

## Warming decreased and grazing increased plant uptake of amino acids in an alpine meadow

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### Keywords

<sup>13</sup>C–<sup>15</sup>N-glycine, amino acid uptake, grazing, plant community, Qinghai–Tibetan Plateau, warming.

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## Introduction

Inorganic nitrogen (N) has long been recognized as dominant plant-available N forms, especially in fertile arable lands. More and more studies, however, indicated that

## Abstract

Organic nitrogen (N) uptake by plants has been recognized as a significant component of terrestrial N cycle. Several studies indicated that plants have the ability to switch their preference between inorganic and organic forms of N in diverse environments; however, research on plant community response in organic nitrogen uptake to warming and grazing is scarce. Here, we demonstrated that organic N uptake by an alpine plant community decreased under warming with <sup>13</sup>C–<sup>15</sup>N-enriched glycine addition method. After 6 years of treatment, warming decreased plant organic N uptake by 37% as compared to control treatment. Under the condition of grazing, warming reduced plant organic N uptake by 44%. Grazing alone significantly increased organic N absorption by 15%, whereas under warming condition grazing did not affect organic N uptake by the *Kobresia humilis* community on Tibetan Plateau. Besides, soil NO<sub>3</sub>–N content explained more than 70% of the variability observed in glycine uptake, and C:N ratio in soil dissolved organic matter remarkably increased under warming treatment. These results suggested warming promoted soil microbial activity and dissolved organic N mineralization. Grazing stimulated organic N uptake by plants, which counteracted the effect of warming.

organic monomers, such as free amino acids (FAAs), can provide plant-available N source in addition to inorganic N forms such as nitrate and ammonium (Chapin et al. 1993; Finzi and Berthrong 2005; Harrison et al. 2007; Averill and Finzi 2011; Geisseler and Horwath 2014; Mansson et al.

2014), and organic N uptake by plants is a significant component of terrestrial N cycle (Schimel and Bennett 2004). It was found that both mycorrhizal and nonmycorrhizal plants were capable of absorbing amino acid-N (AA-N) in situ in various natural and farmland ecosystems in temperate (Owen and Jones 2001; Harrison *et al.* 2007), boreal (Näsholm *et al.* 1998), arctic (Chapin and Schimel 1996; Lipson and Monson 1998), and alpine zones (Raab *et al.* 1996; Näsholm *et al.* 1998; Xu *et al.* 2004). Protein depolymerization, instead of mineralization, is the major limiting step controlling N availability in cold ecosystems, because organic N decomposition and inorganic N supply is usually slow in cold climates due to decelerated microbial activity (Schimel and Bennett 2004; Hobbie *et al.* 2009). Plants thus may have evolved the ability to uptake FAA-N directly and compete well with soil microorganisms (Näsholm *et al.* 2009). It was hypothesized that organic nitrogen would become progressively dominant in plant-N uptake with decreasing temperature (Schimel and Bennett 2004). Using elevation as a proxy of climate, Averill and Finzi (2011) found that N uptake by plants shifted from inorganic to organic sources, and rates of mineralization and nitrification decreased with elevation increment in the cold-temperate forest of the White Mountains in New Hampshire, USA. Shan reported that soil amino acid concentration increased with elevation at a rate of  $0.21 \text{ mg}\cdot\text{kg}^{-1}$  per 100 m, which provides evidence of enhanced importance of amino acids in soil soluble N in north Idaho cold-temperate forest ecosystems with increasing elevation (Shan *et al.* 2014). It should be noted that variation in altitude led to marked changes not only in temperature, but also factors such as moisture and vegetation composition. To date, there have been no direct, in situ tests of organic N uptake by plant community under elevated temperature.

Plant species-specific preference in uptake of different N forms has been drawing attentions (Gruffman *et al.* 2014). Niche differentiation of N forms, that is, individual species used only a limited range of N forms, was even suggested as a mechanism for facilitating species coexistence and maintenance of plant diversity, especially in N limited ecosystems (Mckane *et al.* 2002; Miller and Bowman 2003; Reynolds *et al.* 2003; Weigelt *et al.* 2005). For example, different species of plants in arctic tundra differed in chemical forms as well as timing and soil layer of N uptake (Mckane *et al.* 2002). Key species in *Kobresia* meadows, such as *K. pygmaea* and *K. humilis*, showed a greater capacity to take up organic nitrogen than nonkey species (Xu *et al.* 2004). Moreover, fast-growing species demonstrated a preference for inorganic and simple organic N forms over more complex amino acids, whereas slow-growing species were near equal in terms of N form preference (Harrison *et al.* 2008). As species from the same community varied widely in the capacity to take up  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and glycine (Miller

and Bowman 2002, 2003), and the same species may vary its N form preference with environmental factors or fungi association levels (Xu *et al.* 2004), it is essential to tackle the issue of organic N importance at community level so as to diminish the uncertainty associated with plant species.

Livestock grazing, as one of the main land-use types of natural grasslands, and its interaction with climate change affects a series of soil and vegetation properties (Klein *et al.* 2007; Post *et al.* 2008), which may further influence the relative importance of organic N source. For example, grazing increases daytime soil temperature through shorter canopy height. Grazing may change soil fertility and presence of soil N forms (Rui *et al.* 2011), which in turn alter the relative importance of soil organic and inorganic N form (Scott and Rothstein 2011). In particular, responses of plant community composition to warming differed greatly with and without herbivore grazing (Klein *et al.* 2007; Post *et al.* 2008). Nevertheless, investigation of soil organic N utilization under grazing is scarce.

