

# Temperature sensitivity thresholds to warming and cooling in phenophases of alpine plants

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**Abstract** The assumption of a linear relationship between temperature and phenophases may be misleading. Furthermore, a lack of understanding of the changes in temperature sensitivity of

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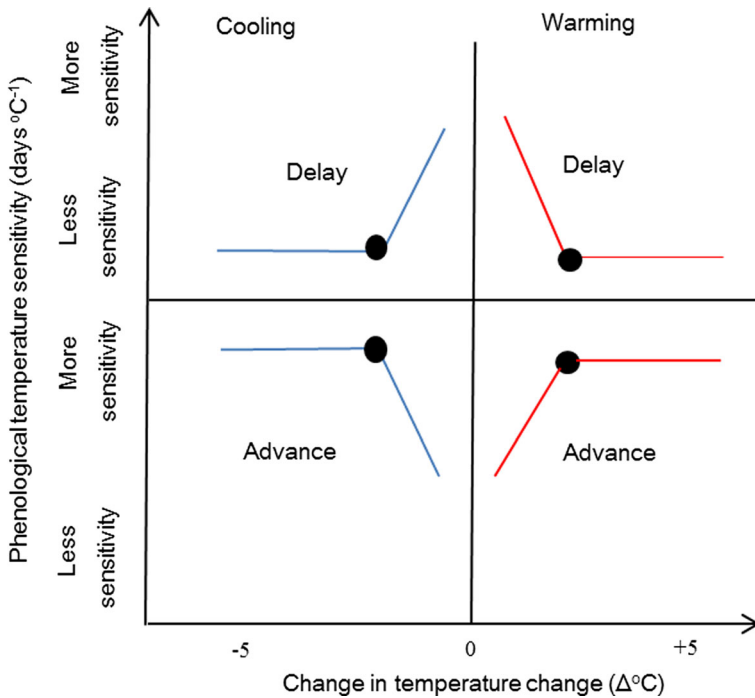
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phenophases to changes in temperature strongly limits our ability to predict phenological change in response to climate change. We investigated the timing of seven phenophases of six alpine plant species to test the hypothesis of nonlinear responses in their temperature sensitivities to warming and cooling, using a reciprocal transplant experiment along a 3200–3800 m mountain slope gradient. Our results supported our hypothesis and showed that there were different thresholds in temperature sensitivity of phenophases to warming and cooling. Moreover, linear temperature sensitivity models significantly underestimated advances and delays of phenophases within the thresholds of temperature change. The nonlinear temperature sensitivity of phenophases is best explained by plastic and adaptive responses of phenophases to temperature change gradients. Therefore, our results suggest that the thresholds of temperature sensitivity for different species should be determined and that nonlinear models of temperature sensitivity may be essential to predict accurately phenological responses to climate change.

