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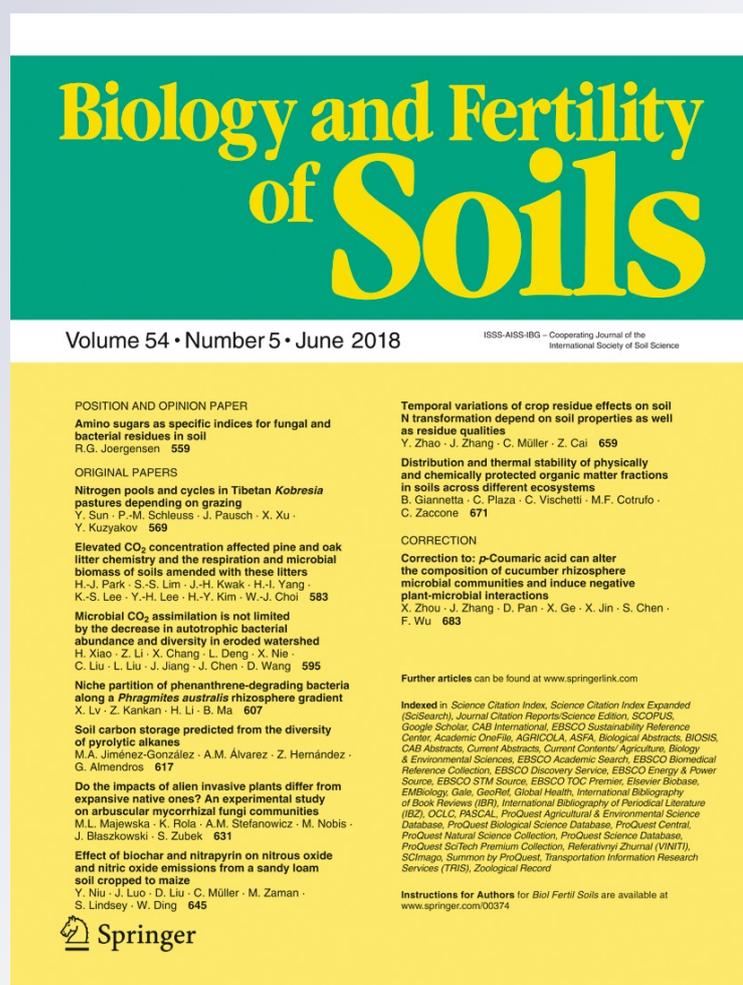
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Nitrogen pools and cycles in Tibetan *Kobresia* pastures depending on grazing

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Abstract

Kobresia grasslands on the Tibetan Plateau comprise the world's largest pastoral alpine ecosystem. Overgrazing-driven degradation strongly proceeded on this vulnerable grassland, but the mechanisms behind are still unclear. Plants must balance the costs of releasing C to soil against the benefits of accelerated microbial nutrient mineralization, which increases their availability for root uptake. To achieve the effect of grazing on this C-N exchange mechanism, a $^{15}\text{NH}_4^+$ field labeling experiment was implemented at grazed and ungrazed sites, with additional treatments of clipping and shading to reduce belowground C input by manipulating photosynthesis. Grazing reduced gross N mineralization rates by 18.7%, similar to shading and clipping. This indicates that shoot removal by grazing decreased belowground C input, thereby suppressing microbial N mining and overall soil N availability. Nevertheless, NH_4^+ uptake rate by plants at the grazed site was 1.4 times higher than at the ungrazed site, because plants increased N acquisition to meet the high N demands of shoot regrowth (compensatory growth: grazed > ungrazed). To enable efficient N uptake and regrowth, *Kobresia* plants have developed specific traits (i.e., efficient above-belowground interactions). These traits reflect important mechanisms of resilience and ecosystem stability under long-term moderate grazing in an N-limited environment. However, excessive (over)grazing might imbalance such C-N exchange and amplify plant N limitation, hampering productivity and pasture recovery over the long term. In this context, a reduction in grazing pressure provides a sustainable way to maintain soil fertility, C sequestration, efficient nutrient recycling, and overall ecosystem stability.

Keywords *Kobresia pygmaea* · Gross mineralization · Nitrogen uptake · ^{15}N labeling · Intensive grazing · Grassland management

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Introduction

The Tibetan Plateau hosts the world's largest and highest grasslands and one of the most sensitive and fragile ecosystems, vulnerable to global environmental change and anthropogenic activities (e.g., Cui et al. 2006; Babel et al. 2014; Chen et al. 2014). The Tibetan grasslands play an important role in ecosystem functioning, e.g., soil carbon (C) storage (ca. 7.4 Pg C), water and soil conservation, and climate regulation (Yang et al. 2008; Lin et al. 2017). Tibetan grasslands support a diversity of plants, livestock, and local pastoral communities (Kang et al. 2007). The alpine Cyperaceae mats of *Kobresia pygmaea* are the major constituent of the Tibetan grasslands and cover an area of 450,000 km², ranging from 3000 to nearly 6000 m in altitude (Miehe et al. 2008, 2017). The grazing lawns grow 2–4 cm high and develop a firm closed root system in the upper 30 cm of soil (termed *Kobresia* turf, Kaiser et al. 2008). Consequently, *Kobresia* root mats are characterized by high quantities of roots and rhizogenic

organic matter that contribute to the large stores of C, nitrogen (N), and other nutrients (Kaiser et al. 2008). Soil C and N dynamics in response to land use change represent a potentially important feedback to climate (Harris 2010; Chen et al. 2013).

The *Kobresia* grasslands have developed as the consequence of over ca. 8 thousand years of moderate grazing (Miehe et al. 2009, 2014, 2017), and nowadays in Northern Tibet, they still provide forage resources for livestock equivalent to more than 1.7 million sheep (Feng et al. 2017). *K. pygmaea* has been evolutionarily selected due to its specific traits reflecting strategies of both resistance (through avoidance) and tolerance (through re-growth capacity): tiny leaves that prevent complete grazing removal and a very large and durable root system that ensures efficient water and nutrient uptake and storage (Xu et al. 2014; Schleuss et al. 2015) and prevents trampling damage (Kaiser et al. 2008); a mixed reproductive strategy also improves their competitiveness compared to other plants (Seeber et al. 2015).

The short-term compensatory growth of *K. pygmaea* may be undermined by serious restrictions on plant re-growth and reproduction due to long-term overgrazing. Indeed, semi-sedentary management has replaced the traditional migratory management and increased the number of livestock since the late 1970s; this has contributed to the degradation of about 30% of the grasslands (Zhou et al. 2005). The subsequent deterioration of the *Kobresia* turf induced further losses of C and N (Wang et al. 2005b; Su et al. 2015). To counter degradation, the central and regional government has launched a series of ecological restoration projects since 2004. Excluding grazing by fencing has been proposed as one restoration practice to balance the stocking rate with grassland productivity. However, the effects on ecosystem recovery of *Kobresia* grassland are not sufficiently understood (Yu et al. 2016).

