

Effects of the frequency and the rate of N enrichment on community structure in a temperate grassland

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Abstract

Aims

Nitrogen (N) enrichment caused by human activities threatens biodiversity and alters plant community composition and structure. It has been found that heavy and infrequent N inputs may overestimate species extinction, but it remains unclear whether plant community structure will equally respond to frequent reactive N enriched conditions.

Methods

We independently manipulated the rates and the frequencies of N addition in a temperate steppe, northern China, between 2008 and 2013.

Important Findings

We found that plant community structure changes, measured by 'Euclidean distance' involving species richness, composition and productivity, were significantly positively related to increasing N enrichment rates rather than frequencies. Changes in aboveground net primary productivity (ANPP), plant species richness and shifts in dominant species were observed. Community ANPP increased with

N enrichment, whereas species richness reduced. The frequency of N enrichment increased species richness but had no impacts on community ANPP and the relative ANPP of the two dominant species, C₃ perennial bunchgrass *Stipa grandis* and C₃ perennial rhizome grass *Leymus chinensis*. The ANPP and relative ANPP of the two dominant species were significantly negatively correlated with each other. Moreover, changes in the relative ANPP of *S. grandis* was negatively associated with the changes in community structure. After 5 years' treatment, direct influence of the frequency of N enrichment on plant community structure was not observed, but the effects of the rate of N enrichment were apparent. Our results suggested that further study in various ecosystems and with long-term and well-controlled comparisons the frequency vs. the rate of N enrichment may still be needed.

Keywords: biodiversity, dominance effect, nitrogen addition frequency, nitrogen deposition, productivity

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INTRODUCTION

Biodiversity largely underpins ecosystem functioning and services on which we all rely (Bleeker *et al.* 2011; Cardinale *et al.* 2012). However, biodiversity is threatened by reactive nitrogen (Nr) enrichment with both intentional (fertilization) and unintentional (atmospheric deposition) addition of Nr generated by human activities worldwide (Canfield *et al.* 2010; Stevens *et al.* 2010; Vitousek *et al.* 1997). In some countries, such as the USA and China, there are regions where atmospheric N deposition has increased about 5- to 20-time over pre-industrial era (Galloway *et al.* 2008), primarily as a consequence of fossil fuel application and N fertilizer used for increasing in food and energy production to keep the pace of rapid world population growing (Gerland *et al.* 2014; Godfray and Garnett 2014). With the predicted continuous growth of the world population in this century (Gerland *et al.* 2014), atmospheric N deposition will continue to increase in the coming decades (Sutton and Bleeker 2013). It has been predicted that Europe, North America and East Asia continue to be hot spots of atmospheric N deposition (Dentener 2006). Recent estimates reported an average annual N deposition rate of $\sim 2 \text{ g N m}^{-2}$ in China (Zhao *et al.* 2017), with larger than 10 g N m^{-2} in some areas of the North China Plain between 2005 and 2007 (He *et al.* 2010). In Europe, although efforts have been made to restrict the emissions of reactive N from human activities, deposition was predicted to continue increasing in coming years (Sutton and Bleeker 2013).

Nitrogen enrichment may alter plant community composition and structure by reducing biodiversity (Bobbink *et al.* 2010; Stevens *et al.* 2004; Talhelm *et al.* 2013), increasing community biomass (Isbell *et al.* 2013; Stevens and Gowing 2014; Stevens *et al.* 2015; Vankoughnett and Henry 2014; Zhang *et al.* 2015) and altering dominant species (Bai *et al.* 2010; Wilberts *et al.* 2013). Some calcifuge and ammonium-loving species with clonal and tall stature traits can grow well with N enrichment in N limited ecosystems (Azevedo *et al.* 2013; Dickson *et al.* 2014; Field *et al.* 2014; Suding *et al.* 2005), whereas other acid and ammonium-sensitive species may be excluded (Azevedo *et al.* 2013; Bobbink *et al.* 2010; Stevens *et al.* 2004; Zhang *et al.* 2014b). Many previous studies have described the changes in plant community structure from the aspects of alteration in biodiversity, species richness, species composition and biomass/productivity separately. However, fewer studies have employed an integrated measure of temporal variability in community structure combining species richness, composition and productivity (Collins *et al.* 2000; He *et al.* 2011).

The frequency of N enrichment may play a vital role in determining the effects of reactive N inputs (Smith *et al.* 2009; Zhang *et al.* 2016b). Large numbers of controlled N application experiments on evaluation the influences of atmospheric reactive N deposition were often added in either dry or liquid form with only a few additions per year (Smith *et al.* 2009). However, annual atmospheric reactive N deposition, with a

relatively small load at each event, frequently occurs in nature (Aneja *et al.* 2001; Asman *et al.* 1998). To date, few field experiments are conducted to tease apart the impacts of the frequencies and the rates of N enrichment on ecosystem properties and functioning. Recent results from previous studies with low frequency of N addition studies suggested that there might cause biased over-estimates of the impacts on the observed plant species richness (Binkley and Högberg 2016; Zhang *et al.* 2014b), new species colonization (Zhang *et al.* 2016b), and C_3 perennial rhizome grass *Leymus chinensis* leaf chlorophyll content (Zhang *et al.* 2013) under the higher frequency of N enrichment, as a result of the excessively infrequent pulses of reactive N added to these ecosystems. By contrast, it has been reported that the frequency of N inputs had few effects on community aboveground productivity (Zhang *et al.* 2015), the temporal stability of community and its component populations (Zhang *et al.* 2016a), and nitrogen uptake efficiencies (Rens *et al.* 2016). The above-mentioned ecological properties can direct and/or indirect affect the plant community structure. However, it remains unclear whether the frequency of N enrichment has an effect on plant community structure.

