

1 **Title:** Phenological responses to multiple environmental drivers under climate change: insights
2 from a long-term observational study and a manipulative field experiment

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19
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23

24 **Abstract**

25 Climate change has induced pronounced shifts in the reproductive phenology of plants,
26 with the timing of first flowering advancing in most species. Indeed, population persistence may
27 be threatened by the inability to track climate change phenologically. Nevertheless, substantial
28 variation exists in biological responses to climate change across taxa. Here, we explore the
29 consequences of climate change for flowering phenology by integrating data from a long-term
30 observational study and a manipulative experiment under contemporary conditions. Dissecting
31 the environmental factors that influence phenological change will illuminate why interspecific
32 variation exists in responses to climate change. We examine a 43-year record of first flowering
33 for six species in subalpine meadows of Colorado in conjunction with a 3-year snow
34 manipulation experiment on the perennial mustard *Boechera stricta* from the same site. We
35 analyze shifts in the onset of flowering in relation to environmental drivers known to influence
36 phenology: the timing of snowmelt, the accumulation of growing degree days, and photoperiod.
37 At our study site, climate change is reducing snowpack and advancing the timing of spring
38 snowmelt. We found that variation in phenological responses to climate change depended on the
39 sequence in which species flowered, with early-flowering species flowering faster, at a lower
40 heat sum, and under increasingly disparate photoperiods in comparison to species that flower
41 later in the season. Furthermore, climate change is outpacing phenological change for all species.
42 Early snow removal treatments confirm that the timing of snowmelt governs observed trends in
43 flowering phenology of *B. stricta* and that climate change can reduce the probability of
44 flowering, thereby depressing fitness. Shorter-term studies would not have captured the trends
45 that we document in our observational and experimental datasets. Accurate predictions of the
46 biological responses to climate change require a thorough understanding of the specific
47 environmental factors driving shifts in phenology.

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53 **Introduction**

54 Across ecosystems worldwide, climate change has induced shifts in the timing of critical
55 life history transitions for a diversity of organisms, from plants to invertebrates, birds, mammals,
56 amphibians and fish (CaraDonna *et al.*, 2014, Charmantier & Gienapp, 2014, Parmesan, 2006,
57 Parmesan & Yohe, 2003, Pilfold *et al.*, 2017, Poloczanska *et al.*, 2013, Wolkovich *et al.*, 2012).
58 Increased temperatures and altered precipitation dynamics have had especially noticeable effects
59 on phenological transitions in the spring, with many species consistently now emerging,
60 migrating, or reproducing 1-3 weeks earlier than historical averages (Amano *et al.*, 2010, Bertin,
61 2008, Menzel *et al.*, 2006, Parmesan & Yohe, 2003, Poloczanska *et al.*, 2013, Sherry *et al.*,
62 2007). In plants, shifts in the onset of flowering are among the most conspicuous and well-
63 documented biological indicators of a changing climate (CaraDonna *et al.*, 2014, Fitter & Fitter,
64 2002, Menzel *et al.*, 2006, Parmesan & Yohe, 2003), yet we know little about which climatic
65 factors are driving phenological responses to climate change. While flowering phenology has
66 advanced over the past several decades for many species, substantial variation exists in responses
67 among taxa (Mazer *et al.*, 2013, Willis *et al.*, 2008). Shifts in phenology alter the abiotic
68 environment under which individuals develop and can disrupt biotic interactions when
69 interacting species time life history events in response to different environmental conditions
70 (Visser *et al.*, 2006). Species unable to track climate change via appropriate phenological
71 changes are at a greater risk of decline (Willis *et al.*, 2008); therefore, we must explore the
72 factors contributing to variation in responses to climate change.

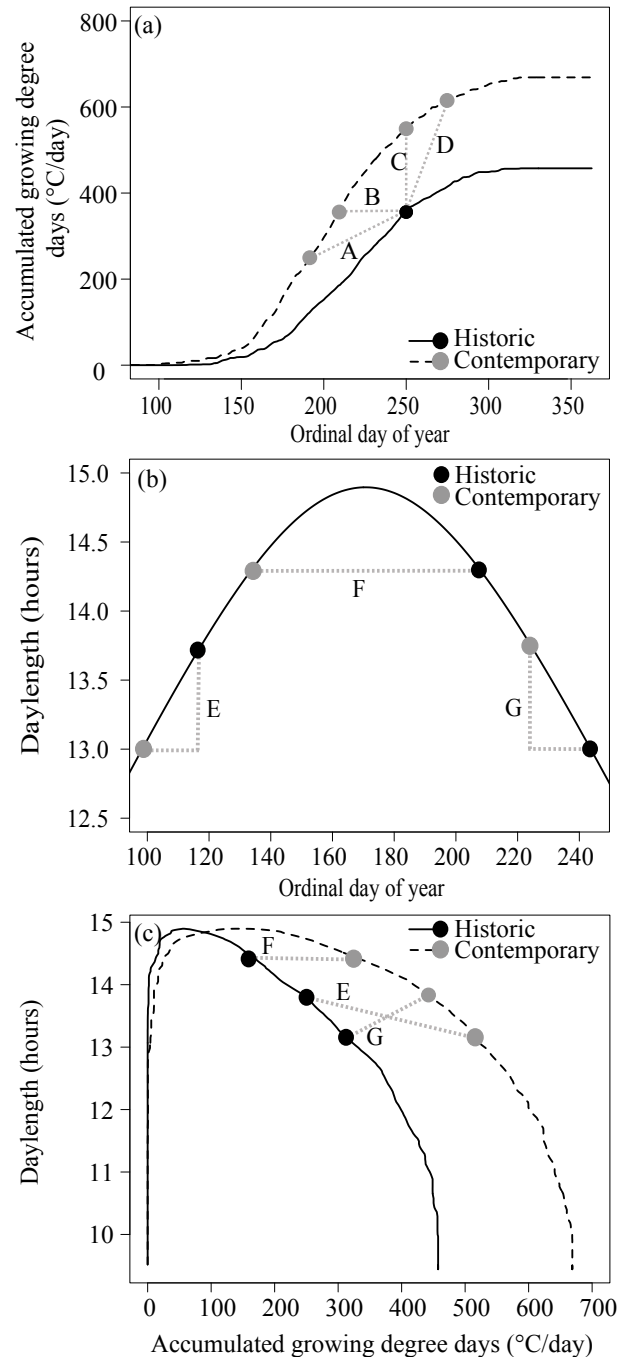
73 Ecological and evolutionary studies typically report phenology as the calendar (ordinal)
74 date of year, but such data may obscure the underlying biological processes that contribute to
75 transitions between life history stages (Cook *et al.*, 2012). Plants time their reproduction based

76 on one or more proximate environment cues, including photoperiod, temperatures, the length of
77 winter (vernalization), and moisture levels (Forrest & Miller-Rushing, 2010, Lacey, 1986). Once
78 a cue is received, these same environmental factors influence the ensuing developmental rate.
79 For instance, some species begin flowering only after having reached a critical photoperiod.
80 Photoperiod correlates strongly with calendar date at a given latitude and is unaffected by
81 climate change. Nevertheless, developmental processes initiated by photoperiod also depend on
82 climatic factors that are changing rapidly, such as temperature and precipitation. Therefore,
83 species that rely on fixed photoperiodic cues may now be undergoing phenological transitions
84 under temperatures or heat sums that are now too high (Fig. 1). Species that respond solely to
85 thermal cues may more effectively track climate change, but will experience novel photoperiods,
86 which will influence their daily exposure to photosynthetically active radiation (Fig. 1). Climate
87 change can decouple previously reliable seasonal conditions, which can constrain phenological
88 responses to climate change.

89 Elevated temperatures during the growing season clearly influence life history traits and
90 reproductive phenology (Wolkovich *et al.*, 2012). In addition, altered winter conditions can also
91 have profound consequences for phenological events that occur in the spring and summer. In
92 high elevation and high latitude regions, the timing of snowmelt is a key environmental cue
93 triggering life history transitions (Anderson *et al.*, 2012). In snow-dominated regions, climate
94 change has reduced winter snowpack and advanced snowmelt (Høye *et al.*, 2013, Iler *et al.*,
95 2013, IPCC, 2014, Pederson *et al.*, 2011), but the timing of frost events has not changed,
96 exposing developing floral tissue to frost damage that would not have occurred historically under
97 the insulation of snow (Inouye, 2008); thus damaged, plants must ‘restart’ floral development. In
98 addition, increased evapotranspiration from spring and summer warming in concert with

99 declining winter snowpack heightens drought conditions in the summer (Rangwala *et al.*, 2012).
100 Thus, multiple interacting environmental cues could elicit variable phenological responses
101 among plant species that grow and flower in different portions of the growing season (Fig. 1)
102 (Marchin *et al.*, 2015).

