Nitrogen enrichment and grazing accelerate vegetation restoration in degraded grassland patches

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ARTICLE INFO

Article history:
Received 6 April 2014
Accepted 28 November 2014
Available online 9 December 2014

Keywords:
Degradation
Grazing
Nitrogen deposition
Restoration
Species richness

ABSTRACT

A rapid increase in grazing intensity since the 1980s has caused large areas of the Inner Mongolian grasslands to become degraded. Increasing atmospheric nitrogen (N) deposition might exert an important influence on vegetation restoration in these degraded grasslands by increasing available N and relieving N limitations on productivity. However, no previous studies have tested the assumption that increasing N deposition promotes vegetation restoration in degraded grasslands. By conducting a 4-year field restoration experiment with four N addition treatments (0, 5, 10, and 20 g N m\(^{-2}\) year\(^{-1}\)) and two grazing treatments (grazed and ungrazed), we investigated the effects of N enrichment and grazing on the restoration of patches in which vegetation had been degraded. N addition significantly accelerated the vegetation restoration of degraded grassland patches regarding both plant cover and diversity. Moderate grazing also promoted the restoration of degraded-vegetation patches in term of both plant diversity and species similarity. Importantly, the positive effects of N addition on the restoration of degraded patches may be augmented by grazing. This study demonstrate that low levels of N enrichment (or increasing atmospheric N deposition) positively impact vegetation restoration in degraded grassland patches, particularly under moderate grazing practices. Our findings provide new insights into the management of severely degraded grasslands through the regulation of N inputs and grazing practices.

1. Introduction

In recent decades, the Inner Mongolian grasslands have been subjected to large-scale degradation as a result of harmful human activities (e.g., heavy grazing and land reclamation) and climate change (e.g., warming climate and severe drought) (Han et al., 2008; Hu et al., 2012). The initial stage of grassland degradation is generally characterized by the severe vegetation degradation, then followed by soil degradation. How to restore these newly degraded grassland patches has become an important issue for grassland managers and government policymakers. Many factors may affect the restoration of degraded grasslands, such as grazing intensity (Wiesmeier et al., 2012; Zhao et al., 2004), available soil nutrition (Holst et al., 2009; Zhang et al., 2012), seed dispersal (Bullock et al., 2003; Czarnecka, 2005; Dyer, 2002; Wurm, 1998), and precipitation (Li et al., 2011). Scientists have previously analyzed the restorative effects of various techniques on degraded grasslands, including fencing, controlled grazing (Dormaar and Willms, 1998; Krummelbein et al., 2009; Wan et al., 2011) and nitrogen (N) and water addition (Brueck et al., 2010; Fanselow et al., 2011; Lu and Han, 2010; Ruan et al., 2012). However, few studies have investigated the effects of N enrichment (or increasing atmospheric N deposition) and grazing on the restoration of degraded grassland patches.

Atmospheric N deposition in terrestrial ecosystems is currently 2–10 times higher than that during the preindustrial period due to the increased consumption of fossil fuels and fertilization of agricultural lands (Galloway et al., 2008). N deposition is currently estimated as 2–3 g m\(^{-2}\) year\(^{-1}\) in the Inner Mongolian grasslands (Jia et al., 2014). Moreover, N deposition is expected to increase at a similar rate in the 21st century in Asia and South America (Galloway et al., 2008; Liu et al., 2013). Lu and Han (2010) have demonstrated that N enrichment enhances plant growth by overcoming the limitation of available N, particularly in the Inner Mongolian grasslands, and the soil available N is generally deficient in the region (Holst et al., 2009; Zhang et al., 2012). Therefore, we assumed that (1) increasing N deposition positively promotes the
restoration of severely degraded grassland patches by enhancing N availability, (2) moderate grazing accelerates the restoration of degraded grassland patches by facilitating seed dispersal and colonization (Papanastasis, 2009).

Here, we established a field restoration experiment containing pairs of plots (degraded plots vs. natural vegetation plots) with four levels of N addition and two grazing management conditions (grazed and ungrazed) in the Inner Mongolian grasslands. The vegetation was removed from one set of plots to simulate the severely vegetation destruction that occurs in grassland patches during the initial stage of grassland degradation. We then annually investigated the plant community composition in each experimental plot from 2009 to 2012. This study aimed to explore whether (1) N enrichment and grazing accelerate vegetation restoration in severely degraded grassland patches and (2) the effect of N enrichment on grassland restoration is regulated by grazing regimes.

