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Flowering season of vernal herbs is shortened at elevated temperatures with reduced precipitation in early spring

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Vernal herbs are exposed to the risk of climate change under spring frost and canopy closure. Although vernal herbs contribute to the biodiversity of the understorey layer in temperate forests, few studies assessed the effect of climate change on the phenology of the herbs. To examine phenological shifts in flowering seasons of vernal herb species caused by climate change, a greenhouse experiment was conducted using four species (*Adonis amurensis*, *Pulsatilla nobilis* var. *japonica*, *Viola phalacrocarpa*, and *Pulsatilla cernua*) under two temperature conditions (ambient or elevated temperature) and two precipitation conditions (convective or reduced precipitation). Experimental warming advanced overall aspects of the flowering timing including the first and last day of flowering. The growth of flowering stalk was also promoted by elevated temperature. Effects of decreased precipitation varied among species, which advanced the last day of the flowering of the later flowering species. Consequently, a decrease in overall flowering period length was observed. These results indicate that overall, climate change results in a shortening of the flowering season of vernal herb species, specifically at a higher temperature and under conditions of less precipitation.

Global climate change affects ecosystems in diverse manners, ranging from changes in individual metabolism to structural or functional changes in populations and communities^{1–4}. Not only the average temperature but also the probability of extreme weather events have been estimated to be higher^{5,6}. Due to altered atmospheric circulation, the probability of extreme precipitation such as heavy precipitation, drought, and precipitation deficits has also been predicted to increase^{1,5,6}. Soil moisture, which plays a role as one of the key factors affecting the plant growth, has also been predicted to decrease^{7–9}. Researchers have predicted that the distribution range of plant species will change in response to altered climate conditions^{10,11}. Every plant species and ecotype express phenotypic plasticity in response to altered climate conditions^{12–15}.

Climate not only affects the morphological plasticity but also temporal traits such as the timing of phenological events¹⁶. Appropriate timing of phenological events is important for plant growth and reproduction under specific environments and interspecific interactions^{17,18}, and higher temperatures can induce faster growth and earlier phenological transition¹⁹. Soil moisture also affects phenological transition such as bud break of trees, germination^{20,21}, and summer flowering of alpine shrubs or forbs^{22,23}. Nonetheless, the combined effects of temperature and soil moisture changes on phenological transitions remain unclear^{4,24,25}. Altered climate does not have a homogeneous effect on phenological shifts in all plant species because the timing of crucial growth or phenological events differs from species to species¹⁴. The varied effects make it difficult to understand the effects of climate change on plant phenology. Advances and delays in phenological transitions (hereafter “phenological shift”) not only affect the population dynamics of each species but also biological diversity and community shifts^{1,2,10,26}. The phenological shift also alters species-species interactions, which depend on the responses of each species^{3,27}. For example, temperature not only affects the flowering periods of entomophilous flower species but also pollinator assemblage²⁸. It is hypothesized that the phenological shift might affect the fitness of flowering plants and pollinator insect species^{3,19,28}.

In general, altered climate mainly affects the transitions between seasons, especially between winter and spring. Climate and photoperiod in spring are crucial factors that determine germination from seed or below-ground parts and leaf emergence from tree species^{22,29,30}. Vernal or spring ephemeral herbs, emerge and flower in early spring in forest understory before canopy closure and the emergence of aboveground parts contribute to

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the biodiversity of forests and meadows through temporal niche separation^{31–33}. They usually exhibit a relatively short growth period and rapid phenological transitions in spring because of the decline in light intensity caused by leaf emergence of trees³³. The belowground parts of vernal herbs are awakened from winter dormancy by sensing soil temperature and/or photoperiod^{14,32}. Vernal herbs should also emerge, flower, and produce seed after the spring frost and before canopy closure, which causes shade-induced senescence^{26,33}. Therefore, vernal herbs are exposed to extreme climate events such as frost, drought, and abnormally high temperatures in early spring^{31,32}, and these risks seem to be higher in altered climate conditions. For example, warmer early spring shortens the vernalization period of some vernal herb species, the necessary period of exposure to the cold before emergence¹⁷. However, assessments of the effect of altered climate conditions on the phenology of vernal herb species have been mainly conducted in extreme climate regions, such as tundra and alpine ecosystem^{14,33}.

Effect of the changing environment on the flowering of vernal herb species could be assessed in diverse ways such as the success of flowering (flowering rate), growth of flowering part, and flowering season. Both flowering rate and growth of flowering stalk are known to be affected by the elevated temperature and drought^{34,35}. Differences in flowering stalk length can be assessed using phenotypic plasticity indices^{36,37}. On the other hand, methods of the assessment on the flowering season, which could be used for the assessment of the phenological shift, are still being conducted in a diverse way.

