

Effects of clipping and shading on $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ recovery by plants in grazed and ungrazed temperate grasslands

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Abstract

Aims In natural ecosystems, plants generally promote the acquisition of nitrogen (N) through the input of carbon (C) into the soil. The present study aimed to clarify how changes in C input affect N uptake by plants.

Methods In situ ^{15}N labeling ($(^{15}\text{NH}_4)_2\text{SO}_4$ and K^{15}NO_3) was performed in grazed and ungrazed *Leymus chinensis* grasslands located in Inner Mongolia, northern China. Clipping and shading treatments were used to reduce C allocation to roots by limiting photosynthesis because grazing can produce such similar effect through defoliation.

Results Grazing increased the allocation of photosynthate to roots and the relative biomass of *Carex duriuscula* but decreased the relative biomass of *L. chinensis*. Grazing decreased $^{15}\text{N-NH}_4^+$ recovery (grazed: 30.9% vs.

ungrazed: 39.3%) but increased $^{15}\text{N-NO}_3^-$ recovery (grazed: 26.9% vs. ungrazed: 17.6%), which may be due to changes in the $\text{NH}_4^+/\text{NO}_3^-$ ratio and species composition. Clipping and shading reduced ^{15}N recovery, mainly because they decreased C supply for N uptake by reducing photosynthesis.

Conclusions These findings indicate that the reduction of the C input to soil decreases ^{15}N recovery by plants in temperate grasslands, which contributes to our understanding of the trade-off between C and N and grassland N cycles.

Keywords $^{15}\text{N-NO}_3^-$ · $^{15}\text{N-NH}_4^+$ · Temperate grassland · Grazing · ^{15}N isotope labeling · ^{15}N recovery

Introduction

Nitrogen (N) is one of the most important essential nutrients limiting net primary production in many terrestrial ecosystems (LeBauer and Treseder 2008). Ammonium (NH_4^+) and nitrate (NO_3^-) are two major forms of inorganic N for plant growth (Masclaux-Daubresse et al. 2010) and are primarily derived from soil organic matter (SOM) mineralization by soil microorganisms (Schimel and Bennett 2004). Numerous studies have revealed differences in the uptake of NH_4^+ and NO_3^- by plants due to the chemical properties of nitrate vs. ammonium, e.g., energy consumption (Gessler et al. 1998), ionic toxicity (Lotze and Schramm 2000), and ionic mobility (Owen and Jones 2001). As NH_4^+ can be directly incorporated into amino acids, its uptake is

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faster and requires less energy than that required for NO_3^- , as this ion must be reduced to NH_4^+ by nitrate reductase before its assimilation (Gessler et al. 1998). However, high NH_4^+ uptake can cause cell toxicity (Lotze and Schramm 2000). Compared to NO_3^- , NH_4^+ has lower mobility in soil solution, because it attaches easily to negatively charged soil colloids (Owen and Jones 2001). The uptake of NH_4^+ and/or NO_3^- can alter pH in the root zone, affect the relative uptake of nutrients, and further disturb plant growth (Guo et al. 2002; Brück and Guo 2006). As a result, NH_4^+ and NO_3^- often have distinct fates in the ecosystem. Thus, clarifying the fates of NH_4^+ and NO_3^- , especially their retention by plants, is beneficial for understanding terrestrial N cycling and can help inform the development of strategies to mitigate N loss.

Grasslands are widely distributed terrestrial ecosystems, accounting for 26% of the ice-free land worldwide (Foley et al. 2011). These ecosystems can influence carbon dioxide (CO_2) levels in the atmosphere as they are potential carbon (C) sinks (Li et al. 2008; Smith 2014). Grazing is the most extensive land use mode for grasslands, and is the major driver of grassland degradation. Generally, grazing affects grasslands through herbivory, physical impact, and deposition. Because herbivores consume plant leaves, stems, and other tissues (Díaz et al. 2007; Zheng et al. 2011a), grazing can alter plant community composition, structure, and productivity (Fig. 1). For example, grazing negatively affected the aboveground production in a *Stipa grandis*/*Leymus chinensis*-dominated steppe community (Schönbach et al. 2011), while it increased the allocation of C and N to the belowground biomass and promoted N retention and C input to the soil (Hui and Jackson 2006). Additionally, grazing often changes the composition and chemical properties of plant litter and affects litter decomposition (Carrera et al. 2008; Semmartin et al. 2008), thus influencing nutrient cycling in the soil (Bakker et al. 2004; Quedstedt et al. 2007). For example, grazing retarded N cycling through a reduction in N-rich and palatable species and an increase in N-poor species with low litter quality (Shan et al. 2011). Thereby, grazing can change C and N inputs from the roots to the soil (Piñeiro et al. 2009). Herbivores (e.g., cattle and sheep) can also trample plants, disturb soil surfaces, and compact soils through hoof action. As a result, trampling can affect soil microbial activity and SOM mineralization by reducing soil water content and aeration (Lavado and Taboada 1988). Grazing animals affect

nutrient cycling by depositing N-rich urine and dung (McNaughton et al. 1997). Overall, grazing can affect the availability of nutrients in the soil by changing the interactions between plants and soil and influencing the utilization and retention of NO_3^- and NH_4^+ by plants, and thus their fate (Fig. 1). A prerequisite to understanding and managing the effects of N limitation on plant growth, as well as C sequestration in grasslands, is to clarify the utilization and retention of NO_3^- and NH_4^+ by plants.

Most previous studies have been conducted in alpine grasslands. For example, our previous studies investigated the fates of NH_4^+ and NO_3^- in alpine grasslands and found that their fates were distinctly different approximately two months after ^{15}N addition. More ^{15}N was recovered in plants than in microorganisms, but plants and soil microorganisms recovered more $^{15}\text{N-NO}_3^-$ than $^{15}\text{N-NH}_4^+$ (Xu et al. 2003). Similar results were observed 11–13 months after ^{15}N labeling, but SOM recovered more $^{15}\text{N-NH}_4^+$ than $^{15}\text{N-NO}_3^-$ (Xu et al. 2004). A study comparing the effects of overgrazing on the fates of inorganic and organic N in alpine grasslands showed that overgrazing-induced crusts led to lower total ^{15}N recovery from inorganic N than from organic N (Zhang et al. 2017). Compared to alpine grasslands, research on the N cycle in temperate grasslands has mostly focused on net N mineralization (Wang et al. 2006; Liu et al. 2010), N_2O emission (Du et al. 2006), and plant-microbe competition for N (Liu et al. 2016; Ouyang et al. 2016). The fates of NH_4^+ and NO_3^- in semi-arid temperate grasslands in China remain largely unknown, although these ecosystems comprise nearly 12.5% of the global grassland area (Liu et al. 2010). Given that the climate and water conditions in temperate grasslands are very different from those in alpine grasslands, it is unreasonable to estimate N retention by plants in temperate grasslands based on the results obtained from alpine grasslands.

