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Effects of nitrogen addition on plant biomass and tissue elemental content in different degradation stages of temperate steppe in northern China

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Abstract

Aims

Grassland degradation has become a common problem worldwide. Several studies have analyzed the effects of nitrogen (N) addition on plant growth in grasslands, but few have considered its effects on plant growth in degraded grasslands. The aim of this study was to evaluate the effects of N addition on plant growth in grasslands with different levels of degradation in Inner Mongolia, China.

Methods

A 2-year field experiment was conducted to evaluate plant growth response to N addition in degraded grasslands on the Inner Mongolian Steppe. Grasslands with four levels of degradation were selected for N-addition treatments (0, 10, 20, 30, 40, 50 g N m^{-2} $year^{-1}$).

Important Findings

Aboveground biomass was increased by N fertilization in degraded grasslands, and N addition was significantly related to increased biomass in grasslands with severe degradation. However, N fertilization did not significantly affect belowground biomass. The effects of N addition on foliar nutrient concentrations in the same species differed among grasslands with different degradation levels. There was an inconsistent response to N addition between *Sanguisorba officinalis* and *Vicia sepium* in non-degraded grassland. There was a significant positive correlation between foliar N content and aboveground biomass in grasslands with different levels of degradation. Our results indicate that the effects of N addition on plant growth in grasslands differ according to the severity of degradation. We conclude that N fertilization may be an effective management technique for degraded grasslands in this area and may improve forage productivity in the short term.

Keywords: nitrogen addition, degraded grassland, aboveground biomass, belowground biomass, foliar nitrogen concentrations, root nitrogen concentrations

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INTRODUCTION

Nitrogen (N) deposition strongly affects terrestrial ecosystem productivity and biomass accumulation, thereby affecting biogeochemical cycles such as ecosystem carbon (C) sequestration (Le Quere *et al.* 2009; Thomas *et al.* 2012). Atmospheric N deposition has increased as result of fossil fuel combustion and anthropogenic N fertilization over the past few decades (Aber *et al.* 2003; Galloway *et al.* 2004; Galloway *et al.* 2008). As extra N enters grassland ecosystems, increased N availability will affect important C-cycling processes, such as ecosystem productivity (Li *et al.* 2011) and the concentrations of various nutrient elements in plant tissues (Li *et al.* 2015). Thus, the studies on the effects of N addition on the productivity of grassland ecosystems and the concentration of nutrient elements in plant tissues will allow us to better understand and predict the role of terrestrial ecosystems in global climate change (Asner *et al.* 2004; Scurlock and Hall 1998).

Grassland is second-largest green vegetation ecosystem next to forests in terrestrial ecosystems (White *et al.* 2000). It is the most widespread vegetation type in China, occupying ~40% of total land area (Kang *et al.* 2007). About 78% of Chinese grasslands are located in northern temperate arid and semi-arid areas (Chen and Wang 2000). Severe climatic conditions together with anthropogenic activities have caused desertification or degradation in most of these areas, and have led to N-deficient conditions in these grasslands (Cao *et al.* 2004; Hooper and Johnson 1999; Zhang and Han 2008). At present, 61.49% of the northern grasslands in China are degraded to some degree (Zhou *et al.* 2014) and N fertilization is used as an effective management technique to enhance their productivity (Contant *et al.* 2001). However, little is known about the responses of grassland biomass to increased N availability along a degradation gradient.

Several previous studies have shown that N addition to grasslands increases plant growth and grassland biomass (Bai *et al.* 2010; Chen *et al.* 2011; Gong *et al.* 2011). With increasing amounts of added N, the aboveground biomass may initially increase and then decrease (Fang *et al.* 2012; Li *et al.* 2015). The initial increase in aboveground biomass may be because the added N alleviates ecosystem N limitation and improves the soil nutrient status (Ladwig *et al.* 2012; Zhang *et al.* 2004). An increase in the availability of soil N leads to a higher foliar N content (Iversen *et al.* 2010), enhanced photosynthesis (Pons and Anten 2004) and ultimately increased plant growth (Ellsworth *et al.* 2004). However, as the amount of applied N reaches a certain threshold, the limiting factor may become soil moisture (He *et al.* 2013) or light (Knops and Reinhart 2000), or phosphorus (Yang *et al.* 2016), rather than N. Also, plant biomass may be reduced because N fertilization can lead to acidification of soils (Yao *et al.* 2014) and inhibition of plant root growth (Liu *et al.* 2013). Consequently, aboveground biomass may decrease when the amount of added N exceeds a certain threshold. Several studies have focused on the effects of N addition on grassland biomass, but few have investigated the effects of N addition to degraded grasslands, or compared biomass responses among grasslands with different levels of degradation.

