

Testing biodiversity-ecosystem functioning relationship in the world's largest grassland: overview of the IMGRE project

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Abstract

Context The relationship between biodiversity and ecosystem functioning (BEF) is a central topic in ecology on local, regional, and global scales. A powerful approach to BEF studies is large-scale field manipulative experimentation.

Objectives The Inner Mongolian Grassland Removal Experiment (IMGRE) was designed to examine the mechanisms of the BEF relationship in the world's largest grassland, explicitly considering multiple trophic levels and grazing by grasshoppers and sheep.

Methods IMGRE followed a randomized block design, with a total of 512 plots (6 m × 6 m each). The project involved massive field campaigns and laboratory analyses, and unprecedentedly employed two removal protocols in parallel: complete removal (eradicating all targeted functional types) and partial removal (an equal-disturbance removal scheme).

Results We summarize key findings on aboveground and belowground primary production, functional richness, identity, and composition, compensation at the species, PFT, and community levels, soil water and N retention, net N mineralization, microbial biomass, and grazing by grasshoppers and sheep. Comparing and contrasting results from the two removal protocols, we have found that the responses of ecosystem processes depend on plant functional richness and identity, as well as disturbance characteristics.

Conclusions As part of the special issue on the ecological patterns and processes in the Inner Mongolian Plateau, this article provides an overview of the IMGRE project. The findings of this project shed new light on the BEF relationship in natural grasslands, and have important implications for ecosystem management in the Mongolian Plateau.

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Keywords Biodiversity and ecosystem functioning relationship · BEF removal experiments · Ecological stoichiometry · Plant functional types · Inner Mongolian grasslands

Introduction

Global sustainability and human prosperity ultimately depend on ecosystem goods and services that result from biodiversity and associated ecological functions such as primary productivity, nutrient cycling, and soil formation (MEA 2005; Wu 2013). Land use change, pollution, climate change, and species invasions have significantly altered ecosystem functioning and the services ecosystems provide (Loreau et al. 2002; Naeem et al. 2009, 2012; Cardinale et al. 2012; Hooper et al. 2012). In a world of rapidly changing landscapes, it is critically important to better understand the relationship between biodiversity and ecosystem functioning (BEF) as well as their relationship to ecosystem services and human well-being (Wu 2013). While research during the past few decades has provided much insight into the BEF relationship, our current understanding of how biodiversity loss influences ecosystem functions and services amidst myriad anthropogenic disturbances is neither precise nor complete (Loreau et al. 2002; Hooper et al. 2005, 2012; Srivastava and Vellend 2005; Naeem et al. 2009, 2012; Cardinale et al. 2012).

Several reasons underpin this incomplete and imprecise understanding. First, BEF experiments have been done mostly in European or North American grasslands. It is not clear if the findings from these studies from old fields, meadows, wetlands, and pasturelands can be generalized to natural grasslands or to other ecosystems. Second, numerous studies suggest that the main mechanism of biodiversity effects on ecosystem function is niche complementarity, but it is quite challenging to explicitly quantify complementarity among plant species and to clearly explain its underlying mechanisms. The essential idea of niche complementarity is that, in an ecosystem with mixed species whose niches complement one another, resources will be used more fully, thus leading to higher productivity than the corresponding monocultures (Loreau and Hector 2001; Naeem 2002a, b; Flynn et al. 2011; Cadotte 2013). Third, past experimental

approaches had several limitations for applying results to natural systems because they typically removed the vegetation and topsoil physically or sterilized them with chemicals, and then seeded to regenerate vegetation, all of which introduce major disturbances to the study system. Removal experiments seem to be the logical means to get around this problem (Wardle et al. 1999; Díaz et al. 2003), but they have other shortcomings (including introduced physical disturbances) and have been used only in a limited way (Díaz et al. 2003). Fourth, much of grassland BEF research has focused on plants and aboveground production, but how increases in production through niche complementarity interact with other factors, such as herbivory or grazing by insects and livestock, remain unclear.

To address these limitations of previous BEF experiments, we designed the Inner Mongolian Grassland Removal Experiment (IMGRE) in 2005, and the project officially started in the summer of 2006 with research funding from the US National Science Foundation (NSF), the National Natural Science Foundation of China (NSFC), and the Chinese Academy of Sciences. The main purpose of this paper is to provide an overview of the IMGRE project, with a particular focus of its conceptual framework, research hypotheses, experimental design, and field implementation. As some of the major discoveries of this project are yet to be published, a particular emphasis here is placed on the research hypotheses, field implementations, and major results in relatively broad categories. Thus, rather than a comprehensive synthesis of the IMGRE project, this article provides an overview of the experimental study for this special issue of Landscape Ecology on the ecological patterns and processes in the Inner Mongolian Plateau, and also serves as the primary background paper for other IMGRE-related publications.