As the same with arctic, boreal, alpine, and other cold ecosystems, nitrogen is a limiting nutrient for plants in the alpine meadow on the Qinghai-Tibetan Plateau (Liu *et al.* 2013). Xu *et al.* (2004) found that alpine plants on this plateau could uptake organic N, which may play an important part in N nutrient. Besides, historical climate records show that the Qinghai-Tibetan Plateau has been experiencing substantial warming (i.e.,  $0.32^\circ\text{C}$  per decade; Liu and Chen 2000), and this trend will likely continue in the future (Wang *et al.* 2014). This study examines the effects of warming and grazing on plant FAA-N uptake in a manipulated field system in an alpine meadow on the Qinghai-Tibetan Plateau (Fig. 1). We hypothesized that plant uptake of organic N decreased under warming and grazing probably due to plant-microbe competition on substrate (DON and DIN).



**Figure 1.** Plant community in the manipulated field system. Photo credit: Jichuang Duan.

## Materials and Methods

### Experimental site

The experimental site is located at the Haibei Alpine Meadow Ecosystem Research Station (HBAMERS; 37°37'N, 101°12'E), lying in a large valley surrounded by the Qilian Mountains on the northeast of the Qinghai-Tibetan Plateau. The mean elevation of the valley bottom is 3200 m. The soil is classified as Mat-Crylc Cambisols. The site has a typical plateau continental climate with short and cool summer, long, and severely cold winter. Mean annual temperature is  $-2^{\circ}\text{C}$ , and the annual precipitation is 500 mm, over 80% of which falls during the summer monsoon season. During the growing seasons (1 May to 20 September) from year 2006 to 2010, mean temperature was 8.4, 8.5, 8.1, 8.5, and  $9.1^{\circ}\text{C}$ , and total rainfall was 449.2, 397.6, 339.4, 282.6, and 376.2 mm, respectively (Wang et al. 2012). The plant community at the experimental site is dominated by *K. humilis*, *Elymus nutans*, *Carex scabrostris*, *Festuca ovina*, *Potentilla nivea*, *Poa pratensis*, *Scripus distigmaticus*, *Gentiana straminea*, *G. farreri*, and *Blysmus sinocompressus* (Wang et al. 2012).

### Controlled warming–grazing experiment

A controlled warming with grazing experiment was established at the HBAMERS in 2006. As the effects of global warming was predicted to be larger at night than during daytime, temperature differences between heated and corresponding reference plots were set as  $1.2^{\circ}\text{C}$  during daytime and  $1.7^{\circ}\text{C}$  at night in summer (Luo et al. 2010), which fell within limits of predicted temperature increases for this century ( $1.1$ – $6.4^{\circ}\text{C}$ , IPCC, 2007). The heating system was namely free-air temperature enhancement method with infrared heaters as described by Kimball et al. (2008).

A two-way factorial design (warming and grazing) was used with four replicates for each of four treatments: no warming with no grazing (control, CK), no warming with grazing (G), warming with no grazing (W), and warming with grazing (WG). In total, 16 plots of 3 m diameter were deployed in randomized block design in the field. The total area of each plot was about  $7\text{ m}^2$ .

A moderate grazing intensity was employed as follows. One adult Tibetan sheep was fenced in each of the grazing plots on the morning of 15 August 2006, the first year, for approximately 2 h. Thereafter, two adult Tibetan sheep for 1 h was adopted instead on the mornings of 12 July, 3 August, and 12 September in 2007, 8 July and 20 August in 2008, 9 July and 24 August in 2009, and 7 July and 23 August in 2010. The canopy height of the vegetation was measured at 50 random points within the plots

before and after grazing. The sheep were removed from the grazing plots when the canopy height was reduced to approximately half of the initial height. In 2011 and 2012, mowing was conducted to simulate the grazing effect of foraging. In November 2011, when the growing season ended, grasses were mowed until the canopy height is 6–7 cm. In April 2012, when the growing season started, grasses were mowed until 2–3 cm left. The forage removal rate by mowing was similar to sheep grazing.

### Soil temperature and moisture measurements

The soil temperature at depths of 5, 10, and 20 cm was monitored automatically using type-K thermocouples (Campbell Scientific, Logan, UT), which were connected to a CR1000 datalogger. Meanwhile, the soil temperatures at 0 and 40 cm depth were manually measured using mercury-in-glass thermometers. The effects of warming and grazing on soil temperature and soil moisture from 2006 to 2008 were reported by (Hu et al. 2010; Luo et al. 2010). Both warming and grazing significantly increased seasonal mean soil temperature at 10 cm by 1.9 and  $1.0^{\circ}\text{C}$  from May to September during the 5 years after 2006. Only warming significantly decreased soil moisture at 10 cm by 15.6%, 19.1%, and 17.8% in 2008, 2009, and 2010 during the growing season.

### Labeling and sampling

On 3 October 2012, a 20-cm diameter mini-quadrat with uniform species composition and coverage was chosen in each of the 16 plots. Inside each mini-quadrat, 20 injection points were evenly spaced. Injection was made by 20-mL syringes with 10-cm-long needles. A needle was pushed 10 cm deep in the soil. Labeling solution of 10 mL was evenly injected along the soil profile during smoothly pulling out of the needle. Thus, 200 mL glycine solution in total was applied to one mini-quadrat. The concentration was  $12\text{ mmol glycine-N L}^{-1}$ , where the glycine was double-labeled with 99.98 atom% of both  $^{13}\text{C}$  (for both C-1 and C-2 atoms) and  $^{15}\text{N}$  (Icon Services, Summit, NJ).

