



Differential response to warming of the uptake of nitrogen by plant species in non-degraded and degraded alpine grasslands

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Abstract

Purpose Chemical niche differentiation and changes in the dominance of plant species have been proposed as mechanisms for the coexistence of different types of plants. We explored how dominant plant species take up ammonium (NH_4^+), nitrate (NO_3^-), and glycine under conditions of warming and soil degradation in alpine grasslands.

Materials and methods Open-top chambers were used to simulate warming in degraded and non-degraded plots in an alpine grassland ecosystem on the Tibetan Plateau. Plant species were selected in both non-degraded (*Kobresia pygmaea* and *Aster tataricus*) and degraded (*Aster tataricus* and *Chenopodium glaucum*) plots. Short-term ^{15}N -labeling experiments with NH_4^+ -N, NO_3^- -N, and glycine ($^{13}\text{C}^{15}\text{N}$ -glycine) were conducted in each of four subplots with water alone as the control.

Results and discussion All of the selected plant species took up both organic and inorganic forms of N in the *K. pygmaea* grassland. Warming increased the uptake of glycine-N by the dominant species, whereas the uptake of NH_4^+ -N by the non-dominant species increased in both non-degraded and degraded grasslands. Warming changed the N uptake preference of *A. tataricus* from NO_3^- -N to NH_4^+ -N in non-degraded grasslands, but did not change the preference of *A. tataricus* under degraded conditions. This suggests that degradation can affect the preference of different plant species for particular forms of N as a response to warming.

Conclusions Plants in *K. pygmaea* grasslands take up organic N, although inorganic N is the dominant form of N used. The effects of warming on the uptake of N by plants varied with species, the form of N, and the degradation of the grassland. Degradation modified the effect of warming on the preference of plants for different forms of N. Different plant species developed different patterns for the uptake of N in both non-degraded and degraded plots, which may facilitate their coexistence in alpine grasslands.

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1 Introduction

Plant growth is limited by the availability of nitrogen (N) in many terrestrial ecosystems (Vitousek and Howarth 1991; Näsholm et al. 1998). Many plant species take up both inorganic and organic forms of N, including NH_4^+ , NO_3^- , and free amino acids (Warren 2006; Jones et al. 2005; Näsholm et al. 2009; Mansson et al. 2014). Many studies have shown that free amino acids are an important source of N for plant growth in cold and wet environments where N mineralization rates are low, such as in arctic, boreal, and alpine grassland ecosystems (Vitousek and Howarth 1991; Miller and Bowman 2003; Jones et al. 2004; Xu et al. 2006; Gärdenäs et al. 2011). In such N-limited ecosystems, plant species use different forms of N as an important mechanism in maintaining the coexistence of different plant species and net primary production at the ecosystem level (McKane et al. 2002; Lin et al. 2011; Jiang et al. 2016). Clarifying the N uptake patterns of plant species in N-limited ecosystems will therefore be beneficial in understanding plant diversity and production.

Alpine grasslands are an important vegetation type on the Tibetan Plateau and cover about 35% of the surface area of the plateau. Large areas of alpine grassland have been degraded to varying degrees in the past few decades as a result of heavy grazing (Zhou et al. 2005; Zhao et al. 2009; Wang et al. 2012). This has led to a series of ecological consequences and has affected the livelihood of local farmers. Many studies have shown that degradation strongly decreases net primary production and plant species richness. Degradation changes the composition of species in the plant community (Wang et al. 2009), increases the distribution of annual plant species (Che et al. 2017; Cui et al. 2017), and decreases the sequestration and storage of carbon (C) and the availability of soil water in alpine grasslands (Babel et al. 2014). Such distinct differences in plant composition in degraded and non-degraded grasslands provide an opportunity to explore the mechanisms responsible for plant diversity in alpine grasslands. Given that N is a major nutrient limiting plant growth in this N-limited alpine grassland, we have proposed that the dominant plant species change their N uptake patterns under non-degraded and degraded conditions to maintain plant diversity (Jiang et al. 2017).

In addition to soil degradation, the Tibetan Plateau has experienced stronger climate warming than other regions. The average surface temperature of the Tibetan Plateau is expected to increase by 2 °C by 2050, which is higher and faster than the global mean value (Thompson et al. 2000; Kuang and Jiao 2016). A rapid increase in surface temperature can significantly accelerate the mineralization of soil organic

matter (Luo et al. 2010) and increase the availability of soil nutrients (Lin et al. 2011). At the same time, the form of N in soils (Kuster et al. 2016) and the supply of substrate may also change, which may affect the uptake of N by plants and the coexistence of different plant species. Temperature also affects the uptake of N by plants (Warren 2009), e.g., plants take up more glycine at low temperatures and more NO_3^- at warm temperatures because of low nitrogen mineralization rate resulting in low N availability (Warren 2009). Hydroponic experiments have shown that some plant species take up more NO_3^- than NH_4^+ and free amino acids under warming conditions (Chapin et al. 1986; Kuster et al. 2016). Other studies have suggested that the uptake of N by some plant species is insensitive to temperature (Henry and Jefferies 2003). These studies indicate that there is no general pattern between temperature and the uptake of different forms of N (Chapin et al. 1986). In this study, we hypothesize that the uptake of available N by plants is more sensitive to warming under degraded conditions than under non-degraded conditions because fast-growing plants are dominant in the degraded alpine meadow and often have rapid uptake rates (Wang et al. 2012).

To test these two hypotheses, we conducted an in situ ^{15}N -labeling experiment with warming and non-warming treatments in degraded and non-degraded alpine grasslands. Because glycine is a very common free amino acid in alpine soils (Jiang et al. 2016), we used glycine dual-labeled with both ^{13}C and ^{15}N to quantify the uptake of intact glycine. This experiment aimed to determine which form of N (NH_4^+ -N, NO_3^- -N, and glycine-N) is preferred by the dominant plant species in alpine grasslands and whether degradation and warming change the N preference of different plant species.

2 Materials and methods

2.1 Study site

The study was performed at the Nagqu Ecological and Environmental Observation and Research Station, Tibet, China (31° 17' N, 92° 06' E; 4501 m a.s.l.), located in the Nagqu river basin. The research station is at the center of the major area of distribution of *Kobresia pygmaea* on the Tibetan Plateau. The experimental site is characterized by a typical alpine grassland climate with strong solar radiation, long, cold winters, and short, cool summers. The mean annual air temperature is -2.1 °C, and the annual mean precipitation over the past 10 years is 406 mm. Most of the precipitation falls

between June and September. *K. pygmaea* is the dominant plant species in this area (Jiang et al. 2017). The soil is classified as an alpine grassland soil, corresponding to a stagnic Cambisol (IUSS Working Group WRB 2006). The soil contains 4% organic carbon, 0.34% total N, and consists of 36% sand (Li et al. 2016).