Most of the studies on the phenological shift have been focused on the first day of the phenological event such as the first flowering day³⁸. The last date of flowering is also important about the duration of the flowering^{9,38}. As many species do not exhibit the uniform shifts of first, peak, and last flowering³⁸, assessment of the phenological shift based on an only first flowering date could have the bias on the flowering season. The length of the flowering period could affect the overall reproductivity such as the number of flowers produced^{39,40}. The warmer temperature is known to extend the flowering duration²⁴ and this could positively affect the reproductive opportunity for plants⁹. The species-specific response of the phenological shift with a heterogeneous shift of first, peak, and last flowering under altered climate conditions are hypothesized to affect the community-level flowering duration which is referred to as “flowering season”¹⁴.

Higher temperatures and drier soil due to reduced precipitation in spring appear to critically affect the phenology of vernal herb species as well as flowering rate and growth; the phenological shift responses of each species vary based on intrinsic life cycle traits, which is represented as “flowering niche” and the flowering season range for each species¹⁴. Even though vernal herb species are sensitive to altered spring climate conditions, their specific responses to ongoing climate change are poorly understood, in particular, in the temperate region. The species-specific phenological shift under the altered climate conditions affects the community-level flowering season. In this study, we conducted a simple factorial experiment under conditions of elevated temperature and reduced precipitation in spring to assess change in the phenological transitions of four early-flowering vernal herb species in the temperate region (*Adonis amurensis*, *Hepatica nobilis* var. *japonica*, *Viola phalacrocarpa*, and *Pulsatilla cernua*). Warmer temperature and reduced precipitation could influence not only the first day of flowering but also the last day of flowering, thereby affecting the flowering season^{9,38}. This study addresses the following four research questions.

1. How are the flowering rate and flowering stalk growth of vernal herb species affected by elevated temperature and reduced precipitation?
2. How are the flowering seasons of vernal herb species affected by elevated temperature and reduced precipitation?
3. Do the effects of temperature and precipitation on flowering seasons differ among species?
4. Does the altered spring climate condition affect the overall flowering season?

Results

Flowering rate under experimental conditions. During the experimental period, 20 ~ 25 individuals of *Adonis amurensis* emerged from the total individuals ($n = 26$ for each experimental condition). From 10 to 15 individuals of *Hepatica nobilis* var. *japonica* emerged from the total individuals ($n = 20$) under different experimental conditions. The number of emerged individuals of *Viola phalacrocarpa* and *Pulsatilla cernua* ranged from 11 to 14 ($n = 17$) and 10 to 13 ($n = 14$), respectively. Flowering rate, which is the rate of the flowering individuals among the survived individuals, exhibited variation among different species and the experimental conditions (Fig. 1). *Adonis amurensis* demonstrated the lowest and similar flowering rate under different experimental conditions, ranging from 24.0% to 28.6% (Fig. 1a). Flowering rate of *H. nobilis* var. *japonica*, *V. phalacrocarpa*, and *P. cernua* under ambient temperature with convective precipitation was 73.3%, 78.6%, and 92.3%, respectively. Under the convective precipitation condition, the flowering rate under ambient temperature was similar to the rate under condition of elevated temperature (average 1.6 °C increase). On the other hand, under ambient temperature, flowering rate of *H. nobilis* var. *japonica*, *V. phalacrocarpa*, and *P. cernua* decreased with reduced precipitation (half of the convective precipitation) from 73.3% to 66.7%, from 78.6% to 63.6%, and from 92.3% to 80.0%, respectively (Fig. 1b–d). Reduced precipitation also decreased the flowering rate of *H. nobilis* var. *japonica* from 80.0% to 66.7% under elevated temperature.

Phenotypic plasticity of stem and flowering stalk growth. Phenotypic plasticity, which is referred as relative distance plasticity index (RDPI)³⁷ under experimental conditions were differed by species, as well as the experimental condition itself (Fig. 2). Stem length RDPI showed the obvious inter-specific difference under three experimental conditions (Fig. 2a). Flowering stalk length showed the relative higher RDPI rather than the stem length (RDPI range of stem length: 0.156 ~ 0.278; flowering stalk length: 0.210 ~ 0.361; Fig. 2b). *Hepatica nobilis* var. *japonica* showed the highest RDPI in both stem length (0.278 under Ele.Tem; 0.252 under Ele.Tem

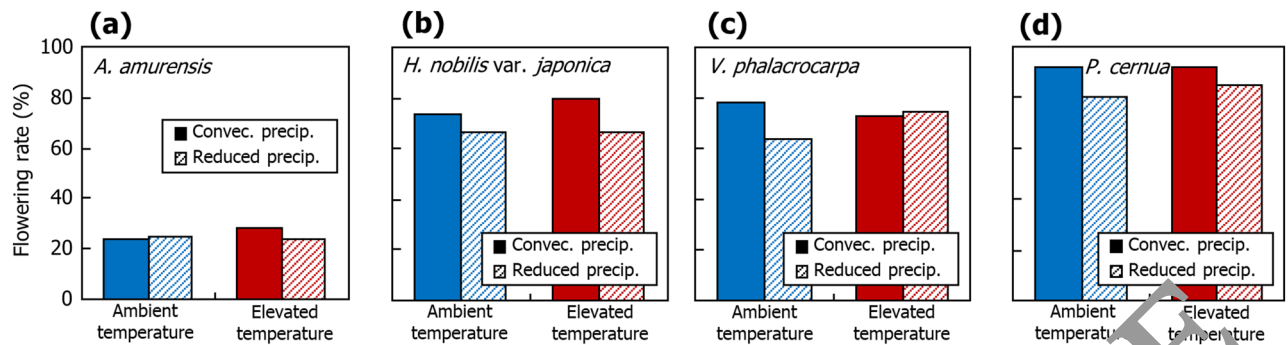


Figure 1. Proportion of flowering individuals (among survived individuals) of four studied species: (a) *Adonis amurensis*; (b) *Hepatica nobilis* var. *japonica*; (c) *Viola phalacrocarpa*; (d) *Pulsatilla cernua*. Convective = convective; Precip. = precipitation.

