Testing the scaling effects and mechanisms of N-induced biodiversity loss: evidence from a decade-long grassland experiment

Zhichun Lan, G. Darrel Jenerette, Shuxia Zhan, Wenhui Li, Shuxia Zheng and Yongfei Bai

1 State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China; 2 University of Chinese Academy of Sciences, Beijing 100049, China; and 3 Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124, USA

Summary

1. Although extensive studies demonstrate that nitrogen (N) enrichment frequently reduces plant diversity within small quadrats (0.5–4 m\(^2\)), only a few studies have evaluated N effects on biodiversity across different spatial scales.

2. We conducted the first experimental test of the scale dependence of N effects on species richness from a 10-year N treatment (1.75–28 g N m\(^{-2}\) year\(^{-1}\)) in a typical steppe. We used species–area relationship (SAR) to analyse the scale dependence of species loss with power model \(S = cA^{z}\) (S is species number, A is area, c is intercept, and z is slope).

3. Absolute species loss decreased at sampling area > 8 m\(^2\). Proportional species loss (compared to control) decreased and critical threshold (\(N_{\text{crit}}\)) for biodiversity losses increased with sampling areas.

4. These scale dependences were quantified as increasing slope (z-value) of SAR with N addition. Through SAR decomposition, we found that this overall positive effect was in response to positive effects of changes to the species abundance distribution over negative effects of overall species richness losses.

5. Synthesis. As nitrogen (N) enrichment typically occurs at scales much larger than individual plots, understanding how N enrichment affects the scaling patterns of biodiversity is necessary for biodiversity conservation and ecosystem management in response to anthropogenic N deposition.

Key-words: critical threshold, determinants of plant community diversity and structure, N enrichment, number of individuals, SAR decomposition, scaling effect, species abundance distribution, species aggregation, species–area relationship (SAR)

Introduction

While terrestrial primary production is widely limited by nitrogen (N) availability (Vitousek & Howarth 1991), the increasing rate of nitrogen pollution is changing biodiversity and ecosystem functioning world-wide (Vitousek et al. 1997; Phoenix et al. 2006; Galloway et al. 2008; Bobbink et al. 2010; Pardo et al. 2011). Decreases in biodiversity are commonly observed following N input (Suding et al. 2005; Clark & Tilman 2008; Hautier, Niklaus & Hector 2009; Clark et al. 2013), and N deposition is recognized as one of the major causes of biodiversity decline in terrestrial ecosystems (Sala et al. 2000; Stevens et al. 2004a). However, these findings are limited to quadrant scale responses and are not necessarily representative of the regional N deposition. Only a few studies have evaluated scaling relations of N-induced species loss (Crawley et al. 2005; Hillebrand et al. 2007; Chalcraft et al. 2008; Leps 2014). These findings suggested that the patterns of N-induced biodiversity loss may be changed at larger scales, and few studies have tested mechanisms underlying these patterns (Chalcraft et al. 2008; Leps 2014). The scale dependence of biodiversity change also suggests that a scale-dependent critical threshold (\(N_{\text{crit}}\)) for N enrichment may exist, below which N might have minimal effects on biodiversity (Clark & Tilman 2008; Bai et al. 2010; Lan & Bai 2012). It is still unclear how the sensitivity and magnitude of biodiversity loss after N addition vary with spatial scales. A mechanistic understanding of scale-dependent N-induced species loss is needed to extend findings from plot level and develop more efficient management strategies for biodiversity conservation (Chalcraft et al. 2008; Cleland & Harpole 2010).
Species–area relationship (SAR) is one of the most frequently used approaches to analyse scale dependence of biodiversity changes (Keil et al. 2011; Sandel & Corbin 2012; Powell, Chase & Knight 2013). The scale dependence of biodiversity is commonly modelled as a power function (Preston 1962; Tjørve 2003), $S = c A^z$, where $S$ is species number, $A$ is area, and $c$ and $z$ are constants (Arrhenius 1921). The slope ($z$-value) and intercept ($c$-value) of the SAR in log–log space are frequently used to quantify how species richness varies across multiple spatial scales (Tjørve 2003; Powell, Chase & Knight 2013). For example, the commonly observed reduction of species richness at the plot level with increased N may be associated with decreases in the intercept ($c$-value) and/or increases in the slope ($z$-value) of the SAR (Crawley et al. 2005; Chalcraft et al. 2008). If $N$ increases the slope ($z$-value) of SAR, the rate of species loss would be smaller at broader scales, whereas if $N$ decreases the slope of SAR, then species reduction will be larger at broader scales (Fig. 1). However, the majority of research into understanding variation in SARs has been observational and experimental approaches, though more challenging, are needed to directly test potential mechanisms (Jenerette & Shen 2012).