103 **Fig. 1.** Conceptual diagrams demonstrating how climate change can decouple previously reliable
104 seasonal cues and influence the environmental
105 conditions that plants experience directly through
106 shifts in climate and indirectly through shifts in
107 phenology. (a) Accumulated growing degree days
108 (GDD) over the growing season may shift in
109 response to a warming climate. The historic curve
110 shows accumulated GDD in our study region from
111 1975 while the contemporary curve portrays data
112 from 2016. The reaction norms illustrate scenarios
113 comparing the historic flowering onset dates for
114 species (black circles) to that observed in
115 contemporary conditions (grey circles), where we
116 see (A) shifts towards earlier flowering onset date
117 and decreased heat sum at flowering, (B) shifts
118 towards earlier flowering onset date alone, (C) shifts
119 towards increased heat sum at flowering alone, and
120 (D) shifts towards later flowering onset and
121 increased heat sum at flowering. (b) Shifts in the
122 day length experienced at first flower depend on the
123 degree of phenological change and when in the
124 growing season a species flowers. The curve reflects
125 the photoperiodic regime occurring in our study site.
126 Historic shifts in flowering onset date (black circles)
127 result in a (E) shorter, (F) equivalent, or (G) longer
128 day length at first flower than in contemporary
129 conditions. (c) The relationship between shifts in
130 accumulated GDD shown in (a) and the constant
131 photoperiods in (b). These data demonstrate how
132 climate change and shifts in phenology may disrupt
133 previously dependable combinations of temperature
134 and photoperiod. The same shifts in day length
135 depicted in (b) are reflected here and illustrate that
136 shifts in flowering onset date for species that flower
137 at different points throughout the growing season
138 will result in variable combinations of accumulated
139 GDD and day length at flowering.
140



141 By examining phenological trends in relation to relevant environmental drivers,
142 contemporary studies can reveal whether climate change could outpace phenological events in
143 natural communities. Plants could display perfect calendar day advances in flowering time that
144 mirror climate change, such that individuals flower one day earlier for every day advance in the
145 timing of spring. In that case, we would predict that phenological plasticity to lower photoperiod
146 and increased frost damage would hasten biological responses to climate change. If, instead, first
147 flowering accelerates at a rate slower than the advancement of the spring, then other
148 environmental cues likely constrain the phenological response to climate change (Iler *et al.*,
149 2013). For example, an examination of first flowering data for 490 species in the south-central
150 United Kingdom and eastern United States revealed that flowering phenology of species thought
151 to be insensitive to climate change were in fact responding to the nullifying effects of insufficient
152 winter chilling and warmer spring temperatures (Cook *et al.*, 2012). We suggest that constraints
153 on future phenological shifts may already be evident in current records.

154 We hypothesize that climate change is decoupling previously reliable environmental cues
155 for flowering, which will constrain future phenological responses to ongoing climatic changes.
156 Furthermore, we propose that this decoupling has more profound consequences for species that
157 flower early in the season, when phenological shifts may increase exposure to frosts than shifts
158 during the summer season. As a corollary, we posit that increased temperatures are not the sole
159 mechanism underlying phenological shifts. To evaluate the environmental drivers of shifts in
160 flowering onset dates, we first examined long-term trends in climate variables important for plant
161 phenology. Then we analyzed shifts in the timing of first flowering for six plant species relative
162 to plausible environmental drivers of phenological change from a long-term observational study.
163 Finally, we conducted a manipulative experiment in contemporary environments to test the

164 influence of snow depth and snowmelt date on flowering phenology in an ecological model plant
165 (*Boechnera stricta*, Brassicaceae). We assess whether advancing patterns of first reproduction
166 reflect changes in the rate of floral development and the heat sum or photoperiod at which
167 individuals flower. Alternatively, phenological shifts could simply reflect a passive response to
168 advancing spring conditions.

169 The observational dataset provides an exemplary long-term record of the extent to which
170 reproductive phenology has changed over a period of rapid climate change and can evaluate
171 correlations between the timing of flowering and various environmental factors. However, data
172 from natural populations cannot test causal links between environmental conditions and
173 phenology, nor can those datasets distinguish between plasticity and possible genetic responses
174 to environmental change. Therefore, we manipulated snowpack dynamics in our experimental
175 study of *B. stricta* to test the extent to which climatic factors other than temperature could be
176 implicated in changes in reproductive phenology. These experimental data enable us to evaluate
177 whether plants can keep pace with changing seasonal dynamics via plasticity. Furthermore, this
178 experiment can provide much needed information about the fitness consequences of ongoing
179 climate change (Anderson, 2016) by assessing whether reduced snowpack and earlier snowmelt
180 dates depress fitness by reducing the probability of flowering. Manipulative field experiments
181 complement long-term observational studies by determining the specific environmental factors
182 that promote phenological change, identifying limits to shifts in phenology, and testing whether
183 climate change will reduce fitness. By comparing long-term records with experimental data from
184 a common garden, we can make more robust inferences about the extent to which phenology will
185 continue to advance with ongoing climate change.

186

187 **Methods**

188 *Study system*

189 We examined shifts in reproductive phenology of perennial forbs in dry rocky meadows
190 around the Rocky Mountain Biological Laboratory (RMBL; 38°57'N, 106°59'W; approximately
191 2900 m; Gothic, Colorado). Climate change has exposed subalpine meadows in this snow-
192 dominated system to increased winter temperatures, decreased snowpack, and advancing timing
193 of snowmelt, which has caused significant shifts in the timing of reproduction of multiple species
194 in the plant community over the last four decades (Anderson *et al.*, 2012, CaraDonna *et al.*,
195 2014, Dunne *et al.*, 2003, Gezon *et al.*, 2016, IPCC, 2014, Pederson *et al.*, 2011). This system is
196 ideal for evaluating the phenological consequences of climate change owing to long-term records
197 and experimental studies in contemporary climates. Furthermore, observations and simulations
198 point to increased warming at higher elevations such as the Rocky Mountains (Mountain
199 Research Initiative, 2015).

200

201 *Trends in climate*

202 We acquired snowmelt data from two sources. First, from 1975-2016, billy barr has
203 measured the annual date of snowmelt at the RMBL as the date of 100% bare ground in a
204 permanent 5 x 5 m plot. Second, we estimated snowmelt dates for each plot from the long-term
205 observational study (see below) in 2007-2016 from light intensity and temperature data recorded
206 by HOBO pendant temperature and light data loggers (Part # UA-002-64, Onset Computer Corp,
207 Bourne, MA, USA). We estimated snowmelt dates for the observational plots in preceding years
208 (1973-2006) by regressing plot-level snowmelt dates on snowmelt dates measured by b. barr
209 ($r^2=0.77$, $F_{1,33}=110.6$, $p<0.0001$; Plot date = barr date / 0.81* 1.168 - 33.047). Previously, data

210 on observed dates of snowmelt were combined with historic runoff values in the nearby East
211 River to evaluate trends in the timing of snowmelt from 1935 to 2012 (Anderson et al. 2012). We
212 update that analysis here with data from 2013-2016 (Fig. S1).

213 We acquired records of maximum and minimum daily temperatures from 1973-2016
214 from the land-based NOAA station located approximately 9 km from the RMBL (station
215 USC00051959, <https://www.ncdc.noaa.gov/data-access/land-based-station-data>). Temperature
216 data taken at the RMBL were available from permanent weather stations established in 2004
217 (<http://www.gothicwx.org/>). To estimate daily temperatures in years prior to 2004, we separately
218 regressed minimum and maximum daily temperatures taken at the RMBL against those from the
219 NOAA station (Minimum: $r^2=0.88$, $F_{1,4905}=36960$, $p=0$, RMBL min = $0.76 \cdot \text{NOAA min} + 0.46$;
220 Maximum: $r^2=0.85$, $F_{1,4905}=29101$, $p=0$, RMBL max = $0.83 \cdot \text{NOAA max} + 0.82$). This 43-year
221 record of spring temperatures lacked minimum or maximum temperatures for only 31 days. In
222 these cases, we substituted the average of the temperatures recorded on the previous and
223 subsequent days. We used these temperature data to estimate growing degree days for each day
224 during the study period.

225 Growing degree days (GDD) are typically calculated by adjusting average temperatures by
226 a baseline temperature below which growth cannot occur ($(\text{maximum} - \text{minimum})/2 - \text{base}$).
227 However, this method overestimates the number of growing degree days available on days when
228 minimum temperatures are below the baseline temperature (Arnold, 1960). Here, we assign a
229 baseline temperature of 8°C based on a resource allocation model applied to *Boechera stricta*
230 (Colautti *et al.*, 2016). In our study region, springtime minimum temperatures are consistently
231 below this threshold (Fig. S2d). To assess GDD accurately during the spring, we modeled heat
232 sum accumulations each day using the sine-wave method, which applies sine curves to daily

233 maximum and minimum temperatures to approximate diurnal thermal curves (Baskerville &
234 Emin, 1969). For both the long-term and experimental phenology data, we estimated the heat
235 sum acquired by plants each year upon reproduction as a summation of GDD values from that
236 year's date of snowmelt to the ordinal date of first flower.