Additionally, plants under N-limited conditions provide a large amount of available C as root exudates to fuel rhizosphere microorganisms. In return, rhizosphere microorganisms produce extracellular enzymes to accelerate SOM mineralization and release available N to meet their own needs, as well as those of plants (Kuzyakov and Xu 2013; Sun et al. 2014). Such changes in labile C input via root exudation are very common in grasslands, because grazing animals can strongly affect photosynthesis and production through defoliation (Jaramillo and Detling 1988;

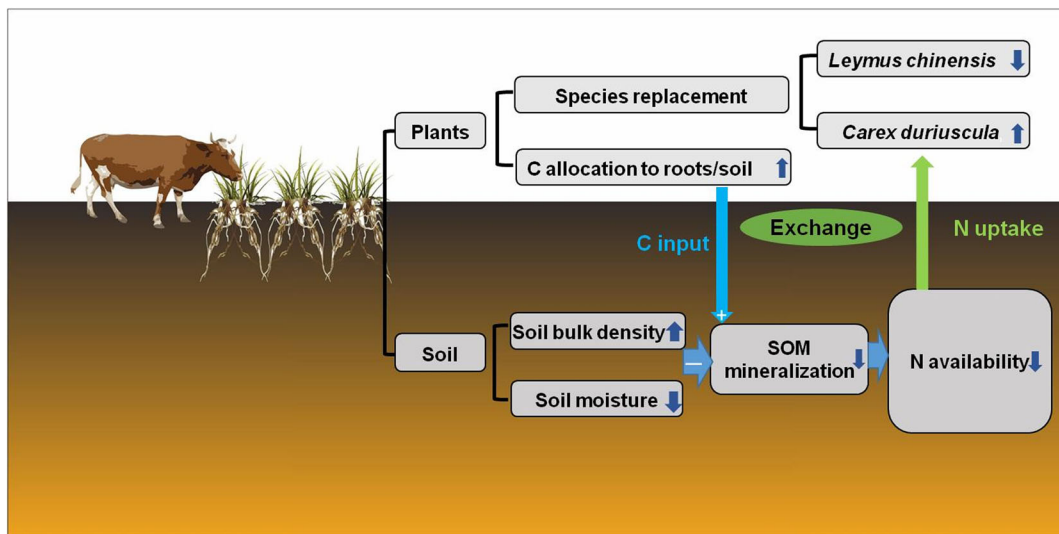


Fig. 1 A scheme for the effect of grazing on the exchange of carbon input and nitrogen uptake in a temperate grassland. Grazing removes part of the aboveground biomass and reduces litter coverage. This process accelerates the evaporation of soil surface water, and thus decreases litter decomposition and SOM mineralization. Substantially livestock trampling decreases soil porosity and limits the diffusion of CO_2 and oxygen, thereby inhibiting SOM

mineralization and the availability of soil N in the grazed grassland. The arrows indicate the direction of changes in ecological processes and its thickness indicates the magnitude of the changes. White plus sign (+) indicates that carbon input can increase mineralization of soil organic matter (SOM), while white minus sign (–) indicates that increased soil bulk density and reduced soil moisture due to trampling decrease SOM mineralization

Ferraro and Oesterheld 2002). These findings indicate that grazing could affect the uptake of N by plants by modifying root exudation (Sun et al. 2018), but this hypothesis remains unexplored in temperate grasslands.

It is well known that grazing can reduce plant height and leaf area directly by defoliation (Díaz et al. 2007; Zheng et al. 2011a). As a result, grazing can affect light availability and plant photosynthesis, thus altering C allocation and fluxes in grasslands. In contrast to grazing, clipping (removing photosynthetic organs) and shading (reducing light intensity) are two important manipulation approaches to reduce the substrate supply to soil from the photosynthesis due to reduced leaf area and light availability (Wan and Luo 2003). Therefore, clipping and shading treatments are often used to simulate grazing effects on grasslands, especially clipping treatment (Dahl et al. 2016). To explore the potential effect of reduced C input via root exudation on NH_4^+ and NO_3^- retention by plants, a ^{15}N -labeling field experiment together with clipping and shading treatments was conducted in grazed and ungrazed temperate grasslands in Hulunbeier, Inner Mongolia, for 28 days. The present study aimed to test the following hypotheses: (1) Plants can recover more ^{15}N in grazed than in ungrazed grasslands due to compensatory growth and changes in

species composition; (2) Clipping and shading reduce ^{15}N recovery by plants because they decrease the production of the photosynthate used as energy for NH_4^+ and NO_3^- uptake by roots; and (3) Plants recover more $^{15}\text{N-NH}_4^+$ than $^{15}\text{N-NO}_3^-$, because NH_4^+ is the dominant N form in the soil and plants prefer to absorb the dominant N form.

Materials and methods

Study site

The experiment was conducted at the Hulunbeier Grassland Ecosystem Observation and Research Station (Hulunbeier station) in Inner Mongolia, North China ($49^\circ22'42''\text{--}49^\circ22'92''\text{N}$, $120^\circ01'54''\text{--}120^\circ02'32''\text{E}$, 628 m above sea level). The climate is semi-arid inland, with an annual mean temperature ranging from -2 to 1°C and annual mean precipitation ranging from 380 to 400 mm. Most precipitation occurs in summer, from May to August (Meng et al. 2009). The grassland vegetation is dominated by *L. chinensis*, and the associated species are mainly *Carex duriuscula*, *Cleistogenes squarrosa*, *Stipa baicalensis*, *Potentilla bifurca*, and

Bupleurum scorzonerifolium. This vegetation type is generally called “*Leymus chinensis*” grassland. The soil is chestnut soil (IUSS 2014), corresponding to Calcic-orthic Aridisol according to The United States Department of Agriculture (USDA) Soil Taxonomy (Soil Survey Staff 2003).