2. Materials and methods

2.1. Study sites

The experimental plots (43°26' N, 116°04' E) were located in the Xilin River Basin in the Inner Mongolia Autonomous Region of China. The experimental plots were administered by the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), Chinese Academy of Sciences. This region is part of the continental semiarid grasslands of the Central Asian steppe ecosystem and is located at an elevation of approximately 1200 m above sea level. The area has a dry and cold middle-latitude climate, with cold, dry winters and warm, moist summers. The mean annual temperature (1980–2009) is 1.1 °C, with a maximum temperature of 19 °C in July and a minimum temperature of approximately −21 °C in January. The mean annual precipitation is approximately 345 mm (He et al., 2008), and the precipitation during the experimental period (2009–2012) is shown in Fig. 1. The soil type is classified as a calcic Chernozems, and the soil texture is 63.23% sand, 32.54% silt and 4.23% clay (He et al., 2009). The dominant plant species are Stipa grandis, Leymus chinensis, Cleistogenes squarrosa, and Agropyron michnoi gaertn.

2.2. Experimental design

The same experimental treatments were established in both grazed and ungrazed grasslands (i.e., fenced grasslands) in mid-September, 2008 (Fig. 2). The grazed grasslands had subjected to long-term free grazing by sheep and cattle, which were slightly degraded in terms of the aboveground community and plant diversity. The ungrazed grasslands were established in 1999 by IMGERS by fencing off a section of previously grazed grassland for long-term monitoring and experimental studies. Theoretically, the grazed and ungrazed plots possess the same vegetation and soil type, and their artificial division into two blocks was only conducted to facilitate field monitoring and scientific experimentation. Therefore, the changes in vegetation and soil properties between the two plots were assumed to result from grazing, because the plots were floristically and topographically similar, and distributed adjacent to one another (Fig. 2).

First, six blocks and 24 experimental plots (8 m × 8 m) were established in both the grazed and ungrazed grasslands, respectively. Four levels of N (urea) addition regimes were selected (0, 5, 10, and 20 g N m⁻² yr⁻¹), and N addition plots were randomly chosen in each block (Fig. 2). The fertilizer was thoroughly mixed with sand and then applied in late May of each year from 2009 to 2012.

In each plot, we first set two 1-m diameter circular quadrats at 4 m apart. In the vegetation removal patches (RV), the original

![Fig. 1. Seasonal precipitation from 2009 to 2012 in the study area.](image1)

![Fig. 2. Experimental design of the field plots and experimental treatments.](image2)

CK, 0 g N m⁻² yr⁻¹; N1, 5 g N m⁻² yr⁻¹; N2, 10 g N m⁻² yr⁻¹; N3, 20 g N m⁻² yr⁻¹. The vegetation removal treatment (RV) and original vegetation treatment (OV) were a pair of fixed quadrats used to investigate the changes in plant community composition from 2009 to 2012.
vegetation was removed manually and the roots in the 0–15 cm soil layer were removed by sieving (pore diameter, 2 mm), thereby simulating degraded grassland. The vegetation and soil were retained in the original patches (OV). Therefore, a pair of fixed quadrats was established in each plot (RV vs. OV) and used to investigate the changes in plant community composition between the plot types (Fig. 2).

2.3. Field investigation

Field investigations of plant community composition were conducted annually at the end of August from 2009 to 2012. In brief, all living vascular plants in the RV and OV quadrats were investigated, and vegetation cover, species composition, and the height and density of each species were recorded. Plant cover was determined by visual assessment, while height was measured using a tape. We did not clip the above-ground plants to measure biomass because this practice causes severe disturbance to vegetation restoration.

2.4. Calculations and statistical analyses

Vegetation cover, species richness, and species diversity in the RV patches were used to characterize the direct effects of restoration. We made a census of the number of species in the RV and OV quadrats, which were assumed to represent species richness(S). The Shannon-Wiener diversity index was used to assess the changes in species diversity:

\[ H' = -\sum_{i=1}^{a} P_i \times \ln P_i \]  

where \( S \) denotes the number of species in the RV or OV patches (or species richness), and \( P_i \) represent the relative proportion of the individuals of each species to the total number of individuals of all species.

Through comparing the species composition in the RV and OV patches, it was possible to quantify the degree of restoration in the two grassland condition. Sorenson’s similarity index was used to characterize the degree of restoration:

\[ C_S = 2 \times \frac{j}{(a+b)} \]  

where \( a \) and \( b \) represent the number of species in the RV and OV patches, respectively, and \( j \) represents the number of common species to both grassland types.

One-way ANOVA was performed to test for difference in species diversity, species richness, vegetation cover, and similarity caused by different levels of N addition and grazing regimes in each year. Repeated-measure ANOVA was used to explore the general and interactive effects of the different levels of N addition, grazing regimes and time from 2010 to 2012 and the data from 2009 were used as covariant for covariance analysis. Differences were considered to be significant when \( p < 0.05 \). All analyses were conducted using SPSS statistical software (v. 17.0, SPSS, Chicago, IL, USA).