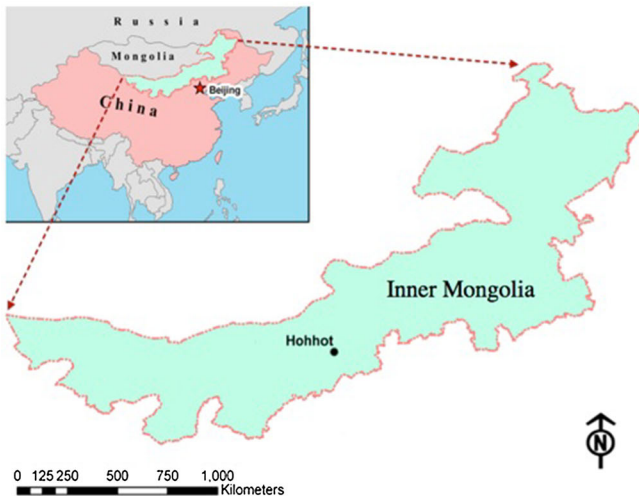
Study area

The field site of the IMGRE project is located in the Xilin River Basin, Inner Mongolia, China (Fig. 1). As part of the Eurasian steppe region, the Xilingol typical steppe extends from 41.4°N to 46.6°N in latitude and from 111.1°E to 119.7°E in longitude, covering an area of 203,000 km² (Wu and Loucks 1992; Bai et al. 2004, 2010). The experimental plots were established within the long-term ecological monitoring area of the

(A) Eurasian Steppe – the largest grassland of the world



(B) Inner Mongolia, China as part of the Eurasian Steppe Region



(C) A landscape view of the Xilingol Grassland



(D) The IMGRE field site



Fig. 1 The location and landscape views of the study area for the Inner Mongolia Grassland Removal Experiment (IMGRE). The Xilingol grassland is located in Inner Mongolia, which in turn is part of the world's largest grassland—the Eurasian

Steppe (a, b). The Xilingol grassland landscape is typically a vast and flat terrain interrupted occasionally by low hills and dominated floristically by the *Leymus chinensis* community and *Stipa grandis* communities (c, d)

Inner Mongolia Grassland Ecosystem Research Station (IMGERS), a field research facility of Institute of Botany of the Chinese Academy of Sciences and a key component of the Chinese Ecological Research Network (CERN).

The study area is characterized by a semiarid climate, with a mean annual precipitation of 346 mm, and a mean annual temperature of 0.3 °C. About 60–80 % of precipitation falls in the growing season between May and August, coinciding with peak temperatures. The coldest month (January) has a mean temperature of −21.6 °C, and the warmest month (July) is 19.0 °C. The site has dark chestnut soil with relatively homogeneous physiochemical properties.

The landscape of Xilingol is dominated by natural grasslands, as agricultural, residential and forested areas together account for less than 10 % of the total land area (Li et al. 2012). The topography of this area is characterized by gently rolling hills, tablelands, and sand dunes, with elevation between about 1,000 and 1,300 m.

The Xilingol steppe is composed mostly of drought-resistant plants, such as bunch grasses, rhizome grasses, forbs, and some dwarf shrubs, most of which are C₃ species. Two characteristic assemblages are widely found in this region: the *Leymus chinensis* community and *Stipa grandis* community. The IMGRE plots were placed within a *Leymus chinensis*

community. Other plant species at the site include *Achnatherum sibiricum*, *Cleistogenes squarrosa*, *Koeleria cristata*, *Agropyron cristatum*, and *Allium tenuissimum*. Overall, more than 85 vascular plant species are found (Wu and Loucks 1992; Bai et al. 2004).

Overall design of the IMGRE project

The conceptual framework

Studies have shown that functional diversity (FD) is more tightly related to ecosystem functioning than is species diversity (Symstad and Tilman 2001; Naeem and Wright 2003; Symstad et al. 2003; Bai et al. 2004; Hooper et al. 2005). A comprehensive framework for examining the functional basis of links between

species and functional diversity and ecosystem processes of energy flow and nutrient cycling has recently emerged in the theory of ecological stoichiometry (Elser et al. 2001; Sterner and Elser 2002). Most BEF experiments adopt a framework in which complementarity is based on phenology (Hooper 1998; Hooper and Vitousek 1998), light (Naeem et al. 1994; Fridley 2003), nutrients (Tilman et al. 1996; Fridley 2003), or resource-based niche axes (Tilman 1997; Loreau 1998). Unlike the other BEF experiments, the overall design of IMGRE was based on a stoichiometric framework. As an organism's elemental composition directly reflects its resource use and represents a quantifiable measure of functional differentiation among species (Elser et al. 2001; Sterner and Elser 2002), our framework links complementarity directly to stoichiometrically defined niche differentiation (Fig. 2).