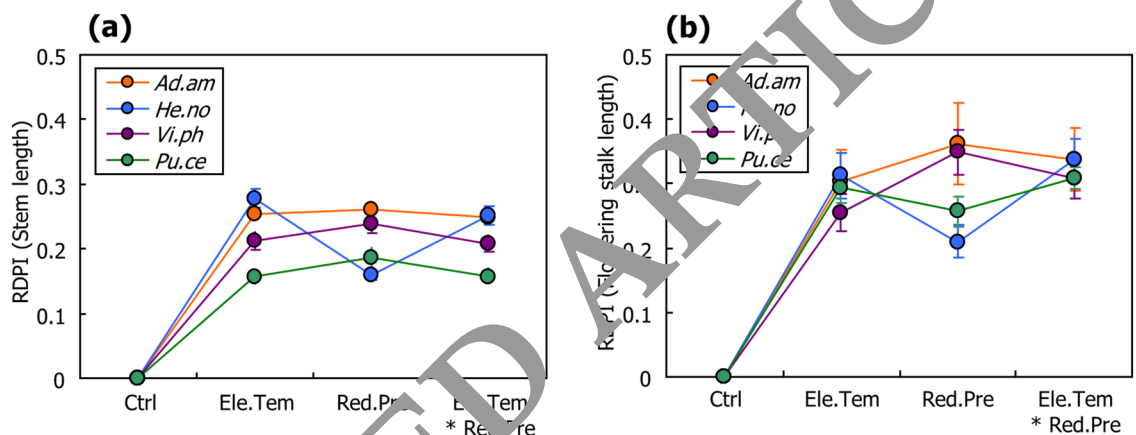


Figure 2. Relative distance plasticity index (RDPI) of each species under three experimental conditions compared to ambient temperature with convective precipitation. (a) RDPI of maximum stem length; (b) RDPI of maximum flowering stalk length. Vertical bars refer the standard error; *Ad.am* = *Adonis amurensis*; *He.no* = *Hepatica nobilis* var. *japonica*; *Vi.ph* = *Viola phalacrocarpa*; *Pu.ce* = *Pulsatilla cernua*; Ctrl = ambient temperature with convective precipitation; Ele.Tem = elevated temperature with convective precipitation; Red.Pre = ambient temperature with reduced precipitation; Ele.Tem * Red.Pre = elevated temperature with reduced precipitation.

* Red.Pre, and flowering stalk length under elevated temperature (0.312 under Ele.Tem; 0.337 under Ele.Tem * Red.Pre) regardless of the precipitation. Meanwhile, *H. nobilis* showed the lowest RDPI value under ambient temperature with reduced precipitation condition, whereas RDPI of *A. amurensis* showed the highest value under the same condition.

Phenological shift on flowering season of vernal herb species. In the ambient temperature environment with convective precipitation condition, the average first flowering (69.7 ± 10.2 day of year [DOY]) and last flowering day (84.3 ± 8.2 DOY) of *A. amurensis* were the earliest among the four studied species. The flowering season of *H. nobilis* var. *japonica* was from 77.6 ± 5.1 to 88.3 ± 2.9 DOY, and that of *V. phalacrocarpa* was from 94.6 ± 3.1 to 100.5 ± 5.3 DOY. *Pulsatilla cernua* showed the latest flowering season (from 93.9 ± 5.0 DOY to 107.4 ± 5.7 DOY).

Elevated temperature majorly influenced the first and the last flowering day (Fig. 3). The first flowering day was significantly advanced to 7.6 days at the elevated temperature in *H. nobilis* var. *japonica* (70.0 ± 4.5 DOY; $p = 0.0001$), 11.1 days in *V. phalacrocarpa* (83.5 ± 3.0 DOY; $p < 0.0001$), and 8.3 days in *P. cernua* (85.6 ± 5.5 DOY; $p < 0.0001$) under convective precipitation. *Adonis amurensis* also showed the advanced first flowering day about 4.0 days without statistical significance (65.7 ± 7.5 DOY; $p = 0.1320$). Also, the four species demonstrated advanced last flowering day. The last flowering day of all the studied species under elevated temperature with convective precipitation also demonstrated advanced last flowering day in 9.8 days, 7.8 days, 7.7 days, and 2.1 days in *A. amurensis*, *H. nobilis* var. *japonica*, *V. phalacrocarpa*, and *P. cernua*, respectively (74.5 ± 12.7 DOY and $p = 0.0262$ in *A. amurensis*; 80.5 ± 9.4 DOY and $p < 0.0001$ in *H. nobilis* var. *japonica*; 92.8 ± 3.1 DOY and $p < 0.0001$ in *V. phalacrocarpa*; 105.3 ± 5.6 DOY and $p = 0.0076$ in *P. cernua*).

