

Contrasting responses in leaf nutrient-use strategies of two dominant grass species along a 30-yr temperate steppe grazing exclusion chronosequence

Xiao-Tao Lü · Grégoire T. Freschet · Elena Kazakou ·
Zheng-Wen Wang · Li-Shi Zhou · Xing-Guo Han

Received: 17 April 2014 / Accepted: 29 September 2014
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Abstract

Aims Grazing exclusion practices can be promising restoration techniques where ecosystem degradation follows from rapidly increasing grazing pressure, as widely observed in northern Chinese grasslands. However, the mechanisms of plant-soil interactions responsible for nutrient cycling restoration remain unclear.

Methods We examined the functional response of the two most dominant grass species with contrasting nutrient economies to a grazing exclusion chronosequence varying greatly in soil moisture and extractable N and P.

Results The relative biomass of the nutrient acquisitive species *Leymus chinensis* increased while that of the nutrient conservative *Stipa grandis* decreased across the

chronosequence. *Leymus chinensis* displayed increasing leaf nutrient concentration and decreasing nutrient resorption with time since grazing exclusion for both N and P. In contrast, *S. grandis* showed decreasing leaf N and P concentrations and largely stable nutrient resorption.

Conclusions These differences in plasticity, with respect to nutrient stoichiometry and resorption, suggest contrasting abilities of these two dominant species to compete for soil resources and/or differences in their affinity to the changing forms of soil available N and P likely occurring along the restoration gradient. Ecosystem trajectory of change after grazing exclusion appears therefore largely dependent on the nutrient use strategies of co-occurring dominant grassland species.

Responsible Editor: Alfonso Escudero.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-014-2282-7) contains supplementary material, which is available to authorized users.

X.-T. Lü (✉) · Z.-W. Wang · X.-G. Han
State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China
e-mail: lvxiaotao@gmail.com

G. T. Freschet
Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden

E. Kazakou
Montpellier SupAgro, UMR Centre d'Ecologie Fonctionnelle et Evolutive 5175, 1919 route de Mende, 34293 Montpellier, France

L.-S. Zhou · X.-G. Han
State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Present Address:
G. T. Freschet
CNRS, UMR Centre d'Ecologie Fonctionnelle et Evolutive 5175, 1919 route de Mende, 34293 Montpellier, France

Keywords Ecological stoichiometry · Grazing exclusion · N:P ratio · Nutrient resorption · Plant-soil feedback · Restoration chronosequence · Semi-arid grassland · Species-specific response

Introduction

Temperate steppe is a productive and floristically diverse grassland type of great economic and ecological importance in Eurasia (Bai et al. 2010). However, nearly 90 % of the steppe in northern China has been degraded to various degrees due to anthropogenic activities (Jiang et al. 2006). The associated reduction in biodiversity and primary productivity (Li et al. 2008) highlight the need for steppe protection and restoration (He et al. 2011). Grazing exclusion appears as a promising method to restore these grassland ecosystems (Jiang et al. 2006; He et al. 2008, 2009) and is therefore becoming widely used in Chinese grasslands (Jiang et al. 2006; Li et al. 2011). However, the short- and long-term consequences of this restoration practice on ecosystem processes and functions are still poorly understood.

Grazing exclusion generally induces large successional changes in plant community structure, primary production, and soil fertility (Li et al. 2008; He et al. 2011). Grazing exclusion after heavy grazing generally induces changes in plant biomass allocation and more precisely to increased aboveground primary production and decreased belowground production, at least partly through its positive effects on water availability (Pucheta et al. 2004; Piñeiro et al. 2009). It also modifies ecosystem nutrient cycling (Piñeiro et al. 2009; Shan et al. 2011; Medina-Roldan et al. 2012), although this appears highly variable across plant communities (Knops and Tilman 2000; Brye and Riley 2009). In the temperate steppe of Inner Mongolia for instance, grazing exclusion significantly enhances total yearly cumulative net N mineralization (Shan et al. 2011). However, slower soil N mineralization rates and lower soil inorganic N availability have been reported in other restored grasslands (Baer et al. 2002; Li et al. 2009; Medina-Roldan et al. 2012).

