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REGULAR ARTICLE

Plant organic N uptake maintains species dominance under long-term warming

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Abstract
Background and aims There is ample experimental evidence for shifts in plant community composition under climate warming. To date, however, the underlying mechanisms driving these compositional shifts remain poorly understood.
Methods The amount and form of nitrogen (N) available to plants are among the primary factors limiting

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productivity and plant coexistence in terrestrial ecosystems. We conducted a short-term ^{15}N tracer experiment in a ten-year warming and grazing experiment in an alpine grassland to investigate the effects of warming and grazing on plant uptake of NO_3^--N, NH_4^+-N, and glycine-N. Four dominant plant species (*Kobresia humilis*, *Potentilla anseria*, *Elymus nutans*, *Poa annua*) were selected. *Results* We found that 10-years of warming decreased plant uptake of inorganic N by up to 80% in all species. In contrast, warming increased the uptake of organic N in *K. humilis*, *P. anseria*, and *E. nutans* but not in *P. annua*. Results showed that plant relative biomass increased hyperbolically with the ratio of the plant species total uptake of available N and plant community uptake of available N. And a significant positive correlation between plant species uptake of soil glycine-N and the uptake of total available N.

Conclusions The stable relative biomass of plant species is largely dependent on organic N uptake by plants. We conclude that plant organic N uptake maintains species dominance under long-term warming.

Keywords Plant N uptake · Alpine grassland · The Tibet plateau · Plant species coexistence

Introduction

Global surface temperature has increased on average by 0.8 °C from 1880 to 2012, and is expected to continue rising for the foreseeable future (Team et al. 2014). Changes in both temperature and rainfall pattern are likely to induce alteration of plant community composition (Walther et al. 2002; Parmesan 2006; Maclean and Wilson 2011; Shi et al. 2015) through shifting species dominance (Wang et al. 2012; Shi et al. 2016), thereby impacting upon a range of ecosystem services. Surprisingly, the underlying mechanisms of how climate warming alters plant species dominance remain largely untested except temperature and its diurnal swings affects seed germination (Pritchard and Wood 1993; Vassilevska-Ivanova and Tcekova 2002). However, changes in the way plants acquire nitrogen (N) may be a latent mechanism, driving variation in plant species responses to warming. (Jiang et al. 2016a, b).

The availability of soluble N in soil is one of the key factors limiting primary productivity and plant species coexistence in terrestrial ecosystems (Näsholm et al. 1998; McKane et al. 2002). Plant-available N forms can include both inorganic (i.e. NH_4^+-N and NO_3^--N) and low molecular weight (MW) organic forms (e.g. urea, amino acids) (Chapin et al. 1993; Näsholm et al. 1998; Lipson and Näsholm 2001; Jones et al. 2005; Ganeteg et al. 2016). Differences among plant species and functional groups in their ability to acquire N forms may prevent niche overlap under high plant species diversity, and therefore reduce interspecific competition for the same limiting N resource (McKane et al. 2002; Kuster et al. 2016). As such, niche partitioning has been suggested as an important mechanism for species coexistence in terrestrial ecosystems (Chapin et al. 1986; Chapin et al. 1993; McKane et al. 2002; Miller et al. 2007; Ashton et al. 2010). Warming can induce changes in the amount and type of N available to plants by altering both above- and below-ground N inputs and rates of microbial N mineralization (Kuster et al. 2016). Additionally, numerous studies showed that warming can change the relative uptake of organic and inorganic N by plants (Henry and Jefferies 2003; Warren 2009b; Kuster et al. 2016; Raab et al. 1996). This indicates that warming has great potential to change both plant N demand and N acquisition which in turn affects plant species dominance. To date, however, this mechanism for driving change remains understudied.

In addition to climate warming, pastoral grazing represents a major land use practice in grasslands and is also known to exert a strong influence on plant composition and soil N availability (Hu et al. 2010; Olsen et al. 2011; Wang et al. 2012). In low-productivity ecosystems, grazing typically decreases soil carbon (C) and N pools and fluxes by reducing soil microbial activity (Zhong et al. 2014; Jiang et al. 2016a; Liu et al. 2016) and directly increases soil nitrate concentration through fecal and urinary inputs (Rui et al. 2011; Wu et al. 2011a; de Vries and Bardgett 2012). Grazing can also indirectly affect the availability of different N forms by altering rates of nitrification or ammonification (Rui et al. 2011; Wu et al. 2011a; de Vries and Bardgett 2012). Further, selective grazing can change plant species composition through decreasing cover of graminoids and legumes and increasing forb cover (Wang et al. 2012; Jiang et al. 2016b; Shi et al. 2016). Importantly, significant interactions have been found between warming and grazing on primary productivity, sward composition and soil N concentration (Jiang et al.2016a; Shi et al. 2015). For example, warming can significantly increase aboveground net primary production (ANPP) (Hobbie and Chapin 1998; Wang et al.

2012; Shi et al. 2015), while grazing may reduce the response of ANPP to warming (Wang et al. 2012). Grazing might also modulate the impact of warming on soil C and N pools and N form through its strong impacts on microbial processes (Rui et al. 2011). However, so far few studies have been conducted to explore how grazing and warming in combination affect plant N acquisition and the shift of plant species dominance in grassland ecosystems.

In this study, we performed a ^{15}N labeling experiment to explore the interactive effects of warming and grazing on plant uptake of inorganic and organic N within a 10-year warming × grazing experiment in an alpine meadow in Tibet, China. Previous studies in this experiment have shown that warming increased ANPP and the cover of graminoids and legumes but decreased the cover of forbs, whereas grazing reduced the response of plant cover and ANPP to warming (Wang et al. 2012). At the same time, warming accelerated the emission of N_2O from the soil, while there was no effect on soil net N mineralization after 5 years of warming (Hu et al. 2010; Wang et al. 2012). Within the soil, warming decreased soluble N concentrations during peak growing season while grazing increased soil NO_3^--N concentrations (Jiang et al. 2016a, b). Therefore, we hypothesized that (1) shifts in plant species dominance in response to warming were due to increased uptake of soil organic N (Fig. 1), and (2) grazing could cancel out the warming effect on plant species dominance by decreasing the uptake organic N by providing more inorganic N as faeces as well as selective grazing.