The temperate steppe, which is located in the east of Eurasian continent, is floristically diverse and productive (Kang *et al.* 2007). Many areas experience low atmospheric N deposition with few industrial and agricultural activities (Zhao *et al.* 2017), making it an excellent model ecosystem for studying the influences of N enrichment via either fertilization or atmospheric deposition on plant community structure and ecosystem functioning. On the basis of a field experiment that included nine rates of annually N enrichment (0 to $50 \text{ g N m}^{-2} \text{ year}^{-1}$) crossed with two frequencies of N enrichment (twice or monthly year^{-1}) as ammonium nitrate (NH_4NO_3) for six years (i.e. 2008–2013) in a temperate grassland, we assessed the effects of the rates and the frequencies of N enrichment on the temporal alterations of plant community structure. We hypothesized that the rate of changes in community structure will be altered by increasing N enrichment rates. Moreover, given the greater negative impacts on plant species richness and species gains of the lower frequency of N enrichment (Zhang *et al.* 2014b, 2016b), we hypothesized that there might be a greater influence on plant community structure at the lower frequency of N enrichment.

MATERIALS AND METHODS

Study site

The field experiment ($116^\circ 14' \text{E}$, $43^\circ 13' \text{N}$) was conducted in a temperate grassland near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS; $116^\circ 42' \text{E}$, $43^\circ 38' \text{N}$), which was located in the Xilin River Basin, northern China. A 50-ha degraded temperate grassland which was overgrazed due to uncontrolled heavy sheep grazing since the early 1980s had been fenced since 1999 to exclude ungulate animal grazing (Bai *et al.* 2010). The topography of the experimental

area was flat, ranging from 1255 to 1260 m. The long-term (1980–2013) mean annual temperature was 0.9°C, with mean monthly temperatures range of –21.4 to 19.7°C. The mean annual precipitation was 351.4 mm, with ~72.8% falling from May to August (i.e. the growing season). The soil was classified as a Haplic Calcisol by the Food and Agriculture Organization of the United Nations (FAO) soil classification system. Two dominant species, C₃ perennial bunchgrass *Stipa grandis* and C₃ perennial rhizome grass *L. chinensis*, together accounted for >60% of the peak community aboveground biomass (Zhang et al. 2015). No fertilizers were added prior to this experiment (Zhang et al. 2014a). The ambient total annual atmospheric N deposition was still <1.5 g N m⁻² in recent years (Zhao et al. 2017).

Experimental design

The field experiment, following a randomized block design, was administrated in September 2008 (Zhang et al. 2017). In brief, nine rates of N enrichment (0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m⁻² year⁻¹) crossed with two frequencies of N enrichment (2 times vs. 12 times N addition year⁻¹) were conducted. An unamended control treatment (control) was also set up. Hence, 19 experimental treatments in total were in this study. Each treatment was presented in each of 10 blocks. The use of higher rates of N enrichment provided a proxy for long-term extreme N enrichment on temperate grassland ecosystem. Moreover, the lower frequency of N enrichment (i.e. 2 N additions year⁻¹) was a kind of proxy for infrequent N enrichment in controlled N experiments (Smith et al. 2009), while the higher frequency treatment (i.e. 12 N additions year⁻¹) was used to mimic the frequent atmospheric N deposition (Aneja et al. 2001; Zhang et al. 2014b). Each treatment plot was 8 m × 8 m. NH₄NO₃ (>99%) application started on 1 September 2008 and continued on the first day of each month thereafter for the higher frequency treatment (i.e. 12 N additions year⁻¹), and started on 1 November 2008 and continued on the first day of June and November thereafter for the lower frequency treatment (i.e. 2 N additions year⁻¹). During the growing season from May to October, NH₄NO₃ was weighed and mixed with distilled water (9.0 l total for all treatments receiving purified water: either 9.0 l once in June for the lower frequency treatments or 1.5 l monthly from May to October for the higher frequency ones), and sprinkled uniformly using a back sprayer to each plot to simulate wet N deposition. There was estimated that <1 mm water was annually added to each plot including the zero N enrichment plots, but no water was added to the control plots. During winter (from November to next April), NH₄NO₃ was mixed with sands (to ensure even broadcast; 0.5 kg total for each treatment receiving sands; either 0.5 kg once in November or 0.08 kg monthly from November to next April) and broadcast evenly by hand. Sands were sieved to <1 mm, dipped in hydrochloric acid for 2 days, washed in purified water for three times, and then oven-dried at 120°C for 24 h.

Field sampling

Aboveground net primary productivity (ANPP) and plant species richness were estimated from peak plant aboveground biomass every year. Peak plant aboveground biomass was an acceptable approximation for ANPP as aboveground plant tissues die during the winter season in this region (Bai et al. 2010). Plant aboveground biomass was annually sampled from 2008 to 2013 between 10 and 15 August using a 0.5 m × 2 m quadrat. The quadrats were randomly placed without spatial overlap in each plot among years. To avoid edge effects, the quadrats were no <50 cm inside the border of each plot. All living tissues were sorted to species and oven-dried at 65°C to constant weight. Plant species richness (number of species per m²) were recorded in the quadrat when aboveground biomass was sampling, and >60% of species in the whole 64 m² plot could be found in the 1 m² quadrat (Zhang et al. 2016b).

