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Relationship of plant diversity with litter and soil available nitrogen in an alpine meadow under a 9-year grazing exclusion

Abstract Grazing exclusion is widely used globally to restore degraded grasslands. Plant diversity has important impacts on grassland ecosystem functions, including grassland productivity and carbon storage. In this study, we selected a *Kobresia* meadow on the Qinghai–Tibetan Plateau to investigate how grazing exclusion affects plant diversity. Inorganic nitrogen (NH_4^+ and NO_3^-) was also measured because its availability impacts plant growth. We found that plant diversity in the meadow was significantly lower under grazing exclusion (fenced meadow) for 9 years compared with moderate grazing. Accumulated litter was significantly higher under grazing exclusion (386.41 g m^{-2}) compared with grazing (58.77 g m^{-2}). Soil inorganic nitrogen at 0–5 cm depth was significantly higher under grazing exclusion (13.60×10^{-2} g kg^{-1}) than under grazing (9.40×10^{-2} g kg^{-1}). The composition of the four functional groups (grasses, sedges, legumes, and forbs) might alter in response to significant changes in the amount of litter and soil available nitrogen content under grazing exclusion compared with grazing. However, the enhanced soil available nitrogen content showed weak feedbacks on plant diversity. In conclusion, light limitation induced by increased amounts of litter may be the main factor causing decreased plant diversity in grazing-excluded meadows compared with moderately grazed meadows.

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Introduction

Biodiversity largely determines ecosystem functions in the terrestrial biosphere (van Ruijven and Berendse 2005; Tilman et al. 2006). Higher plant diversity could improve grassland productivity through complementary effects among different species (van Ruijven and Berendse 2005; Roscher et al. 2008) and thus maintain greater C sequestration in soils (De Deyn et al. 2011).

Worldwide, grazing exclusion is widely used to restore degraded grasslands and to assess the influences of livestock grazing on grasslands. A large number of studies have demonstrated that grazing exclusion has a profound effect on plant diversity in grasslands (Rusch and Oesterheld 1997; Collins et al. 1998; Altesor et al. 2005; Cheng et al. 2011; Rebollo et al. 2013). However, these studies sometimes present opposing results; some studies have shown that grazing exclusion enhances plant diversity in grasslands (Bock et al. 1984; Cheng et al. 2011), while other studies have shown the opposite (Rusch and Oesterheld 1997; Altesor et al. 2005; Rebollo et al. 2013).

These contradictory findings might be largely attributed to differences in grassland type and resource availability (Olff and Ritchie 1998). With the loss of plant species and the colonization of new species, grazing exclusion might alter plant diversity by regulating resource use and competition in ecosystems. In grasslands with low productivity, grazing exclusion generally has positive effects on plant diversity (Cheng et al. 2011). The empty niches in the plant community under livestock exclusion provide opportunities for new species to enter the ecosystem. However, in grasslands with high productivity, species exclusion from resource competition caused by grazing exclusion might lead to species loss and lower plant diversity (Altesor et al. 2005). Grazing exclusion might also interact with precipitation, light, soil nutrients, and other factors, producing a

combined impact on grassland plant diversity. For example, He et al. (2011) showed that plant diversity in different grassland types has diverse responses to litter that accumulates under grazing exclusion. Therefore, to explore the mechanisms that underlie changes in the plant diversity of grasslands, it is necessary to obtain a better understanding of how grazing exclusion affects plant diversity in grasslands with different types of vegetation, climate, nutrients, and other factors. This information would help predict how plant diversity responds to grassland managements.

Grasslands occupy 57 % (1.5×10^6 km^2) of the total area of the Qinghai–Tibetan Plateau (Sun and Zheng 1998). For centuries, grazing of the grasslands has been the traditional land use in this region (Zhou et al. 1987; Zhou 2001). Furthermore, grazing exclusion tends to be one of the main methods used to restore degraded grasslands following overgrazing (Li and Zhou 1998; Yeh 2006). However, several previous studies have demonstrated that plant diversity is higher in areas under grazing compared with areas of grazing exclusion in the alpine meadow (Klein et al. 2004; Chen et al. 2008; Wu et al. 2009). Chen et al. (2008) suggested that traditional winter grazing generates high small-scale richness and spatial variation of plant species in the alpine meadow. Wu et al. (2009) also demonstrated that plant species diversity is lower in meadows that are fenced during the growing season than in meadows subject to year-round grazing. However, Chen et al. (2008) found that species richness and spatial variation are lower in alpine meadows subjected to year-round grazing compared with those under winter grazing only. A long-term grazing study in a shrub meadow showed that non-grazing and grazing with different stocking rates had similar impacts on plant species richness (Zhou et al. 2006). Grazing stocking rates, climate characteristics, and other factors on the plateau influence how grazing exclusion affects plant diversity (Klein et al. 2004; Zhou et al. 2006; Chen et al. 2008; Wu et al. 2009).

To improve our understanding of the mechanisms behind the response of plant diversity to grazing exclusion, we selected a *Kobresia humilis* meadow on the Qinghai–Tibetan Plateau subject to 9 years of grazing exclusion as our study site. We hypothesized that grazing exclusion has a greater negative affect on plant diversity than moderate grazing. Specifically, we investigated: (1) how plant diversity at moderate grazing densities responds to grazing exclusion, and (2) whether changes to plant diversity are related to light and soil nutrient resource competition in the *Kobresia humilis* meadow.

Methods

Site description

The study was conducted at the Haibei Alpine Meadow Ecosystem Research Station (37°29′–45′N, 101°12′–23′E) at an altitude of 3250 m in the northeastern Qinghai–Tibetan Plateau. The climate is dominated by a continental monsoon-type climate, with long, cold winters and short, cool summers. The annual average air temperature is −2 °C. The coldest monthly temperature is −18 °C in January, while the warmest is 10 °C in July. Average annual precipitation in this area ranges from 426 to 860 mm, with 80 % falling in the short summer growing season from May to September. Annual average sunlight is 2462.7 h, with 60.1 % sunlight being available for the photosynthesis and growth of herbage (Zhao and Zhou 1999). The study area is dominated by four important vegetation communities: *Kobresia humilis* meadows, *Dasiphora fruticosa* shrubs, *Kobresia pygmaea* meadows and *Kobresia tibetica* swamp meadows. Plants grow from May to September.