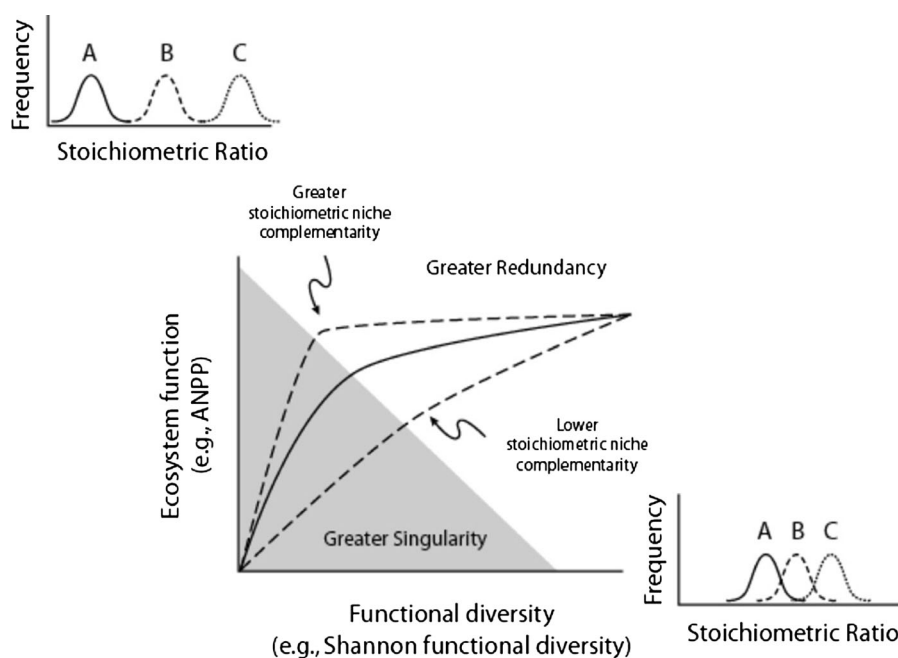


Fig. 2 An ecological stoichiometric framework for the biodiversity and ecosystem functioning relationship. If there are distinctly separate modal ratios (*upper left* in which A, B, and C represent three distinct PFTs), then niche differentiation is high. The converse is illustrated in the *lower right*. Functional diversity (horizontal axis of the middle figure) represents a gradient in diversity from low (one species of a single PFT) to high (all species of all PFTs). For illustration, we use Shannon functional diversity, but other indexes may be used. Ecosystem function is illustrated as annual net primary production (ANPP), but other functions may also be used. The relationship between

plant functional diversity and ecosystem functioning is shown as the commonly observed saturating curve. Initial increases in diversity increase ecosystem function due to the addition of species that make singular contributions to ecosystem function, but subsequent additions make redundant contributions. Using this stoichiometric framework for niche differentiation, alternative relationships are predicted. A shallower relationship occurs where PFTs are more similar in C:N ratio (*lower dashed curve*) due to lower niche differentiation. Conversely, a steeper rise in ecosystem function is expected when stoichiometric niche complementarity is greater (*upper dashed curve*)

Table 1 Plant functional types (PFTs) and their stoichiometric C:N ratios and other properties (PR—perennial rhizome, PB—perennial bunchgrasses, PF—perennial forbs, AB—annuals and biennials, SS—shrubs/subshrubs)

PFT	Number of species per PFT	Leaf ^{13}C value (‰) ^a	C:N (atomic ratio)	Root/shoot ratio	Aboveground biomass (g m^{-2})
PR	1	-26.13	28.99	4.0	102.10
PB	5–7	-22.40	33.53	2.8	139.88
PF	>20	-26.81	22.89	3.1	40.43
SS	2–3	-23.91	18.36	0.6	0.84
AB	3–4	-27.08	15.18	0.2	0.18

^a Leaf ^{13}C values are related to plant water use efficiency (Ehleringer and Dawson 1992)

To combine a functional approach with the ecological stoichiometric perspective, we classified all the plant species in the study area into five plant functional types (PFTs) according to their C:N ratio (Table 1): perennial rhizome (PR), perennial bunchgrasses (PB), perennial forbs (PF), annuals and biennials (AB), and shrubs and semi-shrubs (SS). These stoichiometrically derived PFTs are consistent with life form-based PFTs (Bai et al. 2004), and differ substantially in their stoichiometric ratio, root/shoot ratio, aboveground biomass, and water use efficiency (Table 1). Then, the conceptual framework that we developed relates the stoichiometric characteristics of PFTs to alternative BEF relationships (Fig. 2). The framework assumes that different PFTs have largely distinct modal stoichiometric ratios. As plant functional diversity increases, ecosystem function also increases rapidly initially due to the addition of species that make singular contributions to ecosystem functioning. However, subsequent species additions make progressively smaller contributions due to functional redundancy, thus producing a frequently observed saturation curve (Fig. 2). This stoichiometric framework for niche differentiation predicts alternative BEF relationships: a shallower relationship when PFTs have similar C:N ratios and a steeper relationship when PFTs have more distinct C:N ratios (Fig. 2).