Besides grazing stress, plant growth in such harsh alpine environments is co-restricted by numerous abiotic factors, such as high solar radiation, low temperature and precipitation, a short vegetation period, and nutrient deficiency (Callaway et al. 2002; Miehe et al. 2017). Among the nutrients, N is most limited and its availability has a major impact on net primary production in Tibetan grasslands. This has been confirmed by previous N fertilization experiments (Song et al. 2007; Seeber et al. 2015; Wang et al. 2015; Zong et al. 2016). The prevailing low N availability in Tibetan grassland is mainly because (1) most N is in organic forms and not directly plant available, (2) N mineralization from organic forms is suppressed by low temperature (Wang et al. 2016), (3) N availability has strong temporal and spatial variability during the short growing seasons (Song et al. 2007; Xu et al. 2011a, b), and (4) large amounts of N have been removed from the ecosystem, since intensive grazing hampers the natural livestock-mediated N redistribution: most of the yak dung is used as a fuel source by Tibetan nomads, and a

part of urine is easily lost through volatilization and nitrate leaching (Luo et al. 2010).

K. pygmaea faces two major challenges under grazing: First, N should be captured efficiently by roots; second, mineralization of organic N forms should be enhanced (Kuzyakov and Xu 2013; Dijkstra et al. 2016). In fact, *K. pygmaea* invests a very high proportion of assimilated C in root production (with root to shoot ratios of up to 20) to compete for N with microorganisms and coexisting plants (Xu et al. 2011a, b; Kuzyakov and Xu 2013) and develops chemical (N forms) and/or temporal niche differentiation (Xu et al. 2011a, b; Jiang et al. 2017). In addition, it has become increasingly clear that plants can stimulate microbial growth, activity, and enzyme production by providing labile and energy-rich C compounds. The microbes accelerate soil organic matter (SOM) decomposition and increase N availability through rhizosphere priming effects (Kuzyakov 2002; Jones et al. 2009). Root exudates are known to be the key C inputs in this cold, semi-arid ecosystem (Farrar and Jones 2000; Cleveland et al. 2004). However, grazing alters the amount of photosynthetic assimilates and their allocation belowground, which, in turn, affects SOM mineralization and nutrient uptake by plants, with feedback to net primary production and C sequestration (Paterson and Sim 2000; Sun and Wang 2016a). In this N-limited ecosystem, we assume that *K. pygmaea* interacts with microorganisms for an improved N supply, which is highly important for sustaining regrowth after grazing. Such interactions also help to explain the existence and long-term stability of this unique ecosystem despite natural and human disturbance over the past millennia.

Nonetheless, major changes in pasture management (i.e., intensive grazing) and climate in recent decades have strongly impacted the plant-soil system, with unknown consequences for N pools and cycles (e.g., exchange mechanisms). This could be an important mechanism underlying degradation in *Kobresia* grasslands. Identification of the role of grazing on the exchange mechanisms for the Tibetan grassland is challenging because the grazing activities have inconsistent definitions and various standards. Exclusion experiments provide a feasible way for studying grazing effects based on the assumption of inverse process direction and ecosystem recovery. To further clarify the exchange mechanisms, we investigated gross mineralization, N uptake by plants and N partitioning in the plant-soil system on grazed and ungrazed sites. Clipping (simulating defoliation by grazing) and shading, that reduce the leaf area and the light-dependent reactions, respectively (Detling et al. 1979, Schmitt et al. 2013), were used to manipulate photosynthesis and hence the C allocation into belowground (i.e., root and soil). We hypothesized that (1) grazing-driven removal of shoots lowers photosynthesis and belowground C translocation, reduces microbial activities in the rhizosphere, and thus decreases soil gross N mineralization; (2) N uptake rates of plants and N retention in root

biomass will be higher in the grazed *Kobresia* grassland, in order to ensure N delivery for an efficient regrowth of new shoots. To test these hypotheses, we conducted an in situ ^{15}N labeling study at grazed and ungrazed sites. Considering the long-term grazing impacts on *Kobresia* grasslands, we expected that *K. pygmaea* developed specific strategies for N uptake and partitioning to facilitate plant re-growth after grazing. The ^{15}N dilution approach was used to quantify in situ gross N mineralization at grazed and ungrazed sites.

Materials and methods

Study area

The study site is located in the core area of alpine *K. pygmaea* grasslands adjacent to the Naqu Ecological and Environmental Observation and Research Station (Xizang, China; 31° 16' 45" N, 92° 59' 37" E; 4410 m above sea level). The Research Station was established in 2007 as the “*Kobresia* Experimental Monitoring Area” (KEMA), close to the village Kema, and now managed by the Institute of Tibetan Plateau Research, Chinese Academy of Science. This area is influenced by a moderately continental monsoonal climate with mean summer precipitation of 276 mm (June–September). Average annual temperature was $-1.2\text{ }^{\circ}\text{C}$ in the previous 5 years (Miehe et al. 2011). Our experiment was conducted from July to August 2012 during the growing season, which lasted from mid-May to mid-September. A mean temperature of $10.4\text{ }^{\circ}\text{C}$ was recorded (Babel et al. 2014).

The sites are dominated by *K. pygmaea* (Cyperaceae), which covers more than 80% of the surface area. *K. pygmaea* is accompanied (remaining 20%) by other graminoid species, such as *Carex ivanoviae*, *Carex* spp., *Poa* spp., *Festuca* spp., *Stipa purpurea*, and *Trisetum* spp., as well as a small number of rosette and cushion plants (*Arenaria bryophylla*, *Arenaria kansuensis*, *Androsace tapete*, *Leontopodium nanum*, *Lamiophlomis rotata*). The *K. pygmaea* lawn has an average height of 2 cm at the grazed site, overlying a dense root mat of approximately 14 cm. The soil is classified as a stagnic Cambisol (humic, eutric) (IUSS Working Group WRB 2006). The upper soil horizons (Afe horizons, according to Kaiser et al. 2008) consist of humified organic matter and a large quantity of living and dead roots. Soils are free of carbonates in the upper 15 cm and have mean pH values of 6.4. A detailed description of soil characteristics is reported by Schleuss et al. (2015).

