ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/267287722

Phenological mismatch with bees and unfavorable abiotic conditions reduce Claytonia lanceolata reproduction in…

Conference Paper · August 2014

CITATIONS
0

READS
157

3 authors, including:



Zachariah Gezon
The Walt Disney Company
7 PUBLICATIONS **47** CITATIONS
SEE PROFILE



David Inouye
University of Maryland, College Park
198 PUBLICATIONS **9,738** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:



The effect of repeated, lethal sampling on wild bee abundance and diversity View project



Long-term study of the phenology and abundance of flowering at the Rocky Mountain Biological Laboratory, and how that affects pollinators and seed predators. View project

All content following this page was uploaded by Zachariah Gezon on 04 April 2016.

The user has requested enhancement of the downloaded file.

Global Change Biology

Global Change Biology (2016), doi: 10.1111/gcb.13209

Phenological change in a spring ephemeral: implications for pollination and plant reproduction

ZACHARIAH J. GEZON[1,2,3,a], DAVID W. INOUYE[2,4] and REBECCA E. IRWIN[1,2,b]

[1]*Department of Biological Sciences, Dartmouth College, 78 College St, Hanover, NH 03755, USA,* [2]*Rocky Mountain Biological Laboratory, Crested Butte, CO 81224, USA,* [3]*Florida Museum of Natural History, Gainesville, FL 32611, USA,* [4]*Department of Biology, University of Maryland, College Park, MD 20742, USA*

Abstract

Climate change has had numerous ecological effects, including species range shifts and altered phenology. Altering flowering phenology often affects plant reproduction, but the mechanisms behind these changes are not well-understood. To investigate why altering flowering phenology affects plant reproduction, we manipulated flowering phenology of the spring herb *Claytonia lanceolata* (Portulacaceae) using two methods: in 2011–2013 by altering snow pack (snow-removal vs. control treatments), and in 2013 by inducing flowering in a greenhouse before placing plants in experimental outdoor arrays (early, control, and late treatments). We measured flowering phenology, pollinator visitation, plant reproduction (fruit and seed set), and pollen limitation. Flowering occurred approx. 10 days earlier in snow-removal than control plots during all years of snow manipulation. Pollinator visitation patterns and strength of pollen limitation varied with snow treatments, and among years. Plants in the snow removal treatment were more likely to experience frost damage, and frost-damaged plants suffered low reproduction despite lack of pollen limitation. Plants in the snow removal treatment that escaped frost damage had higher pollinator visitation rates and reproduction than controls. The results of the array experiment supported the results of the snow manipulations. Plants in the early and late treatments suffered very low reproduction due either to severe frost damage (early treatment) or low pollinator visitation (late treatment) relative to control plants. Thus, plants face tradeoffs with advanced flowering time. While early-flowering plants can reap the benefits of enhanced pollination services, they do so at the cost of increased susceptibility to frost damage that can overwhelm any benefit of flowering early. In contrast, delayed flowering results in dramatic reductions in plant reproduction through reduced pollination. Our results suggest that climate change may constrain the success of early-flowering plants not through plant-pollinator mismatch but through the direct impacts of extreme environmental conditions.

Keywords: Claytonia lanceolata, climate change, phenological mismatch, phenology, plant reproduction, pollen limitation, pollination

Received 30 July 2015; revised version received 8 December 2015 and accepted 14 December 2015

Introduction

Phenology, the timing of natural history events, is a critical life-history trait of organisms (Forrest & Miller-Rushing, 2010). For species to perform natural history events during times of the year with favorable abiotic conditions, as well as synchronously with other interacting species, they often cue in on one or more abiotic conditions, such as temperature. These abiotic conditions can vary naturally as well as due to human activities, and recent climate warming is associated with advanced phenologies of both animals and plants (Walther *et al*., 2002; Root *et al*., 2003; Anderson *et al*., 2012). Such changes in phenology can have considerable effects on reproduction and survival (Post *et al*., 2008; Thomson, 2010), and altering flowering phenology in particular can have dramatic, typically negative, effects on plant reproduction (Price & Waser, 1998; Kudo & Ida, 2013). However, the mechanisms behind these changes in plant reproduction are not well-understood and have not been experimentally explored. Given that climate change is affecting the flowering phenology of species across the globe (Parmesan & Yohe, 2003; Wolkovich *et al*., 2012), investigating the consequences of and mechanisms behind changes in plant reproduction as phenology varies is vital to our understanding of how plant communities may respond to a changing climate.

At least three, nonmutually exclusive mechanisms could be responsible for observed changes in plant

[a]Present address: Animal Programs, Disney's Animal Kingdom, Animal Programs Administration, PO Box 10000, Lake Buena Vista, FL 32830, USA

[b]Present address: Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695, USA

Correspondence: Zachariah J. Gezon, tel. +1 603 722 0870, fax +1 407 938 1982, e-mail: zak.gezon@disney.com

© 2016 John Wiley & Sons Ltd

reproduction as phenology varies. First, altering phenology can affect the abiotic conditions experienced by an organism, which may affect reproduction. For example, advanced flowering phenology can increase the risk of early-season frost damage in some species of flowering plants (Inouye, 2008), having a negative effect on seed production that year. Second, phenological shifts can have effects on plant reproduction by altering plant and floral traits that mediate species interactions. Plant and floral traits can exhibit plasticity in response to their environment (Price & Waser, 1998; Anderson & Gezon, 2015), and because altering phenology can expose plants to different abiotic conditions, there is the potential for changes in traits that affect species interactions and plant fitness. For example, floral traits such as petal length and stamen number can vary across individuals with phenology (Ishii & Harder, 2012), which could affect plant-pollinator interactions and subsequent plant reproduction (Strauss *et al*., 1996).

Third, altered phenology may result in phenological mismatches between plants and their associates, such as pollinators, resulting in effects on reproduction. Organisms that use unique environmental cues to time life-history events, or that respond to changes in the same environmental cue at different rates, could have differing phenological responses given the same environmental change (Forrest *et al*., 2010). As a result, environmental change can cause phenological mismatches between organisms that currently co-occur in space and time (Post *et al*., 2008). If mismatches occur between organisms involved in consumer-resource interactions, the interacting species could experience positive or negative fitness consequences. Examples of phenological mismatches disrupting plant-herbivore and predator-prey interactions are growing (Rafferty *et al*., 2013), including from both aquatic (Winder & Schindler, 2004) and terrestrial (Visser & Holleman, 2001) systems. However, the occurrence and consequences of phenological mismatches within mutualisms, such as plants and pollinators, have received less attention (Memmott *et al*., 2007; Forrest & Miller-Rushing, 2010), and experimental field data examining plant-pollinator phenological mismatches are rare (but see Hegland *et al*., 2009; Parsche *et al*., 2011; Rafferty & Ives, 2011). The importance of natural variation in flowering phenology has a rich history of study in pollination biology and plant reproductive ecology (e.g., Robertson, 1895; Mosquin, 1971; Waser & Real, 1979), but a greater understanding is needed of the mechanisms driving how phenological change affects pollination and plant fitness.

The goal of this study was to investigate how experimentally altered flowering phenology affected plant–pollinator interactions and plant reproductive success, as well as to assess the mechanisms involved. We tested how manipulating flowering time affected reproduction of the spring ephemeral *Claytonia lanceolata* and the degree to which changes in pollination and abiotic factors affected the plant fitness response. We focused on *C. lanceolata* because its phenology is highly correlated with snow melt timing, it is the first species to flower after snow melt in our study system (subalpine Western Colorado), and it flowers for a short period of time (Price & Waser, 1998), thus increasing the potential susceptibility of this species to phenological mismatch with its mutualist pollinators (Rafferty *et al*., 2015). Our experiments were designed to manipulate flowering phenology, but not pollinator phenology, at a local level, thus allowing us to test the degree to which the strength of plant-pollinator mutualisms may be labile to variation in flowering time. We focused on manipulating flowering phenology to isolate the effects of flowering time on plant reproduction, but manipulating pollinator phenology and abiotic conditions such as frost damage would provide important additional ecological insights within the context of climate change in future research. To manipulate flowering time, we used two approaches. First, we used a snow manipulation to induce earlier flowering of the plant community (Dunne *et al*., 2003), including *C. lanceolata*. Within the snow manipulation treatment, we also tested the degree to which flowering phenology affected pollination by using a pollen-supplementation experiment. Second, because snow manipulation affects not only snow melt timing but also water and nitrogen availability to plants (Pilon *et al*., 1994), which are known to affect plant–pollinator interactions and plant reproduction (Burkle & Irwin, 2010), we also manipulated flowering time and pollination using a field array of potted plants with constant abiotic resources (akin to Rafferty & Ives, 2011, 2012). We predicted that altering flowering phenology would decrease pollinator visitation by creating a phenological mismatch with pollinators, which would increase pollen limitation and decrease plant reproduction. We also predicted that advancing phenology would put plants more at risk of unfavorable abiotic conditions such as early season frost, which would decrease plant reproduction regardless of changes in pollinator visitation. Together, the two approaches allowed us to isolate the effects of experimentally generated variation in flowering phenology on pollinator visitation, pollen limitation, and plant reproduction.