Changes in soil resource availability and plant nutrient status have potentially large consequences for plant nutrient conservation strategies, at both intraspecific (van Heerwaarden et al. 2003b; Richardson et al. 2005; Kobe et al. 2005; Lü et al. 2012a) c(Aerts 1996; Killingbeck 1996). Generally, it

is expected that increases in soil nutrient availability drive increased plant nutrient content (Xia and Wan 2008) via the ‘luxury consumption’ of N in both leaf and fine root tissues (van Wijk et al. 2003). Although the link between soil P availability and plant P content is more complex (Aerts and Chapin 2000), plant P content often increases with increasing soil fertility (Richardson et al. 2005; Lü et al. 2013). Consequently, the balance between N and P availability in soil generally determines plant N:P ratios (Koerselman and Meuleman 1996). On the contrary, in less fertile environment it has been suggested that the percentage reduction of nutrient content between green and senesced leaves (defined as nutrient resorption efficiency sensu Chapin 1980) decreases with increasing nutrient supply (van Heerwaarden et al. 2003b; Kobe et al. 2005). Finally, soil water availability can also impact on plant nutrient resorption (Minoletti and Boerner 1994). This is especially true for N in semi-arid grassland (Lü and Han 2010) where soil water availability has positive effects on soil N transformation and availability (Wang et al. 2006).

In this study we explore how changes in soil resources associated with grassland restoration via grazing exclusion drive within-species changes in plant nutrition and nutrient conservation strategies of the two most dominant grasses (*Leymus chinensis* and *Stipa grandis*) in the system. These two species show contrasting life strategies. While both are C3 grasses, *L. chinensis* is a perennial rhizome grass, and *S. grandis* is a perennial bunchgrass. *Leymus chinensis* generally dominates the habitats with relatively abundant water and nutrient resources, while *S. grandis* is usually abundant in dryer and N-limited conditions (Chen et al. 2005). Furthermore, foliar N and P concentrations are higher in *L. chinensis* than that in *S. grandis* (Lü et al. 2013). Also, these two species vary in their strategies of N use efficiency (NUE) and water use efficiency (WUE) in that *L. chinensis* achieves high WUE at the expense of NUE whereas *S. grandis* is able to simultaneously maintain relative high NUE and WUE (Chen et al. 2005).

Our first hypothesis is that grazing exclusion would increase soil moisture and soil nutrient availability for plants with positive effects on plant biomass. Secondly, given the contrasting resource economics of *L. chinensis* and *S. grandis* (Chen et al. 2005), we hypothesize that the two species would show different responses to changes in soil resources along the restoration gradient.

We expect therefore different trends/slopes of the relationships between time since grazing exclusion and leaf nutrient concentration, leaf N:P ratios, and leaf nutrient resorption efficiency of the two species.

Materials and methods

Study sites

This study was conducted in 2009 in a temperate steppe ecosystem located near the Inner Mongolia Grassland Ecosystem Research Station (43°38'N, 116°42'E). Mean annual temperature was 1.1 °C (1980–2009) and mean annual precipitation was 345 mm, with about 70 % occurring from June to September (Lü et al. 2014). The soil was chestnut, equivalent to Calcicorthic Aridisol in the US soil taxonomy classification system. The vegetation of the region is dominated by *Leymus chinensis* and *Stipa grandis*, accounting for 40–80 % of the total aboveground biomass (Lü et al. 2014).

The chronosequence of duration of grazing exclusion was represented by five nearby plots within the same temperate steppe ecosystem ranging in size from 5 to 40 ha, which included a control plot being grazed during winter in each year (0 year) and grazing exclosures dating from 2007 (2 years), 2004 (5 years), 1999 (10 years) and 1979 (30 years). Like most chronosequence studies this set-up was subject to pseudo-replication and space-for-time substitution limitations (Walker et al. 2010; Blois et al. 2013). Nevertheless, the five experimental plots were continuously distributed on the same upper basalt platform (He et al. 2008), appeared topographically and floristically similar and generally experienced low frequency and severity of disturbance (see Walker et al. 2010). Therefore, the length of grazing exclusion was identified as the only important factor of change across plots. In July 2009 we established one 20×20 m sub-plot representative of the vegetation present within each plot of distinct grazing exclusion history. In each sub-plot, we set two parallel 20×3 m transects separated by 10 m. Five 1 m² quadrats were then evenly laid across each transect to capture plot heterogeneity.