The alpine grasslands in this region have been degraded over the last 40 years, and annual plant species are present in the most severely degraded areas (Zhao 2011). The dominant species in degraded alpine grasslands is *Aster tataricus*, which has a canopy height of 2 cm and 50% coverage (Cui et al. 2017). The soil organic carbon, total N, and sand contents in the degraded alpine grasslands are about 2, 0.06, and 60%, respectively (Li et al. 2016).

Four open-top chambers were set up in degraded and non-degraded alpine grassland plots in May 2013 to study the influence of warming on the uptake of N by plants. Grazing animals have been excluded from the non-degraded plots for about 10 years. The open-top chambers were made of plastic that could transmit solar radiation and were cylindrical, 0.5 m high and 1.5 and 1.0 m in diameter at the base and top, respectively (Cui et al. 2017) (Fig. 1). Warming significantly increased the seasonal mean soil temperature by about 1.0 °C from May to September 2014 (Cui et al. 2017).

2.2 ^{15}N -labeling

Four subplots measuring 15 cm × 15 cm were established randomly in the warming and non-warming treatments in both degraded and non-degraded plots in August 2014. We selected two plant species (dominant and subordinate species) in each type of plot: *K. pygmaea* and *A. tataricus* in the non-degraded plots and *A. tataricus* and *Chenopodium glaucum* in the degraded plots. *A. tataricus* was the most common species in both non-degraded and degraded plots and was the dominant plant species in the degraded plots without *K. pygmaea*. Each of the four subplots was injected with water (with no added ^{15}N) as a control, $\text{NH}_4^+ \text{-}^{15}\text{N}$ [$(^{15}\text{NH}_4)_2\text{SO}_4$, 98.2%], $\text{NO}_3^- \text{-}^{15}\text{N}$ ($\text{Na}^{15}\text{NO}_3$, 98.2%), or glycine- ^{15}N ($^{13}\text{C}_2 \text{-}^{15}\text{N}$ -glycine, 99.98%). The labels were applied on 13 August 2014. The

amount of N was adjusted to 90 mg N L⁻¹ for each of the three solutions. When labeling, each subplot was divided into nine 3 cm × 3 cm squares, and 1 mL of N solution (or water for the control plots) was injected at 0–8 cm depth in the soil to give a homogeneous distribution of ^{15}N equal to 7.5 μg N g⁻¹ soil (Xu et al. 2011a). Plant above ground biomass was collected within 15 cm × 15 cm quadrat, and plant below ground biomass was collected within 15 cm × 15 cm × 10 cm quadrat.

2.3 Sampling and isotope analysis

Six hours after the injection of the ^{15}N tracer, the above- and below-ground parts of the plants in the plots were collected with scissors. The plant materials were partitioned into species. Soil samples at 0–10 cm depth (> 80% of roots are concentrated within this depth) were collected and immediately transported to the laboratory. The soil sample was mixed by hand, sieved to < 2 mm, and stored at -20 °C for the determination of the soil moisture content and total C and N. The plant roots were carefully removed from the soils, rinsed first with tap water and then for 3 min with 0.5 mmol L⁻¹ CaCl₂ solution and again with distilled water to remove any traces of soil absorbed on the surface. The above- and below-ground parts of the plants were dried at 65 °C for 48 h, weighed, and then ground to a fine powder using a ball mill (MM2, Fa. Retsch, Haan, Germany) to determine the total N and atom% ^{15}N using a Thermo Scientific Flash EA1112 Nitrogen and Carbon Analyzer with a ConFlo III continuous flow universal interface (MAT 253, Finnigan MAT, Germany).

2.4 Analysis and calculations

The ^{15}N atom% excess (APE) was calculated as the difference in atom% ^{15}N between the ^{15}N -labeled plots (atom% $^{15}\text{N}_{\text{labeled}}$) and the control plots (atom% $^{15}\text{N}_{\text{control}}$). The N uptake rate (μg N g⁻¹ dry weight root h⁻¹) was calculated by multiplying the N content (μg N g⁻¹ dry weight soil), the APE, and the total plant biomass (g m⁻²) and divided by the root mass (g m⁻²), time (h), and the ratio of $m_{\text{unlabelled}}$ to m_{labelled} , where m_{labelled} is the total mass (g m⁻²) of ^{15}N for each form of N injected per

Fig. 1 Open-top chambers in alpine grassland plots with and without degradation



plot and $m_{\text{unlabelled}}$ is the mass of the corresponding form of N already in the soil (Xu et al. 2011b):

$$\text{APE}(\%) = \text{Atom}\%_{\text{labeled}} - \text{Atom}\%_{\text{control}} \quad (1)$$

where APE (%) is the atom percent excess, $\text{Atom}\%_{\text{labeled}}$ is the atom% ^{15}N from the ^{15}N -labeled material, and $\text{Atom}\%_{\text{control}}$ is the atom% ^{15}N from the same material in the control treatment.

$$^{15}\text{N}_{\text{uptake}} (\text{mg m}^{-2}) = \frac{\text{total biomass} \times \text{N}_{\text{content}}\% \times \text{APE} \times 15}{\text{Atom}\%_{\text{labeled}} \times 15 + (100\% - \text{Atom}\%_{\text{labeled}}) \times 14} \quad (2)$$

where $^{15}\text{N}_{\text{uptake}}$ (mg m^{-2}) is the amount ^{15}N absorbed by plant at per unit area of soil. The total biomass (g m^{-2}) is the sum of the mass of the root and shoot biomass, and the $\text{N}_{\text{content}}\%$ is the content of N in the plant.

$$^{15}\text{N uptake rate} (\mu\text{g N g}^{-1} \text{ dry weight root h}^{-1}) = \frac{^{15}\text{N}_{\text{uptake}}}{\text{root biomass} \times \text{time}} \quad (3)$$

where ^{15}N is the uptake rate ($\mu\text{g N g}^{-1}$ dry weight root h^{-1}) by the plant roots from the soil, calculated by dividing the ^{15}N uptake (mg m^{-2}) by the root biomass (g m^{-2}) and the ^{15}N labeling time (h) (McKane et al. 2002; Xu et al. 2011b).

$$\text{Actual N uptake rate} (\mu\text{g N g}^{-1} \text{ dry weight root h}^{-1}) = \frac{^{15}\text{N uptake rate} \times \text{MN}}{^{15}\text{N added}} \quad (4)$$

where the actual N uptake rate ($\mu\text{g N g}^{-1}$ dry weight root h^{-1}) is the N uptake rate by plants per unit soil area and per unit dry root biomass. MN (g m^{-2}) is the amount of native NH_4^+ , NO_3^- , or glycine in the soil, and $^{15}\text{N added}$ (g m^{-2}) is the total amount of added ^{15}N - NO_3^- , ^{15}N - NH_4^+ , or ^{15}N -glycine (Xu et al. 2011b).