The study site is traditionally used as a continuously grazed pasture. However, livestock stocking exceeds the carrying capacity, and thus, the pasture in this region is intensively grazed (Ingrisch et al. 2015; Wei and Chen 2001). To assess the effect of grazing exclusion on pasture restoration, a grassland area of $100 \times 250\text{ m}$ was fenced in 2010 (excluding livestock such as

yaks and goats, but allowing minor grazing by small mammals). The experiment was established within the fence (referred to as the ungrazed site) and outside but near to the fenced area (referred to as the grazed site). Two percent more vegetation coverage was observed after grazing exclusion, but there was no significant difference between plant communities.

^{15}N labeling experimental setup, sampling, and analysis

The in situ ^{15}N labeling experiment was started in August 12. On each site (grazed and ungrazed), 16 square plots ($15 \times 15\text{ cm}$) were randomly selected; 12 of them were randomly chosen for three treatments: control, clipping, and shading with four replicates. The remaining four plots were reference plots, which were injected only with water to determine plant and soil properties as well as ^{15}N natural abundance.

The in situ gross N mineralization rate at 0–10 cm depth was estimated by a ^{15}N dilution approach (Davidson et al. 1991; Hart et al. 1994; Murphy et al. 2003). An amount of 4.562 mg ^{15}N was added as $(^{15}\text{NH}_4)_2\text{SO}_4$ (10 atom% ^{15}N) to each plot. We used the same labeling grid with 16 injection points to ensure a uniform distribution of tracer in each plot. $(^{15}\text{NH}_4)_2\text{SO}_4$ was dissolved in water and 1 ml of this tracer solution was injected at 5 cm soil depth for each injection point with a dispensing pipette. The “initial” soil cores (two soil cores per plot, 5 cm diameter \times 10 cm depth) from each ^{15}N -labeled plot were sampled 3 h after labeling (the t_0 point). After that, four labeled plots as replicates were subjected to clipping or shading under both grazing regimes. For clipping, the plant shoots were clipped at 1 cm above the soil surface and thereafter allowed to grow continuously. For shading, the light intensity was reduced by more than 90% by a fine black mesh cover throughout the experimental period of 28 days. The four remaining labeled plots with untreated plants were referred to as control (without clipping and shading, but with tracer injection). Previous studies showed that a period of 6 days allowed an accurate quantification of gross mineralization by ^{15}N pool dilution approach (Mikan et al. 2000; Murphy et al. 2003). Since the N mineralization rate in alpine grasslands is slow, we took two further soil cores from each plot after 6.5 days of in situ incubation (the t_1 point). The soil samples were transferred immediately to the lab and well mixed after removing the roots. Fresh soil subsamples (8 g from each replicate) were extracted for 1 h with 30 ml 0.5 M K_2SO_4 . The concentrations of soil NO_3^- and exchangeable NH_4^+ were determined with an autoanalyzer (AA3, Bran-Luebbe, Germany). NH_4^+ in the extracts was determined by a diffusion process (Murphy et al. 2003), and the ^{15}N enrichment was determined with an isotope ratio mass spectrometer (Mat 253, Finnigan MAT).

Nitrogen uptake by plants and ^{15}N partitioning in the plant-soil system were traced by in situ $^{15}\text{NH}_4^+$ labeling because

NH_4^+ is the dominating mineral N form in the soil. Following the ^{15}N labeling and the treatments (clipping and shading), samples of shoots, roots, and soil were taken from each plot 3 h and 7, 14, and 28 days after labeling. The shoot biomass was cut within an area of 25 cm^2 on each small plot close to the soil surface. After sampling cutting, root and soil samples were taken by soil cores of 0–10 cm soil depth, because over 70% of the total root biomass is concentrated within this horizon (Schleuss et al. 2015). Fresh soil samples were sieved through a 2-mm mesh. The separated roots were washed with tap water and soaked into 0.5 mM CaCl_2 solution for 30 min, then rinsed with distilled water to remove ^{15}N absorbed on the root surface. Plant materials and soil samples were dried at $65\text{ }^\circ\text{C}$ for 72 h and ground to a fine powder using a ball mill (MM2, Fa. Retsch, Haan, Germany). They were weighed into tin capsules to analyze total C and N contents and stable isotope signatures using an elemental analyzer (EA 1112, CE Instruments, Milan, Italy), ConFlo III device (Finnigan MAT, Bremen, Germany), and continuous-flow gas isotope ratio mass spectrometry (MAT253, Finnigan MAT, Bremen, Germany). Bulk density was determined by oven-drying undisturbed soil at $105\text{ }^\circ\text{C}$ for 48 h (Van Reeuwijk 2002). Soil pH was measured by a dry soil-water ratio of 1:2. Total C and N stocks of plant and soil as well as the contents of NO_3^- and exchangeable NH_4^+ (unlabeled) were determined on the reference plots.

Calculations and statistics

Soil gross N mineralization rate was calculated from the measured NH_4^+ pool and atom % ^{15}N of NH_4^+ as follows (Murphy et al. 2003):

$$m = \frac{M_1 - M_0}{t_1 - t_0} \cdot \frac{\ln \frac{AP_0 - AP_C}{AP_1 - AP_C}}{\ln \frac{M_1}{M_0}}$$

where m is the gross mineralization rate ($\text{mg N kg}^{-1} \text{ day}^{-1}$), M_0 is the initial exchangeable NH_4^+ pool (mg N kg^{-1}), M_1 is the post-incubation exchangeable NH_4^+ pool (mg N kg^{-1}), AP_0 is the initial atom-%- ^{15}N of NH_4^+ , AP_1 is the post-incubation atom-%- ^{15}N of NH_4^+ , AP_C is the atom-%- ^{15}N of NH_4^+ in unlabeled control, and t_0 and t_1 are the time points (in day) of initial and post-incubation soil core sampling, respectively. Net mineralization and net nitrification were determined from the difference in the N pool of exchangeable NH_4^+ and of NO_3^- , respectively, after 6.5 days of incubation (Hart et al. 1994).

^{15}N uptake by plants (or ^{15}N allocation to soil) was calculated by an isotope mixing model (Robinson 2001), as the product of the N content of the pool, the mass of the component per square meter, and its APE (atom-%-excess ^{15}N). APE

is the ^{15}N difference between ^{15}N -labeled and unlabeled plants or soil. The proportion of ^{15}N recovered in plants (or soil) was calculated by dividing plant ^{15}N uptake (or ^{15}N incorporation of soil) by total amount of ^{15}N injected into the soil. Based on previous studies (Xu et al. 2011a, b), an assumption was made that the N forms in Tibetan grasslands do not change during the 3-h period of labeling. The NH_4^+ uptake rate by plants was calculated by dividing the actual NH_4^+ uptake amount by time and root biomass. The actual NH_4^+ uptake from soil by plants was calculated as follows:

$$U_{\text{unlabeled}} = U_{\text{labeled}} \cdot (m_{\text{unlabeled}} / m_{\text{labeled}})$$

where U_{labeled} is the amount of $^{15}\text{NH}_4^+$ uptake by plants, $m_{\text{unlabeled}}$ is the content of total exchangeable NH_4^+ in the unlabeled soil, and m_{labeled} is the total mass of injected ^{15}N (McKane et al. 2002; Xu et al. 2011a, b).