Materials and methods

Study site and experimental design

Our study site was located at the Haibei Alpine Meadow Ecosystem Research Station on the Qinghai-Tibetan plateau (37°37′N, 101°12′E). The mean elevation of the experimental site is 3200 m, the mean annual temperature is −1.7 °C and the annual precipitation is 560 mm. Almost 80% of the precipitation falls in the plant growing season (from May to September). The dominant plant species at the site are *Kobresia humilis* (grass), *Potentilla anserina* (forb) and *Elymus nutens* (grass) (Jiang et al. 2016a, b). More than 85% of the root biomass is concentrated in the upper 10 cm of the soil

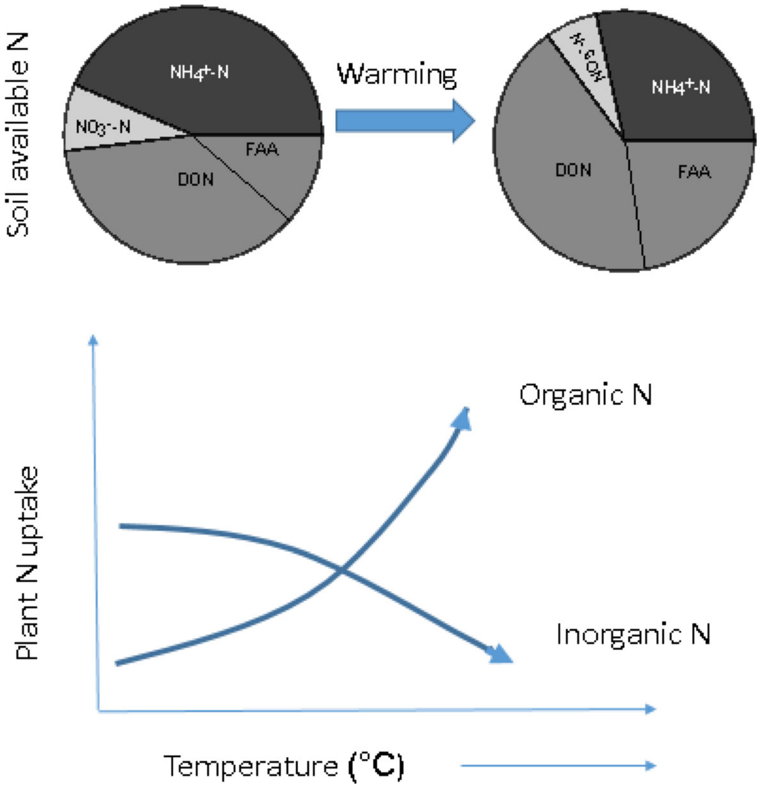


Fig. 1 Schematic diagram showing the effects of warming on plant uptake of different forms of soil available N in alpine meadow. The pie chart shows the change in different soil N forms (DON, soil dissolved organic nitrogen, does not include FAA) under warming. The solid lines show the shift in plant N uptake under warming. DON = dissolved organic nitrogen, FAA = free amino acids (including Glycine)

(Wu et al. 2011b). Soil bulk density is 0.75. The 0–10 cm soil includes 39.5% sand (50–2000 μm), 39.7% silt (2–50 μm) and 20.8% clay (<2 μm) (Du et al. 2007). Hydromica is dominant in the clay accompany with kaolinite and chlorite, having relative weak ammonium fixation (Zhou 2001).

In May 2006, we set up a warming × grazing experiment using a randomized block design. The fully factorial experiment included four treatments; control (no warming, no grazing, NWNG), grazing (no warming with grazing, NWG), warming (warming with no grazing, WNG), warming with grazing (WG). Each treatment was replicated four times, resulting in a total of 16 plots. Each circular plot was 3 m in diameter. Infrared heating was used for warming as described in Jiang et al. (2016a, b). Throughout the growing season, warming increased soil temperature on average by 1.2 °C and 1.7 °C at daytime and nighttime, respectively. At every plot, we used a digital temperature sensor (JM624 digital thermometer, Living-Jinming Ltd., China) and a Time Domain Reflectometer (JS-TDR300, Meridian Measurement, USA) to measure soil temperature and moisture at 0–10 cm soil depth, respectively (Zhu et al. 2015).

Grazing was performed by enclosing one adult sheep in the grazing plots for 2 h or two adult sheep for 1 h on 7th July and 23rd August from 2006 to 2010 (Wang et al. 2012). Sheep were removed when the sward height had been reduced to about half of the initial height before grazing (i.e. ca. 50% of the aboveground biomass). This grazing intensity is equal to a moderate stocking rate in alpine meadows (Jiang et al. 2016a, b). From 2011 to 2015, grazing was replaced by manual cutting, removing ca. 50% of the aboveground biomass in March and October each year (Zhu et al. 2015).

^{15}N labeling and sampling

We performed a short-term ^{15}N labeling experiment to test the effect of warming and grazing on plant N uptake. Four subplots (10 × 10 cm) were set up in each plot on 15th August, 2015. The distance between subplots was >20 cm to avoid potential cross-contamination between plots. The four subplots were injected with ^{15}N at the same time on 15th August, 2015 with one of the following solutions: (1) distilled water (9 ml) containing $(^{15}NH_4)_2SO_4$ (99.2 atom% ^{15}N enrichment; NH_4^+-N treatment), (2) distilled water (9 ml) containing $Na^{15}NO_3$ (99.2 atom% ^{15}N enrichment; NO_3^--N treatment), (3) distilled water (9 ml) containing ^{15}N-glycine (99.98 atom% enrichment; Organic treatment), or (4) distilled water (9 ml) containing no ^{15}N (Control treatment). These keep 0.02 g N m^{-2} was added into soil (Jiang et al. 2016a, b). Two hours after the ^{15}N labeling treatments were applied, we collected 10 × 10 × 10 cm soil samples from each of the subplots. Plant root and shoot material was collected and sorted to species level. Roots were first washed with tap water to remove the soil adhered to the roots. The roots were then washed with distilled water and dipped into 0.5 M $CaCl_2$ solution for 30 min to remove ^{15}N adhered to the root surface (Jiang et al. 2017). The roots were then rinsed again with distilled water to remove any excess $CaCl_2$ solution. Root and shoot material was dried at 70 °C for >36 h and then weighed.

After weighing, the plant material was milled to a fine powder using a ball mill (MM400, Retsch, Haan, Germany) for measuring plant N and ^{15}N using a Flash EA1112 analyzer and a Conflo III device connected to IRMS (MAT 253, Finnigan MAT, Germany). From each soil sample, a subsample was sieved (2-mm mesh) and stored at −20 °C to determine soil inorganic N and soil microbial C and N. Another subsample was air-dried (< 30 °C) for measuring soil glycine concentration. Glycine concentrations were measured using gas chromatography-mass spectrometry as described in Jiang et al. (2017). Soil exchangeable NH_4^+-N and NO_3^--N was extracted with 2 M KCl. Soil microbial biomass C and N were measured using the chloroform fumigation-extraction method (Brookes et al. 1985).