Soil sampling and analysis across chronosequence

Five soil cores (10 cm depth) were collected from each quadrat using a 3 cm diameter soil auger and mixed into

a composite sample in mid-August 2009. Ultrasonic energy was used to disrupt soil aggregates, and particle-size fractionation was performed using bulk soil samples <2 mm according to Chen and Chiu (2003) and He et al. (2009). Gravimetric water content was determined by drying soil subsamples at 105 °C for 24 h. The soil pH was determined with a glass electrode in a 1 : 2.5 soil : water solution (w/v). To analyze inorganic N, 50 ml of 2 M KCl solution was added to a 10 g fresh soil subsample. The mixture was shaken for 1 h on a reciprocal shaker, and then the soil suspension was filtered. The filtered soil extract was used to determine NH₄⁺-N and NO₃⁻-N concentration with a continuous flow spectrophotometer (FIAstar 5000, Foss Tecator, Denmark). Available soil P was estimated by extracting soil with 0.5 M NaHCO₃ (pH=8.5) and analyzed using the molybdenum blue-ascorbic acid method (Olsen et al. 1954). The organic C content in soils was measured using a modified Mebius method (Nelson and Sommers 1982). Briefly, 0.5 g soil samples were digested with 5 ml of 1 M K₂Cr₂O₇ and 10 ml of concentrated H₂SO₄ at 180 °C for 5 min, followed by titration of the digests with standardized FeSO₄.

Leaf monitoring and measurements for nutrient concentrations

In each of the 10 quadrats per plot, 10 to 20 shoots of *L. chinensis* and *S. grandis* were randomly selected from each of which two fully expanded leaves of almost the same size were used. One of the leaves was sampled from all selected shoots in all quadrats in mid-August, when aboveground biomass attained its peak value in this region (He et al. 2008; Lü et al. 2012b). The other was tagged with red thread and labeled. These leaves were checked weekly from late September to mid-October and collected only when fully senesced. Green and senesced leaves (including petioles) were oven dried at 70 °C for 48 h and then weighed. Leaves of each species from the same quadrat were combined and ground using a ball mill (Retsch MM 400, Retsch, Haan, Germany).

Total N concentrations of plants and soils were determined colorimetrically by the Kjeldahl acid-digestion method with an Alpkem auto-analyzer (Kjtek System 1026 distilling unit, Sweden) after extraction with sulfuric acid (Bremner 1996). Total P concentration was measured by the ammonium molybdate after persulfate oxidation followed by colorimetric analysis (Kuo 1996).

Nitrogen and P concentrations were expressed on a mass basis. Nutrient resorption efficiency (NuRE), defined as the proportion of mature leaf nutrient pool resorbed during leaf senescence, was calculated as:

$$\text{NuRE} = (1 - \text{Nutrient}_{\text{senesced}} / \text{Nutrient}_{\text{green}}) \times 100\%,$$

where $\text{Nutrient}_{\text{senesced}}$ and $\text{Nutrient}_{\text{green}}$ are the N or P pools of senesced and green leaves in each quadrat, respectively. For each species in each quadrat the nutrient pool of leaves was calculated as the product of total leaf mass from the sampled shoots with average leaf nutrient concentration (Aerts et al. 2007; Lü et al. 2012a). Because similar cohorts of leaves were sampled at full maturity and after complete senescence, we avoided biases in the calculation of resorption efficiency resulting from mass loss and area shrinkage occurring during leaf senescence (van Heerwaarden et al. 2003a). Nutrient resorption proficiency was defined as the level to which nutrient concentrations were reduced during the resorption process following Killingbeck (1996), with higher resorption proficiency meaning lower nutrient concentration in senesced leaves.

Aboveground green and litter biomass measures

Adjacent to the five 1 m² quadrats used for species sampling in each transect, we selected another five 1 m² quadrats to measure aboveground biomass and litter biomass in mid-August. The aboveground biomass in those quadrats was clipped at the ground level, which was considered approximately equal to the aboveground net primary production of the current year. All living vascular plants in the sample were sorted into species, and then dried and weighed for each quadrat separately. Litter samples were collected subsequently from each 1 m² quadrat. Total dry weight of litter biomass was also measured for each 1 m² quadrat separately.

Data analysis

Normality and homogeneity of variances were verified for all data using Kolmogorov-Smirnov's test and Levene's test, respectively. One-way ANOVAs were used to compare all means of soil characteristics among plots with different grazing exclusion history, with Duncan's test as the post-hoc test for multiple comparisons. Relationships between the duration of grazing exclusion and soil extractable nutrients, soil water

availability, green leaf N:P, green and senesced leaf nutrient concentrations and NuRE were estimated using linear regressions. Duration of grazing exclusion and soil nutrient availability variables were previously log-transformed as $\log(X+1)$ and $\log(X)$, respectively, in order to comply with normality assumptions. Within each plot, paired-sample t-tests were used to compare foliar nutrient concentrations between the two species. All the analyses were carried out with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

