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RESEARCH ARTICLE

Intra-annual species gain overrides species loss in determining species richness in a typical steppe ecosystem after a decade of nitrogen enrichment

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Abstract

1. Increasing deposition of atmospheric nitrogen (N) due to accelerated human activities is a threat to various ecosystems. However, there is a lack of long-term experimental evidence demonstrating the seasonal dynamics of plant species turnover that ultimately determines species richness in natural ecosystems under N enrichment. Moreover, the frequency of N addition also may affect species turnover in a community, but it is rarely studied.
2. To assess the responses of a plant community to N addition, we manipulated the amounts (0–50 g N m^{-2} $year^{-1}$) and frequency (2 vs. 12 times $year^{-1}$) of N addition in an Inner Mongolian typical steppe ecosystem in northern China for 12 consecutive years (2008–2020). We measured species richness and density of plant in the growing seasons (May–September) from 2018 to 2020, starting 10 years after the initial N addition treatment.
3. Both species gain and species loss decreased with increasing amounts of N addition, resulting in a lower plant species turnover rate and greater similarity in the community between two adjacent months throughout the growing season. Species loss and species gain increased modestly under high N addition frequency. Species gain was more important than species loss in determining species richness after a decade of N application. In addition, plant density increased at high N amounts, mainly driven by enhanced clonal growth of the dominant species, *Leymus chinensis*.
4. *Synthesis*. Together, these results suggest that high levels of N deposition may suppress species richness due to aggravated soil chemical properties and may favour growth of a limited number of N-tolerant species compared to systems that experience low levels of N deposition. To conserve biodiversity and to facilitate restoration of degraded grassland ecosystems exposed to long-term N

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deposition, amelioration of the acidified soils induced by N deposition may be an important strategy to use.

KEYWORDS
biodiversity, community dynamics, determinants of plant community diversity, nitrogen addition frequency, nitrogen deposition, plant density, soil acidification, species turnover

1 | INTRODUCTION

Increasing applications of inorganic nitrogen (N) fertilizers to agricultural lands and increased combustion of fossil fuels have drastically increased the concentration of active N compounds in the atmosphere (Erisman et al., 2008; Galloway et al., 2004; Sutton et al., 2011). Consequently, it is anticipated that both terrestrial and aquatic ecosystems throughout the world will experience multifaceted impacts from atmospheric N deposition (IPCC, 2022; Sutton & Bleeker, 2013). N deposition is known to affect the structure, function and stability of ecosystems (Bai et al., 2010; Isbell et al., 2013; Vitousek et al., 1997; Zhang et al., 2019). For example, it has been found that N deposition generally enhances annual net primary productivity (ANPP), but at the same time reduces plant diversity across various ecosystems, particularly those that are N limited (Sala et al., 2000; Seabloom et al., 2021). N-induced decreases in biodiversity, in turn, have negative impacts on an array of ecosystems services, such as increased temporal variability of ANPP or decreased ecosystem stability (Hautier et al., 2014, 2020; Isbell et al., 2011), alteration of biogeochemical and hydrological cycles (Asner et al., 2001; Widdig et al., 2020), impoverishment of soil biota (Widdig et al., 2020), and increased susceptibility of ecosystems to multiple stressors such as pest infestation and extreme climate events (Beierkuhnlein et al., 2011; Pato & Obeso, 2013).

A host of hypotheses have been proposed to explore the fundamental mechanisms of biodiversity losses in varying ecosystems subjected to N deposition. These hypotheses include, but are not limited to three categories. The first category of hypotheses emphasizes the importance of competition among co-occurring species (Boyer & Zedler, 1999; Broadbent et al., 2018). It is generally accepted that N addition intensifies below-ground competition for nutrients and water (Rajaniemi et al., 2003). Similarly, enhanced competition for light may occur among species with different heights, where tall species have advantages over short, understorey species (Hautier et al., 2009). The second category of hypotheses pertains to changes of soil physicochemical properties that will favour the performance of some species but not others. For instance, increases in concentration of ammonium (NH_4^+) followed by enhancement of nitrification (Dorland et al., 2003) can exacerbate soil acidification (de Graaf et al., 1998) and cause aluminium (Al^{+3}) toxicity (Abedi et al., 2013), and N deposition can create imbalanced changes in N:P ratios (Elser et al., 2009), thereby leading to changes in community composition. The third category of hypotheses focuses on niche dimensionality, which suggests that addition of limiting resources would reduce the number of coexisting species by reducing the number of non-overlapping ecological niches (Harpole et al., 2016; Harpole & Tilman, 2007). The above-mentioned hypotheses, which are independent but not exclusive, have often been applied to explain the causes of reduced plant diversity in N-enriched ecosystems. However, most of these studies have been based on short-term experiments that used addition of limited amounts of N (Stevens, Bunker, et al., 2004; Tulloss & Cadenasso, 2016). In fact, long-term field experiments that consider accumulative effects across a wide range of amounts of N added are needed. The use of a large gradient of N addition allows for the detection of thresholds of abrupt changes in parameters of plant population demography and for disentangling the relative importance of species gain and species loss in shaping the community structure (Norghauer & Newbery, 2010).