237

238 *Long-term phenology study*

239 Since 1973, David Inouye and colleagues have quantified flowering phenology in the
240 natural plant communities within 23 2 × 2 m plots near the RMBL; here, we analyze records
241 from seven plots in dry rocky meadows ranging in elevation from 2928–2970 m over 1973–2016
242 (data were not taken in 1978 and 1990, $N = 41$ years). Inouye *et al.* visited each plot
243 approximately every other day during the growing season to record the number of open flowers
244 of each plant species (or the number of capitula with open florets for species in the Asteraceae),
245 thereby generating an extensive long-term record of the flowering schedule of 120 species
246 (CaraDonna *et al.*, 2014, Inouye, 2008). To examine the environmental drivers of shifts in the
247 timing of first reproduction, we focused on six species: *Mertensia fusiformis* (Boraginaceae),
248 *Delphinium nuttallianum* (Ranunculaceae), *Boechera stricta* (Brassicaceae), *Lathyrus leucanthus*
249 (Fabaceae), *Vicia americana* (Fabaceae), and *Erigeron speciosus* (Asteraceae) (referred to in the
250 results as *Mf*, *Dn*, *Bs*, *Ll*, *Va*, and *Es*, respectively). We selected these species because they are
251 well represented in the rocky meadow plots throughout the study period, their flowering onset
252 dates range from early spring to midsummer, and they are members of plant families
253 representative of the communities near the RMBL. These species are pollinated by native bees,
254 hummingbirds, or butterflies, except for *B. stricta*, which is primarily selfing.

255 We examined flowering phenology in relation to the timing of snowmelt by calculating
256 the number of elapsed days from snowmelt to first flower for each species in each year, with data
257 pooled across the seven Inouye plots. To evaluate the heat sum acquired by first flowering, we
258 estimated the number of accumulated GDD from snowmelt to first flower. We obtained day
259 lengths (www.sunrise-sunset.org.) for each day of the study period to examine whether the day
260 length at first flower has changed over time.

261

262 *Experimental phenology study*

263 To examine the mechanistic relationship between flowering time and altered winter
264 climates, we analyzed flowering phenology data from a multiyear snow manipulation
265 experiment. From 2014-2016, we monitored flowering phenology in experimental transplants of
266 *B. stricta* in a common garden experiment in a dry rocky meadow near the RMBL (2891 m,
267 38°57.086’N, 106°59.4645’W; 2890 m elevation). *Boechera stricta* is a primarily self-
268 pollinating perennial forb that inhabits elevations from 700-3900 m in its native Rocky Mountain
269 range (Al-Shehbaz & Windham, 2010, Rushworth *et al.*, 2011, Song *et al.*, 2006). Previously,
270 we demonstrated that *B. stricta* flowers ~13 days earlier in contemporary years than in the mid-
271 1970s; this shift likely resulted from both plasticity and adaptation to strong directional selection
272 (Anderson *et al.*, 2012). This mustard shows plasticity in flowering phenology to temperature,
273 winter length, and the timing of snowmelt (Anderson & Gezon, 2015, Anderson *et al.*, 2012,
274 Anderson *et al.*, 2011).

275 In the fall of 2013 (hereafter: 2013 cohort), we planted $N = 1691$ 3-month-old juvenile
276 rosettes from 100 maternal families originating from 43 source populations into 16 randomly
277 arrayed 2 x 1 m experimental blocks in the common garden (average = 8.4 individuals/treatment;

278 Table S1 has exact sample sizes per genotype, source population, and numbers of individuals
279 that flowered per growing season from 2014-2016). We replicated this experiment in the fall of
280 2014 (hereafter: 2014 cohort), when we planted $N = 1839$ juvenile rosettes from 154 maternal
281 families originating from 48 source populations into 18 different experimental blocks the same
282 common garden (average=6.0 individuals/treatment; Table S2 has sample sizes). Before
283 transplanting these cohorts, we grew seeds from natural populations in the greenhouse for a
284 generation to reduce maternal effects and generate maternal families consisting of selfed full
285 siblings. The source populations spanned a broad elevational gradient (2013 cohort: 2694–3690
286 m; 2014 cohort: 2520–3530 m), which allowed us to investigate the phenological consequences
287 of climate change in families with divergent evolutionary histories.

288 We exposed half of the transplants to contemporary climates and half to early snow
289 removal, which reduces snowpack, accelerates the timing of snowmelt, and reduces soil
290 moisture. In the spring of 2014-2016, we implemented the snow removal treatment, shoveling
291 snow off of half of the experimental blocks in mid-April, when snowpack receded to 1 m deep,
292 following our published protocol (Anderson & Gezon, 2015). To prevent damage to vegetation,
293 we shoveled snow to 10 cm depth and allowed the remaining snow to melt naturally over the
294 subsequent days. We placed shoveled snow outside of the vicinity of the experimental garden
295 and shoveled a 0.5 m buffer area around each snow removal plot. We were careful to leave
296 control plots intact. We recorded date of snowmelt (100% bare ground) for all control and snow
297 removal plots.

298 As soon as snow melted, we monitored all experimental plants for flowering 2-4 times per
299 week. At each census, we recorded the phenological status (vegetative, bolting, or flowering).
300 We defined flowering onset as the appearance of the first flower. On reproductive plants, we

301 measured plant height, and recorded the number of flowers, number of developing siliques
302 (fruits), and the length of the siliques. For plants that flowered on days in between censuses, we
303 used elongation rates of siliques and growth rates of flower production to estimate the exact
304 ordinal date of first flowering (Table S3), as we have done previously (Anderson *et al.*, 2011,
305 Wadgyamar *et al.*, 2017). For the 2013 cohort, more than half of the experimental individuals
306 flowered during their first growing season (2014: 947 individuals), with fewer flowering in
307 subsequent years (2015: 475 individuals; 2016: 124 individuals, Table S1). For the 2014 cohort,
308 flowering success was lower, with 18.8% of individuals flowering in their first year (2015: 346
309 plants) and only 5.3% flowering in 2016 (96 individuals, Table S2).

310

311 *Data analysis*

312 *Trends in climate.* We analyzed climatic series using both generalized additive mixed
313 models and linear mixed models. Additive models are not constrained by the assumption of
314 linear relationships between predictor and response variables. In this way, additive models can
315 easily incorporate seasonal fluctuations or outlier events without compromising the fit of the
316 predicted function. In contrast, linear models can accentuate underlying trends and yield
317 coefficients that are easy to interpret and compare among analyses. Here, we use additive models
318 to inspect fluctuations and anomalies in climatic or phenological data, but we focus the majority
319 of our discussion on the linear trends uncovered from our data.

320 We used generalized additive models and linear models to assess changes in the date of
321 snowmelt; the snow depth on April 1 (hereafter, snowpack); and May maximum, average, and
322 minimum temperatures over time. We also used these models to examine the extent to which
323 snowpack predicts the date of snowmelt, which in turn determines the spring temperatures that

324 plants will experience. We fit additive models using unpenalized regression splines, which yield
325 more accurate p -values and with the degree of smoothing selected by generalized cross-
326 validation (Wood, 2017). For these models, significant predicted functions indicate that the data
327 are nonlinear at some point, although they do not reveal whether there are overall increasing or
328 decreasing trends. To gauge which portions of the predicted functions are significantly
329 increasing or decreasing, we computed the first derivative and its confidence intervals of spline
330 function supplied by our additive models. In all figures, portions of the dependent variable where
331 the derivative function indicated that the slope of the predicted function was significantly
332 different than zero are emphasized with a bold trend line. We utilize linear models to examine
333 general trends in the data. In all linear models, we assessed the significance of quadratic terms
334 for dependent variables, but only linear terms were retained in the final models. In both additive
335 and linear models, we applied autoregressive autocorrelation structures and included error
336 variance covariates to remedy residual heterogeneity where appropriate. We used the *mgcv*
337 (Wood, 2004) package and *lm* base function in R (R Core Team, 3.2.2, [https://www.R-](https://www.R-project.org)
338 [project.org](https://www.R-project.org)) to apply the generalized additive models and linear models, respectively, and used
339 the *tsgam* package (Simpson, 2017) to estimate the derivatives of the predictive functions from
340 the additive models.

341 *Phenology.* For both the observational and experimental phenology data sets, we assessed
342 temporal trends or treatment effects on four aspects of flowering phenology: the ordinal date of
343 first flower, the number of days from snowmelt to first flower, the number of accumulated GDD
344 at first flower, and the day length at first flower. To meet assumptions of residual normality and
345 homoscedasticity, we used a lognormal distribution for accumulated GDD at first flower for both
346 observational and experimental data.

