

# Effects of grazing on the acquisition of nitrogen by plants and microorganisms in an alpine grassland on the Tibetan plateau

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## Abstract

*Background and aims* Nitrogen (N) limitation leads to intense competition between plants and soil

microorganisms for available N. However, it is unclear how grazing affects the acquisition of N by plants and microorganisms.

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**Methods** We conducted short-term  $^{15}\text{N}$  tracer experiments during the growing season (June, early growing season; July, mid-growing season; and September, late growing season) in an alpine grassland on the Tibetan Plateau to investigate the effects of grazing on the acquisition of  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, and glycine-N by plants and soil microorganisms. Three dominant plant species (one graminoid, *Kobresia pygmaea*, and two forbs, *Potentilla bifurca* and *Potentilla multifida*) were selected for the study. As these species represented >90% of the vegetation, the plant recovery of  $^{15}\text{N}$  reflected competition at the plant community.

**Results** Grazing decreased the recovery of  $^{15}\text{N}$  by soil microorganisms and plants by 46 and 69%, respectively, indicating that grazing strongly reduced the uptake of  $^{15}\text{N}$  by plants and microorganisms and altered the partitioning of  $^{15}\text{N}$  between them. Significant interactions were found between grazing, season and the different forms of N. In the absence of grazing, plants acquired relatively more N than soil microorganisms for the three forms of N in July and September, whereas the microorganisms obtained relatively more  $^{15}\text{N}$  glycine in July and all three forms of N in September under grazing conditions. Under grazing, the plant root biomass played an important role in controlling plant–microbial N acquisition.

**Conclusions** Grazing alters the partitioning of inorganic and organic N between plants and soil microorganisms by reducing microbial  $^{15}\text{N}$  recovery to a lesser extent than plant  $^{15}\text{N}$  recovery. This indicates that heterotrophic microorganisms play an important part in N cycling in N-limited ecosystems.

**Keywords** Organic N · Inorganic N · N partitioning · Grazing · Tibetan plateau

## Introduction

Plant growth in many terrestrial ecosystems is limited by the availability of nitrogen (N) (Vitousek and Howarth 1991). Soil microbes compete for the same soil resources as plants and they are mutually dependent on each other (Kuzuyakov and Xu 2013; Liu et al. 2016). Plants need mineralized N from soil microorganisms and the latter need labile organic substances from plant roots or litter (Kuzuyakov and Xu 2013). Clarifying the acquisition of N by plants and microorganisms is

essential to understanding the N cycle in ecosystems (Chen et al. 2015a).

Grazing decreases the pools of soil organic C (SOC) and N (Hafner et al. 2012; Jiang et al. 2016), changes plant community composition (Milchunas et al. 1993; Lu et al. 2017) and often decreases the soil microbial biomass (Jiang et al. 2016). Many studies have investigated the effects of grazing on the interactions between plants and soil microorganisms, including how grazing influences the soil microbial biomass by altering both the above- and below-ground functional traits of plants (Greenwood and McKenzie 2001; Xu et al. 2011a). However, comparatively little is known about the effects of grazing on the uptake of N by soil microorganisms and the partitioning of N between soil microorganisms and plants. Because N is a limiting nutrient for plant growth in alpine grasslands, elucidating how grazing affects the partitioning of N between plants and microbes will improve our understanding of how plant productivity responds to grazing.

Grazing can also change the forms of soil N through fecal and urinary inputs and can increase the amount of ammonium in the soil. Increasing the amount of ammonium could stimulate nitrification, leading to increased concentrations of nitrate (Rui et al. 2011; Wu et al. 2011), which, in turn, may affect the composition and diversity of the plant community (Wang et al. 2012; Yan et al. 2013) because dominant plant species prefer to absorb  $\text{NO}_3^-$  (Song et al. 2007; Xu et al. 2011b). Grazing in alpine grasslands generally decreases the cover of graminoids and increases the cover of forbs because grazing animals prefer to eat graminoids and their grazing can disturb graminoid flowering and seed formation (Wang et al. 2012). However, both positive and negative effects of grazing on the soil microbial biomass have been observed (e.g., Rui et al. 2011), suggesting complex effects of grazing on the partitioning of N acquisition between plants and soil microorganisms.

Plant-available N includes both inorganic (i.e.,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) (Chapin et al. 1993; Mansson et al. 2014) and organic forms (e.g., peptides and free amino acids; Hill et al. 2011). Coexisting plant species show resource partitioning in the uptake of various forms of N (McKane et al. 2002; Jiang et al. 2016). For instance, McKane et al. (2002) found that the dominant plant species in an arctic tundra environment preferentially took up the most abundant form of N (free amino acids), whereas the less dominant species used the less abundant forms of N ( $\text{NH}_4^+$ ). Similarly, the partitioning of

different forms of N between plants and soil microorganisms has been observed. For example, it has been shown that many dominant plant species in alpine grasslands prefer to take up  $\text{NO}_3^-$  (Song et al. 2007; Xu et al. 2011b), whereas soil microorganisms have a preference for  $\text{NH}_4^+$  (Xu et al. 2003; Xu et al. 2004).