## 1 Introduction

Phenology is one of the most sensitive indicators of responses to temperature change, especially at high latitudes and altitudes (Walker et al. 2006; Piao et al. 2011; Wolkovich et al. 2012; Inouye and Wielgolaski 2013; Wielgolaski and Inouye 2013; Wang et al. 2014a; Wang et al. 2014b; Shen et al. 2015; Jiang et al. 2016; Li et al. 2016). Studies of phenological responses to temperature change should allow prediction of the magnitude of these responses and how they will affect other ecosystem functions and services under future warming conditions (Lucht et al. 2002; Walker et al. 2006; Piao et al. 2008; Iler et al. 2013; Wang et al. 2014a). These responses can be studied through long-term observations (Sparks et al. 2000; Amano et al. 2010; Iler et al. 2013), remote sensing (Yu et al. 2010; Shen et al. 2011), manipulative warming experiments (Dunne et al. 2003; Walker et al. 2006; Sherry et al. 2007; Dorji et al. 2013; Wang et al. 2014a), and climate-phenology models (Kaduk and Los 2011). Most studies imply that phenological temperature sensitivity is a constant due to simple linear regression (i.e., using the slope of the regression as temperature sensitivity) of phenological dates against temperature variation from long-term observations (Parmesan and Yohe 2003; Menzel et al. 2006; Wolkovich et al. 2012), or observed phenological and temperature changes between warmed and control plots in manipulative warming experiments (Wolkovich et al. 2012; Wang et al. 2014a). However, linear changes in the timing of life-history events cannot continue indefinitely (Sparks et al. 2000; Iler et al. 2013), and inherently segmented linear phenological change (Sparks et al. 2000; Schleip et al. 2008; Primack et al. 2009; Sparks et al. 2009; Haggerty and Galloway 2011; Ishizuka and Goto 2012; Iler et al. 2013; Wang et al. 2014a) suggests that temperature sensitivity (ratio of changes of phenophase date and temperature, days °C<sup>-1</sup>) should vary with temperature change (°C) in a segmented linear fashion (Fig. 1). Therefore, we hypothesize that the responses of timing of phenophases to temperature change are non-linear (Fig. 1). Prediction of changes of phenophases under temperature change includes two basic components: temperature sensitivity and magnitude of temperature change (Fig. 1). However, a lack of understanding of how temperature sensitivity changes with temperature strongly limits our ability to predict phenological change. The aim of our study was (1) to determine the response of temperature sensitivity of phenophases to temperature change (i.e., warming and cooling gradients), and (2) to assess the differences between linear and segmented linear (i.e., nonlinear) temperature sensitivity for different phenophases.



**Fig. 1** Conceptual diagram of response of phenological temperature sensitivity ( $\text{days } ^\circ\text{C}^{-1}$ ) to temperature change gradients ( $^\circ\text{C}$ ). *Red* and *blue* lines demonstrate how we expect temperature sensitivity to vary with the magnitude of temperature change under warming and cooling, respectively. Positive and negative values of temperature sensitivity mean delay and advance days per degree for the timing of the phenophases ( $\text{days } ^\circ\text{C}^{-1}$ ), respectively. We hypothesize that phenological temperature sensitivity increases with increasing temperature change for the advanced phenophases or decreases with decreasing temperature change for the delayed phenophases before the breakpoints ( $\bullet$ ) (i.e. thresholds) of temperature change, and then reaches a relative plateau beyond the threshold

## 2 Materials and methods

### 2.1 Experimental site, design and phenophase monitoring

Details of the experimental site and design and monitoring of phenophases have been reported previously (Wang et al. 2014a, b). In brief, the experiment was conducted at Haibei Alpine Meadow Ecosystem Research Station (HBAMERS) of the Chinese Academy of Sciences, located at latitude  $37^\circ37'\text{N}$ , and longitude  $101^\circ12'\text{E}$  along a 3200 to 3800 m elevational gradient (i.e., 3200, 3400, 3600 and 3800 m) on the south slope of the Qilian Mountains in Qinghai China. The four sites included four different plant communities within nine km of one another. In total, forty-eight intact soil blocks ( $100 \times 100$  cm wide  $\times$  30–40 cm deep) with attached vegetation were reciprocally transferred across the elevation gradient after the soils started to thaw in early May, 2007 (Wang et al. 2014a). First, twelve intact soil blocks were dug out at each elevation, and three blocks were randomly chosen per elevation and then replaced at the original site to produce experimental control blocks (i.e., 12 soil blocks per elevation  $\times$  4 elevations, for a total of 48 soil blocks or plots). Thus, three replicates were transferred from each elevation and the intact soil blocks were fully randomized throughout the study site.

