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Nitrogen addition alters the phenology of a dominant alpine plant in northern Tibet

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Abstract

Among the various plant traits, phenology is one of the most sensitive to environmental changes. Nitrogen (N) has been reported to be an important factor constraining plant growth, while N deposition is an increasing concern due to social and economic development. However, studies exploring influences of atmospheric N deposition on alpine plant phenology have been barely reported. We conducted a N addition gradient experiment in an alpine meadow on the plateau to examine plants' phenological response. The results suggest that N addition plays an important role in regulating plant phenology, but only for the dominant species; specifically, the budding time of *Kobresia pygmaea* advanced in response to N addition. The prolonged lengths of flower phase and reproductive period of *K. pygmaea* were mainly ascribed to an earlier start of the growing season and more resources being allocated to reproductive growth in the N-rich soil. The N addition caused no effect on the phenology of the accompanying species. There were no obvious phenology differences among each N addition treatment. The earlier budding time and the prolonged growing season of the dominant species in response to the N addition means a longer provision duration of food sources to local herders, which can facilitate local economic development.

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Introduction

Phenology is the study of periodic plant and animal life cycle events and how they are influenced by seasonal and interannual climate variations (Parmesan, 2006). Plant phenology, usually composed of plant budding, leaf unfolding, anthesis, fructification, and senescence, is one of the most sensitive traits in response to environmental change (Parmesan and Yohe, 2003; Cleland et al., 2007; Post and Inouye, 2008). In minimizing competition for pollinators and other resources, plants employ diversified phenology, which is an important mechanism for plants to maintain their coexistence in plant communities (Rathcke and Lacey, 1985; Martinkova et al., 2002). The timing of growth onset and senescence may also determine the growing season length, thus driving annual carbon uptake in terrestrial ecosystems (Cleland et al., 2007; Forrest and Miller-Rushing, 2010).

In recent decades, effects of warming on vegetation phenology have been plentifully reported (Ahas et al., 2000; Sparks and Menzel, 2002; Piao et al., 2007; Zhang et al., 2013a). Besides temperature, other factors like soil moisture, nitrogen (N) availability, light, and elevated CO₂ are all likely to affect plant phenology (Nord and Lynch, 2009). What is more, other environmental factors may confound the temperature sensitivity of plant phenology in natural ecosystems (Wolkovich et al., 2012).

N is a basic nutrient for plants. However, an excessive usage of N can lead to eutrophication, thus changing ecosystem structure and function. The consequences can be altered growth, altered organism chemistry composition, shifted phenology, changed nutrient cycling and, ultimately, alteration in plant community composition (Xia and Wan, 2008; Yang et al., 2012). It is estimated that the global land surface has received approximately doubled N input as a result of the worldwide application of artificial N fertilizers (Gruber and Galloway, 2008). Some researchers have reported advanced or delayed plant phenology due to N addition (Cleland et al., 2006; Power et al., 2006; Lupi et al., 2012; Xia and Wan, 2013). But related studies have been barely reported for ecologically fragile areas, such as the alpine meadows of the Tibetan Plateau (Smith et al., 2012).

The Tibetan Plateau is among the most sensitive regions to global changes because of its unique geographical environment, including high altitude, strong solar radiation and low air pressure (IPCC, 2007). During the past 30 years, the N deposition has climbed up from 1 kg N ha⁻¹ to 5 kg N ha⁻¹ (Liu et al., 2013). The Tibetan Plateau alpine meadow system is strongly restricted by N availability, partially due to a slow N mineralization. Exogenous N input can increase resource availability and lessen the nutrient restriction for the alpine ecosystem (Bowman et al., 2006). Previous studies have reported changed biomass allocation strategy and de-

TABLE 1
The traits of each species monitored in this study.

Species name	Family	Functional type	Life cycle	Biomass (g m ⁻²)	Coverage (%)	Height (cm)	Frequency (%)
<i>Kobresia pygmaea</i>	Cyperaceae	Sedge	Perennial	87.21	52.40	2.01	99.80
<i>Potentilla saundersiana</i>	Rosaceae	Forb	Perennial	18.50	4.64	1.16	51.40
<i>Festuca coelestis</i>	Poaceae	Grass	Perennial	2.35	1.50	4.67	13.00
<i>Saussurea stolicikai</i>	Asteraceae	Forb	Perennial	2.15	0.98	1.57	15.80
<i>Potentilla cuneata</i>	Rosaceae	Forb	Perennial	1.62	0.74	0.89	5.80
<i>Youngia simulatrix</i>	Asteraceae	Forb	Perennial	1.25	1.06	0.32	21.20
<i>Potentilla bifurca</i>	Rosaceae	Forb	Perennial	0.15	0.28	1.43	5.30