Materials and methods

Study system and focal species

We conducted this study in subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL), Gothic,

Gunnison County, Colorado, USA (elev. 2950 m). Climate at this site follows a cyclic pattern similar to the Atlantic Multidecadal Oscillation (Mccabe *et al*., 2007), but climate forecasts predict that conditions will break from this cyclical pattern. Temperatures are predicted to increase and winter snow fall and total precipitation to decrease (Stewart *et al*., 2005; Overpeck & Udall, 2010), potentially resulting in earlier snow melt (Saunders *et al*., 2008). Flowering phenology of many species in this region is often strongly correlated with the timing of snow melt (Wielgolaski & Inouye, 2013). Although killing frosts historically have occurred every 3 to 5 years in the meadows near the RMBL (Inouye, 2000), they have been occurring more frequently in recent years (Inouye, 2008).

Claytonia lanceolata (Portulacaceae) is the first herbaceous plant to bloom in the spring at this study site, and is a protandrous, obligately out-crossing perennial herb (Douglas & Taylor, 1972; Mistretta & Liston, 1992). *C. lanceolata* typically produces 3–15 flowers per inflorescence, with one to three inflorescences connected to an underground corm (Inouye, pers. obs.). For this study we selected single inflorescences from separate genets, which are hereafter referred to as 'plants.' The white-to-pink actinomorphic flowers are visited primarily by bees collecting nectar and/or pollen, including *Lasioglossum* spp., *Hylaeus* spp., and *Ceratina* spp. (Douglas & Taylor, 1972). Flowering phenology of *C. lanceolata* is highly correlated with date of snow melt in this area, and has advanced by an average of 0.68 ± 0.22 days per year from 1979 to 2012 (CaraDonna *et al*., 2014).

Snow manipulation experiment

We conducted snow manipulations from 2011 to 2013. We haphazardly established a series of 4-m^2 plots in an open meadow (38 °57′58.61″N, 106 °59′25.17″W; elev 2987 m), and randomly assigned each plot to one of two treatments: snow removal and control. We used five plots per treatment in 2011 and 10 plots per treatment in 2012 and 2013. In 2011 and 2013 when natural snow pack melted to approx. 100 cm in depth, we used shovels to reduce snow pack to approx. 5 cm in depth within snow removal plots (Fig. 1a). We included a 0.25 m buffer zone around each plot. Snow depth at the time of snow removal in 2012 was approx. 40 cm due to extremely low snow pack in that year. Control plots were left to melt out naturally, although we used snow shoes and shovels to disturb the ground when the snow naturally melted to approx. 5 cm as a sham treatment. In 2011, we also included five additional plots that we allowed to melt out naturally with no disturbance to test whether disturbing the plots at 5 cm snow depth with shovel and snow shoe activity affected flowering phenology and plant reproduction. We found no effect of disturbance on *C. lanceolata* flowering time or plant reproduction (Appendices S1 and S2). Thus, data from these additional plots were combined in 2011 for a total of 10 control plots.

Flowering phenology and floral abundance. We visited all plots every other day to record snow depth, percent bare ground (estimated visually to the nearest 5%), and flowering phenology of *C. lanceolata* (Fig. 1b). We recorded the number of *C. lanceolata* plants in flower and counted the number of open flowers per plant on up to 10 haphazardly chosen plants per plot per census. To estimate *C. lanceolata* flowering phenology, we calculated four variables per plot per year: 'first flower' (the day of the year when *C. lanceolata* first flowered within a plot), 'peak flower' (the day of the year with the highest total number of flowers within a plot), 'last flower' (the day of the year that the last flower remained within a plot), and 'flowering duration' (the number of days that at least one *C. lanceolata* flower was present in a plot) (CaraDonna *et al*., 2014).

To test for an effect of snow manipulation on date of snow melt, we used a repeated-measures (rm) ANOVA with snow manipulation (snow removal vs. control), year (repeated term), and their interaction as factors, and time of snow melt (day of the year the plot was 100% snow free) as the response variable. We used similar rm-ANOVAs to test for effects of snow removal on flowering phenology (date of first, peak, and last flower), maximum flower density (highest observed value of flowers per m^2 for each plot), and flowering duration. In all analyses, plot was used as the unit of replication. All analyses here and below were performed using JMP®, Version 11.0 (SAS Institute Inc.) unless otherwise noted.

To ensure our snow manipulation treatments induced a realistic phenological response, we compared the flowering phenology of *C. lanceolata* in our experimental plots to long-term *C. lanceolata* flowering phenology data collected from 1979 to 2014 (except 1990) near the RMBL (CaraDonna *et al*., 2014). We used long-term data only from dry, rocky meadow plots because these plots had the most similar habitat to our study site and were geographically close to our study site. We obtained date of bare ground over the 35 years monitoring period from a site at the north end of Gothic, CO (data provided by b. barr, www.rmbl.org). To compare the relationships between flowering phenology and date of bare ground in our study to long-term data, we regressed first, peak, and last flower on date of bare ground, and included in the analysis the source of the data (experiment vs. long-term data) and the bare ground by data source interaction using R version 3.1.0 (R Development Core Team, 2014). We performed similar linear models for maximum flower density, total flower production, and flowering duration.

Floral morphology and water potential. In 2012 we recorded petal width and flower diameter using digital calipers (to the nearest 0.01 mm) for up to three, fully open flowers per plant on up to 16 marked plants per plot. Each flower was measured only once, and measurements were taken every other day until up to three flowers per plant had been measured. We calculated plant and then plot mean values for each metric of morphology. We used the multivariate Hotelling's T^2 to test for differences in petal width and flower diameter between snow removal and control plots. A significant multivariate analysis was followed by univariate *t*-tests for each metric of floral morphology.

Because snow removal can also affect water availability in the soil (Sherwood, 2013), in 2012 we measured leaf water potential (Ψ_l). Measurements of Ψ_l were obtained using a

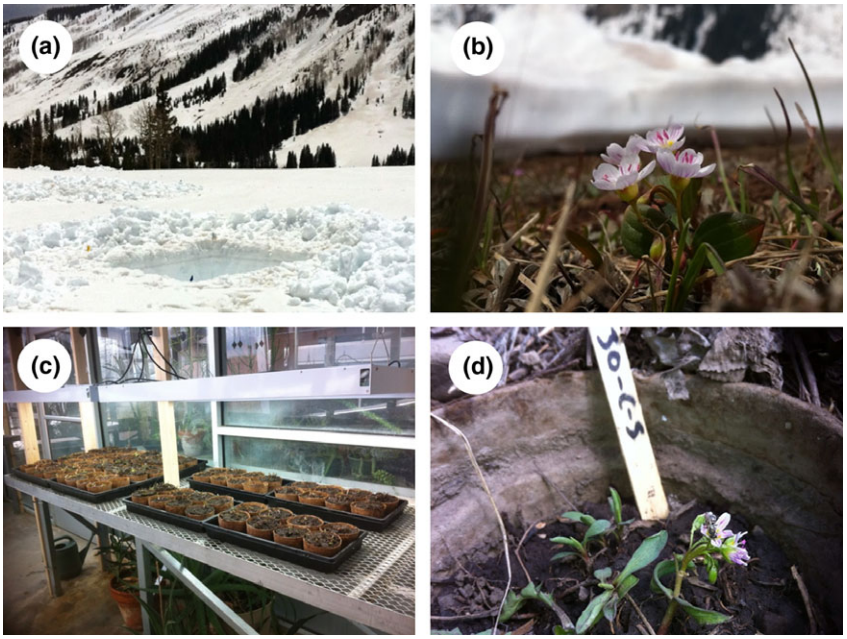

Fig. 1 We used two experimental approaches to alter the phenology of *Claytonia lanceolata*: snow removal within 2 × 2 m plots (a), causing *Claytonia* to flower early (b), and putting potted plants into a greenhouse (c) before placing the plants into an outdoor array (d). Note the *Lasioglossum* bee visiting the potted *Claytonia* (d).

hand-pump pressure chamber (PMS Instruments Co., Corvallis, OR, USA) and were taken at the time of peak flowering in each plot. All measurements were taken once each pre- and postdawn. We destructively sampled one entire inflorescence for each measurement (i.e., the entire inflorescence was placed in the pressure chamber). We collected predawn measurements at approx. 0500 (before the plants were photosynthesizing for the day) and postdawn at approx. 1400 (when the sun was at the highest point in the sky). After measuring Ψ_l, we dried the inflorescences at 60 °C for 48 h and measured their dry mass (to 0.01 g). To test for differences in leaf water potential between snow removal and control plots, we used a general linear model with snow treatment, measurement time (predawn vs. postdawn), and their interactions as factors and dry mass as a covariate.

Pollinator visitation. In 2011 and 2012, we observed pollinator visitation to *C. lanceolata* every other day. Each census day, we observed each plot for 20 min, for a total of 80 h of observation. We randomized the order in which we observed the plots to ensure that visitation was not confounded with time of day. For each floral visitor to *C. lanceolata*, we recorded the number of flowers probed per plant, time spent visiting each flower, and total bout time per plot. We identified the visitor to the lowest taxonomic resolution possible on the wing, often to genus, but sometimes to family or order. We collected representative specimens of visitors to assist in on-the-wing identification. We calculated the total number of plants visited, mean flowers visited per plant, mean time per flower, and total visitation time (summed across bouts). Reductions in total plants visited and total visitation time with earlier flowering may be most indicative of phenological mismatch. To test how snow manipulation affected metrics of pollinator visitation, we used ANOVA with snow treatment, year, and their interaction as fixed effects; we used separate analyses for each response variable. We included plot within year as a random effect to account for temporal autocorrelation. Total visitation time was $\log(x + 1)$ transformed to meet the assumption of normally distributed residuals. We used orthogonal linear contrasts to test the *a priori* hypothesis that pollinator visitation would be lower in snow removal than control treatments within each year.