The plant relative biomass (%) was calculated by dividing the total plant community biomass by the plant species biomass. The plant N stock was calculated by multiplying the plant biomass (g m^{-2}) by the plant N concentration (%). The percentage of different N forms was calculated as the uptake of individual forms of N (NH_4^+ -N, NO_3^- -N, or glycine-N) divided by the total plant N uptake (Lin et al. 2011).

The regression of excess ^{13}C to ^{15}N of the plant material was used to conservatively estimate the fraction of intact amino acid uptake in the total uptake of amino acid N (Näsholm et al. 1998). We used the values of the atom percent and concentrations of C and N to calculate molar excess ^{13}C and ^{15}N . The molar excess ^{15}N and ^{13}C were calculated using Eqs. (5) and (6), which are based on the description given by Näsholm et al. (2000) and Warren and Adams (2007):

$$^{15}\text{N-excess} (\mu\text{mol g}^{-1} \text{ dry mass}) = \frac{\text{biomass} \times \text{N}_{\text{content}}\% \times (\text{Atom}\%_{\text{Gly-N}} - \text{Atom}\%_{\text{CK-N}})}{\text{Atom}\%_{\text{Gly-N}} \times 15 + (100\% - \text{Atom}\%_{\text{Gly-N}}) \times 14} \quad (5)$$

$$^{13}\text{C-excess} (\mu\text{mol g}^{-1} \text{ dry mass}) = \frac{\text{biomass} \times \text{C}_{\text{content}}\% \times (\text{Atom}\%_{\text{Gly-C}} - \text{Atom}\%_{\text{CK-C}})}{\text{Atom}\%_{\text{Gly-C}} \times 13 + (100\% - \text{Atom}\%_{\text{Gly-C}}) \times 12} \quad (6)$$

For the calculation of excess ^{15}N , the atomic standard of ^{15}N (N_2 0.3363 atom%) was used as the reference and the $\text{atom}\%_{\text{CK-N}}$ as the atomic standard for ^{15}N . The mean values of the abundance of ^{13}C in the ^{15}N -ammonium-labeled and ^{15}N -nitrate-labeled plants were used as references for the calculation of the excess ^{13}C (Näsholm et al. 2000; Warren and Adams 2007), which is the $\text{atom}\%_{\text{CK-C}}$. Intact amino acid N uptake was conservatively estimated using ^{15}N uptake rate \times the slope of the regression of excess ^{15}N to excess ^{13}C (Näsholm et al. 1998).

Two-way ANOVA was used to test the differences in warming and degradation on the community biomass and soil properties. One-way ANOVA followed by Duncan's multiple range test was used to examine separately the differences in biomass, N stock, and root-to-shoot ratios among the two species in the non-degraded and degraded plots. The effects of treatment on the rate of uptake of N were tested using linear mixed models with the plant species (*K. pygmaea* and *A. tataricus*), form of N (NH_4^+ -N, NO_3^- -N, and glycine-N), and warming (unwarmed and warmed) as the fixed factors in the non-degraded plots and with the plant species (*C. glaucum* and *A. tataricus*), form of N (NH_4^+ -N, NO_3^- -N, and glycine-N), warming (unwarmed and warmed) as fixed factors in the degraded plots. Duncan's new multiple range test was used for post hoc comparisons.

The data are expressed as the excess ^{13}C and ^{15}N with standard errors. Regression analysis for excess ^{15}N and ^{13}C by dual-labeled glycine was performed to calculate the fractions of intact amino acids in the uptake of amino acid N (Näsholm et al. 1998; Näsholm et al. 2000).

For all the ANOVAs, normality was checked with the Kolmogorov–Smirnov test, and the assumption of homogeneity of variances was checked using Levene's test. If the assumptions of normality and homogeneity of variances were not met, the data were log- or square-root-transformed prior to analysis. Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA), and the significance was considered at the $p < 0.05$ level.

3 Results

3.1 Effect of degradation and warming on soil and plant traits

Degradation significantly increased the bulk density of the soil by 44% and the soil pH by 6% (Table 1). Degradation significantly decreased the concentration of soil organic carbon (C),

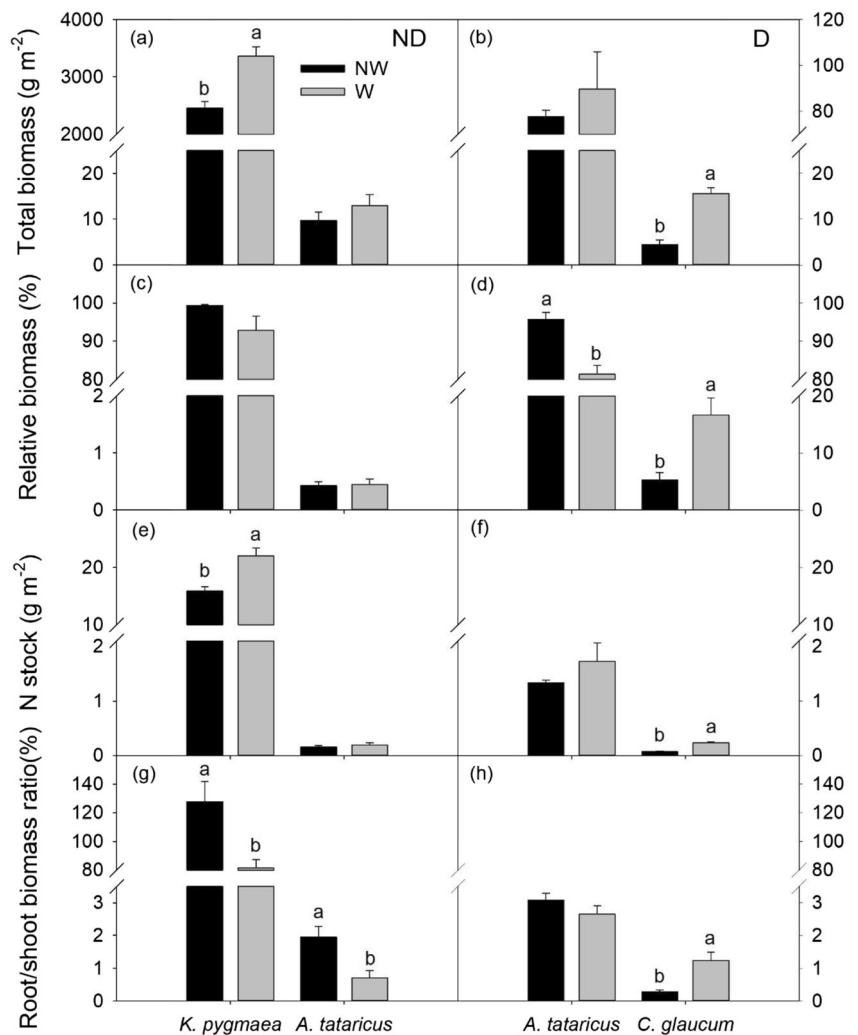
Table 1 Effects of degradation and warming on soil properties (0–10 cm) in an alpine meadow