Note: The biomass and coverage were calculated as the average of the aboveground biomass, coverage, height, and frequency, respectively, in the control plots in the middle of August 2013.

creased species abundance due to an extra N input and increased N mineralization around the world (Luo et al., 2006; Niu et al., 2008; Bai et al., 2011). However, little is known about how plant phenology responds to N addition, especially in alpine environments. To date, most published findings about the phenology response on the Tibetan Plateau have been focused on effects of warming at a regional scale (Yu et al., 2010; Piao et al., 2011; Zhang et al., 2013a) or a site scale (Klein et al., 2014).

Plant phenology plays an important role in maintaining species coexistence (Miller-Rushing and Inouye, 2009; Forrest and Miller-Rushing, 2010). Studies on the responses of phenology to N deposition can shed light on its effects on community structure. To this end, we conducted a gradient fertilization experiment and monitored phenological phases of seven species from different functional types. Specifically, we are trying to answer the following two questions. (1) How does N addition affect the plant phenology of an alpine meadow on the Tibetan Plateau? (2) Will multiple-level N additions cause differences in phenology responses?

Material and Methods

STUDY SITE

The experiment was conducted in a typical alpine meadow (31°38.513'N, 92°0.921'E, 4585 m a.s.l.) on the Tibetan Plateau. The long-term (1955–2012) annual mean temperature and precipitation are –1.16 °C and 430 mm, respectively. The annual cloud-free solar radiation is about 2788 h. There is no absolute frost-free period throughout the year. The snow period or soil freezing period is from October through following May. The duration of the growing season is from June to August. The vegetation, typical of Tibetan alpine meadows, is dominated by *Kobresia pygmaea*, accompanied by *Potentilla saundersiana*, *Youngia simulatrix*, and *P. cuneata*, etc. The soil is classified as meadow soil with sandy loam. To exclude livestock grazing effects, the experimental site has been fenced since October 2011.

EXPERIMENTAL DESIGN

The experimental plots were established on a fairly flat terrain to minimize the precipitation leaching effects on added N. The experiment used a complete block design with four N treatment levels, including N addition at 0 (control), 2.5, 5, and 10 g N m⁻² yr⁻¹ levels

(added as urea). Hereafter, the treatments were denoted as N₀, N_{2.5}, N₅, and N₁₀. The four treatments were randomly arranged within a block and replicated five times. Thus, twenty 6 × 6 m plots were arranged in a 4 × 5 matrix, with a 1 m buffer zone between adjacent plots and a 2 m buffer zone between adjacent blocks. The pelletized fertilizer was hand-broadcasted onto plots twice a year, each time with 50% of the total applied before rain events in the middle of June and July, respectively. The plots have been fertilized since 2012. As monitored, there was no rainstorm right after fertilizer application. Also, the flat terrain can minimize fertilizer leaching. To check the fertilizing efficiency, we measured ecosystem carbon exchange (net ecosystem exchange [NEE], ecosystem respiration [R_{eco}], and gross primary productivity [GPP]) for each treatment.

CLIMATE CONDITIONS AND SOIL MOISTURE

We used precipitation and air temperature data from a meteorology station ~18 km away from the experimental site. Soil moisture was measured every seven days from June to September at 3.8, 7.6, and 12 cm depths in all 20 plots using the TDR 300 (Spectrum Technologies, Plainfield, Illinois, U.S.A.).

PHENOLOGICAL OBSERVATION

We monitored phenological phases of seven species, including five forbs (*P. saundersiana*, *P. cuneata*, *P. bifurca*, *Y. simulatrix*, and *Saussurea stolicikai*), one graminoid specie (*Festuca coelestis*), and one sedge (*K. pygmaea*) (Table 1). *K. pygmaea* accounts for about 70% of the total community aboveground biomass and 90% of the total community coverage, respectively. They are all anemophilous flowers. The shallow-rooted *K. pygmaea* begins to blossom in June. It has a fibrous rooting system, which is mostly confined to the top 10 cm surface soil. Also, as a shallow-rooted plant, *Potentilla* begins to blossom in June. They have small taproots (generally smaller than 3 mm in diameter), which normally do not penetrate into soil deeper than 10 cm. The deep-rooted *F. coelestis* begins to blossom in July. It has a strong fibrous rooting system. *Y. simulatrix* and *S. stolicikai*, as deep-rooted plants, begin to blossom in late July. These two species have strong taproots, which can reach 30 cm deep into soil (Lv et al., 2009; Dorji et al., 2013).