Previous studies have shown that N addition significantly affects the concentration of elemental nutrients in plant foliage and roots (Iversen *et al.* 2010; Li *et al.* 2015; Magill *et al.* 1997; Pastor and Bridgham 1999). For instance, Ren *et al.* (2011) studied the effects of N addition on five typical grassland plants (*Agropyron cristatum*, *Cleistogenes squarrosa*, *Melilotoides ruthenica*, *Potentilla tanacetifolia* and *Stipa krylovii*), and found that foliar N concentrations increased by 22.3% while the C:N ratio decreased by 20.7% under N fertilization. Li *et al.* (2015) reported that N addition resulted in a significant increase in plant root N concentrations in a temperate grassland.

Several studies have suggested that N fertilization might enhance foliar N concentrations and stimulate photosynthetic activity, ultimately leading to increased grassland biomass (Ren *et al.* 2011). Different species have shown different responses to N addition in various studies. For example, Wei *et al.* (2014) found that N fertilization resulted in increased plant tissue N concentrations in *Leymus chinensis*, but decreased N concentrations in *Caragana microphylia*, *Carex korshinskii*, *C. squarrosa*, *Potentilla bifurca* and *Stipa grandis*. Furthermore, the responses of the same species to N addition may differ with the severity of degradation. However, few studies have compared the responses of the same species to N addition among grasslands with different levels of degradation.

Grassland degradation is severe worldwide (Dickie and Parsons 2012; Pereira *et al.* 2013; Mukhopadhyay and Maiti 2014) and N deposition is expected to increase in 21st century (Galloway *et al.* 2008; Liu *et al.* 2013). Thus, the response to N addition likely differ due to interactions with the degree of grassland degradation. Here, we conducted a controlled experiment on the Ulan Buton Steppe at the southeastern edge of the Inner Mongolian Plateau, China. We selected four grasslands with different degrees of degradation, and applied N at six different levels. We then analyzed the responses of grassland biomass and plant elemental composition to N addition. The aims of this study were as follows: (1) to determine whether the effects of N addition on grassland biomass differ depending on the level of degradation; and (2) to assess how N addition affects the contents of certain elemental nutrient in plants in grasslands with different levels of degradation.

MATERIALS AND METHODS

Site description

The study was conducted on the Ulan Buton Steppe of the Inner Mongolian Plateau, China. The mean annual temperature and precipitation were −1.4°C and 400 mm, respectively. Sand and silt dominate the surface soil layer of the Chernozem soil. Four 100 m × 100 m experimental fields were fenced on level land in 2011. These fields were separated by no more than 10 km, allowing the different fields to share similar environmental conditions (i.e. temperature and precipitation). The fields were selected because they had the original vegetation types.

Among all vegetation and soil features, plant species composition and community structure are good indicators of the degree of grassland degradation. Liu *et al.* (2008) found that the herb species in grasslands in this region could be categorized into three groups: annuals indicative of seriously degraded grassland, species indicative of moderate degradation and climax species in mature grassland on the Steppe. Xu *et al.* (2015) showed that the degradation level of grasslands could be estimated by determining the relative cover of climax species. Thus, we quantified the degree of grassland degradation as described by Xu *et al.* (2015). Specifically, extremely degraded grassland (EDG) had the highest proportion of annuals among the four fields. Non-degraded grassland (NDG) had the highest proportion of climax species, and there were high proportions of species indicative of moderate degradation in the other two fields. The relative cover (ranging from 0 to 1) of climax species was 0.34 in EDG, 0.40 in severely degraded grassland (SDG), 0.54 in moderately degraded grassland (MDG) and 0.74 in NDG.

The plant species composition in each of grasslands is shown in supplementary Table S1. There was local grazing in EDG, resulting in low species richness. The SDG was a high-quality pasture 20 years ago, but it become degraded as a result of overgrazing until 2011. The MDG had managed grazing and a relatively low biomass. The NDG had been fenced to prevent since 2000 and had high species richness.

Experimental design

We divided each of the fields into three blocks separated by a 2-m buffer zone. In each block, we selected 12 plots of 6 × 6 m separated by a 1-m buffer zone for the different treatments. The N addition treatment began in May 2011 using urea as the fertilizer. The six amounts of added N were as follows: 0 (CK, control check), 10, 20, 30, 40 and 50 g N m^{-2} $year^{-1}$. We applied N four times during the first 10 days of May, June, July and August using a quarter of the annual amount each time.