Statistical analyses

Although sampling quadrats were not in fixed locations within the plots, on the basis of a relative even distribution of species in the sampling quadrats and a strong statistical confidence with 10 repetitions for each treatment (Zhang et al. 2016b), Euclidean distance (*ED*) was employed as measure of plot-scale structural variability of the community (Collins et al. 2000). $ED = \sqrt{\sum_{j=1}^N (x_{ij} - x_{ik})^2}$, where x_{ij} is the ANPP of the i th species in the j th sample, and x_{ik} is the ANPP of the i th species in the k th sample, and N is the total number of species. A larger *ED* means more rapid temporal changes in community structure (Collins et al. 2000). A time-lag analysis of *ED* in the community was performed to determine the rate of changes in plant community structure by denoting the temporal variability of species richness, composition, and productivity as a whole over years (Collins et al. 2000). A data set of the community sampled over 6 year intervals (i.e. from 2008 to 2013) had five 1-year time-lags (e.g. 2008 vs. 2009, 2009 vs. 2010, ... 2012 vs. 2013), four 2-year time-lags (e.g. 2008 vs. 2010, 2009 vs. 2011, etc.) through one 5-year time-lag (i.e. 2008 vs. 2013), giving a total of fifteen time-lags. Linear regressions were calculated for time-lag changes in community structure with the increasing in the time-lag. The time-lags were square root transformed to reduce the probability of bias analysis from the unequal points between smaller and larger number of time-lags. Based on previous suggestions (Collins et al. 2000), if the linear regression is significant and positive, then it implies that the plant community is undergoing directional change. Time-lag analysis of *ED* in plant community can be used to illustrate the pattern and rate of temporal alterations in community structure for the communities whose sampling durations were too short to use traditional approaches (Collins et al. 2000). A response ratio of the effects of N enrichment on the plant community structure was calculated as $\frac{\text{Community structure}_{\text{Treatment}}}{\text{Community structure}_{\text{Control}}}$, in which the

control treatment was completed control (without N, water, and sands additions).

For exploring the effect of the rates and the frequencies of N enrichment on the dominant species, the relative ANPP of species in the plant community was calculated as $\frac{ANPP_i}{ANPP_{COM}} \times 100\%$, where $ANPP_i$ is the i species ANPP in the plant community, and $ANPP_{COM}$ is the plant community ANPP. The relative ANPP of the two dominant species (i.e. *S. grandis* and *L. chinensis*) was calculated.

Repeated-measures analysis of covariance was used to test influences of the rates and the frequencies of N enrichment, year, and their interactions on the relative ANPP of *L. chinensis* and *S. grandis* via using the value in 2008 as a continuous variable.

As community species richness and community structure co-varied with N enrichment, the residuals from regressions of plant community structure with N enrichment (or plant species richness) were used to explore the remaining effects of plant species richness (or N enrichment; Zhang *et al.* 2016a).

Structural equation modeling (SEM) was employed to estimate the strength of the rates and the frequencies of N enrichment, respectively, on plant community structure through the changes in relative ANPP of two dominant species, *S. grandis* and *L. chinensis*, species richness and community ANPP. For homogeneity, the ANPP and relative ANPP of the two dominant species, community ANPP, and community structure were \log_{10} transformed. Data were fitted to the model using the maximum likelihood estimation method. Adequacy of the model was determined by using χ^2 test, root square mean errors of approximation (RMSEA), and Akaike Information Criteria (AIC). Adequate model fits were indicated by a non-significant χ^2 test ($P > 0.05$), low RMSEA (< 0.08) and AIC (Grace 2006). As there were no significant impacts of the frequency of N enrichment on the relative ANPP of *S. grandis* and community ANPP (Zhang *et al.* 2015), direct relationships between N enrichment frequency and these two measures were not included in the SEM of the effect of the frequency of N enrichment.

The AMOS 22.0 (Amos Development Co., Greene, Maine, USA) was employed for SEM analyses. The SPSS software package (SPSS 18.0 for windows, SPSS Inc., Chicago, IL, USA) was used to perform the remaining statistical analyses.

RESULTS

Effects of the rates and the frequencies of N enrichment on two dominant species

There were significant effects of the rates of N enrichment on the relative ANPP of *L. chinensis* (Fig. 1a and c) but not of the frequency of N enrichment on either the relative ANPP of *L. chinensis* (Fig. 1a; Table 1; $F_{1,161} = 1.2$, $P = 0.271$) or *S. grandis* (Fig. 1b; $F_{1,161} = 0.0$, $P = 0.8243$). The relative ANPP of *S. grandis* was significantly increased at the lower frequency of N enrichment, i.e. 2 N additions year⁻¹ (Fig. 1d;

$R^2 = 0.78$, $P < 0.01$), but not at 12 N additions year⁻¹ (Fig. 1d; $R^2 = 0.18$, $P > 0.1$). Repeated-measure analysis of variance showed that there was also a significant annual variability in the relative ANPP of *L. chinensis* (Table 1; $F_{4,644} = 73.7$, $P < 0.001$) and *S. grandis* ($F_{4,644} = 6.7$, $P < 0.001$). Significant interactions between the rates of N enrichment and year for relative ANPP of *L. chinensis* ($F_{32,644} = 3.7$, $P < 0.001$) and *S. grandis* ($F_{32,644} = 3.0$, $P < 0.001$) were detected. The frequencies of N enrichment and year interacted on the relative ANPP of *L. chinensis* ($F_{4,644} = 2.4$, $P = 0.072$). No other interactions were detected on the relative ANPP of *L. chinensis* and *S. grandis* (all $P > 0.1$). The ANPP (Fig. 2a; $R^2 = 0.16$, $P < 0.001$) and the relative ANPP (Fig. 2b; $R^2 = 0.27$, $P < 0.001$) of *L. chinensis* and *S. grandis* were significantly negatively correlated.

