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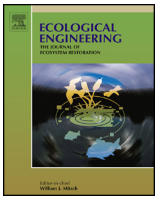

Effects of nitrogen addition and mowing on reproductive phenology of three early-flowering forb species in a Tibetan alpine meadow

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ABSTRACT

Nitrogen (N) deposition and land use are likely to alter plant phenology, with subsequent effects on community structure and ecosystem function. Phenological responses of three early-flowering species, *Anemone trullifolia* var. *linearis*, *Caltha scaposa*, and *Trollius farreri* to N addition and hay mowing were investigated in a Tibetan alpine meadow over three years. N addition significantly delayed the first flowering time of *A. trullifolia* var. *linearis* (by 11.7 days) and *C. scaposa* (by 11.1 days), but did not affect that of *T. farreri*. Mowing prolonged the first flowering time and first fruiting time by 4.7 and 7.4 days across all three species. Significant interactions between mowing and N addition on reproductive phenology characteristics were detected. Mowing induced changes in the first and last flowering times were 6.5 and 4.3 days earlier in the N addition plots than those in the N non-addition plots. The changes in reproductive time were mainly attributed to the variations in standing litter in each treatment, i.e. reproductive phenology (timing of flowering and fruiting) was positively associated with litter accumulation. Our results indicate that N deposition and land use can affect plant phenology by changing the accumulation of standing litter in Tibetan alpine meadows.

1. Introduction

Plant phenology is the seasonal timing of environmental-mediated phenomenon, and therefore sensitive to environmental changes (Cleland et al., 2006; Neil and Wu, 2006; Wolkovich et al., 2012; Guo et al., 2013; Fu et al., 2015; Ge et al., 2015). Responses of plant phenology to environmental changes can alter community structure (Encinas-Viso et al., 2012), inter-species interactions (Rafferty and Ives, 2011), and ecosystem carbon exchange (Xia et al., 2015). Plant phenology is thus widely regarded as an important indicator of global change (Menzel and Fabian 1999; Peñuelas and Filella 2001; Chuine et al., 2004; Keenan, 2015).

As an important aspect of global change, nitrogen (N) deposition is regionally becoming serious (Galloway et al., 2004; Liu et al., 2013). N deposition has been demonstrated to facilitate plant photosynthesis (Fleischer et al., 2013) and stimulate plant growth (Xia and Wan, 2008; Cao et al., 2011; Lü et al., 2011; Zhang et al., 2013), which may further alter plant reproductive phenology (Cleland et al., 2006; Smith et al., 2012; Xia and Wan, 2013; Xi et al., 2015). For example, Cleland et al. (2006) reported that N addition prolonged flowering times of forbs but delayed those of grasses in an American annual grassland. Smith et al. (2012) found contrary results in an alpine tundra. Xia and Wan (2013) showed that N addition in a temperate steppe did not affect flowering time at the community level. Therefore, the effects of N deposition on plant reproductive phenology remain controversial across plant functional groups and species in different ecosystems.

Mowing for hay is an important human activity in grazing pastures, and can change microclimates (Wan et al., 2002) and associated plant traits such as plant height and specific leaf area (Kahmen and Poschlod, 2002; Diaz et al., 2007; Klimesova et al., 2008), which are closely related to plant reproductive phenology (Sun and Frelich, 2011; Wolkovich et al., 2012). However, there is little evidence for the effects of mowing on plant reproductive

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Abbrevations: FFlT, First flowering time; LFlT, last flowering time; FFrT, first fruiting time; LFrT, last fruiting time; AT, *Anemone trullifolia* var. *linearis*; CS, *Caltha scaposa*; TF, *Trollius farreri*.

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phenology. We know only several cases in which mowing has been shown to advance plant flowering time to avoiding to be cut off because of the changes of selective forces during evolution (Reisch and Poschlod, 2008), or delay plant reproductive phenology by increasing the time to regenerate lost tissue (Freeman et al., 2003) and reduce the growth rate (Trtikova, 2008). Therefore, the effects of mowing on plant reproductive phenology are also unclear.

The eastern part of the Tibetan plateau is characterized by extensive alpine meadows. This area has experienced universal N deposition (0.53 kg N ha^{-2} yr^{-1}) in the last few years and it is predicted to consistently increase in the future (Liu et al., 2013). Meanwhile, these meadows provide large amounts of high-quality forages, supporting millions of livestock (Xiang et al., 2009). Mowing for hay is the only way for Chinese herdsman to store forages in winter (Cao et al., 2009). Mowing removes the aboveground part of the plant, which may alleviate the N induced changes in plant growth and alter associated plant reproductive phenology. Thus, N deposition and mowing might interactively affect plant reproductive phenology in these meadows. However, to the best of our knowledge, no study has examined the interaction between N addition and mowing in this area.

In the present study, we conducted a manipulative 3-year field experiment involving N addition (addition vs. non-addition) and mowing (mowing vs. non-mowing) in a Tibetan alpine meadow. We quantified plant reproductive phenology traits including the first and last flowering times, the first and last fruiting times, and community standing litter height as well as flowering height of three early spring flowering species. These data were used to examine the effects of both N deposition and mowing on plant reproductive phenology of early-flowering forb species to predict responses of plant phenology to global change.