There were no differences in soil pH and soil texture among the five plots with different grazing exclusion history (Table 1). Soil bulk density was the highest in the grazed plot (Table 1). While there was no difference for total soil P in the five plots, total soil C and N increased with the duration of grazing exclusion (Table 1). Soil inorganic nutrient concentrations and moisture varied greatly across the 30-years grazing exclusion chronosequence. Soil inorganic N concentration was highest in the plots with the longest exclusion history, whereas soil extractable P concentration in soil was highest in plots without exclusion and with shortest exclusion history. Soil moisture was lowest in plots without exclusion and highest in the plots of 10-years exclusion (Table 1). Soil moisture was marginally correlated to soil inorganic N availability along the restoration chronosequence ($P=0.053$; Fig. S1; Supplementary materials).

Both total aboveground plant biomass and litter biomass increased along the chronosequence (Fig. 1a and b). The biomass of *L. chinensis* increased whereas that of *S. grandis* remained stable across the chronosequence (Fig. 1c). The relative contribution of *L. chinensis* to total plant biomass increased while that of *S. grandis* decreased across the whole chronosequence (Fig. 1d). There was no change in the N:P ratio of *L. chinensis*, while this of *S. grandis* tended to decrease across the chronosequence (Fig. 2). Total aboveground biomass of the communities was positively correlated with soil inorganic N concentrations ($P=0.02$, Fig. S2, Supplementary materials) and negatively correlated with soil extractable P ($P<0.001$, Fig. S2, Supplementary materials).

Leaf nutritional traits responded differently to grazing exclusion between species (Fig. 3). Green leaf N

Table 1 Soil characteristics (0–10 cm soil layer) across the 30-years grazing exclusion chronosequence in a temperate steppe of northern China

Duration of grazing exclusion	Soil pH	Soil texture			Bulk density (g cm ⁻³)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Inorganic N (g m ⁻²)	Available P (g m ⁻²)	Soil moisture (%)
		Sand (%)	Silt (%)	Clay (%)							
0 year	7.3±0.1	63.7±0.6	33.9±0.5	2.4±0.2	1.4±0.1a	19.0±0.5a	1.8±0.1a	0.3±0.0a	0.5±0.0b	0.7±0.1a	4.0±0.2c
2 years	7.2±0.1	63.9±0.3	33.7±0.3	2.4±0.1	1.3±0.1b	20.7±0.5b	2.0±0.0b	0.4±0.1a	0.7±0.1b	0.7±0.1a	5.6±0.1b
5 years	7.2±0.1	63.8±0.2	33.7±0.3	2.5±0.2	1.3±0.1b	22.2±0.4c	2.2±0.0c	0.3±0.0a	0.7±0.1b	0.4±0.1c	5.6±0.1b
10 years	7.1±0.1	64.4±0.3	33.0±0.2	2.5±0.3	1.2±0.1c	23.7±0.6c	2.3±0.0d	0.3±0.0a	0.6±0.0b	0.4±0.0bc	6.5±0.1a
30 years	7.2±0.1	64.1±0.3	33.4±0.2	2.5±0.2	1.2±0.1c	25.2±0.5d	2.6±0.1e	0.3±0.0a	1.1±0.1a	0.5±0.0ab	5.4±0.2b

Values represent mean ± SE (n=10)

Different letters in the same column indicate significant differences at $P < 0.05$

concentration of *L. chinensis* increased ($P=0.01$), whereas that of *S. grandis* decreased ($P=0.07$) across the restoration chronosequence (Fig. 3a). Similarly,

senesced leaf N concentration of *L. chinensis* increased ($P=0.01$), while that of *S. grandis* did not vary along the chronosequence (Fig. 3c). The response patterns of P

