



Nitrogen Addition Alters the Phenology of a Dominant Alpine Plant in Northern Tibet

Authors: Xi, Yi, Zhang, Tao, Zhang, Yangjian, Zhu, Juntao, Zhang, Geli, et al.

Source: Arctic, Antarctic, and Alpine Research, 47(3) : 511-518

Published By: Institute of Arctic and Alpine Research (INSTAAR),
University of Colorado

URL: <https://doi.org/10.1657/AAAR0014-054>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

Yi Xi^{1,2,3}

Tao Zhang^{1,2,3,4}

Yangjian Zhang^{1,2,4}

Juntao Zhu^{1,2}

Geli Zhang^{1,2} and

Yanbin Jiang^{1,2}

¹Lhasa Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A, Datun Road, Chaoyang District, Beijing, 100101, China

²Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences, No. 16, Lincui Road, Chaoyang District, Beijing, 100101, China

³University of Chinese Academy of Sciences, 19A, Yuquan Road, Shijingshan District, Beijing, 100049, China

⁴Corresponding authors:

Lhasa Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A, Datun Road, Chaoyang District, Beijing, 100101, China. Tao Zhang (zhangt.11b@igsnr.ac.cn), Yangjian Zhang (zhangyj@igsnr.ac.cn)

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.

DOI: <http://dx.doi.org/10.1657/AAAR0014-054>

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-

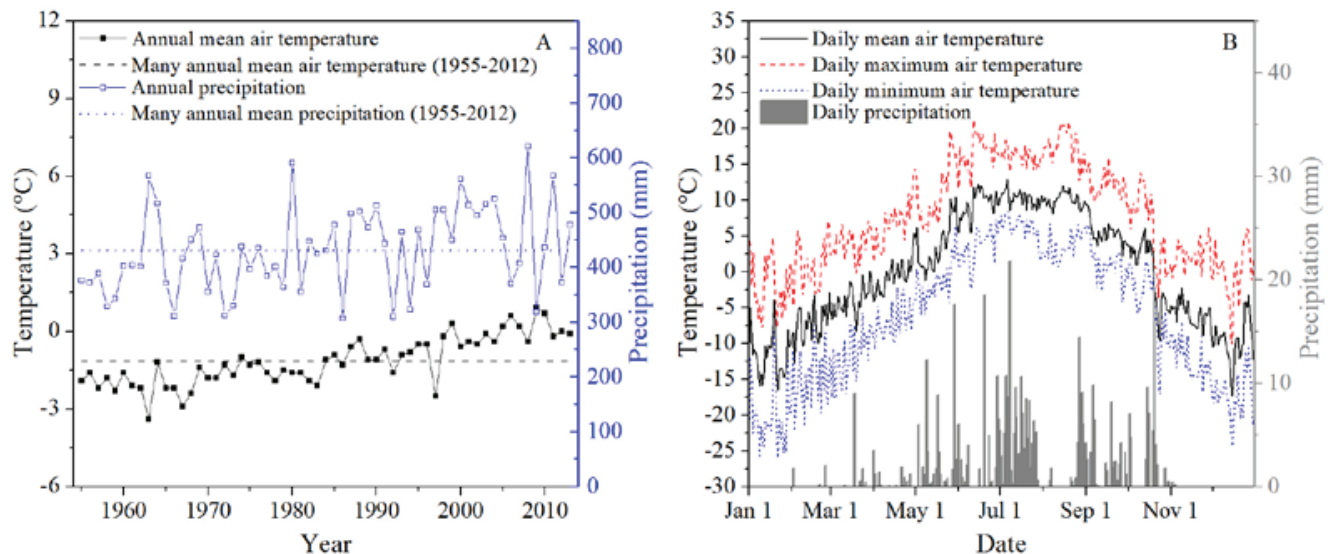


FIGURE 1. (A) Annual mean air temperature and annual precipitation from 1955 to 2013. (B) Temporal dynamic of air temperature and precipitation in 2013.