Recent SAR decomposition approaches provide an analytical framework to quantify changes in the $c$- and $z$-values as caused by a combination of changing species pool, number of individuals, species abundance distribution and/or species aggregation (spatial distributions) (He & Legendre 2002; Tjørve et al. 2008; Scheiner et al. 2011; Powell, Chase & Knight 2013). $N$ addition may affect overall SAR and consequently regulate the scale dependence of biodiversity changes through its distinct influences on each of the four individual factors above. First, $N$ addition may lead to decreased species pool through loss of rare species (H1: reduced richness) (Crawley et al. 2005; Chalcraft et al. 2008). Through the reduced richness hypothesis, we expect $N$ additions to decrease both the $c$- and $z$-value of the SAR, and $N$-induced species loss would increase with sampling area. Secondly, $N$ addition increases biomass production, causes assemblage-level thinning and thereby decreases the number of individuals because of increased plant size and intensified light competition (H2: reduced individuals) (Goldberg & Miller 1990; Oksanen 1996). Through reductions in the number of individuals, we expect $N$ addition would decrease the $c$-value but increase the $z$-value of SAR (Oksanen 1997), and thus, the effects of $N$ enrichment on species richness would diminish with increasing sampling area. Thirdly, $N$ addition increases the abundance of a few $N$-favoured species at the expense of less favoured species (H3: altered abundance distributions) (Suding et al. 2005; Bai et al. 2010), which will decrease the evenness of community (Gilliam 2006; Hillebrand et al. 2007). Through decreased evenness of species abundance distribution, we expect $N$ additions would decrease the $c$-value but increase the $z$-value, and thereby the $N$-induced reduction in species richness would become increasingly moderated with sampling area. Finally, $N$ addition intensifies recruitment limitation (Stevens et al. 2004b), which will reduce immigration between different communities and increase species aggregations (H4: species aggregation). Through increases in species aggregation, we expect $N$ addition would decrease the $c$-value but increase the $z$-value of SAR; hence, the effects of $N$ enrichment on species richness would decrease with increasing sampling area (Table S1 in Supporting Information).

Our objective in this study was to experimentally quantify the scale dependence of $N$-induced species loss and test the underpinning mechanisms. We established a 10-year field}

![Fig. 1. Three scenarios of how $N$ addition affects the slope of species–area relationship (SAR) and the corresponding scale effects on proportional species loss (PSL). In each scenario, the discrepancy between the two SARs represent $N$-induced species loss, that is $\Delta S = \log (S_{\text{control}}) - \log (S_N)$, or $\exp (\Delta S) = S_{\text{control}}/S_N$. Proportional species loss (PSL) was defined as proportion of species loss in $N$ treatments compared to control, that is $\text{PSL} = 1 - S_N/S_{\text{control}} = 1 - 1/\exp (\Delta S)$. Therefore, PSL was positively correlated with $\Delta S$. (a) $N$ addition has no effects on the slope of SAR, and $\Delta S$ and PSL are independent of spatial scale; (b) $N$ addition increases the slope of SAR, and $\Delta S$ and PSL decrease with increasing sampling area; (c) $N$ addition decreases the slope of SAR, and $\Delta S$ and PSL increases with sampling area.](image-url)
experiment with six levels of N addition rate in a semi-arid Inner Mongolia grassland, China. The Inner Mongolia grassland both historically and currently received little N deposition (Galloway et al. 2008) and has not yet experienced a rapid loss of plant species induced by chronic N input. This low N deposition background allows testing N effects on biodiversity. Specifically, we address two questions: first, how do the N-induced biodiversity loss (i.e. plant species richness, absolute and proportional species loss and critical threshold) change with spatial scales? Secondly, what are the relative contributions of alternative mechanisms in terms of species pool, number of individuals, species abundance distributions and species aggregation, mediating the scale dependence of N-induced species loss?

**Materials and methods**

**STUDY SITE**

The study was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS; 116°42’ E, 43°38’ N and 1250 m a.s.l.), which is located in the Xilin River Basin, Inner Mongolia Autonomous Region of China (Bai et al. 2010). The mean annual temperature in the study area is 0.3 °C, with mean monthly temperature ranging from −21.6 °C in January to 19.0 °C in July. The mean annual precipitation is 346.1 mm, with about 80 per cent occurring in the growing season from May to September. The field experiment was carried out on a natural steppe community, which was fenced to prevent grazing since 1979. At the beginning of the experiment, there were 48 vascular plant species and 80 per cent of them were rare species (relative abundance < 1%) (Lan & Bai 2012). The dominant and common species in plant community include Leymus chinensis (perennial rhizomatous grass, PR), Stipa grandis (perennial bunchgrass, PB), Agropyron cristatum (PB), Achnatherum sibiricum (PB), Carex korshinskyi (sedge), Kochia prostrata (perennial forbs, PF), Allium tenuissimum (PF), Chenopodium glaucum (annual forbs, AF), Axyris amaranthoides (AF) and Salsola collina (AF) (Bai et al. 2004).