Since deposition of atmospheric N occurs at a high frequency with chronic input of small amounts of reactive N into ecosystems, the frequency of N addition also should be considered in N deposition studies (Galloway et al., 2008; Zhang, Loreau, et al., 2016). For example, Zhang, Loreau, et al. (2016) found that the effects of N input on the temperate grassland ecosystem were regulated by the frequency of N addition, with greater losses of species at low frequency (pulsed N addition) and high amounts of N addition. This effect was mainly due to the high concentration of NH_4^+-N in the soil (Zhang, Stevens, et al., 2016). However, the effects of frequency of N addition on species turnover (species gain and species loss) remain to be comprehensively investigated using long-term studies. Moreover, most of studies of plant diversity have focused mainly on counting the total number of species at one sampling event during the growing season or only at the stage of peak biomass. There is a lack of information on the seasonal dynamics of plant communities affected by N deposition (Stevens, Dise, et al., 2004), which is important in evaluating the emergence and survival of the species during the course of a growing season. Thus, more frequent sampling is needed to address the intra-annual turnover of all plant species, including both dominant and rare species in plant communities (Avolio et al., 2015, 2019).

Before formulation of specific hypotheses, we assumed that N enrichment might have an influence on species richness of the community through its effects on species turnover based on intra-annual monitoring of species gain and loss in experimental plots. There are four possible scenarios that can describe changes in species gain, loss and turnover at any point in time with N addition, especially under high N addition amounts. First, species loss is high, but species gain is not affected by N deposition, resulting in increased species turnover under N enrichment (Figure 1a). Short-term studies have shown that establishment of plant is limited due to intensified competition for

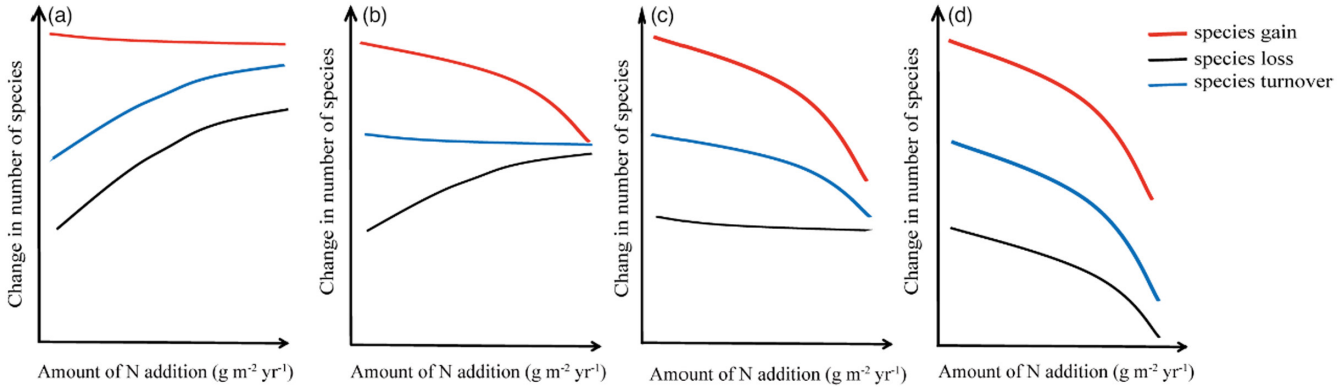


FIGURE 1 Possible scenarios of species gain, species loss and species turnover in response to long-term nitrogen addition during the growing season. The red lines are the species gain, the black lines are the species loss, and the blue lines are the species turnover in the community.