## 2.2 Measurement of phenophases

Six common plant species were chosen from each plot along the elevation gradient for phenological monitoring during the growing seasons between 2008 and 2010. The plant species observed were two early-spring flowering (ESF) species (*Kobresia humilis* (Kh) and *Carex scabrivostris* (Cs)) that flower before May, and four mid-summer flowering (MSF) species (two grasses, *Poa pratensis* (Pp) and *Stipa aliena* (Sa), and two forbs, *Potentilla anserine* (Pa) and *Potentilla nivea* (Pn)), which flower between late June and July (Wang et al. 2014b). During the previous autumn, ten individuals for forbs and ten stems of graminoids for each plant species in each plot were randomly marked along the elevation gradient so that individual plants could be followed throughout the growing season. We made observations every three or four days from early April to the end of October in each year and recorded the onset dates of seven phenophases, including emergence of first leaf (EFL), first bud/boot stage (BS; transition from the vegetative to the reproductive stage); first flowering (FF), first fruit-set for forbs or seeding-set for graminoids (FFS), vegetative stage after fruit/seeding (VAFS), first leaf-coloring (FFL), and the date of complete leaf-coloring (CLC) (i.e., corresponding to its senescence) (Wang et al. 2014b). For each species the duration of each phenophase was calculated as the average number of days between successive phenophases for all individuals or stems. The entire reproductive stage included three observed developmental phenophases (i.e., a bud event, a flowering event, and a fruiting event), but excluded the event of seed ripening because this was difficult to monitor in the field. No data were obtained at 3600 m in 2010 because mice destroyed the plots.

At each site, HOBO weather stations (Onset Computer Corporation, Bourne, Massachusetts, USA) were used to monitor air temperature, soil temperature (ST) at 5-cm depth and soil moisture (SM) at 20-cm soil depth. Data were sampled at 1-min intervals, and then 30-min averages were stored in the data logger (Wang et al. 2014b). The annual average soil temperatures (Ts) at 5-cm soil depth were 3.9, 2.5, 2.0 and 0.4 °C, and annual average soil moistures at 20-cm depth were 11.8, 11.3, 12.7 and 10.2 % at 3200, 3400, 3600 and 3800 m, respectively (Wang et al. 2014a; Wang et al. 2014b).

## 2.3 Data analysis

### 2.3.1 Calculation of phenological temperature sensitivity

Timing and durations of all phenophases for each species were averaged across the 10 individuals from each soil block, and the three replicate soil blocks for each elevation and warming/cooling transfers. The ratios between the differences of phenophases (i.e., temporal differences in days of year) and temperature change between control sites and transferred sites (i.e., downward/warming and upward/cooling respectively) were used to calculate the phenological temperature sensitivity for each species. Thus, negative and positive values in temperature sensitivities of the phenophases indicate days of advance and delay of the phenophase per degree soil temperature change, respectively (Wang et al. 2014a).

### 2.3.2 Linear and segmented estimation

Phenological temperature sensitivity to soil temperature change was fitted with two types of model: linear and segmented linear regression (Wang et al. 2014a). The

segmented linear regression was implemented with R package `segmented` 0.5–1.0 (Muggeo 2003, 2008). Three steps are involved in conducting segmented regressions. First, each subset of both warming and cooling was fitted with a linear regression with R command `lm()`. Second, function `davies.test()` was used with each of the linear models to determine the existence of a breakpoint. The null hypothesis of `davies.test()` was zero difference-in-slopes (i.e. no breakpoint). Third, if the null hypothesis was rejected at  $p = 0.05$ , which suggested that the data were better fitted by a segmented model than by a linear model, `segmented()` was implemented to the linear model in order to (1) determine the position of the breakpoint, and (2) fit a model for the parts separated by the breakpoint. To initiate an iterative process of estimating the breakpoint, a starting value should be specified for function `segmented()`, and it was set to 1.0 °C for warming or -1.5 °C for cooling subset. If `segmented()` was completed successfully, `confint.segmented()` and `slope()` were used to extract the estimated parameters, i.e., the breakpoint and the two slopes for the segmented linear regressions.

The model fit was compared between the linear model and the segmented linear regression, and both models were compared with Akaike's Information Criterion (AIC). Regression models with  $\Delta\text{AIC} > 2$  were designated as either linear or segmented linear, and no difference indicates that  $\Delta\text{AIC} < 2$  between a linear and a segmented linear regression model. Analyses were performed for individual species and for pooled species, respectively. R-3.2.1 was used in the procedures of model-fitting and estimation (The R Development Core Team 2015).