Shifts in the first and last flowering days of each species were not the same under each experimental condition (Fig. 3b). Therefore, it was apparent that the overall length of the flowering period was significantly affected by elevated temperatures in two of the four species. Under elevated temperature, the flowering period was

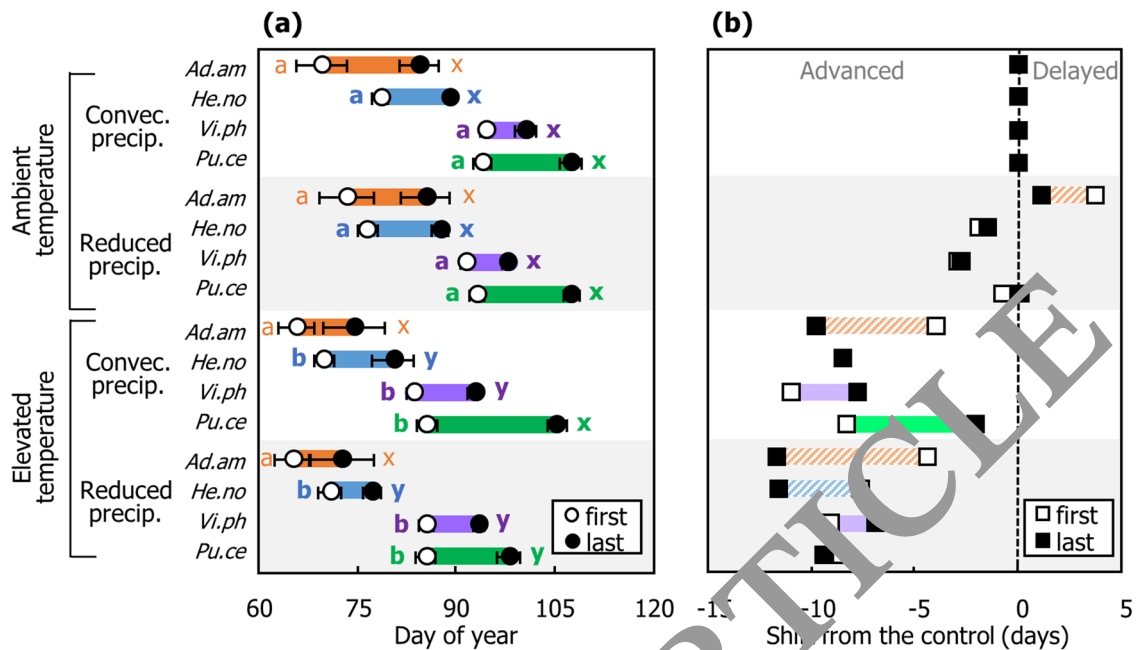


Figure 3. (a) Flowering season (the first- and last flowering day) of four studied species under the four different experimental conditions and (b) shift of the flowering season. Lower cases in (a) refer to the statistically significant group based on Duncan's post-hoc test in each species. Horizontal bars in (a) refer to the standard error. *Ad.am* = *Adonis amurensis*; *He.no* = *Hepatica nobilis* var. *japonica*; *Vi.ph* = *Viola phalacrocarpa*; *Pu.ce* = *Pulsatilla cernua*; Conv. = Convective; Precip. = precipitation; first = first day of flowering; last = last day of flowering. Detailed information of analysis of variance are presented in Supplementary Table 2.

shortened from 14.7 ± 3.4 days to 8.8 ± 5.9 days in *A. amurensis* ($p = 0.0344$), whereas the period was longer in *V. phalacrocarpa* (from 6.0 ± 0.7 to 10.6 ± 3.8 days; $p = 0.0416$). The flowering period of *H. nobilis* var. *japonica* and *P. cernua* were not significantly affected by temperature elevation ($p = 0.11$ and $p = 0.12$, respectively; Supplementary Table 2).

Decreased precipitation had no significant effect on the first flowering day of any of the four studied species ($p \geq 0.05$; Fig. 3a), and the last flowering day was advanced by 7.3 days only in *P. cernua* under elevated temperature (98.0 ± 6.2 DOY; $p = 0.0250$). In some cases, reduced precipitation influenced the traits of the flowering season differently from the effects of temperature; for instance, the interaction was observed between temperature and precipitation on the first flowering date of *V. phalacrocarpa*. Specifically, decreased precipitation slightly advanced 2.2 days of the first flowering day in *V. phalacrocarpa* under ambient temperature (91.6 ± 2.5 DOY) and delayed 1.9 days under elevated temperature (85.4 ± 3.6 DOY; $p = 0.0271$; Supplementary Table 2). In *P. cernua*, reduced precipitation advanced 7.3 days of the last flowering day only under elevated temperature conditions (from 105.3 ± 5.6 DOY to 98.0 ± 6.2 DOY; $p = 0.0356$). As a result, the length of *P. cernua*'s flowering period was shortened by 7.2 days with reduced precipitation only under elevated temperature (from 19.8 ± 6.4 days to 12.6 ± 4.2 days under reduced precipitation; $p = 0.0167$).