347 *Long-term phenology study.* We tested for temporal trends in flowering phenology from
348 the observational study using generalized additive mixed models and linear mixed models with
349 plot included as a random effect. We assessed whether shifts in snowmelt date and phenology
350 were progressing at the same rate by regressing the ordinal date of first flower against the date of
351 snowmelt for each species. To distinguish between the influence of snowmelt and thermal
352 conditions on phenology, we include spring temperature as a covariate in these models. We used
353 the same R packages and functions as described earlier to apply these models. We used the
354 Benjamini-Hochberg (1995) false discovery rate procedure to correct for multiple testing and
355 only present corrected p-values.

356 *Experimental phenology study.* For the manipulative experiment of *B. stricta*, we
357 conducted repeated measures analyses in a generalized linear mixed model framework to
358 evaluate the extent to which snow removal altered flowering phenology (Proc Glimmix, SAS
359 ver. 9.4). We conducted models separately for each cohort. Our models included fixed effects for
360 season, treatment, the season by treatment interaction, and repeated effects for season with an
361 autoregressive correlation structure. We accounted for the evolutionary history of transplanted
362 individuals through a fixed effect of source elevation and a random effect of genotype nested
363 within population. We also included a random effect for experimental block. We focused these
364 analyses on three response variables: flowering time (ordinal date; Poisson distribution with log
365 link), elapsed days from snowmelt to flowering (Poisson distribution with log link), accumulated
366 growing degree days at first flowering (lognormal distribution), and photoperiod at first
367 flowering (normal distribution in Proc Mixed). Analyses of raw daylength data generated highly
368 heteroscedastic residuals. Therefore, we evaluated statistical significance using randomization
369 tests with 1000 permutations for this variable with the %rand_gen and %rand_anl macros

370 (Cassell, 2002). Owing to different statistical distributions, we could not use a multivariate
371 repeated measures framework to analyze all response variables separately. We applied the
372 Benjamini-Hochberg (1995) false discovery rate to correct for multiple testing within each
373 cohort.

374 Finally, for both cohorts, we conducted repeated measures logistic regression analyses to
375 determine whether snow removal depressed the probability of flowering across growing seasons
376 (Proc Glimmix, SAS, ver. 9.4) using the same fixed and random effects described above.

377

378 **Results**

379 *Trends in climate*

380 Annual measurements of snow depth on April 1 declined by 13.3 ± 0.61 cm per decade,
381 resulting in a 33% decrease in snowpack from 1973-2016 (spline: $F_{9,30}=1.34$, $p=0.26$; linear
382 trend: $F_{1,38}=4.72$, $p=0.036$, Fig. 2a, Table S4). The spring snowpack strongly predicts the date of
383 snowmelt, with each 10 cm reduction in the snow depth on April 1 accelerating snowmelt by
384 2.36 ± 0.025 days (spline: $F_{9,30}=9.50$, $p<0.0001$; linear trend: $F_{1,38}=89.2$, $p<0.0001$, Fig. 2b,
385 Table S4). Indeed, the ordinal date of snowmelt has advanced at a steady pace by 1.34 ± 0.053
386 days per decade over the past 82 years, occurring approximately 11 days earlier in 2016 than in
387 1935 (updated from Anderson et al. 2012; spline: $F_{9,72}=1.04$, $p=0.42$ linear trend: $F_{1,80}=6.31$,
388 $p=0.014$, Fig. S1, Table S4). Although variable, spring temperatures have not changed
389 appreciably across the study period (Fig. S2, Table S4). However, changes in the timing of
390 snowmelt expose plants to different temporal segments of spring, with each 10-day advancement
391 in the date of snowmelt resulting in a $0.55 \pm 0.013^{\circ}\text{C}$ decrease in the average daily temperature
392 experienced in the first 50 days after snowmelt (spline: $F_{9,32}=2.05$, $p=0.065$; linear trend:

393 $F_{1,40}=16.8, p=0.0002$, Fig. 2c, Table S4). Our study region is accumulating less snow over time,
394 resulting in earlier spring seasons and a cooler thermal environment for plants.

395

396 **Fig. 2.** (a) Temporal trends in snow pack on April 1st, (b) the relationship
397 between snowpack and date of
398 snowmelt, and (c) the influence of the
400 date of snowmelt on the average daily
401 temperature experienced in the first 50
402 days of spring over 1973-2016 at the
403 Rocky Mountain Biological
404 Laboratory in Colorado. The smooth
405 trend lines with 95% confidence
406 intervals were derived via penalized
407 regression splines from general
408 additive mixed models. Portions of the
409 predicted functions that are
410 significantly increasing or decreasing
411 are shown in bold (see Fig. S3). When
412 significant, we also display the linear
413 trend line (dashed line) from linear
414 mixed models.

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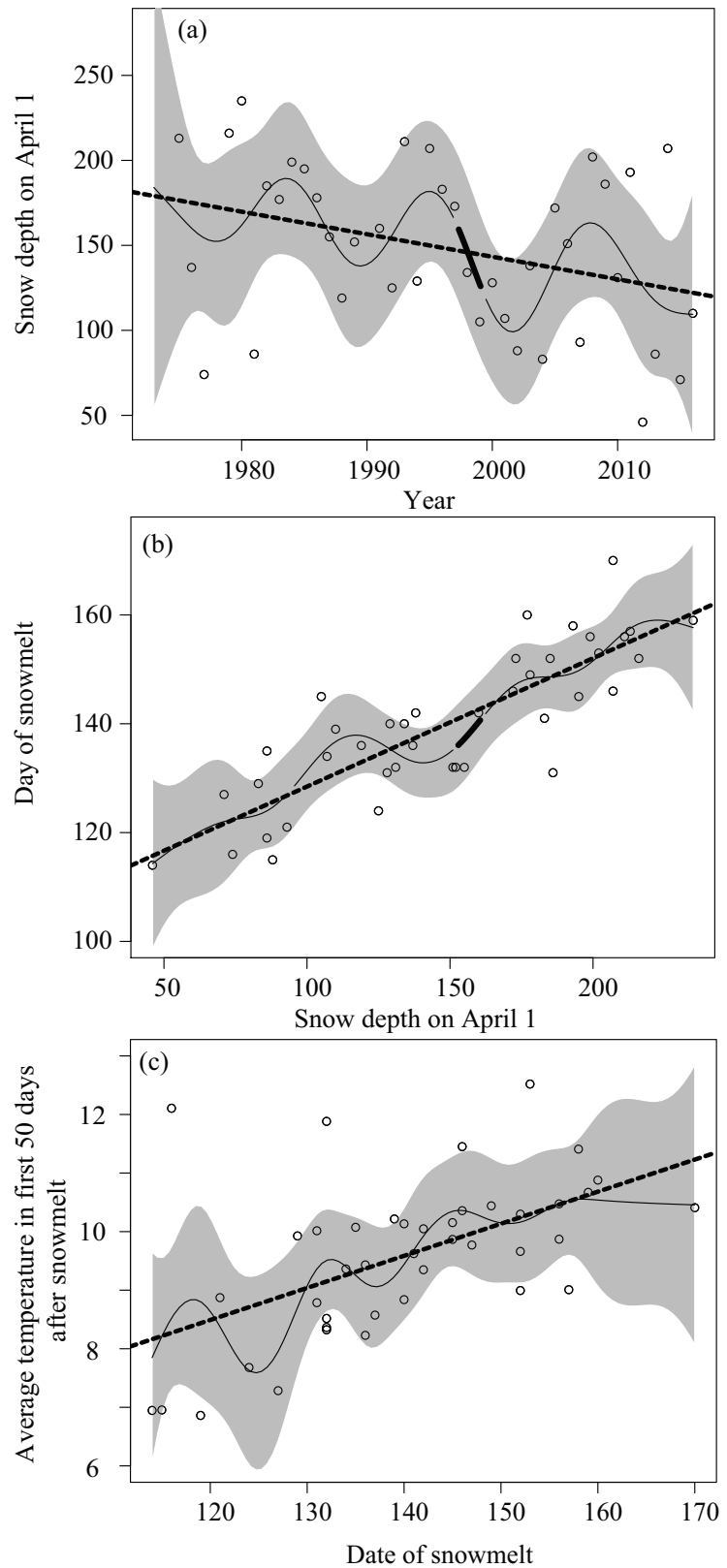
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425 *Long-term phenology study*