Experimental settings

In June 2013, one ungrazed temperate grassland and one grazed temperate grassland were selected for the ^{15}N labeling experiment. Both grasslands were about 5 km away from Hulunbeier station. The ungrazed grassland was developed from the grazed grassland by enclosing a portion of the grazed grassland in 2007 to exclude grazing in that portion. This grassland was dominated by *L. chinensis* and covered approximately an area of 36 ha. The grazed grassland was exposed to perennial grazing mainly by Sanhe cattle in the past 30 years. Soil properties in the upper layer (15 cm) in grazed and ungrazed grasslands are presented in Table 1. Four quadrants (100 × 100 cm) were randomly selected in the grazed or ungrazed grasslands for species investigations (Table 2).

To test whether clipping and shading affect $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ recovery by plants, we randomly established sampling plots (20 × 20 cm), separated by at least 1 m, in the grazed and ungrazed grasslands. The sampling plots were provided a clipping treatment, a shading treatment, or control treatment. For the clipping treatment, aboveground parts were harvested 1 cm above the soil surface, and the plants grew freely during the remaining experimental period. The shading treatment consisted of covering the plots with a black mesh

Table 1 Properties of the top 15 cm soil at the study site (means ± SE, $n = 12$), and the lowercase letters represent significant difference of the characteristics between grazed and ungrazed grasslands at $P = 0.05$

<i>Leymus chinensis</i> grassland	Grazed	Ungrazed
Soil bulk density (g cm^{-3})	1.19 ± 0.03 ^a	0.99 ± 0.03 ^b
NH_4^+ -N concentration ($\mu\text{g N g}^{-1}$ soil)	5.84 ± 0.50 ^b	21.20 ± 2.04 ^a
NO_3^- -N concentration ($\mu\text{g N g}^{-1}$ soil)	2.93 ± 0.04 ^b	4.16 ± 0.30 ^a
Water content (%)	0.28 ± 0.01 ^b	0.34 ± 0.02 ^a
Microbial biomass carbon ($\mu\text{g C g}^{-1}$ soil)	473.01 ± 35.81	376.42 ± 25.53

to reduce available light intensity up to 90%. At the time of introducing the abovementioned treatments, plant species composition and growth stage were similar in either of the two grasslands. For each treatment, we labelled the plants using two ^{15}N -forms (i.e., $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$). There were four replicates for either of two ^{15}N forms under each abovementioned treatment. To calculate the recovery of different N-forms, we also established four plots as references of the natural abundance of ^{15}N of plants in each of the two grasslands. In total, we have 56 plots (3 different treatments [1 clipping + 1 shading + 1 control] × 2 grasslands × 2 ^{15}N -forms × 4 replicates + 4 references of the natural abundance of ^{15}N in plants × 2 grasslands).

Based on previous measurements on inorganic N concentrations in the investigated soil, ^{15}N was applied at 1 $\mu\text{g N g}^{-1}$ soil to avoid a fertilization effect. The tracers ($(^{15}\text{NH}_4)_2\text{SO}_4$, 10% ^{15}N enrichment or K^{15}NO_3 , 10% ^{15}N enrichment) were dissolved in distilled water and injected into the soil at 2.5-cm depth. To ensure an equal distribution of ^{15}N tracers, each plot was divided into nine blocks, and the ^{15}N solution was injected in each block. The reference plots indicating the natural abundance of ^{15}N in plants were injected with the same amount of water.

Sampling and analyses

Three hours after ^{15}N labeling, plant and soil samples were harvested from both treated and control plots, and these were considered as initial soil cores. At the same time, clipping and shading treatments were implemented in grazed and ungrazed grasslands. To test whether sampling time affects the biomass and N recovery, plant and soil samples were collected on days 7, 14, and 28 after ^{15}N labeling. Plants were harvested from a 7 × 7 cm area within each plot using scissors. Soil cores (5 cm in diameter; 10 cm depth) were taken from these areas.

Fresh soil samples were immediately transferred to the laboratory. Living roots were carefully removed from soil cores, and the soil was sieved through a 2-mm mesh and stored at -20°C . The characteristics of soils including moisture content, inorganic N (NH_4^+ and NO_3^-) and microbial biomass C (MBC) were measured within three days, while soil bulk density was determined within two weeks after soil collection.

To determine soil moisture content, fresh soil (15 g) samples were placed in an oven at 105°C for 24 h. Ten

Table 2 Relative biomass (%) of dominant plant species in grazed and ungrazed grasslands and the lowercase letters denote difference of various species in both grasslands. The numbers in brackets are SD of four replicates

Latin name	Section	Grazed Relative biomass (%)	Ungrazed Relative biomass (%)
<i>Leymus chinensis</i>	Gramineae	7.93(3.71)^b	76.17(11.46)^a
<i>Stipa baicalensis</i>	Gramineae	1.47(1.47) ^a	0.60(0.30) ^a
<i>Cleistogenes squarrosa</i>	Gramineae	13.31(3.56) ^a	0.00(0.00) ^a
<i>Carex duriuscula</i>	Cyperaceae	67.05(6.63)^a	9.67(4.75)^b
<i>Thalictrum squarrosum</i>	Ranunculaceae	0.47(0.47) ^a	0.67(0.45) ^a
<i>Pulsatilla turczaninowii</i>	Ranunculaceae	1.10(0.68) ^a	0.00(0.00) ^a
<i>Potentilla tanacetifolia</i>	Rosaceae	1.17(1.17) ^a	0.03(0.03) ^a
<i>Potentilla acaulis</i>	Rosaceae	3.58(2.26) ^a	0.00(0.00) ^a
<i>Potentilla bifurca</i>	Rosaceae	0.00(0.00) ^a	0.87(0.87) ^a
<i>Sanguisorba officinalis</i>	Rosaceae	0.00(0.00) ^a	0.18(0.18) ^a
<i>Adenophora elata</i>	Campanulaceae	1.40(0.17) ^a	7.55(3.63) ^a
<i>Galium verum</i>	Rubiaceae	0.11(0.11) ^a	0.00(0.00) ^a
<i>Bupleurum scorzonerifolium</i>	Umbelliferae	0.57(0.57) ^a	0.57(0.57) ^a
<i>Saposhnikovia divaricata</i>	Umbelliferae	1.27(1.27) ^a	0.62(0.28) ^a
<i>Plantago depressa</i>	Plantaginaceae	0.15(0.15) ^a	0.00(0.00) ^a
<i>Lychnis sibirica</i>	Caryophyllaceae	0.17(0.17) ^a	0.00(0.00) ^a
<i>Heteropappus altaicus</i>	Compositae	0.00(0.00) ^a	2.39(1.78) ^a
<i>Lilium pumilum</i>	Liliaceae	0.00(0.00) ^a	0.14(0.14) ^a
Others		0.26(0.26) ^a	0.56(0.56) ^a
Plant cover (%)		26.67 ^b	70.00 ^a

grams of fresh soil was extracted with 40 mL 0.05 M K₂SO₄ for 1 h, under continuous shaking at 150 r min⁻¹. Filtered extracts were then used to measure NO₃⁻-N and NH₄⁺-N using an auto-analyzer (AA3; Bran-Luebbe, Norderstedt, Germany). Bulk density was determined from undisturbed soil cores treated at 105 °C for 48 h (Van Reeuwijk 1993).

