

Different inter-annual responses to availability and form of nitrogen explain species coexistence in an alpine meadow community after release from grazing

MING-HUA SONG^{*†}, FEI-HAI YU[‡], HUA OUYANG^{*}, GUANG-MIN CAO[§], XING-LIANG XU^{*} and JOHANNES H.C. CORNELISSEN[¶]

^{*}Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, 100101, China, [†]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China, [‡]College of Nature Conservation, Beijing Forestry University, Beijing, 100083, China, [§]Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 59 Xiguan Dajie, Xining, 810008, China, [¶]Systems Ecology, Dept. of Ecological Science, Faculty of Earth and Life Sciences, VU University, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands

Abstract

Plant species and functional groups in nitrogen (N) limited communities may coexist through strong eco-physiological niche differentiation, leading to idiosyncratic responses to multiple nutrition and disturbance regimes. Very little is known about how such responses depend on the availability of N in different chemical forms. Here we hypothesize that idiosyncratic year-to-year responses of plant functional groups to availability and form of nitrogen explain species coexistence in an alpine meadow community after release from grazing. We conducted a 6 year N addition experiment in an alpine meadow on the Tibetan Plateau released from grazing by livestock. The experimental design featured three N forms (ammonium, nitrate, and ammonium nitrate), crossed with three levels of N supply rates (0.375, 1.500 and 7.500 g N m⁻² yr⁻¹), with unfertilized treatments without and with light grazing as controls. All treatments showed increasing productivity and decreasing species richness after cessation of grazing and these responses were stronger at higher N rates. Although N forms did not affect aboveground biomass at community level, different functional groups did show different responses to N chemical form and supply rate and these responses varied from year to year. In support of our hypothesis, these idiosyncratic responses seemed to enable a substantial diversity and biomass of sedges, forbs, and legumes to still coexist with the increasingly productive grasses in the absence of grazing, at least at low and intermediate N availability regimes. This study provides direct field-based evidence in support of the hypothesis that idiosyncratic and annually varying responses to both N quantity and quality may be a key driver of community structure and species coexistence. This finding has important implications for the diversity and functioning of other ecosystems with spatial and temporal variation in available N quantity and quality as related to changing atmospheric N deposition, land-use, and climate-induced soil warming.

Keywords: aboveground biomass, alpine meadow, compensatory dynamics, long-term experiment, niche differentiation, species richness

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Introduction

Anthropogenic activities have greatly changed global nitrogen (N) cycling, and contributed profoundly to N enrichment (Vitousek *et al.*, 1997; Gruber & Galloway, 2008). Also, N mineralization in soils is expected to increase with soil temperature due to climate warming (Nadelhoffer *et al.*, 1990; Hobbie, 1996; Mack *et al.*, 2004). Increasing N availability can significantly affect plant species diversity and composition of communities (Stevens *et al.*, 2004; Hillebrand *et al.*, 2008), productiv-

ity (Wedin & Tilman, 1996; Gough *et al.*, 2000) and stability of ecosystems (Tilman & Downing, 1994; Tilman *et al.*, 2006; Grman, 2010). Worldwide, both increasing atmospheric N deposition and experimental addition of N resulted in an increase in nitrophilous species and a decrease in N-sensitive ones (Morecroft *et al.*, 1994; Lee & Caporn, 1998; Diekmann *et al.*, 1999; Suding *et al.*, 2005; Xia & Wan, 2008; Bobbink *et al.*, 2010).

The sensitivity of terrestrial ecosystems to N enrichment may also depend on the physical environmental conditions. In this regard, high-elevation mountain ecosystems are potentially sensitive to the increase in N availability, because climatic controls on decomposition greatly constrain the supply of N to plants (Aerts &

Correspondence: Fei-Hai Yu, tel. + 86 10 62336173, fax + 86 10 62336173, e-mail: feihaiyu@bjfu.edu.cn

Chapin, 2000). In such nutrient-poor ecosystems, plants exhibit inherently low resource uptake, tissue turnover, and growth rates (Aerts & Chapin, 2000), constraining changes in community biomass (Zhou, 2001). However, such ecosystems often contain species that differ in their capacity to respond to changes in resource availability (Soudzilovskaia *et al.*, 2005). As a result, changes in the composition of alpine plant species and functional groups may precede the changes in other properties such as community biomass when N is added (Bowman *et al.*, 2006). So far, very few long-term studies have examined the effects of N on alpine plant communities and ecosystems.

In contrast to the many studies that examined the effects of N supply rates on species diversity and composition of plant communities and ecosystem functioning (e.g., Bobbink *et al.*, 2002; Bowman *et al.*, 2006; Clark & Tilman, 2008; Xia & Wan, 2008; Bai *et al.*, 2010), little is known about the effects of different N chemical forms on plant species coexistence and ecosystem functioning during long-term external N supply (Stevens *et al.*, 2011). This is at odds with evidence that plant species co-occurring in N-limited communities differ greatly in the capacity to uptake N in different chemical forms from the soil (i.e., ammonium, nitrate, and free amino acids; Kielland, 1994; Näsholm *et al.*, 1998; McKane *et al.*, 2002; Miller & Bowman, 2002; Kahmen *et al.*, 2006). As a consequence, the abundance of a species in a given community may be strongly correlated with its capacity to use a specific N form (Miller *et al.*, 2007) or its plasticity in using different N forms (Ashton *et al.*, 2010). Thus, different availabilities of N in different chemical forms at fine spatial scales may provide additional niche space for different species, which may promote species coexistence and diversity, as was shown for arctic tundra (McKane *et al.*, 2002). Moreover, such niche diversity may vary from year to year, because annual differences in weather patterns may provide different niches of available N in different forms in a given place over time (Dunnett *et al.*, 1998).

Also, the effects of N chemical forms at different levels of organizational hierarchy, such as plant species, plant functional groups, and community levels, still need to be discovered. Whereas a meta-analysis found neither consistent overall effects of types of fertilizer nor N oxidation status (oxidized, reduced, or combined) on aboveground productivity in fertilized vs. control plots (Lebauer & Treseder, 2008), this does not exclude the possibility of strong effects on community biomass, composition, and diversity within particular ecosystems in different parts of the world. Finally, herbivory is another strong control on vegetation net productivity and species and functional type composition (Olf & Ritchie, 1998; McIntyre *et al.*, 2003), and grazing

regimes may interact with the community response to different N niches in space and time.

