Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient

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Summary

1. The Eurasian steppe has long been subject to grazing by domestic ungulates at high levels, resulting in widespread deterioration of biodiversity and ecosystem services. While abundant evidence demonstrates that heavy grazing alters the ecosystem structure and function of grasslands, research on how grazing specifically affects ecosystem functioning and stoichiometry on broad scales is scarce because of a lack of adequate ungrazed reference sites.

2. We examined the effects of grazing on ecosystem functioning and C : N : P stoichiometry across a precipitation gradient along the 700 km China–Mongolia transect (CMT), covering three community types: meadow steppe, typical steppe and desert steppe.

3. Long-term grazing has dramatically altered the C, N and P pools and stoichiometry of steppe ecosystems along the CMT. Grazing reduced the C, N and P pools in above-ground biomass and litter, while the responses in below-ground biomass and soil C, N and P pools to grazing differed substantially among community types.

4. Grazing increased N content and decreased C : N ratios in all plant compartments, suggesting accelerated N cycling. The altered C : N : P stoichiometry may be explained by changes in the composition of species and functional groups as well as increased foliar N and P contents for the same species in grazed communities.

5. *Synthesis and applications.* Plant stoichiometric responses to grazing ranged from large in the meadow steppe to small in the typical steppe to generally insignificant in the desert steppe, implying that different underlying mechanisms operated along the regional precipitation gradient. Our findings suggest that reducing the stocking rate and restoring the vastly degraded steppes are essential to sustain native steppe biodiversity, ecosystem functioning and biological capacity for mitigating the impact of climate change in the Inner Mongolia grassland.

Key-words: China–Mongolia transect, N and P co-limitation, N cycling, plant and soil C, N and P pools, plant functional group composition, primary production, stoichiometry, the Eurasian steppe

Introduction

Grasslands have experienced dramatic shifts in structure and functioning driven primarily by human disturbances and global climate change (Milchunas & Lauenroth 1993; White, Murray & Rohweder 2000; Knapp *et al.* 2002; Wittmer *et al.* 2010). The Eurasian Steppe, which extends over 8000 km across northeastern China, Mongolia, Russia, Ukraine and Hungary (Coupland 1993), has been historically subjected to continuous grazing by domestic ungulates at increasingly high levels (White, Murray & Rohweder 2000). The long-term grazing has resulted in widespread declines in biodiversity and ecosystem functioning and services (White, Murray & Rohweder 2000; Bai *et al.* 2007). This is triggered by the direct and indirect effects of grazing and often mediated by the complex interactions between vegetation and environmental

drivers (Gillson & Hoffman 2007; Golluscio *et al.* 2009; Shan *et al.* 2011). Thus, it is critical to obtain a better understanding of how grazing, abiotic factors and biotic–abiotic interactions influence key properties of ecosystem functioning and sustainability and thereby provide guideline for improving grassland management practices in the Eurasian steppe.

Abundant evidence indicates that grazing affects plant diversity, species composition and primary production (Bai *et al.* 2007; Sasaki *et al.* 2008), as well as soil properties (e.g. soil C and N pools) in many arid, semi-arid and sub-humid grasslands (Frank *et al.* 1995; Reeder *et al.* 2004; Han *et al.* 2008). Few studies, however, have examined the mechanisms of ecosystem responses to grazing, and, in particular, the linkages between above-ground and below-ground components have received little attention (Bardgett & Wardle 2003). The grazing optimization hypothesis predicts that moderate grazing increases primary production through compensatory growth and recycling of limiting nutrients (McNaughton 1979), highlighting links and feedbacks between plant responses and nutrient cycling. Several previous studies propose that moderate grazing accelerated nutrient cycling by promoting the tissue loss of grazing-tolerant species with higher nutrient concentration, stimulating the compensatory growth and enhancing the dominance of these species, and by directly inputting urine and faeces into the system (McNaughton 1985; Frank & Evans 1997). Other studies, however, have shown that grazing decelerated nutrient cycling by inhibiting the growth of palatable and nutrient-rich species with high litter quality and promoting the dominance of those nutrient-poor or chemically defended species with low litter quality that slow rates of nutrient cycling (Ritchie, Tilman & Knops 1998). These seemingly contradictory hypotheses suggest that the effects of grazing on nutrient cycling and ecosystem functioning are contingent on a host of processes, including vegetation type (dry vs. mesic grasslands), evolutionary history of grazing (grazing tolerance of plants), species composition (e.g. plant tissue nutrient concentration, palatability), grazing intensity and N inputs and outputs of the system (Milchunas, Sala & Lauenroth 1988; Ritchie, Tilman & Knops 1998; Singer & Schoenecker 2003).

Ecological stoichiometry provides a powerful framework for studying how grazing affects the balance of essential nutrients (e.g. C, N and P) at different trophic levels and over a wide range of spatial and temporal scales (Sterner & Elser 2002). Two mechanisms of how grazing affects the C, N and P contents and stoichiometry at the community level have been proposed (Bardgett & Wardle 2003). First, grazing may change the contents and stoichiometry of the plant community through a cascade of plant–soil feedbacks (McNaughton 1985; Frank 2008). Grazing often increases C-rich root exudates that stimulate microbial activity and turnover, ultimately resulting in an increase in soil nutrients available to plants (Bardgett, Wardle & Yeates 1998; Hamilton & Frank 2001). However, these stimulatory feedbacks cannot be sustained at high grazing intensities and may be absent or weak in resource-poor conditions. Second, grazing may alter the species composition and thus stoichiometry of the community because species differ in their nutrient contents (Ritchie, Tilman & Knops 1998; Bardgett & Wardle 2003).

Spatial scale is an important dimension for understanding mechanisms underpinning the observed responses in ecosystem functioning (e.g. primary production, C, N and P pools and stoichiometry). This is because the direction and magnitude of these effects are system and/or scale dependent (Levin 1993; Milchunas & Lauenroth 1993), and they often interact with environmental conditions, such as water and N availability (Maschinski & Whitham 1989; Wise & Abrahamson 2005). Few studies have examined the effects of grazing on ecosystem functioning and C : N : P stoichiometry across broad geographic regions. Understanding these complex relationships requires large-scale reference sites that have not been grazed. The border between China and Mongolia, with a demilitarized fenced area preventing grazing, provides an ideal transect to address this deficiency because the buffer zone has not been grazed since the 1950s. The arid and semi-arid grasslands on the Mongolia plateau, representative of the Eurasian steppe region, are extremely water-limited, with a similar evolutionary history of grazing (Coupland 1993; Bai *et al.* 2008). However, it remains unclear to what extent the water availability affects functioning and stoichiometric responses of grasslands to long-term grazing across broad regions.