light among plants under high amounts of N addition, while seed germination was less affected by N addition amounts (Stevens, Bunker, et al., 2004). Second, increases in species loss are accompanied by a decrease in species gain at similar rates, resulting in unchanged species turnover with N addition amount (Figure 1b). This may suggest that N addition, even in the short term, will inhibit species gain and simultaneously accelerate species loss due to intensified above-ground competition for light and deteriorated soil chemical properties such as reduced soil pH and increased cation toxicity (Hautier et al., 2009; Zhang, Loreau, et al., 2016). Third, N deposition significantly reduces species gain, but its effect on species loss is negligible, resulting in reduced species turnover (Figure 1c). This has been demonstrated by seed bank and seedling establishment results from a long-term N addition experiment in which N enrichment caused a drastic reduction in the density of forb seedlings due to litter accumulation and changes in soil physical and chemical properties, with seedling survival from May through August remaining unchanged among treatments (Kitajima & Tilman, 1996). Fourth, both species gain and species loss decreased with N addition, leading to decreased species turnover in N enrichment (Figure 1d). These four scenarios, as abovementioned, may depend on the duration of the N addition experiment. Scenarios 1 and 2 may operate under the circumstances of a short-term N addition experiment, while scenarios 3 and 4 may become more prominent in a long-term N addition experiment. Previous studies have shown that long-term N deposition progressively depletes the soil seed bank and suppresses seed germination and asexual reproduction of plants, resulting in a reduction of species gain (Basto et al., 2015; Zhong et al., 2019). Thus, the N-sensitive species may have been lost long ago (Phoenix et al., 2012). Therefore, several years after N application, there will be only a pool of a few species remaining that have the potential to be lost, and furthermore they are likely tolerant to high N (Milbau et al., 2017).

Based on the above arguments, we adopted the fourth scenario as the basis for formulating the working hypothesis of our study because our field measurements took place from the 10th to the 12th year of N treatment. We conducted a long-term field N addition experiment using nine levels of N addition (0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m^{-2} $year^{-1}$) with two frequencies of N addition (2 vs. 12 additions $year^{-1}$) to examine the effects on species gain and species loss in a temperate steppe ecosystem. By doing so, we were able to examine the dosage (N addition amounts) and frequency (pulsed N addition vs. equally divided N addition throughout the year) effects of N addition on the seasonal dynamics of species richness.

Specifically, we aimed to test three hypotheses: (1) Both species gain and species loss on an intraannual basis would decrease across a large gradient of N addition. (2) Low-frequency N addition (2 times $year^{-1}$) would have a stronger effect on rates of species gain and loss than high-frequency addition (12 times $year^{-1}$). 3. Species gain would be more important than species loss in determining species richness of communities under varying amounts of N addition.

2 | MATERIALS AND METHODS

2.1 | Study site

The N addition experiment was established in 2008 in a steppe grassland adjacent to the Xilinhot Grassland Ecosystem Research Station in the Inner Mongolia Autonomous Region, China (43°32′45″N, 116°40′30″E) (Figure S1). This grassland ecosystem is a part of the Eurasian steppe, which is one of the largest contiguous grassland ecosystems in the world (Kang et al., 2007). Thus, studying the structure and function of Eurasia grasslands can provide important information about the future direction of vegetation dynamics under the impacts of human disturbance and climate change. Based on the meteorological records from this station for the past 34 years (1980–2014) (http://nmg.cern.ac.cn/meta/metaData), mean annual precipitation is 349.6 mm, with 72.0% of it occurring from May to August during the growing season, and mean annual temperature is 0.9°C, ranging from −21.1°C in January to 19.8°C in July. Mean monthly temperature and the total monthly precipitation during the study period (2018–2020) are given in Figure S2. The soil is classified as Calcic-Orthic Aridisol according to the US soil taxonomy classification (Baillie, 2001). Annual N deposition in the experimental area is

approximately 1.8 g m^{-2} $year^{-1}$ (Giese et al., 2013). The study site was fenced to exclude livestock grazing for 9 years prior to the initiation of the experiment and no fertilizers were applied until the experiment began in 2008. The dominant species, the grasses *Leymus chinensis* and *Stipa grandis*, together accounted for more than 60% of the total peak above-ground biomass (Zhang, Stevens, et al., 2016).