Soil inorganic and organic N in terrestrial ecosystems often show strong seasonal variations (Farrell et al. 2011; Chen et al. 2015b; Jiang et al. 2016). Plant growth and the soil microbial communities also change over the season (Jaeger et al. 1999; Xu et al. 2011a; Gao et al. 2014) as does the uptake of N by plants and soil microorganisms (Jaeger et al. 1999; Liu et al. 2016). For example, plants take up more N than soil microorganisms early in the growing season, whereas soil microorganisms take up more N later in the growing season in alpine grasslands (Jaeger et al. 1999). The chemical niche differentiation of the N uptake of plants and microorganisms and competition for N also shows seasonal dependence (Kaye and Hart 1997; Liu et al. 2016). However, it is still unknown how grazing affects the acquisition of N by plants and microorganisms across the growing season. Therefore, it is important to take into account seasonal variations and the form of N in trying to understand N acquisition by plants and soil microorganisms under grazing conditions (Hodge et al. 2000).

The Tibetan Plateau is often referred to as the third pole of the Earth and has the highest mean elevation in the world; >30% of the area consists of alpine grassland (Piao et al. 2006; Shen et al. 2011). Low temperatures and low productivity make the alpine grassland of the Tibetan Plateau especially sensitive to human disturbance (Piao et al. 2006; Yang et al. 2013). Grazing is the main land use in the alpine grassland of the Tibetan Plateau and the intensity of grazing is forecast to gradually increase in the future (Wiener et al. 2003). Testing the effects of grazing on plant–microbial competition for inorganic and organic N may improve our understanding and predictive capability of changes in grassland productivity and sustainable development in this region.

To investigate this issue, we performed  $^{15}\text{N}$  tracer experiments under field conditions over one growing season. We tested the following hypotheses: (1) grazing decreases soil microbial N uptake – this was based on previous observations that grazing decreases the microbial biomass (Jiang et al. 2016); and (2) grazing changes the seasonal pattern of soil microbial and plant N

acquisition – this was based on previous observations that grazing changes plant growth and the available form of N in soils in a season-dependent manner (Jiang et al. 2016).

## Materials and methods

### Study site and target species

This study was conducted in an alpine grassland in Nagqu County on the Tibetan Plateau ( $30^\circ 31' - 31^\circ 55' \text{ N}$ ,  $91^\circ 12' - 93^\circ 02' \text{ E}$ , 4450 m a.s.l.). This location experiences a short growing season (from June to August). The average annual temperature is  $-2.1^\circ \text{ C}$  and the mean annual precipitation is approximately 400 mm, most of which falls from July to September. The mean annual evaporation is 1810 mm and the mean relative humidity is 51%. *Kobresia pygmaea* is the dominant plant species in the area (Jiang et al. 2016).

### Experimental design

Our experiment consisted of five random alpine meadow blocks of ungrazed and grazed plots ( $15 \times 15 \text{ m}$ ), respectively. The initial vegetation cover and species composition were uniform across all ungrazed and grazed plots (Jiang et al. 2016). The ungrazed plots had not been grazed since 2005. The grazed plots were subjected to free grazing by sheep at a density of approximately 0.36 sheep per hectare, which is considered as moderate grazing (Wang et al. 2005). At each block, four random  $^{15}\text{N}$  labelling plots ( $10 \times 10 \text{ cm}$ ) were established in each block. To avoid the potential confounding effects of residual N in microbial and/or plant biomass from previous labeling events, care was taken that there was at least 2 m between each plot.

Each of the four plots in the blocks were injected simultaneously on 27 June (early growing season), 20 July (mid-growing season) and 5 September (late growing season) with one of the following solutions: (1) a 9-ml volume of water without the addition of  $^{15}\text{N}$ ; (2) water supplemented with  $(^{15}\text{NH}_4)_2\text{SO}_4$  (99.2 at.%  $^{15}\text{N}$  enrichment); (3) water supplemented with  $\text{Na}^{15}\text{NO}_3$  (99.2 at.%  $^{15}\text{N}$  enrichment); or (4) water supplemented with glycine- $^{15}\text{N}$  (98.7 at.%  $^{15}\text{N}$  enrichment). The concentration of  $^{15}\text{N}$  was adjusted to  $90 \text{ mg N L}^{-1}$  for each of the three solutions. We selected  $^{15}\text{N}$ –glycine as an isotopic label for organic N uptake because glycine is

one of the most common amino acids found in alpine grassland soils (Jiang et al. 2016). We used  $(^{15}\text{NH}_4)_2\text{SO}_4$  and  $\text{Na}^{15}\text{NO}_3$  as the labelling material for two main reasons: Firstly, they are often used as fertilizers, and secondly, they are often used in  $^{15}\text{N}$  labeling experiments. Additionally, addition of  $\text{Na}^+$  has no appreciable effect on plant growth (Xu et al. 2014).