To test these hypotheses and predictions, we examined the effects of grazing on ecosystem functioning and C : N : P stoichiometry along the 700 km China–Mongolia transect (CMT) using consistent methods. The CMT, which covers a wide range of biotic and abiotic conditions, enables us to observe the total effects of multiple mechanisms that probably operate simultaneously but vary in their relative strengths across regions. Specifically, we address the following questions: (i) How has grazing affected ecosystem functioning (i.e. species richness, above- and below-ground biomass and litter biomass) and C : N : P stoichiometry of grasslands along the regional precipitation gradient during the last 50 years? (ii) How do the responses of plant and soil C, N and P pools and stoichiometry to grazing differ among community types? (iii) What is the relative importance of plant functional group (PFG) composition and species plasticity in influencing ecosystem functioning and stoichiometry?

Materials and methods

STUDY AREA AND EXPERIMENTAL DESIGN

This study was conducted in the Inner Mongolia grassland, a central part of the Eurasian steppe (Bai *et al.* 2007). The study area is located at 44·73º–46·74ºN in latitude and 111·96º–118·89ºE in longitude, and elevation is between 760 and 1450 m. The mean

annual temperature ranges from −1·8 to 1·6 °C, with the lowest mean monthly temperatures in January (−21·0 to −24·0 °C) and the highest in July (18·7–21·6 °C). The mean annual precipitation (MAP) is between 170 and 370 mm, about 80% of which falls in the growing season from May to August (Table S1, Supporting information). Soil types include dark chestnut, typical chestnut, calcic brown and light brown soils, corresponding to the different community types (Table S1, Supporting information).

We established a regional transect across a precipitation gradient along the China–Mongolia border, which runs 700 km and covers three major community types: meadow steppe, typical steppe and desert steppe (Table S1, Supporting information and Fig. 1). The meadow steppe, located at the eastern part of the transect, is dominated by *Stipa baicalensis* Roshev., *Leymus chinensis* (Trin.) Tzvel. and *Carex pediformis* C. A. Mey., with the highest species richness and above-ground productivity among the three community types. The typical steppe is dominated by *Stipa grandis* P. Smirn., *L. chinensis*, *Stipa krylovii* Roshev. and *Caragana microphylla* Lam., with intermediate levels of species richness and productivity. The desert steppe at the western part of the transect is dominated by *Stipa klemenzii* Roshev. and *Allium polyrhizum* Turcz. ex Regel, with low species richness and productivity. Within the transect, a total of nine pairs of grazed and ungrazed sites were selected, including two pairs for meadow steppe, five pairs for typical steppe and two pairs for desert steppe, with the number of sites being proportional to its area. The ungrazed (control) sites were located within the fenced buffer zone along the China–Mongolia border, which has not been grazed by large herbivores for more than 50 years. In contrast, the grazed sites were inside the Chinese border, which have been subjected to grazing of different intensities since the 1950s (Table S1, Supporting information).

FIELD SAMPLING AND MEASUREMENTS

Field sampling was carried out during 14–30 August 2003, corresponding to annual peak-standing biomass (Bai *et al.* 2008). At each site, above-ground standing biomass of herbaceous plants was sampled by ten 1 × 1 m quadrats located randomly within a 100 × 100 m area. For each quadrat, live and dead above-ground biomass was clipped at the ground level, and dead parts were removed and combined with litter. Vascular plants in five of the ten quadrats were sorted to species, with those other five kept as bulk samples. All plant materials in each quadrat were oven-dried at 65 °C for 48 h and weighed. Above-ground biomass for shrubs was measured by collecting leaves and current-year twigs of each species using five 5 × 5 m quadrats. The averaged total dry mass, number of species and biomass of each species were used to estimate above-ground biomass, species richness and functional composition for each site. Leaf samples of 15 dominant and subdominant species were collected in the same area for C, N and P analysis. At each site, according to leaf size, 50–200 fully expanded and mature leaves from 30 to 50 adult individuals of each of the dominant and subdominant species were picked and divided into five samples, each with 10–40 leaves. For all the grazed sites, no livestock grazing occurred during the growing season of our field sampling in 2003.

Four of the five bulk biomass quadrats at each site were selected randomly for below-ground plant biomass and soil sampling. Below-ground biomass was sampled by randomly taking a 30-cm-diameter soil core from 0 to 60 cm depth inside each quadrat, which samples 80–90% of total below-ground biomass in the top 100 cm of soil (Jiang *et al.* 1985). Soil was rinsed out from roots under running water over a 1-mm screen, and below-ground biomass was oven-dried at 65 °C and weighed. Soil samples were collected by taking three 5-cm-diameter soil cores from 0 to 20, 20 to 60 and 60 to 100 cm depths at each quadrat of below-ground biomass sampling. The three cores from each quadrat were mixed *in situ* as one composite sample and hand-sorted to remove rocks and visible plant materials. Masses and volumes of rocks were also measured for correcting soil C, N and P storage. Bulk density at each soil depth for each site was obtained by using a cylindrical sampler (50·46 mm in diameter and 50 mm in length). Soil cores were oven-dried at 105 °C to constant weight.

All samples were processed and analysed in the laboratories of the Inner Mongolia Grassland Ecosystem Research Station of Chinese Academy of Sciences (Bai *et al.* 2004). A subsample of
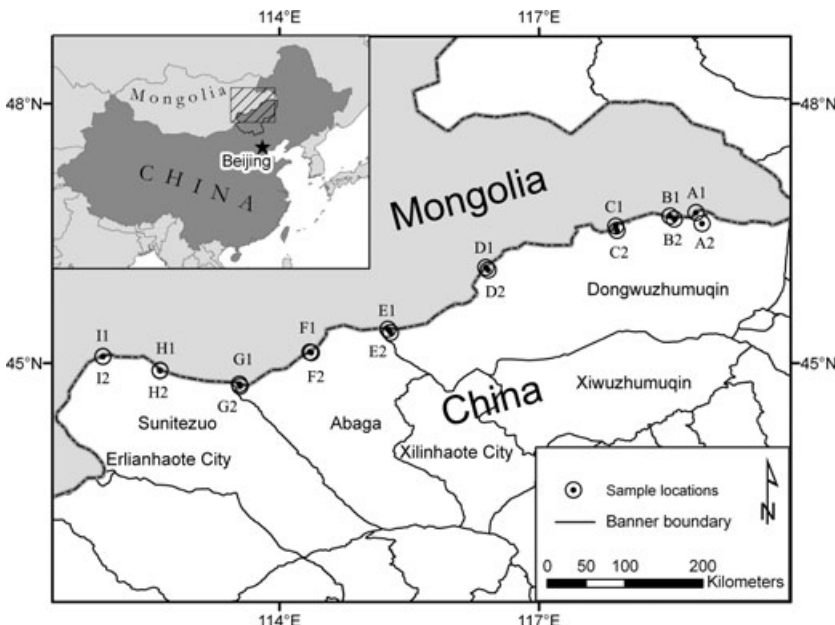

Fig. 1. Location map of the 18 paired study sites along the China–Mongolia transect.

