Plant Soil
DOI 10.1007/s11104-017-3404-9

REGULAR ARTICLE

Recovery succession drives the convergence, and grazing versus fencing drives the divergence of plant and soil N/P stoichiometry in a semiarid steppe of Inner Mongolia

Zhaoping Yang · Taogetao Baoyin · Hugjiltu Minggagud · Haipeng Sun · Frank Yonghong Li

Received: 11 May 2017 / Accepted: 28 August 2017

Abstract
Background and aims Understanding the plant and soil elemental stoichiometry during grassland dynamics is important for developing measures to enhance the restoration of degraded grassland. A number of restoration practices have been applied in the degraded typical steppe grassland in Inner Mongolia, either for research purpose or as actual restoration projects. However, the effects of different restoration measures on soil and plant N/P stoichiometry remain unclear.
Methods Here we explored the effects of three restoration measures (i.e., natural recovery, NR; shallow ploughing, SP; and harrowing HA) on the N, P stoichiometry of plant and soil in a typical steppe of Inner Mongolia, by comparing plant and soil N, P content and N/P ratio among the grasslands restored through NA, SP and HA, and that under sustained animal grazing (GR).
Results Long-term restoration increased aboveground plant biomass, litter accumulation and changed soil and plant N/P ratio. Soil N and P contents in restored grassland (NR, SP or HA) were higher than those under grazing (GR); the restored grasslands shared a common slope of N-P linear regression, which was significantly greater than that of grazing grassland. Plant N content and N/P ratio decreased firstly and then increased during the restoration of degraded grassland.
Conclusion Soil N limitation is greater than soil P limitation in typical steppe of Inner Mongolia. Soil N limitation is smaller in naturally recovered grassland and grazing grassland than in the restored grassland following shallow ploughing and harrowing. The restoration succession over 26-years after the exclusion of animal grazing have changed the N-P coupling relation in grassland soil, with a common N-P relation converged under grassland that are treated with different restoration measures.

Keywords Ecological restoration measures · Grazing · Ecological stoichiometry · Community succession · Semiarid steppe · Inner Mongolia

Responsible Editor: Alfonso Escudero.

Zhaoping Yang and Taogetao Baoyin contributed equally to this work.

Z. Yang
Jiangsu Collaborative Innovation Center of Atmospheric Environment and Equipment Technology, School of Environmental Science and Engineering, Nanjing University of Information Science & Technology, Nanjing 210044, China

Z. Yang · T. Baoyin · H. Minggagud · H. Sun · F. Y. Li (✉)
Key Laboratory of Grassland Ecology, School of Ecology and Environment, Inner Mongolia University, Hohhot 010021, China
e-mail: lifyhong@126.com

Introduction

The Eurasian Steppe is the largest contiguous grassland region in the world, extending from northeastern China, through Mongolia and the former Soviet Union, to Hungary in the west (Li 1979). The Inner Mongolia grassland is a key component of the Eurasian Steppe, and rich in species diversity and ecosystem types (Wu and Loucks 1992; Bai et al. 2008). The Xilingol grassland, located in the central part of Inner Mongolia, is well known for its extensive distribution, representativeness of the Eurasian steppe vegetation, and long history of land use by nomadic herders. The grassland dominated by *Leymus chinensis* is one of the most widely distributed grassland types in the eastern part of Eurasian Steppe region, covering Northern and Northeastern China, Mongolia and Southern Siberia of Russia, and is a dominant zonal vegetation type in Xilingol region (Li 1978; Xiao et al. 1995). *L. chinensis* is a drought-enduring and saline-alkaline resisting rhizomatous native perennial plant with high palatability and nutrient values (Li 1978; Wang et al. 2003). The health of the *L. chinensis* grassland is of great importance for both the pastoral agriculture and environment sustainability in the region and beyond (Li et al. 1988; Jia et al. 2006).

Grazing has a long history and is still the main land use regime in Inner Mongolia grassland (Li et al. 2008a; Yu et al. 2004). Light or moderate grazing intensity is generally beneficial to maintain biodiversity and plant production of grassland ecosystems (Schönbach et al. 2011). However, overgrazing has been shown to destroy the structure and composition of plant communities and decrease plant production (Han et al. 2008; Schönbach et al. 2011), soil C and nutrient contents (Han et al. 2008; Steffens et al. 2008), leading to the degradation of *L. chinensis* grassland. It is reported that 90% of the *L. chinensis* grassland areas in Inner Mongolia has been degraded during the past several decades due to the long-term overgrazing and improper management (Li et al. 2008b; Baoyin and Li 2009), which greatly threatens the ecological security and social-economic sustainability in the region. The need for restoring the degraded grasslands has thus been stressed frequently. Grazing exclusion and agricultural techniques such as harrowing, reseeding and fertilization are among the most practical options for restoring degraded *L. chinensis* grassland in Inner Mongolia (He et al. 2009; Baoyin and Li 2009; Qian et al. 2014; Baoyin et al. 2015). These measures enhance the restoration processes of degraded grassland by improving soil physicochemical properties and nutrient cycling, facilitating vegetative propagation of rhizomatous grass and enhancing nutrient availability (Baoyin and Li 2009; Baoyin and Liu 2003; Baer et al. 2002). The restoration processes of the *L. chinensis* grassland communities, in terms of species composition, density, diversity, and productivity are well described (Wang et al. 2000; Baoyin and Liu 2003; Baoyin et al. 2011). However, most of the long-term studies on the succession of the degraded *L. chinensis* grassland focuses primarily on the community structure and diversity (Baoyin and Li 2009; Baoyin and Wang 2006; Baoyin et al. 2015; Li et al. 2008b), with less attention paid to the nutrient processes. A successful ecosystem restoration practice must be successful in the restoration of both ecosystem structure and ecological processes that sustain its function (Reay and Norton 1999; Hobbs and Norton 1996). The restoration of ecosystem structure only does not necessarily indicate a successful ecosystem restoration (Reay and Norton 1999; Wilkins et al. 2003). More attention need to be paid to the nutrient processes in the restoration dynamics of the degraded *L. chinensis* grassland.

Ecological stoichiometry, the study of the balance of multiple elements in ecological interactions and processes, suggests that it is not simply a limiting concentration of elements but the relationship between them that determines many important ecological processes (Elser et al. 2000; Wang and Moore 2014). Concurrently, as organisms in terrestrial ecosystems are frequently limited by the availability of nitrogen (N) and phosphorus (P) (Güsewell 2004), N and P are the core elements in the ecological stoichiometry studies. By investigating N and P contents and N/P ratios of organisms and substrates, ecological stoichiometry can not only help determine the nutrition status of an individual organism and communities but also advance our understanding of nutrient cycling and biological processes in the ecosystem (Sterner and Elser 2002; Heuck et al. 2015). Ecological restoration measures can alter the soil N and P contents (Jiao et al. 2013), and consequently affect plant N and P contents and their stoichiometric characteristics. Many restoration projects have been carried out in Inner Mongolia to study the mechanisms underlying the restoration succession of the degraded typical steppe grasslands (mostly on the *L. chinensis* grassland), and to distinguish effective measures for restoring these degraded grasslands in Inner Mongolia. However, little is known on the response of plant and soil N/P stoichiometric characteristics to different restoration measures in these grassland, which limits our capability to select the best restoration measures.