We randomly flagged 15 individuals of each species in total at the start of the growing season, with 3 individuals in each plot for each species, and monitored their phenological phases at ap-

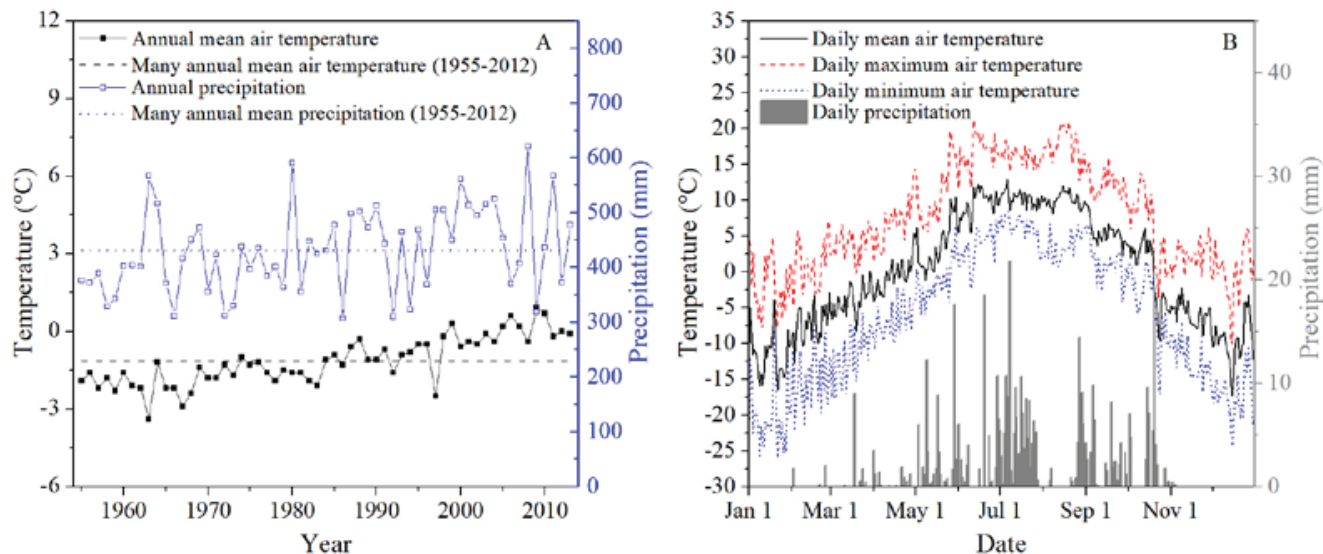


FIGURE 1. (A) Annual mean air temperature and annual precipitation from 1955 to 2013. (B) Temporal dynamic of air temperature and precipitation in 2013.

proximately 4 day intervals. We used the scoring method to record their phenology (Price and Waser, 1998; Dunne et al., 2003). For forbs, we used six phenological stages: “0” for plant turning green, “0.5” for most culms being visible, “1” for unopened buds, “2” for open flowers, “3” for initiated fruit, “4” for enlarged fruit, and “5” for dehiscence fruit. For grass and sedge, we used five phenological stages: “0” for plant turning green, “1” for appearance of spikelets, “2” for exerted anthers and styles from the spikelet florets, “3” for developed seed, and “4” for disarticulated seeds. Because most of *P. bifurca*, *S. stoliczkae*, and *Y. simulatrix* have no complete reproductive growth in our study area, we defined vegetative growth from 0 to 0.9, and leaf withering from 5.1 to 6.

PHENOLOGICAL ANALYSIS

It is difficult to continuously monitor the exact timing of each phenological stage. We obtained them by fitting the growth curvatures of the Richards growth equation against the day in Julian units (X) for each plot. Based on our monitoring, *P. saundersiana*, *P. cuneata*, *F. coelestis*, and *K. pygmaea* almost completed their reproductive growth. So we fitted the observation scores to the Richards growth equation directly (Richards, 1959; Sherry et al., 2007; Xia and Wan, 2013).

For the Richards growth equation:

$$Y = K [1 + a \cdot \exp(-b \cdot X)]^{-m} \quad (1)$$

where Y is the phenological score of each species; X is the day in Julian units; K is the maximal growth (here the last phenological stage, 5 or 4); a is the initial parameter whose value is related to the first observation date; b is the growth rate over time X ; and m is a variable curve shape parameter.