**N ADDITION EXPERIMENT**

To explore the effects of N addition on plant species diversity and ecosystem functioning, an N addition experiment was initiated in 2000 and is still ongoing (Bai et al. 2010; Lan & Bai 2012). The experiment has six treatments and nine replicates for each treatment, with a total number of 54 5 m × 5 m plots. Experimental plots were arranged in a randomized block design, with 1-m-width walkways between plots. N treatments included six levels of N addition rate, 0, 1.75, 5.25, 10.5, 17.5 and 28.0 g N m⁻² year⁻¹ as commercial granular NH₄NO₃ (Bai et al. 2010). The low N input rates (e.g. 0, 1.75 and 5.25 g N m⁻² year⁻¹) allow us to approximate N deposition and estimate critical thresholds for species loss (Bobbink et al. 2010). The high N input rates (i.e. 10.50, 17.50, 28.00 g N m⁻² year⁻¹) allow us to approximate management techniques (e.g. N fertilization) and assess the effects of N enrichment on plant diversity (Schellberg et al. 1999; Crawley et al. 2005; Socher et al. 2012). For all treatments, to ensure that N was the only limiting nutrient, based on the soil census data, we also added the same amount of P (10 g P₂O₅ m⁻² year⁻¹), S (0.2 mg m⁻² year⁻¹) and trace elements (Zn, 190 mg m⁻² year⁻¹, Mn, 160 mg m⁻² year⁻¹ and B, 31 mg m⁻² year⁻¹). Each year, nutrients were uniformly applied to each treatment plot with manual broadcasting in the mid-growing season (July 1–5) coinciding with high temperature and precipitation, as in other N addition studies (Tilman 1987).

**SPECIES-AREA RELATIONSHIP**

To test the scale dependence of N effects on species richness, we collected species abundance data from fifty-four 5 m × 5 m plots for constructing species-area relationship (SAR). Each 25 m² plot was divided into twenty-five spatially explicit 1 m × 1 m quadrats. In each quadrat, we determined the abundance (number of individuals) for each species in August 2010. For non-clonal species with no physical connections among different stems (e.g. C. glaucum), an individual was defined as a separately rooted above-ground shoot. For caespitose species with densely packed ramets, such as bunchgrasses (i.e. S. grandis), an individual was defined as a basal. For rhizomatous species with widely spaced ramets (e.g. L. chinensis), an individual was defined as a ramet (Pérez-Harguindeguy et al. 2013).

Based on our data sets, the empirical SAR in each plot was constructed with type IIA design (Scheiner 2003), that is using a set of spatially explicit quadrats arrayed in a continuous grid (Fig. S1 in Supporting Information). The set of quadrat sizes in each 5 m × 5 m plot includes 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 15, 16, 20 and 25 m², respectively. For a given quadrat (sampling area), we aggregated contiguous 1 m² quadrats and counted the number of species. For a given quadrat size within each plot, the species richness was the average of number of species across all quadrats with the same size. For example, the species richness of 1 m² quadrats within each plot was the average of number of species across twenty-five 1 m × 1 m quadrats. In the same way, we determined the species richness of 2, 3, 4 m² and other quadrat sizes within each plot.

SARs were fitted and model parameters were estimated using the `msar` R-package (Guilhaumon, Mouillot & Gimenez 2010). Specifically, we fitted all the eight candidate models of SARs in the `msar` R-package (Fig. S2). These models include power, exponential, negative exponential, Monod, rational function, logistic, Lomolino and cumulative Weibull functions of SAR (Guilhaumon, Mouillot & Gimenez 2010). The preliminary analysis indicated that, among the eight models, power, logistic and Monod functions were three best fit models with the highest R² value but lowest AIC, AICc and BIC values. However, based on the experimental design and sampling scheme of this study, the shape of the species–area curve is expected to be convex upward, which can be best fitted by the power model (Tjørve 2003). It can also be used to determine the predicted Nₘₑ and extrapolate the results to larger scales. Therefore, we used the power model S = cAα (S, species; A, sampling area) to assess the scale dependence of N effects on species richness.

**ESTIMATING EFFECTS OF N ADDITION ON SPECIES LOSS**

Absolute species loss was calculated as the difference of species richness between control and each N treatment (i.e. Richness_{control} – Richness_{Ntreatment}). To allow direct comparison across sampling areas, proportional species loss was defined as loss proportion compared to control and calculated as one minus species richness response ratio (i.e. 1 – Richness_{Ntreatment}/Richness_{control}). For each N treatment, the percentage of overall species richness was calculated as the ratio of species richness at a given sampling area to the overall species richness of the plot (25 m²), with the values ranging from 0% to 100%.

of individuals included in the simulation. z-

between the mean rarefaction curve (non-spatially explicit) and empir-

eation curves. These processes were performed using the software
age of 1000 simulated rarefaction curves, while the mean rarefaction
Knight 2013). The mean rarefaction curve for each plot was the aver-

of rarefaction species richness and sampling area (Powell, Chase &

For a given simulation, parameters with check marks (✓✓✓) were included in the simulation, whereas parameters without check marks were not included in the simulation.