The experiment was carried out in a typical alpine *Kobresia humilis* meadow. The soil of the *Kobresia humilis* meadow is classified as Mat Cry-gaelic Cambisols according to the Chinese National Soil Survey and Classification System (Chinese Soil Taxonomy Research Group in Institute of Soil Science of Chinese Academy of Sciences 2001). The plant community is dominated by *Kobresia humilis*, *Stipa aliena*, and *Festuca ovina*. The aboveground productivity of vegetation peaks in late August each year. A large proportion of root biomass is found in the upper 30 cm of the soil profile (Wang et al. 1995; Jackson et al. 1996; Zhao 2009).

Experimental design

The experimental site is situated along a valley floor. It is a winter pasture subjected to grazing from January to March each year, with a moderate grazing intensity of 3.51 sheep ha^{-1} season^{-1}. The 50- × 50-m fenced meadow excludes yaks, sheep, and goats throughout the year and was established at the experimental site in 2005. Outside of the fenced area to one side, the grazed meadow exceeds 50×50 m in size, and is separated from the fenced area by at least 5 m. This area was used as the control in our study. Before grazing exclusion, the vegetation and other environmental conditions inside and outside the fence were homogeneous. Four independent plots (1×1 m) were selected at random in each treatment area to investigate the effects of grazing exclusion on plant diversity in the *Kobresia* meadow. The mean distance between the plots in each treatment was about 3 m. The experimental plots in each treatment were in a segregated area, and were considered as pseudoreplications. However, the homogenous vegetation and environmental conditions in both treatments before grazing exclusion should reduce or eliminate the effects of potential bias from different resources. Thus, differences in the values between the two treatments were assumed to be due to the effects of the 9-year grazing exclusion.

Plant diversity survey and data analysis

The vegetation survey in the fenced and grazed sites was conducted using one 50- × 50-cm quadrat in each plot for the fenced and grazed treatments in late August, 2014. Plant diversity was investigated using the point intercept method (Walk 1996). In brief, the fixed frame (50 × 50 cm) with 100 squares divided by nylon strings, each measuring 5 × 5 cm, and was placed over the vegetation in the selected plot. Wire pins (2 mm in diameter) were inserted vertically from the canopy top down to the ground in each square (n = 100 pins per quadrat). For each plant species, the point at which it touched the pin was recorded as a hit, and the percentage of the hits relative to the total 100 points was calculated as the cover of each plant species. In addition, the natural height of the foliage that the pin touched was recorded as the height of each plant species. After the investigation, the total aboveground biomass in each quadrat was harvested. The aboveground vegetation was separated by species, and the biomass of each species was estimated separately. Four vegetation functional groups were identified: grasses (Gramineae), sedges, legumes, and forbs.

Plant diversity was determined for each quadrat under the two treatments. The Simpson index (D) and Shannon–Wiener index (H')were used to illustrate plant diversity in our study. These indices were calculated by the following equations:

$$D = 1 - \sum P_i^2,$$
$$H' = - \sum P_i \ln P_i,$$

where P_i represents the relative important value (IV) of species i, which was calculated as:

$$IV = (\text{relative height} + \text{relative cover} + \text{relative biomass})/3.$$

The Evenness index (E) was calculated as:

$$E = H' / \ln S,$$

where S is the total number of species in the selected quadrat.

Plant biomass and soil nutrient analysis

The aboveground biomass was estimated by summing the aboveground biomass of all species harvested in the 50- × 50-cm quadrat. The aboveground biomass recorded in late August was used to calculate the annual plant production. To assess root biomass, the top 30 cm of soil (25 × 25 cm) was divided into three layers (0–5, 5–15, and 15–30 cm), and sampled after harvesting the aboveground vegetation within the quadrat. All roots and soil in the samples were carefully extracted and sieved with a 2-mm mesh. The roots were carefully washed with tap water using a 0.45-mm mesh, and were then oven-dried for 48 h at 75 °C for root biomass measurement.

Another three soil cores of 4-cm diameter were sampled from each plot in the fenced and grazed areas. Each soil core was divided into three layers (0–5, 5–15, and 15–30 cm). The three soil cores from each layer in the plot were mixed into a single sample for soil nutrient analysis. Soil samples were passed through a 2-mm mesh, and the roots were extracted. The samples were then frozen for subsequent laboratory analysis. To determine the inorganic nitrogen content (NH_4^+ and NO_3^-) in the soil, fresh soil was extracted with 2 M KCl. The NH_4^+ and NO_3^- in the extracts were then determined using an auto-analyzer (AA3, Bran-Luebbe, Germany).

Statistical analyses

The Shapiro–Wilk test was used to confirm the normal distribution of the data. The non-parametric Mann–Whitney U test was used to reveal significant differences in the estimated values between the fenced and grazed meadows. Statistical analysis was carried out using SPSS 19.0 software. In the following sections, $P < 0.05$ is considered statistically significant for differences between the fenced and grazed meadows.

Results

Effects of grazing exclusion on plant diversity

A total of 42 species belonging to 12 families were recorded in the experimental area, with 27 species present in both the fenced and grazed meadow. Out of the 27 species, 6, 3, 2, and 16 species were classified into the four functional groups (grasses, sedges, legumes, and forbs), respectively (Table 1).

Fewer species were recorded in the fenced meadow (n = 32) than in the grazed meadow (n = 36). Species richness, D, and H' values indicated that plant diversity was significantly lower in the fenced meadow compared with the grazed meadow (Fig. 1).