2.2 | Experimental design

A completely randomized block design was used for the N-addition experiment. A total of 10 blocks was used, and each block was 45 × 70 m, with a walkway 2 m wide between the blocks. The experiment included nine amounts of N (0, 1, 2, 3, 5, 10, 15, 20 and 50 g N m^{-2} $year^{-1}$; N_0, N_1, N_2, N_3, N_5, N_{10}, N_{15}, N_{20} and N_{50}, respectively) applied at two frequencies (N addition 2 times $year^{-1}$ vs. N addition 12 times $year^{-1}$), with each treatment having 10 replicates. NH_4NO_3 addition started on 1 September 2008 and was continued on the first day of each month thereafter for the high-frequency treatment (N addition 12 times $year^{-1}$). For the low-frequency treatment (N addition 2 times $year^{-1}$), NH_4NO_3 addition was started on 1 November 2008 and was continued on the first day of June and November thereafter. The addition amounts of NH_4NO_3 are shown in Table S1. That is, between September of 2 year and August of the next, the amount of N added per year in both frequencies of the N addition (2 and 12 $year^{-1}$) was the same. No permits were required to conduct fieldwork.

During the growing season (May–October) when N application was carried out, we first mixed NH_4NO_3 with 9 L of purified water, and then uniformly sprayed with a sprayer on the surface of each treatment plot. During the non-growing season (November–April), NH_4NO_3 was mixed with 0.5 kg clean sand (sieved through less than 1 mm sieve, dipped in hydro-chloric acid, washed in purified water and heated at 120°C for 24 h in the oven) and the mixture was manually spread evenly on the soil surface of each treatment plot. The 0 g N m^{-2} $year^{-1}$ (with water, or sand depending on the season) was designated as the control to assess any potential confounding effects coming from additions of sand and water. Hence, there were 18 experimental treatments (9 amounts × 2 frequencies of N addition) with 10 replicates, yielding a total of 180 experimental plots (8 m × 8 m). By 2020, all blocks had received N addition treatments for 12 consecutive years. More details on the experimental design can be found in Zhang et al. (2014) and Wang et al. (2019).

2.3 | Sampling and measurements

To evaluate the effect of long-term N addition on plant species turnover in the community on an intra-annual basis, the number of plant species and plant density (number of individual plants per square meter for sexually reproducing species and number of ramets for clonal species) was counted in each experimental treatment plot, once a month from May to September. The field measurements were repeated for three consecutive years from 2018 to 2020. In April of each year, a 0.5 m × 2 m quadrat was established in each plot, and this quadrat was used to non-destructively count the number of species and plant density at monthly intervals from May to September. To minimize the impact of the field survey on the plant community, new quadrats were laid out in the second and the third year of the study. The quadrats in each plot had no spatial overlap among years, at least 50 cm apart from each other to avoid edge effects.

We compared the species in each quadrat for each month from May to September, to determine which species were newly emerged and which ones were lost. The number of species gained (June–August) and the number of species lost (June–August) in a quadrat were calculated based on the number and identity of the species from two sampling intervals (1 month) in the same quadrat. We did not differentiate whether species in quadrats were produced by seed germination or vegetative regeneration. We defined species gain as the number of newcomers that were not found in the plot in prior months, which does not necessarily mean that the species was not there in the previous year. The reappearance of previously emerged species that had disappeared was excluded from species gain for any of the subsequent months, because the temporary disappearance of a species may be an artefact of sampling or population stochasticity rather than a biologically meaningful event (Anderson, 2007). Similarly, the temporary disappearance of a species also was excluded from species loss. Species loss was defined as the number of species present in a quadrat in previous months but not present in later months during the growing season of that particular year. For example, if a species appeared in May, but it disappeared in June and never showed up in any of the following months (July–September), we consider this species as being lost in June. Since the growing season ends in September, we did not calculate the species gain and species loss in this month.