Twenty-four hours after labeling, a soil core of 20-cm diameter was sampled in each mini-quadrat to depth of 10 cm, in which layer over 80% of the roots were concentrated in *Kobresia* meadows (Wang and Shi 2001). Soil cores with plants were transported to the laboratory immediately within 20 min. The whole plants (shoots and roots) were separated from the soil cores carefully to keep plants as intact as possible. Plants were gently washed in water to clear off soil attached, then immersed in  $0.5\text{ mmol}\cdot\text{L}^{-1}\text{ CaCl}_2$  solution for 30 min, and rinsed with

distilled water to remove tracers absorbed on the surface. The remaining soils were mixed well by hand and passed through 2-mm sieve. Soil samples were stored under  $-20^{\circ}\text{C}$  before analyzing for moisture, total C and N, dissolved organic C and N (DOC and DON), soluble inorganic nitrogen (DIN;  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ),  $^{13}\text{C}$  content,  $^{15}\text{N}$  content, microbial biomass C (MBC), and microbial biomass N (MBN).

Plants were grouped into species and weighed for shoots and roots separately. However, due to the limited amount of total biomass, shoots and roots were mixed for analysis of plant total N, total C,  $^{13}\text{C}$ , and  $^{15}\text{N}$  content. Plant and root-free soil samples were dried ( $60^{\circ}\text{C}$ , 48 h) and ground with mortar and pestle. Isotopic analysis was performed by a Finnigan MAT DELTAplusXP (Thermo Fisher, San Jose, CA, USA) isotope ratio mass spectrometer.

### Sample analysis and calculation

Uptake of the labeled atoms is expressed as moles  $^{13}\text{C}$  and/or  $^{15}\text{N}$  in excess of background levels. To make the least disturbance to the long-term warming–grazing experimental platform, we did not set up unlabeled sampling mini-quadrats inside the plots as control. The International Atomic Energy Agency standards of  $^{15}\text{N}$  ( $\text{N}_2$ : 0.3663 atom%) and  $^{13}\text{C}$  (PDB: 1.11 atom%) were used as background for calculating  $^{15}\text{N}$  excess. The error introduced by such substitution would be negligible (Jing Zhang, Baoming Ji, unpublished data). The  $\delta^{15}\text{N}$  values in plants were  $-1.55 \pm 1.4\text{‰}$  (means  $\pm 1$  SD) for *K. humilis*,  $0.00 \pm 0.79\text{‰}$  for *E. nutans*,  $0.07 \pm 1.4\text{‰}$  for *C. scabriorstris*,  $-0.09 \pm 0.98\text{‰}$  for *P. nivea*. The moles of  $^{13}\text{C}$  or  $^{15}\text{N}$  in excess of background were then calculated by multiplying the atom% at excess by moles C or N in the sample. Comparisons were made between moles  $^{13}\text{C}$  and  $^{15}\text{N}$  at excess to determine if intact amino acid uptake occurred.

One subsample of soil was dried (24 h at  $120^{\circ}\text{C}$ ) to determine gravimetric soil water content. Two subsamples were used to determine MBC and MBN by chloroform fumigation technique (Brookes et al. 1985; Vance et al. 1987). One subsample was immediately extracted with  $0.5 \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{SO}_4$  (10.0 g fresh weight (FW) soil: 50 mL  $\text{K}_2\text{SO}_4$ ). While the other was fumigated with  $\text{CHCl}_3$  for 4 days in an evacuated desiccator and then extracted with  $0.5 \text{ mol}\cdot\text{L}^{-1} \text{ K}_2\text{SO}_4$  (8.0 g FW soil: 40 mL  $\text{K}_2\text{SO}_4$ ). Aliquots of the fumigated and unfumigated  $\text{K}_2\text{SO}_4$  extracts were measured by TOC/TN Analyzer (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany). The fourth subsample was extracted by  $2 \text{ mol}\cdot\text{L}^{-1} \text{ KCl}$  with soil mass to extractant ratio of 1:5. In all cases, after shaking at 220 rpm for 30 min, the extracts were filtered and stored at  $-80^{\circ}\text{C}$  before analysis. The concentration of

$\text{NH}_4^+$  and  $\text{NO}_3^-$  in soil extracts was measured on an auto-analyzer (Auto Analyzer 3 System; SEAL Analytical GmbH, Norderstedt, Germany). Soil total dissolved N (TDN) was measured by TOC/TN Analyzer (Liqui TOC II; Elementar Analysensysteme GmbH). DON was calculated by the difference between TDN and DIN.