Once the parameters of the Richards equation were obtained for each plant, budding, flowering, or fruiting timing can be calculated from the equation, and then also the plot-average. The budding and flowering timing was calculated as $Y = 1$ and $Y = 2$ for all

species, respectively. The fruiting time was calculated as $Y = 3$ and $Y = 2.5$ for forbs and grass/sedge, respectively. The phenological duration equaled the phase $Y = 4$ for the forbs minus the phase $Y = 1$ (or phase $Y = 3$ minus phase $Y = 0.5$ for grass and sedge).

Few individuals of *P. bifurca*, *Y. simulatrix*, and *S. stoliczkae* started or completed their reproductive growth, so we fit the Richards growth equation model on the phenological scores for vegetative growth and leaf withering, separately. Using the Richards equation, we fitted the phenological scores to the two models. The phenological duration was obtained as $Y = 5.1$ from the second model minus $Y = 0.5$ from the first model.

STATISTICAL ANALYSIS

Treatment differences were tested using the one-way analysis of variance (ANOVA), and the least significant difference (LSD) test was employed for the multiple factor comparisons. Soil moisture difference during each month was compared by the independent-sample-T-test. The fittings of the Richards equation were carried out in Matlab (Mathworks, Natick, Massachusetts, U.S.A.) with the contraction-expansion algorithm (Gu et al., 1998), and all statistical analyses were conducted using SPSS software (SPSS, Chicago, Illinois, U.S.A.).

Results

CLIMATE CONDITION AND SOIL MOISTURE

In comparison with the long-term (1955–2012) mean (-1.16°C), the mean air temperature in 2013 (-0.1°C) was about 1°C higher. Meanwhile, total precipitation in 2013 (478 mm) was 11.2% higher than the long-term mean (430 mm) (Fig. 1, part A). However, total precipitation over the entire growing season (June to August) in 2013 (274 mm) was 3.5% lower than the long-term mean (284 mm).

The seasonal dynamics of air temperature followed a unimodal pattern, with a peak value in summer (Fig. 1, part B). The