Here, we argue that the complexity of all these interacting environmental drivers may provide a large variety of niche space over spatial, temporal, and functional scales (cf. Lang *et al.*, 2009), which can correspondingly be exploited by a large diversity of species and functional types, each of which may show different ecophysiological or phenological responses to multiple nutrition (Pornon *et al.*, 2007) and disturbance regimes (Questad & Foster, 2008). This could be a mechanism for inhibiting dominance by a few strong generalist competitors for resources. Thus, we hypothesize that idiosyncratic year-to-year responses of different plant functional groups to availability and form of nitrogen are a mechanism promoting species coexistence and diversity. We test this hypothesis on the Tibetan Plateau, where we conducted a 6 year N addition experiment in an alpine meadow where net productivity is recovering from the removal of grazers. We employed a unique experimental factorial design to study the year-to-year effects of three N chemical forms and three N supply rates on vegetation composition and aboveground biomass.

Materials and methods

Experimental site

The Tibetan Plateau has an average altitude of more than 4,000 m asl., and covers about 2.5 million km², of which 35% is occupied by alpine meadows (Zheng, 2000). In these meadows, low temperature restricts decomposition and thus most N is bound in organic forms (Cao & Zhang, 2001), which greatly constrains the N supply to plants. It has been suggested the Tibetan Plateau is experiencing climatic warming (Thompson *et al.*, 1993) and the change in temperature in this area will be greater than the global average (Giorgi *et al.*, 2001). As a result, the rate of soil organic matter mineralization is expected to increase, leading to N enrichment in alpine meadow soils. In addition, increasing atmospheric nitrogen deposition could also enhance nitrogen availability in this region (Zhang *et al.*, 2008). Jiang (2010) measured the atmospheric wet N deposition through precipitation in the alpine meadow from May 2008 to May 2009, and the value was $0.46 \pm 0.03 \text{ g m}^{-2} \text{ yr}^{-1}$. However, the current atmospheric wet N deposition may be higher in this region because precipitation from May 2008 to May 2009 was lower than usual (Jiang, 2010). There is no data about atmospheric dry N deposition in this region, but it is expected that both atmospheric wet and dry N deposition will increase due to the increased impacts of anthropogenic activities (Jiang, 2010).

The experiment was carried out in an alpine meadow at the Haibei Alpine Meadow Ecosystem Research Station (37°37'N, 101°12'E, 3240 m asl.) located in the northeast of the Tibetan Plateau in Qinghai Province, China. Mean annual temperature

Table 1 Soil (upper 15 cm) and plant community properties at the study site in Haibei, Qinghai-Tibet Plateau. Mean \pm SE are shown ($n = 6-8$)

Variable	Value
(a) Soil properties	
pH	8.0 \pm 0.1
Bulk density (g cm ⁻³)	0.70 \pm 0.05
Water content (%)	24.0 \pm 1.3
Organic carbon (kg m ⁻²)	11.8 \pm 0.3
Total N (kg m ⁻²)	0.60 \pm 0.04
C:N	19.6 \pm 0.3
Microbial biomass N (g m ⁻²)	6.5 \pm 0.3
Dissolved organic nitrogen (g m ⁻²)	1.8 \pm 0.1
Extractable inorganic N (g m ⁻²)	1.4 \pm 0.4
Atmospheric wet N deposition (g m ⁻² yr ⁻¹) ¹	0.46 \pm 0.03
Symbiotic N ₂ -fixation rate by the legumes (g m ⁻² yr ⁻¹) ²	1.00 \pm 0.12
(b) Community properties	
Richness (in 1 \times 1 m plot)	24.3 \pm 1.7
Aboveground biomass (g m ⁻²)	274 \pm 10
Cover of grasses (%)	37 \pm 5
Cover of sedges (%)	14 \pm 2
Cover of legumes (%)	18 \pm 2
Cover of forbs (%)	31 \pm 4

¹From Jiang (2010).²From Yang *et al.* (2011).

(MAT) is -1.7 °C, and annual precipitation is 560 mm, 85% of which falls in the growing season (from May to September). The soil is classified as Mat Cryo-gelic Cambisols (Chinese Soil Taxonomy Research Group, 1995) corresponding to Gelic Cambisol (WRB, 1998) (Table 1).

In 2005, an area of 80 \times 60 m was marked out and half of the area was used for this experiment. The area has uniform vegetation and is within the permanent research area of the research station, which has not been fertilized since the foundation of the station in 1976. Since then this area has been lightly grazed by domestic animals only in winter. The dominant species are the perennial tussock sedge *Kobresia humilis* Serg., and the grasses *Elymus nutans* Griseb, *Stipa aliena* Keng. and *Festuca ovina* Linn.. The abundant species include *Poa* sp., *Saussurea superba* Anth., *Gentiana lawrencei* Burk. var *farreri* T. N. Ho, *Gentiana straminea* Maxim., *Potentilla nivea* Linn., *Potentilla saundersiana* Royle, *Scirpus distigmaticus* Tang et Wang, *Kobresia pygmaea* C.B. Clarke in Hook and *Carex* spp. (Table 1). This meadow is N limited, and the net N mineralization rate is -10.3 \pm 4.1 mg kg⁻¹ day⁻¹ during the plant growth peak in August (Zhou, 2001). Vegetation cover is over 95% (Table 1).

Experimental design

Within the experimental alpine meadow area, nine unfertilized plots on the outside were not fenced off and therefore continued to be exposed to light winter grazing by yak and sheep; this was referred to as winter light-grazed alpine

meadow treatment (LGM). An inner area was fenced off at the start of the fertilization experiment and thereby released from such grazing. Within the ungrazed area we established 30 plots of 2 \times 2 m, arranged in three rows (blocks) with ten plots each. Plots were 1 m apart. Iron sheets of 35 cm high were inserted along the four edges of the plots to a depth of 30 cm to separate the plots from the surrounding vegetation, with the other 5 cm above the soil surface, which was unlikely to hamper seed dispersal. The fertilization experiment had nine N treatments and an unfertilized control, with three replicate plots each. In the control treatment no N was added. The other nine treatments were a factorial combination of three N chemical forms and three levels of N rates. The three N forms were (1) ammonium-N (NH₄-N), (2) nitrate-N (NO₃-N), and both NH₄-N and NO₃-N, coded as Am, Ni, and AN, respectively. (NH₄)₂SO₄ was used for the Am treatment, NaNO₃ for the Ni treatment, and NH₄NO₃ for the AN treatment. The three N rates were 0.375, 1.500, and 7.500 g N m⁻² yr⁻¹, coded as LN (low), MN (medium), and HN (high), respectively. Thus, the ten treatments were AmL, AmM, AmH, NiL, NiM, NiH, ANL, ANM, ANH, and Control. The N was supplied twice a year during the growing season (on 10–15 July and 10–15 August) of 2006–2010, with half of the total annual N amount each. It was added only once with the total amount on 10 July 2005. The N was applied in aqueous solution and 5 L of solution was evenly sprayed into each plot. For the control plot, 5 L of water was supplied.