426 The ordinal dates of first flower for the six focal species were staggered throughout the
427 growing season, with the rank order of flowering times maintained across the study period (Fig.
428 3a). The flowering onset dates across species fluctuate in synchrony every 8-10 years, as
429 evidenced by the smoothing functions derived from generalized additive mixed models and their
430 derivatives (spline: *Mf* $F_{10,253}=826$; *Dn* $F_{10,261}=2217$; *Bs* $F_{10,136}=2415$; *Ll* $F_{10,155}=1362$; *Va*
431 $F_{10,40}=1717$; *Es* $F_{10,217}=2438$; $p<0.0001$ in all cases; Fig. S3, Table S5). All species show
432 significant advancement across the study period in flowering time as measured by ordinal day of
433 first flowering, with greater advances in the earliest-flowering species (linear trend: *Mf* $F_{1,261}=56$,
434 $p<0.0001$; *Dn* $F_{1,269}=62$, $p<0.0001$; *Bs* $F_{1,144}=42$, $p<0.0001$; *Ll* $F_{1,163}=18$, $p=0.0001$; *Va* $F_{1,48}=9$,
435 $p=0.0086$; *Es* $F_{1,225}=21$, $p<0.0001$; Fig. 3b-g, Table S5). These patterns parallel those of
436 declining snow pack and advancing snowmelt date in this region (Fig. 2a-b).
437 When examined as days from snowmelt to first flower, the timing of flowering has remained
438 fairly constant in all but the earliest flowering species (Fig. 3h-n), with the onset of flowering for
439 the early-flowering *M. fusiformis* advancing by 1.07 ± 0.043 days per decade (linear trend:
440 $F_{1,261}=6.10154$, $p=0.024$, Table S5). For all species, flowering occurred later with respect to
441 snowmelt in a year of abnormally early snowmelt (1977, Fig. S1). There is a similar delay in
442 flowering for the three later-flowering species in a year with an aberrantly early and cool spring
443 (1992). The lack of a trend in flowering time when measured as elapsed days since snowmelt
444 demonstrates that flowering generally commences at a fixed duration after snowmelt for most
445 species.

446

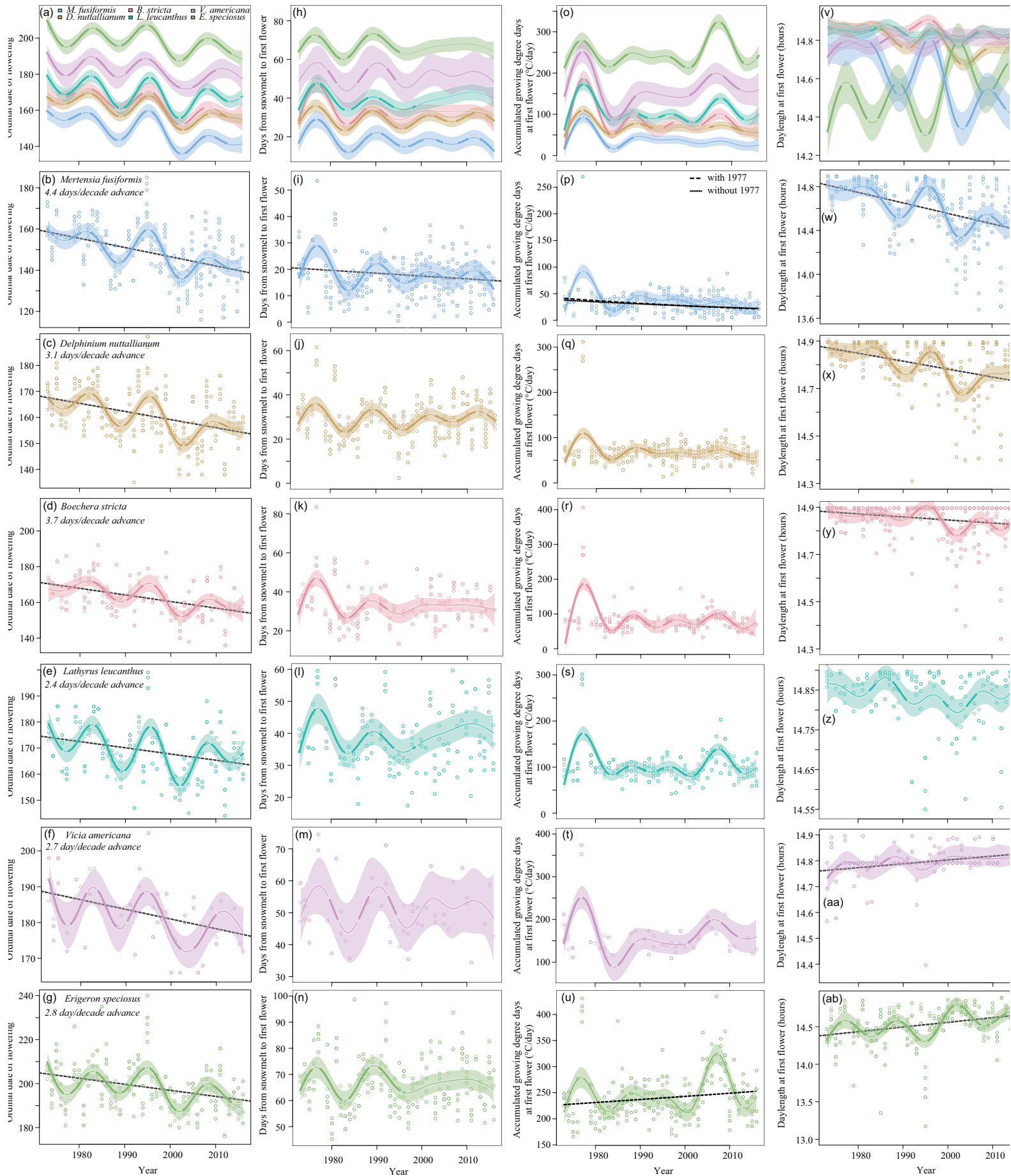
447

448 **Fig. 3 (see p. 20).** Temporal trends in (a-g) ordinal flowering onset dates, (h-n) the number of
449 days from snowmelt to first flower, (o-u) the number of accumulated growing degree days
450 (GDD) at first flower, and (v-ab) the day length at first flower for six plant species monitored in
451 a long-term phenology study over 1973-2016 at the Rocky Mountain Biological Laboratory in
452 Colorado. Smooth trend lines with 95% confidence intervals were derived via penalized
453 regression splines from general additive mixed models. The top set of panels show predicted
454 functions for all six species together, while the raw data for each species are displayed as points
455 in the panels beneath them in the order that species come in to flower. Portions of the predicted
456 functions that are significantly increasing or decreasing are shown in bold (see Fig. S4). When
457 significant, we also display the linear trend line (dashed line) from linear mixed models.

458
459 Please note: Owing to the size of this figure, we have opted to provide it as the first supplemental
460 file as well as embedding it in the manuscript file (per *Global Change Biology* guidelines).
461 Please review the supplement for a larger version of this figure.

462

463



465 With snowmelt gradually occurring earlier (Fig. S1), and cooler spring temperatures in
466 years of early snowmelt (Fig. 2c), we would expect species that are advancing their ordinal
467 flowering onset dates to initiate reproduction at a lower heat threshold over time (Fig. 1a).
468 However, the number of accumulated GDD at first flower have largely remained constant over
469 time for all but the earliest flowering species (Fig. 3o-u), with peaks in 1977 (due to an
470 exceptionally early snowmelt date, Fig. S1), and in 2007 for the later flowering species (where
471 record maximum daily temperatures occurred on 16 of the 30 days from mid-June to mid-July).
472 The number of accumulated GDD at flowering for the early-flowering *M. fusiformis* have
473 decreased over the study period (spline: $F_{10,253}=9.73$, $p<0.0001$; linear trend: $F_{1,261}=29.18$,
474 $p<0.0001$), and this decline is still evident when data from 1977 are excluded (spline:
475 $F_{10,250}=12.94$, $p<0.0001$; linear trend: $F_{1,258}=22.05$, $p<0.0001$, Fig. 3p, Table S5).

476 As ordinal dates of first flower shift over time, species may encounter new photoperiodic
477 regimes, the novelty of which will depend on their latitude and when in the growing season they
478 flower relative to the summer solstice (Fig. 1b). The day length during the summer solstice
479 (ordinal day 172) is 14.9 hours at our study site. Species that flower closest to the summer
480 solstice experienced modest or no changes in day length over time despite advances in the
481 ordinal date of first flowering (Fig. 3v-ab, Fig. S3, Table S5). In contrast, early- and late-
482 flowering species are experiencing shorter or longer day lengths at flowering over time,
483 respectively, in accordance with advances in the ordinal onset date of flowering (linear trend: *Mf*
484 $F_{1,261}=45.3$, $p<0.0001$; *Es* $F_{1,225}=24.7$, $p<0.0001$). The earliest flowering species, *M. fusiformis*,
485 will continue to encounter increasingly shorter day lengths at first flower as its ordinal flowering
486 onset date advances (Fig. 3w). In contrast, the latest flowering species, *E. speciosus*, will face

487 more consistent day lengths as its ordinal flowering onset date
488 shifts nearer to the summer solstice (Fig. 3ab).