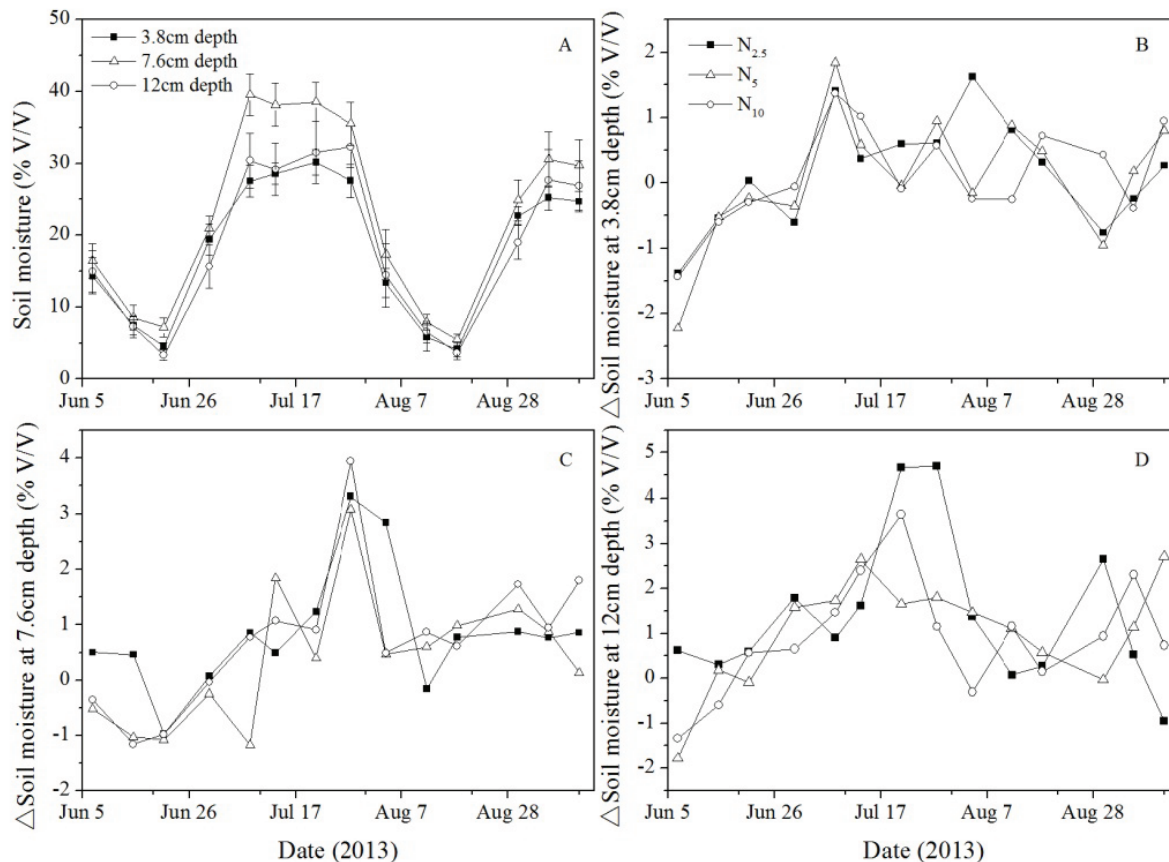


FIGURE 2. Temporal dynamic of (A) soil moisture at 3.8, 7.6 and 12 cm depths in the control plots (N_0), and fertilization-induced changes in soil moisture at (B) 3.8 cm depths, (C) 7.6 cm depths, and (D) 12 cm depths in response to $2.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ ($N_{2.5}$), $5 \text{ g N m}^{-2} \text{ yr}^{-1}$ (N_5), $10 \text{ g N m}^{-2} \text{ yr}^{-1}$ (N_{10}) addition.

mean maximum air temperature (16.8°C) is almost four times the minimum (4.4°C) over the growing season. Precipitation mostly occurred in July as rainfall and September as snowfall.

Soil moisture fluctuated with precipitation during the growing season (Fig. 2, part A). Soil moisture was significantly higher in July than in June and August at each depth ($P < 0.01$). Soil moisture at the 7.6 cm depth was slightly higher than in other depths. The multiple-level N additions had no distinct effects on soil moisture (Fig. 2, parts B, C, and D).

NITROGEN ADDITION EFFECTS ON ECOSYSTEM CARBON EXCHANGE

During peak growing seasons in 2013, NEE under $N_{2.5}$, N_5 , and N_{10} treatment significantly increased by 64% ($P = 0.011$), 54% ($P = 0.027$), and 92% ($P < 0.001$), respectively. Different N additions also significantly increased GPP by 44% ($P = 0.002$), 42% ($P = 0.003$), and 71% ($P < 0.001$), respectively. Only the N_{10} treatment significantly increased ecosystem R_{eco} by 49% ($P = 0.045$).

NITROGEN ADDITION EFFECTS ON FLOWER PHASE

Among the seven species observed, most of *K. pygmaea*, *F. coelestis*, *P. saundersiana*, and *P. cuneata* completed their reproductive growth and the N addition resulted in no obvious difference on the flowering time of the four species (Fig. 3, part A). Different levels of N additions failed to cause distinct flowering time responses from the four species. Two response patterns were detected in the flower phase

length. The length of *K. pygmaea* was elongated by 0.7 d and the elongation was statistically significant ($P < 0.05$); the length of *F. coelestis*, *P. saundersiana*, and *P. cuneata* showed no responses (Fig. 3, part B).

NITROGEN ADDITION EFFECTS ON FRUIT PHASE

There was no significant difference of fruiting time and fruit phase length between the N additions and the control treatments (Fig. 4, part A). In the meantime, there were no significant difference of fruiting time and fruit phase length among each level of N addition treatment (Fig. 4, part B).

NITROGEN ADDITION EFFECTS ON GROWTH DURATION

The reproductive phase is composed of bud, flower, and fruit phases. Among the seven studied species, *K. pygmaea*, *F. coelestis*, *P. saundersiana*, and *P. cuneata* had reproductive phases, while most of *P. bifurca*, *S. stolicikai*, and *Y. simulatrix* failed to start reproductive growth in 2013 (Fig. 5). Different levels of N additions failed to cause distinct growth duration responses from the seven species.

For the budding time or the start of vegetative phase of the seven species, only *K. pygmaea* showed significantly positive responses to N addition (Fig. 5, part A), whose budding time advanced by 5.9 d ($P < 0.05$).

In terms of ending the vegetative phase, only *S. stolicikai* exhibited statistically different responses to N addition (Fig. 5, part A). It advanced the end of the vegetative growth by 3.3 d ($P < 0.05$).