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

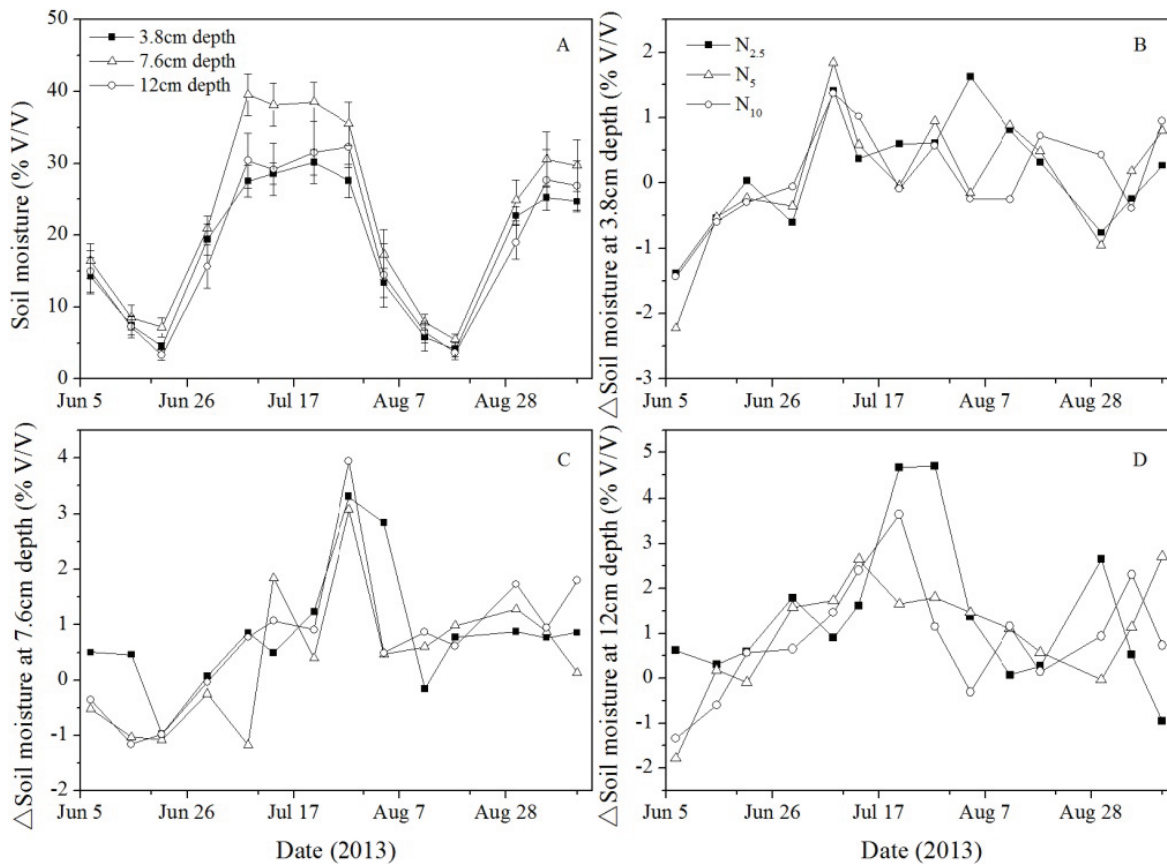


FIGURE 2. Temporal dynamic of (A) soil moisture at 3.8, 7.6 and 12 cm depths in the control plots (N_0), and fertilization-induced changes in soil moisture at (B) 3.8 cm depths, (C) 7.6 cm depths, and (D) 12 cm depths in response to $2.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ ($N_{2.5}$), $5 \text{ g N m}^{-2} \text{ yr}^{-1}$ (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 ($P < 0.05$).