The relative growth rate (RGR) of shoots (or roots) for each treatment was determined by their initial and final biomass during 28 days of experiment period, calculated as follows (Hunt et al. 2002):

$$\text{RGR} = [(\log \text{ final shoot biomass}) - (\log \text{ initial shoot biomass})] / \text{time}$$

Normality (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variance (Levene test, $p > 0.05$) were examined before the statistical analysis. Data were log-transformed prior to analysis if they were non-normally distributed. Repeated measures analysis of variance (ANOVA) with the sampling date as the within-subject factor and grazing and treatments (control, clipping, and shading) as the between-subject factors was calculated for shoot and root biomass; ^{15}N recovery in shoot, root, and total biomass; and NO_3^- and exchangeable NH_4^+ contents. One-way ANOVA was used to assess the effect of grazing and then the effects of control, clipping, and shading nested within each of the grazing treatment on the parameters of plants (C and N stocks, RGR of shoot/root, total biomass, NH_4^+ uptake rate, total plant ^{15}N recovery) and those of soil (C and N stocks, the mineral N contents, gross and net N mineralization, net N nitrification, bulk density, and pH). ANOVAs were followed by post hoc test for multiple comparison using least significant differences. All statistical analyses were performed with SPSS 16 (SPSS Inc., Chicago, IL, USA) and considered significance at a $p < 0.05$ level.

Results

Above- and belowground C and N stocks

In general, shoot biomass made only a minor contribution to the overall stocks of C (1.5%) and N (0.9%) on average, whereas the majority of the total C and N was stored belowground, mainly in root mats (root + soil). The root biomass

contributed 13.5 and 4.0% of the total C and N storage, and the soil accounting for 85.0 and 95.2%, respectively.

Three years after grazing exclusion, C and N stocks in shoots increased by 46.2% ($p < 0.01$) and 29.7%, respectively, compared to those in the grazed site. In contrast, C and N stocks of the root biomass decreased by 14.6 and 10.4% ($p < 0.05$; Fig. 1). Grazing exclusion increased soil C and N stocks by 14.7 and 18.2%, respectively ($p < 0.05$). As a result, the overall total C and N stored in the upper 10-cm root mats (root + soil) were 10.2 and 16.9% larger in the ungrazed site than those in the grazed site ($p < 0.05$; Fig. 1).

Mineral N and soil N transformation processes

NH_4^+ was the dominant mineral N form in this *Kobresia* pasture, and its content in the unlabeled soil was about four times higher than that of NO_3^- (Fig. 1). Grazing exclusion increased the content of NO_3^- and exchangeable NH_4^+ in unlabeled soil by 32 and 72%, respectively ($p < 0.05$; Fig. 1). The combined mineral N pool (exchangeable NH_4^+ , NO_3^-) was on average 1.4 times higher in ungrazed site than that in grazed site ($p < 0.05$). After tracer addition, the NO_3^- and exchangeable NH_4^+ contents were similar during 28 days after labeling under both grazing regimes, despite their differences before labeling (Fig. S1; Table S2). The NO_3^- content fluctuated with time under both grazing regimes (ranged from 2.1 to 4.7 $\mu\text{g N g}^{-1}$ soil), but that of exchangeable NH_4^+ strongly decreased (ranged from 80.4 to 12.5 $\mu\text{g N g}^{-1}$ soil).

The gross N mineralization rate was averagely lower for the grazed site compared to that for the ungrazed site among all treatments ($p < 0.05$; Fig. 2). In the grazed site, the highest N mineralization was for control (10.4 mg N kg^{-1} soil day^{-1}), followed by clipping (8.7 mg N kg^{-1} soil day^{-1}) and by shading (5.6 mg N kg^{-1} soil day^{-1}). A similar pattern was evident for the ungrazed site: 12.8, 10.7, and 9.2 mg N kg^{-1} soil day^{-1} for

control, clipping, and shading, respectively (Fig. 2). Linear regression between gross N mineralization rates and the mean increase in N stock in plant biomass showed a weak relationship at the grazed site ($r^2 = 0.3$, $p = 0.07$), but the relationship was much stronger at the ungrazed site ($r^2 = 0.6$, $p = 0.003$; Fig. 2).

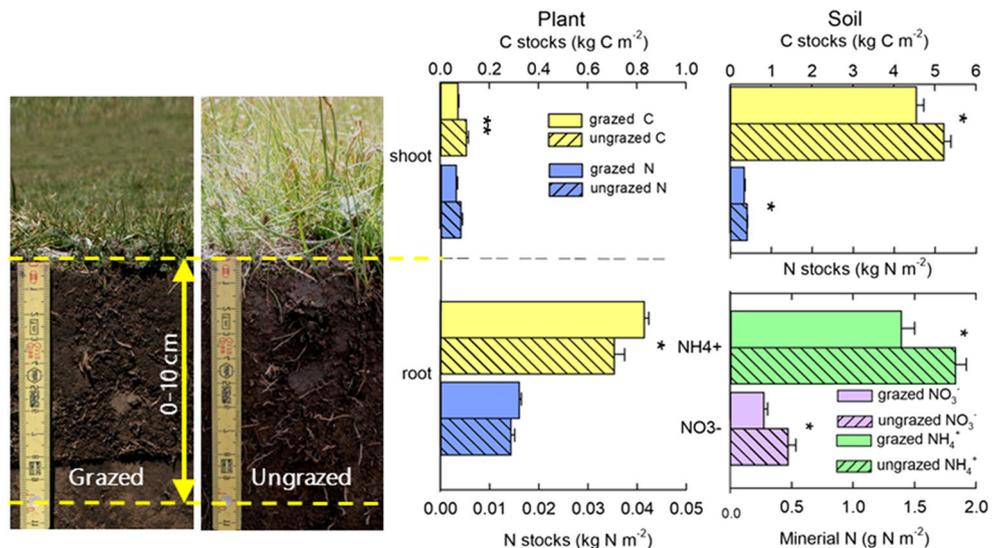
The negative values of net N mineralization under both grazing regimes (Table S1) indicated that NH_4^+ consumption (including immobilized NH_4^+ , NH_4^+ oxidization, gaseous losses, and plant uptake) exceeded gross mineralization. The highest average net mineralization rate was observed for control, which was followed by clipping and then shading, under both grazing regimes. Net N nitrification rates at the grazed site were on average 1.7 times higher than at the ungrazed site (Table S1), indicating higher potential N losses by emission, denitrification, and leaching.