Based on the relationships between proportional species loss and N addition rate, we extrapolated our fitted curve and calculated the predicted critical threshold of N rate (N_{crit}), below which there are no reductions in species diversity (Clark & Tilman 2008). Therefore, for a given sampling area, when N addition rates < N_{crit}, species loss would not occur as the extant community is capable to sequester the added N; when N rates > N_{crit}, species richness would be decreased in response to N addition (Bai et al. 2010).

**SAR DECOMPOSITION**

To partition the relative contribution of the four potential mechanisms responsible for N-induced changes in the slope (z-value) and intercept (c-value) of SAR, we created a set of null models based on sampled data using methods of Collins & Simberloff (2009) and Powell, Chase & Knight (2013). For each 25 m² plot of each treatment, SARs were determined and simulated by four factors: species aggregation overall, species abundance distribution, total number of individuals and species richness (Table 1).

We used rarefaction bias to quantify the effect of species aggregation (i.e. inter- and intraspecies aggregation) on changes in the z- and c-values of SAR, and a greater bias indicated higher spatial aggregation (Collins & Simberloff 2009; Powell, Chase & Knight 2013). For each N treatment, the null model was constructed using spatially explicit species abundance data collected in nine 25 m² plots. For each 25 m² plot, we randomized individuals within and among the twenty-five 1 m² quadrats, determined species richness at each sampling area (quadrat size) and constructed the rarefaction curve based on the set of rarefaction species richness and sampling area (Powell, Chase & Knight 2013). The mean rarefaction curve for each plot was the average of 1000 simulated rarefaction curves, while the mean rarefaction curve for each treatment was the average of 9000 simulated rarefaction curves. These processes were performed using the software EstimateS. Rarefaction bias (RB) was calculated as the difference in area between the mean rarefaction curve (non spatially explicit) and empirical SAR (spatially explicit) divided by the area under the empirical SAR, that is RB = (Area_{rarefaction} - Area_{empirical})/Area_{empirical} (Collins & Simberloff 2009; Powell, Chase & Knight 2013). For a given sampling area, per cent rarefaction bias (PRB) was calculated as the difference of species richness between mean rarefaction and empirical SAR divided by the species richness of empirical SAR, that is PRB = (SR_{rarefaction} - SR_{empirical})/SR_{empirical} (Collins & Simberloff 2009).

To examine the effect of species abundance distribution on shifts in the z- and c-values of SAR, we fitted the ranked species abundance distributions for each 25 m² plot by using a log-series model: 

\[ Y = (1/\log(1-c)) \times e^{X/X} \]

where Y is species’ relative abundance, X is rank of species’ relative abundance, and c is coefficient (Powell, Chase & Knight 2013). In the null models, species abundance was calculated by multiplying total number of individuals collected in each 25 m² plot and the relative abundance of each species obtained from the log-series distribution of the same plot. We randomly filled twenty-five 1 m² quadrats based on the number of individuals in each plot and reassigned all individuals randomly in space for each 25 m² plot. We determined the average species richness at each sampling area (i.e. 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 15, 16, 20 and 25 m²). Then, the average species richness was divided by (1 + per cent rarefaction bias) to get the simulated species richness at each sampling area (Collins & Simberloff 2009). By the set of simulated species richness and sampling areas, we constructed the simulated SAR using power model (S = cA²). We replicated each simulation 1000 times and then calculated the mean simulated SAR. All simulations were conducted with r 2.14 (R Development Core Team).

As our experiment was a random block design with nine blocks, we compared simulated SARs between N treatment plots and control plot (zero N) from the same block for examining the N effects on the slopes and intercepts of SARs in log-slog space. For a given pair of N treatment and control plots, we generated five simulations using the parameters as shown in Table 1. Simulations 1 and 2 represent observed SARs. Simulations 3, 4 and 5 changed the parameters to test the contribution of each mechanism. The contribution of each mechanism was calculated by subtracting the z- or c-values of the simulations (Powell, Chase & Knight 2013). Specifically, the contribution of the four potential mechanisms to changes in the z-value of SAR was calculated as follows: overall species richness = S_0 - S_1; total number of individuals = S_1 - S_3; species abundance distribution = S_4 - S_5; species aggregation = S_5 - S_6; and total effect = S_6 - S_1 (S_1 - S_3 represent the c-value of simulated SAR from simulations 1–5, respectively). We repeated these processes to partition the mechanisms contributing to the changes in the c-value of SAR.

---

**Table 1.** Parameters of 25 m² plots used for each simulation to partition mechanisms contributing to changes in the slope and intercept of species–area relationship (SAR). Parameters were calculated from control and N addition plot. Simulations 1 and 2 represented observed SAR of control and N addition plot, respectively. Simulations 3, 4 and 5 change parameters to test the contribution of each mechanism. The contribution of the four potential mechanisms to changes in the z- and c-value of SAR was calculated as follows: overall species richness = S_0 - S_1; total number of individuals = S_1 - S_3; species abundance distribution = S_4 - S_5; species aggregation = S_5 - S_6; and total effect = S_6 - S_1 (S_1 - S_3 represent the z-value of simulated SAR from simulations 1–5, respectively).