10 g soil from each composite sample was dried at 105 °C for soil moisture measurement. The remaining soil was air-dried and ground to pass a 1-mm sieve for C, N and P analysis. Total organic C in soil and plant samples was analysed by the Walkley–Black modified acid-dichromate $FeSO_4$ titration method (Sparks *et al.* 1996). Total N in plant and soil samples was determined following Kjeldehl digestion by a Nitrogen Analyzer System (KJELTEC 2300 AUTO SYSTEM II, Foss Tecator AB, Höganäs, Sweden). Total P was determined by the H_2SO_4-$HClO_4$ fusion method (Sparks *et al.* 1996).

Stoichiometric ratios in plant, litter and soil samples, that is, C : N, C : P and N : P ratios, were calculated on mass basis. To examine the effects of grazing on C, N and P pools of plant and soil compartments, the total N, P and organic C contents (%) in soil and plant samples were converted from mass content basis ($mg\ kg^{-1}$) to land area basis ($g\ m^{-2}$). To present a quantitative measure independent of grazing-induced changes in soil bulk density, soil total N, P and organic C pools from each original depth interval were adjusted on an equivalent mass basis (Ellert & Bettany 1995). To assess the effects of shifts in functional group composition on ecosystem functioning, we classified all plants into five functional groups based on life forms, including perennial rhizome grasses (PR), perennial bunchgrasses (PB), perennial forbs (PF), shrubs and semi-shrubs (SS) and annuals (AS) as in Bai *et al.* (2004).

METEOROLOGICAL DATA

Meteorological data from 1961 to 2000 were obtained from 48 weather stations within and around the study area. Mean annual precipitation and mean annual temperature for each site were interpolated using a Geographic Information System (GIS)-based multiple regression method (Bai *et al.* 2007).

STATISTICAL ANALYSES

Statistical analyses were performed using SAS version 9·1 (SAS Institute, Cary, NC, USA, 2003). The effects of grazing on C, N and P contents and C : N : P stoichiometry in plant and soil pools,

above-ground biomass, relative biomass of PFGs and below-ground biomass were tested with ANOVA, using Treatment (grazed or ungrazed), Community type, Site and all interactions as fixed-effects. These ANOVAs were followed by Duncan's multiple-range test to compare the grazing effects for each property and community type. To test the dependency of responses in stoichiometric ratios to grazing on precipitation, we further analysed the relationships between the responses of C : N, C : P and N : P ratios to grazing (i.e. C : N_{grazed}/C : $N_{ungrazed}$, C : P_{grazed}/C : $P_{ungrazed}$ and N : P_{grazed}/N : $P_{ungrazed}$) and MAP across the CMT. The responses of stoichiometric ratios to grazing were transformed with the natural logarithm before regression analysis to improve the normality.

Results

ABOVE- AND BELOW-GROUND BIOMASS, SPECIES RICHNESS AND PFG COMPOSITION

Plant species richness, above- and below-ground biomass and litter biomass at ungrazed sites all increased linearly with MAP across the CMT, and MAP alone explained 55–86% of the variance in these variables (Fig. 2). Species richness and standing above-ground biomass at the grazed sites showed a hump-shaped relationship with MAP, and both peaked at the intermediate level of MAP (Fig. 2). Below-ground biomass at the grazed sites followed the same pattern as that at the ungrazed sites, with litter biomass showing no significant relationship with MAP (Fig. 2).

When data were classified into community types, above-ground standing biomass and litter were significantly reduced by grazing in the three community types (data not shown). On average, the above-ground standing biomass decreased by 61% ($1280\ kg\ ha^{-1}$) in the meadow steppe, by 27% ($390\ kg\ ha^{-1}$) in the typical steppe and by 23% ($170\ kg\ ha^{-1}$) in the desert steppe. Species richness
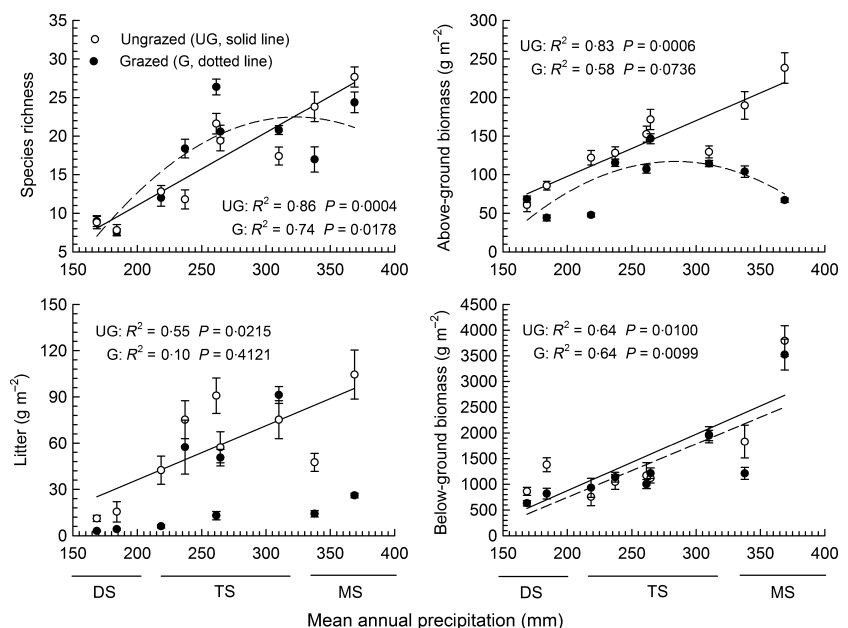

Fig. 2. Relationships of species richness, above- and below-ground biomass, and litter biomass with mean annual precipitation across the ungrazed (UG) and grazed (G) sites along the China–Mongolia transect. MS, meadow steppe; TS, typical steppe; and DS, desert steppe.

declined in the meadow steppe, increased in the typical steppe and remained relatively constant in the desert steppe. Below-ground biomass exhibited little change in the meadow and typical steppes, but decreased substantially in the desert steppe. At plant functional group level, responses of relative biomass to grazing were quite different among the three community types. In particular, relative above-ground biomass decreased for perennial forbs and increased for perennial bunchgrasses in the meadow steppe (both were dominant functional groups). The opposite trends were observed in the typical and desert steppe communities (Fig. 3). In general, perennial rhizomatous grasses decreased, annuals increased and shrubs and semi-shrubs either did not change or decreased in grazed sites from meadow, typical, to desert steppes across the regional precipitation gradient (Fig. 3).