Key hypotheses

The primary goals of the IMGRE project were to experimentally determine how plant functional diversity (as delineated by stoichiometric parameters) would affect ecosystem processes, and to assess how these effects would be influenced by grazing by grasshoppers and sheep in the Inner Mongolian Grassland. To achieve these goals, our research

program was structured around three key hypotheses that are discussed below.

Hypothesis 1

Our central hypothesis is that ecosystem function variables (e.g., ANPP, soil respiration, N retention) are affected by plant functional diversity (e.g., PFT richness, evenness, and divergence) as predicted by the framework of stoichiometric niche complementarity (Fig. 2). This framework predicts responses of ecosystem function and stability to changes in plant functional diversity. We hypothesize that stoichiometric niche differentiation controls both complementarity (differences in resource use or greater singularity) and compensation (the ability of one species to replace another or redundancy) at the community level. Divergence in C:N ratios among plant functional types increases complementarity but decreases redundancy (and thus compensation). The reverse occurs for convergence in niche differentiation. This leads us to suggest that the rate of change in ecosystem function, and thus the shape of the BEF relationship, depend on both the functional composition (identities and relative abundance) and stoichiometric characteristics (e.g., C:N ratio) of PFTs (Table 1; Fig. 2). This hypothesis is based on the assumption that the mechanistic basis of the BEF relationship is reflected, at least partially, in the relative nutrient utilization strategies of the dominant taxa, as captured in biomass C:N ratios (Fig. 2).

Hypothesis 2

Changes in plant functional diversity affect the structure and function of additional trophic levels (e.g., belowground microbial communities and aboveground

herbivory). While most research in BEF has focused on aboveground ecosystem functioning, changes in plant diversity should also influence belowground biodiversity and ecosystem processes as well as higher trophic levels (Mulder et al. 1999; Wardle et al. 1999; Wardle and Grime 2003; Heemsbergen et al. 2004; Wardle et al. 2004b). Numerous studies in freshwater, marine, or laboratory microcosm studies have demonstrated linkages between diversity and functioning at other trophic levels (Norberg 2000; Duffy et al. 2001; Downing and Liebold 2002; Duffy 2002; Naeem 2002b; Petchey et al. 2004; Solan et al. 2004). It is uncertain whether these findings can be extrapolated to grasslands. Also, these studies have shown that aboveground and belowground relationships can be positive, negative, or nonexistent (Hooper et al. 2005; Wardle 2002; Wardle et al. 2004a), indicating that a variety of mechanisms may be involved and that further studies are urgently needed. Previous studies in the Inner Mongolian Grassland seem to corroborate this general observation (Bai et al. 2004, 2007; Pan et al. 2011), but further scrutiny of its underlying mechanisms is needed.

Hypothesis 3

Changes in plant functional diversity affect the resilience of steppe ecosystems in response to livestock grazing. Resilience here refers to the ability of an ecosystem to return to its previous state or trajectory after a disturbance (Pimm 1984; Wu and Loucks 1995). A number of studies have suggested that biodiversity reduces variability in ecosystem productivity through compensatory effects in which a species increases its abundance in response to the reduction of another in a fluctuating environment (McNaughton 1977; Naeem and Li 1997; Tilman 1997, 1999; Loreau 2000). However, this view has been challenged on several grounds (Huston 1997; Pfisterer and Schmid 2002). For example, Pfisterer and Schmid (2002) found that less diverse plots are more resistant and resilient than more varied communities. Most of these studies were based on artificially constructed grasslands and had short durations. Recently, based on a 24-year study we showed that ecosystem resilience of the Inner Mongolian Grassland was positively related to plant diversity, which was explained, at least in part, by compensatory interactions among major species and functional types (Bai et al. 2004; Wu et al. 2005).

These correlational results need to be verified through manipulative experiments.