In the present study, the *L. chinensis* grassland restored from a previously degraded grazing land using different restoration measures are studied in comparison with the grassland in a severe deterioration status under heavy grazing over years in Xilingol region of Inner

Mongolia. We investigate how the soil and plant N, P and N/P stoichiometry respond to different restoration measures used in the ecological restoration projects. We expect this research will enrich our understanding of the nutrient cycling mechanisms underlying the restoration of the degraded grassland, and provide more insights for the development of restoration measures.

Materials and methods

Study sites

Our study was conducted in a typical steppe region (43°26′–44°08′ N, 116°04′–117°05′E) located in Xilingol League of Inner Mongolia. Long-term (1980–2008) mean annual temperature in the study area is 0.3 ± 0.1 °C, with mean monthly temperature ranging from −21.6 °C (January) to 19 °C (July). The mean annual precipitation is 345 ± 86 mm, about 80% of which occurs in the plant growing season from May to September. The soil is chestnut according to Chinese classification, which is equivalent to Calcic-orthic Aridisol in the United States Soil Taxonomy classification system (Yuan et al. 2005). The content of calcium carbonate accounts for 0.81% and 1.29% of total soil in 0–20 cm and 20–40 cm soil layers (Wang and Cai 1988).

The experimental grassland with fairly uniform vegetation was fenced in 1983 as a permanent monitoring and demonstration site of the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) of the Chinese Academy of Sciences. Before enclosure, the plant community at this site was a degraded formation of the *L. chinensis* grassland (Wang et al. 1996). Three restoration measures were included in this study: shallow ploughing (SP), harrowing (HA) and natural recovery (NR). A 650 × 400 m exclosure area with flat terrain and good drainage was divided into five sections, among which three adjacent plots were designed for restoration by shallow ploughing (400 × 75 m), harrowing (400 × 75 m), and natural recovery (400 × 150 m) (Baoyin and Li 2009). Restoration measures were applied in July 1983. Shallow ploughing depth was set to 18–20 cm, and harrowing depth to 8–10 cm. Distance between harrow blades was 10 cm. The three restoration plots were contiguously distributed on the same basalt platform, appeared topographically and floristically similar and experienced the same duration of grazing exclusion. Therefore, the difference in restoration measure was identified as the only important factor across the plots. The grassland outside the exclosure has been open for public grazing (GR). A grazing area about 10–30 m distant from the long side of the restoration exclosure and opposite of the inside restored grassland was also studied for comparison purposes. The stocking rate on the open grazing area was unknown, but the average stocking rate of the farm where the experimental grassland was located was 0.7 sheep units.ha^{-1} in 1983, which increased to about 1 sheep unit in 1990 and then varied around that level for the rest of the study period (Baoyin and Li 2009).

Field sampling and measurements

Aboveground biomass was sampled at the time of peak biomass (mid-August 2009) by clipping all plants above the soil surface using five 1 m × 1 m quadrats. The quadrat was randomly placed at least 10 m inside the border of each plot to avoid edge effects. The distance between two quadrats in each plot was over 20 m. Individuals of all vascular plant species in quadrats were identified to determine plant density before clip. Litters were collected from each quadrat after clip. All living vascular plants were sorted to species, oven-dried at 65 °C for 48 h to a constant weight, and then weighed. Nutrient analyses were completed on the nine dominant plant species in the system: one perennial rhizome grass, *Leymus chinensis*, three perennial bunchgrasses, *Stipa grandis*, *Agropyron cristatum* and *Cleistogenes squarrosa*, two shrubs and semi-shrubs, *Artemisia frigida* and *Kochia prostrata*, two perennial forbs, *Potentilla tanacetifolia* and *Carex korshinskyi*, and one annual forb, *Artemisia sieversiana*. Together, these species accounted for over 90% of the total aboveground biomass in all plots. Soil samples were taken from 0 to 10 cm, 10–20 cm, 20–30 cm, and 30–40 cm depth in each of those quadrats following plant sampling. For each quadrat, three soil cores from each of the four soil depths were collected using a 5-cm-diameter soil auger and mixed into one composite sample. All soil samples were sieved through a 2 mm mesh size to remove roots.

Samples of plant and soil were ground and homogenized with a mill (MM400, Retsch, Germany) and then digested using Kjeldahl digestion for determining total soil N and plant N concentration by a semi-auto analyzer (Kjeltec 2300 Analyzer Unit, Foss Tecator, Sweden). Total P concentration was measured by persulfate oxidation followed by colorimetric analysis (Schade et al. 2003). N and P concentrations were expressed on a mass basis. Mass ratios of N/P are used here to facilitate comparisons with previous studies (Güsewell 2004; Sardans et al. 2012).

Data calculation and statistical analysis

To calculate the community-level nutrient concentrations for each quadrat, the sum of total nutrient contents of the sampled species in each quadrat were divided by the sum of their total aboveground biomass (Han et al. 2014). Consequently, nutrient concentration at community-level is defined as the biomass-weighted mean concentration of all sampled species in the quadrat. Community N and P pools were calculated for each plot, based on the biomass and element concentration of each species presented in the plot (Ren et al. 2015).

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 and plant nutrient. We used least-significant-difference (LSD) tests for multiple comparisons when the variances were homogeneous; otherwise, we used Tamhane's T2 test. Linear regression was employed to investigate the relationships between soil N and P concentration. In all analyses, the differences and correlations were considered statistically significant when $P < 0.05$. The differences in slopes and intercept of linear regression between soil N and P concentration were estimated using the SMATR (Standardized Major Axis Tests & Routines) library (version 1) (Falsterb et al. 2006; Warton et al. 2006). All other data analyses were conducted using SPSS (v18.0) software (SPSS Inc., Chicago, IL, USA).

Results

Aboveground biomass, litter accumulation and community density

Restoration measures significantly improved the aboveground biomass ($P < 0.05$), litter accumulation ($P < 0.05$) and community density ($P < 0.05$) of the degraded *L. chinensis* grassland, and they had a consistent rank order, i.e., NR > HA > SP > GR (Fig. 1). There was no significant difference in aboveground biomass between SP and HA ($P > 0.05$), and their aboveground biomass was significantly lower than NR ($P < 0.05$). Litter accumulation was significantly lower in SP than in HA and NR ($P < 0.05$), but no difference existed between HA and NR ($P > 0.05$). The community density was significantly different among all treatments ($P < 0.05$).