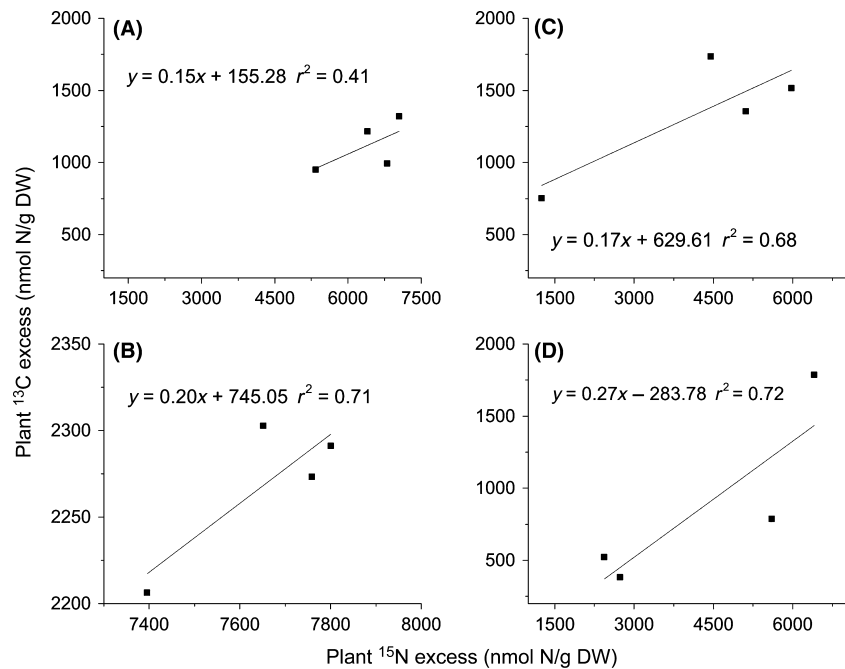
### Statistical analysis

The four mini-quadrats of the same treatments were considered replicates, and statistical analysis was based on  $n = 16$  (4 treatments  $\times$  4 replicates). Differences among treatments for plant total carbon (TC) and total nitrogen (TN),  $^{13}\text{C}$  and  $^{15}\text{N}$  excesses of plant,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , DON, FAA–N, soil total dissolved C (TDC), soil TDN, MBC, and MBN of soil were analyzed by two-way ANOVA with warming and grazing as the main factors. Multiple stepwise regression analysis was carried out using  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , DON, TDC, TDN, soil  $^{13}\text{C}$ , soil  $^{15}\text{N}$ , soil TC, soil TN, soil FAA–N, soil glycine–N, plant TC and plant TN as independent variables, and plant mix uptake of  $^{15}\text{N}$  as dependent variables. All analyses were carried out using SAS version 9.0 (Cary, NC, USA). All significant differences were set at  $P < 0.05$ , and highly significant differences were at  $P < 0.01$ .

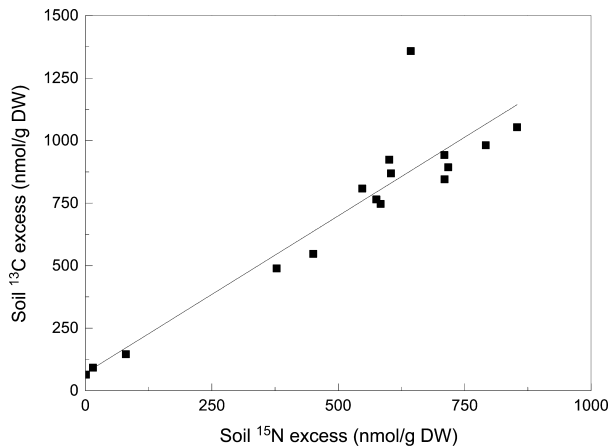
### Results

Enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  in plant samples was detected in mini-quadrats supplying  $^{13}\text{C}/^{15}\text{N}$  glycine at all of the four treatments (CK, G, W, and WG; Fig. 2). The slopes of regressions of  $^{13}\text{C}$  excess against  $^{15}\text{N}$  excess ranged 0.15–0.27, which indicated that 7.5–13.5% of the  $^{13}\text{C}/^{15}\text{N}$  labeled glycine was absorbed intact into plants (Näsholm et al. 1998). Correlation coefficients were high for all treatments, except for CK, due to one value with low  $^{13}\text{C}:^{15}\text{N}$  ratio. For sieved soil from the treated plots (Fig. 3), significant regression was found between excess  $^{13}\text{C}$  and  $^{15}\text{N}$  ( $P < 0.0001$ ), and 37% of the  $^{13}\text{C}$  label was lost from the plots.

The uptake rate of organic N by *K. humilis* meadow under different treatments lay in the range of  $0.11\text{--}0.33 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  (Fig. 4). Glycine uptake rate was significantly lower under warming treatments ( $P < 0.05$ ; Fig. 4). Compared to control treatment, warming decreased glycine uptake rate by 37% ( $P < 0.05$ ; Fig. 4). Under the condition of grazing, warming reduced plant organic N uptake by 44% ( $P < 0.05$ ; Fig. 4). Grazing itself increased glycine uptake rate (between CK and G treatments,  $t$ -test,  $P < 0.05$ ; Fig. 4), whereas under warming condition grazing did not affect organic N uptake by the *K. humilis* community (i.e., between W and WG treatments).

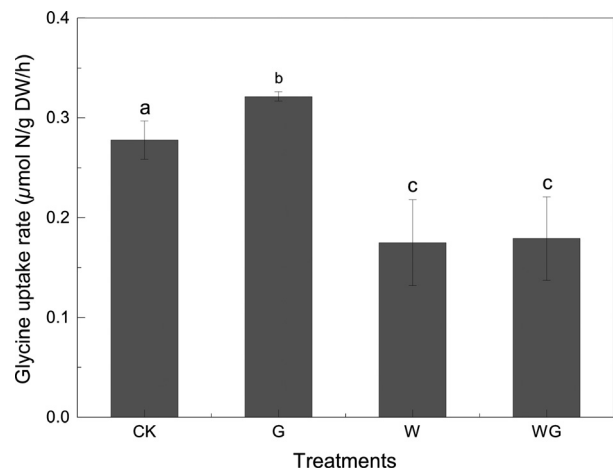


**Figure 2.** The relationship between  $^{13}\text{C}$  excess and  $^{15}\text{N}$  excess in roots and shoots of plants supplied with  $^{13}\text{C}$ - $^{15}\text{N}$ -glycine. The regression of  $^{13}\text{C}$  excess on  $^{15}\text{N}$  excess of glycine-treated plants is shown. The lowercase letters a, b, c, d represent CK, G, W, WG, respectively.