At the end of August each year, litter was collected from another 1 m × 1 m quadrat of each plot and weighed to determine litter mass. In the same month, three soil cores (0–10 cm depth and 50 cm apart) were collected using a 3-cm-diameter soil auger from the assigned sampling area adjacent to the quadrats. Soil samples were thoroughly mixed and one composite sample was sieved through a 2-mm mesh for analysing the concentrations of soil ammonium N (NH_4^+-N) and nitrate N (NO_3^--N). The subsamples were air-dried for analysis of soil pH. To measure soil NH_4^+-N and NO_3^--N concentrations, a 10 g aliquot of fresh soil was extracted with 50 ml KCl (2.0 M) solution and then shaken for 30 min with a reciprocal shaker. Afterwards, the soil solution was filtered through Whatman No. 32 filter paper. The filtrate was analysed for soil NH_4^+-N and NO_3^--N using a flow injection auto analyser (FLAstar 5000 Analyser; Foss Tecator, Hillerød, Denmark). The concentrations of NH_4^+-N and NO_3^--N were expressed on a dry weight basis. Soil pH was measured in water suspension (soil: water = 1: 2.5) using a pH meter (FE20-FiveEasy).

We calculated the species gain rate (G_p), species loss rate (L_p) and species turnover rate (T_p) for each month (June–August) based on the equations proposed by Anderson (2007):

$$G_P = S_{gain} / \left[(1/2)\left(S_1 + S_2\right)\right], \quad (1)$$

$$L_P = S_{loss} / \left[(1/2)\left(S_1 + S_2\right)\right], \quad (2)$$

$$T_P = \left(S_{gain} + S_{loss}\right) / \left(S_1 + S_2\right). \quad (3)$$

where S_{gain} and S_{loss} represent species gain and species loss, respectively, between any two adjacent sampling intervals. S_1 and S_2 are species richness at two adjacent samplings.

We also calculated species similarity at each sampling interval using Jaccard's similarity index (Real & Vargas, 1996):

$$SJ = S / \left(S_1 + S_2 - S\right), \quad (4)$$

where S_1 and S_2 are species richness at two adjacent samplings, respectively, and S is number of species shared by two sampling events.

Total species gain (TS_{gain}) during the entire growing season was calculated by summing up species gains from June to August. Total species loss (TS_{loss}) was the sum of monthly species losses from June to August. Plant species were classified into common and rare species based on their relative abundance. A species was defined as 'common' if its mean relative abundance in the control plots exceeded 1%, and as 'rare' if its mean relative abundance was <1% (Clark & Tilman, 2008). We also counted total gain and total loss of the common species and rare species, respectively, across the growing season. The proportion of common species gain (PSC_{gain}) and rare species gain (PSR_{gain}) to total species gain and proportion of common species loss (PSC_{loss}) and rare species loss (PSR_{loss}) to total species loss were calculated.

2.4 | Statistical analysis

All statistical analyses were implemented in R version 3.4.0 (R Core Team, 2018) using the NLME and VEGAN packages. Figures were produced using the GGPLOT2 package (Wilkinson, 2011). To meet the assumption of data normality for further analyses, we used natural logarithms to transform dependent variables including G_p and L_p prior to performing the analyses. We used mixed-effects models to test treatment effects and temporal trends while accounting for repeated measurements following the lme function:

$$\begin{aligned} &\text{lme}\ (Y \sim N \times F \times \text{Time}; \text{random} \\ &\quad = \sim 1|\text{Plot}; \text{correlation} = \text{corCAR1}(\text{form} = \sim 1, \text{Plot})), \end{aligned} \quad (5)$$

where Y was the response variable (species richness, S_{gain}, S_{loss}, density of plant, G_p, L_p, T_p, SJ, TS_{gain}, TS_{loss}, PSC_{gain}, PSR_{gain}, PSC_{loss} and PSR_{loss}), N (amount of N added), F (N addition frequency) and time (month and year) were treated as fixed effects and the plot was included as a random variable. All models were assumed to follow a first-order autoregressive temporal covariance structure, which was accounted for by repeated measurement. We treated amount of N addition as a continuous variable and N addition frequency as a categorical variable. We also used variance partitioning (VEGAN package in R) and Venn diagrams to determine the total variance in species richness at the peak of the growing season that was explained by a predictor matrix of total species gain and total species loss (uniquely and jointly) (Ding et al., 2020).

Soil NH_4^+-N and NO_3^--N concentrations were $\log_{10}$ transformed to meet the assumption of normality. Linear regressions were used to estimate the effects of soil parameters on total species gain and total species loss, by including N addition frequency as a categorical variable that interacted with soil parameters. A significance ($\alpha = 0.05$) of the interactive effect indicated the differences between the two slopes. All statistical analyses were implemented in R.