Sample collection and measurements

Soil samples (0-10 cm depth) were collected from each replicate plot in mid-August 2012 using a 5.8-cm diameter soil corer (72 samples in total). Roots, litter and small stones were removed by hand and the soil was then sieved through a 2-mm mesh sieve. The samples were separated into several sub-samples for analysis of soil total carbon (STC), soil total nitrogen (STN) and plant C and N. The STC and STN were measured by an element analyzer (Vario EL III, Elementar, Hanau, Germany).

Aboveground and root biomass were sampled in mid-August. Aboveground biomass was collected by clipping plant within a 50 × 50 cm sampling frame, and samples from each replicate plot were then dried and weighed. Root biomass was collected from a soil depth of 30 cm using a 5.8-cm diameter soil corer with three repetitions. The roots were separated from the soil by washing, dried at 60°C for 48 h, and then weighed.

Fresh foliage samples were randomly collected from dominant species with three replicates in each field in mid-August. The plant species were *S. officinalis* and *V. sepium* in NDG, *Carex rigescens* and *L. chinensis* in MDG, *C. rigescens*, *C. squarrosa* and *L. chinensis* in SDG and *L. chinensis* in EDG. Samples were dried at 60°C to constant mass. All roots and fresh foliage samples were ground and subjected to chemical analyses. The C and N concentrations of all samples were measured using an elemental analyzer (Vario EL III).

Statistical analysis

All statistical analyses were performed using SPSS statistical software ver. 17.0 (SPSS Inc., Chicago, IL, USA). Repeated-measures ANOVA was used to analyze the effects of different amount of added N on aboveground biomass in each field over time. Specifically, year was a within-subjects effects and degradation level and N addition level were between-subjects effects. The main effects and their interactions were tested. Belowground biomass and root nutrient element content data were analyzed with two-way ANOVA with degradation level and amount of added N as the factors. For foliar nutrient element content data, one-way ANOVA was performed to test the response of species among N-treatments at each degradation level. Significant differences among treatment means were detected using Tukey's multiple comparison test (Haynes 2013). Standard major axis (SMA) regression analysis were performed to determine the relationship between grassland biomass and foliar N concentrations. Significant effects were determined at $P < 0.05$ unless otherwise stated.

RESULTS

Effects of N addition on above- and belowground biomass in degraded grasslands

The aboveground biomass in NDG, MDG, SDG and EDG ranged from 391.1 to 819.9, 237.0 to 462.1, 212.0 to 634.4 and 233.6 to 671.8 g m^{-2}, respectively, in our 2-year experiment (Fig. 1). The amount of aboveground biomass were significantly affected by N addition ($P < 0.001$; Table 1). The size of difference in aboveground biomass between non-degraded grassland and degraded grassland was decreased by N enrichment (Fig. 1A–D and E–H). The aboveground biomasses in MDG, SDG and EDG supplemented with 40 g N m^{-2} $year^{-1}$ were higher than that in NDG in 2013 (Fig. 1E–H). With N enrichment at 50 g N m^{-2} $year^{-1}$, the aboveground biomass in EDG was significantly higher than that in NDG in 2013 ($P = 0.014$; Fig. 1E and H). The aboveground biomass increased with increasing amount of N addition to degraded grasslands. In NDG, the aboveground biomass tended to initially increased and then decrease with increasing amounts of added N. Conversely, belowground biomass was not significantly affected by N addition or by the degree of grassland degradation ($P > 0.05$; Table 2). However, there was a strong reduction in belowground biomass in NDG as the amount of added N increased (Fig. 2). The effects of N addition were stronger on aboveground biomass than belowground biomass (Fig. 3).

Effects of N addition on elemental nutrient contents in plant tissues

The addition of N significantly affected foliar N concentrations by N addition in grasslands with different levels of degradation (Fig. 4). After N addition, the N concentration in fresh foliage either increased or remained unchanged. Specifically, for *S. officinalis* in NDG, *C. squarrosa* in SDG, *C. rigescens* in