### 2.3.3 Calculation of predicted linear and segmented linear temperature sensitivity

The temperature sensitivity of phenological events was calculated using slope 1 with intercept 1 before the temperature change reached its breakpoint, and it was calculated using slope 2 with intercept 2 beyond the breakpoint under warming (Table S3). Under cooling it was calculated using slope 2 with intercept 2 before the temperature change reached its upper breakpoint, and it was calculated using slope 1 with intercept 1 when beyond the breakpoint (Table S3). The same plant species were compared for linear and segmented linear temperature sensitivity under warming and cooling, respectively (Table S4). In some cases the regressions were not significant between the differences of phenophases and temperature change (Fig. S8-S14), or no breakpoints were found at all (Table S3), in which case the comparisons could not be performed.

### 2.3.4 Statistical analyses

Linear mixed models with repeated measurements were used for analysis of variance with SPSS version 22.0 (SPSS Inc. Chicago, USA). Type III Sum of Squares was used to test the difference in temperature sensitivity of phenophases between transferred and original sites. For the transfer-caused warming and cooling, plot was taken as the repeated variable; the fixed factors were year, species, donor and acceptor sites, the random factor was plot, and the dependent variables were temperature sensitivities of the timing of phenophases. The differences between temperature sensitivity calculations using linear regressions and estimated through segmented linear regressions at

different temperature changes were compared at different phenophases for individual flowering functional groups (i.e., early-spring and mid-summer flowering plants) because of their different responses to the warming gradient (Fig. S2-S7). Significant differences were reported at 0.05 level in the text.

### 3 Results

#### 3.1 Linear and segmented linear temperature sensitivity under temperature change gradient

The temperature sensitivities of the starting dates of most phenophases were significantly affected by plant species, original site, transferred site, phenophases, year, and their interactions (Table S1). Two models (i.e., linear and segmented linear regression) were evaluated for all species (Table S2 and Fig. S2-S7), and we found that the segmented linear (i.e., nonlinear) regression varied with species (Table S2). For example, segmented linear regression provided a better fit for the starting date of most phenophases for Kh, Pp and Sa, except CLC for Kh and FFS for Sa under cooling, and FFS for Pp under warming and cooling (Table S2). However, FBS, FF and FFS provided a better fit using linear regression for Pa and Pn under warming and cooling (Table S2).

Generally, nonlinear regressions were best except for first flowering for both warming and cooling, and first fruit-set for cooling by pooling data from the six plant species (Table 1 and

**Table 1** Breakpoints (°C) of the segmented linear regression between temperature sensitivity of phenophases and soil temperature change gradients based on Akaike's Information Criterion (AIC) analysis for the pooled all species. Summary data are shown for models with  $\Delta AIC > 2$  as either linear or segmented linear, and no difference indicates that  $\Delta AIC < 2$  between a linear and a segmented linear model

Phenology	Temperature change	AIC_lin	AIC_seg	Breakpoint (°C)
EFL	W	1491.4	1381.2	1.50
	C	1526.2	1347.7	-0.60
FBS	W	1865.8	1825.9	0.53
	C	1797.8	1744.9	-0.51
FF	W	1859.1	/	/
	C	1833.1	/	/
FFS	W	1773.2	1763.5	1.10
	C	1793.9	/	/
VAFS	W	2047.5	1942.9	1.10
	C	1913.6	1907.1	-1.25
FLC	W	1778.6	1637.0	1.08
	C	1961.2	1809.5	-1.09
CLC	W	1745.3	1617.4	1.45
	C	1766.9	1668.4	-0.68

*EFL* emergence of first leaf; *FBS*: first bud/boot stage, *FF* first flowering *FFS* first fruit-set for forbs or seed-set for graminoids *VAFS* vegetative stage after fruit/seeding *FLC* first leaf coloring and *CLC* complete leaf-coloring. *W* warming, *C* cooling

/ not significant at 0.05 level or there is no significant linear regression

Fig. S1). In these cases, there were nonlinear responses of temperature sensitivities of the first flowering for Kh, Pp and Sa, and of the first fruit-set for Kh, Sa and/or Cs to the soil temperature change gradient under warming and cooling (Table S2 and Figs. S2–S7).