	No degradation	Degradation
Bulk density (g cm ⁻³)	0.781 ± 0.041 ^b	1.392 ± 0.043 ^a
pH	6.609 ± 0.042 ^b	7.033 ± 0.034 ^a
Soil organic carbon (%)	4.170 ± 0.060 ^a	1.960 ± 0.050 ^b
Total nitrogen (%)	0.339 ± 0.008 ^a	0.061 ± 0.008 ^b
Ammonium (µg N g ⁻¹)	4.216 ± 0.537 ^a	0.755 ± 0.142 ^b
Nitrate (µg N g ⁻¹)	5.180 ± 1.576	7.943 ± 1.242
Glycine (µg N g ⁻¹)	0.200 ± 0.006 ^a	0.165 ± 0.003 ^b

Data are presented as mean ± SE values (*n* = 4). Different letters indicate significant differences at the 0.05 level. Lowercase letters show the difference between degraded and non-degraded plots

total nitrogen (N), ammonium, and glycine by 53, 82, 82, and 17%, respectively (Table 1). Degradation did not significantly affect the concentration of nitrate (Table 1; *p* > 0.05). Warming also did not significantly affect all the measured soil traits (Table 1; *p* > 0.05).

Fig. 2 Separate effects of warming on (a, b) plant species biomass, (c, d) relative biomass, (e, f) N stock, and (g, h) the root-to-shoot ratios in plots with and without degradation. Data are presented as mean ± SE values (*n* = 12). Letters above bars indicate a significant difference (*p* < 0.05) between plots with and without warming. Error bars represent the standard error. ND, no degradation; D, degradation; NW, no warming; W, warming



Degradation changed the composition and dominance of plant species. *K. pygmaea* was the dominant plant species in the non-degraded plot, and *A. tataricus* was a subdominant plant species. By contrast, *A. tataricus* was the dominant plant species, and *C. glaucum* was a subdominant plant species in the degraded plot (Fig. 2). Degradation significantly decreased the biomass of the plant community (*p* < 0.05). However, degradation significantly increased the total biomass, relative biomass, N stock, and root-to-shoot ratio of *A. tataricus* (Fig. 2; *p* < 0.05).

The response of the plant species traits to warming was dependent on the plant species identity. Warming significantly increased the total biomass and N stock of *K. pygmaea* and significantly decreased its root-to-shoot ratio, whereas warming significantly only decreased the root-to-shoot ratio of *A. tataricus* in the non-degraded plot (Fig. 2). Warming significantly decreased the relative biomass of *A. tataricus* and significantly increased the total biomass, relative biomass, N stock, and root-to-shoot ratio of *C. glaucum* in the degraded plot (Fig. 2).

3.2 Effect of degradation, warming, and form of N on the actual uptake of N by different plant species

There were significant interactions among the variables plant species, warming, and the form of N and their effect on the uptake of N by different plant species in both non-degraded and degraded plots. There was also a significant interaction between plant species and warming on the total uptake rate of N in both non-degraded and degraded plots (Tables 2 and 3). In the non-degraded plot, warming significantly decreased the uptake of ammonium and nitrate and increased the uptake of glycine by *K. pygmaea* (Fig. 3a). By contrast, warming significantly increased the uptake rate of ammonium and decreased the uptake rate of nitrate and glycine by *A. tataricus* in the non-degraded plot (Fig. 3c). The pattern for *A. tataricus* in the degraded plot is consistent with the response to warming of the dominant plant species (*K. pygmaea*) in the non-degraded plot (Fig. 3d). Warming significantly increased the uptake of ammonium and significantly decreased the uptake rate of nitrate and glycine for the subdominant plant species (*C. glaucum*) in the degraded plot (Fig. 3b). The pattern for the uptake of intact glycine was similar to that for ^{15}N -glycine (Fig. 3).

Ammonium and nitrate made up a significantly higher percentage of the total plant uptake of N for *K. pygmaea* in the non-degraded plot with no warming, whereas ammonium and glycine made up a higher percentage of the total plant N uptake under warming conditions (Fig. 4a). There was a significantly higher percentage of nitrate in the total plant uptake of N for *A. tataricus* with no warming and a higher percentage of ammonium in the total plant uptake of

N with warming (Fig. 4c). However, warming significantly changed the percentage of N in the total plant uptake of N by *A. tataricus* in the degraded plot (Fig. 4d). The same pattern was observed for the subdominant plant species (*C. glaucum*) in the degraded plot as for the subdominant plant species *A. tataricus* (Fig. 4b).

Warming significantly decreased the measured total N uptake rate in both non-degraded and degraded plots for all plant species (Fig. 5). The correlation between the ^{13}C and ^{15}N excesses was significant (Fig. 6). The carbon excess was 1.72 and 1.39 times the N excess for roots in the non-degraded (Fig. 6a) and degraded (Fig. 6b) plots, respectively.