Soil N, P and N/P

Soil N content ($F_{3,16} > 12.23$, $P < 0.001$) and P content ($F_{3,16} > 12.34$, $P < 0.001$) showed a decreasing trend with the depth (Fig. 2). For the same restoration measure, among different soil layers below 20 cm, significant difference in soil P content only existed between 20 - 30 cm and 30-40 cm layers of SP ($P < 0.05$). N and P contents were lower under GR plot in all soil layers than in the restoration plots (i.e. SP, HA, NR); and were generally lower in HA and SP than in NR plots. Soil N/P ratio is lower in GR plot than in restoration plots in top soil layer only. Soil N/P in 0-10 cm layer of GR plot was significantly lower than in restoration plots ($P < 0.05$). However, there was no significant difference in other soil layers among all treatments ($P > 0.05$).

Soil N and P contents were significantly correlated in both the restoration plots (i.e. SP, HA, and NR) ($P < 0.001$) and GR plot ($P = 0.015$) (Fig. 3). Further slope homogeneity test showed that there was no significant difference in the slope of N-P stoichiometric relation among the three restoration plots ($t = 1.541$, $p = 0.456$), with a common slope of 7.62 ($P < 0.001$), though the intercept of the N-P linear relation of SP and HA plots was significantly greater than that of NR plot ($P < 0.05$).

Plant N, P and N/P at species level

Restoration measures had significant effects on plant N, P contents and N/P ratios (Table 1). Plant nutrient contents in NR plot were significantly higher than in HA and SP plots except for *Agropyron cristatum* that showed no significant difference in N content between NR and SP plots. *Cleistogenes squarrosa* had higher N content in HA than SP plots. Nevertheless, there was no significant difference in the N content of other species between HA and SP plots. Plant N content in GR plot was significantly higher than in SP and HA plots. Across common species, no significant difference in the N content was observed between GR and NR plots.

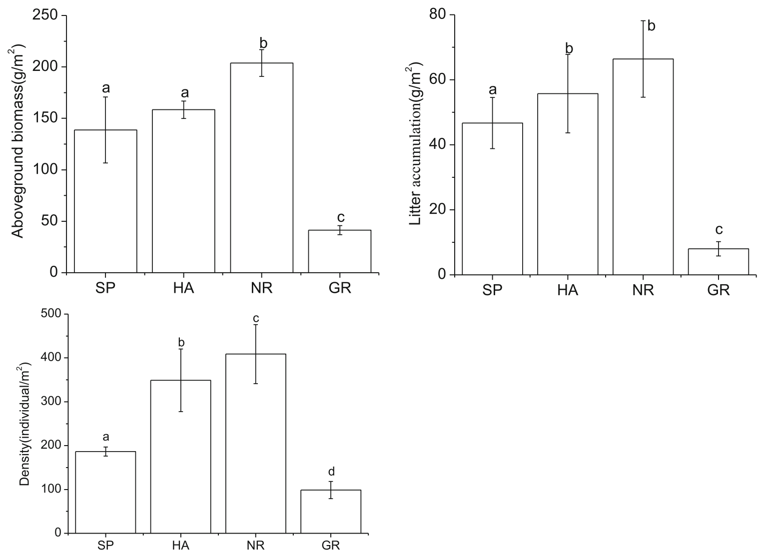

Fig. 1 The aboveground standing biomass, litter accumulation and plant density of *Leymus chinensis* communities restored using different measures. SP, shallow ploughing; HA, harrowing; NR, natural recovery; GR, grazing. Different lowercase letters denote significant differences at a significance level of $P < 0.05$

The P contents of *Stipa grandis* and *Cleistogenes squarrosa* in HA plot were significantly lower than those in the SP plot, while the P contents of other common species were not significantly different between HA and SP plots. Plant P contents in NR plot were either significantly higher than (e.g. *Leymus chinensis*, *Agropyron cristatum*, *Artemisia sieversiana*, and *Potentilla bifurca*), or no significant difference from (e.g. *Stipa grandis*, *Carex korshinskyi*, and *Bassia prostrata*) those in HA or SP plots. The P content of *Agropyron cristatum* was significantly lower in GR than NR plots. The P content of *Cleistogenes squarrosa* was significantly lower in GR than HA and SP plots.

There was no significant difference in the N/P ratio of *Agropyron cristatum* among the NR, HA, and SP plots, and the N/P ratio was significantly lower in GR than in the three restoration plots. For *Stipa grandis*, N/P was significantly higher in GR than in HA and SP plots, and in NR than SP lots; but no significant difference observed between GR and NR plots. The N/P of *Cleistogenes squarrosa* in HA plot was significantly higher than in SP plot, and in HA and SP plots were significantly lower than in GR plot. The N/P of *Leymus chinensis*, *Artemisia frigida*, *Artemisia sieversiana*, *Carex korshinskyi*, *Bassia prostrate*, and *Potentilla tanacetifolia* has the same change pattern among the

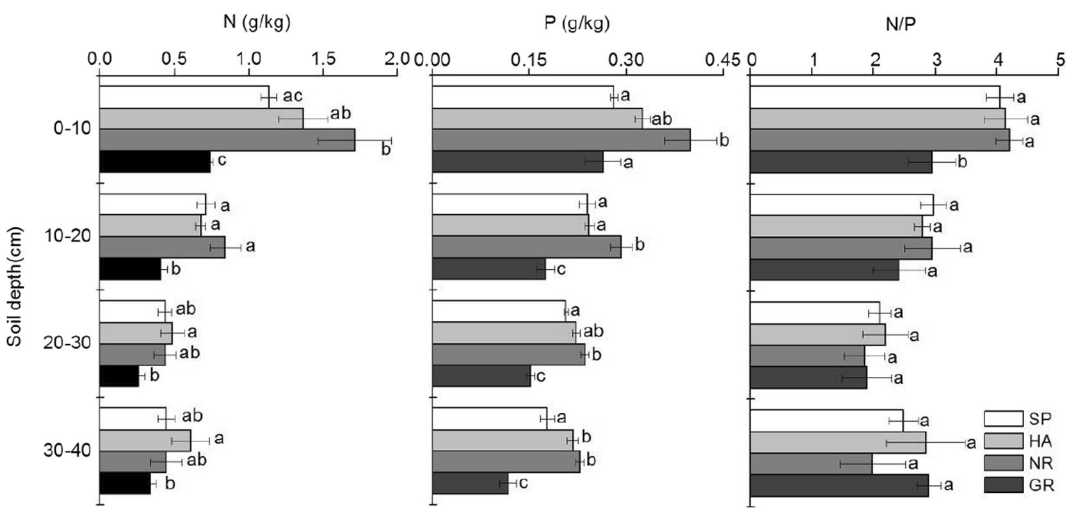

Fig. 2 Vertical distribution of soil N and P contents in the grasslands restored by different measures. The values are mean ± SE (n = 5). SP, shallow ploughing; HA, harrowing; NR, natural recovery; GR, grazing. Different lowercase letters denote significant differences in soil depths at a significance level of $P < 0.05$