Field experimental setting

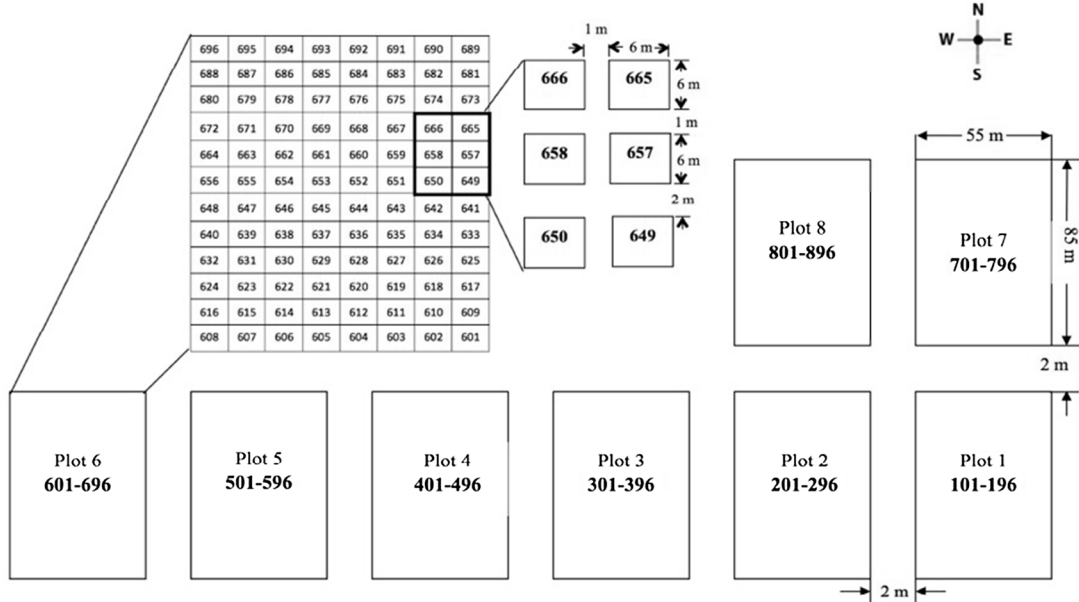
The IMGRE experiment was established in 2005 and consisted of 8 blocks (55 m × 85 m each), and each block was divided into 96 plots (6 m × 6 m each), resulting in a total of 768 plots (Fig. 3a, b). The 8 blocks were arranged horizontally in a topographically uniform area to avoid the effects of anisotropy (directional dependence). The 768 plots were divided into 3 sets, two of which were used for the current experiment: the first set of plots was for the complete removal protocol, the second set for the partial removal protocol, and the third set was left intact for future studies (the two removal protocols will be discussed in detail below). Plots were separated by a 1-m wide path, and sets of plots by a 2-m wide walking path (Fig. 3a, b, c). Each plot was further divided into 16 quadrats (1 m × 1 m), which were themselves separated by 40 cm from each other to avoid edge effects.

This BEF removal experiment took a full combinatorial design approach. As mentioned earlier, we originally categorized all plant species into 5 functional groups: PB, PR, PF, AB, and SS. As the project began to start, however, we discovered that only the first four PFTs existed in appreciable numbers in our experimental plots. Thus, by removing 0–4 functional groups, we had a total of 16 different removal treatments, each having 16 replicates (Table 2). Each of the two parallel removal protocols (complete and partial) needed 256 plots, and the total number of plots for the IMGRE project was 512 (Table 2). The 16 treatments were assigned to each plot according to a randomized block design scheme.

Complete and partial removal protocols

Testing the hypotheses proposed in this study required establishing a gradient of plant functional diversity. Thus, how to create this biodiversity gradient was the most critical part of the field experimental design. Removal experiments have been criticized for introducing physical disturbances that tend to increase with the number of plants removed (Wardle et al. 1999; Díaz et al. 2003). To alleviate this problem and to take full advantage of this large-scale BEF experiment, we

(A) A schematic of the experimental plot layout of the IMGRE project



(B) An aerial view of the experimental field site of the IMGRE project



(C) Field manipulations in summer 2006



(D) Sheep grazing experiment in 2010

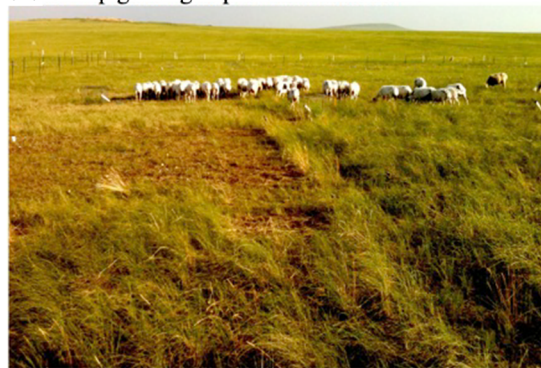


Fig. 3 The field experimental setting of the IMGRE project: **a** the plot layout, **b** an aerial view of the experimental field, **c** a close landscape view of the experimental plots with graduate

students doing plant biodiversity manipulations, and **d** sheep grazing experimentation in action (July 2010)

Table 2 Full combinatorial design of the IMGRE BEF experiment (PR—perennial rhizome, PB—perennial bunchgrasses, PF—perennial forbs, AB—annuals and biennials)

Treatment number	Number of PFTs removed	PR	PB	PF	AB	Number of replicates
1	0					16
2	1	x				16
3	1		x			16
4	1			x		16
5	1				x	16
6	2	x	x			16
7	2	x		x		16
8	2	x			x	16
9	2		x	x		16
10	2		x		x	16
11	2			x	x	16
12	3	x	x	x		16
13	3	x	x		x	16
14	3		x	x	x	16
15	3	x		x	x	16
16	4	x	x	x	x	16
Number of removal protocols: 2 (complete and partial removal)						
Total number of plots						512

employed, painstakingly, two contrasting removal protocols: complete and partial removal. To our knowledge, using the two protocols simultaneously in the same BEF experiment has never been done.