Living roots were rinsed with tap water, submerged in 0.5 mM CaCl₂ solution for 30 min, and again rinsed with distilled water to remove the ¹⁵N adsorbed on the root surface (Xu et al. 2011). Shoots and roots were then dried at 75 °C for 48 h and ground into a fine powder using a ball mill (MM2; Retsch, Haan, Germany). Plant materials (about 2 mg) were weighed into tin capsules to analyze total N and ¹⁵N/¹⁴N ratio using a continuous-flow gas isotope ratio mass spectrometer (MAT253; Finnigan MAT, Bremen, Germany), coupled with a

ConFlo III device (Finnigan MAT) and an elemental analyzer (EA 1112; CE Instruments, Milan, Italy).

Calculations and statistics

The ¹⁵N atom percent excess (APE) was calculated as the difference between the atom% ¹⁵N in shoots or roots of plants from ¹⁵N labeled and control plots:

$$APE(\%) = atom\%_{plant(labeled)} - atom\%_{plant(control)} \quad (1)$$

The ¹⁵N uptake (mg ¹⁵N m⁻²), defined as the amount of ¹⁵N recovered from different N pools (shoots and roots), was calculated by multiplying APE (%) by the moles of N in the plants as follows:

$$^{15}\text{N}_{\text{uptake}} = APE * \frac{N\%_{\text{plant}} \times \text{Biomass}}{atom\%_{\text{plant}(labeled)} \times 15 + (100\% - atom\%_{\text{plant}(labeled)}) \times 14} * 15 \quad (2)$$

The percentage of ^{15}N recovered in plants ($^{15}\text{N}_{\text{recovery}}$) was calculated using the following equation:

$$^{15}\text{N}_{\text{recovery}}(\%) = \frac{^{15}\text{N}_{\text{uptake}}}{^{15}\text{N}_{\text{added}}} \times 100\% \quad (3)$$

Where $^{15}\text{N}_{\text{uptake}}$ (g m^{-2}) refers to the ^{15}N mass uptake by plants (shoots and roots), and $^{15}\text{N}_{\text{added}}$ (g m^{-2}) refers to the total ^{15}N mass added to the soil per square meter.

Considering the rapid turnover of NO_3^- and NH_4^+ , N uptake rates ($\mu\text{g N g}^{-1}$ dry weight root h^{-1}) was only calculated over three hours by multiplying $^{15}\text{N}_{\text{uptake}}$ by the amount of native NH_4^+ or NO_3^- in the soil and then dividing it by the labeling time in hours and the amount of added $^{15}\text{N-NH}_4^+$ or $^{15}\text{N-NO}_3^-$ (Xu et al. 2011).

All results are presented as mean values \pm standard errors of the mean. We used the repeated measures analysis of variance (ANOVA) to estimate the effects of time, grazing, treatment (clipping, shading, and control) and their interactions on aboveground biomass, belowground biomass, and total biomass, and to test the effects of time, grazing, treatment, N form (NH_4^+ and NO_3^-), and their interactions on ^{15}N recovery in plants. A one-way ANOVA was used to assess the effect of treatment on the recovery of ^{15}N by plants according to sampling time. All analyses were performed after checking for normality and homogeneity of variance and were conducted using the SPSS 16.0 software package (IBM Corp., Armonk, NY, USA). Significant differences were tested at $P < 0.05$.

Results

Species composition and above- and belowground biomass

Long-term grazing considerably changed plant species composition and vegetation coverage (Table 2). The dominance of plant species shifted from *L. chinensis* in the ungrazed grassland to *C. duriuscula* in the grazed grassland. Vegetation coverage was reduced from 70% in the ungrazed grassland to 26.7% in the grazed grassland (Table 2).

Grazing altered the aboveground and belowground biomass of temperate grasslands ($P < 0.001$, Table 3). The ungrazed grassland had higher values of total aboveground biomass than the grazed grassland, while the belowground biomass showed the opposite pattern

(Fig. 2, $P < 0.001$, Table 3). This resulted in a higher root/shoot ratio in untreated (control, CK) grazed grassland (CK: root/shoot ratio = 4.32 ± 0.27) than in CK ungrazed grassland (CK: root/shoot ratio = 3.06 ± 0.48) (Fig. 2, $P = 0.02$).

Sampling time had no effect on the aboveground biomass ($P = 0.54$, Table 3) but significantly affected belowground biomass ($P < 0.001$, Table 3). Changes in aboveground and belowground biomass showed similar trends under each of the three treatments in both grazed and ungrazed grasslands.

Furthermore, aboveground and belowground biomasses were lower under the clipping and shading treatments than under the control treatment ($P < 0.001$ for aboveground biomass, $P = 0.02$ for belowground biomass, $P < 0.001$ for total biomass, Table 3, Fig. 2).

Plant N uptake rate and soil microbial biomass C

Uptake rates of NH_4^+ -N were higher than those of NO_3^- -N in grazed and ungrazed grasslands (Fig. 3a, $P < 0.05$). The uptake rate of NO_3^- -N by plants was about 10 times higher in the grazed grassland ($19.84 \pm 1.36 \mu\text{g N h}^{-1} \text{g}^{-1}$ root) than in the ungrazed grassland ($1.92 \pm 0.22 \mu\text{g N h}^{-1} \text{g}^{-1}$ root) (Fig. 3a, $P < 0.001$). Uptake rates of NH_4^+ by plants were similar in grazed ($30.02 \pm 3.33 \mu\text{g N h}^{-1} \text{g}^{-1}$ root) and ungrazed ($36.69 \pm 6.96 \mu\text{g N h}^{-1} \text{g}^{-1}$ root) grasslands (Fig. 3a, $P = 0.60$). There was a significant difference in MBC between shading and control treatments in the grazed grassland (Fig. 3b, $P < 0.05$).

