



## Research

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## Global change biology

# Interactions between warming and soil moisture increase overlap in reproductive phenology among species in an alpine meadow

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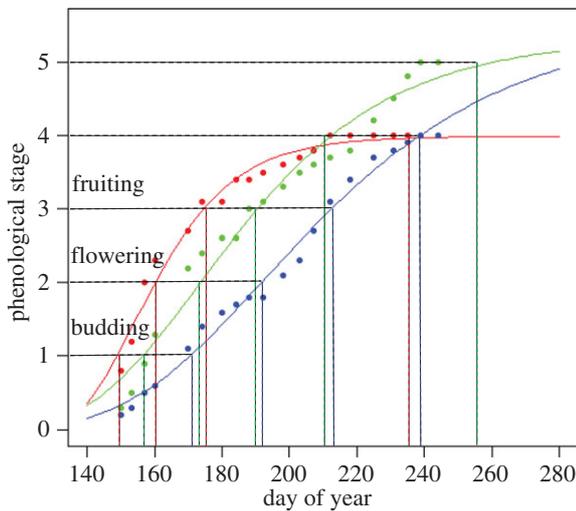
Climate warming strongly influences reproductive phenology of plants in alpine and Arctic ecosystems. Here, we focus on phenological shifts caused by experimental warming in a typical alpine meadow on the Tibetan Plateau. Under soil water stress caused by warming, most plants in the alpine meadow advanced or delayed their reproductive events to be aligned with the timing of peak rainfall. As a result, warming significantly increased the temporal overlap among reproductive stages of early- and late-flowering species. In addition, we found that some species, for example the late-flowering species, were unable to produce flowers and fruits under warming with failed monsoon rains. The potentially warmer- and drier-growing seasons under climate change may similarly shift the phenological patterns and change species composition of these alpine systems.

## 1. Introduction

Reproductive phenology of plants is strongly influenced by temperature in alpine and Arctic ecosystems. Plant species may respond differently to temperature change, depending on their life-history traits [1–3]. For example, warming can have divergent consequences on plant phenology, including advanced [4,5], delayed [6,7] or unchanged [8] phenological events. Under warming, some species respond immediately, whereas other species might take several years for the cumulative effects of warming to emerge [8].

By shifting phenological stages, warming can significantly expand or shorten the overlapping periods of reproductive stages between successively blooming species [9], and disperse the reproductive events of different species away from the peak of summer heat [10]. Results have also been reported that there were no evident divergences in the advance or delay of the flowering date of some species [11]. The rapidly warming temperatures in high-latitude and alpine regions have the potential to alter the phenology of Arctic and alpine plants, and plants of different communities and growth forms differed for some phenological responses [12].

In alpine meadows on the central Tibetan Plateau, warming can result in soil water stress, particularly in the premonsoon growing season [2]. In this study, we put forward a conceptual model regarding responses of reproductive phenology to warming as showed in electronic supplementary material, figure S1. Under this hypothesis, warming would delay the reproductive phenology of



**Figure 1.** The fitted curves of phenological stage changes for *Kobresia pygmaea* (early-flowering species, red line), *Potentilla saundersiana* (mid-flowering species, green line) and *Stipa purpurea* (late-flowering species, blue line) in the control plots. Parameters ( $K$ ,  $a$ ,  $b$  and  $m$ ) describing the shape of the curve were obtained by fitting the observed phenological scores to the Richards equation for each species in each treatment.

shallow-rooted, early-flowering species, advance the phenology of deep-rooted, late-flowering species and cause no effect on shallow-rooted, mid-flowering species, consequently expanding the reproductive overlap among species. With the help of a manipulative experiment, we aimed to test the above conceptual hypothesis by exploring phenological shifts caused by warming for an alpine meadow system on the Tibetan Plateau.