For the pooled plant species, the breakpoints of temperature sensitivity ranged from 0.53 to 1.50 °C for the emergence of first leaf (EFL), first bud-set (FBS), first fruiting-set (FFS), vegetative event after fruiting-set (VAFS), first leaf coloring (FLC) and complete leaf coloring (CLC) under warming (Table 1). They ranged from –0.51 to –1.25 °C for EFL, FBS, VAFS, FLC and CLC under cooling, respectively (Table 1). These breakpoints varied with plant species and phenophases (Table S2). Thus, our results suggest that there were different thresholds of temperature sensitivity for different phenophases and different plant species (Table S2 and Figs. S2–S7).

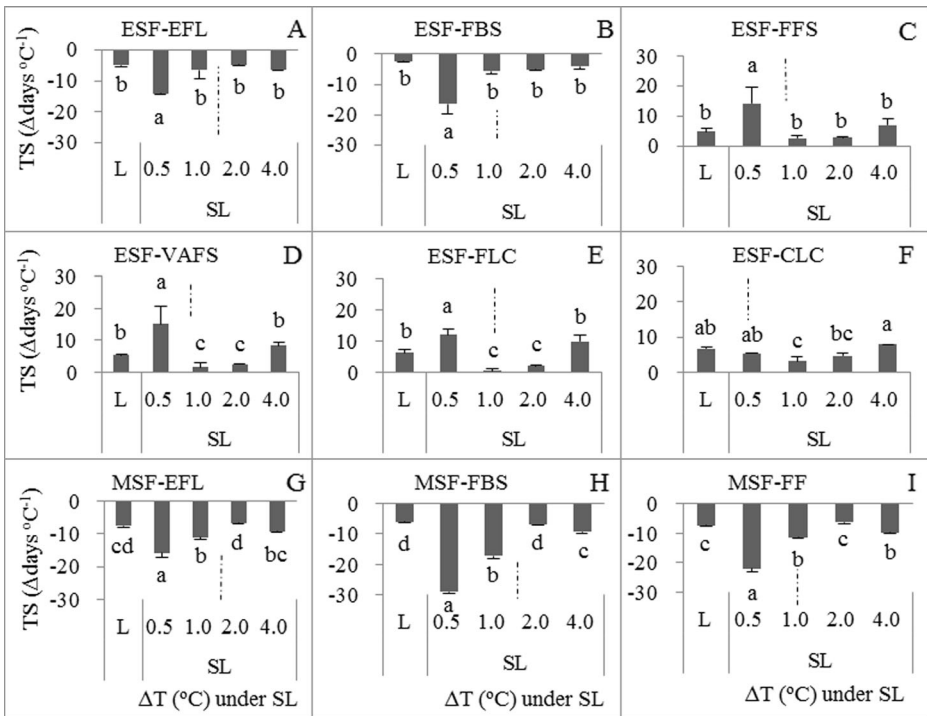
### 3.2 Comparison between linear and segmented linear temperature sensitivity

The slope of the linear regression between changes in phenophases and soil temperature for the different plants under warming and cooling was used as the linear temperature sensitivity (Figs. S8–S13), assuming a constant relationship between temperature and phenological response. For segmented linear temperature sensitivity of phenophases, four soil temperature change gradients (i.e., 0.5, 1.0, 2.0 and 4.0 °C) were set up, and their segmented linear temperature sensitivities were calculated separately (Table S4) for the early-spring and mid-summer flowering plants under warming and cooling (Table S3). We found that the segmented linear temperature sensitivities varied with temperature change gradients for both warming and cooling (Figs. 2 and 3). Moreover, their absolute values reached a maximum before the temperature changes reached their breakpoints under warming (Fig. 2) and cooling (Fig. 3).