Recovery of ^{15}N by plants

In the control treatment, $^{15}\text{N-NH}_4^+$ recovery in the aboveground biomass was higher in the ungrazed ($34.43 \pm 9.92\%$) than in the grazed grassland ($23.85 \pm 2.56\%$) 28 days after labeling (Fig. 4a, b, $P < 0.05$). On the contrary, grazing increased $^{15}\text{N-NO}_3^-$ recovery (grazed: $21.45 \pm 4.28\%$; ungrazed: $14.59 \pm 2.10\%$) (Fig. 4c, d). Overall, ^{15}N recovery in the aboveground biomass increased over time in grazed and ungrazed grasslands (Fig. 4a–d).

Significant effects and interactions were found for time ($P < 0.001$), treatment ($P < 0.001$), and N form ($P < 0.001$), while grazing had no effect ($P = 0.06$) on ^{15}N recovery in aboveground biomass (Table 4). Clipping and shading treatments decreased ^{15}N recovery in

Table 3 Repeated measures analysis of variance (ANOVA) results for the effects of time, grazing, treatment and their interactions on aboveground biomass and belowground biomass. Number in bold indicates that the effect was significantly different at a $P < 0.05$ level

Sources of Variation	Aboveground biomass		Belowground biomass		Total biomass	
	<i>F</i> values	<i>P</i> values	<i>F</i> values	<i>P</i> values	<i>F</i> values	<i>P</i> values
Time	0.68	0.54	9.04	$P < 0.001$	7.49	0.001
Grazing	33.66	$P < 0.001$	55.45	$P < 0.001$	6.39	0.02
Treatment	9.33	$P < 0.001$	4.49	0.02	10.05	$P < 0.001$
Grazing × Treatment	0.97	0.39	1.70	0.19	0.28	0.76

the aboveground biomass in grazed and ungrazed grasslands, which was consistent with the changing trend in the aboveground biomass (Figs. 2 and 4a–d). $^{15}\text{N-NH}_4^+$ recovery was higher than $^{15}\text{N-NO}_3^-$ recovery under control treatments in both grazed and ungrazed grasslands (Fig. 4a–d).

Grazing had a remarkable effect on ^{15}N recovery by roots ($P < 0.001$, Table 4). Concretely, ^{15}N recovery by roots under the control treatment was higher in the grazed grassland ($^{15}\text{N-NH}_4^+$ recovery: $7.03 \pm 1.43\%$; $^{15}\text{N-NO}_3^-$ recovery: $5.47 \pm 0.21\%$; Fig. 4e, g, Table 4) than in ungrazed grassland ($^{15}\text{N-NH}_4^+$ recovery: $4.89 \pm 0.30\%$; $^{15}\text{N-NO}_3^-$ recovery: $3.01 \pm 0.21\%$; Fig. 4 f, 4 h, Table 4) 28 days after the ^{15}N labeling.

Treatment had a significant effect on ^{15}N recovery by roots 28 days after labeling ($P = 0.01$, Table 4), and ^{15}N recovery was highest under clipping treatment in the grazed grassland ($^{15}\text{N-NH}_4^+$ recovery: $7.26 \pm 0.38\%$; $^{15}\text{N-NO}_3^-$ recovery: $6.42 \pm 0.31\%$; Fig. 4e, g) and lowest in the ungrazed grassland ($^{15}\text{N-NH}_4^+$ recovery: $1.95 \pm 0.82\%$; $^{15}\text{N-NO}_3^-$ recovery: $2.23 \pm 0.65\%$; Fig. 4f, h). Under both grasslands, the value of ^{15}N recovered ($^{15}\text{N-NH}_4^+$ and $^{15}\text{N-NO}_3^-$) by roots under shading treatment was lower than that under control treatment, but it was higher than that under clipping treatment (Fig. 4).

Under the control treatment, the effect of grazing on the total ^{15}N recovery (shoots + roots) of the two N

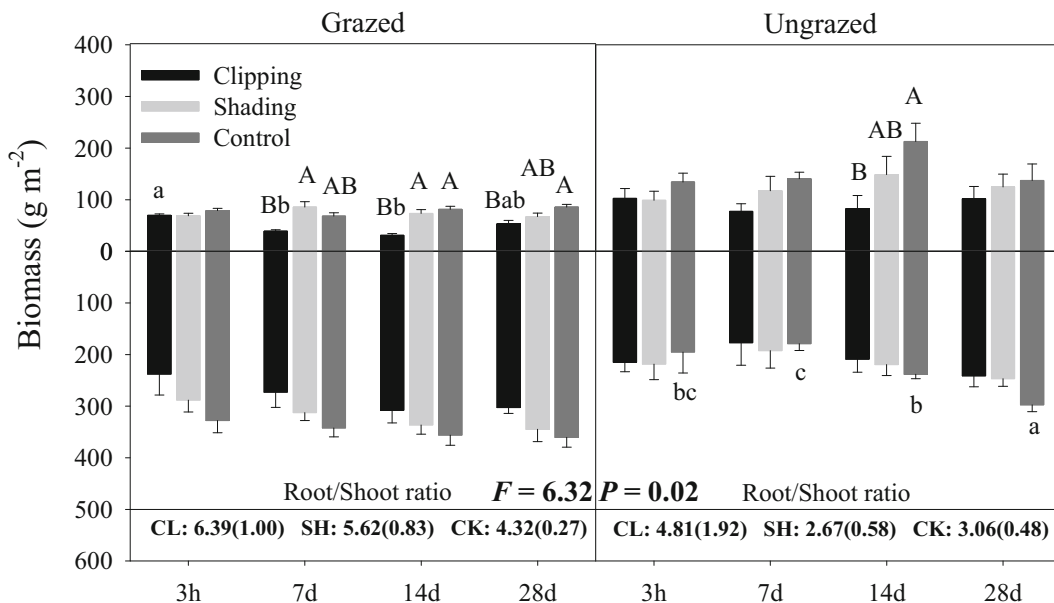


Fig. 2 Aboveground and belowground plant dry mass differences (mean ± SE) among the different times and treatments in grazed and ungrazed grasslands (n = 8). Root/Shoot ratio represents the last sampling (The *P* value represents the difference of root/shoot

ratio between grazed and ungrazed grasslands). Uppercase letters indicate the significant difference of the biomass among three treatments, while lowercase letters represent significant difference of the biomass at different times for either of treatment at $P = 0.05$