Effects of the rates and the frequencies of N enrichment on community structure

Community structure changes were positively related with the increasing N enrichment rates at both 2 (Fig. 3a) and 12 (Fig. 3b) N additions year⁻¹. All the slopes of community-structure vs. time-lag were positive (Fig. 3c) and significantly associated with the increasing rates of N enrichment at both 2 (Fig. 3c; $R^2 = 0.77$, $P < 0.01$) and 12 (Fig. 3c; $R^2 = 0.70$, $P < 0.01$) N additions year⁻¹, respectively. In addition, the slopes of plant community-structure vs. time-lag were positive in both control and enriched N conditions (Fig. 3a and b). The community structure, averaged over time-lags, was significantly increased with the increasing N enrichment rates (Fig. 3d). The response ratio for community structure also significantly increased with the increasing N enrichment rates at either 2 (Fig. 3e; $R^2 = 0.74$, $P < 0.01$) or 12 (Fig. 3e; $R^2 = 0.89$, $P < 0.001$) N additions year⁻¹, respectively.

Results from SEM showed that the rates of N enrichment had both direct and indirect effects on community structure changes (Fig. 4a). Nitrogen enrichment had a significant positive direct effect on community structure (Fig. 4a; standardized effect size: 0.20, $P = 0.031$). Nitrogen enrichment also had significant positive indirect effects (standardized effect size: 0.30) on community structure by altering community ANPP (Fig. 4a; standardized effect size: 0.20), the relative ANPP of *L. chinensis* (standardized effect size: 0.04), and species richness (standardized effect size: 0.03), respectively. The significant direct strengths were smaller than the significantly indirect effects under N enrichment conditions. Overall, N enrichment had positive direct and indirect impacts on the changes in the plant community structure.

The slope of the regression for community-structure vs. time-lag was greater in 2 (solid slope and 95% confidence intervals: 0.61 [0.47 to 0.72]) than that in 12 N additions year⁻¹ (dashed slope and 95% confidence intervals: 0.43 [0.38 to 0.48]; Fig. 3b). Results of SEM showed that the