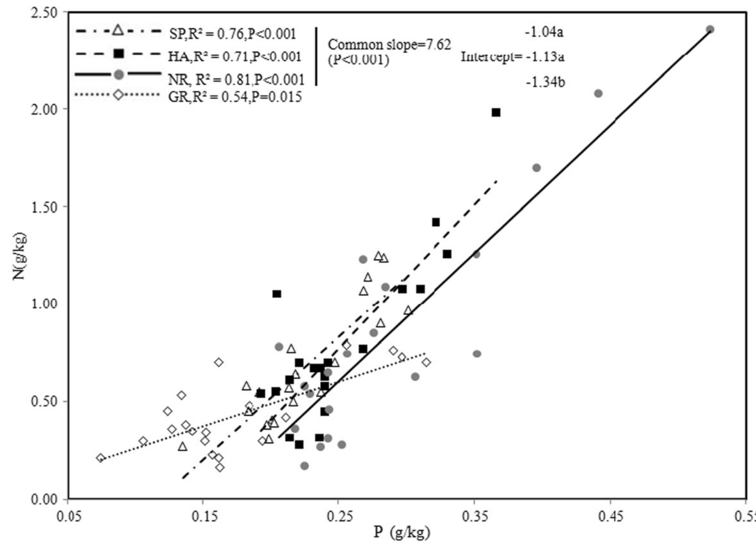

Fig. 3 Relationships between soil N and P contents for different improvement measures. SP, HA, and NR had a common slope of 7.62. Different lowercase letters denote significant differences ($P < 0.05$) in intercept of soil N, P stoichiometric relationships among SP, HA, and NR (SP, shallow ploughing; HA, harrowing; NR, natural recovery; GR, grazing)

NR, HA, and SP plots, and in line with the change pattern in N content. That is, their N/P was higher in NR plot than HA and SP plots, and no difference between HA and SP plots.

Plant N, P and N/P at community level

There was no significant difference in plant N content at community level between the SP (5.13 g/kg) and HA plots (6.12 g/kg) ($P > 0.05$), but they both were significantly lower than those in NR (13.79 g/kg) and GR plots (12.47 g/kg) ($P < 0.05$) (Fig. 4). Plant P content in the NR plot was significantly higher than in other two restoration plots. Plant N content in SP plot (1.17 g/kg) was significantly higher than in HA plot (1.00 g/kg) ($P < 0.05$). Plant N/P in GR (11.87 g/kg) and NR (10.15 g/kg) plots were significantly higher than those in SP (4.39 g/kg) and HA (6.14 g/kg) plots (Fig. 4). N and P pool of aboveground living organism in NR plot were significantly higher than that in SP, HA and GR plots ($P < 0.05$) (Fig. 5).

Discussions

Soil N and P contents in restoration plots (i.e. SP, HA and NR) were higher than those in GR plot (Fig. 2). The input of soil N is mainly dependent on the return of plant residues, and a small part comes from atmospheric deposition (Galloway et al. 1995; Goulding et al. 1998). In this study, all plots are in the same geographical position and receive the same atmospheric N deposition; therefore the difference in soil N content among the plots is mainly a result of different plant residue return. Grazing can reduce the capability of plant fixing N and P in grasslands (Schuman et al. 1999), which causes the decreased soil N and P contents under long-term grazing. Standing biomass (Fig. 1), N and P pools of aboveground living organism in GR plot are lower than those in restoration plots (Fig. 5), which indicates that the nutrients return in GR plot is significantly less than in the restoration plots. Animal grazing has been excluded in the restored grasslands after the application of different measures, thus experienced favorable conditions for plant growth, biomass accumulation (Foster et al. 2009; Ruthrof et al. 2013) and litter accumulation, which facilitates soil organic matter accumulation, including humus (Andersson et al. 2004). In general, the patterns in N and P contents in plant standing biomass and litters across all plots were consistent with that of soil N and P contents. Vegetation restoration and litter accumulation may reduce nutrients loss due to erosion (Li et al. 2004), which may also contribute to the higher N and P content in restoration plots than in GR plot.

Soil N/P was lower, especially in the 0-10 cm soil layer, in GR plot than in restoration plots (Fig. 2), possibly indicating a N limitation for plant growth in GR plot, and an improvement in soil N supply for plant growth by the restoration measures that excluding animal grazing. The results show that there is a positive correlation between soil N and P ($P < 0.05$, $R^2 > 0.54$)

Table 1 The N, P contents and N/P ratio of the dominant plants in the grasslands under different treatments

		NR	HA	SP	GR
Leymus chinensis	N	14.31 ± 1.66a	5.37 ± 0.84b	5.45 ± 0.83b	—
	P	1.45 ± 0.03a	1.05 ± 0.02b	0.95 ± 0.08b	—
	N/P	9.91 ± 1.29a	5.14 ± 0.82b	5.80 ± 0.94b	—
Stipa grandis	N	12.07 ± 1.45a	6.13 ± 1.44b	4.85 ± 0.60b	12.53 ± 1.16a
	P	1.16 ± 0.05ab	0.80 ± 0.04a	1.22 ± 0.34b	0.99 ± 0.04ab
	N/P	10.40 ± 1.12ab	7.84 ± 1.75bc	4.61 ± 0.99c	12.63 ± 1.16a
Agropyron cristatum	N	9.04 ± 1.46 ac	5.33 ± 0.27b	6.31 ± 1.44ab	11.55 ± 1.55c
	P	1.28 ± 0.02a	0.87 ± 0.06b	1.02 ± 0.13b	0.95 ± 0.04b
	N/P	7.02 ± 1.04a	6.18 ± 0.34a	6.73 ± 1.81a	12.27 ± 1.80b
Cleistogenes squarrosa	N	—	8.47 ± 1.05a	5.09 ± 0.45b	14.49 ± 1.27c
	P	—	1.43 ± 0.06a	1.58 ± 0.33b	1.33 ± 0.06c
	N/P	—	5.93 ± 0.66a	3.50 ± 0.52b	10.89 ± 0.77c
Artemisia frigida	N	—	6.86 ± 1.09a	5.97 ± 0.61a	—
	P	—	1.66 ± 0.08a	1.50 ± 0.17a	—
	N/P	—	4.15 ± 0.70a	4.14 ± 0.74a	—
Artemisia sieversiana	N	15.94 ± 2.05a	—	5.20 ± 0.59b	—
	P	1.96 ± 0.39a	—	1.56 ± 0.05b	—
	N/P	8.50 ± 1.02a	—	3.34 ± 0.41b	—
Carex korshinskyi	N	15.90 ± 1.09a	6.74 ± 1.84b	5.09 ± 0.13b	—
	P	1.21 ± 0.05a	0.84 ± 0.10a	1.11 ± 0.31a	—
	N/P	13.23 ± 0.56a	8.73 ± 2.54b	5.45 ± 1.06b	—
Kochia prostrata	N	16.79 ± 2.70a	8.27 ± 1.61b	5.32 ± 0.68b	—
	P	1.25 ± 0.18a	1.14 ± 0.05a	1.08 ± 0.04a	—
	N/P	13.35 ± 0.19a	7.14 ± 1.19b	4.93 ± 0.65b	—
Potentilla tanacetifolia	N	12.58 ± 2.22a	5.62 ± 0.37b	—	—
	P	1.64 ± 0.21a	0.90 ± 0.05b	—	—
	N/P	7.42 ± 1.84a	6.33 ± 0.58b	—	—