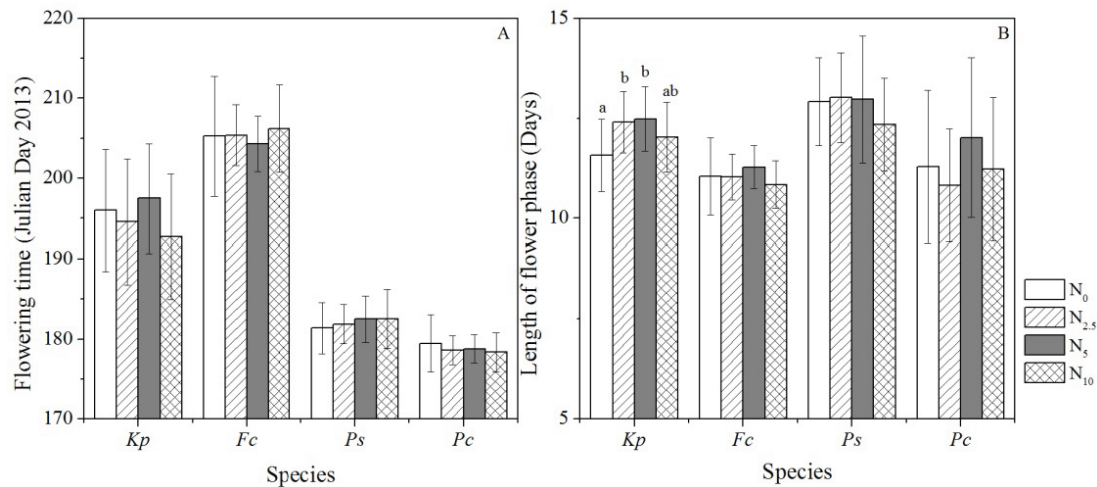


FIGURE 3. (A) Flowering time and (B) length of flower phase of *Kobresia pygmaea* (Kp), *Festuca coelestis* (Fc), *Potentilla saundersiana* (Ps), and *Potentilla cuneata* (Pc) under 0 (N₀), 2.5 (N_{2.5}), 5 (N₅), 10 (N₁₀) g N m⁻² yr⁻¹ addition. Different lowercase letters indicate significant differences ($P < 0.05$) among bars.

0.05). Other species showed no change in ending the vegetative growth in response to N addition.

Due to the changes in budding timing and reproductive growth termination (the start and the end of vegetative growth), the reproductive (vegetative) duration of the seven species responded differently to N addition (Fig. 5, part B). Among the seven species, only *K. pygmaea* stretched the duration by 6.0 d in response to N addition ($P < 0.05$), while other species showed no change.

Discussion

NITROGEN ADDITION EFFECTS ON SPECIES-LEVEL PHENOLOGY

As revealed in this study, plant species responded differently to the short-term N addition. The responses may depend on their morphological, physiological, and life-history traits (Kliker and

Eckert, 2004). Specifically, the phenology of *K. pygmaea* responded to the N addition in a more obvious manner than other species.

We monitored phenological phases of seven species, including five forbs (*P. saundersiana*, *P. cuneata*, *P. bifurca*, *Y. simulatrix*, and *S. stoliczkae*), one grass (*F. coelestis*), and one sedge (*K. pygmaea*). Some other phenological studies have observed N-addition delayed flowering in grasses but accelerated flowering in forbs (Cleland et al., 2006; Smith et al., 2012; Xia and Wan, 2013). In our study, the phenology of the grass and forbs showed no response to N additions. The plant traits associated with resource acquisition, such as rooting depth and life history might mediate plant phenology responses to changing nutritional conditions. *K. pygmaea*, as a shallow-rooted and dominant species, could be more benefited by fertilizing-induced increases in soil N compared to other species. However, also as shallow-rooted plants, *Potentilla* exhibited no response to N additions. The reason might lie in their disadvantage in competing for nutrition resources compared to the