C, N AND P POOLS AND STOICHIOMETRY IN PLANT MATERIALS

The C pools of all plant compartments, including above-ground standing biomass, litter and below-ground biomass, declined significantly in response to long-term grazing in the meadow steppe, typical steppe and desert steppe, except the below-ground biomass C for the typical steppe (Fig. 4). The N pools in above-ground biomass and litter were also reduced by grazing across the three community types. As compared with ungrazed sites, the above-ground biomass N decreased by 49% for the meadow steppe, 17% for the typical steppe and 41% for the desert steppe. Below-ground biomass N did not change in the meadow steppe, increased slightly in the typical steppe and decreased significantly in the desert steppe in response to grazing (Fig. 4). In the meadow and typical steppes, total P decreased in the above-ground biomass and litter but increased in below-ground biomass in response to grazing. In the desert steppe, grazing had no effect on above-ground biomass P, but significantly reduced litter and below-ground biomass P (Fig. 4).

The C : N ratios of all plant compartments were decreased by grazing in the meadow and typical steppes, but generally unaffected in the desert steppe (Fig. 5). The magnitude of the change was largest in the meadow steppe because of decrease in C and increase in N contents (%) in plant tissues (Fig. S1, Supporting information). In the typical steppe, reduced C : N was due primarily to increase in N content. In contrast to the relatively consistent responses of C : N in above- and below-ground, changes in the C : P ratio mainly occurred below-ground (Fig. 5). There was a sharp decrease in root C : P in the meadow and typical steppes, driven by changes in both C and P contents in the meadow steppe and by changes in P content in the typical steppe (Fig. S1, Supporting information).
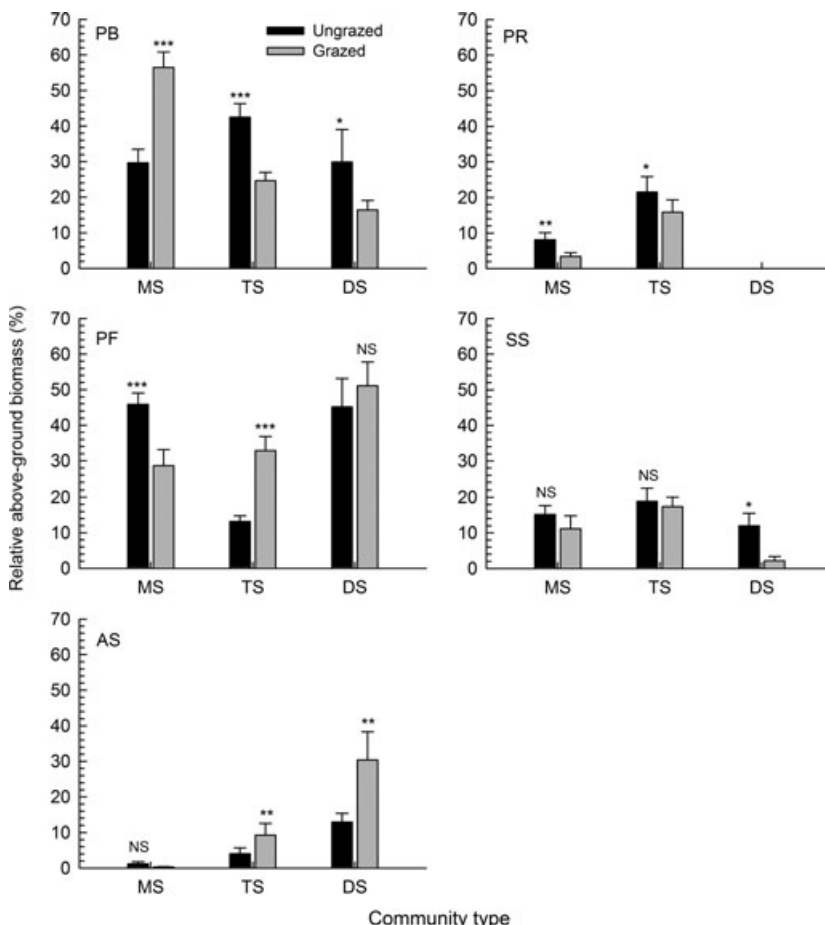