We could not include floral morphology as a covariate in the above model because floral morphology was recorded in 2012 only and the significant difference in morphology between snow treatments (see Results) would violate an assumption of ANCOVA. We therefore performed a correlation analysis across snow removal treatments to assess the relationship between floral morphology (flower diameter) and metrics of pollinator visitation (total visitation time and time per flower) using plot as the unit of replication. We did not include petal width in this analysis because it was positively correlated with flower diameter ($r = 0.79$, $P < 0.0001$).

Pollen limitation and plant reproduction. We performed supplemental hand pollinations on every flower of each marked inflorescence within snow treatments. In each plot, up to 16 *C. lanceolata* were randomly assigned to either supplemental or open pollination treatments (eight plants per treatment). We performed hand pollination every other day throughout the flowering period. Each flower of pollen-supplemented plants was given supplemental pollen using

anthers collected from at least three donor plants within 10 m of the plot. Each flower of open-pollinated plants was similarly handled but not supplemented with pollen.

We collected all fruits when they were fully expanded and counted seeds within each fruit, visually separating mature vs. aborted seeds (similar to Williams & Winfree, 2013). Aborted fruits were collected and recorded as producing no seeds. For each plant, we calculated four metrics of plant reproduction: total seed set (number of mature seeds), proportion seed set (number of mature seeds/total ovules), total fruit set (number of expanded fruits), and proportion fruit set (number of seed-bearing fruits/total flowers produced per plant). Proportion seed and fruit set can be highly sensitive to differences in pollination, while total seed and fruit set may reflect underlying resource status and other differences in the abiotic environment as well as pollination. For each response variable, we calculated the mean per plant per treatment per plot. While counting seeds, we also recorded visual signs of predispersal seed predation (frass or larvae inside fruits). Rates of predispersal seed predation were <1% of fruits and so we did not consider seed predation further in statistical analyses (here and in the array experiment).

To test for differences in reproduction between pollination treatments within snow treatments (snow removal vs. control) and among years, we used a MANOVA with snow treatment, pollination treatment nested within snow treatment, year, and their interactions as fixed effects. A significant MANOVA was followed by univariate analyses for each metric of reproduction (Scheiner, 1993). In the univariate analyses, we included unique plot nested within year as a random effect to account for temporal autocorrelation. Seed set was $\log(x + 1)$ transformed to meet the assumption of normality. We used orthogonal linear contrasts to test the *a priori* hypothesis that *C. lanceolata* reproduction would be higher in supplemental- than open-pollination treatments in snow removal but not control treatments. We performed a separate contrast for each snow treatment in each year.

Plant reproduction as a function of frost damage and pollinator visitation. In 2011 and 2012, we recorded visual signs of frost damage (wilted, damaged petals) every other day. Because the probability of experiencing frost varies with phenology (Inouye, 2008), including frost damage in the above models would have been redundant. We therefore performed a second analysis of plant reproduction (log(mature seeds + 1)) as a function of frost damage (% flowers per plant damaged by frost), total visitation time ($\log(x + 1)$ transformed), and pollination treatment as effects, and plot as a random term. We included the interaction between frost damage and visitation time to test the prediction that plant reproduction would increase with visitation, but only in plants that did not experience frost damage. Snow manipulation treatment and year were not included in this model, and instead served to increase variation in the explanatory variables (akin to Gotelli & Ellison, 2006). Frost damage and pollinator visitation were measured in 2011 and 2012, but not 2013; thus, 2013 was excluded from this analysis.

Array experiment

In 2013 we used a greenhouse to induce variation in flowering of 234 plants, which we then subsequently placed in the field. We created three phenology treatments (early, control, and late phenology) by placing 78 plants at a time in the greenhouse at 8-day intervals (Fig. 1c). Although delayed flowering is not expected under climate change for this study system, we included the late flowering treatment to simulate a scenario where pollinators are advanced in phenology relative to *C. lanceolata*. All plants were potted from a single field population (38 °53′1.77″N, 106 °59′0.50″W, elev. 2736 m). We potted plants on 26 April 2013 for the 'early' phenology treatment and 6 May 2013 for the 'control' phenology treatment. Plants were potted into 12.7 cm round peat pots, watered with root stimulator (Scott's Miracle-Gro Liquid Quick Start, Marysville, OH, USA) for the first 2 days and subsequently fertilized twice per week with 5-1-1 NPK fertilizer (Alaska Fish Fertilizer Co., Renton, WA, USA). Plants were placed in a greenhouse with 14 h light/10 h dark at 25–30 °C during the day and 20–25 °C at night. For the 'late' phenology treatment, we potted plants on 27 April 2013 but to delay flowering, we buried the plants under 1 m of snow at a higher elevation site (38 °54′35.4″N, 106 °57′27.6″W; elev. 2988 m). On 13 May 2013 late-phenology plants were removed from snow, watered with root stimulator and placed in the greenhouse. When ≥50% of the plants within each treatment had at least one flower open (on 29 April for early, 7 May for control, and 15 May for late phenology treatments), we moved them from the greenhouse to an outdoor array (38 °53′14.4″N, 106 °57′42.0″W; elev. 2847 m), with plants spaced 1 m apart (Fig. 1d). Plants were randomly assigned to locations within the array. A spatial arrangement of 1 m among neighbors matches natural plant spacing in the field, and the 8-day interval between early, control, and late treatments was similar to the phenological effect from the snow removal experiment (see Results). Pots were buried into the ground, and plants were left in the array until all fruits were collected.

Flowering phenology and pollinator visitation. Every other day, we recorded the number of flowers open on each plant and the number with visible frost damage (wilted, damaged petals). We also observed the array for pollinator visits for 1 h each in the morning and afternoon every other day to flowers and plants in the array. We calculated the mean foraging time per flower for each plant. Visitation rate per plant was calculated as the number of flowers visited per hour of observation. To test for differences in metrics of pollinator visitation among phenology treatments, we used separate zero-inflated negative binomial models using the glmmADMB package in R (Fournier *et al.*, 2012; Skaug *et al.*, 2013). All visitation times were rounded to the nearest integer to meet the assumptions of a negative binomial model. Each model had visitation (either foraging time per flower or visitation rate) as a function of phenology treatment. We then performed Tukey post hoc comparisons using the multcomp function in R version 3.1.0 (Hothorn *et al.*, 2008) to assess how visitation varied with phenology treatments.

Pollen limitation and plant reproduction. We performed supplemental hand pollinations at the whole plant level every other day within phenology treatments. In each phenology treatment, we randomly assigned 50 plants to either supplemental or open-pollination (25 plants per treatment), and left the remaining 28 plants per treatment in the array as pollen donors. We supplied pollen using *C. lanceolata* anthers collected from the source population using the pollen donor plants within the array. Each female-phase flower of open-pollinated plants was handled but not supplemented with pollen. Once plants ceased flowering, we collected all expanded fruits and counted seeds to estimate plant reproduction. Plant reproduction was measured with the same metrics used in the snow manipulation experiment.

To test how phenology and pollination treatments affected reproduction, we first performed a multivariate analysis with phenology and pollination treatments and their interactions as fixed effects followed by univariate analyses (Poisson errors and canonical link function) for each metric of reproduction. We used an overdispersion factor to adjust the SE and test statistics when overdispersion was detected using goodness-of-fit tests. We used orthogonal linear contrasts to test the *a priori* hypothesis that *C. lanceolata* reproduction would be higher in hand than open pollination treatments, and that reproduction in the control phenology treatment would be higher than either early or late treatments. A significant interaction between phenology and pollination treatments would suggest that the benefits of supplemental pollination are moderated by flowering time.

Plant reproduction as a function of frost damage and pollinator visitation. We performed an additional GLM analysis of plant reproduction (seed set) with frost damage (% flowers per plant damaged by frost), pollinator visitation rate (log (x + 1) transformed), and pollination treatment (hand vs. open pollination) as fixed effects (Poisson errors and canonical link function). To test the prediction that plant reproduction would increase with visitation, but only in plants that did not experience frost damage, we included the interaction between pollinator visitation rate and frost damage. Phenology treatment was not included in this model because the probability of frost damage covaries with the phenology treatment (see Results).

Results

Snow manipulation experiment

Phenology and flower abundance. Snow removal advanced date of bare ground by 17.38 ± 0.52 days (mean ± SE) compared to control plots ($F_{1,\ 50}$ = 1115.75, P < 0.0001; Fig. 2a). Snow pack and ambient snow melt also varied significantly among years ($F_{2,\ 50}$ = 2709.19, P < 0.0001; Fig. 2a), with 2012 experiencing record low snow fall and early snow melt. Snow melt was earlier in snow removal than control plots in all years, although a significant year-by-treatment interaction was driven by a greater difference between snow removal vs. control treatments in 2013 ($F_{2,\ 50}$ = 46.72, P < 0.0001; Fig. 2a).