4 Discussion

Using ^{13}C - and ^{15}N -labeled glycine, we showed that the uptake rates of intact glycine by plants varied from 0.20 to 38.83 $\mu\text{g N g h}^{-1}$. This confirms that all the alpine grassland plant species selected can directly take up low molecular weight substances (Xu et al. 2006; Jiang et al. 2017). Inorganic N was generally the prevailing form of N used by the plants in the *K. pygmaea* meadows. The preference of plants for particular forms of N is species-specific (Harrison et al. 2007). *A. tataricus* and *C. glaucum* in the degraded grasslands preferred NO_3^- -N over NH_4^+ -N and glycine-N. These findings show that plant preferences for the form of N is mediated by the dominance of the N form and the traits of the plant species (Miller et al. 2007; von Felten et al. 2009; Xu et al. 2011a, b; Cui et al. 2017). For example, the availability of soil nutrients can alter the relative abundance of N-

Table 2 Multifactorial analysis of variance testing the effects of species, warming and N form and their interactions on the N uptake rate and preferred form of N by different plant species in plots in an alpine meadow with and without degradation

Treatment	Effect	Degree of freedom	N uptake rate ($\mu\text{g g}^{-1} \text{h}^{-1}$)		N (%)	
			F	p	F	p
No degradation	Species	1	414.06	<0.001	0	1
	Warming	1	133.34	<0.001	0	1
	N form	2	231.97	<0.001	712.85	<0.001
	Species \times warming	1	0.73	0.401	0	1
	Species \times N form	2	101.02	<0.001	142.74	<0.001
	Warming \times N form	2	164.06	<0.001	495.15	<0.001
	Species \times warming \times N form	2	125.52	<0.001	90.3	<0.001
Degradation	Species	1	3.07	0.092	0.01	0.922
	Warming	1	97.92	<0.001	0.01	0.922
	N form	2	434.01	<0.001	1267	<0.001
	Species \times warming	1	0.29	0.594	0.01	0.922
	Species \times N form	2	109.02	<0.001	329.53	<0.001
	Warming \times N form	2	64.59	<0.001	33.06	<0.001
	Species \times warming \times N form	2	12.03	<0.001	48.41	<0.001

Table 3 Two-way analysis of variance testing the effects of species, warming, and their interactions on the uptake rate of total N of plant species in plots in an alpine meadow with and without degradation

Treatment	Effect	Degree of freedom	Total N uptake rate ($\mu\text{g g}^{-1} \text{h}^{-1}$)	
			F	p
No degradation	Species	1	300.99	< 0.001
	Warming	1	96.93	< 0.001
	Species \times warming	1	0.53	0.487
Degradation	Species	1	3.10	0.116
	Warming	1	98.87	< 0.001
	Species \times warming	1	0.29	0.602

exploitative versus N-conservative plants, with N-exploitative species predominating in N-rich environments and N-conservative species predominating in N-poor environments (Lhotsky et al. 2016; Maire et al. 2012; Xi et al. 2017). The key species *K. pygmaea* showed low root N uptake capacity than the other two species (Fig. 5) despite with high root mass. This indicates that *K. pygmaea* has N-conservative traits (Xu et al. 2004; Maire et al. 2009).

Previous studies have shown that warming can increase the uptake of N by plants (Warren 2009; Hou et al. 2018). In this work, we found that the effects of warming on the uptake of N by plants varied with the plant species, form of N, and the amount of degradation of the grassland. Warming significantly decreased the uptake rate of NH_4^+ -N and NO_3^- -N and increased the uptake rate of glycine by *K. pygmaea* in the non-degraded grasslands, whereas warming increased the uptake

rate of NH_4^+ -N and decreased the uptake rate of NO_3^- -N and glycine by *A. tataricus*. Warming significantly increased the uptake rate of NH_4^+ -N and decreased the uptake rate of NO_3^- -N and glycine by *C. glaucum* in the degraded grasslands, whereas warming decreased the uptake rate of NH_4^+ -N and NO_3^- -N and increased the uptake rate of glycine by *A. tataricus*. These results suggest that different plant species may develop different N uptake patterns to facilitate their co-existence under warming conditions in both non-degraded and degraded grasslands (McKane et al. 2002; Miller and Bowman 2003; Harrison et al. 2008; Kuzyakov and Xu 2013). A possible explanation is that *K. pygmaea* predominantly took up exchangeable NH_4^+ -N and glycine-N, whereas *A. tataricus* preferentially acquired NO_3^- -N to alleviate competition under warming conditions. When competition was alleviated in the degraded grasslands, *A. tataricus* continued

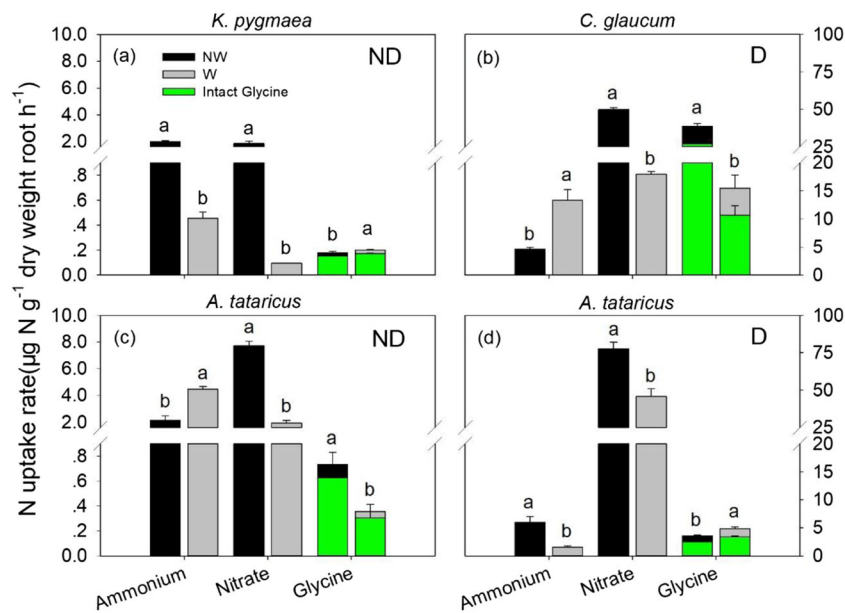
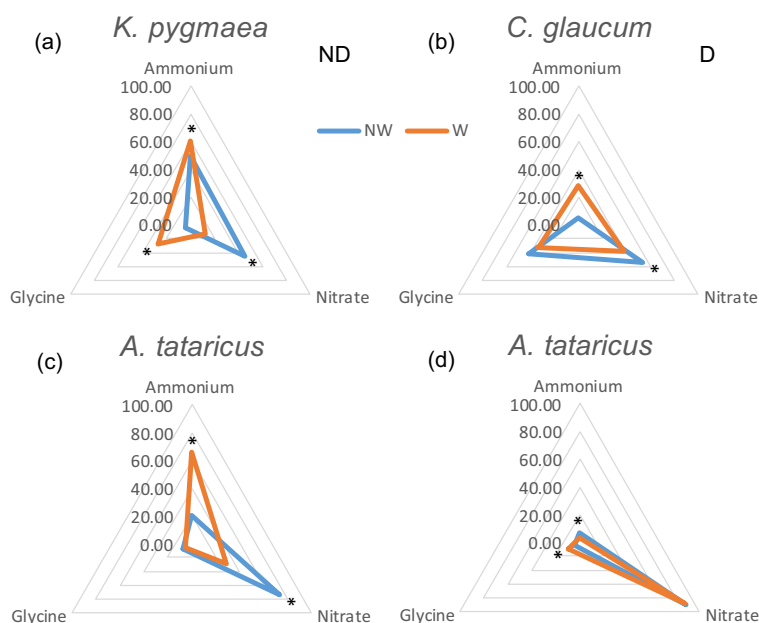