To confirm intact uptake of amino acid, glycine was double-labeled with both ^{13}C (for both C-1 and C-2 atoms) and ^{15}N (Icon Services, Summit, NJ). Glycine was chosen as it represents a model compound for studies of plant organic N uptake (Näsholm et al. 1998; Miller et al. 2007). Under natural conditions, the half-life time of glycine-^{15}N is more than 12 h (Unteregelsbacher et al. 2012). As such, we consider the use of glycine-^{15}N appropriate for testing the uptake of organic N by plants. $(^{15}NH_4)_2SO_4$ and $Na^{15}NO_3$ are commonly used for labeling of inorganic N (Xu et al. 2006), and are also often used as inorganic N fertilizers. The addition of Na has no significant influence on alpine plant growth (Xu et al. 2014).

Data analysis

For each plant species, APE (the Atom Percentage Excess) was calculated as APE = ^{15}N (added) − ^{15}N (control). Here ^{15}N (added) is the ^{15}N added to plots and ^{15}N (control) is the mean ^{15}N of the control plots. For each plant, N uptake rate (μg N g^{-1} dry weight root h^{-1}) was calculated as N uptake rate = plant N content (μg N g^{-1}dry weight soil) × APE × total plant biomass (g m^{-2})/plant root biomass (g m^{-2})/time (h)/ ($M_{unlabeled} \times M_{labeled}$). Here, $M_{labeled}$ is the total ^{15}N mass (g m^{-2}) of each N form injected to soil, and $M_{unlabeled}$ is the mass of the N form already present in the plot (Jiang et al. 2016b). Plant above-ground ^{15}N uptake = plant above-ground biomass × plant N content × APE. Actual plant above-ground N uptake from soil (μg N g^{-1} shoot) = plant above-ground ^{15}N uptake × MN/ total amount of ^{15}N added (NO_3^--N, NH_4^+-N or glycine-N). Where, MN is the amount of ambient NO_3^--N, NH_4^+-N or glycine -N in soil (Xu et al. 2006). Plant relative biomass = plant species biomass/plant total community biomass. Plant N stock = plant biomass (g m^{-2}) × plant N concentration (%). The percentage uptake of different N forms = the uptake of individual forms of N (NH_4^+-N, NO_3^--N, or glycine-N)/total plant N uptake (Jiang et al. 2016b).

We used both ^{13}C and ^{15}N labeled organic N as a tracer in order to distinguish uptake of intact amino N from uptake of mineralized amino acid N (Näsholm et al. 2000). We used the mean values of ^{13}C abundances of NH_4^+-N- and NO_3^--N-treated plants as references for calculating ^{13}C excess (Näsholm et al. 2000). Using NH_4^+-N- and NO_3^--N-treated plants provided the same amount of N as references for each species. So, ^{13}C excess implies levels over the mean of ammonium- and nitrate-treated plants. We aim to consider the shifts in ^{13}C due to N uptake when estimating ^{13}C labeling of glycine-treated plants (Näsholm et al. 2000). We used ^{15}N uptake rate multiplied by the slope of the regression of excess ^{15}N to excess ^{13}C to conservatively estimate the fraction of N taken up by intact amino acid N (Näsholm et al. 1998).

Statistical analyses

We used a two-way ANOVA to test warming and grazing and the interactive effects on the community above-ground biomass and the content of the soil N forms with warming and grazing as the fixed factors. A generalized linear model was used to test the main and all the interactive effects of warming, grazing, plant species (*Kobresia humilis, Potentilla anseria, Elymus nutans, Poa annua*) on plant species relative biomass in the community with the three as fixed factors. Another model was used to test the effects of warming, grazing, plant species, N form (NH_4^+-N, NO_3^--N and glycine-N) and their interactions on total and plant species N uptake rate with the four factors as fixed factors. Duncan's new multiple range test was used for post-hoc comparisons among treatments. We carried out simple regression analyses between uptake of soil glycine-N of each species and uptake of total available N of each species and between plant relative biomass and plant species total N uptake (% plant community total uptake of available N). Statistical analyses were performed using SPSS, v15.0 (SPSS Inc., Chicago, IL).

Results

Plant community biomass was not affected by warming, grazing or their interaction (F = 0.02, $P = 0.72$, Fig. 2a). However, there were significant effects of warming × grazing on plant species relative biomass (F = 3.25, $P =$ 0.023, Fig. 2b). Without grazing, warming decreased the relative biomass of *P. annua* but did not affect the relative biomass of *K. humilis*, *P. anserina* and *E. nutans*. However, with grazing, warming significantly increased the relative biomass of *K. humilis* and decreased the relative biomass of *P. annua* and *P. anserina* (Fig. 2b).

Warming and grazing did not affect significantly soil total C and N concentration ($P > 0.05$; Fig. 3a). And there is no interaction between the effect of warming and grazing ($P > 0.05$; Fig. 3a). One time sample, we found that warming decreased soil NH_4^+-N content with marginal significance (F = 3.22, $P = 0.053$; Fig. 3b) and warming decreased soil inorganic N (the total of NH_4^+-N and NO_3^--N) by 32%, whereas there was no effect of grazing or its interaction with warming on soil NH_4^+-N content (Fig. 3b). On the other hand, grazing significantly increased soil NO_3^--N content (F = 6.88, $P =$ 0.021) whereas there was no effect of warming or their interaction on soil NO_3^--N content (Fig. 3b). There were no significant effects of warming (F = 6.88, $P = 0.021$), grazing or their interaction on soil glycine concentration (Fig. 3b).