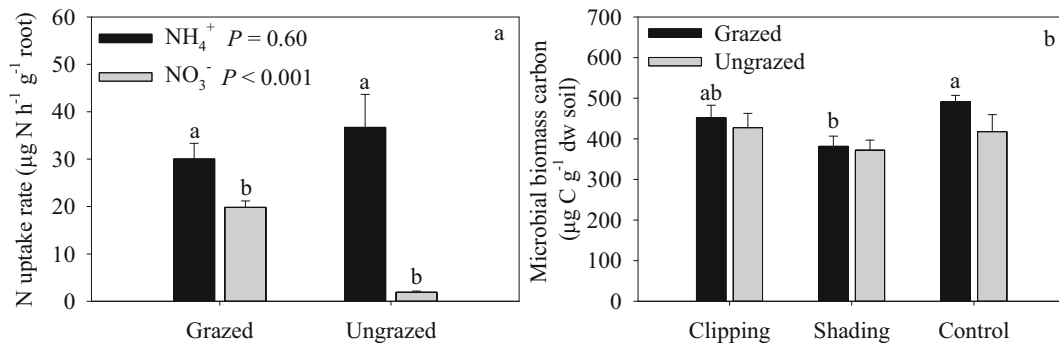


Fig. 3 **a** N uptake rate by plants (3 h) in grazed and ungrazed grasslands. All values are means \pm standard errors ($n=12$). **b** Microbial biomass carbon in soil under clipping, shading and

control treatments on 28 days in grazed and ungrazed grasslands. Values are means \pm standard errors ($n=8$). Lowercase letters represent significant difference level at $P=0.05$

forms differed. Total ^{15}N recovery from NH_4^+ in grazed grassland was lower than in the ungrazed grassland, while total ^{15}N recovery from NO_3^- showed the opposite trend 28 days after labeling (Fig. 5). In addition, total ^{15}N recovery in plants differed significantly between NH_4^+ and NO_3^- ($P < 0.001$, Table 4), with higher values of $^{15}\text{N}\text{-NH}_4^+$ recovery than $^{15}\text{N}\text{-NO}_3^-$ recovery under the control treatment in both grasslands ($P < 0.001$, Table 4, Fig. 5). Overall, clipping and shading decreased total ^{15}N recovery in grazed and ungrazed grasslands (Fig. 5). However, the response of $^{15}\text{N}\text{-NH}_4^+$ and $^{15}\text{N}\text{-NO}_3^-$ recovery by plants to clipping or shading was different in grazed and ungrazed grasslands. Under clipping/shading treatments, there was higher $^{15}\text{N}\text{-NO}_3^-$ recovery in the grazed grassland and higher $^{15}\text{N}\text{-NH}_4^+$ recovery in the ungrazed grassland (Fig. 5).

Discussion

Grazing effects on plant biomass allocation

Our results indicate that aboveground biomass in the grazed grassland was lower than that in the ungrazed grassland (Fig. 2), which is consistent with previous studies (Ren et al. 2012). This is because livestock removes a part of the stems and leaves, thereby decreasing the leaf area index and the photosynthetic rate of grasses (Diaz et al. 2001; Zheng et al. 2010). Moreover, grazing can lead to changes in the dominant species. For instance, grazing inhibits the growth of taller grasses and promotes the rapid growth of shorter plants that are less accessible to grazers (Golodets et al. 2010), thus reducing the primary productivity of the community. In the

current study grazing shifted the dominance of plant species from *C. duriuscula* in the grazed grassland to *L. chinensis* in the ungrazed grassland (Table 2). Such a shift in plant species composition could lead to the observed discrepancy in the aboveground biomass between grazed and ungrazed grasslands, because it is known that *C. duriuscula* is more dwarf than *L. chinensis*.

The response of belowground biomass to grazing varies with grassland type. Grazing may increase (Frank et al. 2002; Patton et al. 2007), decrease (Semmartin and Oesterheld 2001; Leriche et al. 2003), or have no effect (Turner et al. 1993; McNaughton et al. 1988) on belowground biomass. In the present study, grazing induced an increase in belowground production, possibly because the lower soil inorganic N contents (NH_4^+ and NO_3^-) in the grazed grassland (Table 1) lead plants to increase C allocation to roots to acquire more available N (Dijkstra et al. 2008). Additionally, the higher root/shoot ratios in the grazed grassland (Fig. 2) can enhance the resistance of plants to external disturbances (Wang et al. 2003). Therefore, an adaptation of plants to grazing might be to regulate the allocation patterns of photosynthate to shoots and roots (Wang et al. 2003) and increase root density and length to compensate for aboveground shoot removal (Zong et al. 2012).

Aboveground biomass showed different responses to clipping and shading between the two grasslands. The instantaneous photosynthesis of the remaining leaves could increase in response to defoliation (Detling et al. 1979; Nowak and Caldwell 1984; Zhao et al. 2008), possibly due to more chlorophyll synthesized within remaining leaves (Oesterheld