## 2. Material and methods

The study area was located in a typical alpine meadow grassland at Naqu, northern Tibet, China (31°38.513' N, 92°0.921' E), approximately 4600 m in elevation. The long-term mean annual temperature and precipitation is  $-1.2^{\circ}\text{C}$  and 430 mm (1955–2012), respectively. The summer monsoon is the main source of precipitation in this region [2]. The onset of the summer monsoon over the past nearly 60 years has a mean date of 22 May (DOY: 142); however, there is considerable annual variation [13]. The growing season normally starts in mid-May and lasts until mid-September. The vegetation is dominated by *Kobresia pygmaea*, accompanied by *Potentilla saundersiana*, *Potentilla cuneata* and *Stipa purpurea* (figure 1).

Passive warming was achieved using hexagonal open-top chambers (OTCs) based on the International Tundra Experiment (ITEX) design [14]. The treatments included control, and four levels of warming (electronic supplementary material, figure S2). There were four replicates per treatment for a total of 20 plots. The OTCs were set up in October 2013. We measured air temperature and moisture at 10 cm above-ground using the Vaisala HMP155A sensor (Vaisala, Helsinki, Finland). Soil temperature and moisture at 5 cm below-ground were measured in the centre of the plots using Campbell CS655 sensors (Campbell Scientific, Logan, UT). Two air and soil sensors were installed for each treatment, and the data were averaged over the two sensors.

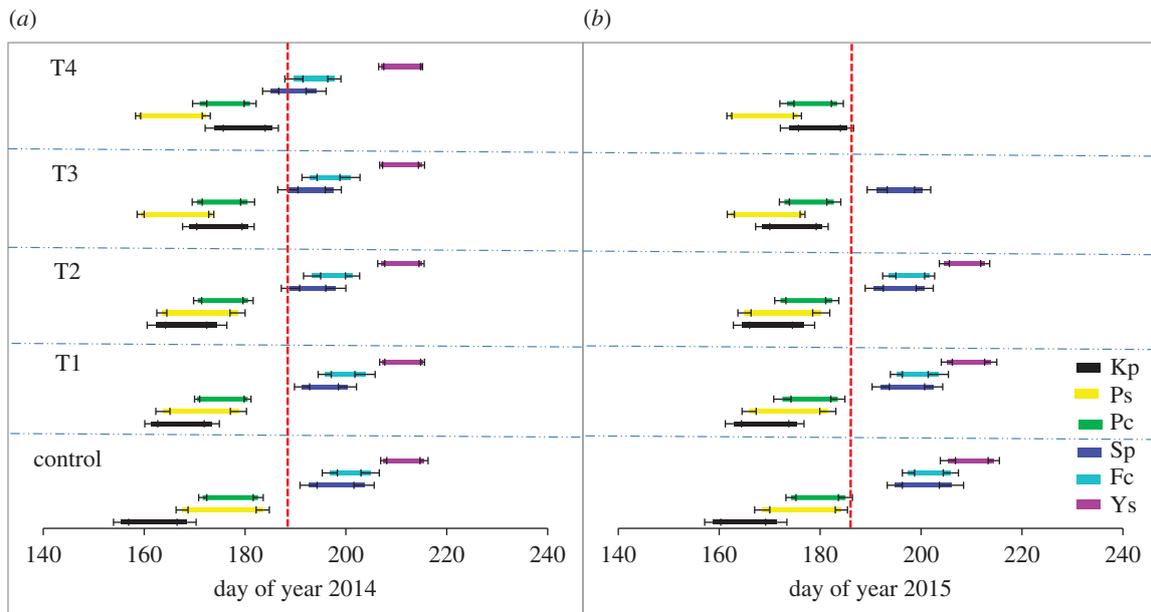
We selected *K. pygmaea*, *P. saundersiana*, *P. cuneata*, *S. purpurea*, *Festuca coelestis* and *Youngia simulatrix* as the focal species, whose coverage and biomass account for more than 80% of the entire community. Twenty individuals of *K. pygmaea* and 10 individuals of other species were selected from each plot in May 2014 and marked with a colour-coded tag. Plant phenology was scored