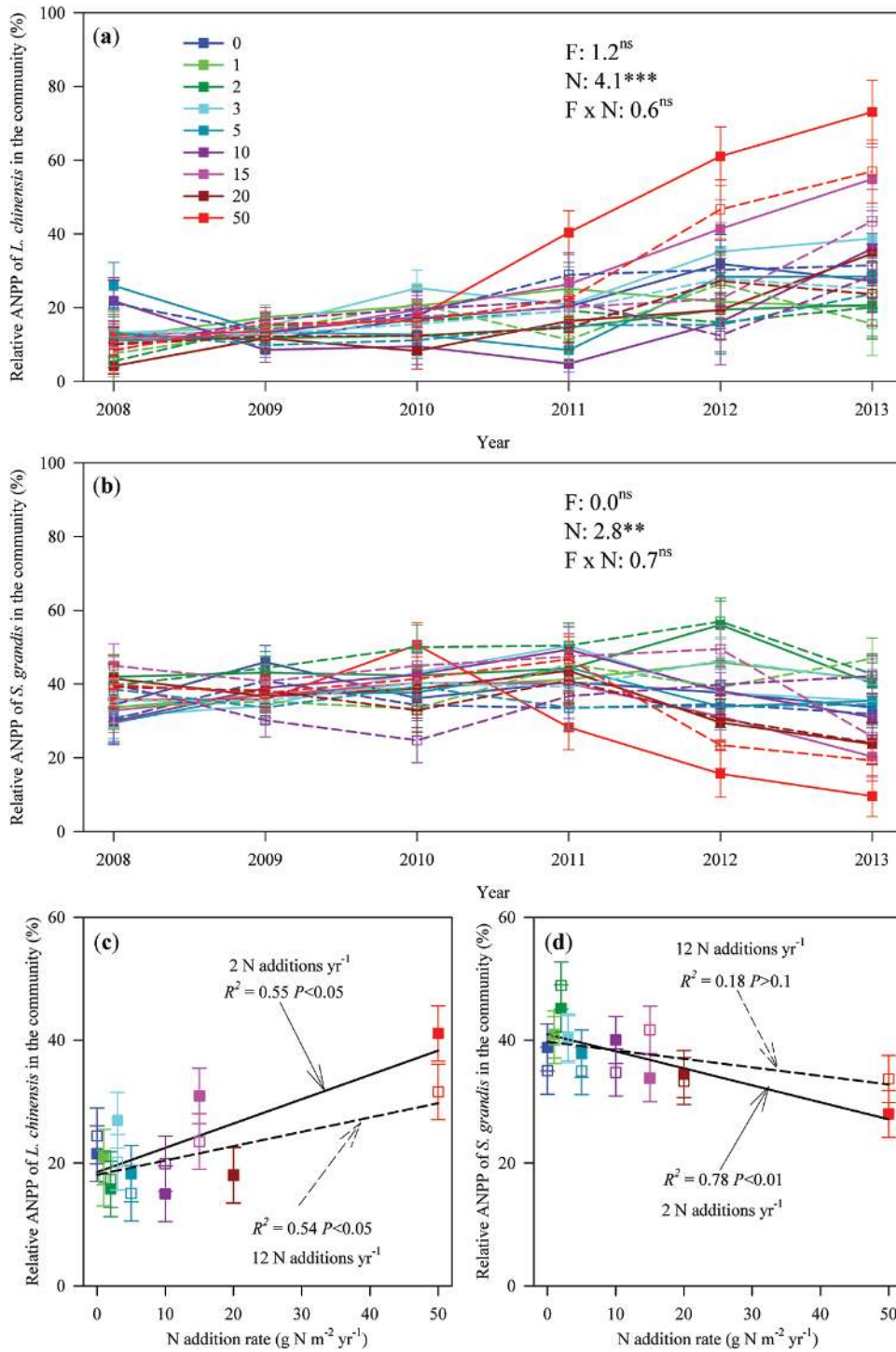


Figure 1: impacts of nitrogen enrichment on the two dominant species (*Leymus chinensis* and *Stipa grandis*). **(a)** Changes in relative aboveground net primary productivity (ANPP) of *L. chinensis* under the frequencies (solid and closed squares = 2 N additions year⁻¹, dashed and open squares = 12 N additions year⁻¹) and the rates (g N m⁻² year⁻¹) of N enrichment. **(b)** Changes in relative ANPP of *S. grandis*. **(c)** Effects of the rates of N enrichment on the relative ANPP of *L. chinensis*. **(d)** Effects of the rates of N enrichment on the relative ANPP of *S. grandis*. F, N enrichment frequencies and N, N enrichment rates. ^{ns}, ** and ***: statistically significant at $P > 0.1$, $P < 0.01$ and $P < 0.001$, respectively. Error bars indicate 1 SE. In a and b, solid and dashed lines corresponding to 2 and 12 N additions year⁻¹, respectively.

frequency of N enrichment had both direct and indirect effects on community structure changes (Fig. 4b). The frequency of N enrichment had a negative direct effect on community structure (Fig. 4b; standardized effect size: -0.10 , $P = 0.088$). The frequency of N enrichment also had significant negative indirect effects on community structure by altering species richness (Fig. 4b; standardized effect size: -0.04 , $P < 0.001$). Although there was no significant effect of the frequency of N enrichment on community structure via the relative ANPP of *L. chinensis* and *S. grandis*, the relative ANPP of *L. chinensis* was significantly positive, either directly or indirectly associated with community structure, while the relative ANPP of *S. grandis* was significantly negatively related to community structure (Fig. 4b).

Table 1: results of repeated-measures analysis of covariance for exploring the effects of the rates of N enrichment (N), the frequencies of N enrichment (F), year (Y) and their interactions on the relative aboveground net primary productivity (ANPP) of two dominant species (i.e. *Stipa grandis* and *Leymus chinensis*) across 2009–2013, using the value in 2008 as a continuous variable (V_0)

	df	<i>Stipa grandis</i>		<i>Leymus chinensis</i>	
		F	P	F	P
V_0	1161	81.9	<0.001	71.8	<0.001
N rates (N)	8161	2.8	0.006	4.1	<0.001
N frequencies (F)	1161	0.0	0.824	1.2	0.271
Year (Y)	4644	6.7	<0.001	73.7	<0.001
N × F	8161	0.7	0.709	0.6	0.767
N × Y	32644	3.0	<0.001	3.7	<0.001
F × Y	4644	1.9	0.117	2.4	0.072
N × F × Y	32644	0.9	0.644	0.8	0.753

F- and P-values were given. df = degrees of freedom.

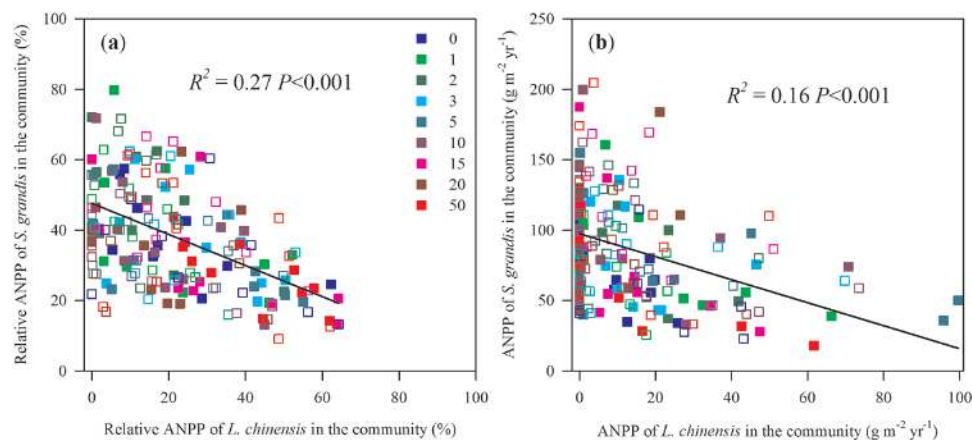


Figure 2: relationship between the two dominant species. (a) Relationship between average relative ANPP of *Leymus chinensis* and *Stipa grandis* in the community. (b) Relationship between the mean ANPP of *S. grandis* and *L. chinensis* under the frequencies (closed squares = 2 N additions year⁻¹, open squares = 12 N additions year⁻¹) and the rates (g m⁻² year⁻¹) of N enrichment.