### Sampling and isotopic analysis

Two hours after the injection of the  $^{15}\text{N}$ -labeled tracer, soil samples  $10 \times 10 \times 10$  cm in size were collected. Above- and below-ground plant material was collected in each of the soil samples. The soil samples were stored in a chilled container and transported to the laboratory to determine the N in the soil microbial biomass. The plant roots were first rinsed with tap water and then rinsed for 30 min with 0.5 mM  $\text{CaCl}_2$  to remove any adsorbed  $^{15}\text{N}$  on the surface of the roots (Xu et al. 2006, 2011b). The roots were then washed again with distilled water to remove any excess  $\text{CaCl}_2$  solution. The above- and below-ground plant material was dried at 70 °C, after which the material was weighed to determine the amount of biomass. The plant material was ground to a fine powder using a ball mill (MM400, Retsch, Haan, Germany), after which total N and  $^{15}\text{N}$  were determined in subsamples of about 2 mg using a Flash EA1112 analyzer and ConFlo III software (MAT 253, Finnigan MAT, Germany). The soil subsamples were air-dried to measure the SOC, soil total N, glycine concentration, and pH. Gas chromatography-mass spectrometry (GC-MS) was used to determine the glycine concentrations in the soil samples (Näsholm et al. 1987). SOC was determined using the  $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$  oxidation method (Nelson and Sommers 1982) and the soil total N was determined using the Kjeldahl digestion method (Kjektec System 1026 Distilling Unit, Sweden). One soil core (3 cm diameter, 10 cm depth) adjacent to every plot was collected during the labeling treatment in June, July, and September in 2013, and then dried for 48 h at 105 °C to determine the soil water content. The soil bulk density was determined using a coring method (Wang et al. 2008). The amount of N in the microbial biomass was determined using the chloroform fumigation-extraction method (Brookes et al. 1985).

Samples were taken 2 h after the injection of the  $^{15}\text{N}$ -labeled tracer because the half-life of glycine in alpine grassland soils is >12 h (Unteregelsbacher et al. 2012). This suggests that glycine mineralization

would be negligible over the 2-h study period and therefore the  $^{15}\text{N}$  levels in plants in response to the glycine treatment should accurately reflect the uptake of intact glycine by the plants within the 2 h time period (Unteregelsbacher et al. 2012).

### Calculations

The immobilization of  $^{15}\text{N}$  by the microbial biomass was calculated based on the difference in the microbial biomass  $^{15}\text{N}$  between the extractable N concentration in the fumigated and the unfumigated samples using a conversion factor (kEN) of 0.45 (Lovell et al. 1995).

The plant atom percentage excess  $^{15}\text{N}$  (APE) was calculated using the following formula:

$$\text{APE} = \text{at.}\%^{15}\text{N}_{\text{added}} - \text{at.}\%^{15}\text{N}_{\text{control}} \quad (1)$$

where  $^{15}\text{N}_{\text{added}}$  corresponds to the atomic fraction in the plots with added  $^{15}\text{N}$  and  $^{15}\text{N}_{\text{control}}$  refers to the mean value in the respective control plots with added water. The recovery of  $^{15}\text{N}$  by plants and microorganisms was calculated by multiplying the N concentration in each plot by the mass per square meter and their APE, divided by the total amount of  $^{15}\text{N}$  added per square meter (Xu et al. 2011b). We measured the  $^{15}\text{N}$  recovery at the plant community level and separately for each of the three dominant plant species: one graminoid (*K. pygmaea*) and two forbs (*Potentilla bifurca* and *P. multifida*).

### Data analysis

Repeated measures mixed models analysis was performed to test the main and interactive effects of grazing (ungrazed and grazed), N form ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and glycine-N) and season (June, July and September) on  $^{15}\text{N}$  recovery by the microbial biomass,  $^{15}\text{N}$  recovery by plants at the community levels, and the ratio of the  $^{15}\text{N}$  recovery by the microbial biomass to the  $^{15}\text{N}$  recovery by the plants at the community levels. Duncan's new multiple range test was used for post hoc comparisons. Post hoc tests were used to examine the differences between ungrazed and grazed plots. For the mixed linear models, assumptions of the homogeneity of variance were determined using Levene's test. If the assumptions were not met, the data were log-transformed prior to analysis. A linear correlation was done between the plant root biomass with ratio of microbial biomass N

recovery to plant biomass N recovery in the grazing and ungrazed plots.

Further, we performed structural equation modeling to test the direct and indirect controls of grazing, biological traits (plant root biomass and soil microbial biomass), soil physical traits (soil water content, soil bulk density), soil chemical traits (soil total N and C) and plant species composition on plant biomass and microbial biomass N recovery (see Fig. S1 for the a priori model). Insignificant relationships were removed through model modification indices and these removals were tested using the Akaike information criterion (IBM SPSS Amos, v.23). Model fit was tested using a likelihood ratio including  $X^2$  and a comparative fit index. Adequate model fits are indicated by a nonsignificant  $X^2$  test ( $P < 0.05$ ), a high probability of a low root mean square error of approximation value ( $P > 0.05$ ) and high comparative fit index ( $>0.95$ ) (de Vries and Bardgett 2016).