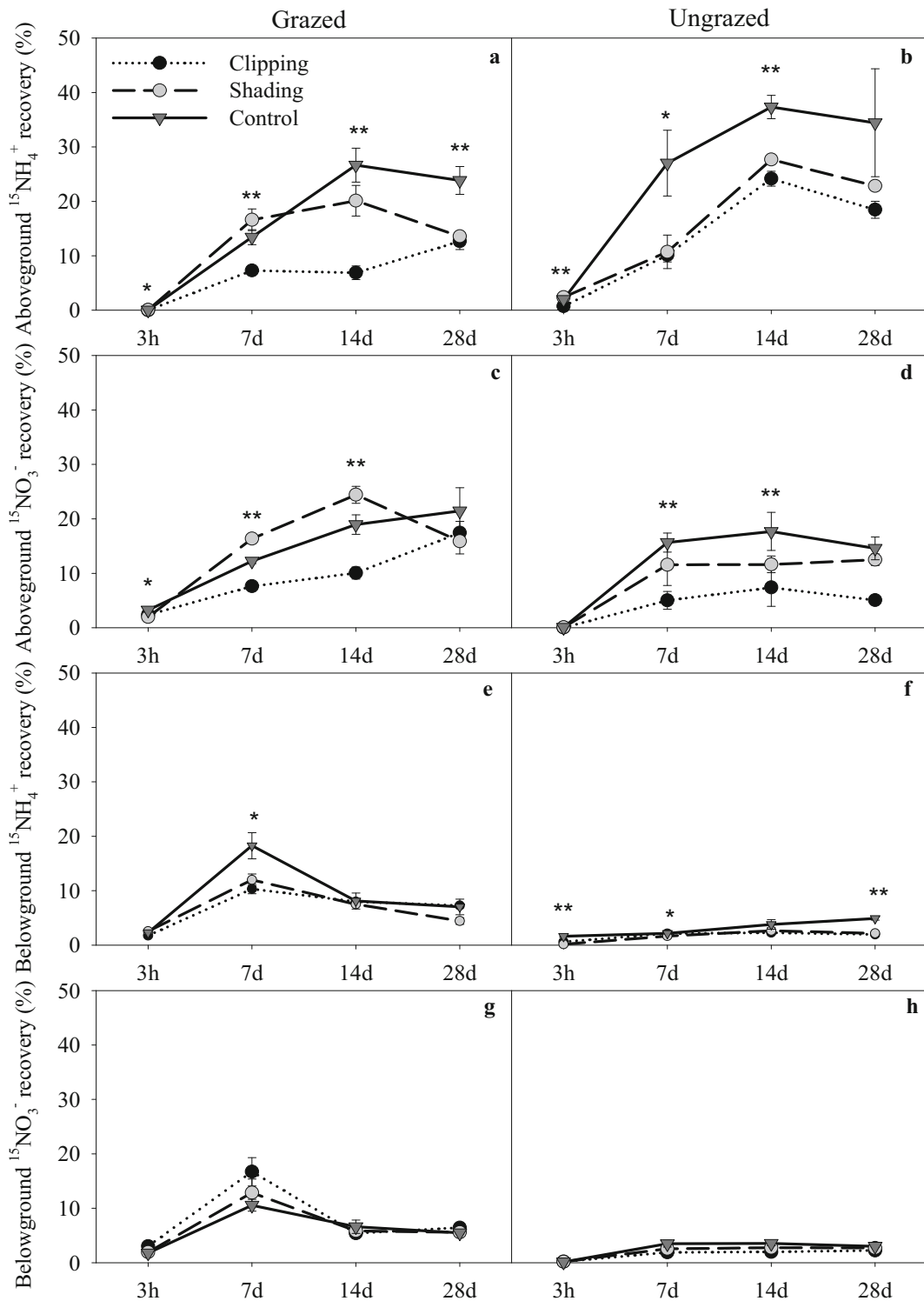


Fig. 4 ^{15}N recovered by plants (% of added ^{15}N) (a–h) at different times in grazed and ungrazed grasslands. The bars and error bars show means \pm SE (n = 4). **, * indicates significance

between shading, clipping and control treatments at $P=0.01$ and $P=0.05$ probability levels, respectively

Table 4 Repeated measures analysis of variance (ANOVA) results for the effects of time, grazing, treatment, N form and their interactions on the ^{15}N recovery of plants. Number in bold indicates that the effect was significantly different at a $P < 0.05$ level

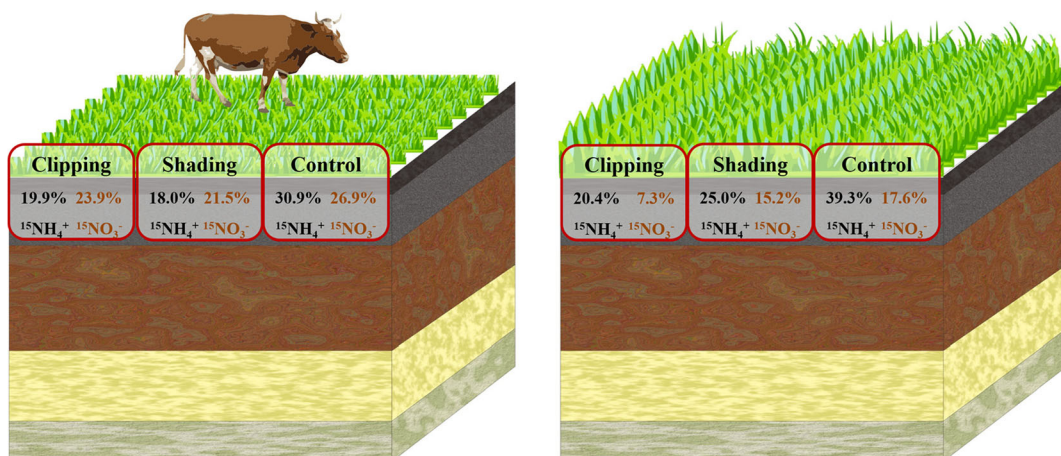
Sources of variation	Aboveground ^{15}N recovery		Belowground ^{15}N recovery		Total ^{15}N recovery	
	<i>F</i> values	<i>P</i> values	<i>F</i> values	<i>P</i> values	<i>F</i> values	<i>P</i> values
Time	66.28	$P < 0.001$	112.45	$P < 0.001$	173.14	$P < 0.001$
Grazing	3.14	0.06	413.44	$P < 0.001$	45.46	$P < 0.001$
Treatment	61.88	$P < 0.001$	5.25	0.01	67.16	$P < 0.001$
N form	46.91	$P < 0.001$	2.15	0.15	54.20	$P < 0.001$
Grazing \times Treatment	5.44	0.009	1.79	0.18	5.45	0.009
Grazing \times N form	65.29	$P < 0.001$	0.98	0.33	57.60	$P < 0.001$
Treatment \times N form	7.32	0.002	7.38	0.002	12.91	$P < 0.001$
Grazing \times Treatment \times N form	0.43	0.65	4.09	0.025	1.44	0.25

and McNaughton 1991). However, the faster photosynthesis of the remaining leaves cannot compensate for the much larger reduction in leaf area such that photosynthesis of the whole plant is decreased. With the growth of new leaves, plant biomass gradually increases.

By comparison, aboveground biomass started to decrease seven days after shading, largely because the continuous decrease in light intensity reduced the photosynthetic rate of plants (Zheng et al. 2011b). Although grazing can increase root biomass to compensate for growth, clipping decreased root biomass. This might be due to a trade-off between aboveground and belowground biomass caused by the compensatory growth of aboveground biomass after clipping treatment (Distelfeld et al. 2014).