With the complete removal protocol, all targeted PFTs in a plot were removed completely. To minimize physical disturbance to soil, plants were removed by clipping off the aboveground parts and tillering nodes at 0–3 cm soil depth. To make sure that the removal treatment completely stopped the growth of targeted plants or at least significantly reduced it, we started the removal treatment in early June of 2006, and then imposed follow-up removal in the following 3 years (2007, 2008, and 2009) when plants started to grow in late May or early June. The complete removal scheme created a clear-cut and “discrete” biodiversity gradient, with the number of remaining PFTs increasing from 0 to 1, 2, 3, and 4.

Under the partial or equal-disturbance removal protocol, the removal of the targeted functional groups in each plot stopped when approximately 50 % of vegetation in that plot was eradicated. Specifically, if the total cover of all targeted functional groups was less than 50 % of the plant cover of the entire plot, all targeted functional groups were completely removed first. Each plot was then divided into 144 small

quadrats (0.5 m × 0.5 m) and a quadrat-based removal method was used for the remaining PFTs. The number of quadrats for further removal was determined by the 50 % vegetation removal rule. All plants were removed within each randomly assigned quadrat and thus a 50 % disturbance in terms of vegetation removed was created. If the total cover of targeted functional groups was larger than 50 %, the rare targeted functional groups (AB, and/or PF) were removed first, and the dominant functional groups (PR and PB) were then removed by quadrat-based removal method until the 50 % of vegetation cover requirement was met. Our choice of 50 % as the vegetation removal target reflected the traditional grazing wisdom in the steppe region—“Take half and leave half”.

Partial removal assured that the physical disturbance due to removal was approximately equal among all treatment plots, thereby eliminating the diversity-disturbance correlation as observed in complete removal experiments (Symstad and Tilman 2001). In contrast with complete removal, partial removal established an approximate and “continuous” biodiversity gradient (in terms of both richness and evenness) because many plots had some PFTs only partially removed. However, this gradient may be a realistic representation of what is likely to be observed

now and in future in the face of myriad anthropogenic drivers of biodiversity change.

Grasshopper and sheep grazing experiments

As part of the IMGRE project, two controlled experiments with grasshopper grazing were carried out in 2007 and 2009. The first was designed to address the question, how does plant functional diversity affect grasshopper biomass, diversity, and abundance? All cages were sampled three times each year in 2007 and 2009 (June 10, July 15, and August 20), using the standard net sweeping method (10 nets per plot). All grasshoppers were counted, weighed, and identified to species and developmental stages. The total number of plots investigated was 512 (including both complete and partial removal plots), and the total number of samples was 3,072 (512 plots \times 3 times \times 2 years). The second BEF-based grasshopper experiment was designed to address the question, how do biodiversity manipulations affect the dietary suitability and stoichiometry of grasshopper species? This experiment contrasted high PFT plots with low PFT plots, and examined their relationships to grasshopper species abundance, diversity, and stoichiometric characteristics. Three dominant grasshopper species (*Dasyhippus barbipus*, *Oedaleus asiaticus*, and *Chorthippus fallax*) were chosen, and their early-instar individuals were put in wired cages (shown as white dots in Fig. 3b). After 15 days, each cage was checked for grasshopper survival rate, and each grasshopper was weighed and then frozen for C, N, P, RNA, DNA, and protein analyses (3 species \times 88 plots = 264 samples). In addition to these two experiments, three related grasshopper experiments were conducted in nearby grasslands next to the IMGRE field.

The sheep grazing experiment started in the summer of 2010. A total of 180 adult native Mongolian sheep (2-year old) were used, following a protocol with different degrees of grazing intensity (Fig. 3d). For both complete and equal disturbance removal treatments, eight blocks were randomly divided into two groups (i.e., 4 blocks each group). One group was treated with light-moderate grazing, and the other with heavy grazing. Grazing activities were managed by two local herders, and extra care was taken to keep all plots within each block grazed evenly. For each block, a removable enclosure was used to help control grazing intensity. To avoid the excessive treading

destruction of the treatment plots, sheep were moved out of the plots along a fenced buffer zone at night. For each plot, aboveground standing biomass was measured at the species level two times per month (before and after grazing) in July and September, respectively. Other ecosystem properties (similar to those mentioned above) were continuously measured with some adjustments according to the grazing protocol.