## Results

Effects of grazing and season on  $^{15}\text{N}$  recovery by microbial biomass

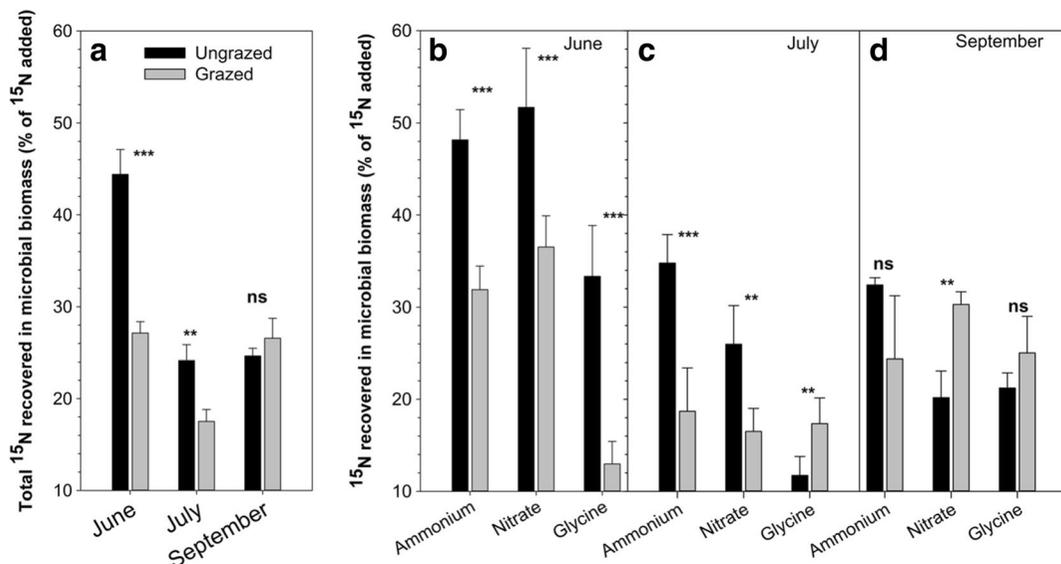
The form of N, grazing and season all affected  $^{15}\text{N}$  recovery in the microbial biomass (Table S1). Overall,

grazing decreased the amount of  $^{15}\text{N}$  recovered in the microbial biomass by 46% (Fig. S2b). Grazing significantly decreased the total  $^{15}\text{N}$  recovered in microbial biomass in June and July ( $P < 0.05$ ), but not in September (Fig. 1a). The effects of grazing on  $^{15}\text{N}$  recovery of different N forms also depended on season. Grazing decreased the recovery of the three forms of  $^{15}\text{N}$  by soil microorganisms in June as well as the recovery of  $^{15}\text{NH}_4^+\text{-N}$  and  $^{15}\text{NO}_3^-\text{-N}$  in July, whereas grazing increased the recovery of glycine- $^{15}\text{N}$  in July and the recovery of  $^{15}\text{NO}_3^-\text{-N}$  in September (Fig. 1b, c and d).

Effects of grazing and season on  $^{15}\text{N}$  recovery by plants

Grazing and season affected the amounts of  $^{15}\text{N}$  recovered in plants (Table S1).  $^{15}\text{N}$  recovery in plants at the community level was lowered by 69% in the grazed plots compared with the ungrazed plots (Fig. S3b). In the ungrazed plots, the lowest amount of  $^{15}\text{N}$  recovered in plants at the community level was found in June, whereas more  $^{15}\text{N}$  was recovered in July and September. In the grazed plots, the lowest amount of  $^{15}\text{N}$  recovered by plants at the community level was found in June and September, whereas the highest amount of  $^{15}\text{N}$  was recovered in July (Fig. S3d).

Analysis of the recovery of different N forms by plant biomass at the community level showed that grazing



**Fig. 1** Total  $^{15}\text{N}$  recovered in microbial biomass (% of added  $^{15}\text{N}$ ) from ammonium- $^{15}\text{N}$ , nitrate- $^{15}\text{N}$  and glycine- $^{15}\text{N}$  two hours after  $^{15}\text{N}$  injection at 0–10 cm soil depth during the growing season (June, July and September). Values are means ( $\pm$  SE) of