489 The timing of snowmelt has a positive effect on ordinal
490 dates of first flower for all species, although snowmelt is
491 consistently advancing at a faster rate than phenology (Figure 4,
492 Table S6). Interestingly, spring temperatures had no effect or a
493 weak negative effect on phenology when accounting for the
494 influence of date of snowmelt (Table S6). These results
495 demonstrate that the date of snowmelt, and not spring
496 temperatures, are driving shifts in phenology.

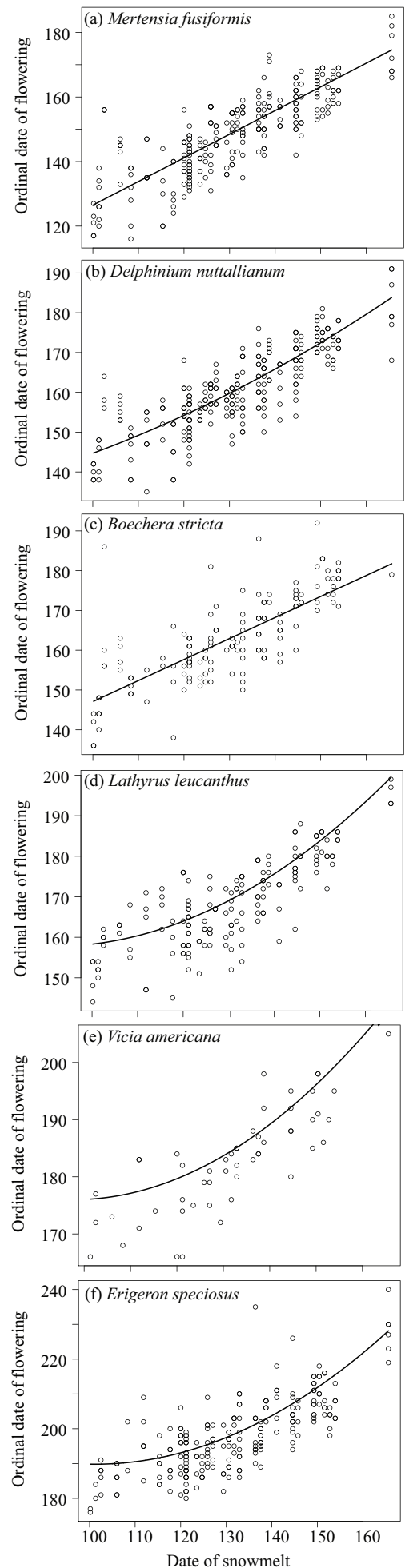
497

498 **Fig. 4.** Relationships between the date of snowmelt and the
499 ordinal date of first flower for species from the long-term
500 phenology study in the order in which they come in to flower:
501 (a) *Mertensia fusiformis*, (b) *Delphinium nuttallianum*, (c)
502 *Boechea stricta*, (d) *Lathyrus leucanthus*, (e) *Vicia americana*,
503 and (f) *Erigeron speciosus*. Linear mixed models include spring
504 temperatures as a covariate and plot as a random effect. Linear
505 and quadratic terms were included for both date of snowmelt and
506 temperature.

507

508

509 A closer examination of phenology data for the focal
510 species in our experimental study, *Boechea stricta*, reveals that
511 patterns of flowering phenology are likely driven by spring
512 snowpack. The ordinal date of first flower is positively
513 associated with spring snowpack, with each 10 cm decrease in



514 snowpack resulting in a 1.4-day acceleration of reproduction (spline: $F_{10,131}=2798$, $p<0.0001$;
515 linear trend: $F_{1,139}=112.1$, $p<0.0001$; Fig. 5a, Table S4). In contrast, low snowpack delays
516 flowering when measured as the number of days from snowmelt to first flower, with each 10 cm
517 decrease in snowpack producing a 1.4-day delay of reproduction relative to snowmelt (spline:
518 $F_{10,131}=185.98$, $p<0.0001$; linear trend: $F_{1,139}=91.15$, $p<0.0001$; Fig. 5b, Table S4). These
519 results point to the importance of winter and early spring climate in determining the timing of
520 flowering in this species.

521

522 **Fig. 5.** The relationship between
523 snow depth on April 1 and (a)
524 ordinal flowering date and (b) days
525 from snowmelt to first flower in
526 *Boechea stricta* over 1973-2016 in
527 the long-term phenology study at the
528 Rocky Mountain Biological
529 Laboratory in Colorado. Smooth
530 trend lines with 95% confidence
531 intervals were derived via penalized
532 regression splines from general
533 additive mixed models. Portions of
534 the predicted functions that are
535 significantly increasing or decreasing
536 are shown in bold (see Fig. S5).
537 When significant, we also display the
538 linear trend line from linear mixed
539 models.

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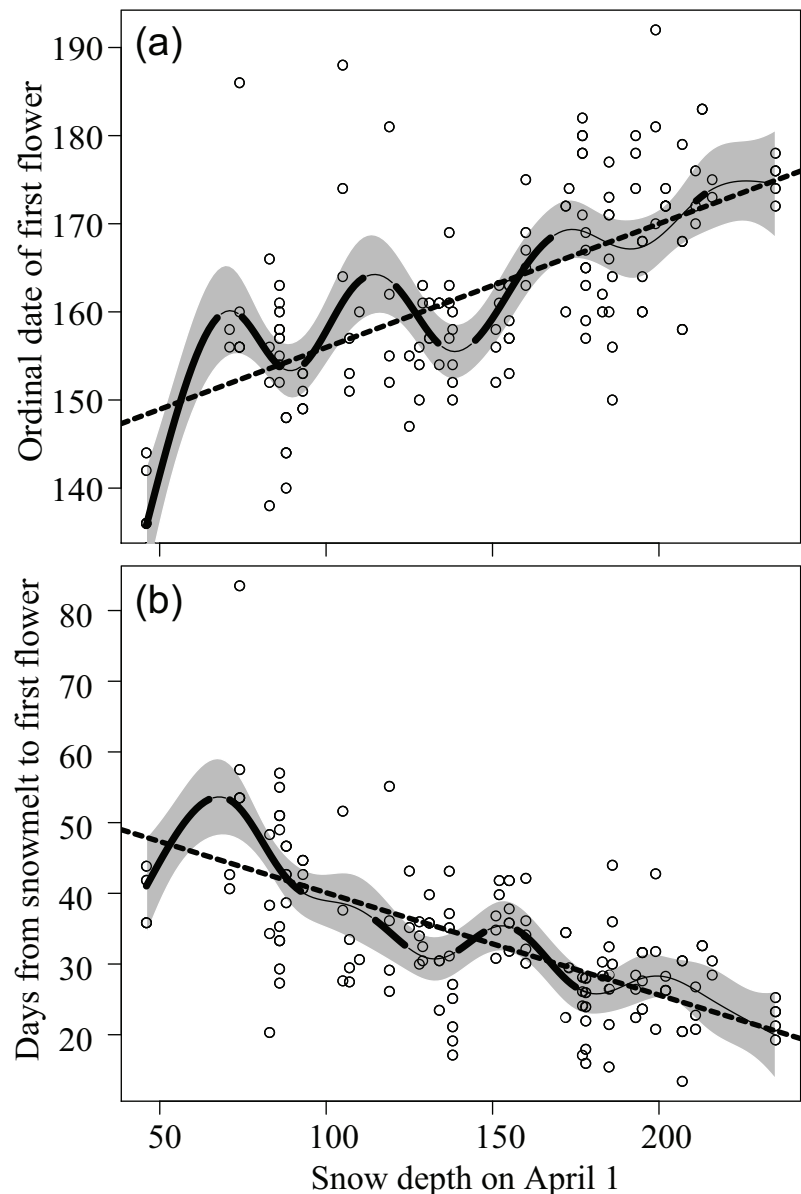
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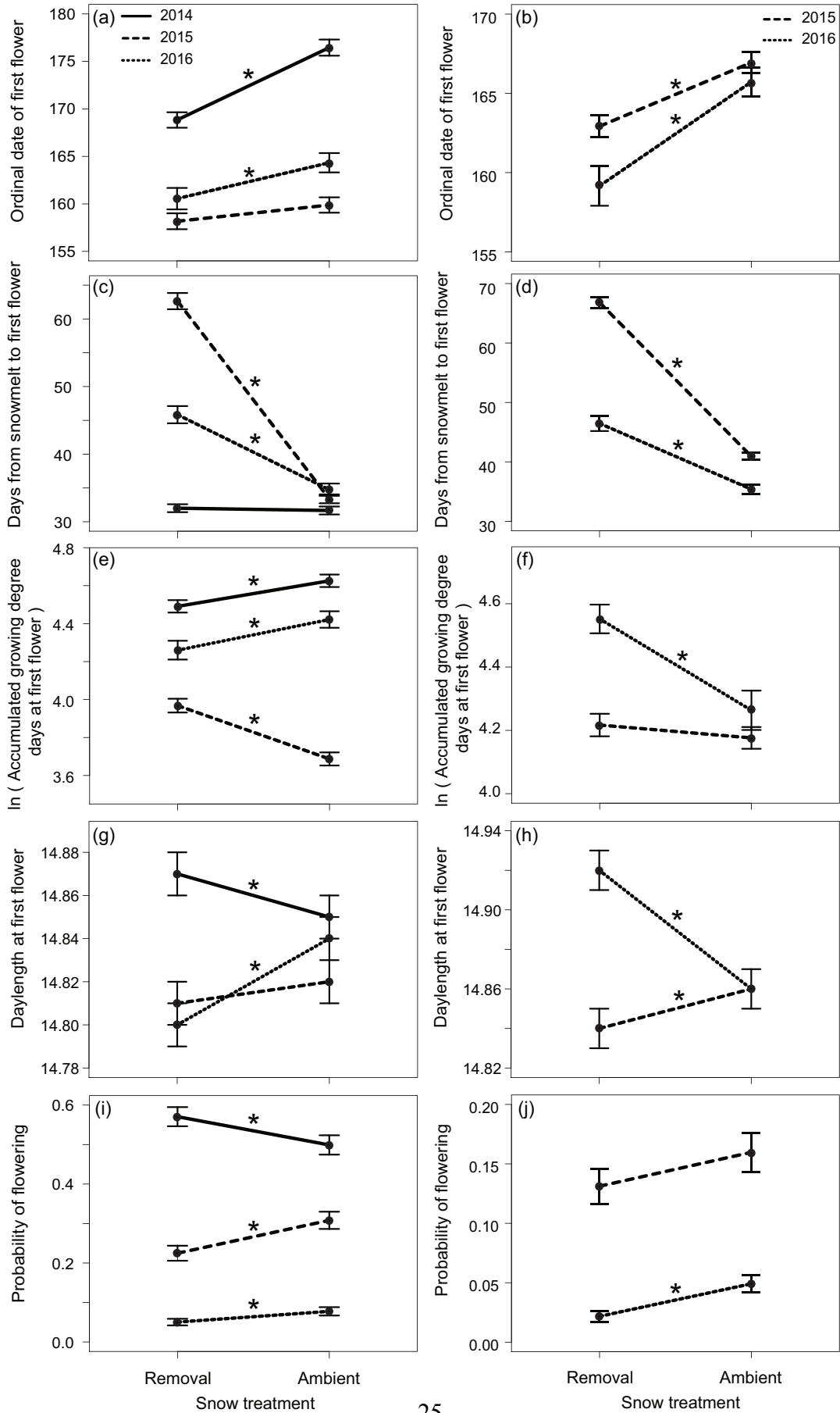
546 *Experimental phenology study*