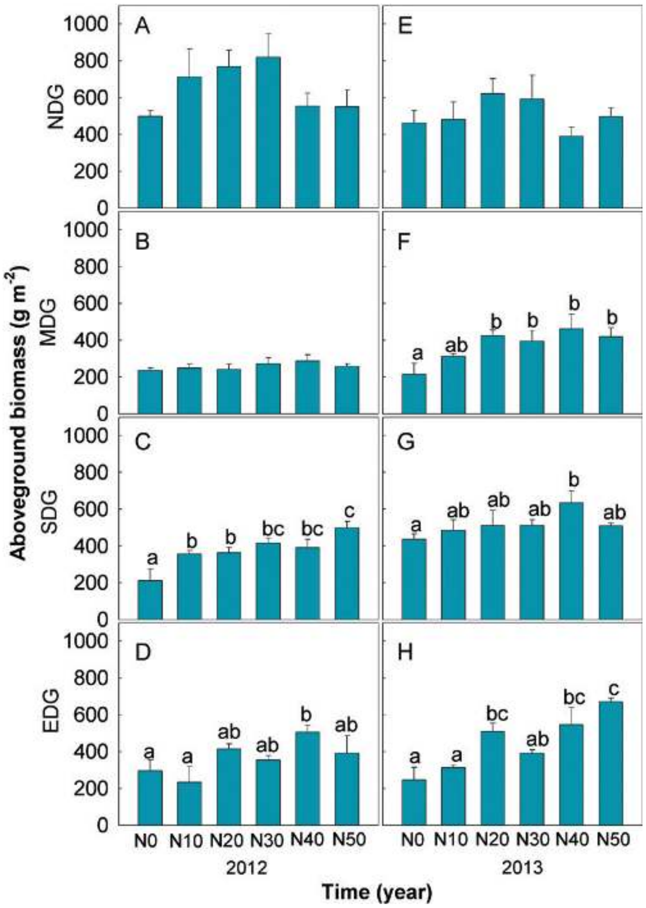

Figure 1: effects of nitrogen (N) addition on aboveground biomass from 2012 to 2013 in different degraded grassland. Significant differences of aboveground biomass among N treatments are indicated by lower case letters. Error bars represent standard error of the means. N0: 0 g N m^{-2} $year^{-1}$, N10: 10 g N m^{-2} $year^{-1}$, N20: 20 g N m^{-2} $year^{-1}$, N30: 30 g N m^{-2} $year^{-1}$, N40: 40 g N m^{-2} $year^{-1}$, N50: 50 g N m^{-2} $year^{-1}$. EDG, extremely degraded grassland; MDG, moderately degraded grassland; NDG, non-degraded grassland; SDG, severely degraded grassland.

Table 1: results of repeated-measures ANOVA with year (Y), nitrogen addition (N) and degradation levels (D) on aboveground biomass (AGB, g m^{-2}) from 2012 to 2013

		AGB	
Source	df	*F*	*P*
Y	1	8.647	**0.005**
N	5	6.963	**<0.001**
D	3	31.603	**<0.001**
Y × N	5	0.890	0.495
Y × D	3	15.492	**<0.001**
N × D	15	2.387	**0.012**
Y × N × D	15	1.379	0.196

Significant effects are shown in bold font ($P < 0.05$).

MDG and SDG and *L. chinensis* in MDG, SDG and EDG, and foliar N concentrations significantly increased with increasing amounts of added N (Fig. 4A and C–H). However, N addition did not significantly affect foliar N concentrations in *V. sepium* in NDG (Fig. 4B).

There was no significant interaction between N addition and the level of grassland degradation. The root C concentration was significantly affected by the degree of grassland degradation, but not by N addition. The root N concentrations were significantly affected by N addition, but not by the degree of grassland degradation (Table 2).

Foliar N and aboveground biomass were significantly positively correlated (Table 3). The SMA regressions showed that aboveground biomass increased with increasing foliar N concentrations. There was a significant linear correlation between aboveground biomass and foliar N concentration in grassland with more severe degradation stages (i.e. SDG and EDG) (Fig. 5E–G).

DISCUSSION

Responses of plant biomass in grasslands with different levels of degradation to N addition

The addition of N increased aboveground biomass by as much as 424 g m^{-2}, much higher than the average value reported in previous studies (150 g m^{-2}) (Bai *et al.* 2004). Our results emphasize that N addition can significantly increase aboveground biomass in this area, as reported in other studies (Bai *et al.* 2010; Gong *et al.* 2011; Li *et al.* 2011). The results showed that the effects of N addition on aboveground biomass differed among grasslands with different levels of degradation. Without N fertilization, the aboveground biomass was significantly higher in NDG than degraded grasslands (Fig. 1A–D). However, as the amount of added N increased, the differences among NDG, MDG, SDG and EDG became smaller. In fact, with N addition at 50 g N m^{-2} $year^{-1}$, aboveground biomass in EDG was significantly higher than that in NDG (Fig. 1E and H).

Previous studies in our study area have shown that in an N-limited ecosystem, degradation increase the severity of N deficiency (Xu *et al.* 2015). Thus, increasing N availability might enhance foliar N concentrations and chlorophyll content and promote photosynthesis leading to rapid plant growth (Pons and Anten 2004; Ren *et al.* 2011). However, among all the degraded grasslands in this study, NDG showed the strongest plant growth in response to low levels of N addition. With additional N, plant growth in NDG peaked at 30 and 20 g N m^{-2} $year^{-1}$ in 2012 and 2013, respectively (Fig. 1A and E); higher rates of N addition inhibited plant growth. Other studies have also reported plant biomass shows an N-saturation phenomenon (Bai *et al.* 2010; Emmett 2007). Bai *et al.* (2010) determined that the N saturation point was 10.5 g N m^{-2} $year^{-1}$ in an area in Inner Mongolia. When additional N is surplus to requirements, plants cannot use the extra N and it negatively affects plant growth.