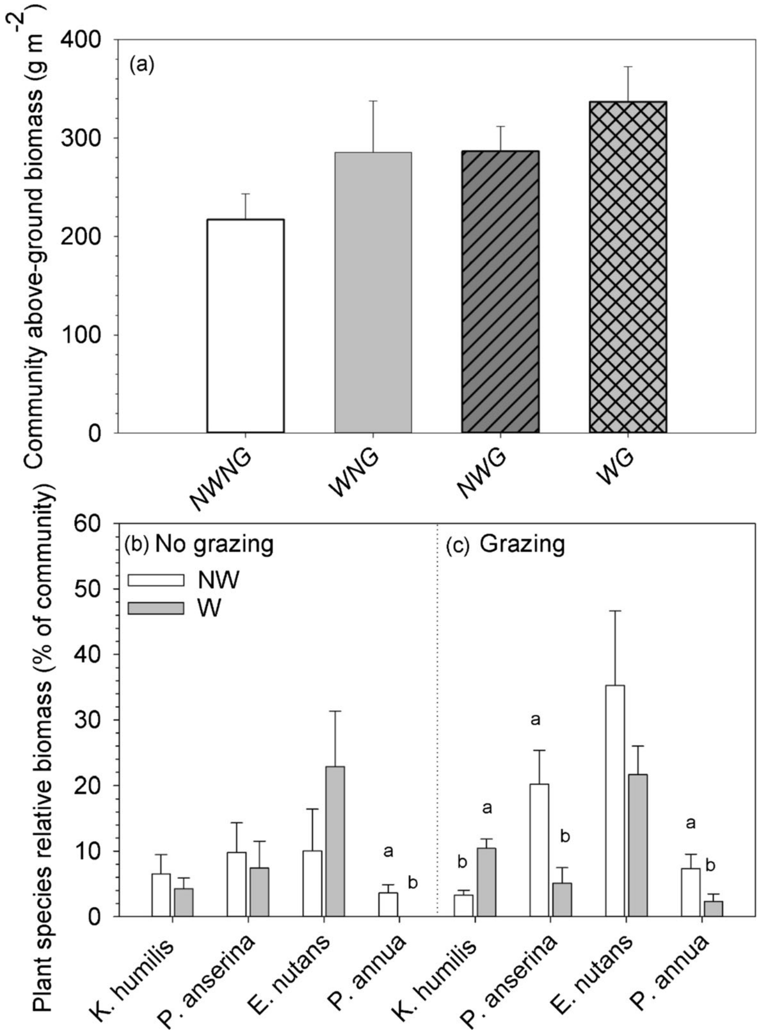


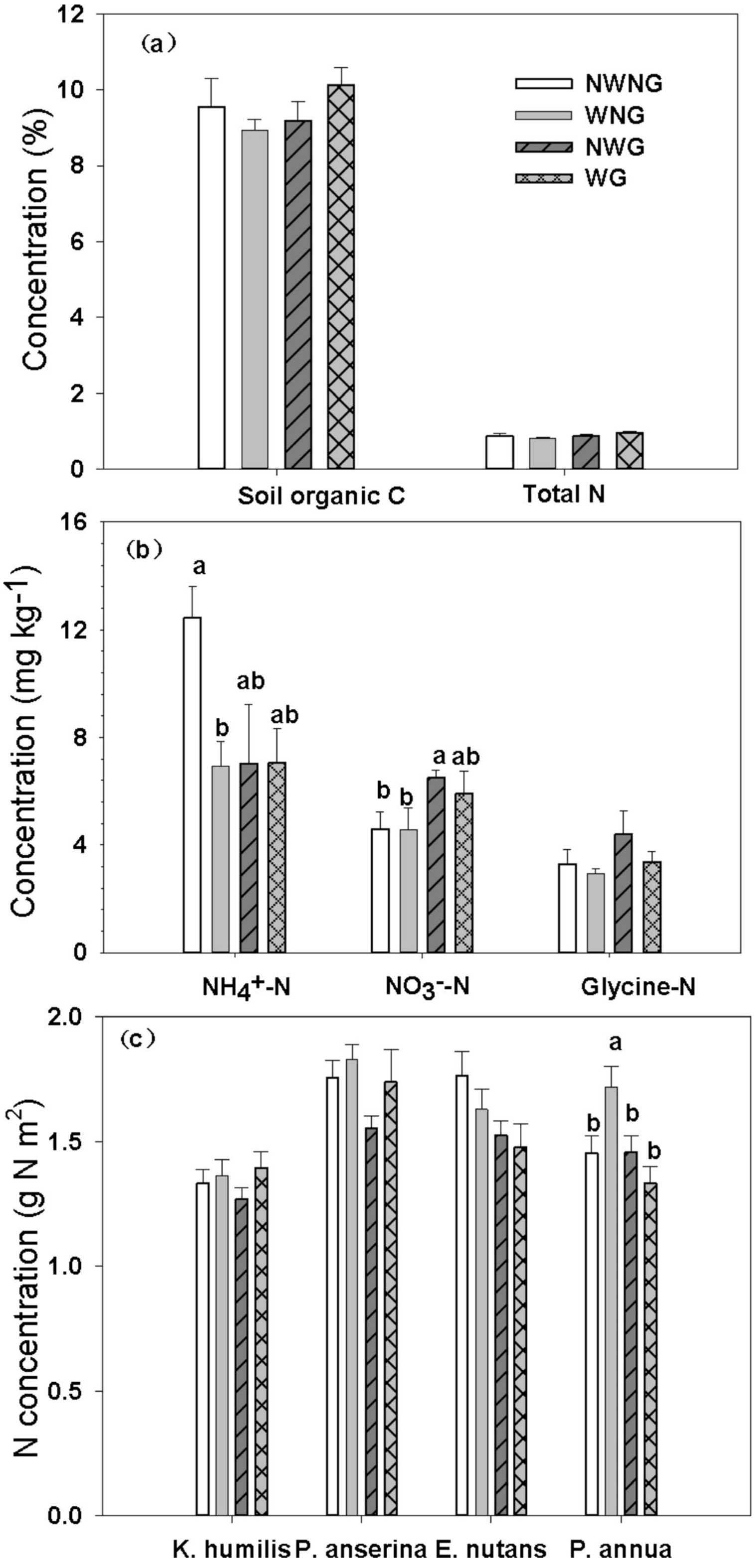
Fig. 2 Effects of warming and grazing on a) plant community above-ground biomass and b) plant species relative biomass. Values are mean ± SE (*n* = 4). Letters above the bars indicate significant differences between no warming and warming plots. NWNG = no warming and no grazing; WNG = warming with no grazing; NWG = grazing with no warming; WG = warming with grazing. NW = no warming; W = warming

There is no significant affection of warming and grazing on the N concentration of *K. humilis* and *P. anserine*. There is no significant affection of warming on the N concentration of *E. nutans* and *P. annua*. Grazing decreased the N concentration of *E. nutans* (F = 5.42, P = 0.02). Warming inecreased the N concentration of *P. annua* (F = 6.96, *P* = 0.01). There is significant interaction between warming and grazing (F = 7.27, P = 0.01).

Warming significantly affected plant uptake rates of different N forms (F = 2.37,*P* = 0.032). For all four species, warming decreased the uptake of NH_4^+-N (Fig. 4a-e) but did not significantly affect the uptake of NO_3^--N. Warming decreased the uptake of total inorganic N (NH_4^+-N and NO_3^--N) by up to 80%. In contrast, warming significantly increased the uptake of glycine-N for *K. humilis* (+152%), *P. anserina* (+600%), and *E. nutans* (+81%) while there was no significant effect on *P. annua* (Fig. 4d). Warming decreased the uptake of NO_3^--N in *P. anserina* (Fig. 4b). There were no significant effects of warming or warming × plant species on total plant N uptake rate (F = 0.25, *P* = 0.855).