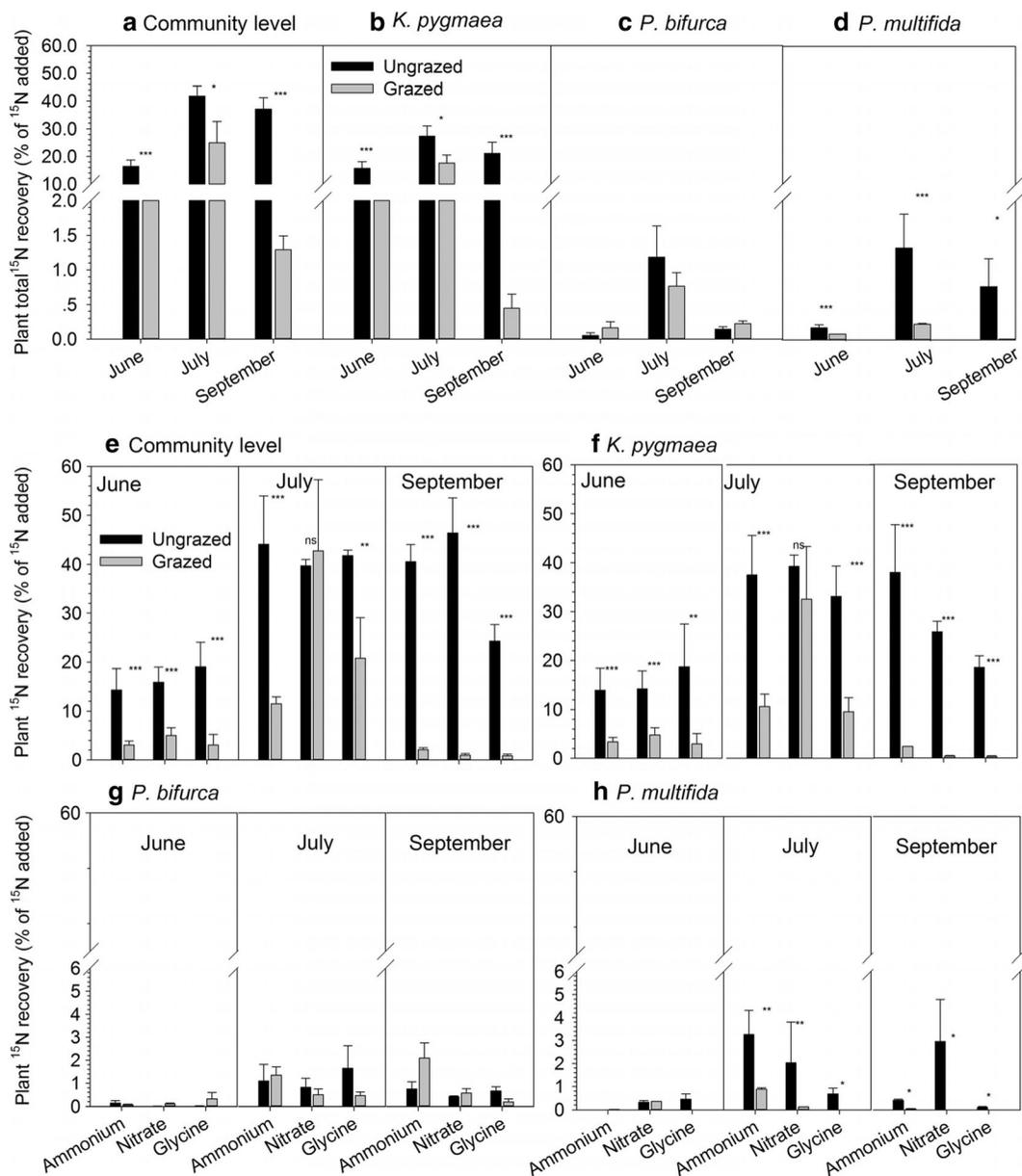
five replicates. Asterisks (\*\*, \*\*\*) indicate significant differences between ungrazed and grazed plots at  $P < 0.01$  and  $P < 0.001$  level respectively while ns indicates no significant difference ( $P > 0.05$ )

decreased the uptake of  $^{15}\text{NH}_4^+\text{-N}$  and glycine- $^{15}\text{N}$  in June, July and September (Fig. 2e), but grazing only decreased the uptake of  $^{15}\text{NO}_3^-\text{-N}$  in June and September (Fig. 2e). The pattern of  $^{15}\text{N}$  recovered in *K. pygmaea* was consistent with the amount of  $^{15}\text{N}$  recovered in the whole plant community (Fig. 2f). Grazing decreased the amount of  $^{15}\text{N}$  recovered in all forms in *P. multifida* in July and September, whereas grazing

had no effect on the amount of  $^{15}\text{N}$  recovered in *P. bifurca* (Fig. 2g and h).