The overall flowering period of studied species, which is calculated from the average first flowering date of the earliest flowering species (*A. amurensis*) to the average last flowering date of the latest flowering species (*P. cernua*) differed between the experimental conditions. With convective precipitation, the overall flowering period was 37.7 days and 39.6 days under ambient- and elevated temperatures, respectively. Reduced precipitation shortened the overall flowering period to 34.1 days and 32.8 days under ambient and elevated temperature, respectively.

Phenological shift on flowering stalk growth. Temperature elevation promoted early flowering stalk growth of *A. amurensis* with decreased t_m (midpoint growth with maximum growth rate⁴¹) from 75.9 to 68.3 DOY under convective precipitation and from 76.0 to 66.8 DOY under reduced precipitation in the sigmoid model (Fig. 4a, Supplementary Table 1). Similar to *A. amurensis*, the t_m of *H. nobilis* var. *japonica* was lower at elevated temperature (69.4 DOY and 68.8 DOY under convective and reduced precipitation, respectively) than at ambient temperature (79.4 DOY and 77.7 DOY under convective and reduced precipitation, respectively; Fig. 4b, Supplementary Table 1).

In *V. phalacrocarpa*, t_m was lower under the reduced precipitation condition (85.2 DOY at ambient- and 77.3 DOY at elevated temperature) than under convective precipitation (88.7 DOY at ambient- and 77.5 DOY at elevated temperature; Fig. 4c, Supplementary Table 1). In *P. cernua*, t_m at reduced precipitation under the elevated temperature condition (90.9 DOY) was slightly lower than that of convective precipitation at elevated temperature (94.9 DOY; Fig. 4d), while t_m values at ambient temperature were similar among the two precipitation conditions (102.0 DOY under convective precipitation and 101.2 DOY under reduced precipitation). Overall, the estimated

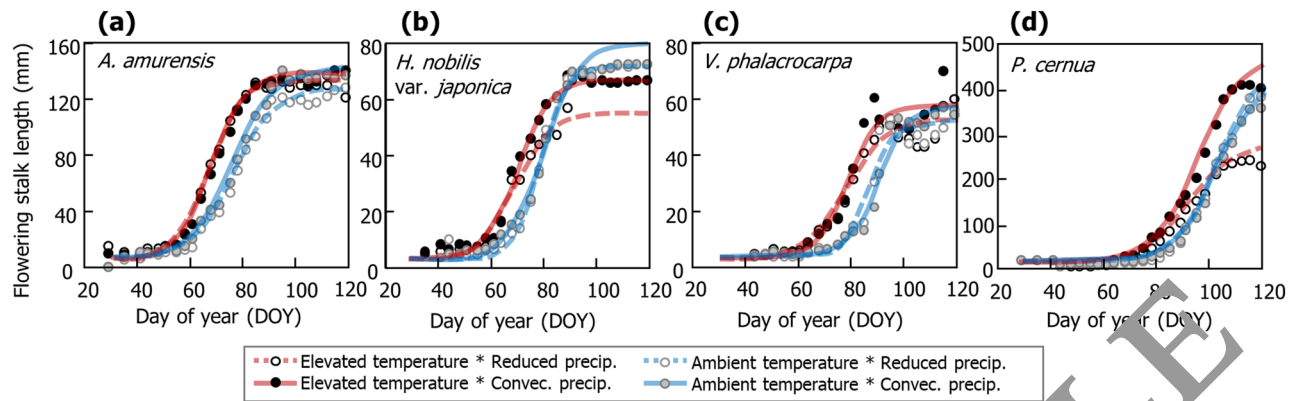


Figure 4. Flowering stalk growth and fitted sigmoid curves of four studied species: (a) *Adonis amurensis*; (b) *Hepatica nobilis* var. *japonica*; (c) *Viola phalacrocarpa*; (d) *Pulsatilla cernua*. Convective = convective; Precip. = precipitation. Information on fitted variables are presented in Supplementary Table 1.

maximum flowering stalk length (h_m) was lower under the reduced precipitation condition except for that of *P. cernua* at ambient temperature (Supplementary Table 1).

Discussion

The overall flowering rate was lower under reduced precipitation rather than the convective precipitation. Phenotypic plasticity of the stem and flowering stalk length seemed to be affected by reduced precipitation under ambient temperature, but not under the elevated temperature (Fig. 2). Even though drought is a commonly known inhibitory factor on the shoot elongation⁴², higher temperature could ameliorate some effects of drought stress³⁴. It means the potential climate change could affect plant traits differentially^{43,44}.