Field sampling and lab analysis

The amount of fieldwork and lab analysis for this project was substantial. A large number of plant, soil, and ecosystem variables were measured, including: plant species composition, vegetation cover, plant functional traits (e.g., specific leaf area and photosynthetic rate), soil temperatures and moistures, plant phenology, aboveground biomass of each species, litter decomposition, root biomass, root turnover, seed bank, soil C, soil N, soil nutrient leaching, N mineralization, nutrient mineralization potential, soil respiration, net ecosystem carbon exchange, soil N/P/C stoichiometry, plant C/N/P stoichiometry, nutrient use efficiency, plant water use efficiency, microbial biomass, microbial community structure, and soil faunal composition and stoichiometry. More than 8,000 soil and plant samples were analyzed in the Inner Mongolia Grassland Ecosystem Station and the Institute of Botany of the Chinese Academy of Sciences in Beijing. More details of field sampling and laboratory analysis associated with the different aspects of IMGRE are found in the individual publications that were based on the project (e.g., Sun et al. 2009; Su et al. 2010; Liu et al. 2011).

Highlights of research findings

To date, the IMGRE project has produced a number of publications, and more are still in preparation. Instead of summarizing all the results already reported elsewhere and those yet to be published, here we briefly highlight some of the major findings from the project.

Aboveground and belowground primary production

With the complete removal scheme, aboveground net primary productivity (ANPP) declined while

interannual variation of ANPP (CV_{ANPP}) increased linearly with the increasing number of PFTs removed. PFT richness explained about 45 % of the variation in ANPP and 16 % of the variation in CV_{ANPP} . The response patterns of ANPP to the PFT removal gradient were consistent over time, although the magnitude of ANPP responses differed among years. Belowground biomass declined in all depths along the PFT removal gradient by the fourth year of the study although no significant responses were observed in the first three years.

For partial removal, ANPP also declined with the increasing number of PFTs removed, with CV_{ANPP} showing no significant response to the PFT removal gradient. PFT richness, however, explained only 6 % of the variation in ANPP. Belowground biomass did not show a significant response to the PFT removal treatments at any depth, although the effects of removal approach and year were generally significant.

Functional richness, identity, and composition

For complete removal, changes in aboveground biomass depended on functional richness, identity, and PFT composition of the community. The percent biomass reduction increased with increasing number of PFTs removed. On average, reductions in aboveground biomass for treatments that removed PB, PR, PF, and AB alone were 60, 21, 15, and 6 %, respectively, in the first two years. By the fourth year, 6 of the 12 treatments that removed PB and/or PR showed a greater amount of aboveground biomass compared to undisturbed controls.

Under the partial removal protocol, the patterns of aboveground biomass change were both qualitatively and quantitatively similar among treatments in the first two years of the study. When averaged across all treatments, the aboveground biomass reduction was 59 % in 2006 and 50 % in 2007. By the third year, biomass reduction was generally larger in the treatments that removed PR alone or PR in combination with other PFTs. By the fourth year, the patterns of aboveground biomass change differed substantially among treatments. However, these patterns were comparable to those of complete removal. Aboveground biomass for 6 of the 12 treatments that included PB and/or PR reached the level of undisturbed control or even higher.

Compensation at species, PFT, and community levels

At the species level, when averaged across all treatments, the observed aboveground biomass for 6 of the 11 dominant and common species (3 perennials and 3 annuals) was 0.4–19 times greater than that of the expected biomass. Accordingly, plant density increased by 0.3–3 times for these species. Our measurements of plant functional traits further revealed that both whole plant traits and leaf traits of these species responded significantly along the PFT removal gradient. Four of the five remaining species (all perennial bunchgrasses) did not show significant responses to the removal treatments. Only one species (i.e., *Allium tenuissimum*) declined in its aboveground biomass, which was caused mainly by the decrease in plant density.

At the plant functional group level, the patterns of observed aboveground biomass differed substantially among extant PFTs. The overall aboveground biomass of PB remained unchanged, whereas that of PR, PF, and AB increased by 92, 33, and 1,700 %, respectively, in the treatment plots where other PFTs were removed. The effects of removal protocol, treatment, year, and their interactions were statistically significant across all PFTs, except for PB and PR of which the effect of removal protocol was insignificant. The response patterns of PB and PR were similar between the complete- and partial removals, and also comparable over time for each treatment.

At the community level, compensatory effects increased through time under both complete and partial removal protocols. For complete removal, plant communities for 7 of the 15 removal treatments fully recovered in terms of total biomass production, 5 of the 8 remaining treatments partly recovered, and only 3 treatments (PF, AB, and PF + AB removals) showed no sign of compensation by the fourth year of the study. The degree of compensation was consistently higher in the treatments including PB than that of others. For partial removal, all treatment communities either fully recovered (7 of 15) or partly recovered (8 of 15) by the fourth year.

Soil water and N retention, net N mineralization, microbial biomass

Soil moisture (0–40 cm) increased linearly with increasing number of PFTs removed for complete

removal, but it did not show a significant response for partial removal. For both complete and partial removals, soil NO_3^- -N and NH_4^+ -N concentrations in the rooting zone increased significantly along the PFT removal gradient. On the average, soil NO_3^- -N concentrations were 3.1–7.5 times higher than those of NH_4^+ -N concentrations across all treatments and removal protocols. However, the rates of change (slopes of regressions) in soil NO_3^- -N and NH_4^+ -N concentrations were consistently greater under complete removal than under partial removal. For both complete and partial removals, soil net N mineralization rates declined with the increasing number of PFTs removed, with complete removal having much greater rates. In contrast, there was no relationship found between soil microbial biomass and PFT richness under either removal protocol.