Sampling and measurements

A 1 \times 1 m quadrat was established in the center of each plot and used for sampling and measurements. Occurrence of each vascular species in each quadrat was recorded in the middle of August each year and species richness was then calculated. Cover% and height of each species were measured in August 2005, 2007, and 2010. For cover measurements, a 1 \times 1 m frame with 100 grid cells (10 \times 10 cm) was put above the canopy in each quadrat and cover of each species was visually estimated in each grid cell. Canopy height of each species was calculated as the average height of at least five randomly selected individuals. For biomass measurement, we clipped aboveground shoots within a 0.25 \times 0.25 m quadrat outside the central 1 \times 1 m quadrat but within the plot in the middle of August every year when biomass peaked. The quadrat for clipping shifted each year within the plot to avoid harvesting the same area in successive years. Shoots were clipped at ground level and sorted into four plant functional groups, i.e., grasses, sedges, legumes, and forbs. All shoots were oven-dried at 60 °C for 48 h, and then weighed.

In each plot a soil sample (15 cm in depth and 5 cm in diameter) was collected in early July, August, and September of 2010, respectively. After removing thick roots and stones by passing through a 2 mm mesh sieve, soil samples were stored in iceboxes and transferred to the laboratory. The fresh samples were extracted with 60 mL of 1 M KCl, and concentrations of available nitrate (NO₃) and ammonium (NH₄) were analyzed with FIASTAR 5000 (Dual Channel Soil Nitrogen Analyzer System, FOSS, Hoana, Sweden). The concentrations

of soil NO₃-N and NH₄-N were the averages of the samples from early July to early September for each treatment.

Statistical analysis

We used response ratio in experimental year 6 to indicate the response sensitivity of biomass and species richness to N addition. The response ratio of aboveground biomass (R_B) in year 6 was calculated as the ratio of aboveground biomass in N addition plots to that in the control plots, and the response ratio of species richness (R_S) in the sixth year of the experiment was the ratio of species richness in the N addition plots to that in the control plots. Similarly, the response ratio of soil available N (R_N) in the sixth year of the experiment was the ratio of soil available N in fertilized plots to that in the control plots.

We used repeated measures ANOVAS to examine the effects of N chemical form and N rate on species richness and aboveground biomass of the whole community as well as each of the four functional groups, with year as the repeated variable. In these analyses, the control treatment was not included. If significant effects of N form and/or N rate by year were found, then for each year Tukey's tests were used to compare the differences in species richness, aboveground biomass and soil available N content (NO₃-N and NH₄-N) among the three N form treatments. Similarly, if significant effects of N rate and/or N rate by year were found, then for each year Tukey's tests were used to compare the differences in species richness, aboveground biomass, and soil available N content (NO₃-N and NH₄-N) among the three N rate treatments.

We used regression analysis to investigate the relationship of biomass response ratio (R_B) and richness response ratio (R_S) with soil available N response ratio (R_N). All analyses were done using SPSS version 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

In all treatments within the fenced-off experimental area, aboveground biomass generally increased and species richness decreased upon the release from grazing between 2005 and 2010 (Figure S1 in supporting information, see Table 2 for the highly significant effects of year), while in the unfenced winter-grazed plots (LGM) biomass remained consistently low and species richness consistently high during this period (Figure S1).

Effects of N fertilization on aboveground biomass

As the overall effects of N chemical form and N supply rate were generally additive both for biomass and species richness (Table 2; slight interaction for richness of sedges being the only exception), we pooled the N form treatments for comparisons of N rate effects and *vice versa* to facilitate the interpretation of the results below (Figs 1 and 2). Neither N chemical

Table 2 Effects of N form, N rate, year and their interactions on (a) aboveground biomass and (b) species richness of communities and of each of the four plant functional groups

Effect	DF	Community	Plant functional group			
			Grasses	Sedges	Legumes	Forbs
(a) Aboveground biomass						
N form	2, 18	0.97 ^{ns}	0.05 ^{ns}	7.00 ^{**}	7.36 ^{**}	0.34 ^{ns}
N rate	2, 18	5.96 [*]	24.64 ^{***}	6.80 ^{**}	12.33 ^{***}	5.39 [*]
N form × N rate	4, 18	0.55 ^{ns}	0.71 ^{ns}	1.14 ^{ns}	1.76 ^{ns}	0.73 ^{ns}
Year	5, 90	125.75 ^{***}	79.11 ^{***}	68.51 ^{***}	32.43 ^{***}	35.59 ^{***}
Year × N form	10, 90	1.17 ^{ns}	2.45 [*]	2.30 [*]	1.25 ^{ns}	1.63 ^{ns}
Year × N rate	10, 90	0.76 ^{ns}	2.31 [*]	6.93 ^{***}	5.97 ^{***}	2.77 ^{**}
Year × N form × N rate	20, 90	1.69 [*]	1.44 ^{ns}	0.73 ^{ns}	1.87 [*]	1.43 ^{ns}
(b) Species richness						
N form	2, 18	3.55 ^{ns}	4.54 [*]	5.73 [*]	2.23 ^{ns}	0.55 ^{ns}
N rate	2, 18	12.65 ^{***}	4.54 [*]	0.42 ^{ns}	12.20 ^{***}	7.12 ^{**}
N form × N rate	4, 18	0.54 ^{ns}	1.91 ^{ns}	3.30 [*]	0.28 ^{ns}	0.28 ^{ns}
Year	5, 90	251.95 ^{***}	99.93 ^{***}	30.34 ^{***}	24.20 ^{***}	212.73 ^{***}
Year × N form	10, 90	4.51 ^{***}	1.22 ^{ns}	1.96 [*]	2.71 ^{**}	3.23 ^{**}
Year × N rate	10, 90	2.50 [*]	0.76 ^{ns}	1.75 ^{ns}	6.80 ^{***}	0.85 ^{ns}
Year × N form × N rate	20, 90	1.09 ^{ns}	1.60 ^{ns}	2.80 ^{**}	2.00 [*]	0.99 ^{ns}

F values and the significance levels are given.