Elevated temperature advanced the overall aspects of the flowering season in the four vernal herb species, regardless of the statistical significance (Fig. 3). Also, the growth of flowering stalk as represented by t_m was advanced by elevated temperature in all the four species (Supplementary Table 1). In diverse climate regions and landscapes, the elevated temperature is known to accelerate the phenological transition such as shoot emergence or flowering^{22,24,26}. Early snowmelt caused by the warmer spring is also known to delay the phenological transition which could shorten the vernalization period^{17,20,30}. In vernal herb species in temperate forests, the flower emerges before vegetative growth or simultaneously, but the elevated temperature is known to delay the emergence of aboveground parts, fruit, seed formation, and flowering^{10,12}. In the present study, the warmer temperature after 30 DOY seemed had no impact on the deficit of the vernalization period. In the former studies, the effect of the day of snowmelt has been mainly studied under tundra or alpine regions^{14,45}. Therefore, elevated temperature in the temperate region did not seem to affect the shortened vernalization or early snowmelt, which causes the delay of the phenological transition.

Decreased precipitation did not significantly affect the overall aspects of the flowering season in the studied species, except for the flowering season of the latest flowering species (Supplementary Table 2). Numerous previous studies have demonstrated that the effect of precipitation on the phenological shift does not reach to the concurrence across species^{46–48}. Under soil drought conditions, the flowering season could be advanced as an 'escape' from drought⁴⁸. However, drought stress during rather than before flower development affects the reproductive yield by the early arrest of flowering or failure to flower³⁵. In this context, the earlier last flowering day and reduced flowering rate seemed to occur in a part of the studied species (Fig. 1).

Interactions between temperature and precipitation occurred on the first day of *V. phalacrocarpa* and the last day of *P. cernua* (Supplementary Table 2). The effect of the drier soil under elevated temperature on the flowering season also did not reach to the concurrence^{4,25}. The phenological shift by altered precipitation was larger under elevated temperature rather than the ambient temperature (Fig. 3). Thus, it is evident that the effect of reduced precipitation on the studied species could vary based on the temperature. Flowering, which is represented by the unfolding of petals, is high energy-consuming metabolism. Elevated air temperature could be the basis of drier soil due to both increased soil transpiration and plant metabolism⁴³. Therefore, it is hypothesized that the drought stress might be higher in elevated temperature conditions even under the same amount of precipitation^{49,50}.

The statistical significance of the phenological shifts under elevated temperature differed among species (Supplementary Table 2). The advance of the first flowering day under experimental conditions was the highest in *V. phalacrocarpa*, followed by *P. cernua*, *H. nobilis* var. *japonica*, and *A. amurensis* (Fig. 3). The phenological shift could vary based on the flowering niche, which is referred from the intrinsic flowering season of each species¹⁴. Also, physiological aspects such as frost resistance of *A. amurensis*⁵¹ may perhaps affect the influence of warmer temperature. *Hepatica nobilis* is also known to be able to flower under the snowfall⁵². Genus *Viola* is regarded as early-flowering species in temperate forest understorey, which phenology is known to be affected by warming⁴⁹. Flowering of the latest flowering species in the present study, *P. cernua*, strongly depends on the light availability⁵³. Therefore, it is expected that the flowering season of each species under the experimental conditions show the heterogeneous shifts.

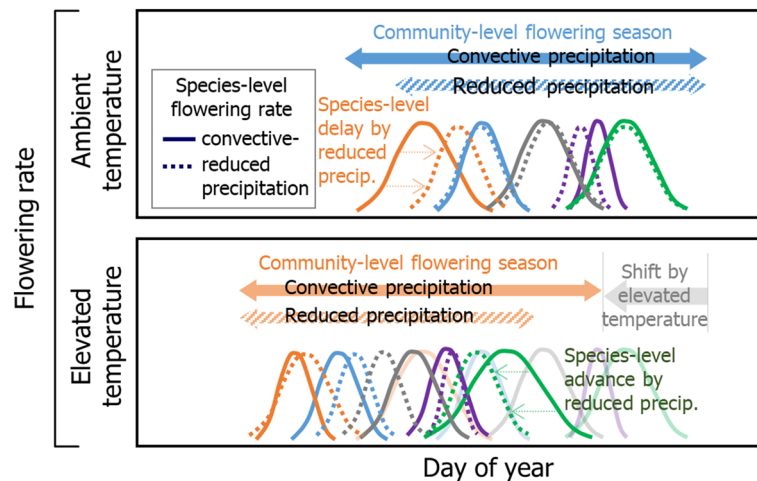


Figure 5. Hypothetical diagram depicting the effects of temperature elevation and precipitation on the flowering season of vernal herb species. Precip. = precipitation.

Reduced precipitation mostly slightly advanced the first and last day of flowering rather than the convective precipitation condition without statistical significance, except in the case of earliest flowering species *A. amurensis* under ambient temperature (Fig. 3b). Soil drought might play a role as a stress factor and suppress flowering and decrease the yield^{35,48}. The lower soil moisture under ambient temperature with reduced precipitation from 40 to 50 DOY seemed to delay the flowering season of *A. amurensis*. On the other hand, the flowering of genus *Hepatica* is known to be resistant to soil drought⁵⁴. Although drought resistance of the studied species was not examined, it is believed that the intrinsic sensitivity of soil moisture at the flowering season could differ among the species. Not only precipitation but increased evapotranspiration by elevated temperature or during the flowering process has been thought to affect the water balance of soil and plant⁴⁹. Consequently, it is believed that the influence of reduced precipitation could vary with species under different temperatures.