The plant diversity of the four functional groups had different responses to grazing exclusion. There was no significant difference in species richness of grasses and legumes between the fenced and grazed meadows (Fig. 1). Species richness of sedges and forbs was significantly lower in the fenced meadow (n = 2.3 and 11.5 species, respectively) than the grazed meadow (n = 3 and 16.3, respectively) (Fig. 1a). However, D for legumes was significantly higher in the fenced meadow (1.00) than the grazed meadow (0.99) (Fig. 1b). In comparison, H' for legumes was significantly lower in the fenced meadow than the grazed meadow (0.02 vs. 0.31) (Fig. 1c). No differences were detected in E between the fenced and grazed meadows (0.58 vs. 0.7)

Table 1 Important values (%) for species in the fenced and grazed meadows

Functional group	Fenced			Grazed		
	Family	Species	Important value	Family	Species	Important value
Grasses	Gramineae	*Elymus nutans*	9.02 (2.80)	Gramineae	*Elymus nutans*	15.85 (10.17)
		Festuca rubra	2.02 (1.60)		*Festuca ovina*	0.14 (0.28)
		Helictotrichon tibeticum	5.92 (11.84)		*Festuca rubra*	1.90 (1.90)
		Koeleria cristata	0.28 (0.34)		*Koeleria cristata*	0.61 (0.76)
		Poa crymophila	9.62 (6.69)		*Poa crymophila*	10.85 (7.00)
		Poa declinata	4.13 (5.68)		*Poa declinata*	1.78 (1.00)
		Ptilagrostis dichotoma	1.88 (2.28)		*Stipa aliena*	28.23 (9.27)
		Stipa aliena	34.32 (5.40)			
Sedges	Cyperaceae	*Carex vulpina*	0.09 (0.18)	Cyperaceae	*Carex vulpina*	1.84 (0.90)
		Kobresia humilis	12.81 (5.94)		*Kobresia humilis*	5.80 (2.10)
		Scirpus distigmaticus	1.17 (1.32)		*Scirpus distigmaticus*	4.23 (3.74)
Legumes	Leguminosae	*Gueldenstaedtia diversifolia*	0.35 (0.44)	Leguminosae	*Astragalus strictus*	0.69 (1.39)
		Oxytropis kansuensis	0.09 (0.17)		*Gueldenstaedtia diversifolia*	5.52 (4.03)
					Medicago ruthenica	2.34 (3.44)
					Oxytropis kansuensis	1.86 (1.86)
Forbs	Asteraceae	*Aster diplostephioides*	1.20 (2.13)	Asteraceae	*Ajania przewalskii*	1.07 (2.14)
		Leontopodium nanum	0.01 (0.01)		*Leontopodium nanum*	0.01 (0.01)
		Saussurea nigrescens	1.38 (1.66)		*Saussurea nigrescens*	1.06 (2.13)
		Saussurea pulchra	1.52 (1.60)		*Saussurea pulchra*	2.86 (4.26)
		Taraxacum mongolicum	0.06 (0.11)		*Taraxacum mongolicum*	2.24 (1.12)
	Caryophyllaceae	*Stellaria umbellate*	1.09 (1.26)	Caryophyllaceae	*Silene conoidea*	0.16 (0.31)
	Gentianaceae	*Gentiana aristata*	0.11 (0.18)		*Stellaria umbellate*	0.05 (0.05)
		Gentiana farreri	0.01 (0.01)	Gentianaceae	*Comastoma pulmonarium*	0.10 (0.11)
		Gentiana straminea	3.58 (2.37)		*Gentiana aristata*	0.02 (0.02)
		Lomatogonium rotatum	0.12 (0.24)		*Gentiana straminea*	1.61 (2.80)
	Iridaceae	*Iris tectorum*	0.93 (1.86)		*Lomatogonium rotatum*	0.01 (0.01)
	Polygonaceae	*Polygonum sibiricum*	0.03 (0.06)	Plantaginaceae	*Plantago depressa*	0.10 (0.14)
	Ranunculaceae	*Delphinium caeruleum*	0.10 (0.21)		*Veronica eriogyne*	0.00 (0.00)
		Ranunculus tanguticus	0.86 (1.20)	Polygonaceae	*Polygonum sibiricum*	0.59 (1.18)
		Thalictrum alpinum	2.69 (0.41)	Ranunculaceae	*Delphinium caeruleum*	0.15 (0.30)
	Rosaceae	*Potentilla anserina*	2.62 (3.04)		*Ranunculus tanguticus*	2.79 (2.35)
		Potentilla bifurca	0.83 (1.19)		*Thalictrum alpinum*	1.60 (1.96)
		Potentilla nivea	0.64 (0.28)	Rosaceae	*Potentilla anserina*	1.19 (0.84)
	Scrophulariaceae	*Tibet lancea*	0.54 (0.62)		*Potentilla bifurca*	0.74 (0.78)
					Potentilla nivea	0.20 (0.18)
				Scrophulariaceae	*Euphrasia pectinata*	0.60 (0.70)
					Tibet lancea	1.21 (0.29)

Data are mean ± standard deviation ($n = 4$). No statistical analyses were completed for these data. Shared species in the fenced and grazed meadows are highlighted in bold

(Fig. 1d). However, E for legumes was significantly lower in the fenced meadow (0.03) than in the grazed meadow (0.36) (Fig. 1d).

Effects of grazing exclusion on community structure

Some changes to community composition occurred in the fenced meadow compared with the grazed meadow. The percentage of legumes significantly decreased by 9.98 % in the fenced meadow compared with the grazed meadow (Fig. 2). In comparison, the percentage of grasses, sedges, and forbs did not change in response to grazing exclusion (Fig. 2). No difference was found in the cover, height, and production of grasses between the fenced and grazed meadows (Fig. 3). The height of sedges increased by 87.4 % in the fenced meadow (17.3 cm) compared with the grazed meadow (9.2 cm; Fig. 3b). In contrast, the production of sedges signifi-

cantly decreased by 27.9 % in the fenced meadow (40.45 vs. 56.14 g m^{-2}) (Fig. 3c). In addition, the cover of sedges did not differ between the fenced and grazed meadows (Fig. 3a). The cover and production of legumes significantly decreased in the fenced meadow (1.8 % and 0.49 g m^{-2}) compared with the grazed meadow (44.5 % and 40.41 g m^{-2}) (Fig. 3a, c). However, there was no change in the height of legumes between the fenced and grazed meadows (Fig. 3b). The cover of forbs in the fenced meadow (45.5 %) was significantly lower than that in the grazed meadow (79.8 %) (Fig. 3a). In comparison, the height and production of forbs showed no difference between the fenced and grazed meadows (Fig. 3b, c).