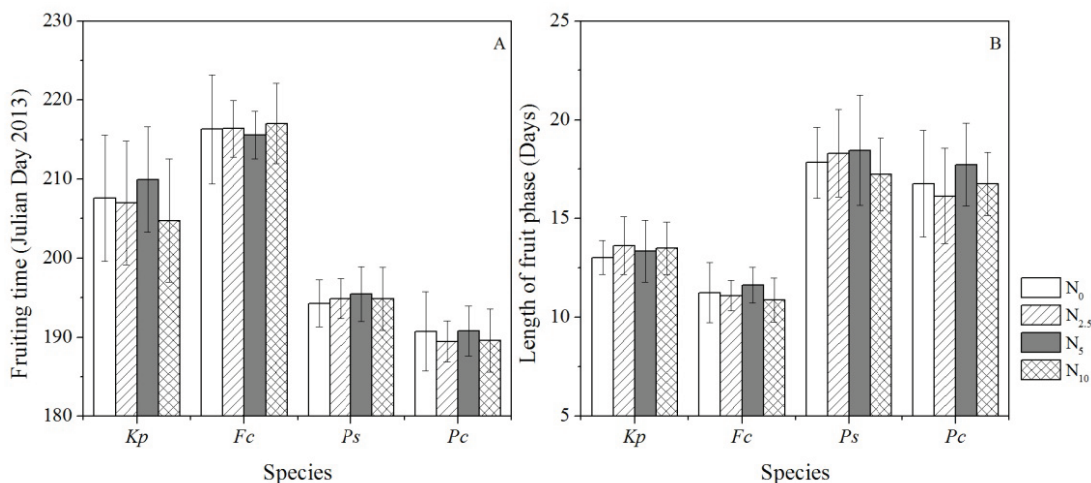


FIGURE 4. (A) Fruiting time and (B) length of fruit phase of *Kobresia pygmaea* (Kp), *Festuca coelestis* (Fc), *Potentilla saundersiana* (Ps), and *Potentilla cuneata* (Pc) under 0 (N₀), 2.5 (N_{2.5}), 5 (N₅), 10 (N₁₀) g N m⁻² yr⁻¹ addition.

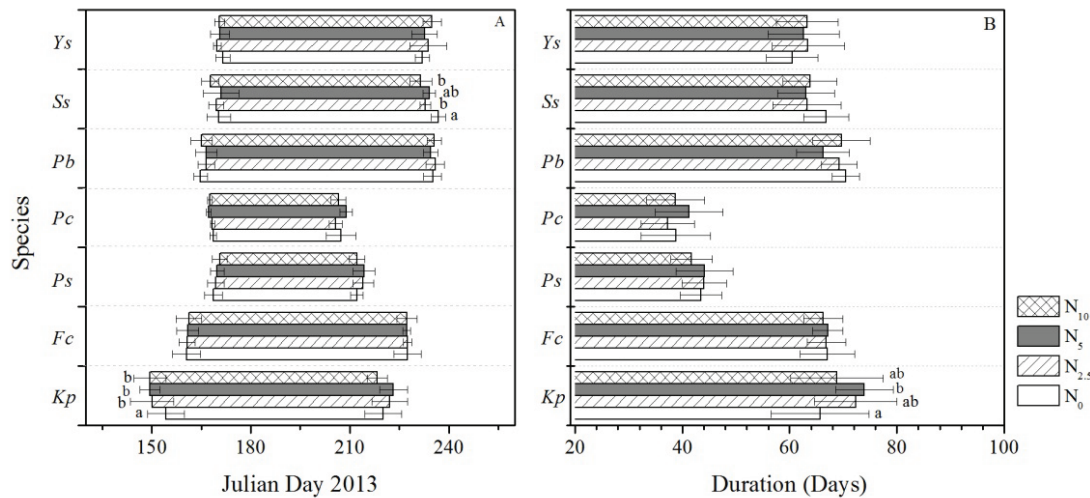


FIGURE 5. (A) Timing and (B) duration of the entire reproductive period composed of three phases (budding, flowering, and fruiting) for *Kobresia pygmaea* (*Kp*), *Festuca coelestis* (*Fc*), *Potentilla saundersiana* (*Ps*), *Potentilla cuneata* (*Pc*), and the entire vegetative period for *Potentilla bifurca* (*Pb*), *Saussurea stoliczkae* (*Ss*), *Youngia simulatrix* (*Ys*) under 0 (N_0), 2.5 ($N_{2.5}$), 5 (N_5), 10 (N_{10}) g N m⁻² yr⁻¹ addition. Different lowercase letters indicate significant differences ($P < 0.05$) among bars.

dominant species (Miller and Bowman, 2003; Miller et al., 2007; Ashton et al., 2010). *F. coelestis*, *Y. simulatrix*, and *S. stoliczkae*, as deep-rooted and late flowering plants, might be slow in absorbing the added N.

On the other hand, the N addition treatment in this study has been conducted only for one year and the studied plants are all perennial. Their belowground reproductive growths can last one or more years, thus delaying an obvious aboveground response to altered environmental conditions (Mark, 1970; Aydelotte and Diggle, 1997; Smith et al., 2012).