All selected plant species in this experiment took up N as intact amino acid (Fig. 5). The mean slopes of the regressions of excess ^{13}C against excess ^{15}N in roots ranged from 0.63 to 0.96 under the four main treatments (NWNG, WNG, NWG and WG; Fig. 5). Because one mole of added glycine contains two moles of ^{13}C per mole of ^{15}N, the slope corresponding to 100% uptake of intact amino acid is 2. Hence, without taking account of ^{13}C lost in plant respiration, slopes ranging from 0.63 to 0.96 indicate that 32–48% of added glycine N was taken up in an intact form.

Fig. 3 Effects of warming and grazing on soil organic C, total N, inorganic N and plant species N concentration. Values are mean ± SE ($n = 4$). Letters above the bars indicate significant differences among treatments. NWNG = no warming and no grazing; WNG = warming with no grazing; NWG = grazing with no warming; WG = warming with grazing



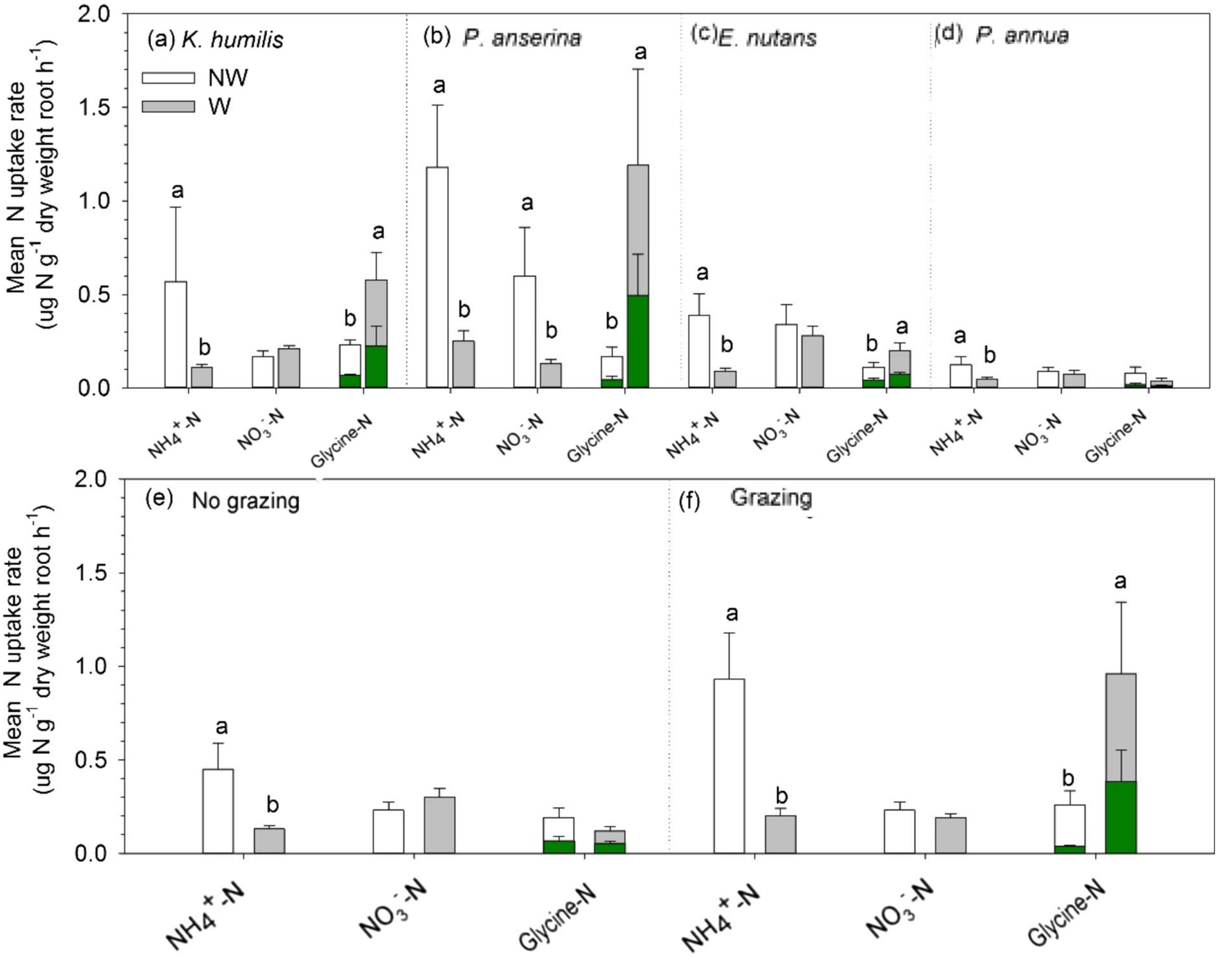


Fig. 4 Effects of warming, grazing and plant species on mean plant N uptake rate. Values are mean ± SE ($n = 4$). The green one in the gray figures about glycine-N uptake indicates the uptake rate of intact glycine. The green one value was calculated using plant species mean N uptake rate multiplied by the slope of regression between excess ^{13}C and excess ^{15}N in roots of plants supplied with dual-labelled $^{13}C^{15}N$-glycine. Letters above the bars indicate significant differences between no warming and warming plots or between ungrazed and grazed plots. NW = no warming; W = warming

There was a significant warming × grazing × N form interaction effect on mean N uptake across species (F = 6.00, $P = 0.003$). Without grazing, warming decreased the uptake of NH_4^+-N, while under grazing, warming increased the uptake of glycine-N but decreased the uptake of NH_4^+-N (Fig.4e, f). *P. anserina* had the highest overall N uptake while *P. annua* had the lowest mean N uptake (Fig. S1). Across plant species, grazing increased plant total N uptake (Fig. S2).

Across all four species, plant relative biomass increased hyperbolically with the ratio of the plant species total uptake of available N and plant community uptake of available N (Fig. 6a). A significant positive correlation was found between plant species uptake of soil available glycine-N and the uptake of total available N (Fig. 6b).

There were significant interactions between warming and grazing and soil temperature and moisture regime ($P < 0.05$). In comparison to the NWNG treatment, soil temperature was significantly higher in the WNG, NWG and NG treatments at 0–10 cm soil depth (Table S1, Fig. S3). Further, soil moisture was significantly lower in the WNG and NG treatments relative to the NWNG control (Table S1, Fig. S3).