Relative to segmented linear temperature sensitivity, linear temperature sensitivity significantly underestimated the advances of the early-season phenophases (i.e., EFL, FBS and/or FF) by about 100–400 %, and delays for the late-season phenophases by about 100–180 % (i.e., VAFS, FLC and CLC except under warming). This occurred for both early-spring (Figs. 2A–F) and mid-summer flowering species (Figs. 2G–I) at 0.5 °C warming, before temperature changes reached their breakpoints (Table S2). However, compared with segmented linear temperature sensitivity, linear temperature sensitivity overestimated delays of VAFS, FLC and CLC for the early-spring flowering plants at 1 and 2 °C warming, whereas it significantly underestimated the advances of ELF, FBS and FF for the mid-summer flowering plants at 1 °C warming (Figs. 2G–I). It also underestimated the advances of FBS and FF for the mid-summer flowering plants at 4 °C warming (Figs. 2H and I).

Compared with segmented linear temperature sensitivity at 0.5 °C cooling, linear temperature sensitivity significantly underestimated the delays of EFL and FBS for the early-spring flowering species by 50–90 % (Figs. 3A and B); it also significantly underestimated the delays of the early phenophases (i.e., EFL, FBS and FF) by 110–310 % (Figs. 3D–3F) and advances of the late phenophases (i.e., FLC and CLC) by 230–290 % under cooling (Figs. 3H and I). However, linear temperature sensitivity significantly overestimated the delays of EFL and FBS for the early-spring flowering species at 1 and 2 °C cooling (Figs. 3A and B), but underestimated the delays of FBS and FF for the mid-summer flowering species at 1 °C cooling (Figs. 3E and F). At 4 °C cooling, linear temperature sensitivity only significantly





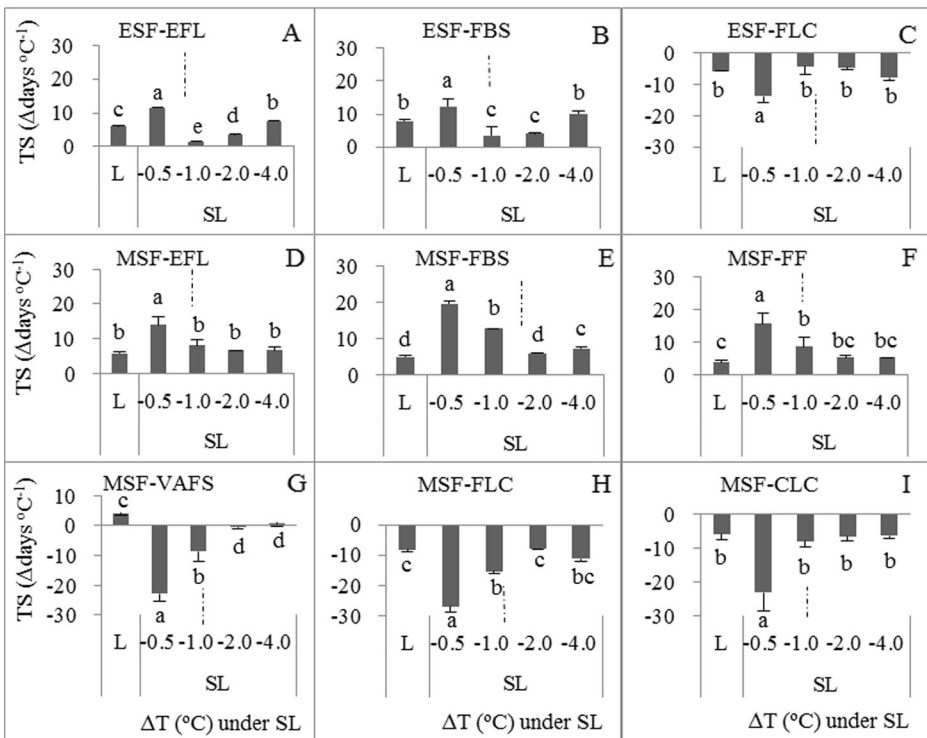
**Fig. 2** Comparison between simulated linear (L) and segmented linear (SL) temperature sensitivity (TS) of phenophases under warming under different soil temperature change gradients. Linear temperature sensitivity was the slope of the linear regression between phenological change and soil temperature change for the different plants under warming and cooling, thus it did not vary with the temperature change gradient. A-F for the early-spring flowering plants (ESF) (i.e. *Kobresia humilis* and *Carex scabrostris*). G-I for the mid-summer flowering plants (MSF) (G for *Potentilla nivea*, *Poa pratensis* and *Stipa aliena*; H and I for *Poa pratensis* and *Stipa aliena*). A and G: emergence of first leaf; B and H: first bud/boot stage; C: first fruit-set for forbs or seed-set for graminoids; D: vegetative stage after fruit/seedling; E: first leaf coloring; F: complete leaf-coloring and I: first flowering. Broken vertical line indicates the mean breakpoint