The onset of stress such as snow layer and photoperiod has been proposed to affect the timing of phenological transition as well as the temperature^{33,54}. Soil drought might also act as cumulative stress thereby affecting the phenological transition as well as the onset stress⁴⁹. In this manner, the relatively later flowering species seems to be affected more than the earlier flowering species. The cumulative effects of temperature and soil moisture also affect the phenological transition timing from flowering to fruiting. Heterogeneous shifts in the first and last flowering days influence the flowering period length of each species (Fig. 3). Flowering period affects the number of flowers, opportunities for fertilization, and seed production⁹. The different responses of the first and last flowering days under the altered climate condition might affect the reproductivity of each species.

In the present study, altered climate condition was found to influence the overall aspects of the flowering season, including the first and last day of flowering, and growth of the flowering stalk (Figs. 3 and 4). Different responses among the aspects and species lead to variations in the length of the overall flowering period as well as the overall shift. These phenological shifts are hard to be observed without the examination of the phenological last (the last flowering day) as well as the phenological first^{9,38}. Elevated temperature advanced the overall timing of the phenological transition, while reduced precipitation shortened the overall flowering period of the studied species (Fig. 5). Besides, a slight decrease in the flowering rate was observed under reduced precipitation (Fig. 1). These results suggest that the overall flowering season length and flowering rate might be altered under climate change, which may affect the community-level phenology in the field. Species-specific response to altered climate change by different flowering niche shortens the community-level flowering season¹⁴. As the aforementioned aspects may perhaps lead to the mismatch of the pollinators²⁶, altered climate conditions might have an impact on species-level fitness.

The flowering niche of vernal herbs in temperate forests is represented by the earlier flowering before the canopy closure by wood species³¹. In altered climate conditions, canopy closure has been reported to advance³³. The species-specific response of vernal herbs could affect the mortality or reproductivity of each species under advanced canopy closure. Moreover, the excessive advance of the flowering season leads to the exposure to the risk of the spring frost³³. Consequently, the community structure of vernal herbs seems to be influenced by altered climate conditions, and reduced precipitation could intensify the effect of the warmer climate condition (Fig. 5). It is hypothesized that the biodiversity of forest understory in the temperate forest could be influenced by altered climate conditions. In the present study, significant phenological shifts of vernal herb species in the species-specific aspects, which could affect the community-level phenological shifts were observed although only the limited species pool was treated in the Asian temperate region. It is believed that further studies on different vernal herb species in temperate forest region could enhance the understanding and the prediction of the understory diversity.

The experimental warming condition advanced the overall aspects of the flowering of the four vernal herb species. Decreased precipitation, which is predicted as the phenomenon as a side effect of climate change in the temperate region, also influenced the flowering seasons and flowering rate as well as the flowering stalk length.

Although it is hard to generalize the effect of experimental warming and decreased precipitation on each vernal herb species, the community-level flowering season could be affected by warmer temperature and half-level precipitation, respectively. Consequently, the biodiversity of the vernal herb species is predicted to differ, which contributes to the biodiversity of the understory layer of temperate forest. For the preservation of the diversity of vernal herb species in the temperate regions, additional studies to uncover the optimal flowering niche under altered climate conditions are necessitated.

Methods

Study species. The study was conducted in Seoul, the Republic of Korea, which is classified as a temperate climate region. The proportion of spring-flowering species has been reported as only 18.4% (560) of a total of 3,037 angiosperm species in the Republic of Korea^{55,56}. Among the 560 spring-flowering species, 28.1% (146) of angiosperm species flower from March to April. For assessment of the flowering response of vernal herb species, only herbal species from 146 angiosperm species were initially screened. Among them, four perennial herb species which flowered in March or April, were easily cultivated, and common in the field or purchasable from the market were screened: *Adonis amurensis*, *Hepatica nobilis* var. *japonica*, *Viola phalacrocarpa*, and *Pulsatilla cernua*. In the Republic of Korea, these are usually observed in forest understory or meadows.

Adonis amurensis (Ranunculaceae) flowers from late February to early March and *H. nobilis* var. *japonica* (Ranunculaceae) flowers in early March. The flowering stalks of both *A. amurensis* and *H. nobilis* var. *japonica* emerge before the emergence of vegetative part⁵⁵. *Adonis amurensis* is known to have cold resistance, which enables it to sprout and flower before snowmelt^{51,55}. In *H. nobilis*, drought does not affect the flowering rate⁵⁴. *Viola phalacrocarpa* (Violaceae) flowers in early April and *P. cernua* (Ranunculaceae) flowers in middle April⁵⁵. Genus *Viola* has both a chasmogamous and a cleistogamous flower; the chasmogamous flower only occurs before canopy closure⁵⁷. Genus *Pulsatilla* occurs in gaps in the forest; the growth depends strongly on light availability⁵³. All the four species make chasmogamous flowers before canopy closure of forest understory.