Table 2: results of two-way ANOVA for the effects of nitrogen addition (N) and degradation levels (D) on belowground biomass (BGB, g m^{-2}), root C concentrations (%) and root N concentrations (%)

		BGB		Root N concentrations		Root C concentrations	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*
N	5	0.76	0.583	3.1	**0.016**	0.4	0.845
D	3	1.5	0.230	1.2	0.332	3.1	**0.037**
N × D	15	1.5	0.136	0.7	0.776	1.0	0.453

Significant effects are shown in bold font ($P < 0.05$).

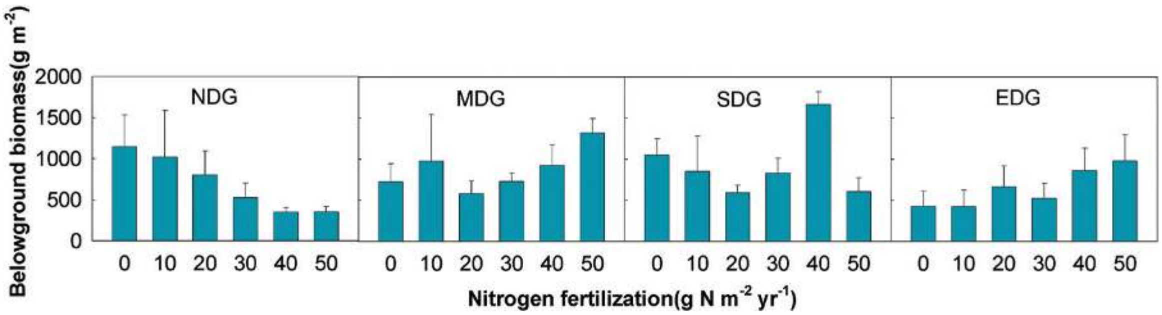

Figure 2: comparison of belowground biomass among different fertilized treatments in 2013. EDG, extremely degraded grassland; MDG, moderately degraded grassland; NDG, non-degraded grassland; SDG, severely degraded grassland.

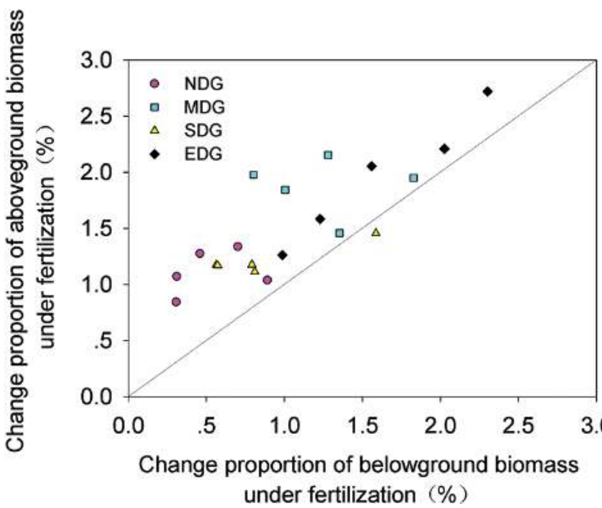

Figure 3: relationship between change proportion of belowground biomass and change proportion of aboveground biomass in different degraded level grasslands. EDG, extremely degraded grassland; MDG, moderately degraded grassland; NDG, non-degraded grassland; SDG, severely degraded grassland.

We observed that N fertilization had a significant positive effect in restoring aboveground biomass in grassland with severe degradation. It has been widely reported that N addition can results in a loss of plant biodiversity (Clark and Tilman 2008; Fang *et al.* 2012; Stevens *et al.* 2004). Xu *et al.* (2015) conducted N-addition experiment in this region for 3 years (2011–2013) and found that N addition caused a loss in species richness in NDG in 2013, but did not significantly affect species richness in degraded grasslands (i.e. MDG, SDG and EDG). These results indicated that N addition did not have negative effects on species richness in degraded grasslands because of relatively short experimental duration (< 3 years), and that NDG would be the first to show harmful effects of N addition on species richness. Thus, N fertilization may be a useful strategy to restore aboveground biomass without reducing plant biodiversity in degraded grassland in a short time (< 3 years) in this region. Our results were based on several field sites in Inner Mongolia. In future research, it will be interesting to scale-up these findings to broader spatial scales. Thus, to better evaluate the effects of N addition on degraded grasslands, we need to conduct further experiments with the same experimental design in more sites, and establish a database to broaden our knowledge on a regional scale.