Plant diversity and community structure

The community structure, averaging over time-lags, was significantly negatively associated with species richness under N enrichment (Fig. 3f; $R^2 = 0.15$, $P < 0.0001$). The residuals (from Fig. 3f) of plant community structure after controlling for the effects of species richness were still enhanced by increases in N enrichment rates at both 2 (Fig. 3g; $R^2 = 0.15$, $P < 0.001$) and 12 (Fig. 3g; $R^2 = 0.05$, $P = 0.048$) N additions year⁻¹, respectively. After controlling for the effect of species richness on community structure, the community structure explained (R^2) by the increasing N enrichment rates was largely reduced at both the low and high frequency of N enrichment. Although species richness was not showed relationships with the residuals (from Fig. 3d) of plant community structure after controlling for the effects of the rate of N enrichment (Fig. 3h; $R^2 = 0.00$, $P > 0.1$), results of the SEMs showed that species richness was significantly negatively associated with plant community structure through community ANPP under N enrichment conditions (Fig. 4; all $P < 0.001$).

DISCUSSION

Consistent with our initial hypotheses, the rate of time-lag alterations in plant community structure was accelerated by the increasing the rates of N enrichment. The significant positive effects of the rate of N enrichment on changes in plant community composition and structure consisted of direct and indirect influences of plant species richness and productivity. The frequency of N enrichment had marginally negative direct and apparently indirect effects through species richness.

Effects of N enrichment

The rate of changes in community structure was significantly accelerated by the increasing N enrichment rates which had

