



Interactive effects of nitrogen and silicon addition on growth of five common plant species and structure of plant community in alpine meadow

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ABSTRACT

Great efforts have been made on the assessment of the effects of nitrogen (N) fertilization or silicon (Si) addition on plant growth and community composition. However, their interactive effects were not well documented. We here tested the effects of N addition (0, 70, 140 and 210 kg ha⁻¹ of N) on the leaf N content, growth, photosynthetic characteristics of five common species and structure of community (biomass, height, abundance and species richness) with or without Si (14.36 kg ha⁻¹) incorporation. Different species and whole community responded different. As compared to without Si, Si incorporation increased the net photosynthetic rate (*P_n*) and biomass of species *Elymus nutans* by 16.7 and 31.1%, *Kobresia capillifolia* by 10.4 and 11.1%, *Oxytropis kansuensis* by 42 and 18.0%, *Anemone rivularis* by 14.3 and 157%, *Potentilla fragarioides* by 12.5 and 91.7%, plant abundance and species richness of community by 6.2 and 11.8% under N addition of 210 kg ha⁻¹. The effect of Si supply on species richness was more efficient under low N addition than high N addition. Together, our results indicate that N fertilization incorporation with Si addition benefits plant growth for higher-photosynthetic activity of individual species and higher-species richness of community in alpine meadow.

1. Introduction

Nitrogen (N) is the primary nutrient that restricts plant growth in many natural environments (Aerts and Chapin, 2000; Koerselman and Meuleman, 1996), especially in alpine meadow, where the temperature and moisture limited plant productivity and nutrient cycling, which lead to a strong N addition in this area (Tamm, 1991; Nadelhoffer et al., 1992). Increasing N addition can affect ecosystem processes and function of communities, and change plant nitrogen use efficiency (Chapin et al., 2002; Lambers et al., 2008). Silicon (Si) is the second most abundant element in soil and it can enhance plant biomass production (Schaller et al., 2012; Schoelynck et al., 2010), improve plant leaf photosynthesis (Tamai and Ma, 2008) and nitrogen use efficiency (Detmann et al., 2012), and can alleviate N fertilization-induced biodiversity loss (Xu et al., 2015a). Silicon addition is expected to play a role in future grassland management.

The responses of plant community and individual plant species to N addition have been well documented. Nitrogen addition significantly increased aboveground biomass but decreased species richness and lead to changes in plant species composition of community (Clark and Tilman, 2008; Isbell et al., 2013). Species responded differently to

increased N availability. Sedges such as *Kobresia humilis*, *Scirpus distigmaticus* and *Carex atrofusca* had a competitive advantage over grasses such as *Elymus nutans*, *Festuca ovina* and *Koeleria cristata* in a nutrient-poor environment, but grasses had a competitive advantage over sedges when soil N was abundant (Jiang et al., 2012). Plant height is a major determinant of a species' ability to compete for light. Increase of inequality in plant heights under nutrient addition is evidence for increased competition for light and a concomitantly greater likelihood of competitive exclusion at community level (Givnish, 1982). Thus, N fertilization can significantly affect competitive capacity by increasing plant height.

On the Qinghai-Tibetan Plateau, alpine meadow is a widespread land type and the most important ecosystem in high altitude regions (Wang et al., 2012). However, alpine meadow vegetation is undergoing significant degradation mainly due to global climate change, overgrazing, rodents and fertilization, especially for N fertilization (Wen et al., 2010). However, to our knowledge, few studies have been conducted on the impacts of N fertilization with Si incorporation, though Si incorporation is becoming a major agronomic practice. Therefore, it is urgent to learn the integrated impacts of N fertilization and silicon incorporation on growth of individual plant and structure of plant

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community. Hence, we conducted an experiment on an alpine meadow community and focused on five common plant species to investigate how species from different functional groups and the community as a whole respond to different N addition with or without Si incorporation. Based on previous fertilization studies, we hypothesized that grasses and sedges species are generally favored by N addition, while legumes and forbs suffer (Niu et al., 2008; Ren et al., 2010), the interaction effect between N and Si are not only benefit for the common individual species, but also for the whole plant community.

2. Materials and methods

2.1. Study site

This study was conducted at the Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University (33°58'N, 101°53'E; 3500 m above sea level), in the east of the Qinghai-Tibetan Plateau, China. The annual precipitation is 620 mm and mean annual temperature is 1.2 °C. Soil was classified as an alpine meadow soil according to the Chinese soil classification and it is similar to a mollisol (soil taxonomy). Soil total nitrogen, total phosphorus, organic matter contents and pH values are 3.72 g kg⁻¹, 0.98 g kg⁻¹, 70.52 g kg⁻¹ and 6.33 respectively. The plant community represents a typical, diverse alpine meadow.

2.2. Experimental design

The experiment was laid out inside a fence to exclude yaks and other herbivores. Six experimental blocks of 16 m × 30 m were established in early May 2011. For each of the blocks, 8 plots of 5 m × 5 m with a 2 m buffer between the plots and the edge were established (48 plots in total over the entire experiment). Eight treatments consisted of four levels of N (0, 200, 400 and 600 kg ha⁻¹ NH₄NO₃, as 0, 70, 140 and 210 kg ha⁻¹ of N using NH₄NO₃) with or without Si incorporation (40 kg ha⁻¹ H₄SiO₄, as 14.36 kg ha⁻¹ of silicon using H₄SiO₄). These addition rates are commonly applied in the study area (Ren et al., 2010; Xu et al., 2015a). Nitrogen and Si fertilizers in solid were applied to the plot surfaces in early June from 2011 to 2013 on a rainy day to avoid the need for watering when rainfall was most abundant in this area. Three quadrats (0.5 m × 0.5 m each) were selected in each of the 40 plots: one quadrat were used for long-term vegetation survey, one was for plant height and the other one for determining aboveground biomass.