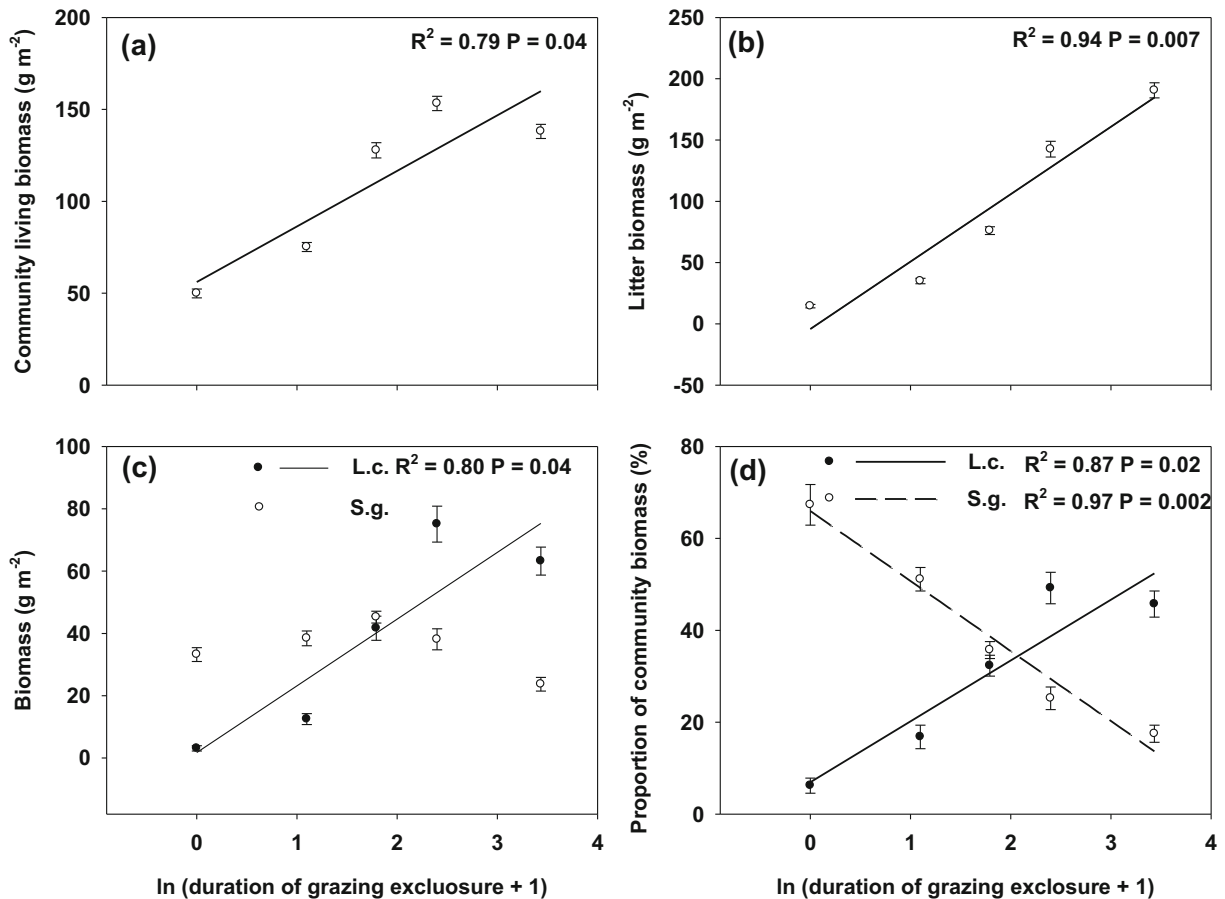
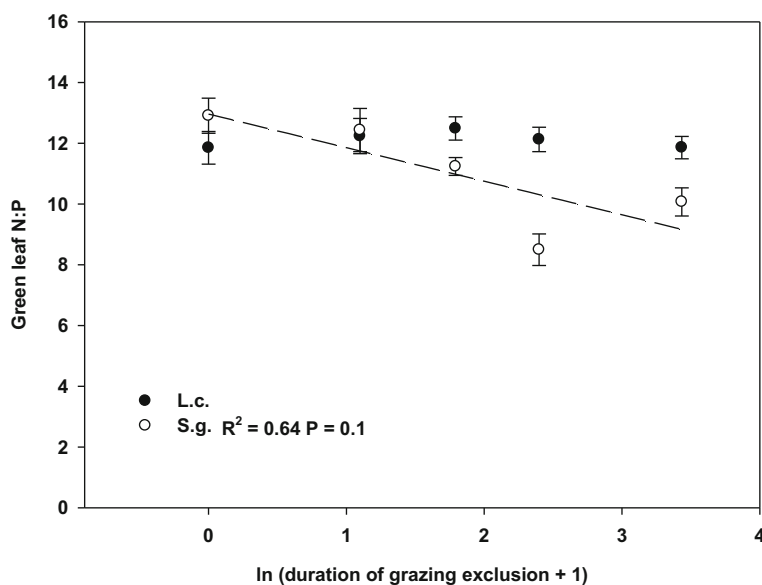


Fig. 1 Total community living biomass (a), litter biomass (b), biomass of *Leymus chinensis* and *Stipa grandis* (c) and their relative contribution to total plant community biomass (d) across

a 30-years grazing exclusion chronosequence in a temperate steppe of northern China. Data are shown as mean ± SE