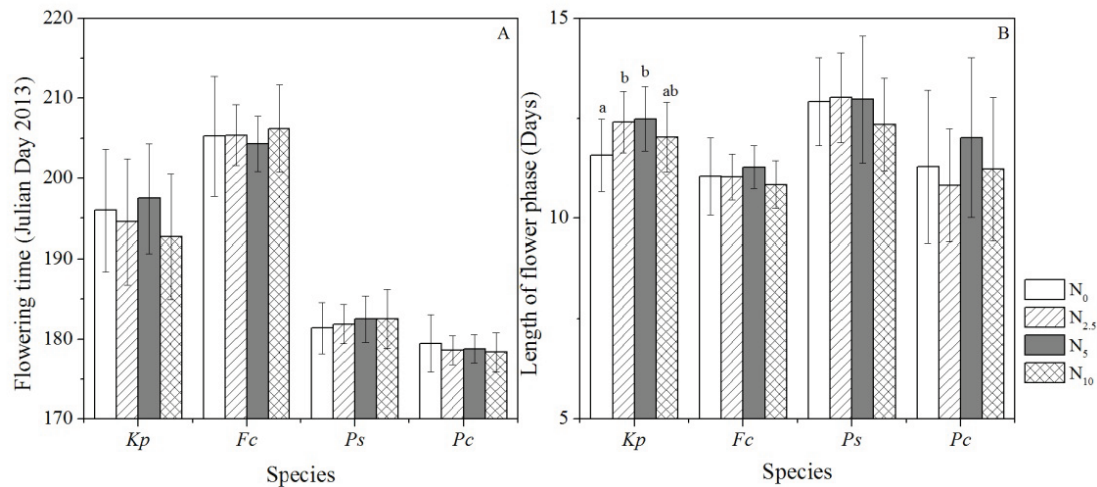


FIGURE 3. (A) Flowering time and (B) length of flower phase of *Kobresia pygmaea* (Kp), *Festuca coelestis* (Fc), *Potentilla saundersiana* (Ps), and *Potentilla cuneata* (Pc) under 0 (N₀), 2.5 (N_{2.5}), 5 (N₅), 10 (N₁₀) g N m⁻² yr⁻¹ addition. Different lowercase letters indicate significant differences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

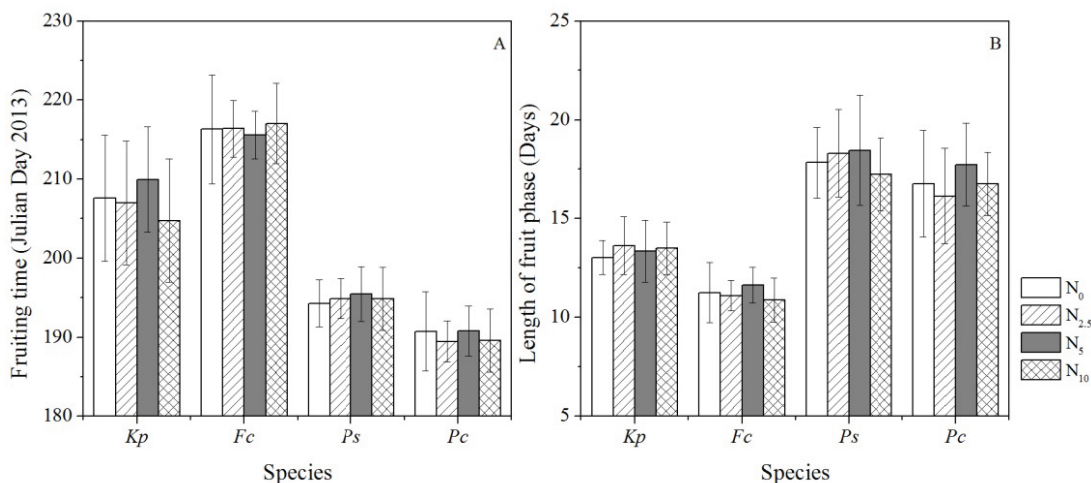


FIGURE 4. (A) Fruiting time and (B) length of fruit phase of *Kobresia pygmaea* (Kp), *Festuca coelestis* (Fc), *Potentilla saundersiana* (Ps), and *Potentilla cuneata* (Pc) under 0 (N₀), 2.5 (N_{2.5}), 5 (N₅), 10 (N₁₀) g N m⁻² yr⁻¹ addition.