The vegetation cover in the fenced meadow (190.5 %) was significantly lower than that in the grazed meadow (321.8 %) (Fig. 3a). The aboveground biomass (257.07 g m^{-2}) in the fenced meadow was 27.1 % lower than that in the grazed meadow (388.18 g m^{-2}; Fig. 3c).
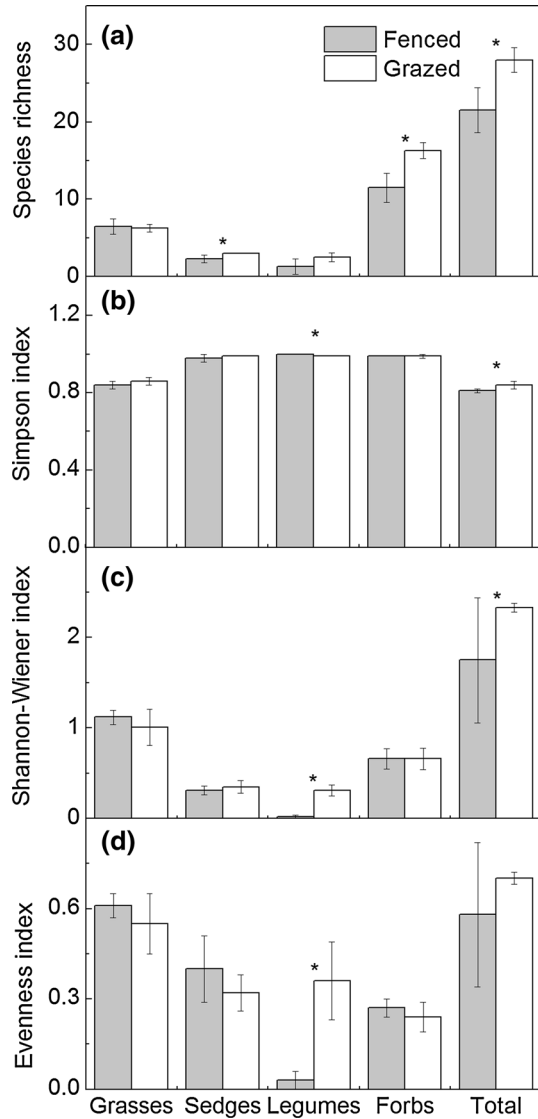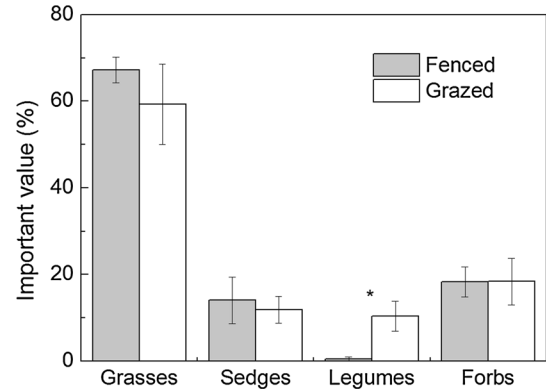
Fig. 1 Species richness (**a**), Simpson index (**b**), Shannon–Wiener index (**c**), and evenness index (**d**) for the functional groups and the vegetation community in the fenced and grazed meadows. *Asterisk* indicates significant differences at $P < 0.05$ between the fenced and grazed meadows. Data are mean $\pm$ standard deviation ($n = 4$)
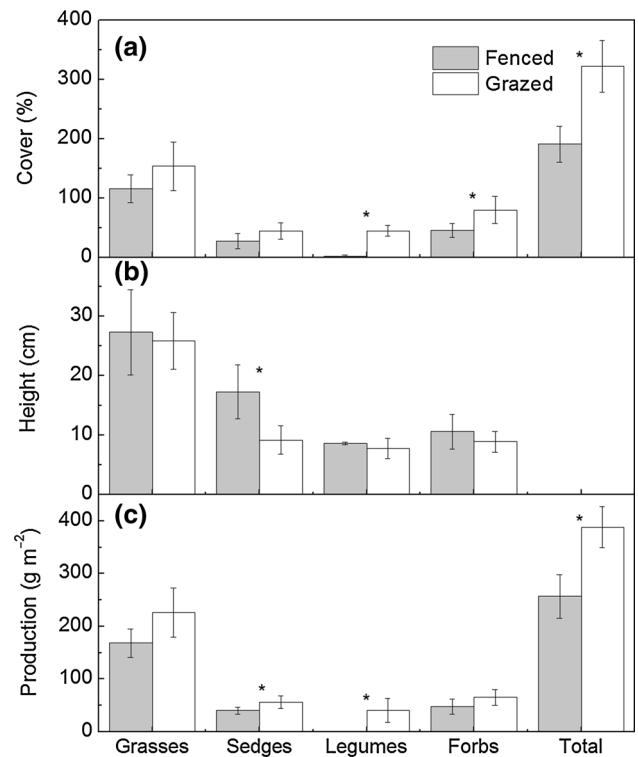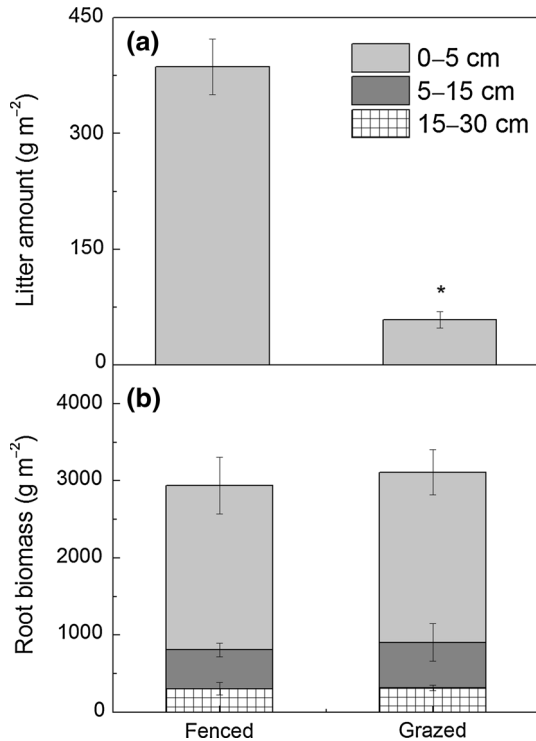