The structural equation modelling (SEM) analyses were based on an initial structural equation model considering all plausible pathways (Figure S3). We tested the effects of experimental treatments (amount and frequency of N addition), soil parameters (Soil NH_4^+-N concentrations, soil NO_3^--N concentrations and soil pH) and litter mass on the total species gain and total species loss using data from 2018 and 2020. To obtain the best SEM, adequacy of the model was determined using a χ^2 test ($p > 0.05$), root mean square errors of approximation (RMSEA) <0.08 and the Akaike information criteria (AIC). Each hierarchical model was simplified by step-wise exclusion of pathways with non-significant regressions ($p > 0.05$). We adjusted the path according to the modified indicators in the statistics until the model passed statistical tests. All SEM models were developed with the AMOS software (version 21; IBM SPSS Inc.).

3 | RESULTS

3.1 | Intra-annual species gain and species loss in the community under varying N addition amounts and frequency

Our experiment was from the 10th to the 12th year of N enrichment. Averaged over 3 years, species richness over the growing season (May–September) was affected by amount but not frequency of N addition (N: $p < 0.0001$, F: $p = 0.071$, Table S2, Figure 2). Species richness declined as N addition amount increased (Figure 2), and the magnitude of this effect differed among months (N × M: $p < 0.0001$, Table S2, Figure 2). Species richness, for a given level of N addition, was more variable among months under low N addition amounts (<5–10 g N m^{-2} $year^{-1}$) relative to high N addition amounts (>15 g N m^{-2} $year^{-1}$) (Figure 2). Species richness was very similar among months under the highest N addition amount (50 g N m^{-2} $year^{-1}$).

Averaged over 3 years, N addition significantly reduced species gain on a monthly basis (N: $p < 0.0001$, Table 1, Figure 3a). This reduction in species gain was more conspicuous in June than in other months. Species gain from June to August over the 3 years was always less than two across all N addition amounts. The effect of N addition frequency on species gain could vary by month only

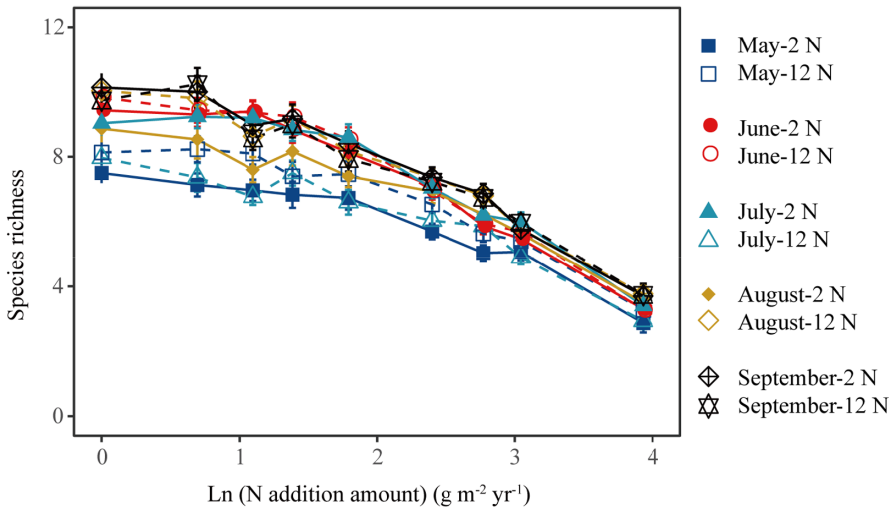


FIGURE 2 Effects of amount and frequency of N addition on monthly species richness during the growing seasons (May–September) averaged from 2018 to 2020. Solid symbols denote 2 N addition year^{-1} and hollowed symbols denote 12 N addition year^{-1}. Data are means of 30 replicates of each treatment ± SE.