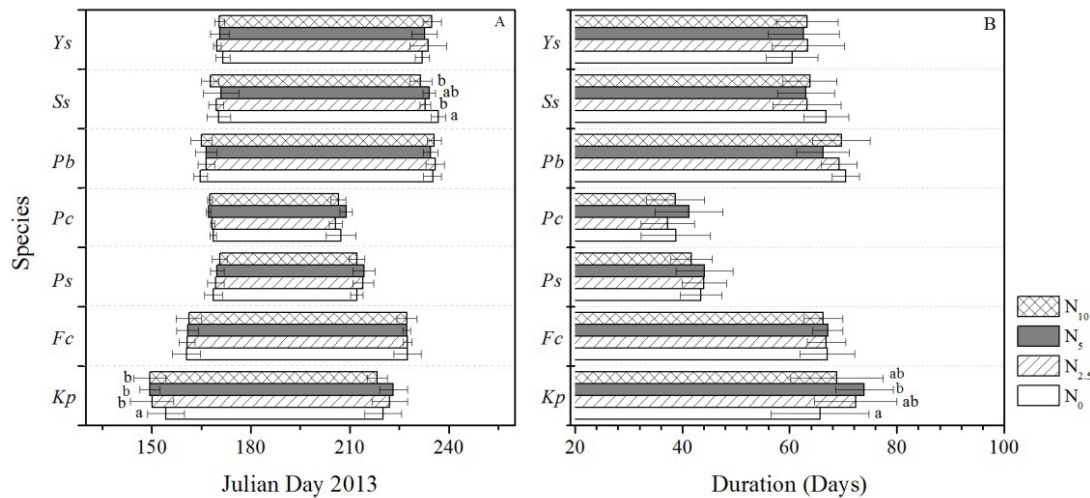


FIGURE 5. (A) Timing and (B) duration of the entire reproductive period composed of three phases (budding, flowering, and fruiting) for *Kobresia pygmaea* (*Kp*), *Festuca coelestis* (*Fc*), *Potentilla saundersiana* (*Ps*), *Potentilla cuneata* (*Pc*), and the entire vegetative period for *Potentilla 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

This research is supported by the 973 Program (2013CB956302) of the Ministry of Science and Technology of China, One Hundred Talents Program of Chinese Academy of Sciences, and National Natural Science Foundation of China (31300356, 41201055).

References Cited

- Aerts, R., and Chapin, F. S., 2000: The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, 30: 1–67.
- Ahas, R., Jaagus, J., and Aasa, A., 2000: The phenological calendar of Estonia and its correlation with mean air temperature. *International Journal of Biometeorology*, 44: 159–166.
- Ashton, I. W., Miller, A. E., Bowman, W. D., and Suding, K. N., 2010: Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology*, 91: 3252–3260.
- Aydelotte, A., and Diggle, P., 1997: Analysis of developmental preformation in the alpine herb *Caltha leptosepala* (Ranunculaceae). *American Journal of Botany*, 84: 1646.
- Bai, J., Xu, X., Fu, G., Song, M., He, Y., and Jiang, J., 2011: Effects of temperature and nitrogen input on nitrogen mineralization in alpine soils on Tibetan Plateau. *Agricultural Science and Technology*, 12: 1909–1912.
- Bayaerta, Jia, P., Yang, X., and Du, G. Z., 2010: Response of dominating species flowering phenology to fertilization in Qinghai-Tibetan alpine meadow. *Acta Prataculturae Sinica*, 19: 233–239.
- Bowman, W. D., Gartner, J. R., Holland, K., and Wiedermann, M., 2006: Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: Are we there yet? *Ecological Applications*, 16: 1183–1193.
- Chapin, F. S., 1980: The mineral-nutrition of wild plants. *Annual Review of Ecology and Systematics*, 11: 233–260.
- Cleland, E. E., Chiariello, N. R., Loarie, S. R., Mooney, H. A., and Field, C. B., 2006: Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences*, 103: 13740–13744.
- Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A., and Schwartz, M. D., 2007: Shifting plant phenology in response to global change. *Trends in Ecology and Evolution*, 22: 357–365.
- Dorji, T., Totland, O., Moe, S. R., Hopping, K. A., Pan, J. B., and Klein, J. A., 2013: Plant functional traits mediate reproductive phenology and success in response to experimental warming and snow addition in Tibet. *Global Change Biology*, 19: 459–472.
- Dunne, J. A., Harte, J., and Taylor, K. J., 2003: Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs*, 73: 69–86.