3. Results

3.1. Changes in vegetation cover, species richness, and species diversity

N addition significantly promoted the restoration of vegetation cover in the RV patches (\( F = 13.744, p < 0.001 \) (Table 1). Grazing produced a significantly reduction in vegetation cover (\( F = 62.993, p < 0.001 \). N addition and grazing together produced a significant interaction effect (\( F = 29.604, p < 0.001 \) (Table 1). Under ungrazed conditions, moderate levels of N addition (5 and 10 g N m\(^{-2}\) year\(^{-1}\)) significantly enhanced vegetation cover; however, high N addition (20 g N m\(^{-2}\) year\(^{-1}\)) depressed vegetation cover (Fig. 3). Under grazed conditions, vegetation cover was enhanced as N addition increased, with maximum cover reached at 20 g N m\(^{-2}\) year\(^{-1}\). Additionally, restoration time significantly affected vegetation cover, which increased in each year (\( F = 6.754, p = 0.002 \) (Table 1).

N addition had no significant effect on plant species richness (\( F = 1.496, p = 0.231 \), and the free-grazing effect was also not significant (\( F = 2.354, p = 0.133 \). However, a significant interaction between N addition and grazing was observed (\( F = 5.091, p = 0.005 \) (Table 1). Richness varied irregularly among years in the ungrazed plots but increased in the grazed condition for all four levels of N addition (Fig. 4).

N addition had a significant effect on species diversity (\( F = 2.958, p = 0.044 \), with moderate N addition levels (5 and 10 g N m\(^{-2}\) year\(^{-1}\)) producing the greatest enhancement (Fig. 5, Table 1).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Cover (%)</th>
<th>Species richness</th>
<th>Shannon–Wiener diversity</th>
</tr>
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<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>N</td>
<td>13.744</td>
<td>0.001</td>
<td>1.496</td>
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<tr>
<td>G</td>
<td>62.993</td>
<td>0.001</td>
<td>2.354</td>
</tr>
<tr>
<td>T</td>
<td>6.754</td>
<td>0.002</td>
<td>3.655</td>
</tr>
<tr>
<td>N x G</td>
<td>29.604</td>
<td>0.001</td>
<td>5.091</td>
</tr>
<tr>
<td>N x T</td>
<td>5.219</td>
<td>0.001</td>
<td>7.942</td>
</tr>
<tr>
<td>G x T</td>
<td>2.814</td>
<td>0.069</td>
<td>60.826</td>
</tr>
<tr>
<td>N x G x T</td>
<td>9.677</td>
<td>&lt;0.001</td>
<td>7.977</td>
</tr>
</tbody>
</table>

N: nitrogen addition; G: free-grazing; T: time.
Table 1. Grazing significantly increased species diversity (F = 109.365, p < 0.001): the species diversity of RV plots was generally higher in grazed plots (from 1.28 to 1.63) than in ungrazed plots (from 0.91 to 1.36) in 2011 and 2012. A significant interaction was observed between grazing and restoration time (F = 34.894, p < 0.001), and this parameter it increased annually from 2009 to 2012 irrespective of N addition levels in grazed plots. In contrast, plant diversity showed irregular variation over time in ungrazed plots (Table 2).

3.2. Effect of vegetation removal

The results showed that N addition significantly decreased the degree of restoration (F = 8.422, p < 0.001), as shown by the level of species similarity in the RV and OV quadrats. Grazing had a highly significant positive effect on the degree of restoration (F = 50.694, p < 0.001). Irrespective of N addition levels, the similarity among the grazed plots was significantly higher than among the ungrazed plots (Fig. 6); however, grazing and addition no apparent interaction effect.