In this study, added N had no significant effect on belowground biomass (Fig. 2; Table 2) and responses of belowground biomass were smaller than those of aboveground biomass (Fig. 3). According to resource allocation hypothesis (Jamieson *et al.* 2012), when the availability of soil N increases, plants tend to allocate more energy to aboveground growth and reduce investment in the belowground root system. In our study, the root:shoot ratio was not significantly affected by N addition but significantly differed with the degradation level of grassland (supplementary Table S2). There was a marked decrease in the root:shoot ratio in NDG with increased N fertilization (supplementary Fig. S1). Other studies have also reported that increased N lead to reduce investment in belowground growth, resulting in reduced root biomass (Bardgett *et al.* 1999; Liu *et al.* 2013). However, we did not observe an obvious decrease of belowground biomass in degraded grasslands. Some studies

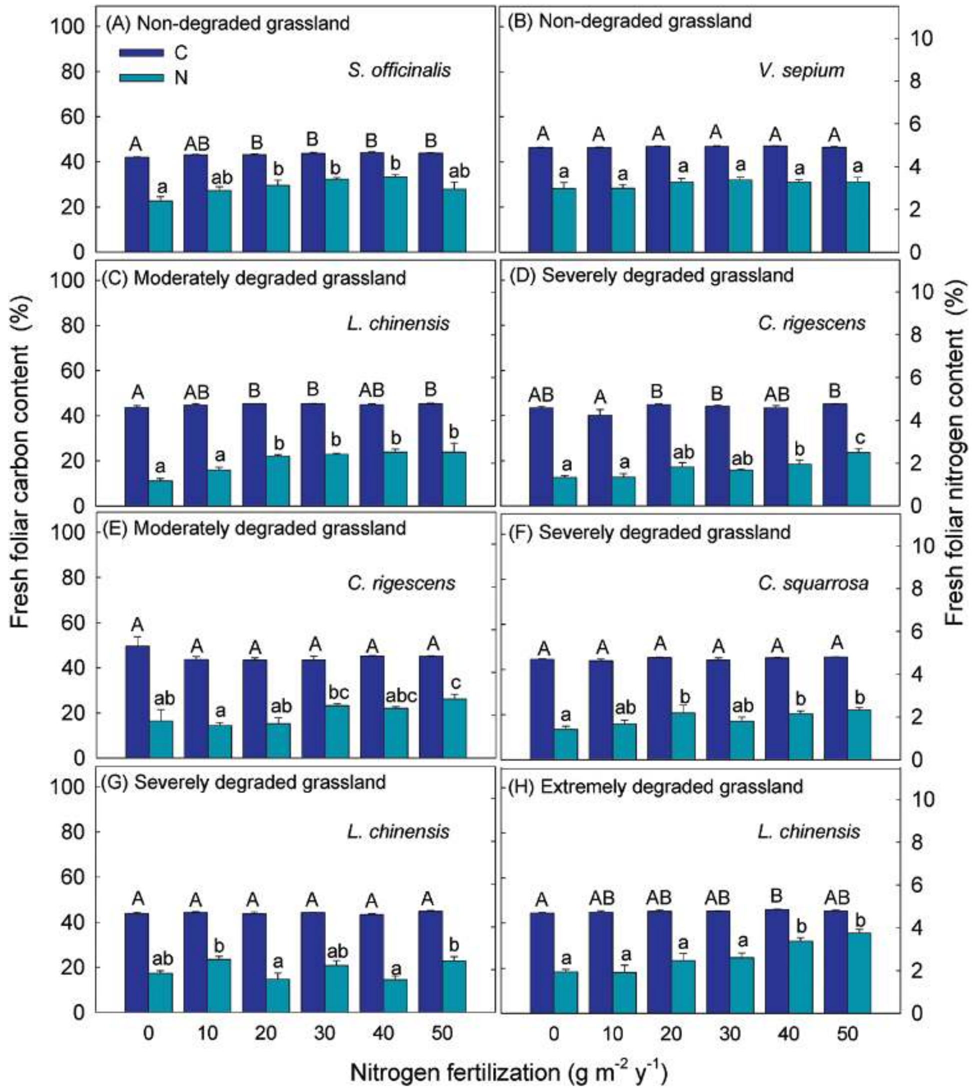

Figure 4: effects of nitrogen (N) addition on carbon (blue) and nitrogen (green) content of fresh foliage. Significant differences among N treatments are indicated by different letters.

