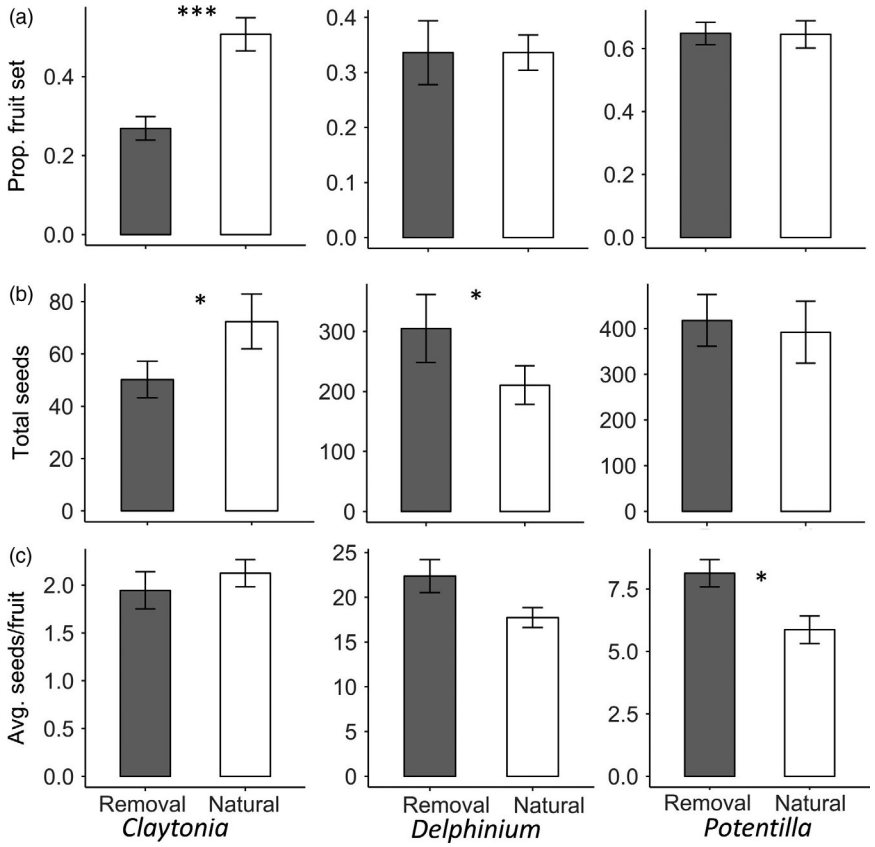


FIGURE 4 Mean plant reproduction for *Claytonia* (left panel), *Delphinium* (centre panel), and *Potentilla* (right panel) between the snow removal (dark bars) and natural snowmelt (light bars) treatments. Panels in row (a) show the proportion of successful fruit, in (b) show the total seeds produced per plant and in (c) show the average seeds produced per fruit. Asterisks denote significance at ***$p < 0.001$ and *$p < 0.05$. Bars are means + *SE*

find any effect of the frost treatment on total number of visitors or number of flower visits ($\chi^2_1 < 2.59$, $p > 0.10$ in all cases), we did find that pollinators spent on average 36.87% more time at flowers in the frost-reduction treatment than flowers in the frost treatment ($\chi^2_1 = 8.04$, $p = 0.004$), and that pollinators spent slightly less time on flowers in 2015 than 2016 ($\chi^2_1 = 3.37$, $p = 0.06$). Further, we found an interaction effect of year on total visitors and total flowers visited in the snow removal treatment ($\chi^2_1 > 13.37$, $p < 0.001$ in both cases), but we did not find any other interaction between the snow removal and frost treatment or year effects on any other metrics of pollinator visitation ($\chi^2_1 < 0.83$, $p > 0.14$ in all cases, Table S4).