547 To examine the direct effects of snowpack depth and snowmelt timing on flowering
548 phenology, we imposed an early snow removal treatment in a common garden containing
549 populations of *Boechnera stricta* from across an elevational gradient. Snow removal accelerated
550 the timing of snowmelt to a varying extent across years, ranging from 8 days in 2014 to 30-31
551 days in 2015 to 13.6-18 in 2016 (Table S7). The ordinal date of first flower consistently occurred
552 earlier in the snow removal treatment in all but the 2015 season for the 2013 cohort, with
553 plasticity ranging from 1.7 to 7.6 days among seasons and cohorts (Fig. 6a,b; Tables S8, S9,
554 S10). When measured as days from snowmelt to first flower, the snow removal treatment
555 delayed flowering in 2015 (a low snowpack year) and 2016 (a moderate snowpack year), but had
556 no effect in 2014 on the 2013 cohort (a high snowpack year; Fig. 6c,d; Tables S8, 9). Plasticity in
557 the number of days from snowmelt to first flower in 2015 and 2016 ranged from 11.1 to 29.3
558 days and was greatest in 2015 for both cohorts (Fig. 6c,d; Tables S8, S9, S10).

559

560 **Fig. 6 (Please see p. 25).** Reaction norms depicting treatment and season effects across years
561 from a manipulative snow-removal treatment on *Boechnera stricta* in the experimental phenology
562 study. Average (\pm SE) (a) ordinal date of first flower, (b) number of days from snowmelt to first
563 flower, (c) number of accumulated growing degree days (GDD) at first flower, (d) daylength at
564 first flower, and (e) the probability of flowering. Stars adjacent to each reaction norm are
565 indicative of significant differences among treatments or seasons.

566
567
568



569 The effects of early snow removal on the number of accumulated GDD mirror shifts in
570 the ordinal date of flowering onset, where years or treatments with earlier flowering correspond
571 with a lower heat sum at first flowering (Fig. 6e,f; Table S8, S9, S10). One exception is in the
572 2015 growing season: For the 2013 cohort, the control treatment had significantly lower
573 accumulated GDD than the removal; in contrast, there was no difference in heat sum across
574 treatments in the 2014 cohort in that year. Patterns of daylength at first flowering were less
575 consistent. In some cases, plants in the removal treatment experienced significantly lower
576 daylengths at first flowering (2013 cohort in the 2016 growing season; 2014 cohort in 2015),
577 whereas in other cases, we found the reverse pattern (2013 cohort in the 2014 growing season;
578 2014 cohort in 2016) or no difference (Fig. 6g,h; Table S8, S9, S10).

579 The common garden experiment also afforded us the opportunity to evaluate the
580 probability of flowering in relation to snowmelt date. In the 2013 cohort, the probability of
581 flowering was greater under snow removal in the first year of the study (2014), when snow
582 removal accelerated snowmelt by only 8 days (Odds ratio 0.749, $t_{495}=0.75$, $p=0.0105$, Table
583 S11). For the 2014 cohort, we found no significant differences in the probability of flowering
584 across treatments in the first growing season (2015). In contrast, both cohorts showed a
585 significant reduction in the probability of flowering in response to early snowmelt in subsequent
586 years (2013 cohort: 2015 Odds ratio 1.536, $t_{495}=3.45$, $p=0.0006$, 2016 Odds ratio 1.585,
587 $t_{495}=2.17$, $p=0.0307$; 2014 cohort: 2016 Odds ratio 2.328, $t_{448}=3.65$, $p=0.0003$; Fig. 6i,j; Table
588 S11).

589

590

591

592 **Discussion**

593 The ordinal dates of first flowering are advancing for species representative of the plant
594 communities in the subalpine meadows of Colorado; this result is similar to findings from most
595 long-term studies of flowering phenology (Amano *et al.*, 2010, Fitter & Fitter, 2002, Miller-
596 Rushing *et al.*, 2008, Peñuelas *et al.*, 2002). Furthermore, flowering phenology is advancing at a
597 more rapid rate in early flowering species than in those that flower later. The greater sensitivity
598 of early-flowering species to climate change has emerged in a variety of plant taxa and across a
599 diverse array of habitats (Dunne *et al.*, 2003, Menzel *et al.*, 2006, Miller-Rushing *et al.*, 2007,
600 Moore & Lauenroth, 2017, Post & Nils Chr, 1999). This interspecific variation in responses to
601 environmental change could result from pollination mode (Fitter & Fitter, 2002), increased
602 plasticity in early-flowering species (Sherry *et al.*, 2007), variation in vernalization, and/or
603 differences in critical photoperiod requirements between early and late flowering species (Cook
604 *et al.*, 2012), and phylogenetic history (Mazer *et al.*, 2013). However, many potentially
605 influential determinants of seasonal variation in phenological change remain underexplored,
606 including variation among species in photoperiodic sensitivity and the importance of interactions
607 among multiple aspects of climatic change (Cook *et al.*, 2012, Dunne *et al.*, 2003, Matthews &
608 Mazer, 2016).

609 Reporting the timing of phenological events as the ordinal dates of year on which they
610 occur is an arbitrary, albeit convenient, choice (Sagarin, 2001). Selecting a biologically
611 meaningful reference point from which to measure first flowering data, such as the date of
612 snowmelt for high latitude and altitude ecosystems, could yield additional insight or entirely
613 different conclusions about the influence of climate change on plant taxa (Bertin, 2008, Dunne *et*
614 *al.*, 2003). In our study, the earliest-flowering species (*M. fusiformis*) displayed a moderate

615 advance in phenology when measured as elapsed days since snowmelt, while we detected no
616 change for the remaining taxa. For all species, flowering commenced later with respect to
617 snowmelt in 1977 and 1992. In these years, snow melted abnormally early, exposing plants to
618 sub-zero temperatures. Counterintuitively, early snowmelt can result in delayed reproduction for
619 plants that were historically insulated by snow but are now exposed to frosts in early spring
620 (Inouye, 2008, Pardee *et al.*). Results from our manipulative experiment confirm that low
621 snowpack and early snowmelt delay flowering relative to snowmelt timing. Our data suggest that
622 species in our system initiate flowering at a fixed time after snowmelt; therefore, shifts in the
623 ordinal date of first flower are not the result of the development of more rapid reproductive
624 strategies.