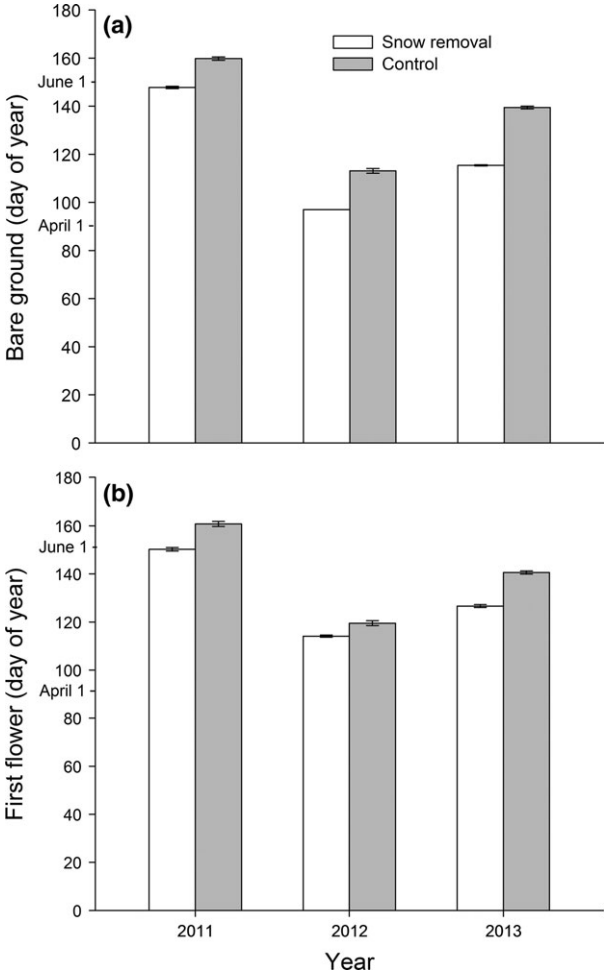

Fig. 2 Snow removal experiment: date of bare ground was significantly earlier in snow removal plots than in control plots in all 3 years of study (a), causing *Claytonia lanceolata* in snow removal plots to flower on average 10 days earlier than control plots (b). Bars represent means ± 1 SE.

Snow removal had a significant effect on *C. lanceolata* flowering phenology. Dates of first, peak, and last flower were 9.98 ± 0.67 (Fig. 2b), 6.81 ± 0.65 (Appendix S3a), and 5.75 ± 0.94 (Appendix S3b) days earlier in snow removal than control plots ($F_{1,\ 50}$ = 220.04, P < 0.0001; $F_{1,\ 50}$ = 109.62, P < 0.0001; $F_{1,\ 50}$ = 37.57, P < 0.0001 respectively). Flowering duration was significantly longer in snow removal than control plots (4.23 ± 0.99 days longer, $F_{1,\ 50}$ = 18.39, P < 0.0001; Appendix S3c) and varied significantly among years ($F_{2,\ 50}$ = 4.48, P = 0.016; Appendix S3c). *C. lanceolata* flowered for the longest period of time in 2012 (20.30 ± 0.80 days) and shortest period of time in

2011 (16.55 ± 0.98 days). Maximum flower density varied significantly among years ($F_{2,\ 50} = 24.77$, $P < 0.0001$), with plots achieving 3.30 times higher flower density in 2011 vs. 2012 (Appendix S4). However, maximum flower density was not affected by snow removal treatment ($F_{1,\ 50} = 2.45$, $P = 0.12$).

When we compared metrics of *C. lanceolata* phenology and date of bare ground between the snow removal experiment and long-term phenology plots, we found that earlier bare ground advanced flowering but the strength of the relationship varied between the two datasets. In particular, we found significant, positive relationships between date of first ($\beta = 0.75 \pm 0.03$, $F_{1,\ 87} = 675.57$, $P < 0.0001$; Appendix S5a), peak ($\beta = 0.66 \pm 0.02$, $F_{1,\ 87} = 734.48$; Appendix S5b), and last flower ($\beta = 0.64 \pm 0.03$, $F_{1,\ 87} = 617.63$, $P < 0.0001$; Appendix S5c) and date of bare ground ($r^2 \geq 0.90$ in all cases). Likewise, we found significant, positive relationships between maximum flower density and date of bare ground ($\beta = 3.97 \pm 0.54$, $F_{1,\ 87} = 54.15$, $P < 0.0001$; Appendix S5D) and between total flower production and date of bare ground ($\beta = 9.29 \pm 1.46$, $F_{1,\ 87} = 40.66$, $P < 0.0001$; Appendix S5E) ($r^2 \geq 0.31$ in both cases). However, for all of these response variables, we found significant interactions between date of bare ground and data source ($F_{1,\ 87} \geq 5.97$, $P < 0.023$ in all cases), driven primarily by the snow removal plots, which had a slower phenological response to bare ground. In addition, we found a significant, negative relationship between flowering duration and date of bare ground ($\beta = -0.11 \pm 0.03$, $F_{1,\ 87} = 17.14$, $P < 0.0001$; Appendix S5f) with no interaction between date of bare ground and data source ($F_{1,\ 87} = 0.08$, $P = 0.78$).

Floral morphology and water potential. Flowers from plants growing in the snow removal plots were significantly smaller than those in control plots (Hotelling's $T^2_{16} = 6.22$, $P = 0.010$). Mean petal width was 10% smaller ($t_{16.98} = 2.95$, $P = 0.009$), and mean flower diameter was 7% smaller ($t_{16.99} = 2.26$, $P = 0.037$) in snow removal compared to control plots. Moreover, leaf water potential varied significantly with snow treatment ($F_{1,\ 35} = 42.57$, $P < 0.0001$) and measurement time (predawn vs. mid-day; $F_{1,\ 35} = 7.44$, $P = 0.025$), with a significant treatment-by-time interaction ($F_{1,\ 35} = 16.60$, $P = 0.0003$; Appendix S6). Predawn water potential measurements were more negative than mid-day measurements in the snow removal plots, with the opposite pattern for control plots, and at mid-day, snow removal plots had less negative water potential measurements than control plots. We found no effect of the covariate, leaf weight, on water potential ($F_{1,\ 35} = 0.07$, $P = 0.80$), and the predawn measurements were not significantly different between the snow removal and control treatments ($t_{35} = 1.70$, $P = 0.10$).

Pollinator visitation. We observed 2172 total seconds of visitation by 159 individual visitors. Across treatments and years, sweat bees *Lasioglossum* (*Dialictus*) were the most common visitors to *C. lanceolata*, comprising 46.6% of all visitors, followed by syrphid flies (18.0%), and bees of the genera *Halictus* (12.4%) and *Osmia* (5.6%). Bees from the genus *Colletes* were only observed visiting *C. lanceolata* in 2011, and bees from the genus *Hylaeus* were only observed visiting in 2012.

We found significant interactions between snow removal treatments and year for every metric of pollinator visitation (Appendix S7), with generally higher pollinator visitation to *C. lanceolata* in snow removal compared to control plots in 2011 but the opposite pattern in 2012 (Fig. 3). Linear contrasts revealed that total visitation time and mean foraging time per flower were 6.88 and 2.60 times greater in snow removal than control plots in 2011 ($t_{31} = 2.29$, $P = 0.023$ and $t_{31} = 2.23$, $P = 0.033$, respectively), but we observed the opposite pattern in 2012 with total visitation time and mean foraging time per flower being 13.50 and 2.40 times lower in snow removal than control plots ($t_{31} = 3.78$, $P = 0.0005$; Fig. 3a and $t_{31} = 2.52$, $P = 0.017$; Fig. 3b respectively). Mean number of plants visited and mean flowers visited per plant did not vary between snow treatments in 2011 ($t_{31} = 1.69$, $P = 0.10$ and $t_{31} = 1.69$, $P = 0.10$, respectively), but were 5.23 and 1.73 times lower in snow removal than control plots in 2012 ($t_{31} = 3.67$, $P = 0.0009$; Fig. 5c and $t_{31} = 2.09$, $P = 0.045$; Fig. 3d respectively). We found no main effect of snow removal treatment on any metric of pollinator visitation, but we did find main effects of year on total visitation time and mean number of plants visited (Appendix S7). Total visitation time was 3.93 times higher and mean number of plants visited was 2.08 times higher in 2011 than 2012.

In 2012, we found a significant, positive relationship between pollinator visitation and floral diameter both in terms of total visitation time ($r = 0.50$, $N = 19$, $P = 0.03$; Appendix S8) and mean time per flower ($r = 0.49$, $N = 19$, $P = 0.03$; Appendix S8). These data suggest that. *C. lanceolata* with smaller flowers in the snow removal plots experienced lower pollinator visitation than plants with larger flowers in the control plots.