every 3–5 days for 2 consecutive years: 2014 and 2015. The phenological stage scoring was modified from Dunne *et al.* [4]. For *K. pygmaea* and forbs, *P. saundersiana*, *P. cuneata* and *Y. simulatrix*, reproductive phenology was divided into six stages: F0, vegetative plants; F1, open buds; F2, open flowers; F3, initiated fruit; F4, expanding fruit and F5, dehisced fruit. For grasses, *S. purpurea* and *F. coelestis*, five reproductive stages were distinguished: G0, plants with flower stalks; G1, presence of spikelets; G2, exerted anthers and styles; G3, past the presence of anthers and styles and G4, disarticulating florets [10]. Based on their life-history traits, we classified *K. pygmaea* as a shallow-rooted (10 cm depth) and early-flowering species (DOY:  $155 \pm 0.8$ , during the early summer monsoon), *P. saundersiana* and *P. cuneata* as shallow-rooted (10 cm depth) and mid-flowering species (DOY:  $168 \pm 0.4$ ; DOY:  $172 \pm 1.1$ ; during the summer monsoon) and *S. purpurea*, *F. coelestis* and *Y. simulatrix* as deep-rooted (30 cm depth) and late-flowering species (DOY:  $193 \pm 0.5$ ; DOY:  $197 \pm 1.2$ ; DOY:  $208 \pm 0.4$ , respectively; during the summer monsoon, based on data from 2014).

We first applied the Richards equation with the contraction–expansion algorithm to fit phenological scores ( $Y$ ) of each species against the day for each plot by using MATLAB (MathWorks, Natick, MA; equation 1 in the electronic supplementary material). Best parameter estimates of  $K$ ,  $a$ ,  $b$  and  $m$  were obtained for each species in each plot. Second, based on equation 2 (see the electronic supplementary material), budding, flowering and fruiting timing were calculated as  $Y = 1$ ,  $Y = 2$  and  $Y = 3$  for all species, respectively. Flowering duration was calculated as the difference between stages 2 ( $Y = 2$ ) and 3 ( $Y = 3$ ). A two-way ANOVA with Turkey's test was used to examine warming, year and their interactive effects on the timing of budding, flowering and fruiting for each species, and the overlap in flowering phase among species. The effects were considered to be significant if  $p < 0.05$ . All statistical analyses were performed using SPSS v. 19.0 (SPSS Inc., Chicago, IL).

## 3. Results

The 2015 growing season (total rainfall = 306 mm) was drier than in 2014 (total rainfall = 460 mm; electronic supplementary material, figure S3). Specially, the total rainfall from 184 to 215 (DOY) in 2015 was 17.3 mm, and 115.4 mm in 2014. Warming increased air and soil temperature, and reduced soil moisture during both growing seasons (electronic supplementary material, figure S3). Warming delayed reproductive phenology (budding, flowering and fruiting) of *K. pygmaea*, advanced phenology of *P. saundersiana*, *S. purpurea* and *F. coelestis* (electronic supplementary material, figure S4 and table S4–S9,  $p < 0.05$ ), but caused no obvious effects on the phenological timing of *P. cuneata* and *Y. simulatrix* in both years ( $p > 0.05$ ). The overlapped flowering period between *K. pygmaea* and *P. saundersiana* increased by 9.4, 10.3, 3.8 days and 6.6, 8.9, 5.1 days in response to the warming treatments (T1, T2, T3) in 2014 and 2015 (electronic supplementary material, table S10 and S11,  $p < 0.05$ ), respectively. The delayed flowering of *K. pygmaea* caused its flowering period to overlap with that of *P. cuneata* by 3.7, 4.3, 10.1, 7.1 days and 2.8, 4.6, 7.5, 9.5 days ( $p < 0.05$ ) under the four experimental warming treatments in 2014 and 2015, respectively (figure 2). Fruits were not produced by *K. pygmaea* in treatment T4 in 2014 and 2015 (see electronic supplementary material, figure S4). In 2015, flowers were not produced by *F. coelestis* and *Y. simulatrix* in treatment T3, nor by *F. coelestis*, *Y. simulatrix* and *S. purpurea* in treatment T4, and fruits were not produced by *S. purpurea*, *F. coelestis* and