*P < 0.05;

**P < 0.01;

***P < 0.001;

nsP ≥ 0.05.

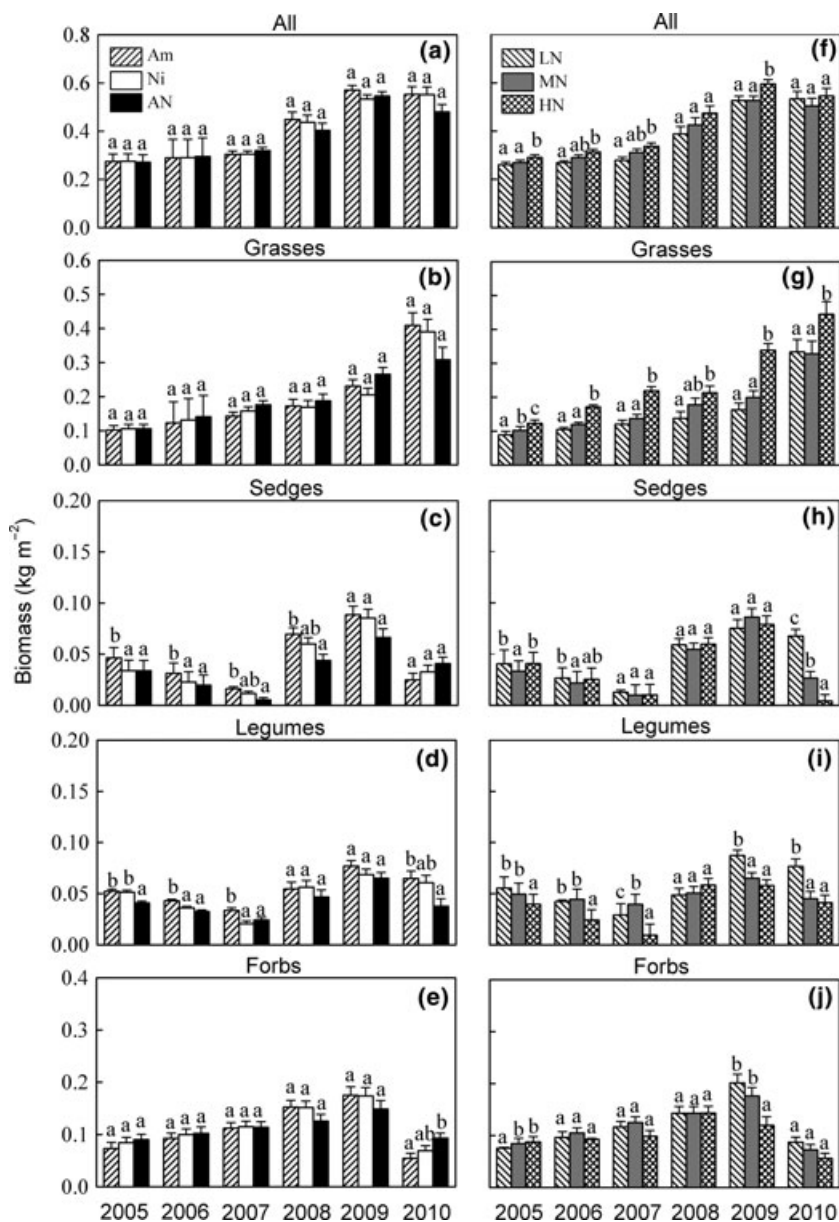


Fig. 1 Aboveground biomass (mean \pm SE) of the communities and of each of the four plant functional groups, i.e., grasses, sedges, legumes, and forbs in an alpine meadow communities from 2005 to 2010 under different N form (a–e) and N supply rate (f–j) treatments. Within the same year, bars sharing the same letters are not different at $P = 0.05$ (Tukey tests). Am represents addition of $\text{NH}_4\text{-N}$, Ni addition of $\text{NO}_3\text{-N}$, and AN addition of both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, L, M, and H represent low ($0.375 \text{ g N m}^{-2} \text{ yr}^{-1}$), medium ($1.500 \text{ g N m}^{-2} \text{ yr}^{-1}$), and high ($7.500 \text{ g N m}^{-2} \text{ yr}^{-1}$) N rate, respectively.

form nor the interaction of N form by year significantly affected aboveground biomass of the whole community (Table 2a), suggesting that N form did not change community aboveground biomass (Fig. 1a). But N form did affect the proportion of the total biomass contributed by the different plant functional groups and the effect of N form also depended on years (Table 2a). For instance, N form did not affect aboveground biomass of grasses (Fig. 1b), but it significantly affected aboveground biomass of sedges

from 2005 to 2008, with higher values in Am and lower values in Ni and AN, except at the end in 2009 and 2010 (Fig. 1c). There was also a significant effect of N form on aboveground biomass of legumes in 2005, 2006, 2007, and 2010, with higher values in Am and lower values in AN, but such differences were absent in 2008 and 2009 (Fig. 1d). Significant effects of N form on aboveground biomass of forbs only occurred in 2010, with lower value in Am and higher value in AN (Fig. 1e).