Pollen limitation and plant reproduction. We observed striking differences in plant reproduction among years (Fig. 4 and Appendix S9), with seed set in 2012 being dramatically lower than in either 2011 or 2013 (8.39 and 2.25-times lower, respectively). Although the strength of pollen limitation varied among snow

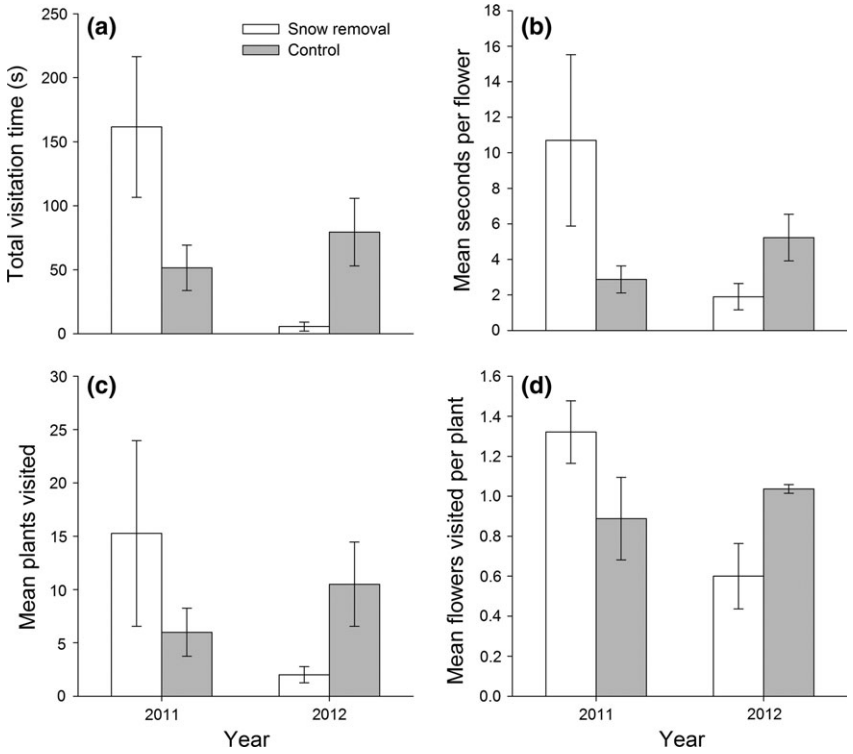

Fig. 3 Snow removal experiment: pollinators varied in their response to snow removal treatments between years, measured as (a) total visitation time (summed across foraging bouts), (b) mean time per flower, (c) mean number of plants visited, and (d) mean flowers visited per plant. Bars are means ± 1 SE.

removal treatments and years (Table 1), the direction of the effect was not predicted based on patterns of pollinator behavior. For example, although we found higher pollinator visitation in snow removal than control plots in 2011, plants were pollen limited for reproduction in both snow removal and control plots (Fig. 4a, Appendix S9a, Table 1, Appendix S10), and the effect of hand-pollination on seed set was 3.76-times higher in the snow removal compared to control plots (Fig. 4a and Appendix S9a). Pollinator visitation was higher in 2011 than 2012, but plants in 2012 were not pollen limited in either snow treatment, suggesting that pollen supplementation did not rescue plants from reproductive failure (Fig. 4b, Appendices S9 and S10). In contrast, in 2013 (which was more average in terms of phenology and snow melt, Fig. 2 and Appendix S3), plants were pollen limited in control plots but not snow removal plots (Fig. 4c, Appendices S9 and S10).

We also found that plant reproduction varied significantly by snow treatment (Table 1), but not always in the expected direction based on pollen limitation. For example, seed set was 55.7% higher in snow removal than control plots in 2011 (Fig. 4a and Appendix S9a), despite being more pollen limited. Conversely, seed set was 80.0% higher in control than snow removal plots in 2013, despite being pollen limited (Fig. 4c and Appendix S9c). Taken together, these results suggest strong interannual variation in how plants in snow removal treatments respond to pollinator visitation and supplemental pollination, and thus an underlying role of the abiotic environment in modifying the response of plants to snow removal and pollination.

Plant reproduction as a function of frost damage and pollinator visitation. In 2011, we observed no plants damaged by frost in either the snow removal or control treatments. In 2012, however, 49.0% ± 8.90 and 35.9% ± 2.89 of flowers per plant were frost damaged in the snow removal and control plots respectively. As predicted, frost damage had a significant, negative effect on plant reproduction ($\beta = -1.25 \pm 0.47$, $F_{1,\ 56.95} = 6.90$, $P = 0.011$) and there was a significant interaction between frost damage and pollinator visitation for plant reproduction ($F_{1,\ 50.64} = 24.59$, $P < 0.0001$), suggesting that frost damage moderates the effect of pollinator visitation on plant reproduction. There was no main effect of pollinator visitation on plant reproduction ($\beta = 0.007 \pm 0.05$, $F_{1,\ 30.73} = 0.02$, $P = 0.88$). Seed set was 31.2% higher in supplemental than open

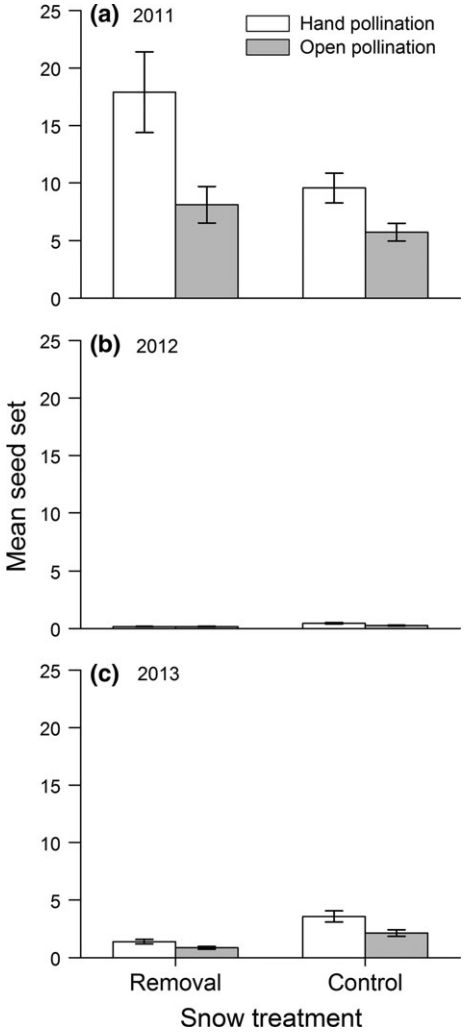

Fig. 4 Snow removal experiment: plant reproduction measured as seed set in (a) 2011, (b) 2012 and (c) 2013 in snow removal and control plants as a function of pollen supplementation. Bars represent means ± 1 SE.

Table 1 Snow removal experiment: MANOVA and subsequent univariate analyses testing for differences in plant reproduction between pollination (supplemental vs. open pollination) treatments within snow manipulations (snow removal vs. control) and among years. Bold values indicate statistical significance

Snow	Wilks' λ	DF	*F* ratio	*P*
Multivariate test		3, 96	8.98	**<0.0001**
Seed set		1, 49	1.23	0.27
Proportion seed set		1, 49	1.39	0.24
Fruit set		1, 49	13.69	**0.0005**
Proportion fruit set		1, 49	11.89	**0.001**
Pollination [snow]				
Multivariate test	0.77	6, 192	4.41	**0.0003**
Seed set		2, 49	11.45	**<0.0001**
Proportion seed set		2, 49	6.84	**0.002**
Fruit set		2, 49	6.28	**0.004**
Proportion fruit set		2, 49	8.94	**0.0005**
Year				
Multivariate test	0.12	6, 192	60.21	**<0.0001**
Seed set		2, 49	180.63	**<0.0001**
Proportion seed set		2, 49	190.35	**<0.0001**
Fruit set		2, 49	4.68	**0.014**
Proportion fruit set		2, 49	18.20	**<0.0001**
Snow × year				
Multivariate test	0.61	6, 192	9.14	**<0.0001**
Seed set		2, 49	10.62	**<0.0001**
Proportion seed set		2, 49	13.10	**<0.0001**
Fruit set		2, 49	9.22	**0.0004**
Proportion fruit set		2, 49	13.21	**<0.0001**
Pollination [snow] × year				
Multivariate test	0.89	12, 254	2.77	**0.006**
Seed set		4, 49	2.42	**0.060**
Proportion seed set		4, 49	1.84	0.14
Fruit set		4, 49	4.91	**0.002**
Proportion fruit set		4, 49	3.30	**0.018**

pollination plants, although the difference was not statistically significant ($F_{1,\ 30.29} = 2.71$, $P = 0.11$). Taken together, these results suggest that pollinator visitation benefited plant reproduction, but only when plants were not frost damaged, whereas frost-damaged plants had low reproduction regardless of pollinator visitation.