Shoot and root biomass production

Grazing exclusion increased shoot biomass ($p < 0.001$, Fig. 3; Table S4). For example, the shoot biomass was about 26% higher in the control of the grazed sites (160 g m^{-2}) compared with that in the ungrazed control (202 g m^{-2} ; Fig. 3). Yet, grazing exclusion had only a small effect on the relative growth rate (RGR) of shoot biomass (for control and shading, Fig. 4), whereas clipping (simulated grazing) increased the RGR of shoot biomass for both grazing regimes ($p < 0.05$). The clipping effect on the RGR was significantly higher at the intensively grazed site compared to that at the grazing enclosure ($p < 0.05$; Fig. 4).

In contrast to the shoot biomass, root biomass decreased by 12% following grazing exclusion (in the upper 10 cm depth; Fig. 3, Table S4). This resulted in a lower root to shoot investment at the ungrazed site (root to shoot ratio 7.9) than at the grazed site (root to shoot ratio 11.4). The mean biomass of the control was 1494 and 1638 g m^{-2} in the ungrazed and the

Fig. 1 The photo (left) shows the characteristics of *K. pygmaea* and of the upper 10 cm of soils at grazed and ungrazed sites. Subfigures (right) illustrate C and N stocks in plant and soil as well as the content of mineral N (exchangeable NH_4^+ , NO_3^-) in the soil. Values are means \pm standard errors ($n = 8$). Asterisk indicates significant difference level (* $p < 0.05$, ** $p < 0.01$)



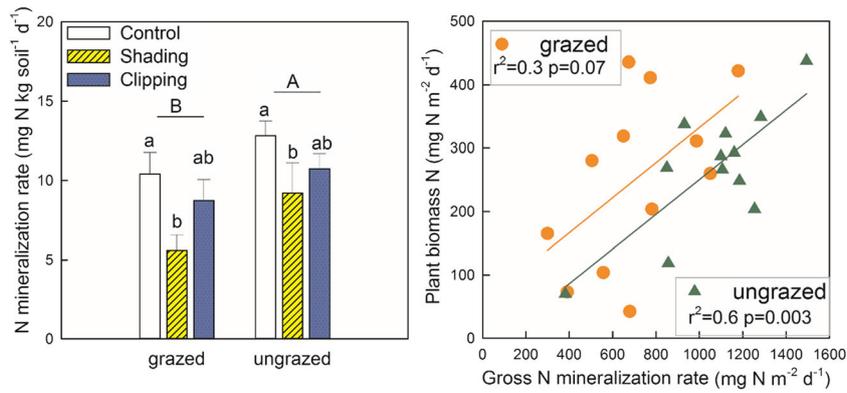


Fig. 2 Left: gross N mineralization rate (mg N kg⁻¹ soil day⁻¹) in soil of the grazed and ungrazed sites. Capital letters indicate significant differences (post hoc LSD test, $p < 0.05$) between grazed and ungrazed sites. Lowercase letters indicate significant differences (post hoc LSD test, $p < 0.05$) between treatments (control, clipping, and shading)

nested within each grazing regime. Right: relationship between gross N mineralization rate (mg N m⁻² day⁻¹) and the mean increment of N stock in plant biomass (mg N m⁻² day⁻¹) for the grazed and ungrazed sites. Each point represents the individual replicates of each treatment (control, clipping, and shading)

grazed sites, respectively. Clipping did not change root biomass under both grazing regimes. Shading inhibited root growth and led to the lowest root biomass under both grazing regimes (Fig. 3). Nonetheless, no significant difference in the RGR of roots was observed between grazed and ungrazed sites (0.02 g g⁻¹ day⁻¹ on average, data not presented).

0.03 μg N g⁻¹ root⁻¹ h⁻¹) than that at the ungrazed sites (0.48 ± 0.04 μg N g⁻¹ root⁻¹ h⁻¹; $p < 0.05$).

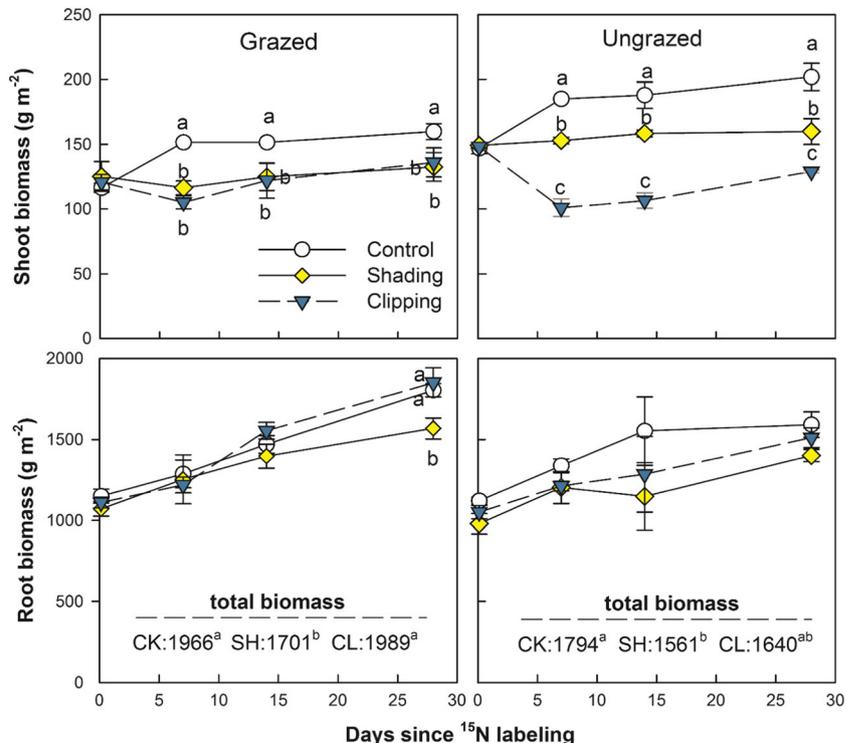
Plant N uptake and ¹⁵N partitioning in the soil-plant system

Overall, 86.3 and 79.7% of the ¹⁵N were recovered in the plant-soil system (the upper 10-cm root mats) at the grazed and ungrazed sites, respectively. The major portion of ¹⁵N remained in soil (0–10 cm; range from 41.8 to 62.6%) 28 days after the labeling. This was independent of grazing and treatments (control, clipping, and shading).