Fig. 3. Influence of grazing on plant functional group composition across different community types (error bars denote SE). MS, meadow steppe; TS, typical steppe; and DS, desert steppe. Abbreviations: PB, perennial bunchgrasses; PR, perennial rhizome grasses; PF, perennial forbs; SS, shrubs and semi-shrubs; and AS, annuals. Significant differences between the grazed and ungrazed sites are reported from ANOVA as NS, $P > 0.1$; $+0.05 < P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
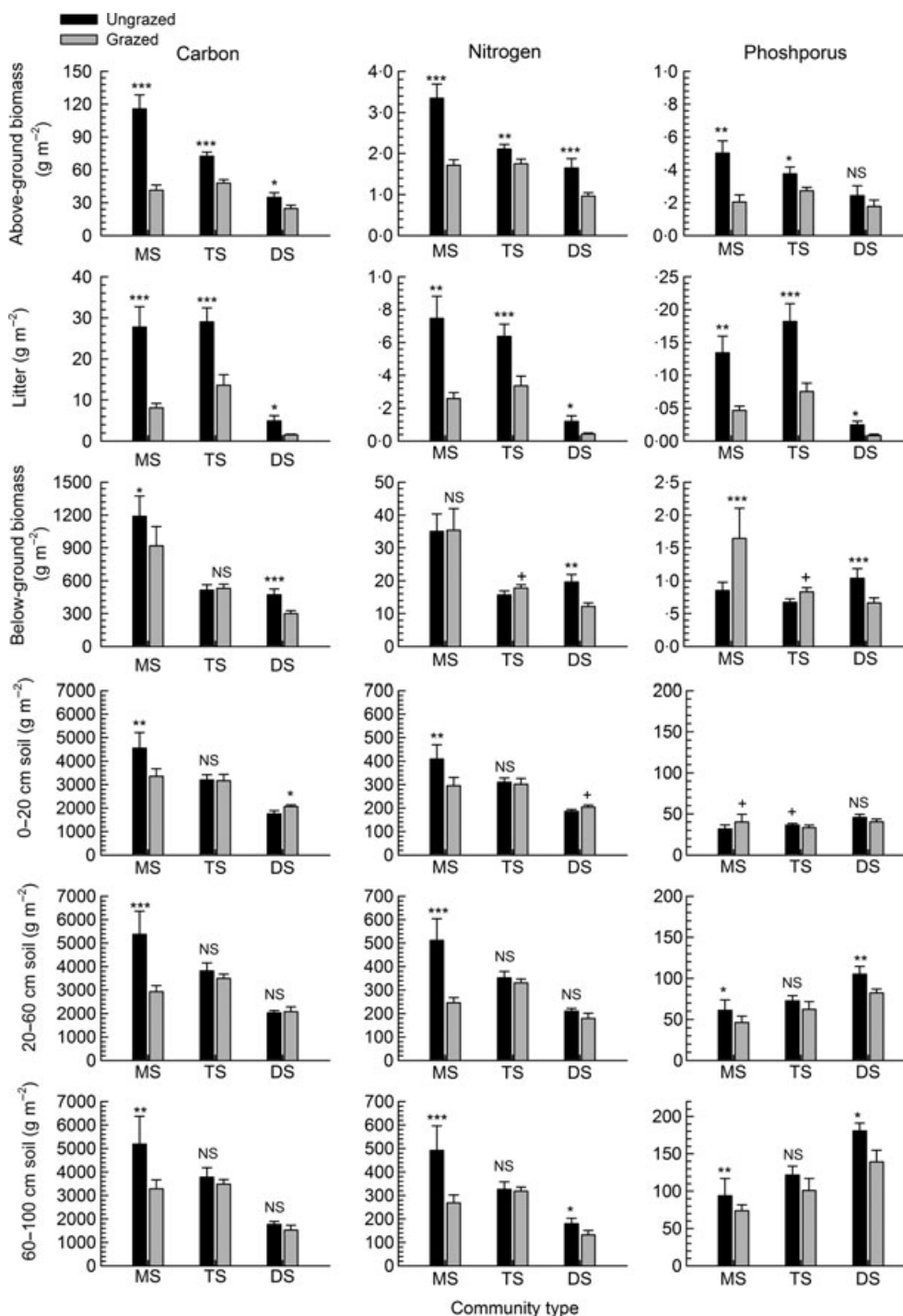
Fig. 4. Effects of grazing on C, N and P pools of plant, litter and soil across different community types on the China–Mongolia transect. Error bars represent SE. MS, meadow steppe; TS, typical steppe; and DS, desert steppe. Soil C, N and P pools at each depth were adjusted on an equivalent mass basis. Significant levels are as in Fig. 3.

At the plant species level, foliar C : N decreased in grazed sites for 11 of the 15 extant species examined (Fig. 6), because of increased N content and unchanged C content (Fig. S2, Supporting information). These changes were more consistent in the meadow steppe (seven of seven species) and typical steppe (eight of 10 species) than in the desert steppe (one of four species).

Species in different communities responded similarly for all stoichiometric ratios (Fig. 6). There were no significant effects of community type and interaction of community type and species (data not shown). Responses of foliar P content to grazing varied considerably among species and showed no consistent trend (Fig. S2, Supporting information).

C, N AND P POOLS AND STOICHIOMETRY IN SOIL

Grazing decreased soil C and N pools in the meadow steppe, but had no effect in both the typical and desert steppes across all depths (Fig. 4). For the desert steppe, soil C and N increased at the depth of 0–20 cm and remained relatively unchanged at the depths of 20–60 and 60–100 cm (Fig. 4). At the depth of 0–20 cm, soil total P increased slightly in meadow steppe, decreased considerably in the typical steppe and did not change in the desert
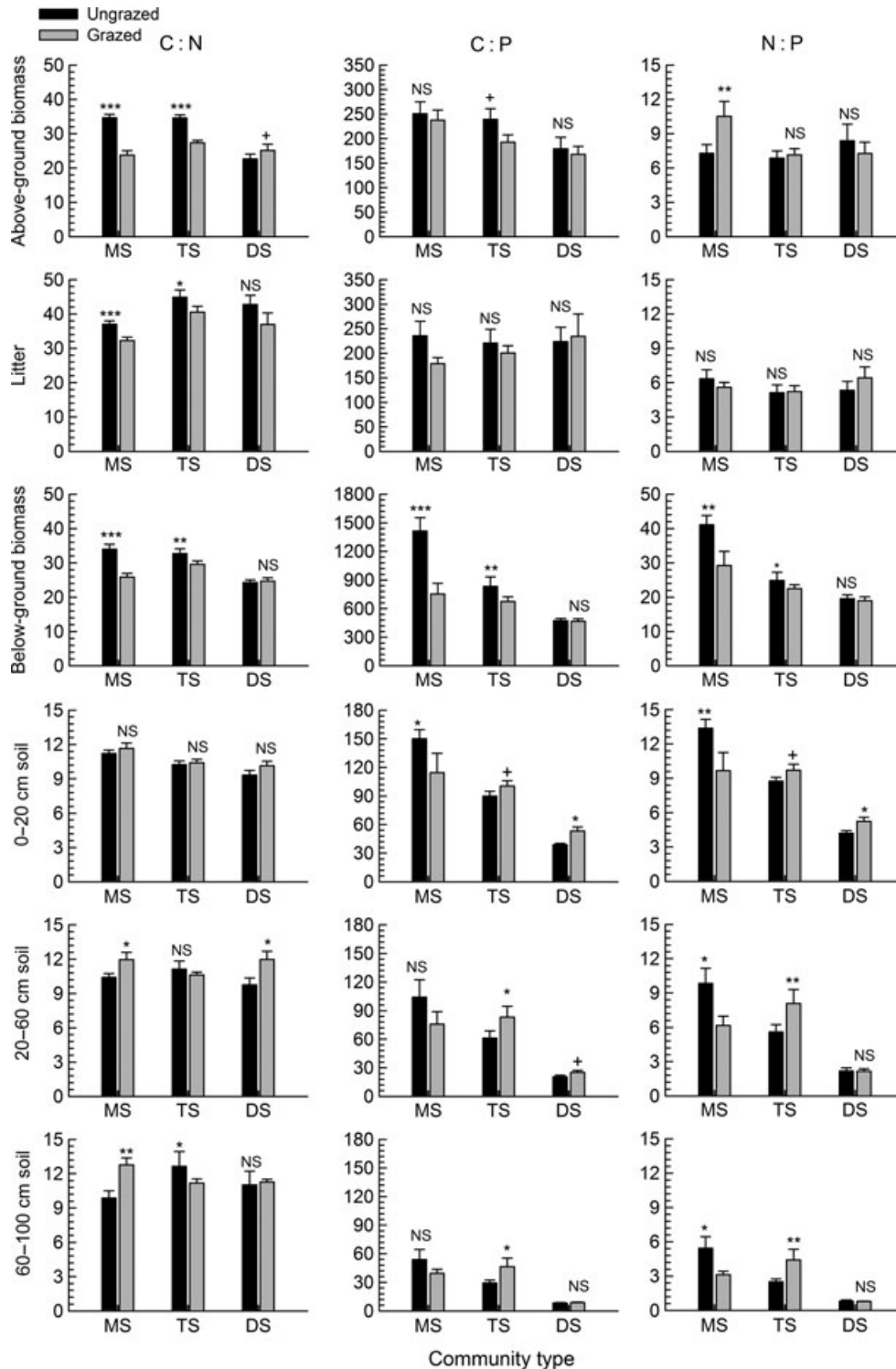