TABLE 1 Results of repeated-measure mixed model ANOVA of N addition frequency (F), N addition amount (N), month (M), year (Y) and their interactions on the monthly species gain (S_{gain}) and species loss (S_{loss}) and density of plant during the growing season in 2018–2020. Values in bold denote significance at $p = 0.05$

		S_{gain}		S_{loss}		Density of plant	
Fixed effect	**df**	***F***	***p***	***F***	***p***	***F***	***p***
N	1528	205.855	**<0.0001**	74.651	**<0.0001**	69.361	**<0.0001**
F	1528	1.965	0.162	5.865	**0.016**	3.286	0.070
M	21,056	46.182	**<0.0001**	69.755	**<0.0001**	41.806	**<0.0001**
Y	2528	11.665	**<0.0001**	1.357	0.258	108.312	**<0.0001**
N×F	1528	1.892	0.170	0.345	0.557	6.546	**0.011**
N×M	21,056	1.158	0.314	17.229	**<0.0001**	5.964	**0.003**
F×M	21,056	1.919	0.147	1.994	0.137	0.140	0.869
N×Y	2528	0.069	0.933	0.057	0.945	26.092	**<0.0001**
F×Y	2528	2.481	0.085	2.902	0.056	0.359	0.699
M×Y	41,056	31.092	**<0.0001**	1.906	0.107	5.604	**0.0002**
N×F×M	21,056	0.550	0.577	0.376	0.687	1.435	0.239
N×F×Y	2528	0.040	0.961	0.437	0.646	1.185	0.307
N×M×Y	41,056	6.368	**<0.0001**	0.961	0.428	13.737	**<0.0001**
F×M×Y	41,056	2.140	0.074	2.161	0.071	3.587	**0.007**
N×F×M×Y	41,056	0.617	0.650	0.337	0.853	2.065	0.083

in certain years (F×M×Y: $p = 0.07$, Table 1). More species were gained under high N addition frequency than under low N addition frequency in the early growing season (F×Y: $p = 0.018$, Table S3, Figure S4). N addition significantly decreased species loss, particularly at high N addition amounts (N_{20}–N_{50}), with species loss dropping to zero in the 50 g N m^{-2} year^{-1} treatment (N: $p < 0.0001$, Table 1, Figure 3b). More species were lost under high N addition frequency (F: $p = 0.016$, Table 1, Figure 3b), being particularly conspicuous in August when plants started to senesce (F: $p = 0.032$, Table S3). N addition not only affected species gain and species loss, but it also affected plant abundance expressed as plant density in the community on a monthly basis (N: $p < 0.0001$, Table 1, Figure 3c). At both low and high N addition frequencies, averaged over 3 years, plant density changed slightly at low to medium levels of N addition and sharply increased at high N addition from 20 to 50 g m^{-2} year^{-1}. Plant density continued to increase with time from June to August, particularly at low N addition frequency.

Based on monthly monitoring of species gain and species loss from 2018 to 2020, rates of species gain, loss and turnover and species similarity of the community were calculated for each sampling time and then the monthly averages were calculated for all quadrats. ANCOVA revealed that N addition amount and year significantly influenced rates of species gain, loss and turnover and species similarity of the community (N: $p < 0.0001$, Y: $p < 0.05$, Table 2, Figure 4) and the effect of N addition amount on rates of species gain and turnover and species similarity of the community also depends on the month