3.2.4 | Potentilla

We found that plants in the snow removal treatment received 21.19% more visitors than plants in the natural snowmelt treatment, and pollinators visited 1.3 times more flowers in the snow removal than natural snowmelt treatment ($\chi^2_1 = 6.48$, $p = 0.01$; Figure 3a, and $\chi^2_1 = 9.00$, $p = 0.002$ respectively; Figure 3b). However, we found that pollinators spent 11.48% more time on average on flowers in the natural snowmelt treatment relative to snow removal treatment ($\chi^2_1 = 6.47$, $p = 0.01$; Figure 3c). We did not find any effect of the frost treatment on patterns of pollinator visitation, and there were no significant interactions between the snow removal and frost treatments ($\chi^2_1 < 1.53$, $p > 0.21$ in all cases). We found an effect of year on all metrics of visitation for *Potentilla* ($\chi^2_1 > 74.60$, $p < 0.001$ in all cases; Table S5), with 2016 having lower metrics of pollinator visits than 2015, but the pollinators spent more time on plants in 2015 than 2016. When we looked at the relationship between bloom duration and number of flower visits per plot, we found that a longer bloom period was associated with increased floral visits ($F_{1,37} = 5.14$, $p = 0.02$).

3.3 | Pollen limitation and plant reproduction

The one common pattern across *Claytonia*, *Delphinium* and *Potentilla* is that none were pollen-limited for plant reproduction in 2016 ($p > 0.18$ 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. Further, we did not find any effects of frost as a main effect or interaction on reproduction for any species ($p > 0.14$ in all cases), except for *Mertensia* (described below).

3.3.1 | Claytonia

We found a significant reduction in proportion fruit set by 22.83% ($F_{1,69} = 31.37$, $p < 0.001$; Figure 4a) and total seeds produced by 46.04% ($\chi^2_1 = 5.46$, $p = 0.01$; Figure 4b) in the snow removal treatment compared to the natural snowmelt treatment, with no difference in average seeds produced per fruit ($F_{1,66} = 0.22$, $p = 0.63$; Figure 4c).

3.3.2 | Mertensia

We did not find any effect of snow removal treatment on proportion fruit set or total seeds ($F < 0.30$, $p > 0.58$ in all cases; Table S3), but we did find a non-significant trend towards 7.23% lower proportion fruit set in the frost compared to frost-reduction 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 fruit set for *Mertensia* ($F_{1,36} = 3.31$, $p = 0.07$) and an interaction on total seeds produced between snow removal and frost treatments ($F_{1,36} = 5.26$, $p = 0.02$), where plants produced 24.40% fewer seeds in the frost treatment compared to the frost-reduction treatment under natural snowmelt conditions, suggesting that snowmelt timing moderates the degree to which plants are damaged by frost with reproductive consequences.

3.3.3 | Delphinium

We found that snow removal resulted in 31.6% higher seeds produced ($\chi^2_1 = 3.83$, $p = 0.05$; Figure 4b), but there was no effect of snow removal on proportion fruit set or mean seeds produced per fruit ($F < 3.54$, $p > 0.06$ in both cases; Figure 4a,c). And there was no effect of frost treatment or interactions between snow removal and frost treatment for any metrics of *Delphinium* reproduction ($F < 3.36$, $p > 0.06$, in all cases; Table S4).

3.3.4 | Potentilla

We did not find any effect of snow removal on proportion fruit set or total seeds produced (Figure 4a,b; Table S5), but we did find that plants in the snow removal treatment produced 20.8% higher average seeds per fruit ($F_{1,71} = 5.87$, $p = 0.01$; Figure 4c) than plants in the natural snowmelt treatment. And there was no effect of frost treatment or interactions between snow removal and frost treatment for any metrics of *Potentilla* reproduction ($F < 1.17$, $p > 0.27$).

4 | DISCUSSION

Using a snow removal experiment, we successfully advanced the date of first bloom, which lengthened the bloom duration of all four focal plant species through early snowmelt. Yet, advanced phenology had differing effects for the four focal species in terms of pollinator visitation and plant reproduction. For *Claytonia*, we found that advanced phenology resulted in higher frost damage, lower flower production, fewer pollinators visiting the flowers and a reduction in seed set. For *Mertensia*, advanced phenology increased frost damage, which resulted in trends towards lower fruit set. For *Delphinium*, advanced phenology increased seed production. And for *Potentilla*, advanced phenology resulted in increased flower visits from pollinators and increased seed set. 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 potentially 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, especially for 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 assess fully the effects of snowmelt timing on flowering for key vital rates.