Effects of grazing and season on the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass  $^{15}\text{N}$  recovery

Grazing and season both affected the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass  $^{15}\text{N}$  recovery at

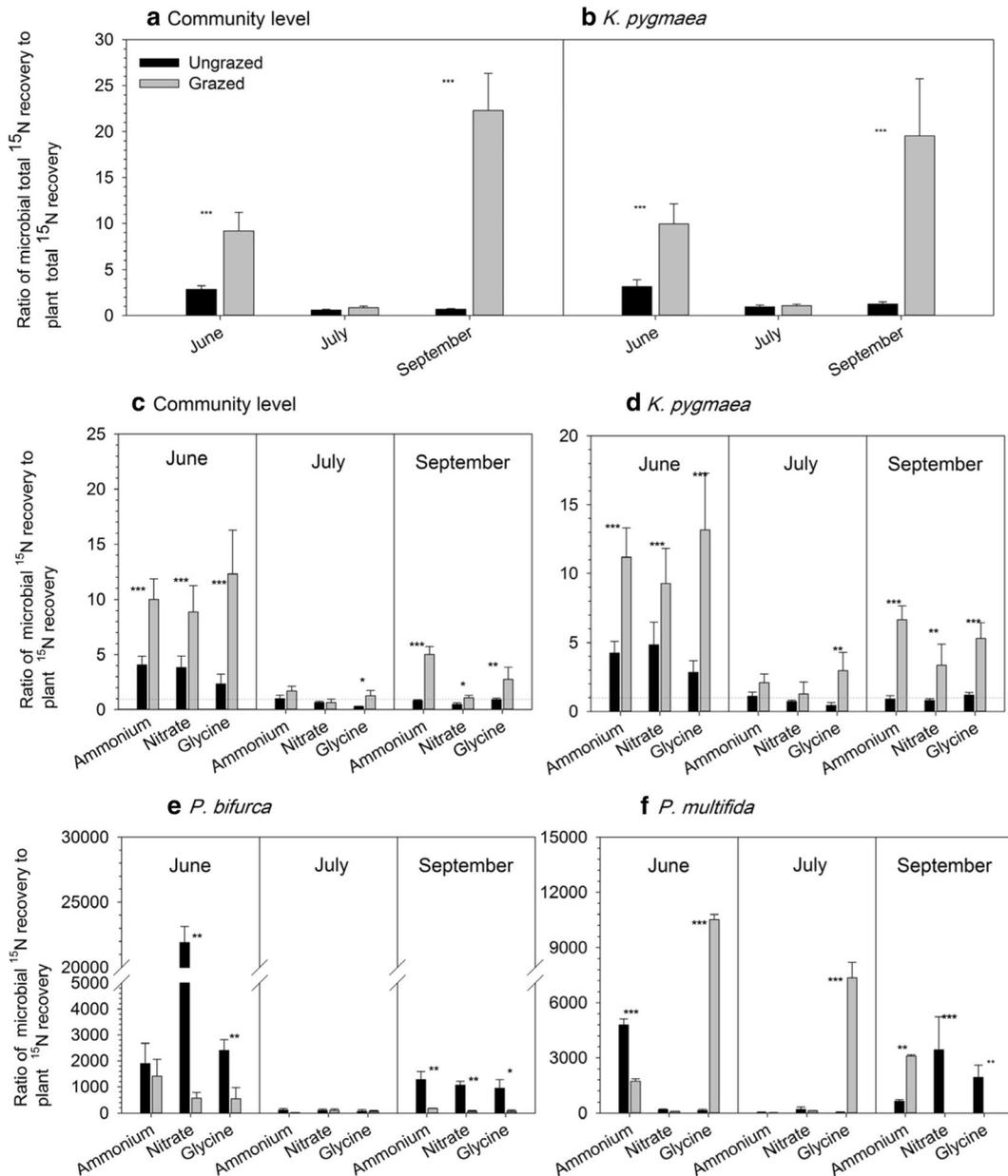


**Fig. 2** Total  $^{15}\text{N}$  recovered by plants (% of added  $^{15}\text{N}$ ) from ammonium- $^{15}\text{N}$ , nitrate- $^{15}\text{N}$  and glycine- $^{15}\text{N}$  two hours after  $^{15}\text{N}$  injection at 0–10 cm soil depth during the growing season (June, July and September). Values are means ( $\pm$  SE) of five

replicates. Asterisks (\*, \*\*, \*\*\*) indicate significant differences between ungrazed and grazed plots at the  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  level respectively

the community level (Fig. S4b, c; Table S1). Grazing increased the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass  $^{15}\text{N}$  recovery at the community level in both June and September; however, no effects of grazing were observed in July ( $P < 0.05$ ) (Fig. 3a and b). Analysis of the results for the three forms of  $^{15}\text{N}$  showed

that grazing increased the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass N recovery of  $^{15}\text{NH}_4^+\text{-N}$  and  $^{15}\text{NO}_3^-\text{-N}$  at the community level in June and September while grazing increased the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass  $^{15}\text{N}$  recovery throughout the season (Fig. 3c, d).