Different lowercase letters denote significant differences ($P < 0.05$) in plant N, P and N/P among shallow ploughing (SP), harrowing (HA), natural recovery (NR) and grazing (GR)

(Fig. 3). However, the regression slope of soil N-P linear relation in GR plot was significantly lower than the common slope of that in the three restoration plots (Fig. 3). These results suggest that ecological restoration in the enclosure have changed soil N-P coupling relation in ecosystem, towards a common N-P coupling relation after 26 years of restoration without animal grazing, even the initial measures for restoration differ. The greater intercept of soil N-P linear relation in SP and HA plots than in NR plot (Fig. 3), signifies a relatively higher N content in HA and SP than in NR plots for the same low P condition, that is, P use efficiency may be higher in SP and HA plots than in NR plots in the initial restoration stage of lower P.

NR plot has the highest aboveground biomass (203.8 g/m^2), significantly higher than that of SP and HA plots ($P < 0.05$) (Fig. 1). NR plot has been considered to restore its native community status after the 13 years of restoration (Wang et al. 1996). The lower population density and litter accumulation in HA and SP plots than in NR plot (Fig. 3) illustrates that HA and SP interventions have slowed the grassland restoration towards a natural community of high function and complexity. Also, the higher soil nutrient content in NR than in SP and HA plots indicates the NR being superior to SP and HA for ecosystem restoration. Although SP, HA and NR plots had a common slope of soil N-P linear relation after long-term restoration (Fig. 3), it still takes a

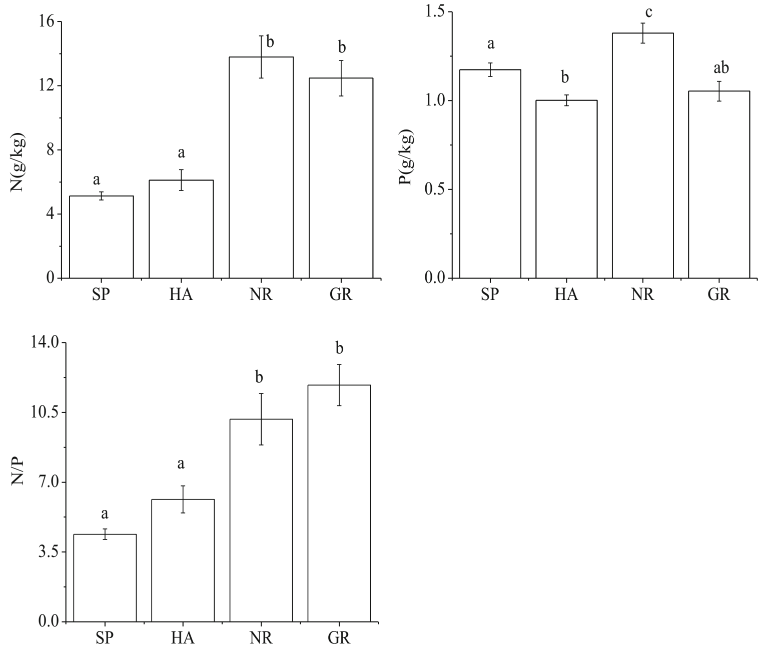

Fig. 4 Plant N and P contents and N/P ratio of the *Leymus chinensis* grassland community under different treatments. Different lowercase letters denote significant differences at $P < 0.05$. SP, shallow ploughing; HA, harrowing; NR, natural recovery; GR, grazing

longer time to reduce the difference in nutrient content between mechanically intevented SP and HA plots and NR plot. Results obtained from community biomass accumulation, community density, and soil nutrient showed that the restoration effect of HA was slightly better than that of SP, and the community of HA plot was closer to the status of native community. This results suggest that a more severe disturbance to the surface soil system (SP > HA) may result in a slower restoration succession of the degraded typical steppe grassland in Inner Mongolia.

N and P are the main nutrient elements limiting plant growth in most terrestrial ecosystem (Aerts and Chapin 1999). N/P ratio of plant tissue is used in many studies as an indicator to judge the nature of nutrient limitation (Koerselman and Meuleman 1996; Verhoeven et al. 1996; Güsewell 2005; Tessier and Raynal 2003). Based on the comprehensive analysis of the fertilization experiment results in a steppe ecosystem of Inner Mongolia, Zhang et al. (2004) concluded that an N/$P < 21$ indicates N limitation, while an N/P ratio > 23 is indicative of P limitation. At N/P ratios between 21 and 23, plant growth is co-limited by N and P together (Zhang et al. 2004). In this study, plant N/P ratios at both species-level and community-level were less than 21 (Table 1, Fig. 4), indicating a N limitation in the studied grasslands. However, we must clearly recognize that the factors influencing plant N/P ratio are complex and comprehensive, and the nature of nutrient limitation for different communities is controlled by a number of factors. Although vegetation N/P stoichiometric characteristics can better reflect the limiting effect of N and P,