Fig. 2 Important values for the functional groups (grasses, sedges, legumes, and forbs) in the fenced and grazed meadows. *Asterisk* indicates significant differences at $P < 0.05$ between the fenced and grazed meadows. Data are mean $\pm$ standard deviation ($n = 4$)

Fig. 3 Cover (**a**), height (**b**), and production (**c**) of the four functional groups (grasses, sedges, legumes, and forbs) and the vegetation community in the fenced and grazed meadows. *Asterisk* indicates significant differences at $P < 0.05$ between the fenced and grazed meadows. Data are mean $\pm$ standard deviation ($n = 4$)
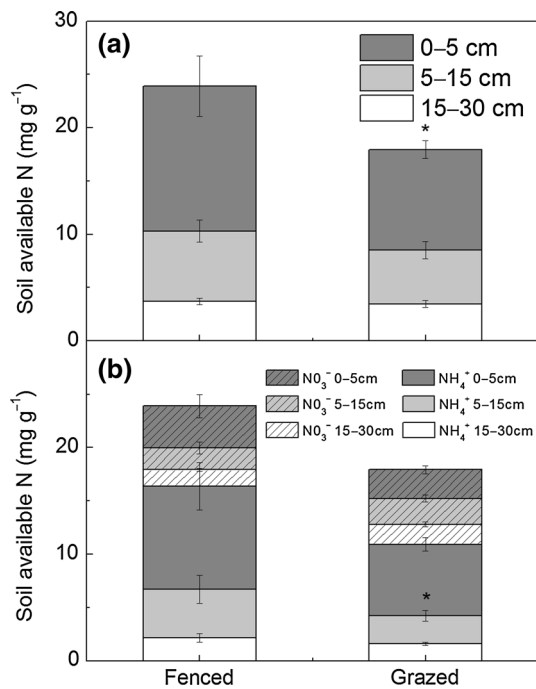
In contrast to the aboveground biomass, the amount of litter in the fenced meadow was significantly larger (386.41 g m^{-2}) (Fig. 4a) than that in the grazed meadow (58.77 g m^{-2}). No differences were found in the root biomass between the fenced and grazed meadows at 0–5, 5–15, and 15–30 cm, and 0–30 cm soil depths (Fig. 4b).

Effects of grazing exclusion on soil available nitrogen

The soil NH$_4^+$ content at 0–5 and 15–30 cm depths was similar between the fenced and grazed meadows (Fig. 5b). However, soil NH$_4^+$ content at 5–15 cm depth was 72.3 % higher in the fenced meadow than in the grazed meadow (Fig. 5b). No differences were de-

tected in soil NO$_3^-$ content between the fenced and grazed meadows at 0–5, 5–15, and 15–30 cm soil depths (Fig. 5b). The results showed that soil available N (NH$_4^+$ + NO$_3^-$) was 44.7 % higher in the fenced meadow (13.60×10^{-2} g kg^{-1}) than in the grazed meadow (9.40×10^{-2} g kg^{-1}) at 0–5 cm soil depth (Fig. 5a). However, there was no difference at 5–15 and 15–30 cm soil depth between the fenced and grazed meadows (Fig. 5a).
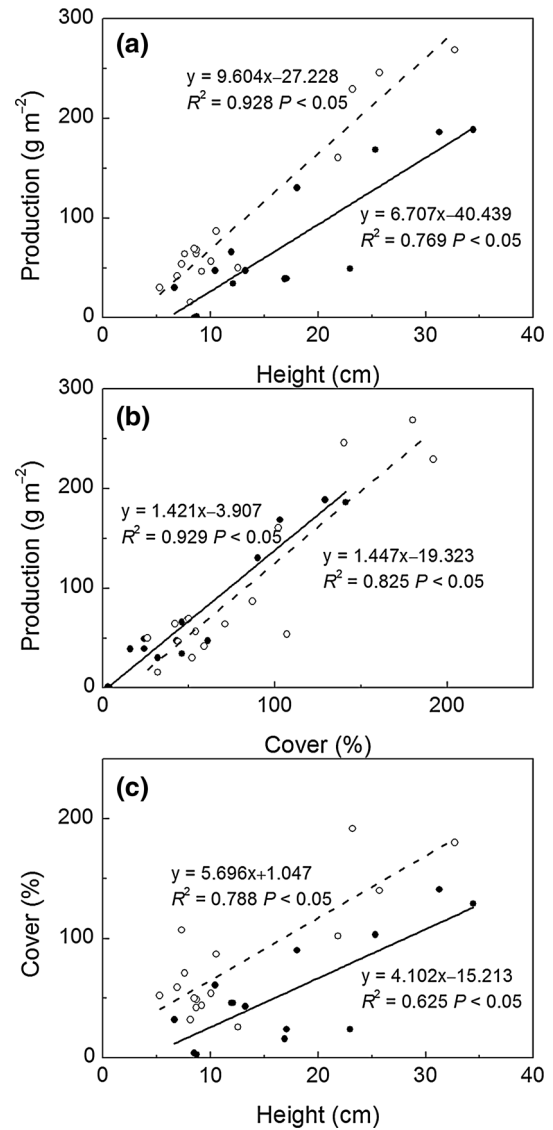
Fig. 4 Amount of litter (a) and root biomass (b) at 0–5, 5–15, 15–30, and 0–30 cm soil depths in the fenced and grazed meadows. *Asterisk* indicates significant differences at $P < 0.05$ between the fenced and grazed meadows. Data are mean ± standard deviation ($n = 4$)