A limited number of studies have reported how sedge phenology responds to N addition. For example, under fertilization, *K. capillifolia* flowered earlier or later (Bayaerta et al., 2010; Zhang et al., 2013b); *K. setchwanensis* shortened the flower phase length; *K. macrantha* showed no response (Han, 2012). The different responses from the similar plant functional types may be due to their distinct growth strategies under distinct environmental conditions.

As the dominant species, each phenological phase of *K. pygmaea* has exhibited a divergent response to N addition. First, *K. pygmaea* budded earlier in response to N addition. Most alpine plants rely on a conservative growth strategy (Chapin, 1980; Aerts and Chapin, 2000). But *K. pygmaea*, as a superior N competitor and shallow-rooted plant, may be able to respond quickly to increased upper-soil N availability and build their growth advantages. For the next phase, the flowering time of *K. pygmaea* showed no change. However, its flowering phase length was prolonged significantly. Its fruit phase exhibited no change either. In N-rich soils, plant species usually allocate more resources to reproductive growth (Tilman and Wedin, 1991). As a result, the entire reproductive period of *K. pygmaea* was significantly prolonged in N-added treatment. The longer growth duration of *K. pygmaea* in N addition plots could be ascribed to an earlier start of growing season and a non-changed end of the reproductive phase.

The phenological phase change of *K. pygmaea*, as the dominant species in the alpine meadow ecosystem, has important implications for various aspects of studies, including ecosystem dynamics, plant demography and community interactions, trophic

dynamics, and ecosystem energy balance. Furthermore, it has fundamental implications for pastoralists and wildlife in the region. In response to increased N, *K. pygmaea* becomes increasingly dominant in the community due to its more effective use of the added nutrition. In alpine ecosystems, species composition would change with altered productivity (Grabherr et al., 1994; Pauli et al., 2012). *K. pygmaea* can become more productive due to its greater dominance under nutrition addition treatment, which in turn would change the community structure and species composition. On the other hand, advanced phenology may make *K. pygmaea* increasingly susceptible to frost damage, which may reduce its production. A further increased N deposition is possible to have significant effects on plant species composition of the alpine meadows, which can cause impacts on pastoral livelihoods and the delivery of key ecosystem services from the Tibetan Plateau (Yu et al., 2012).

MULTIPLE-LEVEL NITROGEN ADDITION EFFECTS ON PLANT PHENOLOGY

Such plant traits as plant phenology, leaf properties, and biomass of different modules (inflorescences, leaves, stems, and rootstocks) have been conventionally investigated to identify global change-related treatment effects on plant development and growth allocation patterns (Franzaring et al., 2008). The majority of the previous N addition treatment studies have focused on such common indicators as biomass or leaf properties (Gough et al., 2000; Zhang et al., 2007; Niu et al., 2008; Xia and Wan, 2008). Only a few have reported on plant phenology (Lupi et al., 2012; Smith et al., 2012; Xia and Wan, 2013), let alone different levels of N supply effect on phenology (Franzaring et al., 2008).

In the present study, the effects of multiple-level N additions on phenology were not obvious. Similar studies have reported effects of different amounts of nutrition supply on plant development timing of *Molinia caerulea* from three origins (Franzaring et al., 2008). However, it is not clear to what extent the phenology responded to the multiple-level N additions. For one origin of *M. caerulea*, leaf senescence decreased with the amount of added nu-

trition, while it increased for another one. For the third origin, leaf senescence is composed of two opposite responses to the two levels of nutrition addition. The divergent plant responses to a gradient amount of added N may depend on plant compositions (Saarinen, 1998; Green and Galatowitsch, 2002; Xia and Wan, 2008).

Knowledge about environmental change effects caused by different levels of N addition might improve our understanding of the above-mentioned phenomena. In this study, the influence of different amounts of N addition on soil moisture was not obvious. Due to the harsh physical environment, the growing season is relatively short over the Tibetan Plateau (Liu et al., 2006; Ernakovich et al., 2014). At our research site, temperature and moisture conditions are suitable for vegetation growth only in July. So it is also possible that phenological responses may require multiple growing seasons to fully materialize.

Conclusions

Our results suggest that N plays an important role in regulating plant phenology of the dominant species. The addition of N significantly advanced budding time and prolonged the flower phase length and reproductive period duration of dominant species, whereas N addition produced no obvious effects on accompanying species. Different amounts of N addition resulted in no distinct phenological responses of each species. Due to the conservative growth strategy of alpine plants, their phenological responses may require multiple growing seasons to fully materialize. To further elucidate on and predict the phenological responses of organisms to nutritional condition modifications, long-term experiments in various ecosystems are required.

Acknowledgments

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