Fig. 5. Effects of grazing on the C : N : P stoichiometry of plant, litter and soil across different community types. All stoichiometric ratios are mass-based. Error bars represent SE. All symbols are derived as in Fig. 4.

Fig. 6. Effects of grazing on the foliar C: N:P stoichiometry of dominant and subdominant species (error bars denote SE.). All stoichiometric ratios are mass-based. The C : N, C : P and N : P ratios for each species were the average of all samples from the grazed and ungrazed fields across the China–Mongolia transect. MS, meadow steppe; TS, typical steppe; and DS, desert steppe. Abbreviations: Lc, *Leymus chinensis*; Sb, *Stipa baicalensis*; Sg, *S. grandis*; Skr, *S. krylovii*; Skl, *S. klemenzii*; Csq, *Cleistogenes squarrosa*; Cso, *C. songorica*; Af, *Artemesia frigida*; Ld, *Lespedeza davurica*; Cm, *Canrogana microphylla*; Gu, *Glycyrrhiza uralensis*; Cp, *Carex pediformis*; Sc, *Serratula centauroides*; Pt, *Potentilla tanacetifolia*; Fs, *Filifolium sibiricum*; SP, species; PFG, plant functional group; and CT, community type. Significant levels are as in Fig. 3.
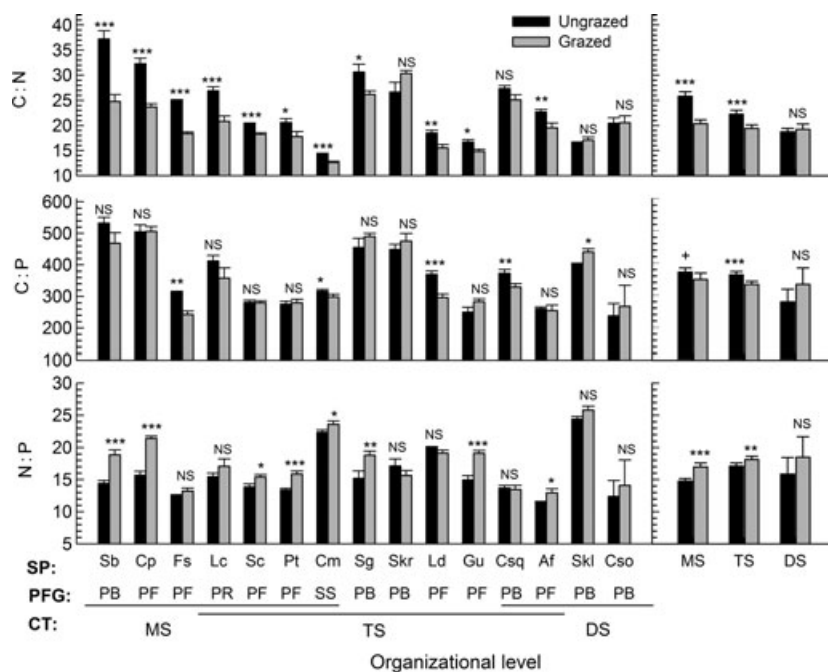
steppe. At the depths of 20–60 and 60–100 cm, total P decreased in the meadow and desert steppes and remained unchanged in the typical steppe (Fig. 4).

The responses of soil C : N ratio to grazing differed between soil depths for the three community types (Fig. 5). In both the meadow and desert steppes, C : N ratio increased in all three soil depths although grazing reduced C and N contents (%) in the meadow steppe but enhanced both in the desert steppe (Fig. S1, Supporting information). In the typical steppe, however, C : N ratio changed little in the top 20-cm soil layer and decreased in soil depths of 20–60 and 60–100 cm (Fig. 5). The responses of soil C : P to grazing differed among community types. In contrast to C : N ratio, C : P ratio decreased in all three soil depths in the meadow steppe. C : P ratio showed a clearly increasing trend in all soil depths in the typical and desert steppes. N : P ratio exhibited a similar response pattern to that of C : P ratio (Fig. 5).

RELATIONSHIPS BETWEEN STOICHIOMETRIC RESPONSES AND PRECIPITATION

In the ungrazed sites, the above-ground biomass C : N ratio and below-ground biomass and soil C : N, C : P and N : P ratios increased linearly with MAP across the CMT ($P < 0.05$, data not shown). The above-ground biomass C : N response to grazing was negatively correlated with MAP (Fig. 7), but the responses of above-ground biomass C : P and N : P ratios had no significant relationships with MAP. The litter C : P response to grazing declined linearly with increasing MAP (Fig. 7). The responses of below-ground biomass C : N, C : P and N : P ratios to grazing all decreased with increasing MAP. The C : P and

N : P responses to grazing also decreased with increasing MAP in the top 20-cm soil layer (Fig. 7). In the soil depths of 20–60 and 60–00 cm, the relationships between the responses of C : N, C : P and N : P ratios to grazing and MAP were not statistically significant (data not shown).