Table 3: correlation between aboveground biomass and C and N content of soil, foliage and root

	Aboveground biomass	
	Correlation coefficient	*P*
Soil total carbon	0.134	0.207
Soil total nitrogen	0.117	0.273
C content of foliage	−0.238*	0.024
N content of foliage	0.384**	<0.001
C content of root	−0.005	0.964
N content of root	0.226*	0.032

*$P < 0.05$; **$P < 0.01$.

have shown that in nutrient-deficient soils, an increase in N availability can promote the growth of fine roots, thereby increasing the plant's ability to take up nutrients and water (Burton *et al.* 2000; Steele *et al.* 1997). Consequently, the degree of grassland degradation might play an important role in the trends of belowground biomass. Additionally, N fertilization might alter soil microbial activity and result in decreased root decomposition (Liu and Greaver 2010), leading to an accumulation of root biomass (Fornara and Tilman 2012).

Response of elemental nutrient concentrations in plant tissues to added N

In this study, N addition resulted in significant increases in plant fresh foliar N concentrations (Fig. 4A and C–H). An increase in foliar N concentrations can result in enhanced photosynthesis (Granath *et al.* 2009), leading to an accumulation of photosynthetic products in the foliage, Thus, foliar C concentrations in some species increased with increased N fertilization (Fig. 4A, C and H). In some species, N fertilization did not significantly affect foliar C concentrations as foliar N concentrations increased (Fig. 4D and E–G). This may be