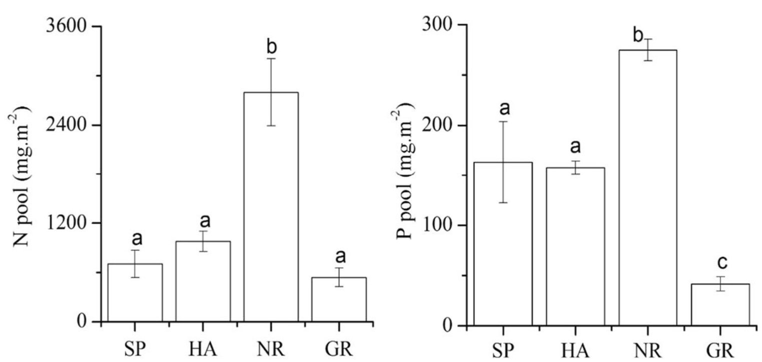

Fig. 5 N and P pool of aboveground living organism, which were calculated as the product of the species biomass (g.m^{-2}) and its nutrient contents (mg.g^{-1}). Different lowercase letters denote significant differences at $P < 0.05$. SP, shallow ploughing; HA, harrowing; NR, natural recovery; GR, grazing

as a numerical variable, it only reflects the relative limitation of N and P and the trend of mutual transformation and is mainly indicative (Güsewell 2004). A lower N/P ratio indicates a N limitation in plant growth, which has been widely accepted in accordance with current research. No significant difference in the plant N/P ratio are detected between SP and HA plots (Table 1), indicating that plant growth in SP and HA plots is similarly N-limited. The higher N/P ratio of plant species in NR plot than in SP and HA plots ($P < 0.05$) (Table 1 except *A. cristatum* and *S. grandis*) indicates a weaker N-limitation in NR than in SP and HA plots.

Nitrogen addition experiment in typical steppe grassland of Inner Mongolia showed that N addition could increase soil N availability and improve plant N content (Lü et al. 2012; Han et al. 2014). In this study, soil N content in restoration plots is higher than in GR plot, especially in 0-20 cm soil layer (major plant root zone) ($P < 0.05$) (Fig. 2). However, the higher soil N content did not result in higher plant N content in restoration plots; instead, plant N content in GR plot is significantly higher than in HA and SP plots ($P < 0.05$), though not higher than in NR plot (Fig. 4). Qi et al. (2016) studied N and P stoichiometric characteristics of plant communities during restoration succession of an abandoned land in forest-steppe zone in hilly area of the Loess Plateau, and showed that plant leaf N content had a decreasing then increasing tend during the restoration. The analysis of Yin et al. (2010) on the stoichiometry of plant nutrients during the restoration succession of a degraded typical grassland in Inner Mongolia indicated that plant N content in overgrazed grassland was higher than in the restored grassland. These results agree to our results that plant N content is higher in grazing (GR) than in restored grasslands. Previous studies have shown that grazing may stimulate the generation of new leaves (Mikola et al. 2009; Ziter and MacDougall 2013) and has a greater effect on increasing plant nutrient content than nutrient addition does (Turner et al. 1993). At the initial stage of restoration succession of severe degraded grassland, the higher plant N content may be attributed to the animal foraging that promotes plant growth and lead to tender plant tissue with relatively higher N content (Ziter and MacDougall 2013). Another reason might be the fast cycling rate of nutrients through grazing and excretion resulting in a higher soil N supply to plant growth (Bussink and Oenema 1998).

Soil N/P ratio is lower in GR plot than restoration plots, while plant N/P ratio is higher in GR than restoration plots. This inconsistence between soil N/P and plant N/P is related with both the balance between plant N or P demand and soil supply. The limitation of an elemental nutrient at any point in nutrient gradient depends not only on the total amount of the element in soil but also on plant community characteristics (Daufresne and Hedin 2005; Miller et al. 2005). Studies on the degraded communities of typical steppe in Inner Mongolia indicates that the relative surplus nutrients in degraded grassland caused by long-term grazing was the driving force for restoration succession after grazing pressure removal (Wang et al. 1996). Community density in GR plot is significantly lower than in restoration plots ($P < 0.05$), and the amount of nutrient required to support the maximum plant growth is also small. Thus, in GR plot, although soil has a great potential N-limitation (low N/P ratio), plant growth is not strongly restricted by soil N as plant growth rate is low.

Early observations on the restoration succession of the studied grassland communities show that plant community in NR plot has greater similarity to the native community than that in HA and SP plots (Wang et al. 1996). Our results based on the changing trends of plant N and N/P in all treatments suggest that plant N and N/P had a trend of decreasing firstly and then increasing during the grassland restoration. The possible mechanisms for this change pattern of plant N and N/P in restoration succession are as follows. At the initial stage of the grassland restoration following the exclusion of animal grazing disturbance, the relative surplus nutrients supply to the grassland plants ensures plant population to colonize quickly (Wang et al. 1996), which lead to an increase in plant species richness and community structure complexity, and an increase in nutrients needs from soils. The increased plant N demand and limited soil N supply lead to the decreased plant N and N/P ratio. Then, soil nutrients supply and cycling rate are increased in the process of vegetation restoration, which lead to an increase in plant N and N/P.

Conclusions

All restoration measures, either natural recovery or mechanical intervention (harrowing or shallow ploughing) after excluding grazing animals from a degraded grassland, have increased aboveground plant biomass and litter accumulation, and increased soil N, P contents, compared with the grassland under sustained animal grazing (GR). Significant correlation existed in soil N and P contents ($P < 0.05$). The restored grasslands share a common N/P ratio (common slope of N-P linear regression), which is significant greater than the N/P ratio of the grazed grasslands. That is, the grassland restored after the application of different measures converged during the long-term restoration in both the plant production and N/P stoichiometry. The results also suggest that the typical steppe grasslands in Inner Mongolia are mostly limited by soil N, and the N limitation in naturally recovered (NR) and grazed (GR) grasslands are smaller than that in the grassland ploughed (SP) or harrowed (HA). Plant N and N/P decreased first and then increased in the restoration of degraded grassland. Long-term in-situ observation on the changes of both soil and plant nutrient contents in the restoration process of grassland communities are warranted for more in-depth understanding of plant stoichiometric pattern during grassland restoration and its implication for grassland management.

Acknowledgements This experimental site has been managed by the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences. The research was funded by China National Basic Research Program (2014CB138805), National Science and Technology Support Program (2015BAC02B04), China Postdoctoral Science Foundation (2016 M601291), Inner Mongolia Natural Science Foundation (2015ZD05, 2017MS0316).