underestimated the delays of EFL for the early-spring flowering species (Fig. 3A) and FBS for the mid-summer flowering species relative to segmented linear temperature sensitivity (Fig. 3E). Moreover, linear temperature sensitivity predicted a delay by 4.1 days °C<sup>-1</sup> for VAFS, but segmented linear temperature sensitivity predicted its advance by about 23 and 9 days °C<sup>-1</sup> at 0.5 and 1 °C cooling for mid-summer flowering plants (i.e., forbs Pa and Pn), respectively (Fig. 3G).

### 4 Discussion

In most cases we found that the absolute value of temperature sensitivity of phenophases decreased sharply in a linear fashion with temperature change until a breakpoint was reached (usually <1.5 °C under warming and > -1.5 °C under cooling), and then remained at a relative plateau or a slight increase when beyond the thresholds (Table 1, Table S2 and Figs. S1-S7). There were significant differences between linear and segmented linear temperature sensitivity within the temperature change thresholds of phenophases (Figs. 2 and 3).





**Fig. 3** Comparison between linear (L) and segmented linear (SL) temperature sensitivity (TS) of phenophases under cooling. A–C for the early-spring flowering plants (ESF) (Kh and Cs). D–I for the mid-summer flowering plants (MSF) (D for *Potentilla nivea*, *Poa pratensis* and *Stipa aliena*; E and F for *Poa pratensis* and *Stipa aliena*; G and H for *Potentilla anserine* and *Potentilla nivea*; I for *Potentilla anserine* and *Stipa aliena*). A and D: emergence of first leaf; B and E: first bud/boot stage; C and H: FLC: first leaf coloring; F: first flowering; G: vegetative stage after fruit/seeding; I: complete leaf-coloring. Broken vertical line indicates the mean breakpoint

Some previous studies have showed plastic and adaptive responses of phenophases to temperature change, especially in high-altitude mountain areas (Vitasse et al. 2010; Wolkovich et al. 2012; Iler et al. 2013; Wang et al. 2014a; Gugger et al. 2015), but there is little known about phenological temperature sensitivity under temperature change gradients in the field (Wang et al. 2014a; Jiang et al. 2016). Wolkovich et al. (2012) reported that temperature sensitivity of first flowering did not vary with warming gradients, however, a breakpoint (i.e., about 1.5 °C) seems to exist if their data are analyzed using segmented linear regression (see Fig. S9 from Wolkovich et al. (2012)). Moreover, we found that in our study the temperature sensitivity of first flowering by 3 out of 6 species (i.e., Kh, Pp and Sa) had segmented linear responses to warming and cooling gradients (Figs. S2–S7).