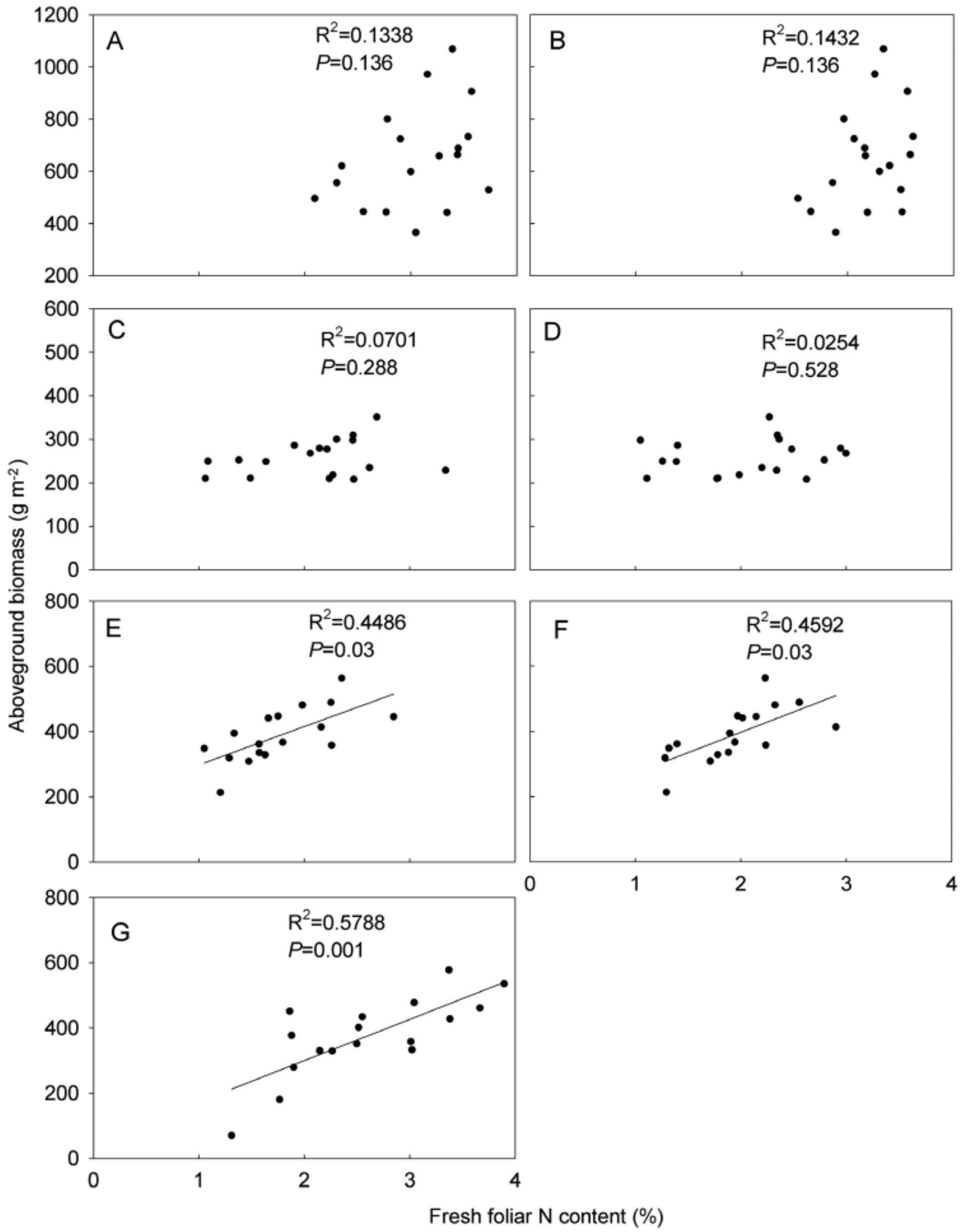

Figure 5: relationships between aboveground biomass and fresh foliage nitrogen content. (A) non-degraded grassland, *S. officinalis*; (B) non-degraded grassland, *V. sepium*; (C) moderately degraded grassland, *L. chinensis*; (D) moderately degraded grassland, *C. rigescens*; (E) severely degraded grassland, *C. rigescens*; (F) severely degraded grassland, *C. squarrosas* and (G) extremely degraded grassland, *L. chinensis*.

because photosynthetic products were rapidly transported to other organs or belowground parts of the plant (Domanski *et al.* 2001; Kuzyakov 2001; Zagal 1994).

The responses of fresh foliar nutrient contents to N addition differed between *S. officinalis* and *V. sepium* in NDG. The foliar N concentrations were higher in the fresh foliage of the legume *V. sepium* than in the other studied plants (Fig. 4B), implying that *V. sepium* does not lack N in its natural environment. It is reasonable to expect that legumes will not respond to N addition (Cui *et al.* 2010). The different responses of the two species may also reflect differences in the ability to compete for nutrients. *S. officinalis* competes strongly for N, responding idiosyncratically to N fertilization in its foliar C and N concentrations. This indicates that different plant species have specific N-use strategies (Zhang *et al.* 2004). Because N addition has different effects among different plant functional groups, the plant community composition may ultimately change after N addition (Clark *et al.* 2007). Also, the same species showed different responses to N addition depending on the degree of grassland degradation. For example, the foliar C and N concentrations in *L. chinensis* increased with increasing N fertilization in MDG and EDG (Fig. 4C and H), while only foliar N concentrations showed significant responses to N addition in SDG (Fig. 4G). *Carex rigescens* showed similar trends in MDG and SDG (Fig. 4D and E). Grassland degradation is considered to be a retrogressive succession and the degradation levels represent different successional stages. Therefore, the responses of nutrient elements in plant tissues to N addition in the same species in grasslands with different degrees of degradation likely represent different nutrient-use strategies during different successional stages (Aidar *et al.* 2003; dos Santos *et al.* 2006).

There was a significant positive relationship between aboveground biomass and fresh foliar N concentrations (Table 3; Fig. 5). The addition of N resulted in an increase in soil N availability, and alleviated the previous N-limited conditions. Therefore, improving plant foliar N concentrations may cause interspecific competition that can, in turn, cause a change from N limitation to other limiting factors. Tilman (1988) found that N addition increased species competition for light resources, and plants could switch their investment in photosynthetic products between aboveground (stems/leaves) and belowground (roots) parts to increase their competitiveness in capturing light. Also, plants can alter the structure of their foliage and chlorophyll content to increase light interception and utilization (Boardman 1977). An increase in foliar N concentrations can result in all of these variations. (Ellsworth *et al.* 2004; Reich *et al.* 1997). Consequently, we concluded that N addition altered foliar N concentrations, thereby promoting photosynthesis and enhancing grassland productivity. In general, there was a positive correlation between aboveground biomass and foliar N concentrations: this effects was not significant in NDG and MDG (Fig. 5A–D), but it was significant in SDG and EDG (Fig. 5E–G). These results suggest that the promoting effect of N addition on aboveground biomass was stronger in grassland with more severe degradation.

CONCLUSIONS

The addition of N resulted in a significant increase in aboveground biomass, and reduced the differences in aboveground biomass between NDG and degraded grasslands. These results indicate that short-term high-N fertilization could restore aboveground biomass in grassland with severe degradation in our study area. Belowground biomass was not significantly affected by N addition, but there was a strong reduction in belowground biomass in NDG with increasing amount of N addition. Different plant species had specific N-use strategies in grasslands with the same of degradation. However, the same plant species showed differences in N-absorption and N-use among grasslands with different levels of degradation. The addition of N altered foliar N concentrations, promoted photosynthesis and enhanced grassland productivity. Thus, N fertilization to degraded grasslands could be an effective strategy to restore grassland biomass in our study area.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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Conflict of interest statement. None declared.

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