**Fig. 3** Ratios of soil microbial total  $^{15}\text{N}$  recovery to plants biomass total  $^{15}\text{N}$  recovery at the community level (panel a) and for *K. pygmaea* (panel b) and ratios of soil microbial  $^{15}\text{N}$  recovery to plants biomass  $^{15}\text{N}$  recovery from ammonium- $^{15}\text{N}$ , nitrate- $^{15}\text{N}$  and glycine- $^{15}\text{N}$  at the community (c) and at the plant species level

(d-f) two hours after  $^{15}\text{N}$  injection at 0–10 cm soil depth during the growing season (June, July and September). Values are means ( $\pm$  SE) of five replicates. Asterisks (\*, \*\*, \*\*\*) indicate significant differences between ungrazed and grazed treatment at the  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  level, respectively

Factors affecting soil microbial and plant  $^{15}\text{N}$  recovery

Structural equation modeling showed that grazing negatively affected the microbial  $^{15}\text{N}$  recovery either directly or indirectly through affecting the soil total N concentration (Fig. 4). Grazing negatively affected the plant  $^{15}\text{N}$  recovery indirectly by affecting the plant root biomass and soil water content (Fig. 4).

Our results showed that grazing changed the effect of the plant root biomass on the ratio of microbial biomass N recovery to plant biomass N recovery at *K. pygmaea* grassland. For example, the plant root biomass was significantly correlated with the ratio of microbial biomass N recovery to plant biomass N recovery under grazing ( $r^2 = 0.42$ ;  $p = 0.01$ ), but there was no significant effect on the ungrazed plots ( $p > 0.05$ ).

## Discussion

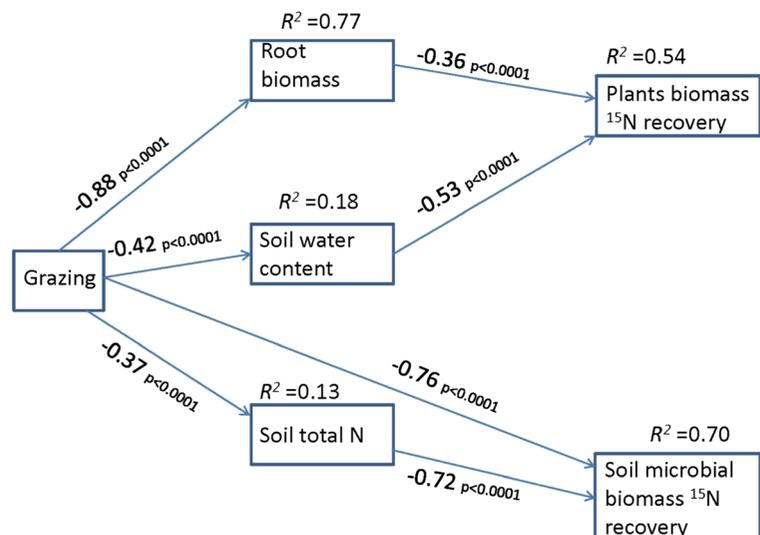
Plant and soil microbial N uptake from inorganic ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) and organic (glycine) forms of N was evaluated by short-term  $^{15}\text{N}$  tracer experiments in an alpine grassland in Tibet, China. The effect of grazing on the soil microbial N uptake and the partitioning between plants and soil microorganisms was investigated at three time points during one growing season.

Effects of grazing on  $^{15}\text{N}$  recovery by microbial biomass

Our results support our first hypothesis that grazing decreased soil microbial  $^{15}\text{N}$  uptake, mostly because grazing decreased the biomass of microorganisms in the soils (Jiang et al. 2016). Our structural equation models also showed that grazing directly affected the recovery of soil microbial  $^{15}\text{N}$  (Fig. 4). However, grazing also decreased soil microbial  $^{15}\text{N}$  recovery through decreased soil total N (Fig. 4). Trampling by livestock compacts the soil and decreases the air permeability, which, in turn, can decrease the soil microbial biomass or change the soil microbial community structure (Yang et al. 2013).

The effect of grazing on soil microbial N uptake depended on the form of N and on the season. Some previous studies have found that the soil microbial community preferentially takes up  $\text{NH}_4^+$  because of the high energy costs associated with the uptake of  $\text{NO}_3^-$  (Tiedje 1982; Recous et al. 1990), although contrasting results have also been found (Kaštovská and Šantrůčková 2011). Our results showed that the preference of the soil microbial community for the form of N depended on both grazing and season. For example, in the ungrazed plots, soil microorganisms showed no preference between the three forms of N in June, but preferred  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in July and  $\text{NH}_4^+$  in September (Fig. 1). On the other hand, in the grazed plots, the soil microorganisms preferred  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in June, but showed no preference between the three forms of N in

**Fig. 4** The most parsimonious model for explaining  $^{15}\text{N}$  recovery of plant biomass and soil microbial biomass. The arrows indicate the relationships, supplemented by standardized path coefficients and  $P$ -values.  $R^2$  values denote the amount of variance explained by the model for the response variables. The model fitted the data well ( $\chi^2 = 13.213$ ,  $df = 12$ ,  $P = 0.486$ ; comparative fit index = 1.000; Akaike information criterion = 4826.5)



July and September. A possible explanation is that grazing increased the soil  $\text{NO}_3^-$  concentration in July, resulting in a change in the soil microbial N preference (Saggar et al. 2004; Rui et al. 2011; Jiang et al. 2016). Kuster et al. (2016) also found that increasing the concentration of soil inorganic N changed the preference of plants and microorganisms for different forms of N.