Discussion

DIFFERENTIAL EFFECTS OF GRAZING ON C, N AND P POOLS IN PLANT AND SOIL COMPARTMENTS

Our results indicate that the reduction in C, N, P pools in above-ground biomass and litter at the grazed sites were attributable mainly to the substantial decrease in standing biomass and litter, although grazing increased the N contents (%) of both above-ground biomass and litter in meadow and typical steppes. However, the responses of below-ground biomass and soil C, N and P pools differed among community types. Specifically, these changes in below-ground pool size were associated primarily with a decrease in C content and increases in N and P content in the meadow steppe, increases in N and P content in the typical steppe, and a reduction in below-ground biomass in the desert steppe.

Our results on the effects of grazing on soil C and N pools in the Inner Mongolia grassland were largely consistent with previous findings (Han *et al*. 2008; Li *et al*. 2008). The effects of grazing on soil C, N and P pools were mainly due to changes in C, N and P contents (Ellert & Bettany 1995). The differential responses of soil C, N and P pools among three community types may be related to the long-term nutrient export through livestock products (e.g. livestock biomass, faeces and urine), which increases with primary productivity (Oesterheld, Sala &
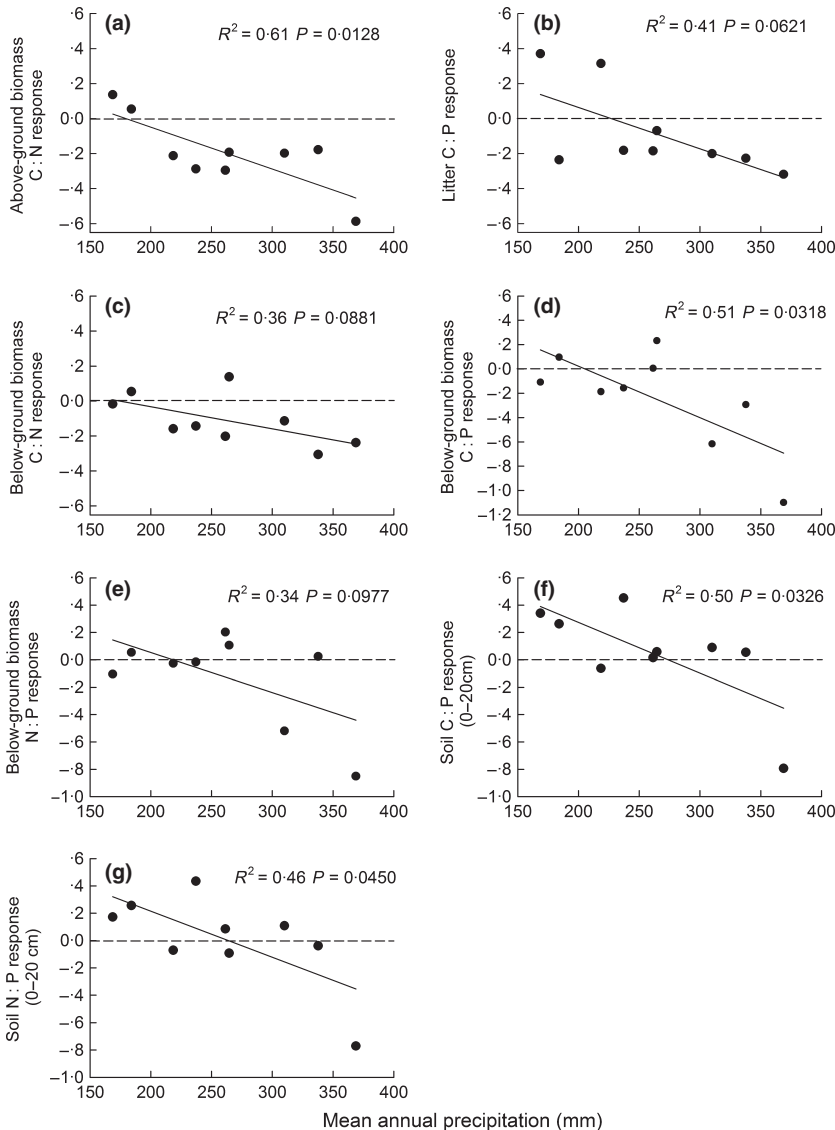
Fig. 7. Relationships between the responses of stoichiometric ratios in plant, litter and soil to grazing and mean annual precipitation across the China–Mongolia transect. The responses of C : N, C : P and N : P ratios to grazing were calculated as Ln (C : N_{grazed}/C : $N_{ungrazed}$), Ln (C : P_{grazed}/ C : $P_{ungrazed}$), and Ln (N : P_{grazed}/N : $P_{ungrazed}$), respectively. The reference line (dashed line) in each panel indicates no change in stoichiometric ratio.

McNaughton 1992). Milchunas, Sala & Lauenroth (1988) hypothesized that differential responses of grasslands to grazing could result from differences in their evolutionary history of grazing and moisture. Given that the three grassland types have similar long-term grazing history, grazing intensity and precipitation may be the two important factors for the observed response patterns.

EFFECTS OF GRAZING ON PLANT AND SOIL C : N : P STOICHIOMETRY

Previous studies proposed that the stimulatory feedbacks between C-rich root exudates, microbial activity, and plant available soil nutrients (Bardgett, Wardle & Yeates 1998; Hamilton & Frank 2001), and shifts in plant species and functional group composition (Ritchie, Tilman & Knops 1998; Bardgett & Wardle 2003) were two major mechanisms underpinning the plant and soil stoichiometric responses to grazing. In our case, we hypothesize that both mechanisms may have operated to different degrees along

the regional precipitation gradient, with the former predominating in the meadow steppe and the latter dominating in the desert steppe, because the proportional nutrient exports by livestock were much higher in the meadow steppe than in the desert steppe. In the meadow steppe, the predominant plant functional group, that is, perennial bunchgrasses, is well adapted to grazing. Grazing under fertile conditions could have stimulated root exudation of C-rich substances, elevated N mineralization, enhanced plant N content and thereby led to a reduction in C : N. This is evidenced by the elevated N contents in all plant apartments in the grazed meadow steppe. In the desert steppe, however, the species are not well adapted to grazing (Milchunas, Sala & Lauenroth 1988; Gillson & Hoffman 2007). Thus, there was a reduction in N pools, leading to an increase in C : N in the desert.