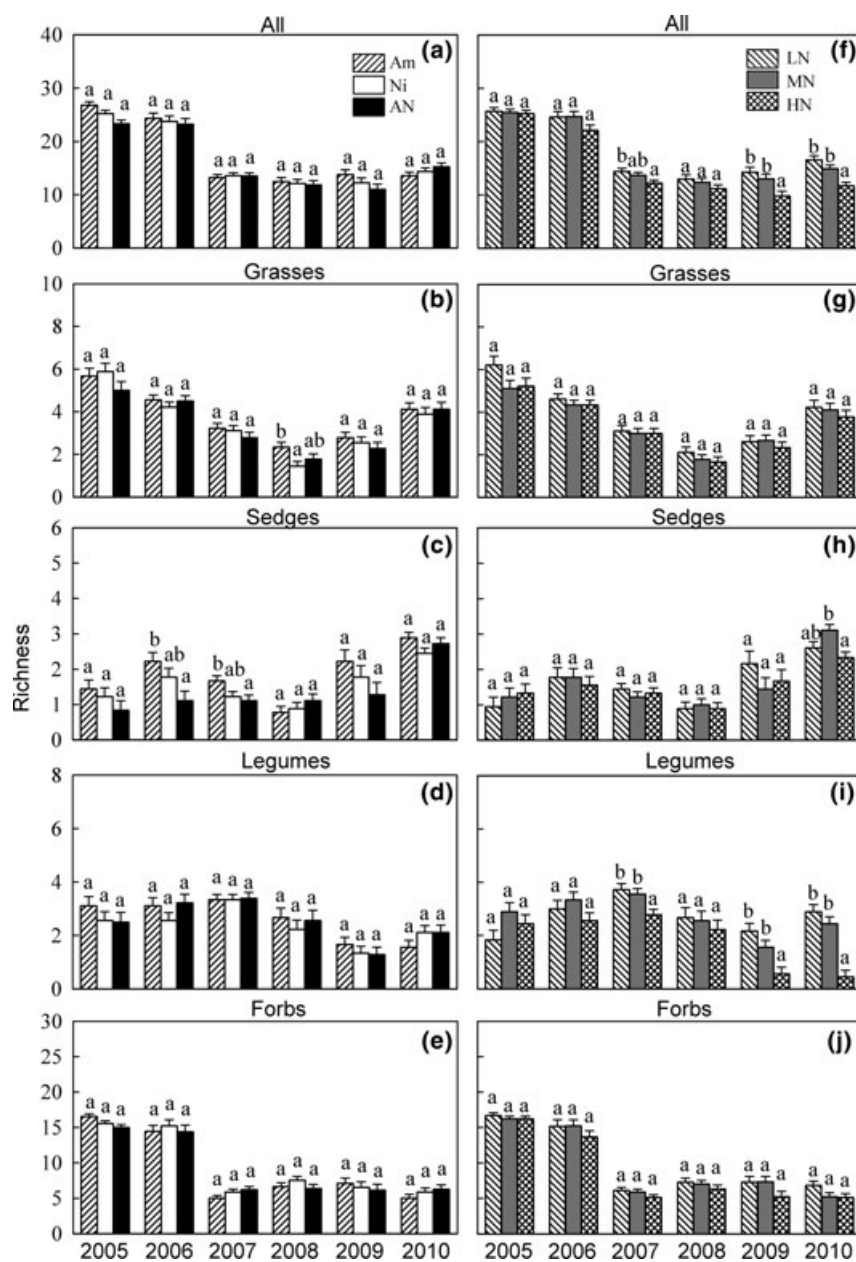


Fig. 2 Species richness (measured in 1 m² quadrats) of the communities and of each of the four plant functional groups, i.e., grasses, sedges, legumes, and forbs in an alpine meadow community from 2005 to 2010 under different N form (a–e) and N supply rate (f–j) treatments. Means and SE are given. Within the same year, bars sharing the same letters are not different at *P* = 0.05 (Tukey tests). Treatment codes are as in Fig. 1.

The N rate significantly affected community aboveground biomass (Table 2a), with higher values in HN and lower value in LN (Fig. 1f). N rate significantly affected aboveground biomass of all the four functional groups, but such effects varied greatly with years (Table 2). For instance, high N rate significantly increased aboveground biomass of grasses in all 6 years, but the amount of the effect differed among years (Fig. 1g). For sedges, significant differences in

aboveground biomass occurred in 2005, 2006, and 2010 (Fig. 1h), with higher value in LN, HN, and lower value in MN in 2005, and higher value in LN and lower value in MN in 2006, and higher value in LN and lower value in HN in 2010. N rate significantly affected aboveground biomass of legumes in all 6 years except 2008, and high N rates decreased their biomass (Fig. 1i). For forbs, significant differences in aboveground biomass occurred in 2005 and 2009 (Fig. 1j).

Effects of N fertilization on species richness

There was a significant, interactive effect of both N form \times year and N rate \times year on richness at the community level (Table 2b), suggesting that effects of both N form and N rate on community richness varied greatly with years. For instance, community richness was significantly higher in LN and lower in HN in 2007, 2009, and 2010, but did not differ among the three N rate treatments in 2005, 2006, and 2008 (Fig. 2f). There was a significant effect of N form \times year on richness of sedges, legumes, and forbs, and also a significant effect of N rate \times year on legumes (Table 2b). For grasses, a significant effect of N form occurred only in 2008, with higher richness in Am and lower in Ni (Fig. 2b), and for sedges a significant effect of N form on richness occurred only in 2006 and 2007 (Fig. 2c), with higher richness in Am and lower in AN. N rate significantly affected richness of legumes in 2007, 2009, and 2010, with higher richness in LN and MN and lower richness in HN (Fig. 2i).

Effects of N fertilization on soil available N

Both N forms and N rates significantly affected soil $\text{NO}_3\text{-N}$ ($F_{9,20}=2507$, $P < 0.001$) and $\text{NH}_4\text{-N}$ ($F_{9,20}=1033$, $P < 0.001$). Soil $\text{NO}_3\text{-N}$ was the highest in the AN and lowest in the Am treatment (Fig. 3a). In addition, soil $\text{NO}_3\text{-N}$ was significantly lower at low N rate, slightly

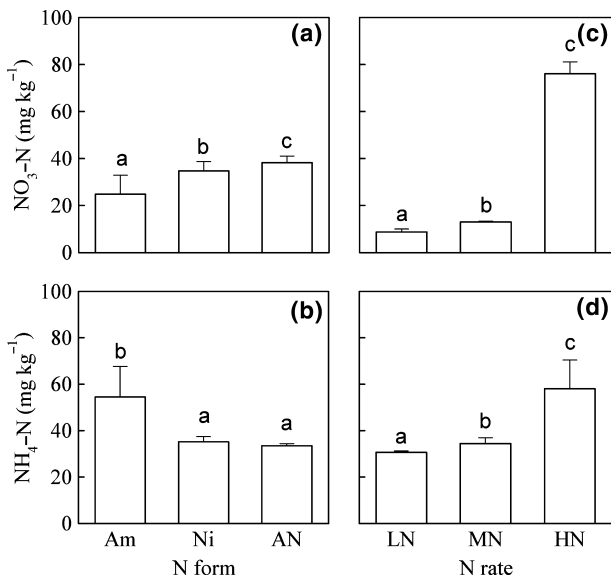


Fig. 3 Soil available $\text{NO}_3\text{-N}$ (a, c) and $\text{NH}_4\text{-N}$ (b, d) under different N form and N supply rate treatments, respectively, in 2010. Means and SE are given. Treatment codes are as in Fig. 1. Bars sharing the same letters are not different at $P = 0.05$ (Tukey tests).

higher at medium N rate, but markedly higher at high N rates (Fig. 3c). On the other hand, soil $\text{NH}_4\text{-N}$ was the highest in Am, but did not differ between Ni and AN (Fig. 3b). High N rates significantly increased soil $\text{NH}_4\text{-N}$ and such effects were more pronounced in AN (Fig. 3d).