Grazing effects on soil mineral N, plant N uptake rate, and soil MBC

Many studies have shown that grazers can influence microbial mineralization and immobilization, leading to changes in N availability for plants (Hamilton and Frank 2001; Rossignol et al. 2006). In the present study, we did not measure mineralization and immobilization, but determined the concentration of mineral N, which was lower in grazed than in ungrazed grassland (Table 1). A possible explanation is that grazing removes part of the aboveground biomass and reduces litter coverage (Schmitt et al. 2013; Wei et al. 2016). This process accelerates the evaporation of soil surface water, and thus decreases litter decomposition and SOM mineralization (Lavado and Taboada 1988; Wang et al.

**Fig. 5** The total ^{15}N recovery of $^{15}\text{N}\text{-NH}_4^+$ and $^{15}\text{N}\text{-NO}_3^-$ in plants (% of added ^{15}N) under clipping and shading treatments on 28 days in grazed and ungrazed grasslands

2010). Additionally, livestock trampling decreases soil porosity and limits the diffusion of CO₂ and oxygen, thereby inhibiting SOM mineralization and the availability of soil N (Li et al. 2013). Grazing increased the NO₃⁻ uptake rate but reduced the uptake rate of NH₄⁺. One possible explanation is that grazing changed plant species composition and the dominant species differ in their preference for N forms (Xu et al. 2011; Wang et al. 2016).

The increasing MBC was consistent with the higher belowground biomass in the grazed grassland under control treatment (Figs. 2 and 3b). This could be ascribed to increased root exudates caused by higher root biomass, which promote microbial growth in the rhizosphere.

Grazing effects on plant ¹⁵N recovery

The increase of ¹⁵N recovery in aboveground biomass over time (Fig. 4a–d) is ascribed to continuous ¹⁵N transfer from soil and roots to shoots (Kuzyakov and Xu 2013). Higher ¹⁵N recovery of roots in grazed than in the ungrazed grassland 28 d after labeling, is consistent with the higher belowground biomass in the grazed grassland.

In the ungrazed grassland, the higher ¹⁵N-NH₄⁺ recovery than ¹⁵N-NO₃⁻ recovery observed under three treatments (Fig. 5) might be related to the N acquisition strategy of the dominant species, *L. chinensis*, which is a type of rhizomatous clonal grass with a well-developed underground rhizome that enables *L. chinensis* to spread quickly and escape rapidly from stressful microenvironments (Li et al. 2007). Therefore, the N absorption strategy of *L. chinensis* will be more flexible and more dependent on the availability of N forms in the soil and *L. chinensis* in ungrazed grassland absorbed more NH₄⁺ because this ion was the main component of the soil N pool.

Higher plant ¹⁵N recovery (¹⁵N-NH₄⁺ + ¹⁵N-NO₃⁻) in the grazed grassland than in ungrazed grassland confirms our first hypothesis. This result could be ascribed to a large amount of N being redistributed for the regrowth of plants after grazing and increasing the N uptake by plants (Fig. 5). Although NH₄⁺ was higher than NO₃⁻ in both grazed and ungrazed grasslands, grazing decreased the total ¹⁵N-NH₄⁺ recovery but increased the ¹⁵N-NO₃⁻ recovery (Fig. 5), partly supporting our third hypothesis. This difference in ¹⁵N recovery of both N forms in grazed and ungrazed grasslands is due to changes in inorganic N ratios as well as

plant composition. For example, both laboratory and field observations showed that the NH₄⁺/NO₃⁻ ratios in the soil affect plant uptake of NH₄⁺ or NO₃⁻ (Houlton et al. 2007). In the present study, the grazing-induced decrease in soil NH₄⁺/NO₃⁻ ratio led to a reduction in NH₄⁺ uptake by plants (Table 1). In addition, plant species in this kind of temperate grassland have distinct access to various forms of N in the soil (Ouyang et al. 2016; Wang et al. 2016). Changes in dominant plant species in ungrazed and grazed grasslands (i.e., *L. chinensis* replaced by *C. duriuscula*) could lead to higher ¹⁵NO₃⁻ recovery in the grazed grassland. More NO₃⁻ than NH₄⁺ taken up by plants under clipping and shading treatments in grazed grassland could also be ascribed to changes in the species composition caused by grazing. Concretely, grazing promotes the growth of *C. duriuscula* (grazing-resistant, root-developed) and reduces the relative biomass of *L. chinensis*. This sedge could preferentially take up NO₃⁻ and increase the ¹⁵N recovery, but this needs further investigation. Besides, a substantial change in C allocation to belowground in grazed and ungrazed grasslands could be another reason for the differences in ¹⁵N uptake by plants.

Compared with the control treatment in both grazed and ungrazed grasslands, clipping and shading treatments reduced ¹⁵N recovery by plants (Fig. 5), confirming our second hypothesis. The strong reduction of leaves or light intensity after clipping or shading led to very low photosynthesis. Consequently, the energy used to pump up nutrients was minimal and resulted in the lower ¹⁵N recovery (Shahzad et al. 2012).

Overall, our results demonstrate that plant C input alters the uptake of N and its retention by plants in temperate grasslands. Grazing increases C input to the soil and promotes plant N acquisition, thereby compensating for the loss of C from the aboveground parts and increasing resistance to grazing due to the shift in plant dominance.

Conclusions

In temperate grasslands, the decrease in N availability in grazed grassland is mainly due to the reduction of SOM mineralization caused by animal trampling and enhanced transpiration caused by a reduction in vegetation coverage. Consequently, the growth of plants in the grazed grassland is more N limited compared with plants in the ungrazed grassland. Moreover, plants take

up more N ($\text{NH}_4^+ + \text{NO}_3^-$) for root compensatory growth to offset the loss of stems and leaves on the ground in the grazed grassland. The effects of grazing on $^{15}\text{N-NH}_4^+$ and $^{15}\text{N-NO}_3^-$ recovery in plants largely depend on soil $\text{NH}_4^+/\text{NO}_3^-$ ratio and changes in plant species composition. Clipping and shading reduce N uptake by plants through decreasing the energy supply for the uptake of mineral N. This study only considered N retention by plants, N retention in SOM and microbial biomass should also be taken into account in future studies to understand the fate of N in temperate grasslands. In addition, the N uptake strategies of dominant species (*L. chinensis* and *C. duriuscula*) in grazed and ungrazed grasslands need to be investigated to understand the effects of grazing and clipping/shading treatments on N uptake by dominant plant species.

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