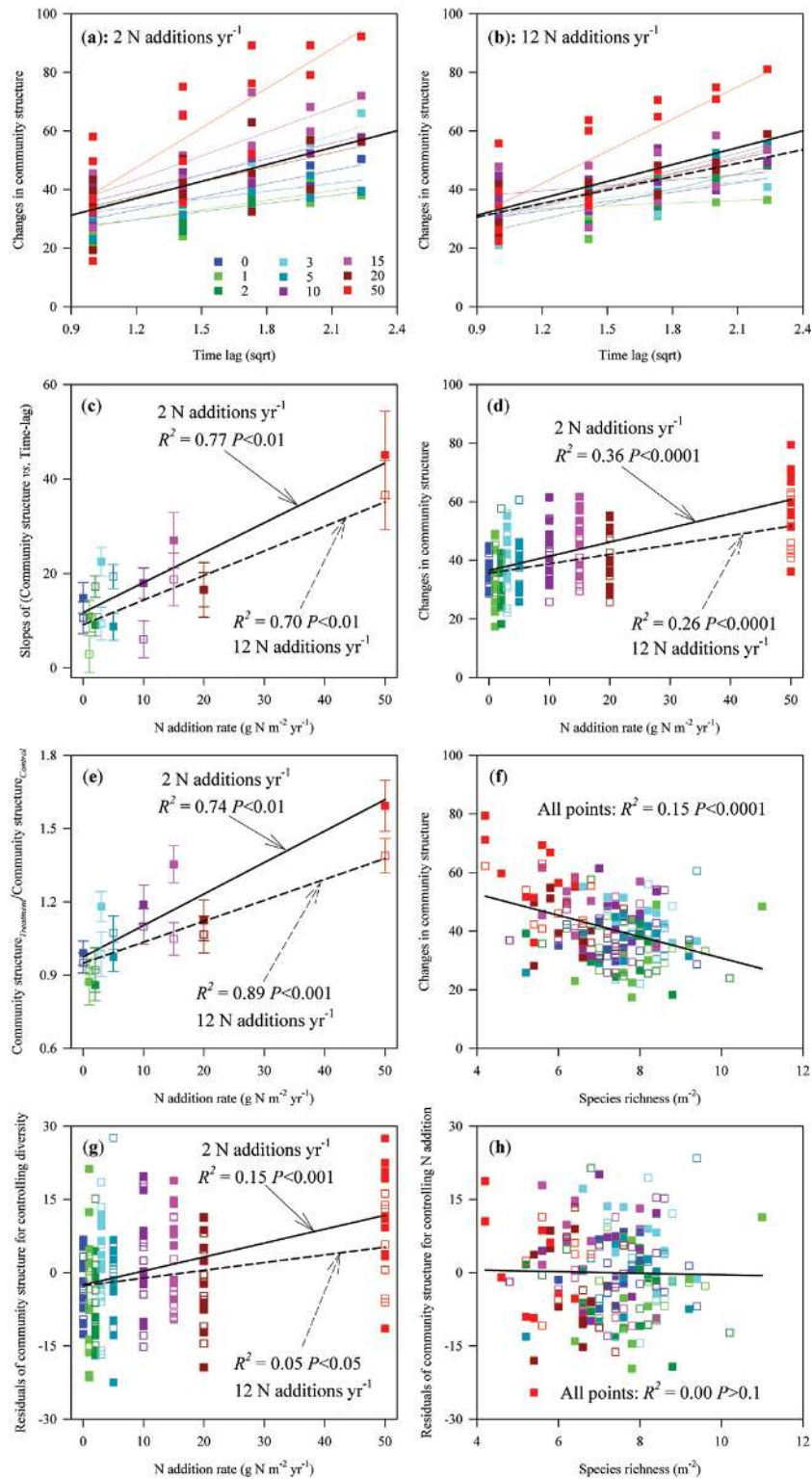


Figure 3: plant community structure and diversity. **(a)** Changes in community structure under 2 N additions year⁻¹. **(b)** Changes in community structure under 12 N additions year⁻¹. In a and b, colored lines depending on the regressions of each N enrichment rate as the function of time-lag. **(c)** The slopes of (Community structure vs. time-lag; from a and b). **(d)** Effects of N enrichment on community structure change. **(e)** The response ratio of (Community structure_{Treatment}/Community structure_{Control}). **(f)** Relationship between species richness and community structure. **(g)** Effects of N enrichment on residuals (from f) of community structure for controlling species richness. **(h)** Relationship between species richness and residuals (from d) of community structure for controlling N enrichment. The frequencies (closed squares = 2 N additions year⁻¹, open squares = 12 N additions year⁻¹) and the rates (g N m⁻² year⁻¹) of N enrichment. Error bars indicate 1 SE.

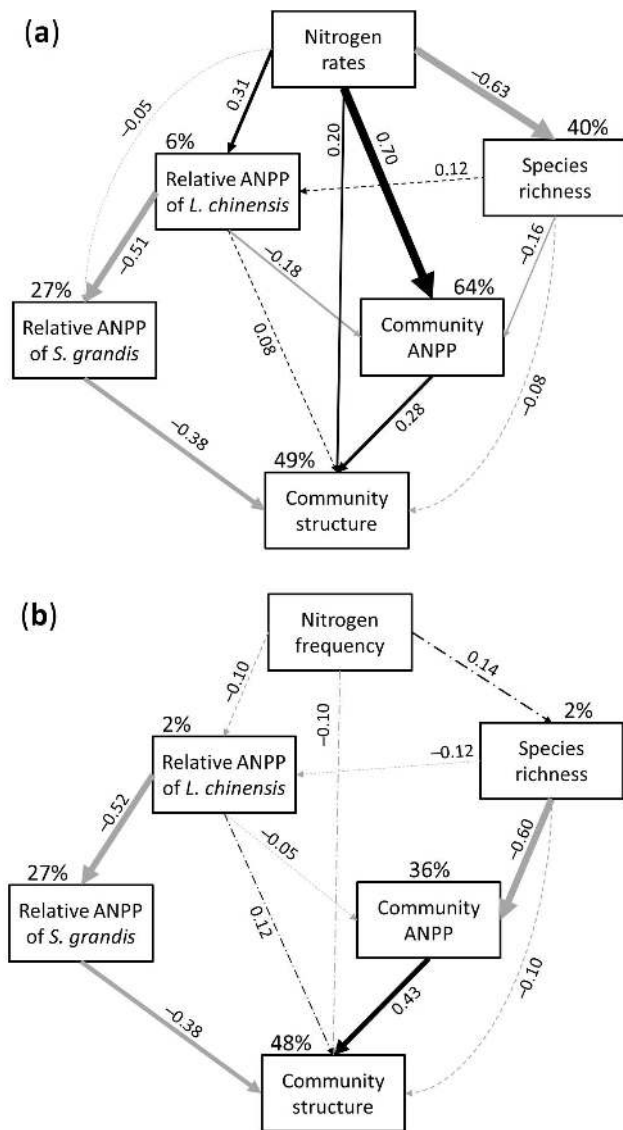


Figure 4: the effect of N enrichment on the alteration in plant community structure relating species richness, relative ANPP of *Stipa grandis*, and *Leymus chinensis*, and community ANPP. (a) The effects of the rate of N enrichment on community structure. The final structural equation modeling (SEM) fitted the data well: $\chi^2 = 0.716$, $df = 2$, $P = 0.699$; RMSEA = 0.000; AIC = 38.716. (b) The effects of the frequency of N enrichment on community structure. The final SEM fitted the data well: $\chi^2 = 4.601$, $df = 4$, $P = 0.331$; RMSEA = 0.029; AIC = 38.601. Numbers adjacent to arrows were standardized path coefficients, and width of the arrows indicated the strength of the relationship. Dashed, long dash dot, and solid arrows indicated $P > 0.1$, $0.1 > P > 0.05$, and $P < 0.05$, respectively. Black and gray arrows indicated positive and negative relationship, respectively. Percentages close to endogenous variables indicated the variance explained by the model (R^2).

significant positive direct and indirect effects by altering community ANPP, species richness and species composition (e.g. the relative ANPP of *L. chinensis*). Our previous reports