When we looked at pollinator visitation as a function of the treatments, we found differences in visitation and foraging behaviour between the earlier-blooming species and the later-blooming species. For *Claytonia*, the number of visitors per plot was significantly reduced in the snow removal treatment compared to the natural snowmelt treatment. This might indicate that either early blooming species in the snow removal treatment were growing under unfavourable weather conditions for pollinators (Polatto, Chaud-Netto, & Alves-Junior, 2014), or their peak flowering may not

have coincided with their pollinators' emergence and peak activity. While these interpretations suggest a mismatch between early blooming species and their pollinators under climate change, we did not find any evidence for pollen limitation in the snow removal plots. 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 differences in visitation rates among years with snow removal.

Considering other patterns of pollinator visitation we observed (Tables S2–S5), our findings of increased pollinator visitation 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 flower visits per plot for *Potentilla*, a longer bloom period in the snow removal treatment likely provided more opportunities for pollinator visitation. Finally, the differences we observed in pollinator visitation between the frost and frost-reduction treatment for *Claytonia* and *Delphinium* 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).

The trends in patterns of pollinator visitation coincided with the trends in plant reproduction; the treatment(s) in which plants that received more visits was also the treatment(s) with higher reproduction. 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 point to the importance of testing for pollen limitation in studies trying to link changes in pollinator visitation patterns to plant reproduction. Our 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 inter-annually (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. Our results for increased seed production of *Delphinium* and *Potentilla* in the snow removal plots could indicate that these species might not be as affected by cooler temperatures during spring plant growth, or that other unmeasured factors associated with early snowmelt and bloom time outweigh any disadvantages for these species.

We found higher frost damage for *Claytonia* between the frost and frost-reduction treatments, and these differences were greater between the snow removal and natural snowmelt 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 night-time 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 inter-annual 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 evidence towards 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 between the frost and frost-reduction treatments for *Mertensia*, our results suggest that frost may have other direct, unmeasured effects on plants that link to seed set and/or that frost may damage flowers and reproductive structures that 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). Although we found higher proportion of plants with frost damage for *Claytonia* in the frost treatment, this did not affect seed set. This might indicate that the flowers and reproductive structures have higher sensitivity to frost than *Mertensia* as the flowers still reproduced despite the plant having visual frost damage. Future studies focused on physiological measurements associated with frost sensitivity or hardiness may provide additional mechanistic insight. Nonetheless, our results show that frost could play a 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, and the mechanisms involved.

Given our findings, early snowmelt timing and advanced phenology negatively impact early blooming species likely through exposure

to harsher growing conditions with the potential to negatively affect reproduction and alter interactions with pollinators. However, species that flower later in the season benefit from advanced phenology through increased reproduction. 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.

ACKNOWLEDGEMENTS

We thank The Rocky Mountain Biological Laboratory for granting us access to the field and members of the Irwin lab for helpful feedback on prior drafts of this manuscript. This work was supported by the following grants and fellowships to G.L.P.: National Science Foundation (NSF) Graduate Research Fellowship DGE-1143953, the Rocky Mountain Biological Laboratory Graduate Research Fellowship, the Garden Club of America Centennial Pollinator Fellowship, the Colorado Native Plant Society Research Grant and the Global Change Fellowship through the Southeast Climate Science Center. This work was also funded by an NSF LTREB award to D.W.I. and R.E.I. (DEB-1354104), and the Luther College Imagine Fellowship and a Rocky Mountain Biological Laboratory Barclay Scholarship awarded to I.O.J. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

AUTHORS' CONTRIBUTIONS

G.L.P., R.E.I. and D.W.I. conceived and designed the study. G.L.P. and I.O.J. conducted the fieldwork. G.L.P. performed the analyses and wrote the paper. All the authors provided edits to prior drafts of the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.kd1r166 (Pardee, Jensen, Inouye, & Irwin, 2019).