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Overall species richness</th>
<th>Number of individuals</th>
<th>Species abundance distribution</th>
<th>Species aggregation</th>
<th>Overall species richness</th>
<th>Number of individuals</th>
<th>Species abundance distribution</th>
<th>Species aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>4</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>5</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS v. 8.0 (SAS Institute, Cary, NC, USA). Two-way ANOVAs were performed on the effects of N treatment, sampling area and their interactions on species richness and proportional species loss. Regression models were used to determine the relationships between N addition rate and biodiversity responses (adjustments for nonlinearity were made as needed).

Results

N EFFECTS ON SPECIES RICHNESS ACROSS DIFFERENT SAMPLING AREAS

Two-way ANOVAs on the effects of N treatment (N), sampling area (A) and their interactions on species richness (SR) and absolute species loss (ASL) showed that the effects of N addition and sampling area on SR and ASL were highly significant ($P < 0.0001$), with their interactions being non-significant (Table 2). Effects of N treatment on proportional species loss were also highly significant ($P < 0.0001$), while the effects of sampling area were not significant ($P = 0.2375$; Table 2). After 10 years of treatment, N addition significantly ($P < 0.05$) decreased species richness at each sampling area for most treatments except the treatment of $1.75 \text{ g N m}^{-2} \text{ year}^{-1}$ (Fig. 2a). There was no significant ($P > 0.10$) difference in species richness between control and treatment of $1.75 \text{ g N m}^{-2} \text{ year}^{-1}$ across all sampling areas in our study (Fig. 2a; Table S2). At the treatment of $5.25 \text{ g N m}^{-2} \text{ year}^{-1}$, species richness was not significantly different ($P > 0.10$) from that of control when sampling area $\geq 20 \text{ m}^2$ (Fig. 2a; Table S2).

<table>
<thead>
<tr>
<th>Response</th>
<th>Term</th>
<th>d.f.</th>
<th>$F$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species richness</td>
<td>N</td>
<td>5</td>
<td>201.92</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>13</td>
<td>89.24</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>N $\times$ A</td>
<td>65</td>
<td>0.54</td>
<td>0.9996</td>
</tr>
<tr>
<td>Absolute species loss</td>
<td>N</td>
<td>4</td>
<td>201.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>13</td>
<td>3.73</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>N $\times$ A</td>
<td>52</td>
<td>0.54</td>
<td>0.9996</td>
</tr>
<tr>
<td>Proportional species loss</td>
<td>N</td>
<td>4</td>
<td>214.86</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>13</td>
<td>1.25</td>
<td>0.2375</td>
</tr>
<tr>
<td></td>
<td>N $\times$ A</td>
<td>52</td>
<td>0.07</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Table 2. Results of two-way ANOVAs on the effects of N treatment (N), sampling area (A) and their interactions on species richness, absolute species loss and proportional species loss.

![Fig. 2.](https://example.com/image2.png) Effects of N addition on (a) species richness, (b) percentage of overall species richness, (c) absolute species loss and (d) proportional species loss across different sampling areas. For a given sampling area, percentage of overall species richness was the ratio of species richness to overall species richness of 25 m² plot. Absolute species loss was calculated as the difference of species richness between control and each N treatment (i.e. Richnesscontrol – Richnessmean). Proportional species loss was calculated as the ratio of species loss in each N treatment to the mean species richness in control (i.e. 1 – Richnessmean/Richnesscontrol). The values for each treatment were the average of nine replicates (error bars denote SEM). Error bands in Fig 1c and d were standard errors at each sampling area in control plots. Arrows indicated the least sampling area that species richness of N addition plots was not significantly different ($P > 0.10$) from control.

Absolute species loss (i.e. \( \text{Richness}_{\text{control}} / \text{Richnesstreat} \)) responded nonlinearly to sampling area \((P < 0.05; \text{Fig. 2c})\). At sampling area <8 m\(^2\), the absolute species loss increased with sampling area; but when sampling area >8 m\(^2\), species loss was relatively constant or even decreased (Fig. 2c). For example, at the sampling area of 1 m\(^2\), mean species richness of control and 5.25 g N m\(^{-2}\) year\(^{-1}\) treatment was 8.36 and 7.04, respectively, and absolute species loss was 1.32. At the sampling area of 8 m\(^2\), mean species richness of these two treatments was 16.41 and 13.36, respectively, and absolute species loss increased to 3.05. At the sampling area of 25 m\(^2\), mean species richness of these two treatments was 20.00 and 17.89, respectively, and absolute species loss decreased to 2.11 (Fig. 2c).