Fig. 2 Changes in green leaf N:P ratios of *Leymus chinensis* and *Stipa grandis* across a 30-years grazing exclusion chronosequence in a temperate steppe of northern China. Data are shown as mean \pm SE



concentration in green and senesced leaves were similar with those of N concentration (Fig. 3b and d). Foliar nutrient concentrations of the two grass species were similar both in the grazed plot and in the plot with short restoration history (2 years) (Fig. 3, Paired samples *T*-test, all $P > 0.05$), except for senesced leaf N concentration, which was slightly higher in *L. chinensis* than that in *S. grandis* in the grazed plot (Fig. 3b, $t = 2.34$, $P = 0.04$). After the ecosystems have been restored for equal to or greater than 5 years though, nutrient concentration in *L. chinensis* was generally much higher than that of *S. grandis* (Fig. 3, Paired samples *T*-test, all $P < 0.001$), except for senesced leaf N in plot with the grazing-exclusion age of 5 years (Fig. 3c, $t = 0.67$, $P = 0.52$).

Nitrogen resorption efficiency (NRE) of *L. chinensis* decreased along the chronosequence ($P = 0.05$, Fig. 3e). NRE of *S. grandis* was much higher in grazed plots than in grazing exclusion plots (One-way ANOVA, $P = 0.05$, Fig. 3e). There was no significant relationship between phosphorus resorption efficiency (PRE) and time since grazing exclusion for both species (Fig. 3f).

Discussion

Changes in soil nutrient availability and soil moisture in response to grazing exclusion

Partly consistent with our first hypothesis, we observed relatively higher soil total and inorganic N

concentrations and soil moisture within the plots with longer duration of grazing exclusion. This, together with the lower soil bulk density observed here on ungrazed plots, confirms previous studies reporting a negative effect of grazing on soil moisture in semiarid grasslands (Zhao et al. 2011; He et al. 2011) owing to lower total pore and macropore volume following animal trampling (Zhao et al. 2011). In the same ecosystems, Reszkowska et al. (2011) found indeed that grazing exclusion led to the progressive recovery of soil pore continuity and hydraulic conductivity, which are critical drivers of soil moisture conditions. Furthermore, we observed higher total vegetation biomass and plant litter cover on ungrazed plots, which can reduce soil temperature and wind-related soil evaporation (He et al. 2011) and therefore contribute to higher soil moisture. Our results also support the report by Wang et al. (2006) of lower soil inorganic N concentration in grazed than in ungrazed plots (4-years and 24-years) within the same region. Our results suggest therefore that grazing exclusion would contribute to the restoration of ecosystem fertility through two partially distinct mechanisms: enhancement of soil moisture, probably the main driver of ecosystem functioning in this semiarid temperate steppe (Lü et al. 2012b), and increased N availability to plants.

In contrast to our first hypothesis, extractable P in soil was higher in plots under grazing and plots with short history of grazing exclusion than in plots with longer exclusion periods. Furthermore, no change in total soil P across the restoration chronosequence was observed. In