Phenotypic plasticity may play a crucial role in the short-term adjustment to novel conditions (Nicotra et al. 2010; Gratani et al. 2012; Richter et al. 2012; Franks et al. 2014; Gugger et al. 2015). When temperature change does not reach the temperature sensitivity thresholds, the required cumulative temperature of phenophases could remain unchanged, thus the temperature increase or decrease could result in a linear advance or delay of phenophases (Forrest and Miller-Rushing 2010; Iler et al. 2013). Although the temperature sensitivity of the phenophases was larger before the breakpoints, the temperature change magnitude was smaller

before the breakpoints (e.g.  $\pm 1.5$  °C in our study), thus advances or delays of the phenophases could be accomplished through adaptation of the plants to the large naturally-occurring variation of climate found in the alpine region. However, when temperature change is beyond the breakpoints, increases or decreases of temperature were synchronized with increases or decreases of degree days requirements when plants were transferred downwards or upwards, respectively (Wang et al. 2014a; Gugger et al. 2015). For example, plants living in higher elevations usually need fewer degree days to start flowering compared with lower elevations (Wolkovich et al. 2012; Wang et al. 2014a; Gugger et al. 2015).

Thus, the balance between the change in degree day requirements and temperature change will determine the responses of phenophases beyond the breakpoints (Haggerty and Galloway 2011; Gugger et al. 2015). For example, in some cases we found that the temperature sensitivity of phenophases increased with temperature change when beyond the breakpoints (Figs. 2 and 3), probably because the magnitude of temperature increase could be larger than the change magnitude of degree days requirements. In contrast, in other cases the temperature sensitivity of phenophases could remain relatively stable beyond the breakpoints (Figs. 2 and 3), probably due to the absence of differences between changes in temperature and degree day requirements. Previous studies have reported lower phenological plasticity for high-elevation perennial herbaceous species relative to mid-elevation species (Gugger et al. 2015), but a reciprocal transplant experiment with three grassland species revealed no difference in plasticity between low- and high-elevation populations (Frei et al. 2014). This discrepancy could derive from different temperature change magnitudes (i.e., within or beyond their breakpoints in temperature sensitivity for different species). Our results suggest that phenophases of alpine plants could have different plastic responses to temperature changes within and beyond their breakpoints, and that their temperature sensitivities could be larger below their breakpoints than above them.

Our results also showed that there were no breakpoints in temperature sensitivity of the early-season phenological events (i.e., FB, FF and FFS) for the mid-summer flowering plants (i.e. Pa and Pn) (Table S2, and Fig. S4 and S5). Plant life type (Gratani 2014) and shade (Valladares et al. 2002) can affect the responses of phenophases to changes in temperature. In our study, the two mid-summer flowering forbs were short and grew in shaded understory habitat, and their thermal accumulation could be furtherly hampered because warming increased the coverage and height of tall grasses such as Sa in alpine meadow (Wang et al. 2012). Thus, limited thermal accumulation could cause their degree day requirements to be within their thresholds, which could result in no breakpoints for their early-season phenological events (Figs. S4, S5 and Table S2). Therefore, differences among species and life forms may reflect different selective pressures on plasticity, different limitations acting upon the maximization of plasticity, or a combination of both (Valladares et al. 2007).

Although our study is based on only six alpine grasses and forbs, which could limit the scope of any generalizations, there is no doubt that our results have two important implications. First, nonlinear responses of temperature sensitivity to temperature change gradients should be taken into account for statistical modeling of plant responses to future climate change. Because most warming manipulation experiments have been in the range of 1–3 °C without temperature gradients and with constant temperature sensitivity (Wolkovich et al. 2012), constraining phenological responses to linear climate models may mask non-linear responses to climate, resulting in shallower linear responses, and underestimations of rates of phenological change, or even failure to detect a phenological response where one exists (Iler et al. 2013). Second, we found that the breakpoints of temperature sensitivity varied with

phenophases for different plant species in the alpine region. Therefore, our results highlight the importance of measuring the breakpoints of temperature sensitivity for different phenophases under different magnitudes of temperature change in the future.

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