Cover height and production of functional groups

We found that there were positive relationships between vegetation cover, height, and production in the fenced and grazed meadows (Fig. 6). Of note, the relationships of production and cover with height differed between the fenced and grazed meadows (Fig. 6). Vegetation height accounted for 63 and 77 % of the variability in vegetation cover and production of the different functional groups in the fenced meadow. In comparison, vegetation height accounted for 69 and 93 % of variability in cover and production in the grazed meadow. Furthermore, cover accounted for 93 and 82 % of variability in production in the fenced and grazed meadows.

Fig. 5 Soil available N content [$NH_4^+ + NO_3^-$ (a), NH_4^+ and NO_3^- (b)] (×10^{-2}; mg g^{-1}) at 0–5, 5–15, and 15–30 cm soil depths in the fenced and grazed meadows. *Asterisk* indicates significant differences at $P < 0.05$ between the fenced and grazed meadows. Data are mean ± standard deviation ($n = 4$)
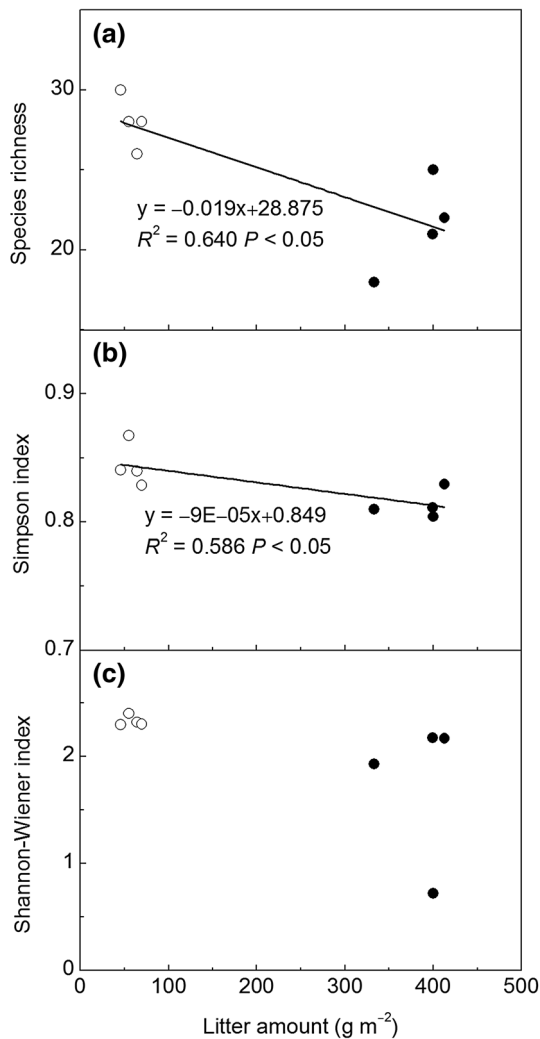

Fig. 6 Linear correlations between the height and production (a), the cover and production (b), and the height and cover (c) of the functional groups (grasses, sedges, legumes, and forbs) in the fenced (*solid line*) and grazed meadows (*dashed line*). Each data point presents the value for each functional group of one plot in the fenced (*filled circles*) and grazed (*open circles*) meadows

Relationships of plant diversity with litter and soil available nitrogen

Negative correlations were found for both species richness and D with the amount of litter in both the fenced and grazed meadows (Fig. 7). However, no linear correlation was found between H' and amount of litter in either of the two meadow types.

The species richness in the fenced and grazed meadows was negatively correlated with NH_4^+, NO_3^-, and soil available nitrogen ($NH_4^+ + NO_3^-$) (Fig. 8a–c). However, there was no correlation between the D or H' values and soil available nitrogen (Fig. 8d–i). There was also no correlation between root biomass and soil available nitrogen.

Fig. 7 Linear correlations between the amount of litter and species richness (**a**), Simpson index (**b**) and Shannon–Wiener index (**c**) in the fenced and grazed meadows. Each data point presents the value for each plot in the fenced (*filled circles*) and grazed (*open circles*) meadows

Discussion

Our results indicate that grazing exclusion negatively affects plant diversity in a *Kobresia humilis* meadow when compared with moderate winter grazing. These results confirm our previous observations of decreased plant species richness in response to 6 years of grazing exclusion at the same research site (Zou et al. 2014). Furthermore, our results are consistent with another study on the response of plant diversity to grazing exclusion on the Qinghai–Tibetan Plateau (Wu et al. 2009). Moreover, our results imply that grazing exclusion is detrimental to plant diversity in alpine grasslands, depending on the vegetation type, grazing history, and other characteristics. Thus, this strategy might not be an effective approach to protecting grassland or restoring plant diversity of the degraded grassland on the Qinghai–Tibetan Plateau, contradicting previous suggestions (Dong et al. 2013).