Our results suggest that the functional response mediated by changes in dominant species and plant functional group composition is a key mechanism underpinning the grazing-induced changes in ecological stoichiometry and

ecosystem functioning. For example, in the desert steppe, grazing increased the absolute and relative abundance of opportunistic and unpalatable annuals with shallow roots, for example, *Neopallasia pectinata* (Pall.) Poljak. and *Convolvulus ammannii* Desr. Annuals commonly have high tissue N levels, although they can still be unpalatable if the N is locked in compounds that dissuade herbivory (Throop & Lerdau 2004). These resource-acquiring annuals have a competitive advantage in depleting available N and water, resulting in increases in root litter input to the surface soil in the grazed sites because of a shift from a perennial-dominated to an annual-dominated community. Indeed, grazing increased the 0- to 20-cm soil carbon although it diminished the total below-ground biomass. These changes in plant species composition could lead to increases in soil C and N pools. In the typical steppe, on the other hand, the predominance of grazing-tolerant species with shallow roots led to a greater proportion of root biomass in the top 20-cm soil layer (Chen *et al.* 2001). As a consequence, soil C and N remained relatively unchanged.

Our findings demonstrate that the impacts of grazing on plant and soil C : N : P stoichiometry are strongly mediated by water availability across the regional transect. Responses of both above- and below-ground biomass C : N ratios to grazing were negatively correlated with MAP, indicating that these ratios were reduced in the meadow steppe but enhanced in the desert steppe. The negative relationship of MAP to both below-ground biomass and topsoil C : P responses suggest that grazing increased root and topsoil C : P ratios in the meadow steppe and reduced them in the desert steppe. Frank (2008) reported that grazing increased plant N contents and shifted the type of nutrient limitation towards N and P co-limitation. In our study, the below-ground biomass and soil N : P responses declined with increasing MAP, implying that long-term grazing is likely to increase the potential for N and P co-limitation in the desert steppe and N limitation in the meadow steppe.

ACCELERATED N CYCLING BY GRAZING

Our results show that grazing accelerated N cycling in the Inner Mongolian grasslands, especially in the meadow and typical steppes. Two contrasting mechanisms exist on the effects of grazing on N cycling. First, grazing may increase nutrient cycling through stimulation of microbial activity owing to input of defoliated fresh plant litter and animal excreta (Holland, Cheng & Crossley 1996; Hamilton & Frank 2001). Second, grazing may decrease nutrient cycling through increases in unpalatable plant species and production of higher concentrations of defensive compounds (Hobbie 1992; Ritchie, Tilman & Knops 1998). Our results suggest that grazing accelerated N cycling as evidenced by increased plant and litter N contents and decreased soil N content. This is particularly conspicuous in the meadow steppe. In the desert steppe, the increase in soil C and N contents suggests that the latter mechanism was in operation. These two opposing mechanisms may coexist in the typical steppe, resulting in only slightly accelerated N cycling.

Under nutrient-deficient conditions, defoliation often causes reductions in root nutrient content and biomass, as C-rich substrates are exuded into the rhizosphere to stimulate microbial activity and stored nutrients are reallocated to support regrowth above-ground (McNaughton 1985). When soil nutrients are abundant, plant nutrient content (especially P) may be unchanged or even increase following defoliation, as plants adapted to grazing are able to recoup losses to grazers (McNaughton & Chapin 1985). The lack of reduction in root or above-ground P content observed in the grazed sites suggests that P availability in the meadow and typical steppes may have been sufficient to support stoichiometrically balanced growth for plants following grazing. This is likely because most of the dominant and subdominant plant species in these steppe communities are colonized by arbuscular mycorrhizal fungi (Tian *et al.* 2009), which could have increased plant P uptake and thus lessened the defoliation-induced P limitation (van der Heijden *et al.* 1998). P availability may also have been sufficient to support balanced growth at higher tropic levels in these communities, as shoot C : P of all three community types across the CMT were below the hypothesized threshold of 250 : 1 required for efficient growth of P-rich herbivores feeding on comparably C-rich plants (Elser *et al.* 2000).

Large changes in N content following grazing in both the meadow and typical steppes, with minor changes in P content, may potentially increase N and P co-limitation in these communities. Grazing increased the N : P ratio of above-ground tissue in the meadow steppe and the soil N : P in the typical and desert steppes, suggesting that grazing accelerated N cycling more than P cycling. However, support for a shift towards P limitation was inconsistent across the different community types, as the N : P ratio of above-ground biomass was unaffected in typical and desert steppe communities, and soil N : P decreased in the meadow steppe, indicating that P cycling increased more than N cycling following grazing in this system. In addition, litter N : P was below all previously documented critical N : P ratios for N vs. P limitation (reported range of 9–25) (Güsewell & Verhoeven 2006), suggesting N-limited systems. Overall, although the increase in N cycling suggests a general shift towards P limitation for plant growth across the three community types, this potential may be buffered in the meadow steppe because of a large increase in P availability and is probably not a dominant process in the non-responsive (and water-limited) desert steppe. Thus, the potential for P limitation may be more likely to occur in the typical steppe. The changes in root P content observed across much of the CMT, however, suggest that the stoichiometric response to grazing may be more complex than previously understood, highlighting the need for more careful interpretation of foliar N : P ratio in determining nutrient limitation.

IMPLICATIONS FOR GRASSLAND MANAGEMENT AND CLIMATE CHANGE

Our study has important implications for the ecology and management of the vast grasslands in the Eurasian Steppe region. First, long-term grazing under high intensities not only alters ecosystem productivity and biodiversity (Bai *et al.* 2007), but also C, N and P pools and stoichiometry across the Inner Mongolian grassland. The results from the current study further demonstrated that both the species richness and above-ground standing biomass at the grazed sites were highest at the intermediate level of precipitation across the CMT, suggesting that the typical steppe seems to be more resilient to grazing than those meadow and desert steppes. Second, our results suggest that the effects of grazing on C : N : P stoichiometry and ecosystem functioning are strongly mediated by precipitation. Future climate change, particularly changes in precipitation amount and seasonality (Easterling *et al.* 2000), may have profound impacts on the ecosystem functioning of the Eurasian Steppe as a whole. Third, the accelerated N cycling reduces N limitation, but may potentially intensify N and P co-limitation. Given that applying fertilizers on large scales is not practical, reducing the currently high stocking rate and restoring the vast degraded steppes are essential to future ecosystem management in the Inner Mongolian grassland region.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Effects of grazing on C, N, and P contents (%) in above- and below-ground biomass, litter, and soil across different community types of the China–Mongolia transect (Error bars represent SE).

Fig. S2. Effects of grazing on foliar C, N, and P contents of dominant and subdominant species (error bars denote SE).

Table S1. Abiotic and biotic characteristics of the 18 paired study sites along the China–Mongolia transect.

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