Experimental conditions. In April 2015, two-year-old *A. amurensis*, *H. nobilis* var. *japonica*, and *P. cernua* were purchased from a market. *Viola phalacrocarpa* was collected in Mt. Gwanak in Seoul, the Republic of Korea. Flowered individuals of four species were transplanted to pots (130 mm diameter × 120 mm height) filled with horticultural topsoil (Nongkyung Co.; pH: 4.7 ± 0.2 ; electric conductivity: $2,400 \pm 255$ μ S/cm; NO₃-N: $1,117 \pm 6.0$ mg/kg; NH₄-N: $1,106 \pm 100$ mg/kg; PO₄-P: 461.8 ± 17.3 mg/kg; K: $7,589 \pm 176$ mg/kg; Ca: $3,415 \pm 192$ mg/kg; Mg: $1,964 \pm 20.4$ mg/kg) and grown in the experimental field located in Seoul, Republic of Korea (37°27'33.92"N, 126°57'13.28"E, 110 m a.s.l.) for a year before treatment for acclimation.

From February 2016 to May 2016, two temperature conditions and two precipitation conditions were applied to four groups: individuals in the ambient temperature condition were grown in a vinyl greenhouse with open sides and a closed roof, and the elevated temperature condition was inside a closed vinyl greenhouse. The size of each vinyl greenhouse was 3 m (W) × 10 m (D) × 2 m (H).

The air temperature in each condition was measured every hour using HOBO Pro V2 (Onset Computer Corporation, Bourne, MA, USA; Supplementary Fig. 1). The differences in the daily mean air and soil temperatures in the two conditions were about 1.6 °C and 1.1 °C during the experiment, respectively. The maximum and minimum difference in air temperature between the two conditions was 30.8 °C and −4.0 °C, respectively. The difference in soil temperature between the two conditions ranged from −3.9 °C to 19.0 °C.

Precipitation was manipulated with weekly watering to simulate a convective rainfall pattern (Supplementary Fig. 2). In the convective precipitation condition, each pot was watered with the amount of average weekly total precipitation from the past three years. In the reduced precipitation condition, the plants were watered with half the amount of the convective condition. Watering volume was calculated by the multiplication of the area of pot and amount of precipitation. Soil moisture under each condition was measured every hour using a PlantCare Mini-logger (PlantCare Ltd., Russikon, Switzerland; Supplementary Fig. 1). The soil in each pot was saturated (100%) after watering and the soil moisture content decreased slowly after watering. The number of pots (individuals) of *A. amurensis*, *H. nobilis* var. *japonica*, *V. phalacrocarpa*, and *P. cernua* in each experimental condition was 26, 20, 17, and 14, respectively.

Observation of the flowering season. The flowering season was considered as the season from the unfolding of the petals to the senescence of petals. In the case of *V. phalacrocarpa*, only the chasmogamous flower was regarded as a flower. The presence of the flower in each pot was observed and recorded two times per week. For each individual (pot), the first day and last day of the presence of flower with unfolded petals were considered as the first flowering day and the last flowering, respectively³⁸. Flowering rate of each species was calculated from the ratio of number of flowering pots to number of total survived pots under each experimental condition. The flowering period of each individual was calculated by the difference in the last and the first flowering day. The length of the stem and flowering stalk was also measured two times per week.

Phenotypic plasticity. For inter-specific comparison of phenotypic plasticity, a relative distance plasticity indices (RDPI) on maximum stem length and flowering stalk length were calculated³⁷. Because of the discontinuity of the experimental condition, relative distances under three treatments (ambient temperature with reduced precipitation, elevated temperature with convective precipitation, and elevated temperature with reduced precipitation) from control treatment (ambient temperature with convective precipitation) were calculated as below:

$$RDPI = \left(\sum \frac{d_{ctrl,i \rightarrow treatment,j}}{x_{ctrl,i} + x_{treatment,j}} \right) / n \quad (1)$$

where n is the total number of distances.

Statistical analysis. Only flowering individuals were statistically analyzed. Two-way analyses of variance were conducted for the first flowering day, last flowering day, and flowering period length. The sigmoid model was applied to flowering stalk growth as the following equation and constants of the fitted model were compared⁴¹:

$$h(t) = \frac{h_m}{1 + e^{-k(t-t_m)}} \quad (2)$$

(t : day of year; h_m : maximum height; k : constant; t_m : midpoint constant).

All statistical analyses were conducted using R 3.3.2⁵⁸.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 5 April 2020; Accepted: 29 September 2020

Published online: 15 October 2020

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Acknowledgements

This work was supported by a grant (NRF-2018R1A2B2002267) of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning (MSIT), Republic of Korea.

Author contributions

B.E.N. participated in the design of the study, data collection, data analyses, and drafted the manuscript. J.G.K. conceived the study, participated in the design of study and data collection, secured the funding, and edited the manuscript draft. All the authors have read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41598-020-74566-z>.

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