Array experiment

Pollinator visitation. We observed 968 total seconds of visitation to the experimental arrays by 79 individual visitors. The composition of floral visitors varied based on phenology treatment (Appendix S11). Early-flowering plants were only visited by *Lasioglossum* (*Dialictus*) bees (Appendix S11a). Control plants were primarily visited by *Lasioglossum* (*Dialictus*), but also by the bees *Lasioglossum* (*Evylaeus*), *Sphecodes*, and *Osmia* as well as bombyliid flies and butterflies (Appendix S11b). Late-flowering plants were visited by *Lasioglossum* (*Dialictus*), *Bombus flavifrons* queens, and syrphid flies (Appendix S11c). Moreover, metrics of pollinator visitation varied significantly among phenology treatments, both in terms of visitation rate ($\chi^2 = 8.52$, df = 2, $P = 0.014$; Fig. 5a) and foraging time per flower ($\chi^2 = 13.75$, df = 2, $P < 0.001$; Fig. 5b). Visitation rate was over five-times higher in early and control treatments than in the late treatment ($z = -2.76$, $P = 0.013$ and $z = -2.92$, $P = 0.007$, respectively), but did not differ between early and control treatments (Fig. 5a and Appendix S12). Foraging time per flower was 3.63-times higher in the control compared to late phenology treatments ($z = -2.94$, $P = 0.007$; Fig. 5b and

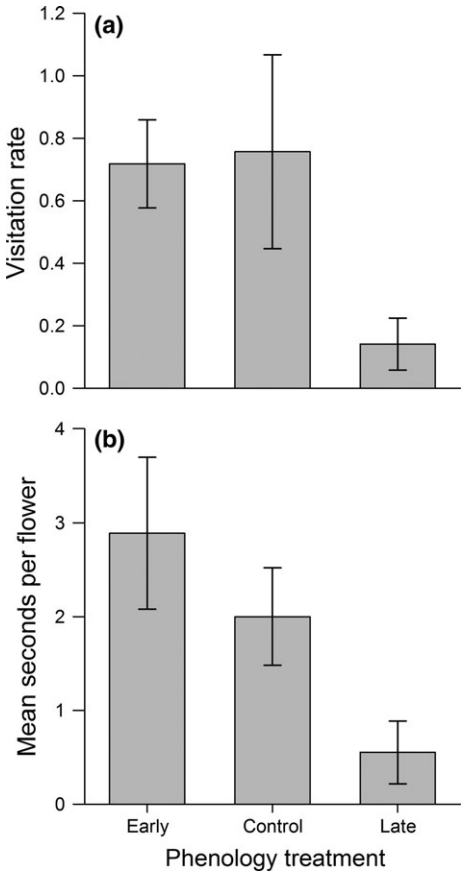

Fig. 5 Array experiment: pollinator visitation to *Claytonia lanceolata* as a function of phenology treatment. Visitation in the late phenology treatment was significantly lower than visitation to plants in the early and control treatments in terms of (a) visitation rate (number of flowers visited per hour per plant), and (b) mean seconds spent foraging per flower. Bars represent means ± 1 SE.

Table 2 Array experiment: MANOVA and subsequent univariate analyses testing for differences in plant reproduction between pollination treatments (supplemental vs. open pollination), phenology treatments (early, control, and late), and their interactions. Bold values indicate statistical significance

Phenology	Wilks' λ	DF	χ^2 or F	P
Multivariate test	0.51	6, 282	18.75	**<0.0001**
Seed set		2	80.28	**<0.0001**
Proportion seed set		2	50.38	**<0.0001**
Fruit set		2	15.88	**0.0004**
Proportion fruit set		2	53.65	**<0.0001**
Pollination				
Multivariate test		3, 141	4.09	**0.008**
Seed set		1	1.54	0.22
Proportion seed set		1	1.82	0.18
Fruit set		**1**	13.54	**0.0002**
Proportion fruit set		**1**	7.94	**0.005**
Phenology × pollination				
Multivariate test	0.88	6, 282	3.17	**0.005**
Seed set		2	5.96	**0.050**
Proportion seed set		2	5.48	0.065
Fruit set		**2**	11.31	**0.004**
Proportion fruit set		**2**	5.79	0.055

Appendix S12), but did not differ significantly between any other treatment comparisons (Fig. 5b and Appendix S12).

Pollen limitation and plant reproduction. Even though early-phenology plants had high pollinator visitation, they were still pollen limited for some metrics of plant reproduction (Table 2 and Appendix S13). Plants in the early-phenology treatment were pollen limited for fruit set and proportion fruit set, but were not pollen limited for seed set or proportion seed set (Fig. 6 and Appendix S13). The plants in the control phenology treatment, however, were not pollen limited for any metric of reproduction (Fig. 6 and Appendix S13) even though they did not receive higher pollinator visitation than the early phenology treatment. Plants in the late phenology treatment received almost no pollinator visitation, and were severely pollen limited for all metrics of reproduction (Appendix S13). For example, seed set in the hand-pollinated plants was 6.42-times higher than in the open-pollinated plants in the late phenology treatment (Fig. 6). Finally, reproduction of the control phenology plants was higher than the other phenology treatments, with seed set of the open pollination plants in the control phenology treatment being over 11.80-times higher than in either the early or late treatments (Fig. 6 and Appendix S13).

Plant reproduction as a function of frost damage and pollinator visitation. In the early phenology treatment, 37.5% of the flowers per plant were frost damaged, but in the control and late phenology treatments, only 2.1% and 0% of the plants were frost damaged respectively. As predicted, there was a significant, negative relationship between frost damage and plant reproduction ($\beta = -2.39 \pm 0.34$, $\chi^2 = 67.88$, df = 1, $P < 0.0001$) and there was a significant interaction between frost damage and pollinator visitation ($\beta = -1.56 \pm 0.60$, $\chi^2 = 7.18$, df = 1, $P = 0.007$), suggesting that frost damage moderates the effect of pollinator visitation on plant reproduction. There was no main effect of pollinator visitation on plant reproduction ($\beta = -0.03 \pm 0.09$, df = 1, $\chi^2 = 0.11$, $P = 0.74$). Seed set was significantly higher in supplemental than open pollination plants ($\beta = -0.22 \pm 0.04$, df = 1, $\chi^2 = 31.09$, $P < 0.0001$), indicating that the plants were pollen limited, in general.

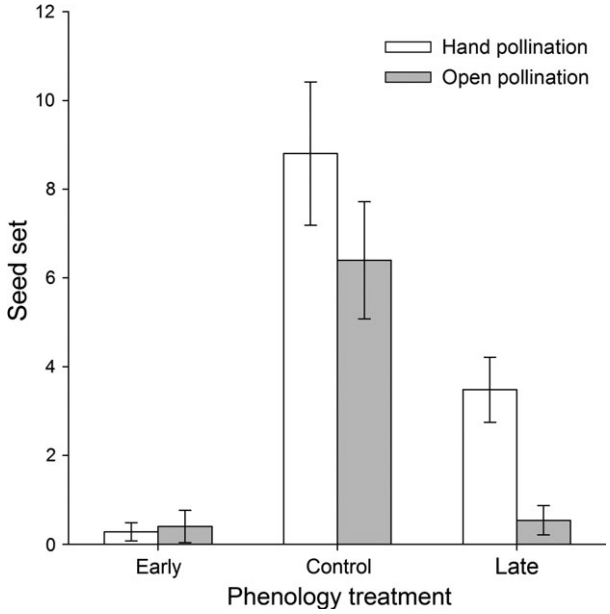

Fig. 6 Array experiment: plant reproduction measured as seed set and the strength of pollen limitation varied as a function of phenology treatment. Bars represent means ± 1 SE.

The results of the array experiment therefore support and build upon those from the snow manipulation experiment by suggesting that pollinator visitation benefited plant reproduction, but only when plants were not frost damaged, and that plants that received little visitation (e.g., the late treatment) suffered low reproduction regardless of other abiotic conditions.

Discussion

By altering the phenology of a spring wildflower, our study elucidated some of the potential consequences of phenological change for plant-pollinator interactions and plant reproduction, as well as revealed some of the mechanisms involved. We found that the degree of pollen limitation varied with phenology as a consequence of changes in abiotic conditions as much as, or more than, changes in interactions with pollinators. Earlier flowering increased the probability of experiencing frost damage, with severe consequences for plant reproduction. On the other hand, increased water potential as a consequence of advanced phenology suggests that the plants were released from resource limitation, increasing plant reproduction and putting more demand on plants for pollination services. However, it is important to note that this release from resource limitation may be an artifact of the snow manipulation and thus may not reflect the true consequences of climate change. We therefore performed the array experiment, which eliminated differences in water potential as a confounding factor, and found strikingly similar results. Although relatively small changes in flowering phenology dramatically altered plant-pollinator interactions, pollinator visitation limited plant reproduction only when abiotic conditions were favorable. Taken together, our results indicate that changes in flowering time can alter the assemblages and visitation rates of pollinators, but we do not find evidence that earlier flowering on the scale that we achieved will result in a phenological mismatch between plants and pollinators. *Claytonia lanceolata* appears well adapted to its current flowering time, with early flowering constrained by extreme weather events and late flowering by low pollination.

Frost damage played an important role in shaping both plant reproduction and the degree of pollen limitation. Our results support previous work showing that experimentally advancing phenology increases the likelihood of frost damage (Williams *et al*., 1992; Molgaard & Christensen, 1997), and that frost events can have catastrophic consequences for plant reproduction (Agrawal *et al*., 2004). In our experiments, plants that experienced frost always suffered low reproduction regardless of other biotic and abiotic conditions, and providing supplemental pollen did not rescue frost damaged plants from low reproductive output. Our results are consistent with other pollen limitation studies, which have shown that frost-damaged flowers cannot take advantage of pollen supplementation to boost reproduction and many fail to reproduce (Jacquemart, 1997). Interestingly, earlier-flowering plants sometimes received higher pollinator visitation, but the benefits of increased pollination were overwhelmed by the negative effects of frost damage. Thus, climate change may constrain the success of early-flowering plants through impacts of extreme environmental conditions rather than plant-pollinator mismatch. Our results are relevant to the growing concern over disruptions of plant-pollinator interactions under climate change scenarios because frost events are expected to become more common and intense as climate change progresses in some systems (Inouye, 2000; Augspurger, 2013). We show strong effects of frost on flower production and plant reproduction. The degree to which frost affects pollinators directly via freezing events and indirectly via lower flower availability requires investigation.