2. Materials and methods

2.1. Study site

The study was performed in Hongyuan County (32°48′N, 102°33′E) of Sichuan province in the eastern Qinghai-Tibetan Plateau. The altitude is about 3500 m a.s.l. The climate is characterized by a short and cool spring, summer, and autumn and a long winter. Mean annual temperature is 0.9 °C, with maximum and minimum monthly means being 10.9 °C and −10.3 °C in July and January, respectively. Mean annual precipitation is 690 mm, almost 80% of which occurs between May and August. The soil is classified as Mat Cry-gelic Cambisol (Chinese Soil Taxonomy Research Group 1995) with high soil organic carbon (250 g kg^{-1}) but low total nitrogen (8 g kg^{-1}) and phosphorus (5 mg kg^{-1}) content at a depth of 0–20 cm (Wu et al., 2011).

The study area is dominated by *Blysmus sinocompressus*, *Carex enervis* ssp. *Chuanxibeiensis*, *Deschampsia caespitosa*, *Anemone trullifolia* var. *linearis*, *Potentilla anserina*, *Haplosphaera himalayensis*, *Aster alpinus* and *Gentiana formosa*. In the growing season (late May to late September), the peak value of total community coverage is over 90% and average plant height is > 30 cm in late-August (Wu et al., 2011). In particular, *Anemone trullifolia* var. *linearis*, *Caltha scaposa* and *Trollius farreri* were the only three early-flowering perennial forb species in the study area (Liu et al., 2011), and occupied 22–61%, 0.23–1.1%, and 0.76–1.85% of the total biomass. The first flowering time of the three species happened from early May to early June, which was in the first two months of the snow-free season at the study site. The first fruiting time of *A. trullifolia* var. *linearis*, and *C. scaposa* occurred in late May, and the first fruiting time of *T. farreri* occurred in early August. We here focused on the responses of reproductive phenology of these three species to N addition and mowing.

2.2. Experimental design

In a fenced area of 1 ha, we conducted an orthogonal two factor-two level experiment in which we crossed N addition (addition vs. non-addition) with mowing (mowing vs. non-mowing), yielding a total of four treatments: (i) neither N addition nor mowing (N-, M-); (ii) N addition only (N+, M-); (iii) mowing only (N-, M+); (iv) both N addition and mowing (N+, M+). Each of these four treatments was replicated five times (n = 20 in total), each replicate was a 1.5 × 0.75 m^2 plot. N (in the form of urea; 5 g N m^{-2} yr^{-1}) was added to the nitrogen addition treatments in early April from 2008 to 2010. We cut and removed aboveground plant parts (leaving 5 cm) in early October after all phenological events were complete in the mowing treatments in both 2008 and 2009. In addition, soil temperature (5 cm underground) was measured in three plots for mowing verse non-mowing treatments using thermometers (model DS1921G, Maxim Integrated Products, Sunnyvale, California, USA). Over a 6-month duration (from January 19th to July 23rd, 2010), the mean daily temperature (over 24 h of measurements made every 30 min) in the mowing plots was 0.84 °C higher than in the non-mowing plots (Fig. A. 1 in Supplementary material).

2.3. Standing litter

The average standing litter height was calculated following the method by Liu et al. (2012). The number of each plant species (species level abundance) was investigated in a subplot of 0.5 × 0.5 m^2 in each plot on 26th to 28th of August in 2009. The standing litter height of each species (from three individuals) was measured in each plot from 21th to 25th of March 2010. The community standing litter height was calculated using the following equation:

$$SLH = \sum_{1}^{n} \left(Hi \times \frac{Ai}{\sum_{1}^{n} Ai} \right)$$

Where Hi and Ai stood for the standing litter height and the abundance of the species i, and n was the number of species.

2.4. Phenological observations

Three flowering stems of each species were randomly taped in each plot and measured on 21th to 25th of March 2010. The flowering height (FlH) of each species was calculated as the mean value of the flowering stems. The number of flower and fruit (if any) of the three species in each plot was investigated over a 5-day interval from 1st April (before the earliest flowering time) to 27 August (after the latest fruiting time) in 2010. The first and last flowering/fruiting proportions (y) were calculated using a quadratic equation, following the protocols of Sun and Frelich (2011), i.e., $y = ax^2 + bx + c$, where x is the Julian date. We defined the time when 10% and 90% of flower were blooming as the first flowering times (FFlT) and the last flowering times (LFlT), respectively. We defined the time when 10% and 90% of fruit were borne as the first fruiting times (FFrT) and the last fruiting times (LFrT), respectively.

2.5. Data analysis

Three-way ANOVAs were employed to determine the effects of species, N addition and mowing on reproductive properties including FFlT, LFlT, FFrT, LFrT, and FlH. Two-way ANOVAs were performed to determine the effects of N addition and mowing on the species level reproductive properties including FFlT, LFlT, FFrT, LFrT, FlH, and the community level standing litter height (i.e., SLH). Once a significant effect was detected, *post hoc* LSD tests were used to further elucidate treatment differences. Where parametric tests