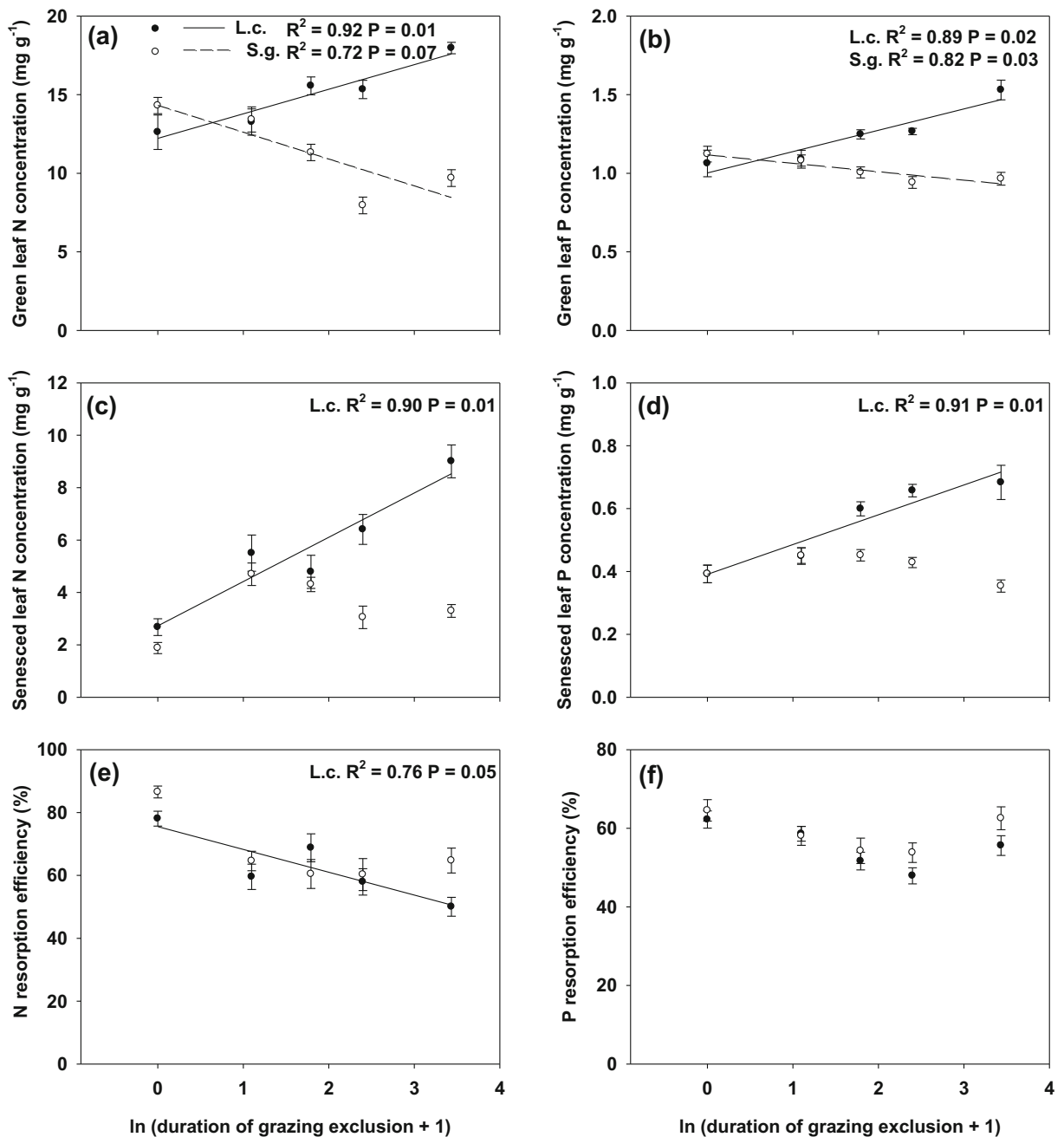


Fig. 3 Changes in N and P concentrations in green (a, b) and senesced (c, d) leaves and in nutrient resorption efficiency (e, f) of *Leymus chinensis* and *Stipa grandis* across a 30-years grazing

exclusion chronosequence in a temperate steppe of northern China. Data are shown as mean \pm SE

opposition to N, which inputs into a system rely mainly on biological processes, P inputs are largely dependent on geochemical processes (Aerts and Chapin 2000). Nevertheless, at shorter time scale, most of soil available P is derived from organic matter recycling (Cross and Schlesinger 1995). The higher total plant biomass and

the greater relative contribution of *L. chinensis* (with higher foliar P concentrations than *S. grandis*) as observed in restored plots indicated an increase of P uptake and retention in plant biomass, and could therefore explain the lower soil extractable P found in plots with longer history of grazing exclusion.

Nutrient use strategies of *L. chinensis* and *S. grandis* across grazing exclusion chronosequence

The positive correlation between community biomass and soil inorganic N indicated that grasslands in this region would be mainly N-limited. Evidence for increasing trends of inorganic N and decreasing trends of extractable P in soil along the restoration gradient suggests that the recovering plant community might progressively become less N limited and instead co-limited by N and P (Soudzilovskaia et al. 2005). However, both leaf N and P concentrations of *L. chinensis* increased along the restoration gradient and its leaf N:P ratio remained constant, indicating higher access of *L. chinensis* to both nutrients and no change in its relative access to N and P. The enhanced P uptake by *L. chinensis* would account for the decreased plant available P in soils across the grazing exclusion chronosequence. Consistent with the growth rate hypothesis (Elser et al. 2000), higher P uptake led to more rapid growth of *L. chinensis*. Further, both leaf N and P concentrations of *S. grandis* decreased along the restoration gradient and its leaf N:P ratio also tended to decrease, suggesting lower access of *S. grandis* to both nutrients and a more strongly limited access to N relative to P. Different plant species preferentially take up different forms of N (McKane et al. 2002) and the same is also likely for P (Turner 2008). This decoupling between soil inorganic nutrients and the leaf N, P and N:P ratios of the two grass species can therefore stem from potential changes in the forms of soil extractable N and P along the gradient as soil organic matter becomes progressively dominated by plant litter rather than by animal faeces (He et al. 2009; Li et al. 2009). Alternatively, these species-specific responses may stem from an increase in plant competition intensity along the restoration gradient (Gao et al. 2009). The decrease in leaf N and P concentrations of *S. grandis* across the chronosequence might therefore stem from its decreasing ability to compete with *L. chinensis* for soil resources as the ecosystem becomes more fertile.