Simultaneous increases in plant reproduction and pollen limitation in the snow removal experiment in 2011 may be due to release from resource limitation. Contrary with our predictions, snow removal plants that did not experience frost (2011) had higher reproduction than control plants, but were severely pollen limited despite receiving greater pollinator visitation. One relevant hypothesis to explain this result is that

these early-flowering plants under frost-free conditions may have been released from resource limitation. An increase in water from surrounding snow melt may have caused higher soil moisture in the snow removal plots while *C. lanceolata* was in flower relative to control plots. While other snow removal studies have found that soil moisture (Sherwood, 2013) and water potential (Pilon *et al*., 1994) decrease, our results suggest the opposite pattern for *C. lanceolata*. *C. lanceolata* is the first forb to flower each spring in the area (CaraDonna *et al*., 2014), typically flowering within days of bare ground. For example, in 2011, *C. lanceolata* in the snow removal plots flowered when the average snow depth of the surrounding meadow was still over 50 cm. Therefore, as the snow melted, the flowering plants in the snow removal plots were likely irrigated by the surrounding snow melt. Although these results do not reflect the probable consequences of climate change, they may mirror early-flowering plant populations, such as those on south facing slopes, which may receive greater early season irrigation from snow melt.

Changes in pollinator visitation that accompanied advanced flowering time affected pollen limitation and plant reproduction. *C. lanceolata* was primarily visited by the small sweat bees *Lasioglossum* (*Dialictus*), which are most abundant early in the season (Gezon *et al*., 2015). We found no difference in pollinator visitation rates between the early and control treatment plants in the array experiment. The early phenology plants were set up before snow had melted over the majority of the focal meadow, and, to the best of our knowledge, before any species of flowering plant were flowering within the foraging range of *Lasioglossum* (*Dialictus*). Nevertheless, *Lasioglossum* (*Dialictus*) began foraging on the experimental *C. lanceolata* as the array was being established. Therefore, despite limited bare ground and a lack of natural flowers for forage, *Lasioglossum* (*Dialictus*) had emerged and were actively looking for floral resources, suggesting that bees may be emerging earlier than their host plants under some climate conditions. Given that *Lasioglossum* (*Dialictus*) typically overwinter underground as adults (Plateaux-Quénu, 2008), their early emergence may imply overwintering in areas that melt out first (e.g., south facing slopes) or movement up-slope soon after emergence. Likewise, in 2011, plants in snow removal plots received significantly higher visitation than plants in control plots, principally by *Lasioglossum* (*Dialictus*). However, in 2012, we found the opposite pattern with lower pollinator visitation rates to plants in snow removal relative to control plots. Because plants in 2012 experienced frost, this lower visitation rate could be due to frost-damaged plants being less attractive to pollinators and/or direct effects of frost on pollinator emergence time or abundance. Preliminary evidence suggests that the number of ground-nesting bees emerging from sites is lower when snow pack is reduced (Z. J. Gezon, D. W. Inouye, R. E. Irwin, *unpublished data*), but further investigation is required.

Turning to the late-flowering treatment in the array experiment, the late-phenology plants likely missed the early-season period of high *Lasioglossum* (*Dialictus*) abundance, resulting in pollen limitation and a near failure to reproduce in the open pollination treatment. This result suggests that a plant-pollinator phenological mismatch occurred. *Lasioglossum* (*Dialictus*) were still dominant visitors to the late-phenology plants in terms of the visitor composition, but these plants were also visited by syrphid flies and bumble bees, and across all three types of visitors, visitation rates were less than one-quarter that of the early and control phenology treatments. Although flowering later in the year seems unlikely given the direction of phenological response to climate change in this study system, our late-phenology treatment in the array experiment simulated a scenario where bees respond to climate change more rapidly than plants. It is important to note that phenological mismatches could occur either when plants emerge before bees, or when bees emerge before plants. Because temperature affects the reaction rate of photosynthesis differently than heterotrophic respiration, and the metabolisms of heterotrophs and autotrophs respond to temperature differently (Brown *et al*., 2004), early insect emergence is a plausible scenario (but see Forrest & Thomson, 2011). An alternative explanation for why our late phenology treatment received such low visitation rates is that the bees were present but choosing to forage outside of the experimental array. Bees in our study system have an average flight period of less than 2 weeks (Eickwort *et al*., 1996; Gezon *et al*., 2015), however, which is shorter than the time period between placement of the early and late plants in the array. Furthermore, we did not observe *Lasioglossum* (*Dialictus*) foraging outside the array while the late phenology plants were present (Z. J. Gezon, *pers obs*). Thus, our observations suggest that a phenological mismatch between *C. lanceolata* and its primary pollinators is the most parsimonious explanation for our results. Unfortunately little is known about the emergence of soil nesting bees (Forrest, 2015), and future research should explore how plant and pollinator emergence compare in response to simulated climate change.

The 3-year snow removal experiment successfully represented the range of variability in date of bare ground and flowering phenology that has been documented in over 34 years of observation in our study area, and our snow removal experiments simulated an additional 11 to 22 years of advanced phenology of *C. lanceolata* due to climate change. Our snow removal

experiment also affected date of first flower more than date of last flower, causing plants to be in flower longer, which is also consistent with long-term trends (CaraDonna *et al*., 2014). Contrary with the long-term data, the snow manipulation suggested that maximum flower density decreases with advanced flowering date. This contrasting result may be a consequence of early season frost events, which damaged flowers and reduced flower density, thus reducing the recorded flower production in the experimental plots. It is worth noting that we observed a slightly slower phenological response to date of bare ground in our snow manipulation experiment than in the long-term dataset, likely due to the early season frost events in 2012. Thus our study may underestimate the effects of earlier snow melt on phenology and reproduction.

Four caveats are important to the interpretation of this study. First, with the exception of a small patch of *C. lanceolata* on a south-facing portion of the focal meadow, *C. lanceolata* in the snow removal plots was the first and only species in flower early. Thus, pollinators from the entire area would be drawn to the relatively small patches where snow was removed, thereby inflating the ratio of bees to flowers and increasing the probability of observing pollinator visitation inside the snow removal plots (Forrest, 2015). In contrast, the entire meadow was in flower when the control plots flowered; thus, the bee : flower ratio was likely lower. However, if floral abundance outside of the experimental plots was driving visitation patterns within the snow removal plots, a similar pollinator visitation pattern relative to the control plots would be expected in all years of observation, which did not occur. In a similar vein, pollen availability may have been lower for plants flowering early given that fewer plants were in-flower during this time period. Such confounding effects of the environment outside of the experiment are an unavoidable consequence for all small-scale phenology manipulations (e.g., Stinson, 2005; Rafferty & Ives, 2012). Second, differences in floral morphology between the snow treatments may have affected pollinator visitation rather than the change in phenology itself. Differences in floral morphology between snow removal vs. control plots may be associated with differences in soil moisture between the treatments, as floral attractive and reward traits can vary significantly with leaf water potential (Carroll *et al*., 2001). Although we found that pollinator visitation was positively associated with floral size, we were unable to establish a causal relationship. Third, although we observed differences in pollinator composition and behavior among the phenology treatments in both experiments, we do not know how pollinator effectiveness varied among the treatments. Other studies have found that decreased pollinator effectiveness negatively affects plant reproduction when phenology is advanced (Rafferty & Ives, 2012). We observed low plant reproduction despite high pollinator visitation in the early phenology treatment of the array experiment; thus, the observed decreases in reproduction could be due to decreased pollinator effectiveness rather than frost damage. Fourth, we used metrics of fruit and seed numbers to estimate plant reproduction and did not measure seed size or viability. Measuring seed size and viability could provide additional ecological insights about plant and population performance for future research.

Our results highlight the importance of both abiotic and biotic consequences of altered phenology for plant reproduction. Phenological mismatches with bees, although widely conjectured, have rarely been documented (Forrest & Miller-Rushing, 2010). We show that phenological mismatches with bees can happen by altering phenology of a single plant species as little as 8 days, even with native species and in a generalist pollination system. However, the phenological mismatch was associated with delayed and not advanced flowering plants and their pollinators. Thus, although plant-pollinator phenological mismatches are a widely hypothesized consequence of climate change, they alone are insufficient to understand how plant populations will respond to climate change. We must consider the abiotic and biotic effects of altered phenology simultaneously to assess adequately how plant reproduction will respond to a changing climate.

Acknowledgments

We thank A. Datta, S. Estes, K. Leimberger, A. Slominski, and H. Bleecker for field assistance, R. Bingham and Western State Colorado University for use of their greenhouse, and J. Anderson, D. Bolger, K. Cottingham, the subject editor, and three anonymous reviewers for comments on the manuscript. The RMBL provided access to field sites. This work was supported by funding from the Dartmouth College Cramer Fund, the RMBL Snyder Memorial Fund, the RMBL Graduate Fellowship, and the National Science Foundation (DEB-0922080 and 1311257). 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.