Proportional species loss (PSL, i.e. \( 1 - \text{Richness}_{\text{treat}} / \text{Richness}_{\text{control}} \)) significantly decreased with increasing sampling area for all N treatments \((\text{mean } R^2 = 0.82, \ P < 0.0001; \ \text{Fig. 2d})\). Relationships between PSL and N rate were well fitted by loglinear models at all sampling rates, that is \( \text{PSL} = \text{Intercept} + \text{Slope} \times \log (N) \) \((R^2 > 0.90, \ P < 0.01; \ \text{Fig. 3a and b})\). Slope of PSL–N relationship significantly decreased with increasing sampling area \((R^2 = 0.54, \ P = 0.0017; \ \text{Fig. 3c})\). We extrapolated the fitted curve of PSL–N relationship and calculated the predicted critical threshold of N rate \((N_{\text{crit}})\) in which no species loss occurred at given sampling area. \( N_{\text{crit}} \) was 1.14 (95% CI 0.52–2.45) and 1.74 (95% CI 0.52–5.44) g N m\(^{-2}\) year\(^{-1}\) at sampling areas of 5 and 25 m\(^2\), respectively (Fig. 3d).

SPECIES–AREA RELATIONSHIPS (SARS)

N addition did not change the shape of SARs, and the SARs were best fitted by power-law model \((\text{mean } R^2 = 0.97; \ \text{Fig. 2a})\). Compared to control, SARs in N addition treatments saturated more slowly (Fig. 2b). For example, to encounter 90% of overall species richness in each plot, the minimum sampling area was about 13, 16 and 19 m\(^2\) for treatment of control, 5.25 and 28.00 g N m\(^{-2}\) year\(^{-1}\) respectively (Fig. 2b).

PLANT COMMUNITY COMPOSITION AND STRUCTURE

Compared to control plots, total number of individuals were slightly decreased at low N addition rates (1.75 and...
5.25 g N m$^{-2}$ year$^{-1}$), but increased at high N addition rates (10.50, 17.50 and 28.00 g N m$^{-2}$ year$^{-1}$) by 36% (Fig. 5b). At the low N addition rates, decreases of individual numbers were mainly due to loss of small-sized species, *Carex korshinskyi* (perennial sedge). This species had the largest number of individuals among all species in control, but it was competitively excluded after N addition, leading to the decreased total individuals in the community. At the high N addition rates, the increase of individual numbers was due to the increase of a few N-favoured annual species (i.e. *C. glaucum* and *A. amaranthoides*).

N addition decreased the abundance of rare species and altered species abundance distribution (Fig. 5c). Shannon evenness firstly increased at lower N addition rates and then declined at higher N addition rates ($R^2 = 0.79, P = 0.0442$; Fig. 5c). To evaluate the effects of species aggregation on SAR, we calculated the rarefaction bias, with greater rarefaction bias indicating greater species aggregation. After N addition, rarefaction bias was significantly increased from 6.48% in control to 10.33% at the three highest N addition rates ($R^2 = 0.91, P = 0.0021$; Fig. 5d), indicating that plant species in N-fertilized communities were more aggregated.

**Discussion**

Our study provides a critical test of the scale dependence of N effects on species richness with a gradient of N addition rates. Although N addition decreased species richness across all scales, the proportional loss of species richness decreased with increasing sampling area. Meanwhile, the predicted critical threshold ($N_{\text{crit}}$) of species loss increased with sampling area and ranged from 1.1 to 1.7 g N m$^{-2}$ year$^{-1}$, which is greater than a previous study based on 0.5 m$^2$ quadrat (0.85 g N m$^{-2}$ year$^{-1}$) (Lan & Bai 2012). Although the predicted $N_{\text{crit}}$ is still within the range of $N_{\text{crit}}$ (0.5–2.0 g N m$^{-2}$ year$^{-1}$) reported from North American and European grasslands (Clark & Tilman 2008; Bobbink et al. 2010), our study clearly demonstrates that both the sensitivity ($N_{\text{crit}}$) and magnitude of species loss in response to N addition are scale dependent. The regional species pool is less sensitive to N addition, and the $N_{\text{crit}}$ could be greater than this range at much larger scales. Our results are consistent with two other studies that N-induced species loss depends on scales (Crawley et al. 2005; Chalcraft et al. 2008). Our findings from long-term experimental studies in the Eurasian steppe, combined with those of related studies in European and North American terrestrial herbaceous plant communities (Crawley et al. 2005; Chalcraft et al. 2008), clearly suggest that N-induced species loss was generally mitigated at larger spatial scales. In addition, previous studies showed that N-induced species loss increased with experiment duration (Clark & Tilman 2008; Lan & Bai 2012). A recent study also found that the N effects on slope of SAR increased with experiment duration (Lepš 2014). Therefore, the effects of spatial scale on biodiversity loss may also depend on the duration of N enrichment.