Results from nutrient resorption efficiency and proficiency of *L. chinensis* and *S. grandis* leaves also support the idea that differences in competitive ability between the two species are responsible for the observed differences in leaf nutrition. The plasticity of nutrient resorption in *L. chinensis* was much higher than that in *S. grandis*. Consistent with previous studies, N resorption efficiency and proficiency of *L. chinensis* decreased

along the restoration chronosequence as soil inorganic N became more abundant (van Heerwaarden et al. 2003b; Richardson et al. 2005; Norris and Reich 2009). Also, P resorption proficiency of *L. chinensis* decreased along the restoration chronosequence, which was in opposite direction of the soil extractable P, suggesting higher access of *L. chinensis* to soil P. In contrast to the pattern observed with *L. chinensis*, nutrient resorption of *S. grandis* did not show any significant changes along the gradient, suggesting relatively stable access of *S. grandis* to N and P despite the soil nutrient availability gradients. These results support therefore the idea that plant species with distinct resource economics can have contrasting responses of nutrient resorption to the same changes in environmental conditions.

At the ecosystem scale, these findings indicate that *L. chinensis* would depend more on plant internal nutrient cycling in grazed ecosystems and more on soil nutrient resources in the restored grasslands while the opposite is true for *S. grandis*. Together with the previous findings by Chen et al. (2005) that *L. chinensis* can achieve higher water use efficiency at the expense of lower N-use efficiency compared to *S. grandis*, which can maintain relatively higher water and N-use efficiency at the same time, our results further explain why *L. chinensis* usually dominates in relatively wet and fertile conditions whereas *S. grandis* has a competitive advantage in dryer and nutrient-poor conditions, as observed along the grazing exclusion chronosequence (see also He et al. 2011). Furthermore, these results suggest a positive feedback between soil nutrient availability and plant nutrient contents, as mediated by increased plant biomass (Piñeiro et al. 2009) and plant litter returned to the soil, as well as increased N and P content of these litters through changes in plant species composition and within-species nutrient content (Freschet et al. 2013). Together, positive quantitative and qualitative changes in plant litter with time since grazing exclusion, especially for the positive changes associated to *L. chinensis*, can accelerate N and P cycling between the above- and below-ground components of ecosystems (Hobbie 1992; Lü et al. 2012a; Freschet et al. 2013) with beneficial effects for degraded grassland restoration in northern China.

Conclusion

Overall, our results demonstrate that species with different nutrient use strategies can have opposite responses

across gradients of grazing exclusion. Distinct responses of plant species to gradients in soil nutrient availability (Lawrence 2003), soil water content (Cornwell and Ackerly 2009) and temperature (Albert et al. 2010) have been previously described for several plant traits. We demonstrate here that such between-species differences also occur regarding nutrient resorption processes. The magnitude of within species plasticity in resorption efficiency, with for instance an average shift from 77 to 50 % in N resorption efficiency of *L. chinensis* along the chronosequence, is even larger than these commonly observed across species of contrasting functional types (Aerts 1996; Kobe et al. 2005; Freschet et al. 2010b) and can therefore have potentially important consequences for plant-soil feedbacks and ecosystem functioning (Hobbie 1992; Lü et al. 2012a; Freschet et al. 2013). The species-specific responses suggest potentially complex responses of plant communities to changing environmental conditions (Kichenin et al. 2013; Kumordzi et al. 2014) potentially leading to large uncertainty in projections of plant assemblages under changing climate and land-use (Thuiller et al. 2008).

Acknowledgements We thank Wu Wei-Jun, Li Qiang, and Li Li for assistance with field and laboratory work. We are grateful to two anonymous reviewers for their constructive comments on this manuscript. This study was funded by National Natural Science Foundation of China (31470505), the National Basic Research Program of China (2015CB150802), and the State Key Laboratory of Vegetation and Environmental Change (Grant No. LVEC-2012kf08).

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