References

Agrawal AA, Conner JK, Stinchcombe JR (2004) Evolution of plant resistance and tolerance to frost damage. *Ecology Letters*, **7**, 1199–1208.

Anderson JT, Gezon ZJ (2015) Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Global Change Biology*, **21**, 1689–1703.

Anderson JT, Inouye DW, Mckinney AM, Colautti RI, Mitchell-Olds T (2012) Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B-Biological Sciences*, **279**, 3843–3852.

Augspurger CK (2013) Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. *Ecology*, **94**, 41–50.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Burkle LA, Irwin RE (2010) Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. *Journal of Ecology*, **98**, 705–717.

CaraDonna PJ, Iler AM, Inouye DW (2014) Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 4916–4921.

Carroll AB, Pallardy SG, Galen C (2001) Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany*, **88**, 438–446.

Douglas GW, Taylor RJ (1972) Biosystematics, chemotaxonomy, and ecology of *Claytonia lanceolata* in Western Washington. *Canadian Journal of Botany*, **50**, 2177–2187.

Dunne JA, Harte J, Taylor KJ (2003) Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs*, **73**, 69–86.

Eickwort GC, Eickwort JM, Gordon J, Eickwort MA (1996) Solitary behavior in a high altitude population of the social sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, **38**, 227–233.

Forrest JRK (2015) Plant-pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? *Oikos*, **124**, 4–13.

Forrest J, Miller-Rushing AJ (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 3101–3112.

Forrest JRK, Thomson JD (2011) An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, **81**, 469–491.

Forrest J, Inouye DW, Thomson JD (2010) Flowering phenology in subalpine meadows: does climate variation influence community co-flowering patterns? *Ecology*, **91**, 431–440.

Fournier DA, Skaug HJ, Ancheta J *et al.* (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods & Software*, **27**, 233–249.

Gezon ZJ, Wyman ES, Ascher JS, Inouye DW, Irwin RE (2015) The effect of repeated, lethal sampling on wild bee abundance and diversity. *Methods in Ecology and Evolution*, **6**, 1044–1054.

Gotelli NJ, Ellison AM (2006) Food-web models predict species abundances in response to habitat change. *Plos Biology*, **4**, 1869–1873.

Hegland SJ, Nielsen A, Lazaro A, Bjerknes AL, Totland O (2009) How does climate warming affect plant-pollinator interactions? *Ecology Letters*, **12**, 184–195.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Inouye DW (2000) The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, **3**, 457–463.

Inouye DW (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, **89**, 353–362.

Ishii HS, Harder LD (2012) Phenological associations of within- and among-plant variation in gender with floral morphology and integration in protandrous *Delphinium glaucum*. *Journal of Ecology*, **100**, 1029–1038.

Jacquemart AL (1997) Pollen limitation in three sympatric species of *Vaccinium* (Ericaceae) in the Upper Ardennes, Belgium. *Plant Systematics and Evolution*, **207**, 159–172.

Kudo G, Ida TY (2013) Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, **94**, 2311–2320.

Mccabe GJ, Betancourt JL, Hidalgo HG (2007) Associations of decadal to multidecadal sea-surface temperature variability with Upper Colorado River flow. *Journal of the American Water Resources Association*, **43**, 183–192.

Memmott J, Craze PG, Waser NM, Price MV (2007) Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, **10**, 710–717.

Mistretta O, Liston A (1992) Genetic diversity, gene flow, and relictualism in *Claytonia lanceolata* var *peirsonii*, a rare montane perennial from Southern California. *Southwestern Naturalist*, **37**, 223–230.

Molgaard P, Christensen K (1997) Response to experimental warming in a population of *Papaver radicatum* in Greenland. *Global Change Biology*, **3**, 116–124.

Mosquin T (1971) Competition for pollinators as a stimulus for evolution of flowering time. *Oikos*, **22**, 398–402.

Overpeck J, Udall B (2010) Dry times ahead. *Science*, **328**, 1642–1643.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.

Parsche S, Frund J, Tscharntke T (2011) Experimental environmental change and mutualistic vs. antagonistic plant flower-visitor interactions. *Perspectives in Plant Ecology Evolution and Systematics*, **13**, 27–35.

Pilon CE, Cote B, Fyles JW (1994) Effect of snow removal on leaf water potential, soil-moisture, leaf and soil nutrient status and leaf peroxicase-activity of sugar maple. *Plant and Soil*, **162**, 81–88.

Plateaux-Quénu C (2008) Subsociality in halictine bees. *Insectes Sociaux*, **55**, 335–346.

Post E, Pedersen C, Wilmers CC, Forchhammer MC (2008) Warming, plant phenology and the spatial dimension of trophic mismatch for large herbivores. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2005–2013.

Price MV, Waser NM (1998) Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology*, **79**, 1261–1271.

R Development Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rafferty NE, Ives AR (2011) Effects of experimental shifts in flowering phenology on plant-pollinator interactions. *Ecology Letters*, **14**, 69–74.

Rafferty NE, Ives AR (2012) Pollinator effectiveness varies with experimental shifts in flowering time. *Ecology*, **93**, 803–814.

Rafferty NE, Caradonna PJ, Burkle LA, Iler AM, Bronstein JL (2013) Phenological overlap of interacting species in a changing climate: an assessment of available approaches. *Ecology and Evolution*, **3**, 3183–3193.

Rafferty NE, Caradonna PJ, Bronstein JL (2015) Phenological shifts and the fate of mutualisms. *Oikos*, **124**, 14–21.

Robertson C (1895) The philosophy of flower seasons, and the phaenological relations of the entomophilous flora and the anthophilous insect fauna. *American Naturalist*, **29**, 97–117.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.

Saunders S, Montgomery C, Easley T, Spencer T (2008) Hotter and drier. In: *The West's Changed Climate*. The Rocky Mountain Climate Organization and Natural Resources Defense Council, New York, NY.

Scheiner S (1993) MANOVA: multiple response variables and multispecies interactions. In: *Design and Analysis of Ecological Experiments*, Vol **94**, p. 112. Chapman & Hall, New York, NY.

Sherwood J (2013) Experimentally simulating environmental change in a montane meadow system via reduced snowpack and passive warming: soil and plant responses. Unpublished M.Sc. thesis, Iowa State University, Ames, IA.

Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker B (2013) Generalized Linear Mixed Models Using AD Model Builder. R package version 0.7.5.

Stewart IT, Cayan DR, Dettinger MD (2005) Changes toward earlier streamflow timing across western North America. *Journal of Climate*, **18**, 1136–1155.

Stinson KA (2005) Effects of snowmelt timing and neighbor density on the altitudinal distribution of *Potentilla diversifolia* in western Colorado, USA. *Arctic, Antarctic, and Alpine Research*, **37**, 379–386.

Strauss SY, Conner JK, Rush SL (1996) Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist*, **147**, 1098–1107.

Thomson JD (2010) Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 3187–3199.

Visser ME, Holleman LJM (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 289–294.

Walther GR, Post E, Convey P *et al.* (2002) Ecological responses to recent climate change. *Nature*, **416**, 389–395.

Waser NM, Real LA (1979) Effective mutualism between sequentially flowering plant-species. *Nature*, **281**, 670–672.

Wielgolaski FE, Inouye DW (2013) Phenology at high latitudes. In: *Phenology: An Integrative Environmental Science* (ed Schwartz MD), pp. 225–247. Springer, Dordrecht.

Williams NM, Winfree R (2013) Local habitat characteristics but not landscape urbanization drive pollinator visitation and native plant pollination in forest remnants. *Biological Conservation*, **160**, 10–18.

Williams WT, Cifuentes S, Delaguila V, Perez R (1992) Rejuvenation of a peach orchard in the highlands of Guatemala through integrated management. *Tropical Agriculture*, **69**, 341–346.

Winder M, Schindler DE (2004) Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology*, **85**, 2100–2106.

Wolkovich EM, Cook BI, Allen JM *et al.* (2012) Warming experiments underpredict plant phenological responses to climate change. *Nature*, **485**, 494–497.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Comparison of flowering phenology and reproduction between two control types.
Appendix S2. Visualization of flowering phenology and reproduction between two control types.
Appendix S3. Visualization of peak flower, last flower, and flowering duration from snow manipulation experiment.
Appendix S4. Visualization of maximum flower density from snow manipulation experiment.
Appendix S5. Visualization of various aspects of phenology from experimental snow manipulation plots and long term data.
Appendix S6. Visualization of leaf water potential between snow removal and control plots and between predawn and mid-day measurements.
Appendix S7. Comparisons of pollinator visitation between snow treatments and years.
Appendix S8. Visualization of the relationships between pollinator visitation and floral morphology.
Appendix S9. Visualization of plant reproduction from snow removal experiment with *y*-axes rescaled for each year.
Appendix S10. Snow manipulation experiment linear contrasts
Appendix S11. Pollinators observed visiting *Claytonia lanceolata* in array experiment
Appendix S12. Post hoc comparisons of pollinator visitation from array experiment.
Appendix S13. Array experiment linear contrasts.

© 2016 John Wiley & Sons Ltd,

View publication stats