Our results demonstrated that the scale dependence of N-induced biodiversity loss was characterized by changes in the slope and intercept of species–area relationship (SAR). Through SAR decomposition, we found that contributions to changes in SAR were highly uneven among four potential mechanisms, including overall species richness, number of individuals, species abundance distribution and species...
aggregation. Changes in overall species richness and species abundance distribution significantly affected SAR after N addition, supporting the findings that these two factors were key to SAR (He & Legendre 2002; Tjørve et al. 2008; Chase & Knight 2013). The observed increases in the slope of SAR were mainly caused by the positive effect of changes in species abundance distribution over the negative effect of decline in overall species richness. Contrary to other studies (He & Legendre 2002; Tjørve et al. 2008; Chase & Knight 2013; Powell, Chase & Knight 2013), changes in total number of individuals had no significant effect on SAR in response to N addition. It seems likely that the total number of individuals in our study were so high (>100 individual m$^{-2}$) that increasing individual numbers after N addition had a few effects on SAR. At low N addition rates (≤ 5.25 g N m$^{-2}$ year$^{-1}$), the total number of individuals decreased because of the decline of C. korshinskyi, a small and short sedge species. However, at the high N addition rates (≥ 10.5 g N m$^{-2}$ year$^{-1}$), the total number of individuals was increased. The increase in the total number of individuals at the high N addition rates was mainly caused by two reasons. First, in the highest N treatments, bunchgrasses (i.e. S. grandis, A. cristatum) were replaced by a large number of tall annual species (e.g. A. amaranthoides, C. glaucum); thus, the total individual number increases. Secondly, without N enrichment, plant above-ground cover was discontinuous in semi-arid grasslands because of strong below-ground competition (e.g. N and water) (Burke et al. 1998; Bai et al. 2008). The high levels of N inputs removed N limitation for plants, reduced below-ground competition and thereby increased the total number of individuals, which decreased community openness and increased above-ground vegetation cover (Bai et al. 2010; Lan & Bai 2012). Therefore, changes in species composition and decreased below-ground competition were attributable to the increased total number of individuals at the three highest N addition rates.

Moreover, the increases in species aggregation in response to N addition also had little effect on the slope SAR, which is consistent to previous findings (Tjørve et al. 2008). Effect size of species aggregation on SAR has been suggested to increase with spatial heterogeneity and the extent of study (Tjørve et al. 2008; Scheiner et al. 2011). The experimental area in our study is comparatively homogeneous, and the extent of study is relatively small (25 m$^2$) for each SAR. These factors could contribute to the limited effects of changes in species aggregation on SAR in our study. The N-induced decreases in the intercept (c-value) of SAR were mainly due to negative effects of changes in species abundance distribution and species aggregation. The decline in
changes in species abundance distribution over the negative effect of losses in overall species richness. The effects of N on overall species richness and species abundance distribution differ among ecosystems (Hillebrand et al. 2007; Chalcraft et al. 2008), and thus, slopes of SARs show context-dependent responses to N addition (Crawley et al. 2005; Lépê 2014). Meanwhile, as sampling increases to include more area and spatial heterogeneity, changes in SAR will be driven more by species aggregation at larger scales (Keil et al. 2011). Future efforts should include more ecosystem types and larger ranges of scales to identify how site-specific characteristics (i.e. moisture, temperature and spatial heterogeneity) influence N effects on slope of SAR, which is the key to understand the scale-dependent effects of N on biodiversity. Will regional species pool be maintained as z-value increases after N addition? Our findings showed that, at the treatments of 5.25 g N m$^{-2}$ year$^{-1}$, species richness decreased at small scales, but it was not significantly different from control when sampling area ≥20 m$^2$. Above this rate of N addition, species richness declined at larger scales. Chalcraft et al. also found that, among 11 experiments which suffered N-induced plant diversity loss at small scales, only three of them maintained diversity at large scales after N addition (Chalcraft et al. 2008). In our study, the proportional species loss (i.e. $1 - \text{Richness}_{\text{treat}}/\text{Richness}_{\text{control}}$) and sampling area were significantly negatively correlated. By extrapolating the fitted curve of proportional species loss and sampling area, we predict that the smallest sampling area with no species loss compared to control is 41, 54, 85, 125 and 165 m$^2$ at the N addition rate of 1.75, 5.25, 10.50, 17.50 and 28.00 g N m$^{-2}$ year$^{-1}$, respectively. Large-scale observational studies along N deposition gradients should be conducted as a first test of these predictions.

Our study has important implications for the biodiversity conservation and ecosystem management of the vast semi-arid grasslands in the Eurasian Steppe region. First, our results suggest that both the critical threshold and magnitude of N-induced biodiversity loss are scale dependant. These findings highlighted the urgency of reassessing the previously estimated values of critical loads, which have been widely used as the backbone of biodiversity conservation and environmental legislation on transboundary pollution in Europe and United States (Posch, Vries & Hettelingh 1995; Sala et al. 2000; Pardo et al. 2011). Secondly, our findings demonstrate that the scale dependence of N-induced biodiversity loss is mainly triggered by reductions in overall species richness and changes in species abundance distribution. It has also been predicted that N deposition will continue to increase in this century (Galloway et al. 2008). Therefore, future biodiversity conservation should put high priorities on the grassland management practices that could efficiently mitigate the negative consequences of N-induced decreases in species richness and shifts in species dominance, such as mowing or grazing (Collins et al. 1998).