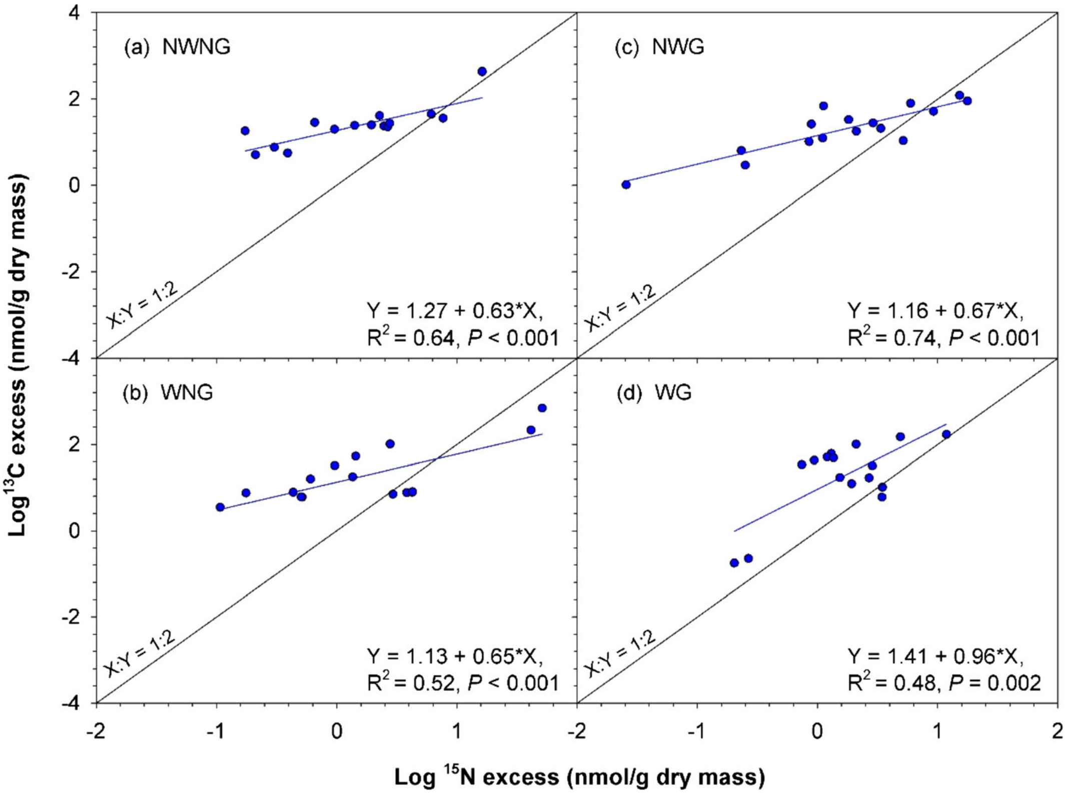


Fig. 5 The relationship between excess ^{13}C and excess ^{15}N in roots of plants supplied with dual-labelled ^{13}C^{15}N-glycine (the mean value of four plant species)

Discussion

To our knowledge, this is the first in situ report to examine how long-term warming in combination with grazing affects plant acquisition of NO_3^--N, NH_4^+-N and organic N (i.e., glycine) from soil in a natural ecosystem. We showed that changes in N uptake pattern under warming could help explain the shifts in plant species dominance and maintenance of alpine grassland productivity.

Nitrogen uptake by plants under warming with no grazing

Considering that the four plant species targeted here represented 60% of the total sward biomass, our findings partly support our first hypothesis that warming increases the uptake of soil organic N while warming decreases soil inorganic N availability. In general, N availability is limited in high latitude or altitude terrestrial ecosystems so that the relative availability of different N forms are of vital importance in determining which forms of N are used by plants (Chapin et al. 2002). If the balance of available N forms is altered this may make the extant vegetation less competitive (Chapin et al. 2002).

In this experiment, warming decreased inorganic N uptake in all four plant species, but it did not significantly affect plant biomass. Our results indicate that increased organic N availability compensates for decreased inorganic N uptake (Warren 2009a). Moreover, increased glycine uptake under warming may be a function of changes in root physiology (e.g. mycorrhizal colonization, transporter expression). As a result, changes in available N composition may select for plant species with high affinity organic N transport systems. Our results are consistent with some hydroponic experiments showing that uptake of glycine was favored at high temperatures (Henry and Jefferies 2003). In contrast to the

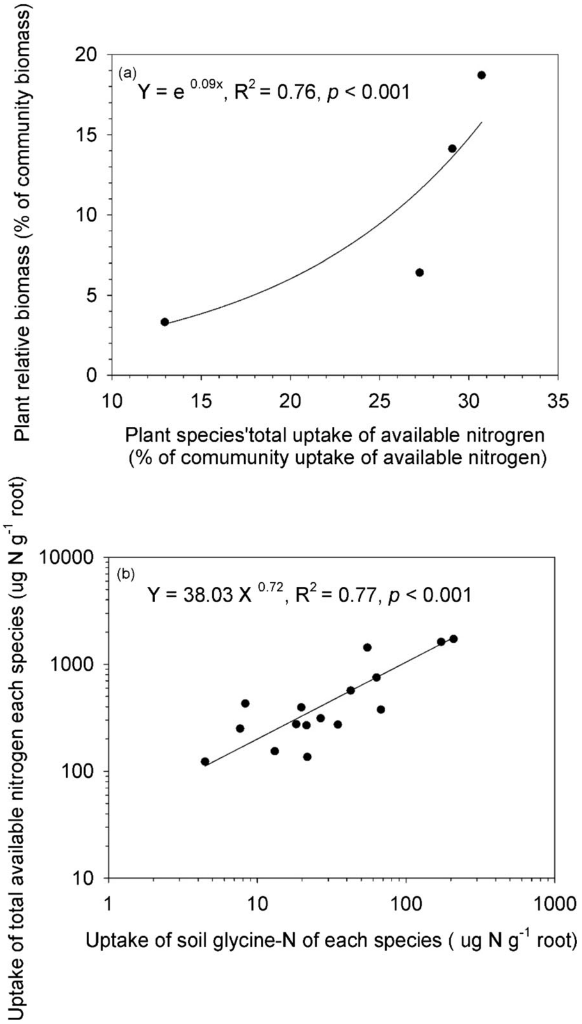


Fig. 6 The relationship between plant species relative biomass in community and the percentage of plant species' total uptake of available nitrogen (% of community uptake of available nitrogen,) (a) and between uptake of total available nitrogen of each plant species and uptake of soil Glycine-N of each species (b). The 4 points in panel A indicate the mean value of 4 different plant species in all treatment; the 16 points in panel B indicate the 4 different plant species in 4 treatments (the treatment: NWNG = no warming and no grazing; WNG = warming with no grazing; NWG = grazing with no warming; WG = warming with grazing)

other three species, warming reduced the ability of *P. annua* to acquire NH_4^+-N but did not affect glycine uptake. And the same time, warming decreased the relative biomass of *P. annua*. This indicates that plant species do not adapt to take more organic N under a long-term warming may decreased their dominance in the communication (Kuster et al. 2016).