Relationships of R_B and R_S with R_N

In the sixth year of the experiment, biomass response ratio (R_B) of communities was not significantly correlated with soil available N response ratio (R_N , Fig. 4a and b). However, richness response ratio (R_S) of communities was significantly negatively correlated with R_N of both soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ (Fig. 4c and d).

The R_B of grasses was significantly positively correlated with R_N of both soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ (Fig. 4e and f), but R_S of grasses was not significantly correlated with R_N (Fig. 4g and h). The R_B of sedges was negatively correlated with R_N of both soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ (Fig. 4i and j). The R_S of sedges was negatively correlated with R_N of soil $\text{NO}_3\text{-N}$, but not with R_N of $\text{NH}_4\text{-N}$ (Fig. 4k and l). The R_B of legumes was not significantly correlated with R_N (Fig. 4m and n), but their R_S was significantly negatively correlated with R_N of both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ (Fig. 4o and p). For forbs, neither R_B nor R_S was significantly correlated with R_N of $\text{NO}_3\text{-N}$ (Fig. 4q and s), but R_B was negatively correlated with R_N of $\text{NH}_4\text{-N}$, and their R_S tended to negatively correlate with R_N of $\text{NH}_4\text{-N}$ (Fig. 4r and t).

Discussion

Our study provides field-based evidence in support of the hypothesis that idiosyncratic and dynamic responses of different plant species and functional groups to nutritional regimes in terms of both N quantity and quality may be a key driver of community structure and species coexistence. Which are the main environmental factors and plant characteristics contributing to these responses?

Dynamic effects of N chemical forms on the performance of different functional groups

In our 6 year N addition experiment in the alpine meadow on the Tibetan Plateau different plant functional groups showed different growth responses to N chemical forms. In general sedges and legumes preferred ammonium addition, forbs preferred nitrate addition, but grasses did not show any preference as evidenced by their biomass response. However, the preference of sedges, legumes, and forbs to the specific N form occurred only in some years, while in other years the

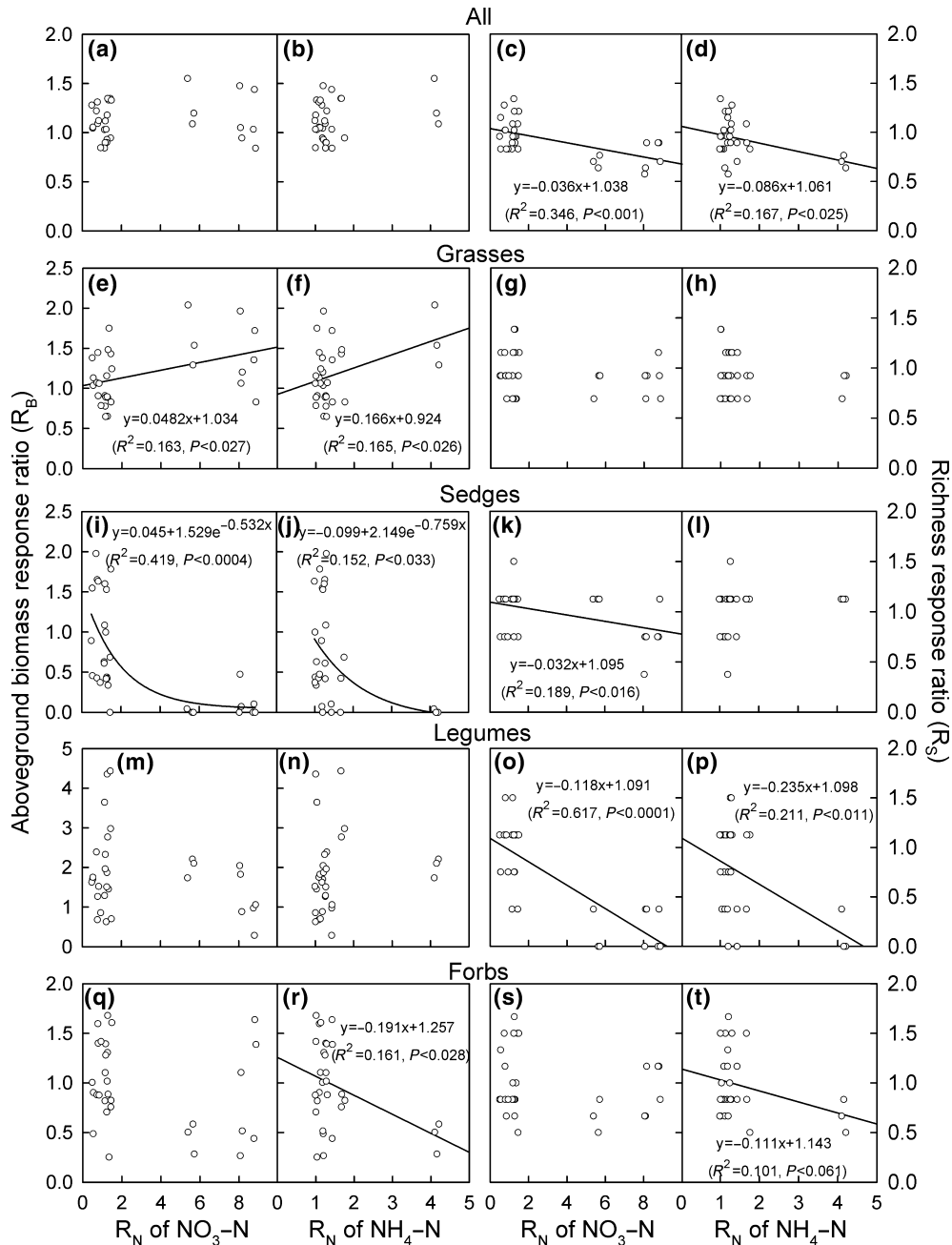


Fig. 4 Relationships of biomass and richness response ratios of communities (a–d) and of the four plant functional groups, i.e., grasses (e–h), sedges (i–l), legumes (m–p), and forbs (q–t), with response ratios of soil available $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$.

effects of N supply rates seemed to overrule the effects of N forms.