ORCID


Gabriella L. Pardee https://orcid.org/0000-0003-1575-3024

REFERENCES

Augspurger, C. K. (2009). Spring 2007 warmth and frost: Phenology, damage and refoliation in a temperate deciduous forest. *Functional Ecology*, *23*, 1031–1039. https://doi.org/10.1111/j.1365-2435.2009.01587.x

Augspurger, C. K. (2013). Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. *Ecology*, *94*(1), 41–50. https://doi.org/10.1890/12-0200.1

Badeck, F.-W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., Schaber, J., & Sitch, S. (2004). Responses of spring phenology to climate change. *New Phytologist*, *162*(2), 295–309. https://doi.org/10.1111/j.1469-8137.2004.01059.x

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Journal of statistical software., *67*, 1–48.https://doi.org/10.18637/jss.v067.i01.

Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A., & Larsson, S. (2005). Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications*, *15*(6), 2084–2096. https://doi.org/10.1890/04-1903

Blois, J. L., Zarnetske, P. L., Fitzpatrick, M. C., & Finnegan, S. (2013). Climate change and the past, present, and future of biotic interactions. *Science*, *341*(August), 499–505. https://doi.org/10.1126/science.1237184

Boggs, C. L., & Inouye, D. W. (2012). A single climate driver has direct and indirect effects on insect population dynamics. *Ecology Letters*, *15*(5), 502–508. https://doi.org/10.1111/j.1461-0248.2012.01766.x

Bosch, M., & Waser, N. M. (1999). Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *American Journal of Botany*, *86*(6), 871–879. https://doi.org/10.2307/2656707

Both, C., & Visser, M. E. (2001). Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature*, *411*, 296–298. https://doi.org/10.1038/35077063

Cahill, J. F., Castelli, J. P., & Casper, B. B. (2002). Separate effects of human visitation and touch on plant growth and herbivory in an old-field community. *American Journal of Botany*, *89*(9), 1401–1409. https://doi.org/10.3732/ajb.89.9.1401

CaraDonna, P. J., & Bain, J. A. (2016). Frost sensitivity of leaves and flowers of subalpine plants is related to tissue type and phenology. *Journal of Ecology*, *104*, 55–64. https://doi.org/10.1111/1365-2745.12482

CaraDonna, P. J., Iler, A. M., & Inouye, D. W. (2014). Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(13), 4916–4921. https://doi.org/10.1073/pnas.1323073111

Chown, S. L., & Klok, C. J. (2003). Altitudinal body size clines: Latitudinal effects associated with changing seasonality. *Ecography*, *26*(4), 445–455. https://doi.org/10.1034/j.1600-0587.2003.03479.x

Cleland, E. E., Allen, J. M., Crimmins, T. M., Dunne, J. A., Pau, S., Travers, S. E., … Wolkovich, E. M. (2012). Phenological tracking enables positive species responses to climate change. *Ecology*, *93*(8), 1765–1771. https://doi.org/10.1002/ecy.2162

Dudash, M. R., & Fenster, C. B. (1997). Multiyear study of pollen limitation and cost of reproduction in the iteroparous *Silene virginica*. *Ecology*, *78*(2), 484–493. https://doi.org/10.1890/0012-9658(1997)078[0484:MSOPLA]2.0CO;2

Dunne, J. A., Harte, J., & Taylor, K. J. (2003). Subalpine meadow and flowering phenology responses to climate change: Integrating experimental and gradient methods. *Ecological Monographs*, *73*(1), 69–86. https://doi.org/10.1890/0012-9615(2003) 073[0069:SMFPRT]2.0.CO;2

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E., & Bernasconi, G. (2007). Time after time: Flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, *22*(8), 432–439. https://doi.org/10.1016/j.tree.2007.05.006