Functional redundancy in the *Kobresia humilis* meadow

Variability in the important value was large for each species in the four functional groups in the plots (Table 1). However, the important value showed low variability for each functional group among the plots. This result indicates that the plant community of the *Kobresia humilis* meadow might have a higher functional redundancy. This may ensure ecosystem reliability, providing higher resistance, with less susceptibility to disturbance (Naeem 1998). In contrast, Wang et al. (2015) reported that all plant species are critical for maintaining ecosystem stability in the alpine meadow. Tang et al. (2015) also reported higher functional redundancy in the alpine meadow due to the higher plant diversity. This phenomenon might buffer the alpine meadow to disturbances, and offer greater ecosystem stability of the plant community through compensatory effects from functionally similar species. Thus, the severity of disturbances to the plant community might determine plant diversity in alpine meadows (Mouillot et al. 2013). In addition, disturbances might affect plant diversity by impacting the optimal patterns of functional traits for resource use in the community (Wu et al. 2009, 2010, 2011; Niu et al. 2010; Yang et al. 2015). Therefore, our results indicate that the negative responses of plant diversity to grazing exclusion might be caused by differences in resource use among the different vegetation functional groups.

Effects of litter on plant diversity

Our results showed that the photosynthesis efficiency of the four functional groups was lower in the fenced meadow compared with the grazed meadow. This difference might be related to the amount of litter. Large
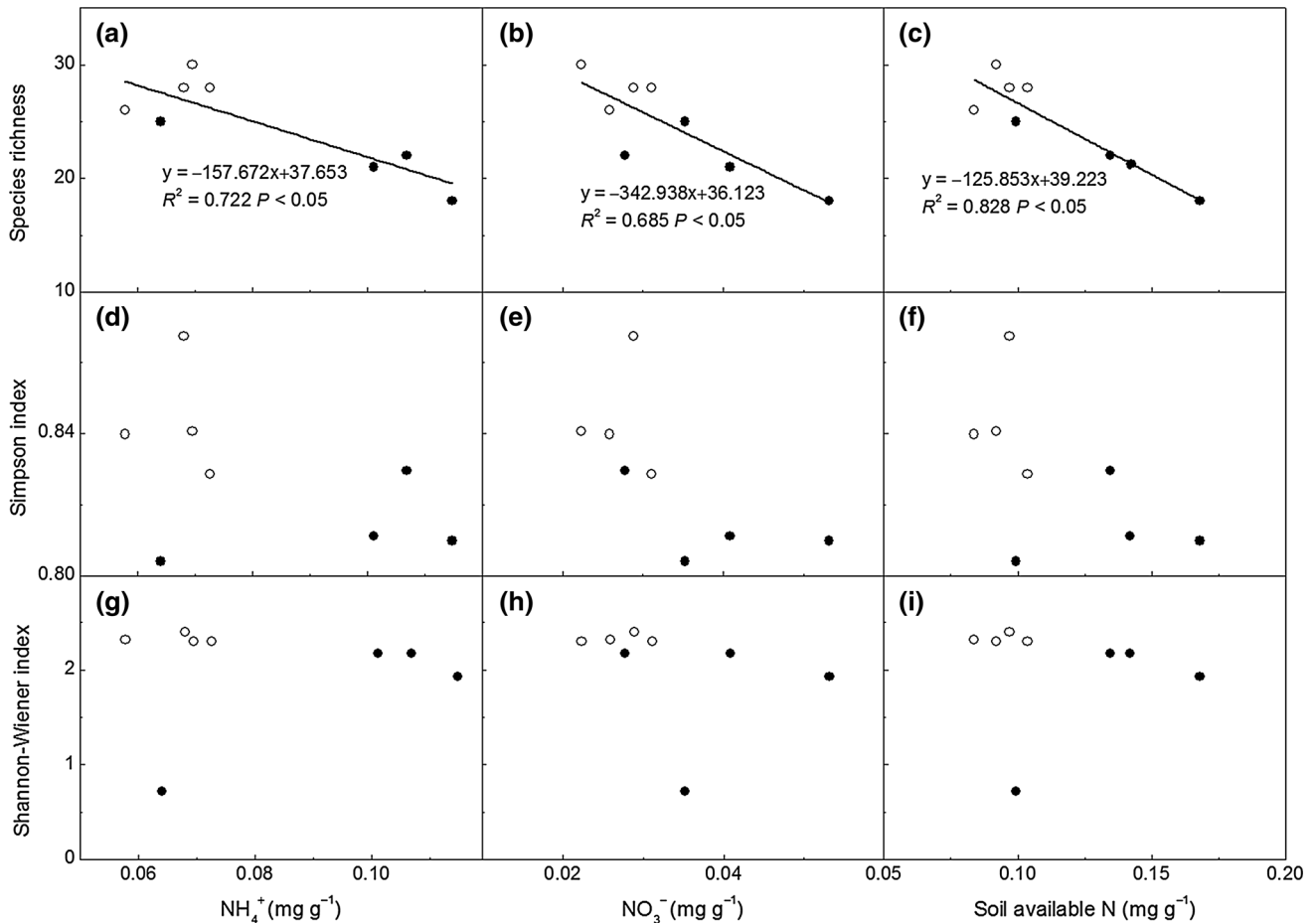
Fig. 8 Linear correlations between species richness (**a–c**) Simpson index d–f and Shannon–Wiener index (**g–i**) and NH_4^+ (**a**, d, g), NO_3^- (**b**, **e**, **h**) and soil available nitrogen (**c**, **f**, **i**) at 0–5 cm in the fenced and grazed meadows. Each data point presents the value for each plot in the fenced (*filled circles*) and grazed (*open circles*) meadows