In alpine meadow, the high soil organic matter content immobilizes N in forms inaccessible to plants (Song *et al.*, 2007). As a result, plant growth is strongly limited by N. A short-term ^{15}N -tracer (a mixture of glycine-N, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ at a ratio of 1:1:1) experiment showed that the common dominant plant species in this meadow had different preferences for different

N chemical forms, and N chemical niche diversification was detected at this low N availability (Xu *et al.*, 2010). Similar niche differentiation was shown in tussock tundra at Toolik Lake, Alaska (Mckane *et al.*, 2002). Therefore, niche differentiation with respect to N form is an important mechanism in promoting the coexistence of the less abundant plant species, especially in nutrient limited habitats. However, N chemical forms did not affect aboveground biomass at community level. This is

because ammonium-N addition increased the aboveground biomass of sedges and legumes, but decreased aboveground biomass of forbs early on in the experiment. In addition, ammonium nitrate decreased aboveground biomass of legumes, but increased that of forbs by the end. The strong compensatory dynamics in biomass of the four plant functional groups contributed to the constancy in aboveground biomass of the community. In addition, although the response of sedges and grasses to ammonium addition occurred in specific years, N chemical forms did not affect species richness at community level, indicating a potential compensatory mechanism taking effect. For example, the annual *Euphrasia pectinata* and *Papaver kansuensis* and the biennial *Gentiana aristata* were common in certain years during the experiment, but in an irregular temporal pattern. However, the negative relationships of the response ratio for species richness (R_S) of sedges with that of nitrate availability (R_N of $\text{NO}_3\text{-N}$) or R_S of forbs with R_N of $\text{NH}_4\text{-N}$, if extrapolated tentatively, suggest that species richness may be decreased in future under continuous N enrichment. Great caution should be taken with external N supply in N-limited alpine meadows, as loss in dominant species of sedges will happen at high nitrate availability, and loss in nondominant forb species at high ammonium availability.

Dynamics of N rate effects on the performance of different functional groups

N addition significantly decreased species richness and increased aboveground biomass, but not drastically and only at the high supply rate ($7.5 \text{ g N m}^{-2} \text{ yr}^{-1}$). Relative to the unfertilized control (both after cessation of grazing) the average increase of 26% in aboveground biomass under high N rate treatment in 2009 in our experiment was slightly lower than the average growth response (an increase by 29%) in most ecosystems across the globe (Lebauer & Treseder, 2008) but was lower than the 35% growth stimulation in tundra or the 50% across seven North American herbaceous ecosystems (Gough *et al.*, 2000), and much higher than the 16% growth stimulation in wetlands (Lebauer & Treseder, 2008). At the high N supply rate species richness also declined steadily relative to unfertilized or very slightly fertilized plots; this adds to the extensive evidence across ecosystems worldwide that, beyond a critical N load, many species disappear either through direct negative effects of high N on their performance or through the expansion of species, often grasses, that capitalize on the high N supply and outcompete other species (Suding *et al.*, 2005; Lebauer & Treseder, 2008; Xia & Wan, 2008; Bobbink *et al.*, 2010). Correspondingly, the declines of sedge and

forb biomass in 2010 suggest a delayed response to asymmetric competition from the strong grass biomass expansion from 2009. In our experiment, some species were particularly sensitive to high N rate, e.g. *Gentiana squarrosa*, *Gentiana aristata*, *Anaphalis lactea*, *Astragalus lecentianus*, *Stellaria umbellata*, *Scirpus distigmaticus*, *Euphrasia pectinata*, and *Oxytropis ochrocephala*; these species disappeared in the high N addition plots during the early years of the experiment. Bowman *et al.* (2006) proposed that changes in plant diversity may be an early indication of alpine meadow productivity response to N. Therefore our results, especially the negative correlations between the response ratios of community species richness and soil N response ratios of both nitrate and ammonium (Fig. 4c and d), may well be a bellweather for more dramatic biomass increase and species richness decline still to come at high N input.

More surprising than the response to high N input is our finding that, even after 5 to 6 years of moderate fertilization ($1.5 \text{ g N m}^{-2} \text{ yr}^{-1}$), community biomass and species richness had not changed significantly compared to unfertilized or slightly fertilized ($0.375 \text{ g N m}^{-2} \text{ yr}^{-1}$) treatments; the grasses did not benefit from moderate fertilization at the expense of the other functional types. Similarly, N addition (2, 4, and $6 \text{ g N m}^{-2} \text{ yr}^{-1}$) to an alpine grassland in Colorado did not significantly change species richness of the vegetation although it increased the Shannon index of diversity (Bowman *et al.*, 2006). In our study, we found some variations in cover of individual species under low and medium levels of N additions. For instance, we registered an increase in cover of *Kobresia humilis*, *Elymus nutans*, *Poa pratensis*, *Carex pachyrrhiza*, and a decrease in cover of *Saussurea superba*, *Thalictrum alpinum*, *Gueldenstaedtia diversifolia*, and *Euphrasia pectinata*. One explanation for the lack of clear response to moderate fertilization may be that the physical environment on the Tibetan plateau exerts strong selection pressures on the component organisms, and most alpine plant species form prostrate rosettes, with low growth rates. The biotic capacity to uptake and use the increased N in growth may be relatively low. Moreover, there is strong competition for available N between plants and soil microbes, especially during the plant growth peak (Xu *et al.*, 2011). Although adding $1.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ did not significantly change community composition over 6 years, this does not exclude the possibility of long-term community shifts in response to cumulative N load. Also, while growth of alpine plants in many regions, including the Tibetan Plateau, may be limited principally by nitrogen alone without fertilization, nutritional constraints on growth may partly shift from N to P limitation after N fertilization (Sturner & Elser,

2002; Soudzilovskaia *et al.*, 2005; Elser *et al.*, 2007, 2009). Therefore, it would be of great interest to include P fertilization treatments in experimental designs with different fertilization treatments of N amounts and forms such as ours.

Similarly to the response to different N forms, the responses of different functional groups to different N supply rates varied greatly from year to year, both in terms of biomass and species richness. While grasses responded positively and sedges and legumes negatively to prolonged high input rates of N, and eventually somewhat to modest N input, the strength and sometimes even the direction of the responses varied from year to year. Also, for sedges and legumes this interaction between years and N supply rate in turn interacted with N form, together leading to the complex response patterns recorded over time (Figs 1 and 2).