The NH₄⁺ uptake rate by plants (within 3 h after ¹⁵N pulse labeling) was higher at the grazed sites (0.67 ±

The total ¹⁵N recovery in plant biomass (shoots + roots) is higher at grazed site than that at ungrazed site 28 days after labeling ($p = 0.01$; Table S5). The total plant ¹⁵N recovery was 36.5 and 42.1% for control at the grazed and ungrazed sites,

Fig. 3 Shoot and root biomass (g m⁻²) during 28 days after labeling. The values of bottom figures show the total plant biomass (shoots + roots) 28 days after the labeling. CK: control, SH: shading, CL: clipping. Values are means ± standard errors ($n = 4$). Letters indicate significant differences at $p < 0.05$ within treatments (control, shading, and clipping) for grazed and ungrazed sites



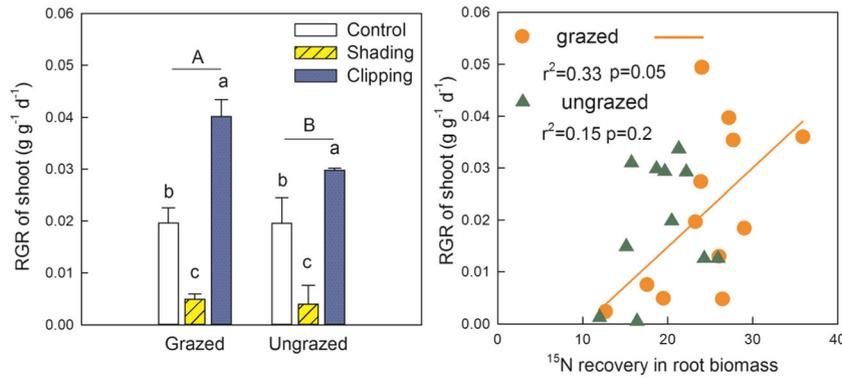


Fig. 4 Left: relative growth rate (RGR) of shoots ($\text{g g}^{-1} \text{ day}^{-1}$). Values are means \pm standard errors ($n = 4$). Capital letters indicate significant differences (post hoc LSD test, $p < 0.05$) between grazed and ungrazed sites. Lowercase letters indicate significant differences (post hoc LSD test, $p < 0.05$) between treatments (control, clipping, and shading) nested

within each grazing regime. Right: relationship between the RGR of shoot ($\text{g g}^{-1} \text{ day}^{-1}$) and ^{15}N recovery in the root biomass (%). Each point represents the individual replicates of each treatment (control, clipping, and shading) for grazed and ungrazed sites

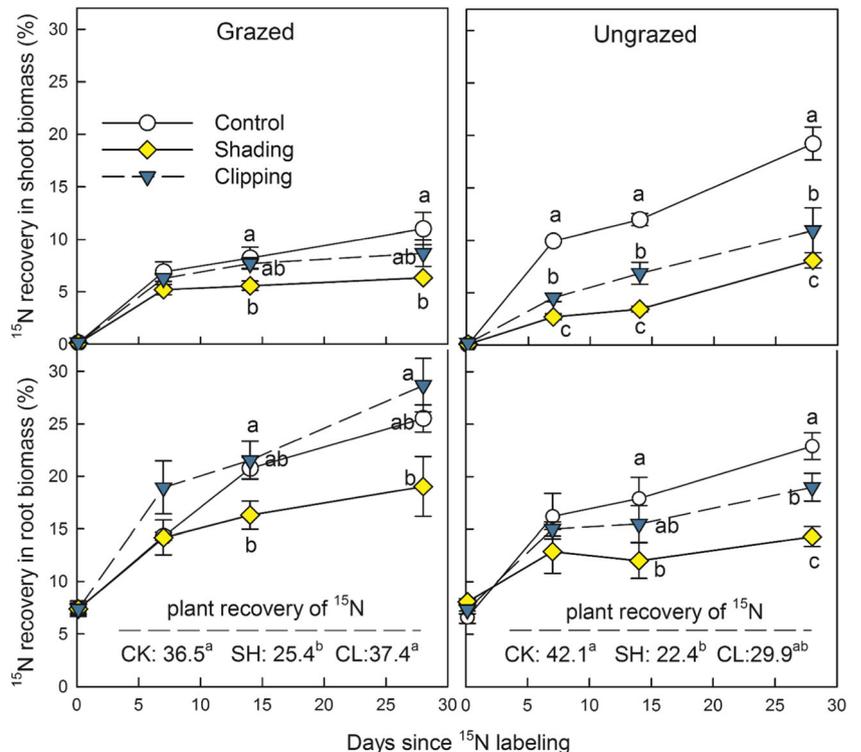
respectively (Fig. 5). The ^{15}N recovery in plants was slightly increased by clipping at the grazed sites, but it was reduced by clipping at the ungrazed sites. Shading had the lowest ^{15}N recovery under both grazing regimes at all times (Fig. 5) and did not differ between grazing regimes. Notably, ^{15}N partitioning between shoot and root biomass showed deviating patterns with grazing regime, which is why both compartments are presented separately (see below).

The ^{15}N recovery in shoots for control was about two times lower at the grazed site (11.0%) than that at the ungrazed site (19.2%). An even lower ^{15}N recovery was observed following clipping and shading for both grazing regimes, essentially due

to the lowered shoot biomass (Fig. 5). The ^{15}N recovery in shoots correlated positively with root biomass, but negatively with soil exchangeable NH_4^+ content under both grazing regimes ($p < 0.001$; Table S3).

^{15}N recovery in roots was higher at grazed sites than that at ungrazed sites for all treatments 28 days after the labeling ($p < 0.05$); the ^{15}N recovery in roots for control was 25.5 and 22.9% at grazed and ungrazed sites, respectively (Fig. 5, Table S5). Compared to the respective control, clipping increased the ^{15}N recovery in roots of grazed plants (28.7%), while it decreased the ^{15}N recovery of the ungrazed plants (19.0%, Fig. 5). Under both grazing regimes, the respective

Fig. 5 The ^{15}N recovery (% of input) in shoot and root biomass during 28 days after labeling. The values at the bottom figures show the ^{15}N recovery (%) in total plant biomass at the last sampling. CK: control, SH: shading, CL: clipping. Values are means \pm standard errors ($n = 4$). Lowercase letters indicate significant differences at $p < 0.05$ within treatments (control, shading, and clipping) under each grazing regime



lowest ^{15}N recovery in roots was observed for shading. The ^{15}N recovery in root biomass was negatively correlated with soil exchangeable NH_4^+ content under both grazing regimes ($p < 0.001$), but its correlation with the shoot biomass was significant only at the grazed sites ($p < 0.05$; Table S3), reflecting that root N uptake was important for the re-growth of photosynthetically active shoots in the grazed *K. pygmaea* grassland. Linear regression between the RGR of shoots and ^{15}N recovery in roots showed a significant relationship for grazed site ($r^2 = 0.33$, $p < 0.05$), but not for the ungrazed one (Fig. 4).