**Figure 2.** Timing and duration of the flowering period for the six species under five treatments in 2014 (a) and 2015 (b). The dotted vertical line indicates the time of rainfall peak (DOY: 187 and 183, in 2014 and 2015). For *Kobresia pygmaea* (Kp), *Potentilla saundersiana* (Ps), *Potentilla cuneata* (Pc), *Stipa purpurea* (Sp), *Festuca coelestis* (Fc) and *Youngia simulatrix* (Ys) changes in timing or duration caused by the warming treatments (T1, T2, T3, T4,  $n = 4$  per treatment) are large enough to significantly affect the overlap between paired species. In 2015, flowers were not produced by *F. coelestis* and *Y. simulatrix* in treatment T3, nor by *F. coelestis*, *Y. simulatrix* and *S. purpurea* in treatment T4. Data are presented as duration  $\pm$  s.e. at the two ends of the flowering periods.

*Y. simulatrix* in all treatments (see electronic supplementary material, figure S4).

## 4. Discussion

Our experiment showed that warming changed the reproductive phenology of alpine plant species and that these responses were not uniform among species. In our study area, warming leads to soil water stress, particularly in the premonsoon growing season [2]. Plants with distinct life-history traits (early versus late flowering) and rooting depths possess different vulnerabilities to the decreased upper-soil moisture caused by increased temperature [2]. Delayed phenological development caused by water limitation under the warming treatment [15] may result in shallow-rooted and early-flowering species, such as *K. pygmaea*, being more sensitive to warming [1] during the wet growing season.

In this study, warming advanced reproductive phenology of *P. saundersiana*, but not of *P. cuneata*. The shallow-rooted and mid-flowering species start flowering in the middle of June when the monsoon has started and can avoid strong water stress, which the early-flowering species has to face [2]. In this case, others have also reported advanced [4,5], delayed [6,7] or unchanged [2,8] phenological responses to warming in other species. The deep-rooted and late-flowering species, such as *S. purpurea* and *F. coelestis*, start flowering during the monsoon season and are also able to use deeper soil water [2]. Thus, their phenological development is less constrained by water limitation when compared with the shallow-rooted and early-flowering species. The increased soil and air temperature may have stimulated their phenological processes [4]. Warming caused no obvious effects on reproductive phenology of the deep-rooted, late-flowering species, such as *Y. simulatrix*. It might take several years for the cumulative effects of warming to appear [8]. However, a continuous drought occurred from 184 to 215 (DOY) in 2015. The timing of flower in late-flowering species occurs at this time. The

warming treatments T3 and T4, combined with a continuous drought, might have decreased deeper soil water, and most late-flowering species were unable to produce flowers and fruits in 2015, which might be a major fitness cost for these species in a warmer, drier alpine environment. Our results indicated that the potential for warm summers and failed monsoon rains would cause the loss of fruiting in the late-flowering species.

Sherry *et al.* [10] found that warming dispersed the reproductive events of different species away from the peak of summer heat, resulting in both expanded and narrowed reproductive overlap period among species in a tallgrass prairie community [10]. In contrast, our targeted alpine meadow ecosystem was mainly constrained by soil moisture as set by the timing of the monsoon. Most plants advanced or delayed their reproductive events closer to the rainfall peak. Therefore, warming caused the convergence of the flowering events of early- and late-flowering species in our study, and increased the flowering period overlap among species. Changes in reproductive period overlap could alter the competitive relationships among species for such resources as water, nutrients and light during the reproduction process [16]. The potential for warmer- and drier-growing seasons with climate change could change the phenological patterns and species composition of these alpine systems.

**Data accessibility.** The datasets supporting this article have been uploaded as part of the supplementary material.

**Authors' contributions.** J.Z. carried out this research, collected field data, drafted the manuscript, carried out data analysis and participated in the design of the study; Y.Z. conceived of the study, designed the study, coordinated the study and helped draft the manuscript; W.W. collected field data; all authors gave final approval for publication; all authors agreed to be held accountable for the content therein.

**Competing interests.** The authors declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical

standards. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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