Fig. 3 Effects of the form of N and warming on the uptake rate of N by plant species in plots with and without degradation. Data are presented as mean \pm SE values ($n=4$). The intact glycine value was calculated using plant species mean N uptake rate multiplied by the slope of regression between excess ^{13}C and excess ^{15}N in roots of plants supplied with dual-labeled $^{13}\text{C}^{15}\text{N}$ -glycine. Letters above bars indicate a significant difference ($p < 0.05$) between plots

with and without warming. Error bars represent the standard error. ND, no degradation; D, degradation; NW, no warming; W, warming. The gray bar for glycine uptake refers to the total uptake of glycine by plants based on ^{15}N calculation. Green bar indicates the uptake of intact glycine based on combined ^{13}C and ^{15}N calculations which excluded the uptake of mineralized glycine from its total uptake

Fig. 4 Changes in the chemical niche for two dominant plant species with no warming and warming treatment in plots with and without degradation. The *x*, *y*, and *z* axes represent the contribution of ammonium, nitrate, and glycine to total N uptake (%). Asterisks indicate significant differences ($p < 0.05$) between the no warming and warming treatments. ND, no degradation; D, degradation; NW, no warming; W, warming



to use NO_3^- -N under warming conditions because of some alpine grassland species preferentially utilize NO_3^- -N (Miller et al. 2007; Jiang et al. 2016), whereas *C. glaucum* changed from NO_3^- -N to NH_4^+ -N uptake. Field observations have shown that *A. tataricus* gradually becomes dominant under degraded conditions (Jiang et al. 2016). When the grassland was degraded, *A. tataricus* becomes dominant and continued to prefer NO_3^- -N. This supports our first hypothesis that plant species change their patterns of N uptake under non-degraded and degraded conditions to maintain plant diversity.

The uptake of N by *K. pygmaea* and *A. tataricus* in non-degraded grasslands responded differently to warming depending on the species. *K. pygmaea* is a slow-growing species and captured more of the available N than the fast-growing *A. tataricus* species. At the same time, warming increased the total biomass and N stock of *K. pygmaea* and *C. glaucum* although it did not increase their total N uptake rate, meaning that plants under warming conditions can acquire more N

from soil through more growth. This indicates *K. pygmaea* and *C. glaucum* had a higher N utilization efficiency under warming conditions (Dorji et al. 2013).

In the non-degraded grasslands, warming changed the preference of *A. tataricus* from NO_3^- -N to NH_4^+ -N, but it did not change its N preference in degraded grasslands. This does not support our hypothesis that the uptake of N by plants is more sensitive to warming under degraded conditions than under non-degraded conditions. Degradation may modify the effect of warming on plant N preferences by changing the availability of N and the composition of plant species (Jiang et al. 2016). In a long-term warming experiment, plant species that increased their uptake of organic N under warming conditions had a relatively higher biomass (Jiang et al. 2018). The increase in uptake of organic N by *A. tataricus* under degraded conditions may reduce competition between *A. tataricus* and *C. glaucum* due to the uptake of more NH_4^+ -N by *C. glaucum*, as demonstrated by Kuster et al. (2016) in a greenhouse experiment.

Fig. 5 Effects of warming and degradation on rate of uptake of total N for different plant species. Data are presented as mean \pm SE values ($n = 4$). The total N uptake rate is the sum of the uptake rate of the three forms of N. Different letters indicate significant differences at the 0.05 level. Error bars represent standard error. ND, no degradation; D, degradation; NW, no warming; W, warming

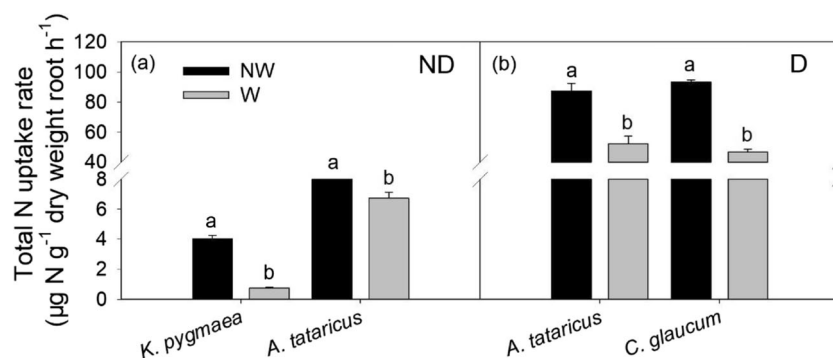
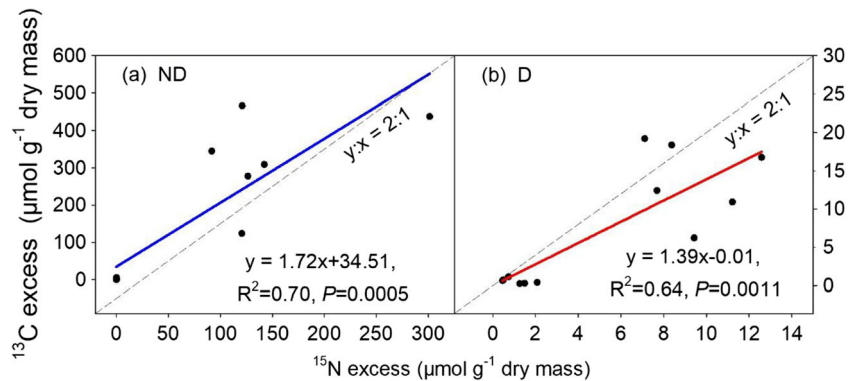