- Ernakovich, J. G., Hopping, K. A., Berdanier, A. B., Simpson, R. T., Kachergis, E. J., Steltzer, H., and Wallenstein, M. D., 2014: Predicted responses of arctic and alpine ecosystems to altered seasonality under climate change. *Global Change Biology*, 20: 3256–3269.
- Forrest, J., and Miller-Rushing, A. J., 2010: Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B—Biological Sciences*, 365: 3101–3112.
- Franzaring, J., Holz, I., and Fangmeier, A., 2008: Different responses of *Molinia caerulea* plants from three origins to CO₂ enrichment and nutrient supply. *Acta Oecologica*, 33: 176–187.
- Gough, L., Osenberg, C. W., Gross, K. L., and Collins, S. L., 2000: Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos*, 89: 428–439.
- Grabherr, G., Gottfried, M., and Pauli, H., 1994: Climate effects on mountain plants. *Nature*, 369: 448.
- Green, E. K., and Galatowitsch, S. M., 2002: Effects of *Phalaris arundinacea* and nitrate-N addition on the establishment of wetland plant communities. *Journal of Applied Ecology*, 39: 134–144.
- Gruber, N., and Galloway, J. N., 2008: An Earth-system perspective of the global nitrogen cycle. *Nature*, 451: 293–296.
- Gu, S., Hui, D., and Bian, A., 1998: The contraction–expansion algorithm and its use in fitting nonlinear equations. *Journal of Biomathematics*, 13: 426–434.
- Han, J. F., 2012: *Effects of Simulated Precipitation Changes and Increased N Deposition on the Phenology and Biomass of Plants in an Alpine Meadow*. Master's thesis, College of Animal Science and Technology, Sichuan Agricultural University, Yaan, Sichuan, China, 23 pp.
- IPCC, 2007: *The AR4 Synthesis Report*. Cambridge: Cambridge University Press.
- Klein, J. A., Hopping, K. A., Yeh, E. T., Nyima, Y., Boone, R. B., and Galvin, K. A., 2014: Unexpected climate impacts on the Tibetan Plateau: local and scientific knowledge in findings of delayed summer. *Global Environmental Change*, 28: 141–152.
- Kliber, A., and Eckert, C. G., 2004: Sequential decline in allocation among flowers within inflorescences: proximate mechanisms and adaptive significance. *Ecology*, 85: 1675–1687.
- Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., and Zhang, F., 2013: Enhanced nitrogen deposition over China. *Nature*, 494: 459–462.
- Liu, X. D., Yin, Z. Y., Shao, X. M., and Qin, N. S., 2006: Temporal trends and variability of daily maximum and minimum, extreme temperature events, and growing season length over the eastern and central Tibetan Plateau during 1961–2003. *Journal of Geophysical Research—Atmospheres*, 11: 11–19.
- Luo, Y. J., Qin, G. L., and Du, G. Z., 2006: Importance of assemblage-level thinning: a field experiment in an alpine meadow on the Tibet plateau. *Journal of Vegetation Science*, 17: 417–424.
- Lupi, C., Morin, H., Deslauriers, A., Rossi, S., and Houle, D., 2012: Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce. *Canadian*