**Figure 3.** The relationship between  $^{13}\text{C}$  and  $^{15}\text{N}$  labeling of sieved soil treated with  $\text{U-}^{13}\text{C}_2$   $^{15}\text{N}$  glycine. Each symbol represents one soil sample. There is a highly significant, positive correlation between the two variables ( $y = 1.26x + 70.75$ ,  $r^2 = 0.85$ ,  $P < 0.0001$ ).

The N content of plants was significantly higher under warming treatment ( $P < 0.05$ ; Fig. 5). However, both DON and DIN contents were significantly lower under warming in 0–10 cm soil layer ( $P < 0.05$ ; Fig. 6). Soil organic matter C:N ratio did not differ among treatments, whereas warming highly significantly increased dissolved organic matter (DSOM) C:N ratio (DOC:DON,  $P < 0.001$ ; Fig. 7). Warming and grazing did not affect soil MBC or MBN (Fig. 8). Only  $\text{NO}_3\text{-N}$  alone was recognized as explanatory variable through the multiple stepwise regression analysis. Correlation analysis showed that

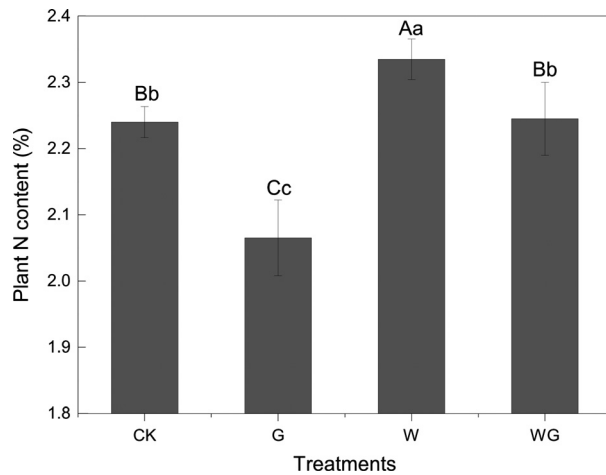


**Figure 4.** Mean uptake rates of the amino acid glycine under CK, G, W, WG treatments, expressed as  $\mu\text{mol-N-g}^{-1}$  DW  $\text{h}^{-1}$  (DW, dry weight). Amino acid uptake was significantly decreased by warming ( $P < 0.05$ ). Bars represent the mean of four replicates per treatment with error bars denoting  $\pm\text{SE}$ . Different lowercase letters indicate a significant difference in uptake rate among treatments ( $P < 0.05$ ).

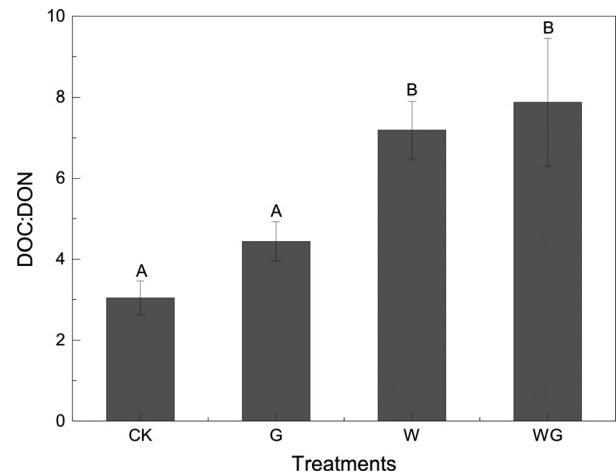
$\text{NO}_3\text{-N}$  explained more than 70% of the variation of plant glycine uptake among treatments (Fig. 9).

## Discussions

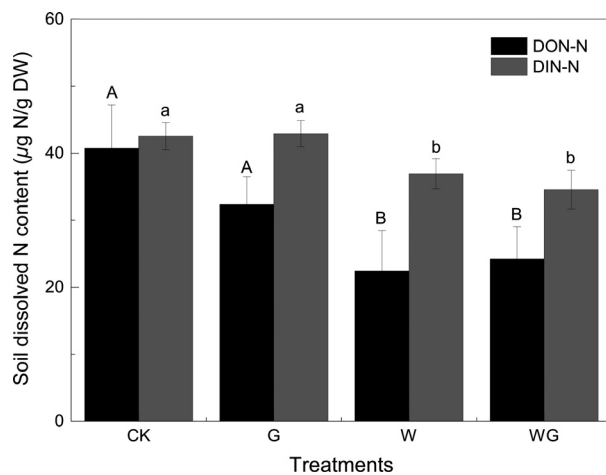
The uptake rates of organic N by *K. humilis* meadow were similar to that in arctic ecosystems (Chapin et al. 1993; Kielland 1994) and in the same alpine ecosystem



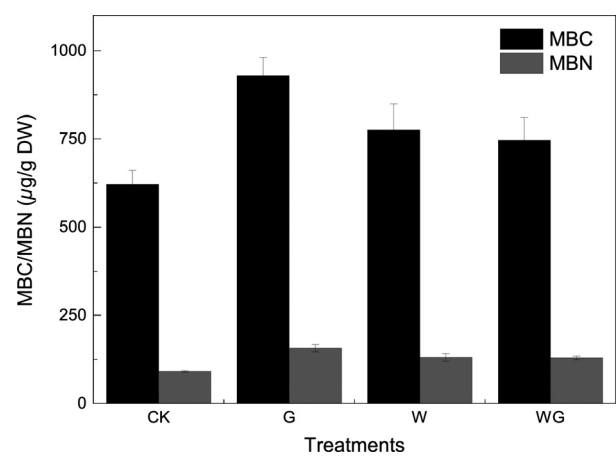
**Figure 5.** Plant N content expressed as percentage of total dry mass. Bars represent the mean of four replicates per treatment with error bars denoting  $\pm$ SE. Different lowercase letters indicate a highly significant difference of grazing effects ( $P = 0.0012$ ). Different uppercase letters indicate a highly significant difference of warming effects ( $P = 0.0090$ ).