Fig. 6 Relationship between excess ^{13}C and excess ^{15}N in roots of plants labeled with $^{13}\text{C}^{15}\text{N}$ -glycine in soils (a) without and (b) with degradation. Each symbol represents one sample. The regression of the ^{13}C excess on the ^{15}N excess of glycine-treated plants is shown. The broken lines show the molar $^{13}\text{C}^{15}\text{N}$ ratios for the nitrogen sources injected (glycine 2:1). ND, no degradation; D, degradation



In addition to competition, warming may indirectly affect the pattern of N uptake by altering other environmental factors. Plant N uptake is influenced by soil abiotic factors, such as moisture, pH, temperature, and the availability of soil N (Xu et al. 2011b; Britto and Kronzucker 2013). Xu et al. (2011a) found that two alpine plant species, *Gueldenstaedtia diversifolia* and *Gentiana straminea*, switched from $\text{NH}_4^+\text{-N}$ / $\text{NO}_3^-\text{-N}$ uptake when the levels of available N were low to glycine uptake when the levels of available N were high. In the current study, degradation decreased the concentrations of $\text{NH}_4^+\text{-N}$ and glycine-N, but had no effect on the concentration of $\text{NO}_3^-\text{-N}$. A similar trend was observed between the concentration of N available in the soil and the uptake rates of different forms of N by *A. tataricus*. This may be a reason for the observed changes in the preference of plants for different types of N.

The main limitation of this study is maybe underestimating the N uptake rate of $\text{NH}_4^+\text{-N}$ and glycine-N. In this experiment, although the study period is short, ammonium may be transformed to nitrate and this transformation could underestimate the value of the N uptake from $\text{NH}_4^+\text{-N}$ label. In addition, the decomposition of glycine to compound serine in plants through 2 mol of glycine are transferred to 1 mol of serine, 1 mol of ammonium, and 1 mol of CO_2 . The CO_2 is lost to the atmosphere. Hence, the glycine metabolism is probably underestimating the value of the N uptake from glycine label.

5 Conclusions

Our results showed that warming can alter alpine plant N uptake rates, but such warming effect is modified by grassland degradation. Two dominant plant species (*K. pygmaea* and *A. tataricus*) increased their organic N uptake in non-degraded or degraded grasslands. This implies that plants can maintain their dominance through using more organic N under future warming conditions. Degradation changes plant species composition but it does not alter the N uptake pattern for the same species *A. tataricus*. This indicates that some dominant plant species in alpine grasslands have the flexibility to take up organic and inorganic N while other species can

remain stable under warming or/and degradation conditions. These findings suggest that the forms and amount of N in soil and plant species should be taken into account for grassland restoration under warming and degradation conditions. It will be beneficial to grassland restoration to reseed those plant species which can prefer NO_3^- and free amino acids.

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References

- Babel W, Biermann T, Coners H, Falge E, Seeber E, Ingrisch J, Schleuß P-M, Gerken T, Leonbacher J, Leipold T, Willinghöfer S, Schützenmeister K, Shibistova O, Becker L, Hafner S, Spielvogel S, Xu X, Sun Y, Zhang L, Yang Y, Ma Y, Wesche K, Graf H, Leuschner C, Guggenberger G, Kuzyakov Y, Miede G, Foken T (2014) Pasture degradation modifies the water and carbon cycles of the Tibetan highlands. *Biogeosciences* 11:6633–6656
- Britto DT, Kronzucker HJ (2013) Ecological significance and complexity of N-source preference in plants. *Ann Bot-London* 112:957–963
- Chapin FSIII, McKendrick JD, Johnson DA (1986) Seasonal changes in carbon fractions in Alaskan tundra plants of differing growth form: implications for herbivores. *J Ecol* 74:707–731
- Che R, Wang F, Wang W, Zhang J, Zhao X, Rui Y, Xu Z, Wang Y, Hao Y, Cui X (2017) Increase in ammonia-oxidizing microbe abundance during degradation of alpine meadows may lead to greater soil nitrogen loss. *Biogeochemistry* 136:341–352
- Cui S, Meng F, Suonan J, Wang Q, Li B, Liu P, Renzeng W, Lv W, Jiang L, Zhang L, Li X, Li Y, Zhang Z, Luo C, Tsechoe D, Wang S (2017) Responses of phenology and seed production of annual *Koenigia islandica* to warming in a desertified alpine meadow. *Agric For Meteorol* 247:376–384
- Dorji T, Totland O, Moe SR, Hopping KA, Pan JB, Klein JA (2013) Plant functional traits mediate reproductive phenology and success in response to experimental warming and snow addition in Tibet. *Glob Change Biol* 19:459–472
- Gärdenäs AI, Ågren GI, Bird JA, Clarholm M, Hallin S, Ineson P, Kätterer T, Knicker H, Nilsson SI, Näsholm T (2011) Knowledge