2.3. Vegetation survey and plant trait determination

We selected five common species that were present in all plots from each of four functional groups for more detailed analysis: *Kobresia capillifolia* C. B. Clarke for the sedges, *Elymus nutans* Griseb. for the grasses, *Oxytropis kansuensis* Bunge. for the legumes, *Anemone rivularis* Buch.–Ham. var. *flore-minore* and *Potentilla fragarioides* Linn. for the forbs. These species accounted for 30–65% of the total vegetation cover.

The vegetation survey in the quadrats was done in late August 2013, including species richness, plant abundance and height. The height of all plant species was measured three times in these quadrats. Among the other two quadrats per plot, one was randomly chosen to estimate aboveground biomass. In the harvested quadrats, all plants were clipped at the soil surface and separated into four functional groups: grasses, sedges, forbs and legumes (also the five species belong to one of the four functional groups). Biomass samples were put in marked paper bags separately, oven-dried at 70 °C for 48 h and weighed. The summed biomass of the four functional groups was used as an estimate of community aboveground biomass.

Net photosynthetic rate (P_n), transpiration rate (Tr) and stomatal conductance (gs) of the five selected species were measured on a fully

expanded young leaf (third leaf from top) on five plants of each species in each treatment from 9:00 to 11:00 on clear days at the end of July, with a Portable Photosynthetic System (Li-6400, Lincoln) photo-synthetically active radiation (PAR) = 1800 μmol·m⁻²·s⁻¹ and flow rate = 350 μmol·s⁻¹. Instantaneous water-use efficiency (WUEi) was defined as mmol of net CO₂ uptake per mol of H₂O lost and was calculated by dividing instantaneous values of P_n by Tr . Leaf area was determined with a portable area meter (Li-3000A, Lincoln), in order to calculate P_n and Tr per unit leaf area.

In each plot, leaves of at least 15–20 plant individuals of the five species were collected and dried at 70 °C for 48 h. Plant leaf total N concentrations were analyzed after samples were finely ground in a Wiley Mill and passed through a 40-mesh sieve, and determined with the Kjeldahl acid-digestion method using a flow auto analyzer (Bran and Luebbe GmbH, Germany).

2.4. Statistical analysis

All statistical analyses were performed using SPSS 13.0 for windows (SPSS Inc., Chicago, IL, USA). The effects of the fertilization treatment on the community and five species were tested by one-way analysis of variance (ANOVA) with least significant differences (LSD test) at $P < 0.05$. Two-way ANOVA was used to determine the interaction effects that are caused by N in combination with Si on the community and the five common species. If main effects or interactions were significant, we then proceeded with multiple comparison tests to compare differences among means using LSD test at $P < 0.05$.

3. Results

3.1. Leaf N concentration in the five species

In control condition, legume *O. kansuensis* had the highest leaf N concentration ($F = 497$, $P < 0.001$) compared to other four species. Except for *O. kansuensis* ($F = 0.184$, $P = 0.906$), plant leaf N concentration increased with increasing N addition rate (Fig. 1). Compared to without Si, Si incorporation significantly increased leaf N concentration by 12.4, 17.9, 10.4 and 11.9% in species of *K. capillifolia*, *E. nutans*, *A. rivularis* and *P. fragarioides*, respectively under N addition of 210 kg ha⁻¹. Two-way ANOVA revealed a significant interaction effect between N and Si on leaf N concentration in species of *K. capillifolia*, *E. nutans*, *A. rivularis* and *P. fragarioides* (Table 1).

3.2. Leaf gas exchange characteristics of the five species

With increasing N addition rate, P_n , gs and Tr of sedges *K. capillifolia* and grasses *E. nutans* increased significantly, while that of legume *O. kansuensis*, forbs *A. rivularis* and *P. fragarioides* decreased significantly. *K. capillifolia* and *E. nutans* had higher P_n , gs , Tr , and WUEi than *O. kansuensis*, *A. rivularis* and *P. fragarioides*. Addition Si alone increased P_n of all five species. The same pattern was also observed for WUEi of *K. capillifolia* ($F = 6.51$, $P = 0.029$) and *E. nutans* ($F = 10.6$, $P = 0.009$), but did not affect that of *O. kansuensis* ($F = 0.058$, $P = 0.841$), *A. rivularis* ($F = 2.77$, $P = 0.127$) and *P. fragarioides* ($F = 4.75$, $P = 0.054$) (Table 2). Leaf gas exchange characteristics of the five species were significantly associated with interaction of N × Si addition (Table 1). Compared to control, Si incorporation significantly increased P_n by 10.4, 16.7, 42.0, 14.3 and 12.5% in *K. capillifolia*, *E. nutans*, *O. kansuensis*, *A. rivularis* and *P. fragarioides*, respectively under N addition of 210 kg ha⁻¹ (Table 2).

3.3. Plant abundance, height and aboveground biomass of the five common species

The addition silicon alone significantly increased plant abundance of four species but not *K. capillifolia* compared to control. Plant

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