- Journal of Forest Research—Revue Canadienne De Recherche Forestiere*, 42: 1277–1288.
- Lv, X., Kang, S., Zhu, L., Zhang, Y., and Han, W., 2009: Phenology characters of dominant plants in the Nam Co Basin and its response to climate, Tibet. *Journal of Mountain Research*, 27: 648–654.
- Mark, A. F., 1970: Floral initiation and development in New Zealand alpine plants. *New Zealand Journal of Botany*, 8: 67–75.
- Martinkova, J., Smilauer, P., and Mihulka, S., 2002: Phenological pattern of grassland species: relation to the ecological and morphological traits. *Flora*, 197: 290–302.
- Miller, A. E., and Bowman, W. D., 2003: Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. *Plant and Soil*, 250: 283–292.
- Miller, A. E., Bowman, W. D., and Suding, K. N., 2007: Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology*, 88: 1832–1840.
- Miller-Rushing, A. J., and Inouye, D. W., 2009: Variation in the impact of climate change on flowering phenology and abundance: an examination of two pairs of closely related wildflower species. *American Journal of Botany*, 96: 1821–1829.
- Niu, K. C., Luo, Y. J., Choler, P., and Du, G. Z., 2008: The role of biomass allocation strategy in diversity loss due to fertilization. *Basic and Applied Ecology*, 9: 485–493.
- Nord, E. A., and Lynch, J. P., 2009: Plant phenology: a critical controller of soil resource acquisition. *Journal of Experimental Botany*, 60: 1927–1937.
- Parnesan, C., 2006: Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics*, 37: 637–669.
- Parnesan, C., and Yohe, G., 2003: A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421: 37–42.
- Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J. L. B., Coldea, G., Dick, J., Erschbamer, B., Calzado, R. F., Ghosn, D., Holten, J. I., Kanka, R., Kazakis, G., Kollár, J., Larsson, P., Moiseev, P., Moiseev, D., Molau, U., Mesa, J. M., Nagy, L., Pelino, G., Puşcaş, M., Rossi, G., Stanisci, A., Syverhuset, A. O., Theurillat, J.-P., Tomaselli, M., Unterluggauer, P., Villar, L., Vittoz, P., and Grabherr, G., 2012: Recent plant diversity changes on Europe's mountain summits. *Science*, 336: 353–355.
- Piao, S. L., Friedlingstein, P., Ciais, P., Viovy, N., and Demarty, J., 2007: Growing season extension and its impact on terrestrial carbon cycle in the northern hemisphere over the past 2 decades. *Global Biogeochemical Cycles*, 21: 1–11.
- Piao, S., Cui, M., Chen, A., Wang, X., Ciais, P., Liu, J., and Tang, Y., 2011: Altitude and temperature dependence of change in the spring vegetation green-up date from 1982 to 2006 in the Qinghai-Xizang Plateau. *Agricultural and Forest Meteorology*, 151: 1599–1608.
- Post, E. S., and Inouye, D. W., 2008: Phenology: response, driver, and integrator. *Ecology*, 89: 319–320.
- Power, S. A., Green, E. R., Barker, C. G., Bell, J. N. B., and Ashmore, M. R., 2006: Ecosystem recovery: heathland response to a reduction in nitrogen deposition. *Global Change Biology*, 12: 1241–1252.
- Price, M. V., and Waser, N. M., 1998: Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology*, 79: 1261–1271.
- Rathcke, B., and Lacey, E. P., 1985: Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, 16: 179–214.
- Richards, F. J., 1959: A flexible growth function for empirical use. *Journal of Experimental Botany*, 10: 290–301.
- Saarenin, T., 1998: Internal C:N balance and biomass partitioning of *Carex rostrata* grown at three levels of nitrogen supply. *Canadian Journal of Botany*, 76: 762–768.
- Sherry, R. A., Zhou, X., Gu, S., Arnone, J. A., Schimel, D. S., Verburg, P. S., Wallace, L. L., and Luo, Y., 2007: Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences*, 104: 198–202.
- Smith, J. G., Sconiers, W., Spasojevic, M. J., Ashton, I. W., and Suding, K. N., 2012: Phenological changes in alpine plants in response to increased snowpack, temperature, and nitrogen. *Arctic, Antarctic, and Alpine Research*, 44: 135–142.
- Sparks, T. H., and Menzel, A., 2002: Observed changes in seasons: an overview. *International Journal of Climatology*, 22: 1715–1725.
- Tilman, D., and Wedin, D., 1991: Plant traits and resource reduction for five grasses growing on a nitrogen gradient. *Ecology*, 72: 685–700.
- Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J. L., Travers, S. E., Pau, S., Regetz, J., Davies, T. J., Kraft, N. J. B., Ault, T. R., Bolmgren, K., Mazer, S. J., McCabe, G. J., McGill, B. J., Parnesan, C., Salamin, N., Schwartz, M. D., and Cleland, E. E., 2012: Warming experiments underpredict plant phenological responses to climate change. *Nature*, 485: 494–497.
- Xia, J. Y., and Wan, S. Q., 2008: Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, 179: 428–439.
- Xia, J. Y., and Wan, S. Q., 2013: Independent effects of warming and nitrogen addition on plant phenology in the Inner Mongolian steppe. *Annals of Botany*, 111: 1207–1217.
- Yang, H., Jiang, L., Li, L., Li, A., Wu, M., and Wan, S., 2012: Diversity-dependent stability under mowing and nutrient addition: evidence from a 7-year grassland experiment. *Ecology Letters*, 15: 619–626.
- Yu, C. Q., Zhang, Y. J., Claus, H., Zeng, R., Zhang, X. Z., and Wang, J. S., 2012: Ecological and environmental issues faced by a developing Tibet. *Environmental Science and Technology*, 46: 1979–1980.
- Yu, H. Y., Luedeling, E., and Xu, J. C., 2010: Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. *Proceedings of the National Academy of Sciences*, 107: 22151–22156.
- Zhang, G., Zhang, Y., Dong, J., and Xiao, X., 2013a: Green-up dates in the Tibetan Plateau have continuously advanced from 1982 to 2011. *Proceedings of the National Academy of Sciences*, 110: 4309–4314.
- Zhang, L. H., Song, C. C., Wang, D. X., and Wang, Y. Y., 2007: Effects of exogenous nitrogen on freshwater marsh plant growth and N₂O fluxes in Sanjiang Plain, Northeast China. *Atmospheric Environment*, 41: 1080–1090.
- Zhang, Z. L., Liu, X. D., Jia, P., and Du, G. Z., 2013b: Effects of nitrogen addition on flowering phenology, functional traits and community structure of alpine meadow in the Eastern Qinghai-Tibet Plateau. *Pratacultural Science*, 30: 728–735.

MS accepted March 2015