Discussion

Grazing effects on N stocks

Soil N stocks in the upper 10-cm root mats were 15% lower under grazing (Fig. 1). This is consistent with several studies reporting negative grazing effects on N stocks, especially within the upper soil horizon (Table S6). In the grazed *Kobresia* pastures, the lower N stocks are mainly attributed to (1) the increasing stocking numbers in recent decades, which increased outflows of nutrients from the soil-plant system by livestock consumption, (2) redistribution of N from the grazing sites to the captive area adjacent to the villages, (3) collection of dung for household fuel, (4) gaseous N loss, and (5) nitrate leaching (Table S1; He et al. 2011; Zhong et al. 2017). Consequently, Soil C sequestration might be limited under grazing due to the tight stoichiometric relationships between C and N (Fig. 1; Hu et al. 2016). However, there are also other studies reporting neutral or even positive grazing effects on N stocks (Table S6). This could be ascribed to grazing intensity and site differences in altitude and regional climate conditions (e.g., temperature, precipitation). For example, moderate grazing intensity has been suggested as a suitable way to preserve the capacity of Tibetan grasslands to store C and nutrients (Hafner et al. 2012; Zhang et al. 2015; Zhou et al. 2016). However, the intrinsic mechanisms controlling N stocks following grazing are less understood in this alpine environment. This requires more detailed investigation of N cycling processes such as microbial N mineralization and plant N acquisition.

Grazing effects on soil N mineralization

Mineral N that highly depends on microbial gross N mineralization (i.e., the ammonification process) and subsequent nitrification constitutes about 80% of plant-available N in Tibetan grasslands (Xu et al. 2006). We found that grazing decreased gross N mineralization (Fig. 2), causing lower contents of exchangeable NH_4^+ and NO_3^- in the soil (Fig. 1). Nitrogen mineralization is a microbially driven process and is largely determined by both quality and quantity of labile C

compounds (Hart et al. 1994). Grazing decreased these labile C inputs because a permanent removal of photosynthetic shoots directly reduces rhizodeposition (Kuzyakov et al. 2002; Schmitt et al. 2013; Wei et al. 2016). Additionally, a higher allocation of C assimilates is required (a) to sustain shoot re-growth (the higher RGR, Fig. 4) and (b) to support the increasing root biomass (Fig. 3), with its associated C maintenance costs, leading to a low rhizodeposition. By means of ^{13}C pulse labeling for the same study site, Ingrisich et al. (2015) showed that most of the ^{13}C was recovered in the roots and only a minor portion (< 10%) remained in the soil after 2 months. Of secondary importance was that grazing caused changes to the chemical composition of roots and litter (e.g., lower cellulose, higher lignin/N ratio, and tannin content), restraining the C release via decomposition (Luo et al. 2009, 2010). We suggest that the lower input of labile C inhibited the growth and activity of microorganisms and enzyme production (Shahzad et al. 2012, 2015). Therefore, these had negative effects on gross N mineralization. Indeed, photosynthetic manipulations via clipping and shading also decreased gross N mineralization compared to the control, for both grazing regimes (Fig. 2). In summary, we confirmed our first hypothesis that intensive grazing suppresses microbial N mining and has a negative impact on gross N mineralization.

Since N mineralization is also restricted by abiotic environmental conditions such as low precipitation and temperature, decreasing gross N mineralization rates might aggravate prevailing N limitations, with respective feedbacks for plant and microbial N uptake (Song et al. 2007, 2011; Wang et al. 2012). In agreement with this, net N mineralization was negative, indicating a strong N limitation to both plants and microorganisms (Table S1; Lang et al. 2016). The correlation between gross N mineralization in soil and the N stock in plant biomass was weak under grazing, but it was strengthened by grazing exclusion (Fig. 2) due to the higher available N supply via the increased rate of gross mineralization. We suggest that increased grazing pressure may disturb the exchange between plant C input and microbial N releases, further exacerbating N limitation in this deficient, vulnerable ecosystem and decreasing C sequestration.

Grazing effects on plant growth

Several studies report that plants respond to grazing by increasing their growth rates to compensate for the lost tissues (Hilbert et al. 1981; Oesterheld and McNaughton 1991; Kuzyakov et al. 2002). This physiological mechanism is referred to as compensatory growth (McNaughton 1979; Wang et al. 2005a). We found that *K. pygmaea* increased the relative growth rate (RGR) of shoots after simulated grazing (clipping) and induced compensatory growth under both grazing regimes. An increase in the RGR of shoots was especially

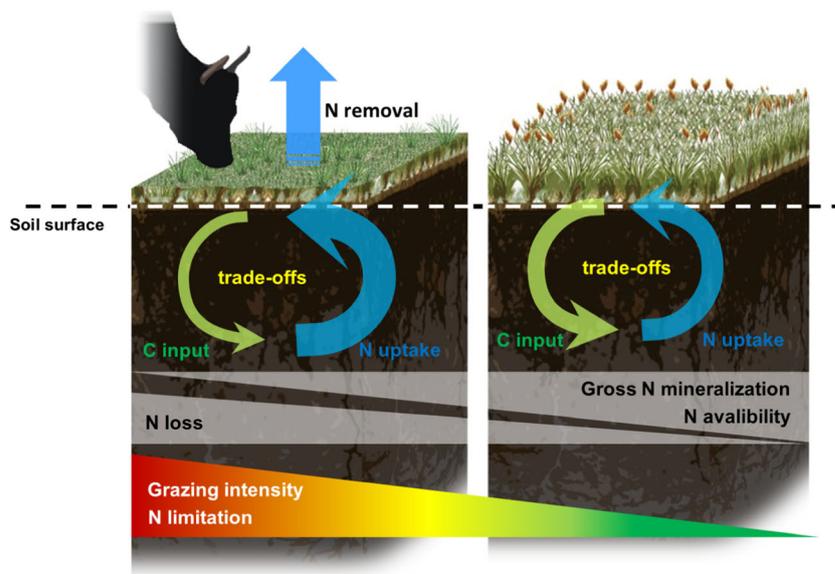
evident at the grazed sites, suggesting that *K. pygmaea* efficiently develops new photosynthetic biomass. A fast regrowth of shoots is an important plant trait to cover the high metabolic C costs of root maintenance (Poorter et al. 1990; Chapin et al. 2002). To sustain the continual renewal of shoot biomass, however, *K. pygmaea* faces increasing demands for resource uptake, which is especially challenging in these N and P co-limited environments (Wise and Abrahamson 2015; Miehe et al. 2017). From this perspective, *K. pygmaea* develops a large amount of root biomass in order to exploit nutrients at the times and depths of their availability. Indeed, *Kobresia pygmaea* has a far higher root than shoot biomass, i.e., root to shoot ratio of ~ 8 for the ungrazed sites (in 0–10 cm), and the ratio can be even higher (up to 20) when the entire rooting zone is considered (ca. 0–25 cm, Li et al. 2008; Schleuss et al. 2015). Such root to shoot ratios are common in other grazing-driven ecosystems (Milchunas and Lauenroth 1993; Mcnaughton et al. 1998; Yan et al. 2013) and in cold and dry climates (e.g., arctic tundra, Callaghan et al. 2005), or for ecosystems with extremely low nutrient status (McConnaughay and Coleman 1999; Lambers et al. 2011). A very higher root biomass production or root activity is a trait of *K. pygmaea* to obtain nutrients (Schleuss et al. 2015) and to facilitate shoot re-growth under prevailing deficient nutrient conditions. Given a lower soil N availability at the grazed sites, we found that the root biomass (0–10 cm) was about 13% higher for grazed site than that for ungrazed site. *Kobresia pygmaea* even sustained root growth after clipping, presumably to counteract nutrient limitation (Chapin 1980; Sun and Wang 2016). In summary, a dense root network for nutrient uptake and efficient compensatory growth are important traits contributing to the high grazing tolerance of *K. pygmaea*. This particularly matters in this pastoral ecosystem, where plants have had to cope with grazing impacts for more