625 Variation in the date of snowmelt governs the spring temperatures that plants experience.
626 While snowmelt is occurring earlier over time, decadal fluctuations in precipitation (Ault & St.
627 George, 2010) and snowmelt date may prevent all but the earliest flowering species from
628 experiencing consistent reductions in the number of accumulated growing degree days at first
629 flower over time, although all species amass large heat sums in years with anomalous snowmelt
630 dates. Recent extreme summer temperatures have increased the heat sum accrued at flowering
631 for the latest species to flower (*E. speciosus*), suggesting that climate change may differentially
632 affect the rate at which thermal energy is accumulated for early and late flowering species.
633 Changes in the photoperiodic regimes encountered upon reproduction also depended on the
634 sequence in which species flowered, with early-flowering individuals experiencing increasingly
635 disparate photoperiods and late flowering individuals experiencing increasingly stable
636 photoperiods. Our focal species all flower in the narrow timeframe of early- to midsummer,
637 when photoperiod is changing at its slowest rate. These species accumulate ~14.5 hours of

638 daylight for photosynthesis per day, whether snowmelt occurs on day 148 (historic) or 137
639 (current).

640 Altogether, our results demonstrate that climate change may decouple historically reliable
641 cues disproportionately among species that differ in seasonal reproductive timing. Consequently,
642 climate change can induce flowering at inopportune times, and may even cause individuals to
643 fail to flower. Subalpine environments have very short growing seasons, meaning that all species
644 flower near mid-summer, when day lengths are relatively constant. Indeed, the daylength at
645 flowering shifted by only minutes in our manipulative experiment and for the mid-summer
646 species in the observational experiment despite significant shifts in the ordinal date of flowering
647 onset. These changes in photoperiod are unlikely to produce biologically relevant effects on
648 phenology for all but the earliest flowering species. In our system, continued phenological
649 change is more likely to be restricted by unmet vernalization requirements or frost damage than
650 by photoperiodic constraints due to the relatively constant day lengths experienced for much of
651 the growing season. Climate change may disrupt thermal and photoperiodic regimes more in
652 regions with longer growing seasons because greater shifts in ordinal flowering onset dates
653 would be possible (Hülber *et al.*, 2010).

654 The transition to flowering involves a suite of interacting developmental pathways that rely
655 on dependable combinations or sequences of cues to signal the appropriate time to reproduce.
656 Recent record-breaking spring temperatures in the eastern United States elicited the earliest
657 recorded flowering onset dates for dozens of species, suggesting that phenological advances may
658 not yet be inhibited by genetic, developmental, or physiological constraints in temperate plant
659 species (Ellwood *et al.*, 2013). Nevertheless, our observational and experimental results reveal
660 constraints on continued phenological responses to climate change. If constraints did not exist,

661 we would have expected plants to keep pace perfectly with climate change; that is, for every one-
662 day advance in snowmelt, we would expect a one-day advance in first flowering. Instead, all
663 species showed a more attenuated relationship, with flowering advancing only 0.5-0.73 days per
664 day acceleration in snowmelt timing. This result is also evident in the manipulative study,
665 wherein snow removal advanced snowmelt by 8-31 days, but only induced flowering to occur
666 1.7-7.6 days earlier than in the control treatment. Across both studies, low snowpack and early
667 snowmelt delay flowering when measured as elapsed days since snowmelt, suggesting that plants
668 exposed to altered snow dynamics suffer frost damage or are slower to accumulate other cues
669 necessary for flowering. Additionally, we found that snow removal reduced flowering success in
670 most years. These results demonstrate that climate change is outpacing plant phenological
671 change and may be depressing fitness in our system, which could have negative consequences
672 for future population growth rates under climate change (Anderson, 2016).

673 The multiyear nature of our observational and experimental studies yielded insights that
674 would not have been apparent from shorter-term studies (Wolkovich *et al.*, 2014). For all
675 species, general additive mixed models indicated significant fluctuations in the trend of earlier
676 flowering through time. Had we collected data for shorter periods of time (e.g., 1990-1994, or
677 2000-2004), we would have erroneously concluded that most species are flowering later every
678 year, instead of the clear pattern of earlier flowering through time that we see in the longer
679 record. Thus, temporal cycles can obscure true patterns that are not captured during a given study
680 (Wolkovich *et al.*, 2014). Multiyear field experiments are similarly valuable (Harte *et al.*, 2015).
681 Consistent with a recent meta-analysis (Anderson, 2016), we found that snow removal decreased
682 flowering success in the experimental study, but this result only emerged after two years of snow
683 removals had been imposed. Similarly, natural temporal variation in snowpack altered the effect

684 of treatment on flowering phenology, resulting in some years with negligible differences in
685 reproductive timing between control and snow removal plots.

686 Our results highlight that factors other than elevated summer temperatures can induce
687 phenological shifts under climate change. In high elevation (Inouye & Wielgolaski, 2013) and
688 high latitude (Wielgolaski & Inouye, 2013) regions, late winter and early spring conditions may
689 play a more prominent role in structuring communities in the context of climate change,
690 particularly for early-flowering species. By evaluating the mechanisms through which climate
691 change alters phenology, we can make more robust predictions about biological responses to
692 ongoing shifts in environmental conditions. Specifically, we argue that constraints will likely
693 prevent plants from keeping pace with novel climates. As climatic conditions continue to diverge
694 from historical values, plants in this community and others are likely to flower at times that are
695 no longer adaptive.

696

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708

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710 J.T.A. did so for the experimental manipulation. D.W.I., J.T.A., J.E.O., and S.M.W collected
711 data. S.M.W. and J.T.A. analyzed data and wrote the first draft of the manuscript. All authors
712 edited that draft.

713

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858 **Supporting Information**

859

860 **Table Captions:**

861

862 **Table S1.** 2013 cohort flowering success data from the experimental phenology study

863

864 **Table S2.** 2014 cohort flowering success data from the experimental phenology study

865

866 **Table S3.** Calculations of flowering onset date for the experimental phenology study

867

868 **Table S4.** Summary statistics for generalized additive (mixed) models and linear (mixed) models

869 for analyses of trends in climate

870

871 **Table S5.** Summary statistics for generalized additive (mixed) models and linear (mixed) models

872 for analyses of trends in phenological change from the long-term phenology study

873

874 **Table S6.** Summary statistics for generalized additive (mixed) models and linear (mixed) models

875 for analyses of trends in phenological change from the experimental phenology study

876

877 **Table S7.** Early snow-removal treatment effects on the timing of snowmelt in the experimental

878 phenology study

879

880 **Table S8.** Early snow-removal treatment and season effects on phenology in the experimental

881 phenology study

882

883 **Table S9.** Least square means of phenological traits in the experimental phenology study

884

885 **Table S10.** The extent of phenotypic plasticity in phenological traits in the experimental

886 phenology study

887

888 **Table S11.** Analyses of the probability of flowering for both planting cohorts in each treatment

889 and across seasons in the experimental phenology study

890

891

892 **Figure Captions:**

893

894 **Fig. S1.** Trends in the date of snowmelt over time using observed dates from b. barr at the

895 RMBL (1975-2016) and runoff measurements from the nearby East River (updated from

896 Anderson et al. 2012).

897

898 **Fig. S2.** (a) A diagram demonstrating the contributions of daily minimum and maximum

899 temperatures to the number of available growing degree days, and (b) Average daily maximum,

900 (c) average, and (d) minimum temperatures in May throughout the study period.

901

902 **Fig. S3.** Derivatives and 95% confidence intervals for the predicted spline functions derived

903 from generalized additive models for (a) temporal trends in date of snowmelt, (b) temporal

904 trends in snow pack on April 1st, (c) the relationship between snowpack and date of snowmelt,
905 and (d) the influence of the date of snowmelt on the average daily temperature experienced in the
906 first 50 days of spring over 1973-2016 at the Rocky Mountain Biological Laboratory in Colorado
907 (see Fig. 2, S1).

908

909 **Fig. S4.** Derivatives and 95% confidence intervals for the predicted spline functions derived
910 from generalized additive mixed models for temporal trends in (a-f) ordinal flowering onset
911 dates, (g-l) the number of days from snowmelt to first flower, (m-r) the number of accumulated
912 growing degree days (GDD) at first flower, and (s-x) the day length at first flower for six plant
913 species monitored in the long-term phenology study over 1973-2016 at the Rocky Mountain
914 Biological Laboratory in Colorado (see Fig. 3).

915

916 **Fig. S5.** Derivatives and 95% confidence intervals for the predicted spline functions derived
917 from generalized additive models for the relationship between snow depth on April 1 and (a)
918 ordinal flowering date and (b) days from snowmelt to first flower in *Boechna stricta* over 1973-
919 2016 in the long-term phenology study at the Rocky Mountain Biological Laboratory in
920 Colorado (see Fig. 5).