Grazers: grasshoppers and sheep

We investigated the effects of various grasshopper species on the abundance, diversity, production and nutrient content of various plant species. Together with projects associated with IMGRE (Yu et al. 2010; Zhang et al. 2011), our research demonstrated significant grazing impacts that differentially affected biomass and C:N:P ratios of aboveground biomass. These findings represent a potentially significant feedback mechanism between plants and herbivores in the Inner Mongolian grassland. We also found that the degree of stoichiometric regulation in the C:N:P ratios of various plant species was highly correlated with their temporal and spatial stability, while the overall dominance of different taxa with different degrees of stoichiometric homeostasis was associated with community-level production and stability (Yu et al. 2010).

In another related project, partly supported through IMGRE, Cease et al. (2010, 2012) found that brown morphs of Asian band-winged grasshoppers (*Oedaleus asiaticus*) have longer wings, larger bodies, and higher metabolic rates compared to green morphs, suggesting that developmental flexibility allows greater capacity for migration and swarming in the brown morph of this locust. In sharp contrast with prevailing paradigms, the research by Cease et al. (2012) showed that host plant N-enrichment and high protein artificial diets decreased the size and viability of the Asian band-winged grasshopper (*O. asiaticus*),

a dominant locust in the region, suggesting that heavy livestock grazing promotes outbreaks of this locust by reducing plant protein content.

Sheep grazing effects on BEF relations

A key component of the IMGRE project was the sheep grazing experiment within the BEF framework. We had to delay our grazing experiment to the summer of 2010, in order to make sure that removal treatment plots were adequately recovered. Field measurements were made before and after the sheep grazing treatments, and the same measurements were repeated for all plots in the summer of 2011. Monitoring of the treatment plots has continued since then. Although no results on the sheep grazing have been reported yet, several publications are expected in the near future. These papers will address a number of research questions: Does plant functional diversity influence the resilience of grasslands to domestic animal grazing pressure? How does plant functional diversity influence ecosystem responses to sheep grazing? Do high diversity plots get grazed more intensely? Do sheep foraging preferences govern their grazing time on plots that differ in plant diversity? Do plant functional diversity and sheep grazing interactively affect ecosystem resilience?

Discussion

The IMGRE project contributes to our understanding of the BEF relationship in several unique ways. First, IMGRE provides the first large-scale BEF field experiment on the Eurasian Steppe—the largest of its kind in the world. Results from IMGRE enable comparisons and possible generalizations of BEF-related findings from previous BEF experimental studies that were conducted mainly in Europe and North America. Second, IMGRE simultaneously employed two parallel biodiversity removal protocols: complete and partial (equal-disturbance) removal. While extremely labor-intensive, the implementation of this strategy provided a unique opportunity to directly compare and contrast these two removal experimental approaches. Third, although functional groups have long been used in ecology, using stoichiometric traits as a basis for defining plant functional types (stoichiometrically defined PFTs), as

demonstrated in the IMGRE project, seemed an effective way of quantitatively assessing the current BEF theory and predictions. Fourth, in IMGRE we also considered both grasshopper and livestock grazing in the context of BEF dynamics in the Inner Mongolian grassland.

The IMGRE project provides a number of important insights into the BEF relationship. For example, decreasing plant functional diversity led to significant reductions in ecosystem productivity but substantial increases in its variability. Removing certain members of plant functional groups (e.g., some treatments with the partial removal scheme) usually had only inconsequential effects on ecosystem functions. On the other hand, the loss of one or two key functional groups may result in a massive loss of ecosystem functions, or even lead to the collapse of the entire ecosystem. Combining the large number of results from both complete and partial removal treatments, we found that the responses of ecosystem processes depended on plant functional richness and identity as well as disturbance characteristics in natural grasslands. The project has also produced several important findings concerning the effects of grasshoppers on plant production, elemental stoichiometry, and the BEF relationship in the Inner Mongolian grassland.

The IMGRE project contributes to several other disciplines, including grassland ecology (e.g., biogeochemical cycling, grazing ecology, and plant population and community dynamics), insect ecology (e.g., physiology and behavioral ecology of grasshoppers), and land management (e.g., impacts of reduced vegetation cover on soil erosion). The results of this study are useful for assessing potential impacts of human land use activities and global climate change on biodiversity and ecosystem functions. Our future research will further examine the possible effects of spatial heterogeneity on the BEF relationship on within-ecosystem scales (Yuan et al. 2015), and explore the impacts of land use and land cover pattern on the BEF relationship on the landscape and regional scales in the Mongolian Plateau.

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