**Figure 7.** Dissolved soil organic matter (DSOM) C:N ratio denoted by the ratio between soil dissolved organic C (DOC) and soil dissolved organic N (DON). Warming highly significantly increased DOC:DON ( $P = 0.0014$ ). Bars represent the mean of four replicates per treatment with error bars denoting  $\pm$ SE. Different uppercase letters indicate a highly significant difference of warming effects ( $P < 0.01$ ).



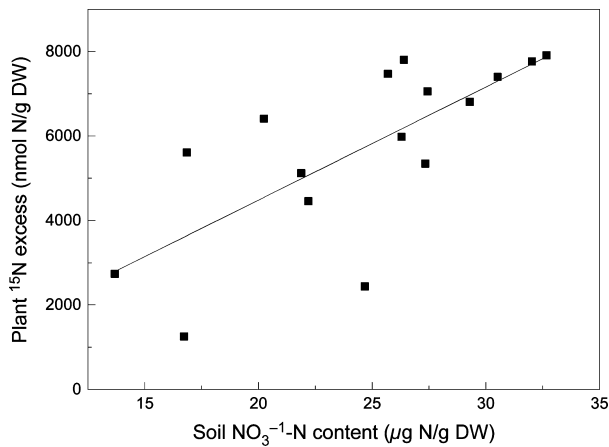
**Figure 6.** The 0–10 cm soil DON content and the 0–10 cm soil DIN content expressed as  $\mu\text{g}\cdot\text{N}\cdot\text{g}^{-1}$  DW. The DON content was significantly lower under warming ( $P = 0.03$ ). Significant decrease in 0–10 cm soil DIN content was observed under warming treatment ( $P = 0.01$ ). Bars represent the mean of four replicates per treatment with error bars denoting  $\pm$ SE. Different lowercase letters indicate a significant difference in DIN-N among treatments ( $P < 0.05$ ). Different uppercase letters indicate a significant difference in DON-N among treatments ( $P < 0.05$ ).



**Figure 8.** Microbial biomass C (MBC) and microbial biomass N (MBN) content ( $\mu\text{g}\cdot\text{g}^{-1}$  DW). Warming highly significantly increased DSOM C:N ( $P < 0.001$ ). Grazing significantly increased DSOM C:N ( $P < 0.05$ ). Bars represent the mean of four replicates per treatment with error bars denoting  $\pm$ SE. Different lowercase letters indicate a significant difference in MBC among treatments ( $P < 0.05$ ). Different uppercase letters indicate a significant difference in MBN among treatments ( $P < 0.05$ ).

reported by Xu et al. (2004). The atomic ratio of  $^{13}\text{C}$  to  $^{15}\text{N}$  in the double-labeled glycine is 2. During the 24 h, the measured atomic ratio in plants ranged 0.15–0.27 in this study, indicating 7.5–13.5% of the  $^{13}\text{C}/^{15}\text{N}$  glycine applied was absorbed intact into plants, close to reports on other herbaceous species (Chapin et al. 1993; Kielland

1994; Näsholm et al. 1998, 2000, 2001; Xu et al. 2004). Our experimental results showed that plants on the Qinghai–Tibetan Plateau can absorb FAA-N intact (Fig. 4). Metabolism of absorbed glycine in roots could result in losses of  $^{13}\text{C}$  through respiration (Schmidt and Stewart 1999). Therefore, the fraction of glycine-N taken up as intact amino acid by the plants was conservative in this



**Figure 9.** The relationship between soil  $\text{NO}_3\text{-N}$  and tracer  $^{15}\text{N}$  recovered in plants. Each symbol represents one soil sample. There is a significant, positive correlation between the two variables ( $y = 267.68x - 870.47$ ,  $r^2 = 0.53$ ,  $P = 0.0115$ ).

study. Furthermore, we found significant correlation between excess  $^{13}\text{C}$  and  $^{15}\text{N}$  ( $P < 0.0001$ ) for sieved soil in the miniplots (Fig. 3), indicating 37% of  $^{13}\text{C}$  added into the miniplots was lost from the soil. This likely resulted from rapid decarboxylation of the carboxyl-C of glycine during microbial metabolism (Fokin et al. 1993; Yan et al. 1996). The same reason also contributed to the lower atomic ratio of  $^{13}\text{C}$  to  $^{15}\text{N}$  in plants. Some researches indicated that the quantity of organic N in shoots was unstable, and mostly lower than that in roots due to quick metabolism of absorbed amino acids in shoots (Näsholm and Persson 2001; Näsholm et al. 2001; Xu et al. 2004; Gruffman et al. 2014). Therefore, the ratio, correlation coefficients (Fig. 2), and uptake rate (Fig. 4) could be higher in this study, if plant roots were used for isotopic analysis and calculation instead of the mixture of shoots and roots.