Effects of climate on the performance of different functional groups

Mean temperature during the growing season and over the full year fluctuated very little between 2005 and 2010 (Figure S2a). However, precipitation during the growing season and over the full year varied considerably. For example, precipitation values during the growing season were lower in 2005 (400 mm) and 2008 (360 mm), respectively, and higher in 2006 (492 mm) (Figure S2b). The highest value of photosynthetically active radiation coincided with the lowest precipitation in 2008 (Figure S2c). With fluctuation of climate conditions from 2005 to 2010, aboveground biomass and species richness in LGM did not vary markedly overall, although aboveground biomass tended to be the lowest and species richness the highest in 2008 (Figure S1). However, N treatments (enclosure and N fertilization) affected patterns of biomass and richness differently over the 6 years, with significant increases in biomass from 2008 and declines in richness from 2007. Moreover, biomass of legumes and sedges increased under N treatments in 2008. The potential reason may be that the dip in precipitation and the rise in PAR in 2008 may have reduced N availability due to soil drought. The legumes are possibly less dependent on soil N due to their ability to fix N₂ in symbiotic association with rhizobia (Yang *et al.*, 2011), while the predominant sedges usually cope well with drought and low nitrogen availability (Zhou, 2001). This example indicates that climate conditions might interact with effects of N treatments on community richness and biomass. As our method of N application is not a true simulation of either wet or dry deposition, further investigation should reveal the real interactions

between climate conditions and N deposition and availability.

The most striking results were not so much the differential responses of plant functional groups to different chemical N forms, but how these different responses varied so strongly from year to year. Apparently, different weather conditions in different years determine the extent to which a certain plant species or functional groups may or may not benefit from input of different forms of N. In-depth study should reveal the extent to which such dynamic interactions also depend on fine-scale variation in soil texture, depth and pH (Grime, 1963; Fridley *et al.*, 2011), all of which could potentially influence the actual availability of these N forms to different plants. Indeed, more attention should be paid to the dynamic effects of N chemical niche differentiation in structuring the community, regulating species coexistence and ecosystem function under long-term N enrichment.

Effects of enclosure on the performance of different functional groups

Obviously, removal of grazers resulted in increase in grasses, such as *Elymus nutans*, *Stipa aliena*, and *Poa pratensis*, resulting in a significant increase in aboveground biomass of the community. However, some rare species disappeared rapidly, notably *Carex pachyrrhiza*, *Scirpus distigmaticus*, *Astragalus lecentianus*, *Aster alpinus*, *Ranunculus pulchellus*, *Ranunculus nephelogenes*, *Rhizoma notopterygii*, *Halerpestes tricuspis*, *Parnassia trinervis*, *Kobresia pygmaea*, *Swertia bimaculata*, and *Viola philippica*. The effects of enclosure on biomass and richness were greater than the effects of N addition (Figure S1). In the acid grassland experiment UKRE-ATE in Mid Wales, an increase in cover of grass species at the expense of bilberry was found following regular additions of N where grazing pressure was reduced (Carroll *et al.*, 2003; Emmett, 2007). However, in more heavily grazed paddocks, the effect of N addition was not apparent, indicating the dominant effect of grazing in some systems (Haines-Young *et al.*, 2003). It has been suggested that grazing is a strong force driving sustainment of *Kobresia humilis* meadow; upon removal of the grazers the dominant species of *K. humilis* would be replaced by grasses. Our findings strongly suggest that N form and N supply rate interact with both removal of grazers and year-to-year climatic variation to affect the biomass and richness of alpine meadow. Thus, a critical next step of our experiment and others in different ecosystems would be to discriminate the contributions of both grazing and climate to the effects of long-term fertilization with N in different forms.

Concluding remarks

The strong interactions of plant responses to chemical N form and/or N supply rate with year of treatment indicate that different functional groups will benefit from different availabilities of N in different forms depending on annual variation in environmental conditions. The negative relationships of R_S of sedges with R_N of nitrate-N or R_S of forbs with R_N of ammonium-N suggest the species composition and richness of alpine meadows such as the one studied here are sensitive to increasing N availability as well as to the form in which this N is available. However, at moderate to low N supply rates these ecosystems may still provide sufficient spatial and temporal heterogeneity for the sensitive species and functional types to find suitable microsites, where they can thrive in certain years when their preferred N form is more available and endure the years when the conditions enhance the relative competitiveness of e.g. certain grasses.

Our findings have important implications for the diversity and functioning of many other ecosystems with spatial and temporal variation in available N quantity and quality. Such variation can be related, for instance, to combinations of fine-scale variation in topography, soil texture, depth (and associated moisture regimes), soil pH, and changing atmospheric N deposition, land-use, and climate-induced soil warming (Fridley *et al.*, 2011; Stevens *et al.*, 2011). It is the complexity of all these interacting drivers of N supply rate and form at local scale that will together determine the species coexistence and diversity of N-limited communities.

A crucial next step will be to investigate the extent to which further opportunities for species coexistence are offered through (1) the stochasticity of seed influx (Bell, 2001; Hubbell, 2001) and spatio-temporal heterogeneity in regeneration niches (Thompson *et al.*, 1996; Harpole & Tilman, 2007; Questad & Foster, 2008) and (2) the interactions of the dynamic responses of different functional groups to N quality and quantity with their responses to moderate grazing, which we showed once more to be another critical driver of community biomass and species richness (Figure S1). All these interactions together could create even more niche heterogeneity in space and time for multiple plant species and functional groups to coexist.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Aboveground biomass (a, c) and species richness (b, d) in N fertilized (N rate: low N, medium N, high N and N form: Am, Ni and AN and control: Control) and winter light-grazed alpine meadow (LGM) during the years of 2005 through 2010. Bars indicate SE of the mean ($n = 9$ for LGM and N fertilization treatments except for control $n = 3$). LGM was lightly grazed by yak and sheep only in winter. N fertilization treatments include control ($0 \text{ g N m}^{-2} \text{ yr}^{-1}$), low ($0.375 \text{ g N m}^{-2} \text{ yr}^{-1}$), medium ($1.500 \text{ g N m}^{-2} \text{ yr}^{-1}$), and high ($7.500 \text{ g N m}^{-2} \text{ yr}^{-1}$) N supply rates within each of the three N forms (Am, Ni and AN) in the winter light-grazing alpine meadow after removal of the primary consumers (yak and sheep) by enclosure.

Figure S2. Temperature (a), precipitation (b), and photosynthetic active radiation (c) during the growing season (from May to October) and the full year (from January to December), respectively, in the 6 years of the experiment. The climatic data collected from Haibei meteorological station. The meteorological station is about 150 m away from our experimental area.

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