**Acknowledgements**

We thank Dunmei Lin, Xinhuang Zhang, Kristin Powell, Xiujuan Qiao, Zhiyao Tang, Lorraine Clarke and Liyin Liang for help with data analysis, and Xue

---

**Fig. 6.** Contribution of changes in overall species richness, number of individuals, species abundance distribution and species aggregation to the changes in (a) slope ($z$-value) and (b) intercept ($c$-value) of the species–area relationship (SAR) after N addition. For each treatment, the values were the average of nine replicates (error bars denote SEM).

---

overall species richness, in contrast, had no significant effects on the intercept of SAR. The result was inconsistent with the predictions of species pool hypothesis that species richness at small scales (associated with intercept of SAR) was determined by species pool size (Rosenzweig 1995; Pärtel et al. 1996; Zobel 1997). Following N addition, the decline of overall species richness was mainly caused by the loss of rare species, which had limited contribution to community species richness (or $c$-value) at small scales. Given that N addition intensified interspecific competition, the results support the hypothesis that strong species interactions would lead to curvilinear local–regional relationships, that is regional species richness changes had little effect on the local assemblage (Cornell & Lawton 1992).

Our results suggest that without identifying the scaling effects, we may substantially overestimate biodiversity losses associated with regional N deposition. For example, at addition rate of 5.25 g N m$^{-2}$ year$^{-1}$, the proportional species loss was 16–20% at the sampling area of 1–4 m$^2$; however, it was only 11% at the sampling area of 25 m$^2$. Increase in the slope ($z$-value) of SAR was in response to the positive effect of...
Species richness and species abundance data are available from the Dryad Digital Repository (Lan et al. 2015).

References


Received 12 October 2014; accepted 9 March 2015
Handling Editor: Will Cornwell

Supporting Information
Additional Supporting Information may be found in the online version of this article:

Figure S1. Comparisons of species area relationships (SARs) built from two different sampling schemes (Scheiner 2003).

Figure S2. Statistics of eight candidate models for the species area relationships (SARs).

Table S1. Hypotheses regarding the effects of N addition on the intercept (c-value) and slope (z-value) of species area relationship.

Table S2. Duncan’s multiple range tests reported from one-way ANOVA among different N addition rates at each sampling area.
Supporting Information

Table S1 Hypotheses regarding the effects of N addition on the intercept (c-value) and slope (z-value) of species area relationship.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Mechanisms</th>
<th>Predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species pool</td>
<td>N enrichment increases the loss of rare species and thus decreases species pool.</td>
<td>c↓</td>
</tr>
<tr>
<td>Individual numbers</td>
<td>N enrichment leads to assemblage-level thinning and thereby decreases the total number of individuals.</td>
<td>c↓,</td>
</tr>
<tr>
<td>Species abundance distribution</td>
<td>N enrichment increases the dominance of a few N-favored species and decreases the evenness of community.</td>
<td>c↓,</td>
</tr>
<tr>
<td>Species aggregation</td>
<td>N enrichment increases recruitment limitation, decreases the success of species dispersal, and thus increases species aggregation.</td>
<td>c↓,</td>
</tr>
</tbody>
</table>

Note: response types: ↓, decreased; ↑, increased.
Table S2. Duncan’s multiple range tests reported from one-way ANOVA among different N addition rates at each sampling area.

<table>
<thead>
<tr>
<th>Area (m²)</th>
<th>N addition rate (g N m⁻² yr⁻¹)</th>
<th>Df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.75 5.25 10.50 17.50 28.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>a     ab   bc   cd   de   e</td>
<td>5</td>
<td>13.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>16.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>18.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>18.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5</td>
<td>a     ab   bc   cd   d    e</td>
<td>5</td>
<td>18.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>18.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>8</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>17.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>9</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>16.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>16.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>15.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>15</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>14.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>16</td>
<td>a     ab   b    c    cd   d</td>
<td>5</td>
<td>13.94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>20</td>
<td>a     a    ab   bc   cd   d</td>
<td>5</td>
<td>12.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25</td>
<td>a     a    ab   bc   cd   d</td>
<td>5</td>
<td>9.94</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: treatments with the same letter were not significantly different in the tests.
Figure S1. Comparisons of species area relationships (SARs) built from two different sampling schemes (Scheiner 2003). For each 25 m² plot, SARs were built from two schemes: type I referred to strictly nested quadrats, and type IIA referred to spatially explicit quadrates arrayed in a contiguous grid. SARs were fitted by power model, i.e. $S = c A^z$; $z$ and $c$ value were the slope and intercept of the model, respectively.
Figure S2. Statistics of eight candidate models for the species area relationships (SARs). The value of each statistic was the average of 54 SARs (error bars denote SEM). Bars with the same letter were not significantly different in Duncan’s multiple range tests reported from one-way ANOVA.