It is emphasized that the change of plant organic N uptake should be evaluated based on the variation of inorganic N absorption, to distinguish specific response of organic N uptake from overall change in TN uptake. A number of studies have examined uptake of  $^{15}\text{N}$  labeled inorganic N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) in comparison with organic N uptake responses. We did not test plant uptake of  $^{15}\text{N}$  labeled inorganic nitrogen or distilled water as a control due to the limited area of the manipulation experimental platform. To make a compensation, we used ANPP and plant nitrogen content to help estimate long-term changes in plant nitrogen uptake. On our platform, warming significantly increased ANPP regardless of grazing, whereas grazing reduced the response of ANPP to warming (from 2006 to 2010, Wang et al. 2012). Meanwhile, based on our measurement, warming highly significantly increased plant nitrogen content, while grazing highly significantly

decreased plant nitrogen content (Fig. 5). We speculate plants' overall nitrogen acquisition from soil increased under warming treatments. The effect of grazing on plants overall nitrogen acquisition was instable as ANPP under grazing varied with year, and in several years (2006, 2009, and 2010) grazing had no effect on ANPP.

We observed that warming decreased plant organic N uptake by 37% as compared to control treatment, and under the condition of grazing, warming reduced plant organic N uptake by 44%. We infer that plant regulated organic N uptake in response to warming, as the overall acquisition of nitrogen increased, not decreased, under warming treatments. Our previous study showed that labile C and N pools were enlarged under warming treatment (Rui et al. 2011). Rothstein (2014) reported that plants took up a higher fraction of amino acid-derived N after soil microorganisms transformed to inorganic forms in a relatively higher N availability site. His finding well supported theoretical proposal that dominant N form for plant shifted from FAA to inorganic N with the increment of soil N availability (Schimel and Bennett 2004). Our results of lowering FAA-N uptake rate in warming treatment backed up the above theory (Fig. 4).

A number of studies have demonstrated that FAA-N can be consumed by soil microorganisms rapidly (Mcfarland et al. 2010; Geisseler and Horwath 2014; Mansson et al. 2014), and the turnover rate may be controlled by overall decomposability of soil C and N limitation to microbial growth (Melillo et al. 1982; Murphy et al. 1998; Mcfarland et al. 2010). An excess of C, rather than N, resulted in a reduced uptake of low molecular weight dissolved organic nitrogen (LMWDON) by soil microbes because LMWDON was taken up primarily to fulfill the C requirement of soil microorganisms (Farrell et al. 2014). Therefore, the higher SOM C:N ratio corresponded to lower FAA-N assimilative rate by soil microbes (Mcfarland et al. 2010). Warming did not alter overall SOM C:N ratio, but increased DSOM C:N by 77–136% in this study (Fig. 7). Nevertheless, this may not mean a slower soil FAA-N turnover rate. On the contrary, FAA mineralization might be accelerated under warming in this study, partly due to direct effects of higher temperature on soil microbial activity. As shown in Figures 6 and 7, increased DSOM C:N stemmed from reduced DON content, but not elevated DOC content under warming. DOC content was nearly constant for all the treatments. As N absorbed by plants increased in warming treatments, labile C supply may be more restricted in this circumstance. Consequently, soil microorganisms used FAA-N more rapidly, partly as its C source, and released more inorganic N for plant uptake.

We observed no significant increase in MBC and MBN under warming treatment, consistent with a warming

study by open-top chambers in three *Kobresia*-dominant alpine meadows on the Qinghai-Tibetan Plateau (Fu et al., 2012). As shown by experimental observations and theoretical modeling, the size of the microbial biomass may acclimate to warming in long term, and microbial biomass was less important than microbial activity in predicting DSOM decomposition rates in such circumstance (Elliott et al. 1993; Waldrop and Firestone 2004).

In this study, grazing itself stimulated plants FAA uptake by 15%, opposite to the effect of warming, whereas under warming condition grazing did not affect organic N uptake (Fig. 4). However, we cannot rule out that the increase in organic N uptake were resulted from a stimulation in plant growth and consequently overall nitrogen demand, as we did not examine the changes in inorganic N uptake, nor can we estimate overall N uptake from changes in ANPP and plant N content. Although grazing also increased soil temperature to some extent (Wang et al. 2012), it had several lines of difference from warming treatment. (1) Grazing by animals tended to compact soil, increase soil temperature, and decrease soil moisture simultaneously. These may reduce soil microbial activity and nitrogen mineralization (Biondini et al. 1998; Bardgett and Wardle 2003), leading to a decrease of soil N supply. (2) Grazing not only reduced litter return, but also removed plant metabolically active and N-rich tissue. Thus, the soil was replenished by less and lower quality organic matter, clearly shown in low N content in grazing treatment (Fig. 5) and high C:N ratio in dung (Table 1). (3) Grazing promoted soil and vegetation heterogeneity by selective browse as well as uneven return of litter, urine, and dung, which were quite different in their C, N content and components. These factors decreased soil N supply overall, and soil microsites with more heterogeneous N supply status compared to nongrazing treatments. Plants in such soil environment absorbed more FAA-N (Schimel and Bennett 2004; McFarland et al. 2010; Rothstein 2014), in accordance with the result here (Fig. 4). It also suggested that grazing counteracted the effect of warming on plant FAA-N use, reflected in the similar FAA-N uptake rate in W and WG treatments (Fig. 4).