One important strategy for plants to adapt to climate change is to adapt in situ. In other words, plants change their morphological and physiological traits (Jump and Penuelas 2005; Hoffmann and Sgrò 2011). Our results support this as suggesting that some plant species change their root physiology to facilitate grater capture of N, e.g. for *K. humilis* and *P. anserina*, N uptake is NH_4^+-N > NO_3^--N > glycine-N under no-warming and NH_4^+-N < NO_3^--N < glycine-N under warming. The pattern of N uptake was coincident with plant relative biomass for *K. humilis* and *P. anserina*. For *E. nutans*, there is no such pattern, but warming increased glycine-N uptake, and it also kept its dominance under warming. This suggests that some subdominant plant species could go extinct under long-term warming if they are unable to increase their uptake of organic N.

The progressive N limitation (PNL) hypothesis suggests that an increase in primary productivity will lead to a reduction in soil available N by increasing root uptake of N, thereby increasing N immobilization in plant biomass and long-lived soil organic matter (Luo et al. 2004; Reich et al. 2006). The PNL hypothesis has traditionally been applied to the availability of inorganic N (NH_4^+, NO_3^-) (Luo et al. 2004; Reich et al. 2006). Interestingly, our results indicate that the deficiency of plant-available DON can also be important in plant community N limitation (Jones et al. 2004, 2005). Consequently, the availability of low MW organic N can be a major constraint of the supply of N to plants in alpine grasslands under cold environments (Jan et al. 2009).

Our results showed that effects of warming and plant species on N uptake were not affected by grazing. However, effects of warming on different N forms varied with grazing. For example, warming increased plant glycine-N uptake only under grazing. This does not support our second hypothesis that grazing may decrease plant species N preference for organic N. In our previous study, grazing significantly increased soil dissolved organic nitrogen (DON) concentration (Jiang et al. 2016a, b). Higher soil DON may be a reason why warming increased plant organic N uptake. It has been found that plants compete well for amino acids in soil when DON concentrations are high (Charpin et al. 2002) and can acquire >60% of their N requirement through direct uptake of DON (Lipson and Näsholm 2001). Due to selective grazing on plant species, plant species responded inconsistently to warming.

Plant traits strongly influence total nutrient uptake after disturbance (Charpin et al. 2002). *K. humilis*, an early-flowering plant species, is especially adapted to heavy grazing and nutrient limitation in alpine ecosystems, having well developed root system. Therefore, the species plays a major role in response to grazing and warming among the plant community.

Our results suggest that a significant proportion of glycine-N was taken up intact. This is supported by the linear relationships between ^{13}C to ^{15}N in roots supplied with ^{13}C, ^{15}N dual-labelled glycine. Comparing the slopes of these lines to the slope of 1:2, derived from the theoretical ^{13}C:^{15}N ratio of the tracer, a conservative estimate of the fraction of N taken up as amino acid can be obtained. After 2 h, this fraction was at least 31%, 32%, 33% and 48% for the grass under NWNG, WNG, NWG and WG, respectively. Glycine metabolism in plants occurs mainly via serine synthesis, whereby 2 mol of glycine are transferred to 1 mol of serine, 1 mol of ammonium and 1 mol of CO_2. The ammonium is reabsorbed, whereas CO_2 is lost to the atmosphere. Hence, our estimates of organic N uptake are probably underestimated, and differences between species could be partly related to differences in rates of decarboxylation. Besides, the underestimate of organic N uptake could be due to 30 min washing roots in 0.5 M $CaCl_2$ solution. Washing the roots with $CaCl_2$ is a traditional method to remove ^{15}N in plant root, but dipped them in it for 30 min may cause the release of ^{15}N from roots.

Conclusion

Warming decreased plant inorganic N uptake but increased plant organic N uptake for three out of four species. However, there was no overall significant effect of warming on total N uptake. We found a significant correlation between plant relative dominance and plant total N uptake and between plant organic N uptake and plant total N uptake. Together, these findings indicate that plant organic N uptake is a driver of plant total N uptake and that plant total N uptake controls plant dominance. This implies that species that are able to take up more organic N under long-term warming retain or increase their dominance at the expense of those species what are not able to adapt their N uptake strategy. However, grazing can change plant dominance under warming because of plant species characteristic response to grazing.

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References

Ashton IW, Miller AE, Bowman WD, Suding KN (2010) Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. Ecology 91:3252–3260

Brookes P, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method tomeasure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Chapin FS, Cleve KV, Tryon PR (1986) Relationship of ion absorption to growth rate in taiga trees. Oecologia 69:238–242

Chapin FS, Matson PA, Mooney HA (2002) Terrestrial decomposition. Springer, New York

Chapin FS, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen for growth by a nonmycorrhizal Arctic sedge. Nature 361:150–153

de Vries FT, Bardgett RD (2012) Plant-microbial linkages and ecosystem nitrogen retention: lessons for sustainable agriculture. Front Ecol Environ 10:425–432

Du YG, Cao GM, Wang Q, Wang CT (2007) Effect of grazing on surface character and soil physical property in alpine meadow. J Mt Sci 25:338–343

Ganeteg U, Ahmad I, Jämtgård S, Aguetoni C, Cambui E, Inselsbacher SH, Schmidt S, Näsholm T (2016) Amino acid transporter mutants of Arabidopsis provides evidence that a non-mycorrhizal plant acquires organic nitrogen from agricultural soil. Plant Cell Environ 10:67–72

Henry H, Jefferies R (2003) Interactions in the uptake of amino acids, ammonium and nitrate ions in the Arctic salt-marsh grass, Puccinellia phryganodes. Plant Cell Environ 26:419–428

Hobbie SE, Chapin FS (1998) The response of tundra plant biomass, aboveground production, nitrogen, and CO2 flux to experimental warming. Ecology 79:1526–1544

Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. Nature 470:479–485

Hu YG, Chang XF, Lin XW, Wang YF, Wang SP, Duan JC, Zhang ZH, Yang XX, Luo CY, Xu GP, Zhao XQ (2010) Effects of warming and grazing on N2O fluxes in an alpine meadow ecosystem on the Tibetan plateau. Soil Biol Biochem 42: 944–952

Jan MT, Roberts P, Tonheim SK, Jones DL (2009) Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biol Biochem 41:2272–2282

Jiang LL, Wang SP, Luo CY, Zhu XX, Kardol P, Zhang ZH, Li YM, Wang CS, Wang YF, Jones DL (2016a) Effects of warming and grazing on dissolved organic nitrogen in a Tibetan alpine meadow ecosystem. Soil Tillage Res 158: 156–164

Jiang LL, Wang SP, Pang Z, Wang CS, Kardol P, Zhou XQ, Rui YC, Lan ZC, Wang YF, Xu XL (2016b) Grazing modifies inorganic and organic nitrogen uptake by coexisting plant species in alpine grassland. Biol Fertil Soils 52:211–221

Jiang LL, Wang SP, Pang Z, Wang CS, Kardol P, Zhong L, Yu Q, Lan ZC, Wang YF, Xu XL, Kuzyakov Y, Luo CY, Zhang ZH, Jones DL (2017) Effects of grazing on the acquisition of nitrogen by plants and microorganisms in an alpine grassland on the Tibetan plateau. Plant and Soil 416:297–308

Jones DL, Shannon D, Murphy DV, Farrar J (2004) Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. Soil Biol Biochem 36:749–756

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

Jump AS, Penuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecol Lett 8: 1010–1020

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 Soil 406:117–129

Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128:305–316

Liu C, Song X, Wang L, Wang D, Zhou X, Liu J, Zhao X, Li J, Lin H (2016) Effects of grazing on soil nitrogen spatial heterogeneity depend on herbivore assemblage and pre-grazing plant diversity. J Appl Ecol 53:242–250

Luo Y, Su B, Currie WS, Dukes JS, C Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–739

Maclean IMD, Wilson RJ (2011) Recent ecological responses to climate change support predictions of high extinction risk. Proc Natl Acad Sci 108:12337–12342

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, 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, Högberg M, Högberg 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

Olsen YS, Dausse A, Garbutt A, Ford H, Thomas DN, Jones DL (2011) Cattle grazing drives nitrogen and carbon cycling in a temperate salt marsh. Soil Biol Biochem 43:531–541

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669

Pritchard HW, Wood JA (1993) Manger influence of temperature on seed germination and the nutritional requirements for embryo growth in Arum maculatum. New Phytol 123:801–809

Raab TK, A Lipson D, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge Kobresia myosuroides: implications for the alpine nitrogen cycle. Oecologia 108:488–494

Reich PB, Hobbie SE, Lee T, S Ellsworth D, West JB, Tilman D, Knops JM, Naeem S, Trost J (2006) Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440:922–925

Rui YC, Wang SP, Xu ZH, Wang YF, Chen CR, Zhou XQ, Kang XM, Lu SB, Hu YG, Lin QY, Luo CY (2011) Warming and grazing affect soil labile carbon and nitrogen pools differently in an alpine meadow of the Qinghai-Tibet plateau in China. J Soils Sediments 11:903–914

Shi Z, Sherry R, Xu X, Hararuk O, Souza L, Jiang L, Xia J, Liang J, Luo Y (2015) Evidence for long-term shift in plant community composition under decadal experimental warming. J Ecol 103:1131–1140

Shi Z, Xu X, Souza L, Wilcox K, Jiang LF, Liang JY, Xia JY, Garcia-Palacios P, Luo YQ (2016) Dual mechanisms regulate ecosystem stability under decade-long warming and hay harvest. Nat Commun 7:11973

Team CW, Pachauri RK, Meyer LA (2014) IPCC, 2014: climate change 2014: synthesis report. Contribution of Working Groups I. II and III to the Fifth Assessment Report of the intergovernmental panel on Climate Change. IPCC, Geneva, Switzerland, p. 151

Unteregelsbacher S, Hafner S, Guggenberger G, Miehe G, Xu X, Liu J, Kuzyakov Y (2012) Response of long-, medium-and short-term processes of the carbon budget to overgrazing-induced crusts in the Tibetan plateau. Biogeochemistry 111: 187–201

Vassilevska-Ivanova R, Tcekova Z (2002) Effect of temperature on seed germination and seedling growth of sunflower (Helianthus annuus L). C R Acad Bulg Sci 55:10–67

Walther GR, Post E, Convey P, Menzel A, Parmesan C, C Beebee TJ, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416: 389–395

Wang SP, Duan JC, Xu GP, Wang Y, Zhang Z, Rui YC, Luo CY, Xu B, Zhu XX, Chang X (2012) Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow. Ecology 93:2365–2376

Warren CR (2009a) Does nitrogen concentration affect relative uptake rates of nitrate, ammonium, and glycine? J Plant Nutr Soil Sci-Zeitschrift Fur Pflan Und Bod 172:224–229

Warren CR (2009b) Why does temperature affect relative uptake rates of nitrate, ammonium and glycine: a test with Eucalyptus pauciflora. Soil Biol Biochem 41:778–784

Wu HH, Dannenmann M, Fanselow N, Wolf B, Yao ZS, Wu X, Brüggemann N, Zheng XH, Han XG, Dittert K, Butterbach-Bahl K (2011a) Feedback of grazing on gross rates of N mineralization and inorganic N partitioning in steppe soils of Inner Mongolia. Plant Soil 340:127–139

Wu YB, Wu J, Deng YC, Tan HC, Du YG, Gu S, Tang YH, Cui XY (2011b) Comprehensive assessments of root biomass and production in a Kobresia humilis meadow on the Qinghai-Tibetan plateau. Plant Soil 338:497–510

Xu XL, 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 XL, Wanek W, Zhou C, Richter A, Song M, Cao G, Ouyang H, Kuzyakov Y (2014) Nutrient limitation of alpine plants: implications from leaf N : P stoichiometry and leaf delta N-15. J Plant Nutr Soil Sci 177:378–387

Zhong L, Du R, Ding K, Kang X, Li FY, Bowatte S, Hoogendoorn CJ, Wang Y, Rui Y, Jiang L (2014) Effects of grazing on N_2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China. Soil Biol Biochem 69:1–10

Zhou XM (2001) Kobresia meadow of China. Science Press, Beijing

Zhu X, Luo C, Wang S, Zhang Z, Cui S, Bao X, Jiang L, Li Y, Li X, Wang Q (2015) Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan plateau. Agric For Meteorol 214:506–514