Fitter, A. H., & Fitter, R. S. R. (2002). Rapid changes in flowering time in British plants. *Science*, *296*(5573), 1689–1691. https://doi.org/10.1126/science.1071617

Forrest, J. R. K. (2014). Plant-pollinator interactions and phenological change: What can we learn about climate impacts from experiments and observations? *Oikos*, *124*(1), 4–13. https://doi.org/10.1111/oik.01386

Forrest, J. R. K. (2015). Plant–pollinator interactions and phenological change: What can we learn about climate impacts from experiments and observations? *Oikos*, *124*, 4–13. https://doi.org/10.1111/oik.01386

Forrest, J. R. K., & Miller-Rushing, A. J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution.

Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, *365*(1555), 3101–3112. https://doi.org/10.1098/rstb.2010.0145

Forrest, J. R. K., Ogilvie, J. E., Gorischek, A. M., & Thomson, J. D. (2011). Seasonal change in a pollinator community and the maintenance of style length variation in *Mertensia fusiformis* (Boraginaceae). *Annals of Botany*, *108*, 1–12. https://doi.org/10.1093/aob/mcr093

Forrest, J. R. K., & Thomson, J. D. (2010). Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower. *American Journal of Botany*, *97*(1), 38–48. https://doi.org/10.3732/ajb.0900083

Gezon, Z. J., Inouye, D. W., & Irwin, R. E. (2016). Phenological change in a spring ephemeral: Implications for pollination and plant reproduction. *Global Change Biology*, *1–15*, https://doi.org/10.1111/gcb.13209

Gibson-Reinemer, D. K., Sheldon, K. S., & Rahel, F. J. (2015). Climate change creates rapid species turnover in montane communities. *Ecology and Evolution*, *5*(12), 2340–2347. https://doi.org/10.1002/ece3.1518

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D. W., & Medina-Elizade, M. (2006). Global temperature change. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(39), 14288–14293. https://doi.org/10.1073/pnas.0606291103

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A.-L., & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, *12*(2), 184–195. https://doi.org/10.1111/j.1461-0248.2008.01269.x

Henry, G. H. R., & Molau, U. (1997). Tundra plants and climate change: The International Tundra Experiment (ITEX). *Global Change Biology*, *3*, 1–9. https://doi.org/10.111/j.1365-2486.1997.gcb132.x

Hollister, R. D., Webber, P. J., & Bay, C. (2005). Plant response to temperature in Northern Alaska: Implications for predicting vegetation change. *Ecology*, *86*(6), 1562–1570. https://doi.org/10.1890/04-0520

Holm, S. (1994). Reproductive variability and pollen limitation in three *Betula* taxa in Northern Sweden. *Ecography*, *17*(1), 73–81. https://doi.org/10.1111/j.1600-0587.1994.tb00078.x

Iler, A. M., Høye, T. T., Inouye, D. W., & Schmidt, N. M. (2013). Nonlinear flowering responses to climate: Are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, *368*, 20120489. https://doi.org/10.1098/rstb.2012.0489

Inouye, D. (2000). The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, *3*(5), 457–463. https://doi.org/10.1046/j.1461.2000.00165.x

Inouye, D. W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, *89*(2), 353–362. https://doi.org/10.1890/06-2128.1

Inouye, D. W., & Wielgolaski, F. E. (2013). Phenology of high-altitude climates. In M. D. Schwartz (Ed.), *Phenology: An integrative environmental science* (pp. 249–272). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Jacquemart, A. (1997). Pollen limitation in three sympatric species of *Vaccinium* (Ericaceae) in the Upper Ardennes, Belgium. *Plant Systematics and Evolution*, *207*, 159–172. https://doi.org/10.1007/BF00984387

Kudo, G., Nishikawa, Y., Kasagi, T., & Kosuge, S. (2004). Does seed production of spring ephemerals decrease when spring comes early? *Ecological Research*, *19*(2), 255–259. https://doi.org/10.1111/j.1440-1703.2003.00630.x