**Table 1.** Initial chemical composition of litter, dung, and soil.

|                | %C                | %N                | C:N                | DOC:DON |
|----------------|-------------------|-------------------|--------------------|---------|
| Litter         | 29.6 <sup>a</sup> | 1.5 <sup>a</sup>  | 19.8 <sup>b</sup>  | –       |
| Dung           | 26.7 <sup>a</sup> | 1.2 <sup>b</sup>  | 23.1 <sup>a</sup>  | –       |
| Soil (0–10 cm) | 9.45 <sup>b</sup> | 0.82 <sup>c</sup> | 11.55 <sup>c</sup> | 5.64    |

Litter, mixed litter of the community under the controlled warming and grazing experiment. Soil, 0–10 cm of the soil under the controlled warming and grazing experiment. Values followed by different letters for the same column means significant difference at 0.05 level. Data of litter and dung are based on Luo et al. (2010).

Previous study demonstrated that warming alone and moderate grazing did not significantly affect soil net N mineralization (Wang et al. 2012), which seemed inconsistent with our suggestion that warming accelerated FAA-N mineralization through increased temperature and increased C:N ratio. It should be cautious to the flaws in measurement and use of net mineralization rate, as pointed out by Schimel and Bennett (2004). Net N mineralization was calculated by initial and final concentration of soil DIN, ignored plant uptake of both FAA-N and inorganic N. On the other hand, both DON and DIN were highly dynamic in soil. They interconverted quickly, especially for DIN fixation (McFarland et al. 2010; Geisseler and Horwath 2014; Mansson et al. 2014).

We found plant glycine uptake rate was strongly positively correlated with NO<sub>3</sub>-N, which explained more than 70% of the variation in plant glycine uptake rate across all treatments (Fig. 9). Mansson et al. (2014) observed amino acid and inorganic N being equally valued in plant uptake and could compensate for each other in changing environments in *Festuca gigantea*. Plants, as relative weak competitors against soil microbes (Kuzakov and Xu 2013), acquired less FAA-N and switched to DIN for nitrogen under warming. Furthermore, plants had higher N content, thus lower C:N ratio, in warming treatment for 6 years (Table 1). This trend, if maintained, would improve labile C supply to soil microbes, accelerating FAA-N mineralization, and ameliorate N competition through litter return in long term. It also inferred that direct contribution of FAA-N to plants would decline under warming. In contrast, the high C:N ratio litter and dung (Table 1) would cut down labile C availability and enhance the direct uptake of FAA-N by plants in the long term. Organic matter decomposition and nutrient release from feces was faster than that of litter in short term (Ruess and McNaughton 1987; Ruess et al. 1989; Hobbs 1996) and was sensitive to warming (Xu et al. 2010). However, the remaining recalcitrant part would decompose more slowly in long run (Allison et al. 2010). Warming restored plant N content caused by grazing (Fig. 5 CK WG). Together with similar values in FAA-N uptake rate (Fig. 4 W WG), soil DON-N, and DIN-N content (Fig. 6), it seemed warming rectified soil N supply depression under grazing. As grazing was the common land-use type in alpine meadow on the Qinghai-Tibet Plateau, FAA-N is an important N source for plants. Future warming is likely to change soil N supply and N use pattern of both plants and soil microorganisms.

As a rule of thumb, the labeled glycine was added to less than 20% of the background level of soil total amino acids to avoid fertilization effect or perturbation of the plant-soil system (Gallet-Budynek et al. 2009). The



dilution effect could be corrected by the equation below (Mckane et al. 2002):

$$U_N = U_N^{15} \times \frac{C_{\text{available N}}}{C_{\text{N applied}}^{15}},$$

where  $U_N^{15}$ , the uptake of  $^{15}\text{N}$  after labeling;  $C_{\text{available N}}$ , the concentration of studied N form in the soil;  $C_{\text{N applied}}^{15}$ , the concentration of applied  $^{15}\text{N}$ -labeled compound in the soil.

We did not correct the uptake rate as the dilution effect was likely small based on the fact that the amount of labeled glycine added was equivalent to  $1.1 \text{ g-N}\cdot\text{m}^{-2}$ , approximately three times of the background value of total extractable AA-N in the upper 10 cm soil layer. Relatively high dose of labeled glycine was used in this research to ensure that enough  $^{13}\text{C}$  was applied and  $^{13}\text{C}$  abundance could be precisely measured in plant root and shoot samples so as to test whether plants took up amino acids directly, according to Chapin and Schimel (1996). This, however, led to some drawbacks including failure to mimic natural conditions of organic N concentration or switch from high-affinity amino acid transporters to low-affinity transporters in plant roots under elevated amino acid content in the soil (Näsholm et al. 2009). In our results, the value of  $^{13}\text{C}$  excess in plants ranged  $380\text{--}2300 \text{ nmol}\cdot\text{N}\cdot\text{g}^{-1} \text{ DW}$ , well above those in some other researches (Näsholm and Persson 2001; Näsholm et al. 2001). Application of lower amount of labeled amino acid to mimic more closely to natural concentrations should be tested in future studies in this area.

The study was carried out in a long-term temperature and grazing manipulation platform on the Tibetan Plateau (Wang et al. 2012). Several experimental treatments as well as sensors and flux monitoring chambers were deployed in each plot with total area of only about  $7 \text{ m}^2$ . We regretfully did not test plant uptake of  $^{15}\text{N}$  labeled inorganic nitrogen ( $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ ) or distilled water as a control due to the limited area and serious disturbance of other ongoing experiments. The information of inorganic nitrogen uptake could facilitate the explanation of the results in this study and untangle the underlying mechanisms.

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## Conflict of Interest

None declared.

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