than 8000 years (Miehe et al. 2009). These traits at least partly explain the wide dominance of *K. pygmaea* on the Tibetan Plateau (Miehe et al. 2017).

Grazing effects on plant N uptake and ^{15}N partitioning

Although $^{15}\text{NH}_4^+$ can be lost via NH_3 volatilization (Cameron et al. 2013) or via leaching after nitrification, more than 80% of added ^{15}N was recovered in the upper plant-soil system after 28 days. About 40% of added ^{15}N was recovered in total plant biomass. This is in a similar range to other studies for the *Kobresia* grasslands (Song et al. 2007; Xu et al. 2004, 2011a, b; Schleuss et al. 2015; Jiang et al. 2017) and indicates efficient plant N uptake in this N-limited ecosystem. Higher NH_4^+ uptake rates at grazed site than at ungrazed site are ascribed to upregulation of NH_4^+ transport systems (i.e., the most dominant N form) in *K. pygmaea* roots in response to grazing (McKane et al. 2002; Jackson et al. 2008; Nacry et al. 2013). Clipping even slightly increased the plant ^{15}N recovery (shoot + root) at grazed sites. This agrees with our second hypothesis that grazing increases plant N acquisition to sustain shoot biomass (i.e., for compensatory growth). A negative correlation between total plant ^{15}N recovery and soil exchangeable NH_4^+ content demonstrated that plants utilized NH_4^+ for re-growth (Table S3). In fact, the mineral soil N contents ($\text{NO}_3^- + \text{exchangeable NH}_4^+$, Fig. 1) were lower for grazed site than those for ungrazed site, presumably due to stimulated plant N uptake and decreasing N mineralization rates following grazing (Figs. 1 and 2). Since low N availability can constrain N absorption by roots, *K. pygmaea* increased root biomass to occupy larger soil volumes and to extend the root surface for acquiring as much N as possible, especially in response to grazing (Fig. 3). Consequently, a positive correlation between

Fig. 6 Grazing effects on plant N uptake and the exchanges between plant C input and microbial N mineralization. The increase in grazing intensity amplified the plant N limitation in *Kobresia* grasslands



the total plant ^{15}N recovery and the root biomass was observed for both grazing regimes (Table S3). Besides root biomass, grazing also increased root N uptake and N retention (as reserves) in roots (root ^{15}N recovery: grazed > ungrazed, Fig. 5) and ultimately caused a higher belowground investment than for ungrazed sites. This is highly beneficial in this N and P co-limited environment because *K. pygmaea* can reallocate N stored in remnant leaves or roots to support re-growth after grazing (Freschet et al. 2015). In agreement with this, a linear correlation between RGR of shoots and root ^{15}N recovery was observed for the grazed sites, but not for ungrazed sites (Fig. 4), which is consistent with our second hypothesis. Overall, our findings support the concept of very efficient above-belowground interaction of *K. pygmaea* in response to grazing (Schleuss et al. 2015). Grazing stimulates a higher N uptake rate for compensatory growth of photosynthetic shoots, to cover the high plant C costs. Simultaneously, *K. pygmaea* develops a dense root network with efficient N uptake mechanisms to meet the high nutrient demands for continually replacing lost shoots.

Conclusions

The soil N stock, N mineralization, and N availability in *Kobresia* pasture were decreased under intensive grazing. The decrease in N mineralization was attributed to the reduced belowground inputs of labile C due to decreased photosynthesis. This aggravated the prevailing N limitation, challenging plant N acquisition. Nevertheless, plant N uptake was higher at grazed site compared to that at ungrazed site, mainly due to the compensatory growth of lost photosynthetic organs. *K. pygmaea* has evolved specific traits (efficient above-belowground interactions) to cope with nutrient limitations and simultaneous grazing pressure. However, intensive grazing disturbs the critical balance between plant C input and microbial N mineralization (Fig. 6). This might establish a negative feedback to plant productivity over the long term. Therefore, a reduction of grazing pressure (moderate grazing or short-term grazing exclusion) benefits soil fertility, C sequestration, and overall ecosystem stability.

Several previous studies have reported positive effects of moderate grazing on N cycles. From this perspective, the traditional migratory grazing system of the Tibetan nomads seems to be well adapted to maintain grassland stability, despite low levels of ecosystem productivity. The increase in stocking numbers due to sedentarization programs on the one hand and the long-term complete grazing exclusion by fencing programs on the other might both have contributed to the vast changes in pasture conditions (i.e., changing vegetation composition, intensified degradation). This study, therefore, provides mechanistic knowledge to understand the existence, resilience, functioning, and future development of

this sensitive and vulnerable ecosystem against the background of land use and climate changes. Further research that compensates the mechanisms of ecosystem vulnerability (e.g., the roles of plant-microbial interactions, microbial communities, and soil fauna) is required, focusing on the tipping point regarding ecosystem resilience under variable degree of grazing intensity and pasture management to avoid serious degradation of these unique alpine ecosystems.

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