- gaps in soil carbon and nitrogen interactions—from molecular to global scale. *Soil Biol Biochem* 43:702–717
- Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* 88:989–999
- Harrison KA, Bol R, Bardgett RD (2008) Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? *Soil Biol Biochem* 40:228–237
- Henry HA, Jefferies RL (2003) Plant amino acid uptake, soluble N turnover and microbial N capture in soils of a grazed Arctic salt marsh. *J Ecol* 91:627–636
- Hou R, Ouyang Z, Xu X (2018) Warming enhances N uptake by winter wheat. *Soil Till Res* 180:116–125
- Jiang L, Wang S, Pang Z, Wang C, Kardol P, Zhou X, Rui Y, Lan Z, Wang Y, Xu X (2016) Grazing modifies inorganic and organic nitrogen uptake by coexisting plant species in alpine grassland. *Biol Fertil Soils* 52:211–221
- Jiang L, Wang S, Pang Z, Wang C, Kardol P, Zhong L, Yu Q, Lan Z, Wang Y, Xu X, Kuzyakov Y, Luo C, Zhang Z, Jones DL (2017) Effects of grazing on the acquisition of nitrogen by plants and microorganisms in an alpine grassland on the Tibetan plateau. *Plant Soil* 416:297–308
- Jiang L, Wang S, Pang Z, Xu X, Kardol P, Li Y, Zhang L, Wang Y, Zhong L, Lan Z, Hill PW, Zhang Z, Luo C, Rui Y, Dong N, Jones DL (2018) Plant organic N uptake maintains species dominance under long-term warming. *Plant Soil* 433:243–255
- Jones DL, Farrar JF, Willett VB (2004) Dissolved organic nitrogen concentration in two grassland soils. Proceedings of the 12th Nitrogen Workshop: Controlling Nitrogen Flows and Losses Wageningen Academic Publishers, Wageningen, the Netherlands, p 441–443
- Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biol Biochem* 37:413–423
- Kuang X, Jiao J (2016) Review on climate change on the Tibetan Plateau during the last half century. *J Geophys Res-Atmos* 121:3979–4007
- Kuster TM, Wilkinson A, Hill PW, Jones DL, Bardgett RD (2016) Warming alters competition for organic and inorganic nitrogen between co-existing grassland plant species. *Plant and Soil* 406(1-2): 117–129
- Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytol* 198:656–669
- Lhotsky B, Kovács B, Ónodi G, Csécséris A, Rédei T, Lengyel A, Kertész M, Botta-Dukát Z (2016) Changes in assembly rules along a stress gradient from open dry grasslands to wetlands. *J Ecol* 104:507–517
- Li Y, Wang S, Jiang L, Zhang L, Cui S, Meng F, Wang Q, Li X, Zhou Y (2016) Changes of soil microbial community under different degraded gradients of alpine meadow. *Agric Ecosyst Environ* 222: 213–222
- Lin X, Zhang Z, Wang S, Hu Y, Xu G, Luo C, Chang X, Duan J, Lin Q, Xu B (2011) Response of ecosystem respiration to warming and grazing during the growing seasons in the alpine meadow on the Tibetan plateau. *Agric For Meteorol* 151:792–802
- Luo C, Xu G, Chao Z, Wang S, Lin X, Hu Y, Zhang Z, Duan J, Chang X, Su A, Li Y, Zhao X, Du M, Tang Y, Kimball B (2010) Effect of warming and grazing on litter mass loss and temperature sensitivity of litter and dung mass loss on the Tibetan plateau. *Glob Chang Biol* 16:1606–1617
- Maire V, Gross N, Pontes LDS, Picon-Cochard C, Soussana JF (2009) Trade-off between root nitrogen acquisition and shoot nitrogen utilization across 13 co-occurring pasture grass species. *Funct Ecol* 23(4):668–679
- Maire V, Gross N, Börger L, Proulx R, Wirth C, Pontes LS, Soussana JF, Louault F (2012) Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytol* 196:497–509
- Mansson KF, Olsson MO, Falkengren-Grerup U, Bengtsson G (2014) Soil moisture variations affect short-term plant-microbial competition for ammonium, glycine, and glutamate. *Ecol Evol* 4:1061–1072
- McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA, Murray G (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* 415:68–71
- Miller AE, Bowman WD (2003) Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. *Plant Soil* 250:283–292
- Miller AE, Bowman WD, Suding KN (2007) Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology* 88:1832–1840
- Näsholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. *Nature* 392:914–916
- Näsholm T, Huss-Danell K, Hogberg P (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. *Ecology* 81:1155–1161
- Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. *New Phytol* 182:31–48
- Thompson LG, Yao T, Mosley-Thompson E, Davis ME, Henderson KA, Lin P (2000) A high-resolution millennial record of the South Asian monsoon from Himalayan ice cores. *Science* 289:1916–1919
- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13:87–115
- von Felten S, Hector A, Buchmann N, Niklaus PA, Schmid B, Scherer-Lorenzen M (2009) Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. *Ecology* 90:1389–1399
- Wang C, Long R, Wang Q, Jing Z, Shi J (2009) Changes in plant diversity, biomass and soil C, in alpine meadows at different degradation stages in the headwater region of three rivers, China. *Land Degrad Dev* 20:187–198
- Wang S, Duan J, Xu G, Wang Y, Zhang Z, Rui Y, Luo C, Xu B, Zhu X, Chang X, Cui X, Niu H, Zhao X, Wang W (2012) Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow. *Ecology* 93:2365–2376
- Warren CR (2006) Potential organic and inorganic N uptake by six Eucalyptus species. *Funct Plant Biol* 33:653–660
- Warren CR (2009) Why does temperature affect relative uptake rates of nitrate, ammonium and glycine: a test with Eucalyptus pauciflora. *Soil Biol Biochem* 41:778–784
- Warren CR, Adams PR (2007) Uptake of nitrate, ammonium and glycine by plants of Tasmanian wet eucalypt forests. *Tree Physiol* 27:413–419
- Xi N, Zhu B, Zhang D (2017) Contrasting grass nitrogen strategies reflect interspecific trade-offs between nitrogen acquisition and use in a semi-arid temperate grassland. *Plant Soil* 418:267–276
- Xu X, Ouyang H, Cao G, Pei Z, Zhou C (2004) Uptake of organic nitrogen by eight dominant plant species in *Kobresia* meadows. *Nutr Cycl Agroecosyst* 69:5–10
- Xu X, Ouyang H, Kuzyakov Y, Richter A, Wanek W (2006) Significance of organic nitrogen acquisition for dominant plant species in an alpine meadow on the Tibet plateau, China. *Plant Soil* 285:221–231
- Xu X, Ouyang H, Richter A, Wanek W, Cao G, Kuzyakov Y (2011a) Spatio-temporal variations determine plant-microbe competition for inorganic nitrogen in an alpine meadow. *J Ecol* 99:563–571
- Xu X, Ouyang H, Cao G, Richter A, Wanek W, Kuzyakov Y (2011b) Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow. *Plant Soil* 341:495–504
- Zhao X (ed) (2011) Restoration of degraded alpine meadow ecosystem and sustainable use on the three Rivers region. Science Press, Beijing
- Zhao X, Cao G, Li Y (2009) Alpine meadow ecosystem and global change, vol 169. Science Press, Beijing, pp 219–221
- Zhou H, Zhao X, Tang Y, Gu S, Zhou L (2005) Alpine grassland degradation and its control in the source region of the Yangtze and Yellow Rivers, China. *Grassl Sci* 51:191–203