#### Effects of grazing on the ratio of microbial versus plant N recovery

Grazing increased the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass  $^{15}\text{N}$  recovery, indicating a relative increase in microbial competition for N. Grazing also decreased the soil water content, soil total N and SOC concentration, all of which contributed to the increase in the ratio of microbial biomass  $^{15}\text{N}$  recovery to plant biomass  $^{15}\text{N}$  recovery.

Our results support our second hypothesis that grazing changes the seasonal pattern of the acquisition of N by plants and microorganisms. We found that two hours after the application of  $^{15}\text{N}$ , the plants had acquired relatively more N than the soil microorganisms for all three N forms in the ungrazed plots in July and September, whereas the soil microorganisms obtained relatively more glycine-N in July and all three forms of N in September in the grazed plots. Plant and microbial N acquisition is modulated by the availability of soil N, the distribution of microorganisms and the temporal mechanism of soil microorganism and plant root turnover (Jonasson et al. 1999; Liu et al. 2016). Drivers of the acquisition of available N by plants and microorganisms include the soil water content and the soil C and N concentrations. Ungrazed conditions are often associated with relatively low soil moisture content (Wang et al. 2012; Jiang et al. 2016). A higher soil moisture content favors the production of low molecular weight organic N compounds from the turnover of organic matter (e.g. amino acids), which can diffuse through the soil to the root surface (Kuzayakov and Xu 2013; Dijkstra et al. 2015).

Our results did not support the hypothesis proposed by Xu et al. (2011a, b) and Kuzayakov and Xu (2013) that the acquisition of inorganic N by plants and microorganisms is driven by plant root variations at a temporal or spatial scale in alpine *Kobresia humilis* grassland. This may be due to differences in the composition of the plant species. In *K. humilis*

grassland, the dominant plant species are *K. humilis*, *Stipa aliena*, *Poa* sp., *Festuca ovina* and *Gentiana aristata*, including both early flowering and late flowering plant species, which results in variation in plant root biomass in different seasons (Xu et al. 2011a, b). However, in our *K. pygmaea* grassland, the root biomass of *K. pygmaea* accounted for almost 80% of the plant community biomass and the early flowering and longevity of *K. pygmaea* resulted in relatively small variations in the root biomass between June and September (Jiang et al. 2016). Grazing decreased the percentage of *K. pygmaea* in the plant biomass and increased the percentage of *P. bifurca* and *P. multifida*, two late flowering plant species, which contributed to the difference in the plant root biomass from June to September (Jiang et al. 2016). Another explanation for this may be that the N limitation in *K. pygmaea* grasslands may not be as severe as in *K. humilis* grassland (Yang et al. 2010).

The increase in the microbial acquisition of glycine-N, but not of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N, in July under grazing conditions indicates that grazing increases the microbial acquisition of organic N compared with inorganic forms of N because soil microorganisms are usually more C-limited than N-limited in terrestrial ecosystems (Demoling et al. 2007; Jones et al. 2008; Sistla and Schimel 2012). It has been suggested that soil microorganisms take up glycine effectively in low C and N grasslands (Bardgett et al. 2003). Glycine provides both C and N for soil microorganisms, which are often limited by the amount of C and energy available (Blagodatskaya and Kuzyakov 2013; Hobbie and Hobbie 2013). In our experiment, grazing decreased the soil C/N ratio as a result of the removal of C (Kalbitz et al. 2000; Bardgett et al. 2003; Jiang et al. 2016) and the microbial biomass of N under grazing was significantly positively correlated with the concentration of SOC ( $F = 3.46$ ;  $P = 0.03$ ). And, our results indicated that grazing can also affect the soil microbial and plant acquisition of N by affecting the amount of SOC and soil total N. These results suggest that the concentration of SOC may be more important for the microbial community in grazed plots than in ungrazed plots. More general, previous studies have shown that heterotrophs control the N cycle in arid and semiarid grasslands (Crenshaw et al. 2008; Jirout et al. 2013; Marusenko et al. 2013) and can play an important part in retaining soil N in low-nutrient ecosystems (de Vries and Bardgett 2012; de Vries et al. 2015).

## Conclusions

Grazing decreased soil microbial N recovery, but increased the ratio of microbial biomass N recovery to plant biomass N recovery which means that microorganisms play an important role in keeping soil N within the ecosystem in low-N ecosystems. Grazing also changed the seasonal patterns of plant and microbial N acquisition. The increased acquisition of organic N by soil microorganisms under grazing provides new evidence that heterotrophic microorganisms may be limited by C under grazing. As such, taking into account the effects of grazing on the partitioning of inorganic and organic N by plants and microorganisms improves our understanding and predictive capability about changes in grassland productivity and the sustainable development of grasslands.

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