Lambert, A. M., Miller-Rushing, A. J., & Inouye, D. W. (2010). Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily; Liliaceae). *American Journal of Botany*, *97*(9), 1431–1437. https://doi.org/10.3732/ajb

Laternser, M., & Schneebeli, M. (2003). Long-term snow climate trends of the Swiss Alps (1931–99). *International Journal of Climatology*, *23*, 733–750. https://doi.org/10.1002/joc.912

Liu, Y., Reich, P. B., Li, G., & Sun, S. (2011). Shifting phenology and abundance under experimental warming alters trophic relationships and plant reproductive capacity. *Ecology*, *92*(6), 1201–1207. https://doi.org/10.1890/10-2060.1

Livensperger, C., Steltzer, H., Darrouzet-nardi, A., Sullivan, P. F., Wallenstein, M., & Weintraub, M. N. (2016). Earlier snowmelt and warming lead to earlier but not necessarily more plant growth. *Annals of Botany*, *13*(8), plw021. https://doi.org/10.1093/aobpla/plw021

Meehl, G. A., Zwiers, F., Evans, J., Knutson, T., Mearns, L., & Whetton, P. (2000). Trends in extreme weather and climate events: Issues related to modeling extremes in projections of future climate change. *Bulletin of the American Meteorological Society*, *81*(3), 427–436. https://doi.org/10.1175/1520-0477(200)081<0427:TIEWAC>2.3.CO;2

Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, *10*(8), 710–717. https://doi.org/10.1111/j.1461-0248.2007.01061.x

Miller-Rushing, A. J., Høye, T. T., Inouye, D. W., & Post, E. (2010). The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, *365*, 3177–3186. https://doi.org/10.1098/rstb.2010.0148

Miller-Rushing, A. J., & Inouye, D. W. (2009). Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species. *American Journal of Botany*, *96*(10), 1821–1829. https://doi.org/10.3732/ajb.0800411

Moller, A. P., Rubolini, D., & Lehikoinen, E. (2008). Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(42), 16195–16200. https://doi.org/10.1073/pnas.0803825105

Pardee, G. L., Inouye, D. W., & Irwin, R. E. (2017). Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers. *Global Change Biology*, *24*(July), 848–857. https://doi.org/10.1111/gcb.13865

Pardee, G. L., Jensen, I. O., Inouye, D. W., & Irwin, R. E. (2019). Data from: The individual and combined effects of snowmelt timing and frost exposure on the reproductive success of montane forbs. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.kd1r166

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*, 37–42. https://doi.org/10.1038/nature01286

Petanidou, T., Kallimanis, A. S., Sgardelis, S. P., Mazaris, A. D., Pantis, J. D., & Waser, N. M. (2014). Variable flowering phenology and pollinator use in a community suggest future phenological mismatch. *Acta Oecologica*, *59*, 104–111. https://doi.org/10.1016/j.actao.2014.06.001

Petraglia, A., Tomaselli, M., Chiari, G., & Carbognani, M. (2014). Responses of flowering phenology of snowbed plants to an experimentally imposed extreme advanced snowmelt. *Plant Ecology*, *215*, 759–768. https://doi.org/10.1007/s11258-014-0368-1

Polatto, L. P., Chaud-Netto, J., & Alves-Junior, V. V. (2014). Influence of abiotic factors and floral resource availability on daily foraging activity of bees. *Journal of Insect Behavior*, *27*, 593–612. https://doi.org/10.1007/s10905-014-9452-6

Price, M. V., & Waser, N. M. (1998). Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology*, *79*(4), 1261–1271. https://doi.org/10.2307/176741

R Development Core Team (2016). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rafferty, N. E., CaraDonna, P. J., & Bronstein, J. L. (2015). Phenological shifts and the fate of mutualisms. *Oikos*, *124*(1), 14–21. https://doi.org/10.1111/oik.01523.Phenological

Rafferty, N. E., & Ives, A. R. (2011). Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecology Letters*, *14*, 69–74. https://doi.org/10.1111/j.1461-0248.2010.01557.x

Saino, N., Rubolini, D., Lehikoinen, E., Sokolov, L. V., Bonisoli-alquati, A., Ambrosini, R., … Møller, A. P. (2009). Climate change effects on migration phenology may mismatch brood parasitic cuckoos and their hosts. *Biological Conservation*, *5*, 539–541. https://doi.org/10.1098/rsbl.2009.0312

Sakai, A., & Larcher, W. (1987). *Frost survival of plants: Responses and adaptation to freezing stress*. Berlin: Springer-Verlag.