References

Aerts R, Chapin FS (1999) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67. https://doi.org/10.1016/S0065-2504(08)60016-1

Andersson M, Kjøller A, Struwe S (2004) Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biol Biochem 36(10):1527–1537. https://doi.org/10.1016/j.soilbio.2004.07.018

Baer SG, Kitchen DJ, Blair JM, Rice CW (2002) Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecol Appl 12(6):1688–1701. https://doi.org/10.1890/1051-0761(2002)012%5B1688:CIESAF%5D2.0.CO;2

Bai YF, Wu J, Xing Q, Pan QM, Huang JH, Yang DL, Han XG (2008) Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. Ecol 89(8): 2140–2153. https://doi.org/10.1890/07-0992.1

Baoyin T, Li FY (2009) Can shallow plowing and harrowing facilitate restoration of *Leymus chinensis* grassland? Results from a 24-year monitoring program. Rangeland Ecol Manag 62:314–320. https://doi.org/10.2111/08-161.1

Baoyin T, Liu ML (2003) The study on biomass composition dynamics of community in degenerated steppe of Leymus Chinensis after shallow ploughing. J Nat Resour 27(2):270–277 In Chinese

Baoyin T, Wang J (2006) Dynamic of plant diversity of degraded Leymus Chinensis steppe after shallowly Ploughing. J Desert Res 26(2):232–237 In Chinese

Baoyin T, Liu ML, Bao QH, Minggagud H (2011) Effect of N addition on plant community structure and index of grassland quality of typical steppe mown grassland. Acta Prataculturae Sinica 20(1):7–14 In Chinese

Baoyin T, Li FY, Minggagud H, Bao Q, Zhong Y (2015) Mowing succession of species composition is determined by plant growth forms, not photosynthetic pathways in Leymus Chinensis, grassland of Inner Mongolia. Landsc Ecol 30(9): 1795–1803. https://doi.org/10.1007/s10980-015-0249-6

Bussink DW, Oenema O (1998) Ammonia volatilization from dairy farming systems in temperate areas: a review. Nutr Cycl Agroecosys 51(1):19–33. https://doi.org/10.1023/A:1009747109538

Daufresne T, Hedin LO (2005) Plant coexistence depends on ecosystem nutrient cycles: extension of the resource-ratio theory. P Natl Acad Sci USA 102(26):9212–9217. https://doi.org/10.1073/pnas.0406427102

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL (2000) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578–580

Falsterb DS, Warton DI, Wright I J (2006) SMATR: Standardised Major Axis Tests& Routines http://www.bio.mq.edu.au/ecology/SMATR/

Foster BL, Kindscher K, Houseman GR, Murphy CA (2009) Effects of hay management and native species sowing on grassland community structure, biomass, and restoration. Ecol Appl 19(7):1884–1896. https://doi.org/10.1890/08-0849.1

Galloway JN, Schlesinger WH, Levy H, Michaels A, Schnoor JL (1995) Nitrogen fixation: anthropogenic enhancement-environmental response. Global Biogeochem Cy 9(2):235–252. https://doi.org/10.1029/95GB00158

Goulding KWT, Bailey NJ, Bradbury NJ, Bradbury NJ, Hargreaves P, Howe M, Murphy DV, Poulton PR, Willison TW (1998) Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. New Phytol 139(1):49–58. https://doi.org/10.1046/j.1469-8137.1998.00182.x

Güsewell S (2004) N: P ratios in terrestrial plants: variation and functional significance. New Phytol 164(2):243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

Güsewell S (2005) High nitrogen: phosphorus ratios reduce nutrient retention and second-year growth of wetland sedges. New Phytol 166(2):537–550. https://doi.org/10.1111/j.1469-8137.2005.01320.x

Han GD, Hao XY, Zhao ML, Wang MJ, Ellert BH, Willms W, Wang MJ (2008) Effect of grazing intensity on carbon and nitrogen in soil and vegetation in a meadow steppe in Inner Mongolia. Agric Ecosyst Environ 125(1):21–32. https://doi.org/10.1016/j.agee.2007.11.009

Han X, Sistla SA, Zhang YH, Lü XT, Han XG (2014) Hierarchical responses of plant stoichiometry to nitrogen deposition and mowing in a temperate steppe. Plant Soil 382(1–2):175–187. https://doi.org/10.1007/s11104-014-2154-1

He N, Wu L, Wang Y, Han XG (2009) Changes in carbon and nitrogen in soil particle-size fractions along a grassland restoration chronosequence in northern China. Geoderma 150(3–4):302–308. https://doi.org/10.1016/j.geoderma.2009.02.004

Heuck C, Weig A, Spohn M (2015) Soil microbial biomass C: N: P stoichiometry and microbial use of organic phosphorus. Soil Biol Biochem 85:119–129. https://doi.org/10.1016/j.soilbio.2015.02.029

Hobbs RJ, Norton DA (1996) Towards a conceptual framework for restoration ecology. Restora. Ecol 4:93–110. https://doi.org/10.1111/j.1526-100X.1996.tb00112.x

Jia B, Zhou G, Wang Y, Wang F, Wang X (2006) Effects of temperature and soil water-content on soil respiration of grazed and ungrazed *Leymus chinensis* steppes, Inner Mongolia. J Arid Environ 67(1):60–76. https://doi.org/10.1016/j.jaridenv.2006.02.002

Jiao F, Wen ZM, An SS, Yuan Z (2013) Successional changes in soil stoichiometry after land abandonment in loess plateau, China. Ecol Eng 58:249–254. https://doi.org/10.1016/j.ecoleng.2013.06.036

Koerselman W, Meuleman AFM (1996) The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. J Appl Ecol 33:1441–1450. https://doi.org/10.2307/2404783

Li JD (1978) *Aneurolepidium chinense* grassland in China. Journal of Northeast Normal University 1:145–159 In Chinese

Li B (1979) General characteristics of the steppe vegetation in China. China's Grasslands 1(1):2–12 In Chinese

Li B, Yong SP, Li ZH (1988) The vegetation of the Xilin river basin and its utilization. In: Sinica A (ed) Inner Mongolia grassland ecosystem Research Station, research on grassland ecosystem, vol. 3. Science Press, China, pp 84–183 In Chinese

Li FR, Zhao LY, Zhang H, Zhang TH, Shirato Y (2004) Wind erosion and airborne dust deposition in farmland during spring in the Horqin Sandy land of eastern Inner Mongolia, China. Soil Till Res 75(2):121–130. https://doi.org/10.1016/j.still.2003.08.001

Li CL, Hao XY, Zhao ML, Han GD, Willms WD (2008a) Influence of historic sheep grazing on vegetation and soil properties of a desert steppe in Inner Mongolia. Agric Ecosyst Environ 128:109–116. https://doi.org/10.1016/j.agee.2008.05.008

Li Y, Wang W, Liu Z, Jiang S (2008b) Grazing gradient versus restoration succession of *Leymus chinensis* (Trin.) Tzvel. Grassland in Inner Mongolia. Restor Ecol 16(4):572–583. https://doi.org/10.1111/j.1526-100X.2007.00332.x

Lü XT, Kong DL, Pan QM, Simmons ME, Han XG (2012) Nitrogen and water availability interact to affect leaf stoichiometry in a semi-arid grassland. Oecologia 168:301–310. https://doi.org/10.1007/s00442-011-2097-7

Mikola J, Setälä H, Virkajärvi P, Saarijärvi K, Ilmarinen K, Voigt W, Vestberg M (2009) Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. Ecol Monogr 79(2):221–244. https://doi.org/10.1890/08-1846.1