(Zhang *et al.* 2014b, 2015; see online supplementary Fig. S1) in this field experiment showed that community ANPP was promoted by the increasing in the rate of N enrichment, whereas species richness was reduced. Species richness was smaller at the lower frequency of N enrichment (Zhang *et al.* 2014b; see online supplementary Fig. S1), while community ANPP was not difference between the two frequencies of N enrichment (Zhang *et al.* 2015; see online supplementary Fig. S1). Nitrogen enrichment was associated with alterations in both species richness and community ANPP, which may directly associate with soil properties (e.g. soil water content [%], soil temperature [°C], soil pH, and N availability [mg kg^{-1} dry soil]) under N enriched conditions (Zhang *et al.* 2014b, 2015; see online supplementary Fig. S2). Consistent with previous reports (Avolio *et al.* 2014; Faust *et al.* 2012; Pauli *et al.* 2002), plant community structure was significantly altered under N enrichment via alteration of species richness, composition and productivity. For example, with the increases in N application rates, aboveground biomass of perennial C_3 grasses, particularly *Elymus repens*, increased and whereas perennial C_4 grasses, particularly *Schizachyrium scoparium*, and rare species, such as *Liatris aspera*, decreased in the grassland experiments in Minnesota (Isbell *et al.* 2013; Wedin and Tilman 1993). Increasing the N addition rates increased the aboveground biomass of a rhizomatous perennial C_3 grass, *L. chinensis*, and tall annuals, *Artemisia sieversiana* or *Chenopodium glaucum*, in Eurasian temperate grasslands (Bai *et al.* 2010; Quan *et al.* 2015). The changes in community structure through alterations in either richness and composition or biomass/productivity separately are relatively well studied under N enrichment conditions with the change direction depending on the extent of N deficit in soil. Our study employed time-lag analysis to illustrate the pattern and rate of temporal changes in community structure (Collins *et al.* 2000). We found that the slopes of community-structure vs. time-lag were positive at both ambient and enriched N conditions, suggesting the changes in community structure might be in the same direction (Collins *et al.* 2000). Hence, adding N may have some benefits to promote ecosystem succession in degrade grasslands (Bai *et al.* 2010). We also found that both the slopes of community-structure vs. time-lag and response ratio of community-structure were significantly promoted by the increasing N enrichment rates. Moreover, this study teased apart the direct and indirect effect sizes of N enrichment on the changes in community structure. We found that N enrichment not only directly affected (i.e. accelerated) the positive direction of changes in community structure but also indirectly altered it by changing the productivity, species richness/composition and shifting in dominant species. In addition, the negative correlation between plant species richness and plant community structure explained by the increasing N enrichment rates was largely reduced at both the low (from 0.36 to 0.15) and high (from 0.26 to 0.05) frequency of N

enrichment. However, we also found that no significant relationship between plant species richness and the residuals of community structure by controlling for the effects of N enrichment was detected. This suggests there could be a stronger effect of N enrichment on plant community structure than that of species richness. With the result, the effect of species richness on community structure might be masked after N enrichment (Loreau 1998; Zhang et al. 2016a).

Effects of the frequency of N enrichment

There was no significant direct ($P = 0.088$) effect of the frequency of N enrichment on the alterations in community structure by changing species richness but the indirect effects were significant. We found that the frequency of N enrichment had significantly positive impacts on species richness but not on both community and population ANPP (Zhang et al. 2014b, 2015; see online supplementary Fig. S1). Thus, the direct effect of the frequency of N enrichment on the plant community structure suggests that assessment of the impacts of N enrichment should consider the changes in species richness, composition, and population- and community-ANPP as a whole. We found that both the ANPP and the relative ANPP of the two dominant species (i.e. *S. grandis* and *L. chinensis*) were negatively correlated, suggesting that appearance of the community may be changed as the increases in both abundance and relative abundance of *L. chinensis* after N enrichment. We also found a greater impact of N enrichment on the relative abundance of two dominant species at a lower frequency of N enrichment. Communities shifted from *S. grandis* and *L. chinensis* co-dominated in the control plots to *L. chinensis* dominated at the higher rates and the lower frequency of N enrichment. *L. chinensis* is favored by higher N enriched conditions (Bai et al. 2010) and its leaf chlorophyll content is higher at the lower frequency of N enrichment (Zhang et al. 2013). In contrast, *S. grandis* is favored by N limited conditions in Eurasian steppe (Lü et al. 2015). These findings suggest that the two co-dominant species of the plant community may ultimately shift to *L. chinensis* under N enriched conditions and such changes would be more rapidly accessed at a lower frequency of N enrichment. The frequencies of N enrichment did not alter that N enrichment eventually caused the losses of rare species (Bai et al. 2010; Cleland and Harpole 2010; Zhang et al. 2014b) and shifted the dominant species (Isbell et al. 2013; Zhang et al. 2015). But the lower frequency of N enrichment had a greater negative impact on the observed plant species richness and the new species gains (Zhang et al. 2014b, 2016b) which affect species composition and then may alter community structure. Thus, in the longer term, the community structure may change faster with a lower frequency of N enrichment.

In conclusion, our study revealed that the rates of alterations in plant community structure were significantly enhanced by the increasing the rates of N enrichment through the positive direct and indirect effects via changing community productivity, species richness and species

composition. With marginally rapid changes in community structure through the frequency of N enrichment, it suggests that the results from previous controlled N fertilizer experiments with large and infrequent N inputs may have similar effect on the rate of changes in community structure by atmospheric N deposition with frequent N inputs for a short term (e.g. shorter than 5 years; Robertson et al. 2012). Our study found the significant impacts on changes in community structure by alterations in dominant species as soil acidification (Zhang et al. 2014b) and/or species competition (Zhang et al. 2015) under a lower frequency of N enrichment, suggesting that, in the longer period, frequent N enrichment may be better for assessment the influence of atmospheric N deposition on ecosystem functioning.

SUPPLEMENTARY MATERIAL

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

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