Sanders-Demott, R., & Templer, P. H. (2017). What about winter? Integrating the missing season into climate change experiments in seasonally snow covered ecosystems. *Methods in Ecology and Evolution*, *8*, 1183–1191. https://doi.org/10.1111/2041-210X.12780

Sherwood, J. A., Debinski, D. M., Caragea, P. C., & Germino, M. J. (2017). Effects of experimentally reduced snowpack and passive warming on montane meadow plant phenology and floral resources. *Ecosphere*, *8*, e01745. https://doi.org/10.1002/ecs2.1745

Stefanescu, C., Penuelas, J., & Filella, I. (2003). Effects of climatic change on the phenology of butterflies in the northwest Mediterranean Basin. *Global Change Biology*, *9*, 1494–1506. https://doi.org/10.1046/j.1365-2486.2003.00682.x

Stinson, K. A. (2004). Natural selection favors rapid reproductive phenology in *Potentilla pulcherrima* (Rosaceae) at opposite ends of a subalpine snowmelt gradient. *American Journal of Botany*, *91*(4), 531–539. https://doi.org/10.3732/ajb.91.4.531

Theobald, E. J., Breckheimer, I., & HilleRisLambers, J. (2017). Climate drives phenological reassembly of a mountain wildflower meadow community. *Ecology*, *98*(11), 2799–2812. https://doi.org/10.1002/ecy.1996

Thomson, J. D. (2010). Flowering phenology, fruiting success, and progressive deterioration of pollination in an early-flowering geophyte. *Philosophical Transactions of the Royal Society Series B: Biological Sciences*, *365*, 3187–3199. https://doi.org/10.1098/rstb.2010.0115

Wahren, C. H. A., Walker, M. D., & Bret-Harte, M. S. (2005). Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Global Change Biology*, *11*, 537–552. https://doi.org/10.1111/j.1365-2486.2005.00927.x

Walker, M. D., Walker, D. A., Welker, J. M., Arft, A. M., Bardsley, T., Brooks, P. D., … Turner, P. L. (1999). Long-term experimental manipulation of winter snow regime and summer temperature in arctic and alpine tundra. *Hydrological Processes*, *13*, 2315–2330. https://doi.org/10.1175/JCLI-D-12-00502.1

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., … Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389–395. https://doi.org/10.1038/416389a

Westra, S., Alexander, L. V., & Zwiers, F. W. (2013). Global increasing trends in annual maximum daily precipitation. *Journal of Climate*, *26*, 3904–3918. https://doi.org/10.1175/JCLI-D-12-00502.1

Wheeler, H. C., Hoye, T. T., Schmidt, N. M., Jens-Christian, S., & Forchhammer, M. C. (2015). Phenological mismatch with abiotic conditions—implications for flowering in Arctic plants. *Ecology*, *96*(3), 775–787. https://doi.org/10.1890/14-0338.1

Wipf, S. (2010). Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. *Plant Ecology*, *207*, 53–66. https://doi.org/10.1007/s11258-009-9653-9

Wipf, S., & Rixen, C. (2010). A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Research*, *29*, 95–109. https://doi.org/10.1111/j.1751-8369.2010.00153.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Pardee GL, Jensen IO, Inouye DW, Irwin RE. The individual and combined effects of snowmelt timing and frost exposure on the reproductive success of montane forbs. *J Ecol*. 2019;107:1970–1981. https://doi.org/10.1111/1365-2745.13152