Miller TE, Burns JH, Munguia P, Walters EL, Kneitel JM, Richards PM, Mouquet N, Buckley HL (2005) A critical review of twenty years' use of the resource-ratio theory. Am Nat 165(4):439–448. https://doi.org/10.1086/428681

Qi DH, Wen ZM, Yang SS, Wang HX, Guo R (2016) Concentration of N,P and stoichiometry characteristics of plant communities and soil under different restoration stages at a forest-steppe zone in hilly and gully region of the Loess Plateau, China. Acta Agrestia Sinica 24(2):322–329 In Chinese

Qian J, Wang Z, Liu Z, Busso CA (2014) Belowground bud bank responses to grazing intensity in the inner-mongolia steppe, china. Land Degrad Dev. https://doi.org/10.1002/ldr.2300

Reay SD, Norton DA (1999) Assessing the success of restoration plantings in a temperate New Zealand forest. Restor Ecol 7: 298–308. https://doi.org/10.1046/j.1526-100X.1999.72023.x

Ren HY, Xu ZW, Huang JH, Lü XT, Zeng DH, Yuan ZY, Han XG, Fang YT (2015) Increased precipitation induces a positive plant-soil feedback in a semi-arid grassland. Plant Soil 389(1–2):211–223. https://doi.org/10.1007/s11104-014-2349-5

Ruthrof KX, Fontaine JB, Buizer M, Matusick G, McHenry MP, Hardy GESJ (2013) Linking restoration outcomes with mechanism: the role of site preparation, fertilisation and revegetation timing relative to soil density and water content. Plant Ecol 214(8):987–998. https://doi.org/10.1007/s11258-013-0224-8

Sardans J, Rivas-Ubach A, Peñuelas J (2012) The C: N: P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. Perspect Plant Ecol 14(1):33–47. https://doi.org/10.1016/j.ppees.2011.08.002

Schade JD, Kyle M, Hobbie SE, Fagan WF, Elser JJ (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecol Lett 6(2):96–101. https://doi.org/10.1046/j.1461-0248.2003.00409.x

Schönbach P, Wan HW, Gierus M, Bai YF, Müller K, Lin LJ, Susenbeth A, Taube F (2011) Grassland responses to grazing: effects of grazing intensity and management system in an inner Mongolian steppe ecosystem. Plant Soil 340(1–2):103–115. https://doi.org/10.1007/s11104-010-0366-6

Schuman GE, Reeder JD, Manley JT, Hart RH, Manley WA (1999) Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. Ecol Appl 9(1):65–71. https://doi.org/10.1890/1051-0761(1999)009%5B0065:IOGMOT%5D2.0.CO;2

Steffens M, Kölbl A, Totsche KU, Kögel-Knabner I (2008) Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (PR China). Geoderma 143(1):63–72. https://doi.org/10.1016/j.geoderma.2007.09.004

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton

Tessier JT, Raynal DJ (2003) Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J Appl Ecol 40(3):523–534. https://doi.org/10.1046/j.1365-2664.2003.00820.x

Turner CL, Seastedt TR, Dyer MI (1993) Maximization of above-ground grassland production: the role of defoliation frequency, intensity, and history. Ecol Appl 3(1):175–186. https://doi.org/10.2307/1941800

Verhoeven JTA, Koerselman W, Meuleman AFM (1996) Nitrogen-or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. Trends Ecol Evol 11(12):494–497. https://doi.org/10.1016/S0169-5347(96)10055-0

Wang JW, Cai YC (1988) Studies on genesis, types and characteristics of the soils of the Xilin river basin. In: Jiang S (ed) Research on grassland ecosystems 3. Science Press, Beijing In Chinese

Wang M, Moore TR (2014) Carbon, nitrogen, phosphorus, and potassium stoichiometry in an ombrotrophic peatland reflects plant functional type. Ecosystems 17:673–684. https://doi.org/10.1007/s10021-014-9752-x

Wang W, Liu Z, Hao D, Liang CZ (1996) Research on the restoring succession of the degenerated grassland in Inner Mongolia. I. Basic characteristics and driving force of the restoration. Acta Phytoecologica Sinica 20:449–459 In Chinese

Wang W, Liang CZ, Liu ZL, Hao DY (2000) Mechanism of degradation succession in *Leymus Chinensis*+*Stipa Grandis* steppe community. Acta Phytoecologica Sinica 24(4):468–472 In Chinese

Wang RZ, Gao Q, Chen QS (2003) Effects of climatic change on biomass and biomass allocation in Leymus Chinensis (Poaceae) along the north-east China transect (NECT)[J]. J Arid Environ 54(4):653–665. https://doi.org/10.1006/jare.2002.1087

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biol Rev 81(2):259–291. https://doi.org/10.1017/S1464793106007007

Wilkins S, Keith DA, Adam P (2003) Measuring success: evaluating the restoration of a grassy eucalypt woodland on the Cumberland plain, Sydney, Australia. Restor Ecol 11(4):489–503. https://doi.org/10.1046/j.1526-100X.2003.rec0244.x

Wu J, Loucks OL (1992) Xilingele (the Xilingol grassland). In: The US National Research Council (ed) grasslands and grassland sciences in northern China. National Academy Press, Washington, DC, pp 67–84

Xiao XM, Wang YF, Jiang S, Ojima DS, Bonham CD (1995) Interannual variation in the climate and above-ground biomass of *Leymus chinense* steppe and *Stipa grandis* steppe in the Xilin river basin, Inner Mongolia, China. J Arid Environ 31(3):283–299. https://doi.org/10.1016/S0140-1963(05)80033-3

Yin XR, Liang CZ, Wang LX, Wang W, Liu ZL (2010) Ecological stoichiometry of plant nutrients at different restoration succession stages in typical steppe of Inner Mongolia, China. J Plant Ecol 34(1):39–47 In Chinese

Yu M, Ellis JE, Epstein HE (2004) Regional analysis of climate, primary production, and livestock density in Inner Mongolia. J Environ Qual 33:1675–1681. https://doi.org/10.2134/jeq2004.1675

Yuan ZY, Li LH, Han XG, Huang JH, Jiang GM, Wan SQ (2005) Soil characteristics and nitrogen resorption in *Stipa krylovii* native to northern China. Plant Soil 273:257–268. https://doi.org/10.1007/s11104-004-7941-7

Zhang LX, Bai YF, Han XG (2004) Differential responses of N:P stoichiometry of *Leymus chinensis* and *Carex korshinskyi* to N additions in a steppe ecosystem in Nei Mongol. Acta Bot Sin 46(3):259–270

Ziter C, MacDougall AS (2013) Nutrients and defoliation increase soil carbon inputs in grassland. Ecology 94(1):106–116. https://doi.org/10.1890/11-2070.1