amounts of litter may have reduced the absorption efficiency of photosynthesis through self-shading, with light limitation having negative effects on plant growth (McNaughton 1983; Knapp and Seastedt 1986; Oesterheld and McNaughton 1991; Semmartin and Oesterheld 1996). Litter negatively impacts ecosystems with heavy precipitation and optimal growing conditions (Knapp and Seastedt 1986). Vegetation growth in *K. humilis* meadows is not limited by precipitation, but might be limited by the accumulation of litter under grazing exclusion (Fig. 3c). The negative effects of litter on plant growth might lead to changes in community composition, and hence species loss, in the alpine meadow (Figs. 2, 7). The negative response of plant diversity to accumulated litter has also been demonstrated in a *Kobresia* meadow on the Qinghai–Tibetan Plateau (Klein et al. 2004). However, our results indicate that the responses of plant diversity to litter differed among the four functional groups (Supplementary data, Fig. S1, Table S1). The diversity of sedges, legumes, and forbs might be negatively related to the amount of litter. However, we found no relationship between the diversity of grasses and the amount of litter. Certain functional groups respond differently to light limitation; for example, grasses have a superior competitive ability in exploiting light resources because of their higher stature (Kull and Aan 1997). The growth of legumes might also be constrained by light limitation. Photosynthetic supply appears to be the most important factor limiting nitrogen fixation in field-grown nitrogen-fixing legumes (Hardy and Havelka 1973; Schubert and Evans 1976). Sedges with lower height and small leaf area exhibit weak competition for light in the community. However, the strategy to access light by sedges differs to that of forbs (Zhao et al. 2009; Niu et al. 2010). Furthermore, many studies have demonstrated that grazing response to species abundance may be predicted by functional traits (Niu et al. 2010). Our results show that the height and cover of the four functional groups exhibited diverse responses to the accumulation of litter (Supplementary data, Fig. S2, Table S1). Thus, the different growth strategies of the four functional groups might contribute to the negative responses of plant diversity to litter in the meadow habitat.

Effects of soil available nitrogen on plant diversity

Plant diversity is closely related to nutrient cycling. Consequently, soil nutrient conditions may have both positive and negative effects on species diversity in grasslands (Stohlgren et al. 1999; Borer et al. 2014; Wang et al. 2014). Our results show that grazing exclusion might enhance soil available nitrogen in the alpine meadow (Fig. 5). More than 80 % inorganic N contributes to soil available N in alpine meadows, with alpine *Kobresia* meadow that have low inorganic N concentrations in the soil being strongly limited by soil available N (Zhou 2001; Xu et al. 2011). Plant diversity in the alpine meadow might be related to inorganic N concentrations in the soil (Wu et al. 2009; Hobbie 2015; Storkey et al. 2015). More fertile habitats could support higher species diversity in the alpine grassland (Wang et al. 2014). Furthermore, many studies have suggested that higher N availability increases aboveground net primary production (Stevens et al. 2004; Suding et al. 2005). However, higher aboveground net primary production might intensify competition excluding effects among species, thus decreasing species diversity (Shaw et al. 2002; Zavaleta et al. 2003; Stevens et al. 2004; Suding et al. 2005; Collins 2009; Hautier et al. 2009). Our results showed a negative linear relationship between species diversity and soil available nitrogen (Fig. 8). However, the decreased diversity in the fenced meadow should not be caused by the competition excluding effects of higher production, but should be largely due to the negative effects of litter accumulation (Fig. 3c). Moreover, soil available nitrogen was mainly absorbed from the soil by the roots. Neutral changes in root biomass under grazing exclusion and the lack of relationship between root biomass and soil available nitrogen indicate that nitrogen absorption activity remains unchanged in the community (Xu et al. 2011). Therefore, enhanced soil available nitrogen concentrations should not have a negative effect on plant diversity, and might be the consequence of decreased diversity and accumulated litter in the alpine meadow under grazing exclusion.

Effects of vegetation on soil available nitrogen and its feedbacks on plant diversity

Our results suggest that the different functional groups with diverse nutrient use strategies have various effects on soil available nitrogen (Supplementary data, Fig. S3–S8, Table S2). A previous study (Wang et al. 2012) showed that grasses (especially *Stipa aliena*) have a high absorption efficiency for NO_3^-, whereas forbs (such as *Potentilla anserine* and *Thalictrum alpinum*) have a high absorption efficiency for NH_4^+. However, legume species (including *Gueldenstaedtia diversifolia* and *Medicago ruthenica*) have strong nitrogen fixation ability, but weak absorption efficiency for NH_4^+ and NO_3^- (Wang et al. 2012). The different uptake patterns of different species have been demonstrated by many studies in alpine meadows and other ecosystems (McKane et al. 2002; Gao et al. 2014; Jiang et al. 2015; Li et al. 2015). Thus, a decrease in the diversity of the functional groups might enhance the contents of different nitrogen forms in the soil. Furthermore, our results showed that enhanced soil available nitrogen might slow the rate of loss of species by supporting the growth of nitrophilic plants (Table 1). For example, the average important values of *Stipa aliena*, *Potentilla anserine*, and *Thalictrum alpinum* were higher in the fenced meadow compared with the grazed meadow, with similarly large spatial variability among the plots. Therefore, the enhanced content of soil available nitrogen had weak positive feedbacks on decreased plant diversity in the fenced meadow. Kull and Aan (1997) also demonstrated that grasses have higher abundance than forbs because of the higher nitrogen use efficiency of grass species. However, this tendency was not obvious under low light resource availability. In conclusion, our study demonstrates that soil available nitrogen had weak feedbacks on plant diversity at our study site, and that the effect of grazing exclusion on plant diversity might be mainly controlled by light limitation in the moderately grazed alpine meadow.

Conclusions

Alpine meadows evolved from livestock grazing over centuries; however, overgrazing has led to the degradation of alpine meadows. Grazing exclusion is often implemented as an efficient method to restore degraded meadows; however, we found that grazing exclusion in alpine meadows reduces species diversity compared with moderate grazing activity. Grazing might regulate resource usage among various species and maintain higher plant diversity in alpine meadows. Our results indicate that plant diversity negatively responded to the 9-year grazing exclusion in the *Kobresia humilis* meadow compared with moderate grazing. The negative responses were related to the availability of light and nitrogen resources in the alpine meadow under grazing exclusion. Light limitation might be the main factor leading to the decline in plant diversity in the alpine meadow. The results of our study also imply that vegetation type and composition should be considered when evaluating how grazing exclusion impacts plant diversity in various ecosystems. A larger amount of data over larger spatial scales must be obtained and analyzed in future studies to improve our understanding of how plant diversity responds to grazing exclusion in different ecosystems.

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