4. Discussion

N addition accelerated the restoration of degraded grassland patches. The findings support our hypothesis that increasing atmospheric N deposition could promote the restoration of degraded grasslands in Inner Mongolia by enhancing N availability or relieving N limitations. As proposed by Zhang et al. (2012), precipitation is also an important limiting factor for plant productivity in Inner Mongolia. Undoubtedly, changes in annual precipitation exert an important influence on vegetation restoration in terms of productivity and cover (Bai et al., 2008; Harpole et al., 2007); however, we assumed the effects of precipitation changes on vegetation restoration to be equal across all plots in the present study because of their adjacent locations. Under N enrichment, the N competition between soil microbes and vegetation should be relieved, resulting in higher water use efficiency and greater aboveground net primary production (Gao et al., 2011; Han et al., 2012; Li et al., 2011). Thus, N addition would promote plant growth and enhance vegetation cover in degraded grassland patches.
Short-term N addition significantly increased plant diversity but had no apparent effect on species richness in the restoration of degraded grassland patches. Several plausible mechanisms may explain these observations. First, by favoring the initial establishment of fast-growing plant species (Bai et al., 2010; Stevens et al., 2004), N addition can apparently promote the biodiversity in degraded grassland patches. Furthermore, the encouragement of fast-growing species by N addition caused significant decreases in species similarity in the RV and OV quadrats. The moderate vegetation cover in newly restored grassland patches may have positive effects on seedling recruitment and establishment. Conversely, previous studies found that, in natural grasslands, N addition resulted in a widespread biodiversity loss in semiarid Inner Mongolian grasslands and in California grassland (Lan and Bai, 2012; Pan et al., 2011; Zavaleta et al., 2003; Zhen et al., 2014). In grasslands with relatively higher productivity, the increased primary production and greater aboveground biomass after N addition decrease species richness and diversity due to the increased intensity of aboveground competition and light limitation, which can result in the loss of poor aboveground competitors and ground-level species that require high light levels (Lan and Bai, 2012). Moreover, the negative effects of N enrichment on species richness and diversity may largely be due to the inhibition of forbs seedling establishment and conditions that favor the dominance of grasses over forbs (Zavaleta et al., 2003; Zhen et al., 2014). In summary, the practice of N addition has inconsistent effects on grassland plant diversity, with positive impacts in the initial phase of grassland restoration and negative impacts in the phases of higher diversity and productivity.

Moderate grazing promotes the restoration of degraded grassland patches although grazing activity by animals reduces aboveground biomass and vegetation cover. Grazing promotes seed dispersal and increases seed sources (Nathan et al., 2008). For example, grazing livestock, such as sheep, can incidentally carry seeds in their wool from other habitats and spread these seeds to degraded patches. In addition, the excretions of grazers also contain seeds, leading to the formation of nutritionally rich patches that promote seed germination (Ma et al., 2007). Moreover, seed germination and establishment are enhanced by the trampling effect of grazing livestock, which leads to the shallow burial of seeds and facilitate germination under dry Inner Mongolian condition. Animal trampling and grazing also force the roots to fracture, thus hastening the tillering (Zhao et al., 2009).

In grazed plots, plant seeds are carried into degraded patches by livestock from nearby natural grasslands, thus increasing the species similarity and biodiversity. Additionally, the feeding preferences of grazing animals both directly and indirectly alter interspecific plant competition, further influencing both species composition and community structure (Dreber et al., 2011; Noymeir et al., 1989; Wan et al., 2011). It should be noted that grazing intensity is a crucial factor regulating the restoration of degraded grassland patches. Heavy grazing clearly decreases above- and below-ground biomass, vegetation cover, species diversity and richness, and soil nutrient contents (He et al., 2008; Wiesmeier et al., 2012; Zhao et al., 2009), which could inhibit the process of grassland restoration. In summary, the effects of grazing on the restoration of degraded grassland patches are complex and require further study.

Interestingly, grazing activity may augment the effects of N addition on the restoration of degraded grassland patches. Our results showed that N promotes the restoration of degraded grassland patches, particularly in grazed grasslands. In ungrazed grasslands, higher N addition levels depressed the plant cover. Extreme N application rates usually cause the direct effects of N accumulation and indirect effects of soil mediated acidification on plant growth (Bobbink et al., 1998; Cleland and Harpole, 2010); the latter impact is unlikely to occur on the calcic chernozem of the present study. High soil N concentration and low annual precipitation can inhibit the growth of some plant species, thereby reducing productivity and diversity. However, in the present study, the plant cover was higher at higher levels of N addition in grazed grasslands. Zhao et al. (2008) have demonstrated that the combination of N addition and grazing promotes revegetation, and triggers the compensatory growth of plants by accelerating the N cycle and eliminating N accumulation. N addition significantly affects the restoration process of degraded grassland patches and might be augmented by moderate grazing, and the combination of these methods should be considered as a new approach for grassland restoration (He et al., 2011).

5. Conclusions

N enrichment positively impacts the restoration of degraded grassland patches, and the positive effect of N might be augmented by moderate grazing intensity. Importantly, increasing atmospheric N deposition, an important source of available N, will benefit the restoration of severely degraded grasslands in Inner Mongolia. The effects of N addition on the maintenance of biodiversity in degraded grassland must be reconsidered. N addition have inconsistent effects on plant diversity in grasslands: addition had a positive effect for grasslands in the initial phase of restoration and a negative effect for grasslands in a phases of higher diversity and productivity. Our study is the first to demonstrated that N input (including increasing atmospheric N deposition) and grazing may accelerate the restoration of severely degraded grasslands.

Acknowledgments

This work was funded by the Natural Science Foundation of China (31270519, 31470506), the National Key Technology R&D Program (2012BAC01B08), and the Program for “Kezhen” Distinguished Talents in Institute of Geographic Sciences and Natural Resources Research, CAS(2013RC102).

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