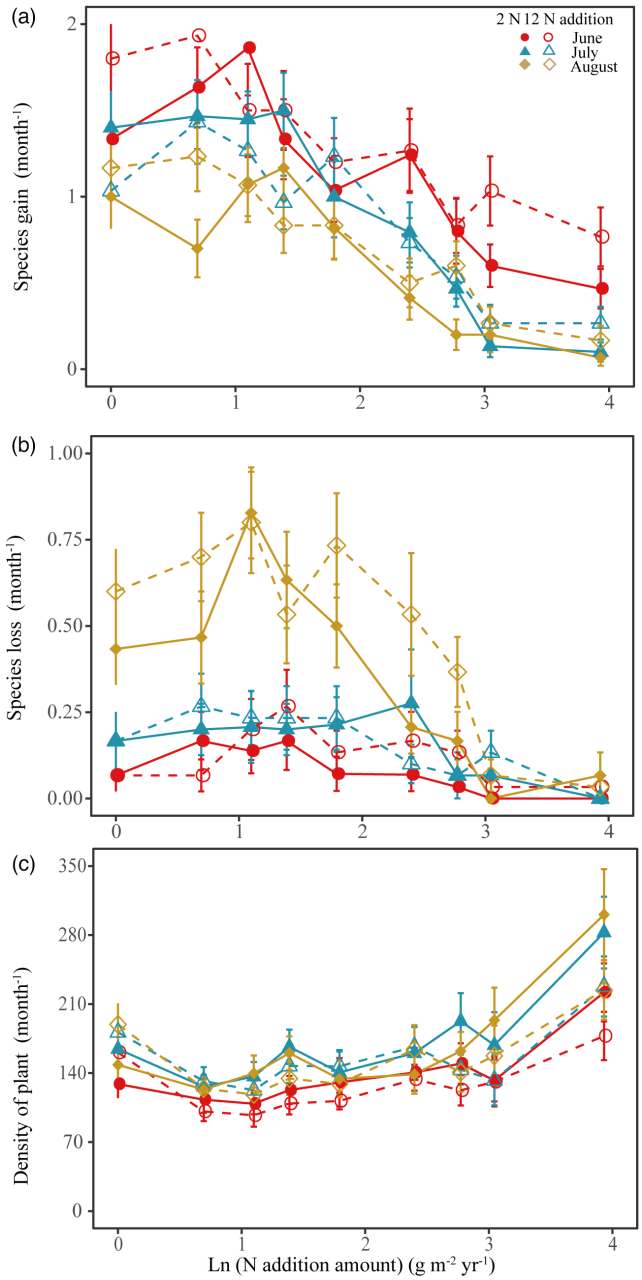


FIGURE 3 Effects of amount and frequency of N addition on monthly species gain (S_{gain}, a), monthly species loss (S_{loss}, b) and monthly density of plant (c) during the growing seasons (June–August) averaged from 2018 to 2020. Solid symbols denote 2 N addition year^{-1} and hollowed symbols denote 12 N addition year^{-1}. Data are means of 30 replicates of each treatment ± SE.

(N × M × Y: $p < 0.01$, Table 2). The frequency of N addition significantly influenced the rates of species gain, loss and turnover and species similarity of the community (F × Y: $p < 0.07$, Table 2, Figure 4), but this interactive effect was only found in certain years (2019 and 2020) (F: $p < 0.08$ Tables S4 and S5). Rates of species gain, loss and turnover were negatively correlated with increasing N addition amount (Figure 4a–l); however, species similarity of community showed an opposite trend (Figure 4m–p). Low N addition frequency had greater effects on these four variables than high N addition frequency.

3.2 | Effects of the total species gain and total species loss on variation in species richness

Total species gain and total species loss had a significantly negative relationship with N addition amounts over the entire growing season (Figure 5a; Table 3), and there was higher total species loss at high than at low frequency of N addition (F: $p = 0.022$, Table 3, Figure 5a). Total species gain decreased more than total species loss with increasing N addition amount. For instance, total species gain decreased from 4.0 in N_0 to 0.6 in N_{50}, while total species loss only decreased from 0.8 in N_0 to 0.1 in N_{50}. We divided the coexisting species in the community into common and rare species based on the number of individuals in a quadrat. The proportion of species gain for rare species decreased, while that for common species increased with increasing N addition amounts in the entire growing season from June to August (Figure 4b). The proportion of species loss for rare species was higher at low N addition ($<N_5$), while that for common species was higher at high N addition ($>N_5$) (Figure 5c; Table 3). Further analysis showed that total species gain independently explained 35% (2 N addition) and 37% (12 N addition) of the variance in peak species richness, whereas total species loss only independently explained 2% (12 N addition) of the variance in peak species richness (Figure S5).

3.3 | Relationship between gain and loss of plant species and soil properties

Averaged over 3 years, total species gain and total species loss were negatively related to soil NH_4^+-N concentration and soil NO_3^--N concentration but positively correlated to soil pH (Figure S6). Litter mass did not have a significant effect ($p > 0.1$) on total species gain and loss. As seen from results of the SEM, N enrichment indirectly decreased total species gain via reduced soil pH and increased soil inorganic N (NO_3^--N) concentrations (Figure 6). Average total species loss was not directly affected by soil properties or N addition amount, but it was positively affected by average total species gain (Figure 6). N addition frequency directly affected total species loss.

4 | DISCUSSION

We clearly demonstrated that both species gain and species loss during the growing season decreased significantly with increasing N addition amounts. The rates of species gain and loss were higher at high (12 times year^{-1}) frequency than at low (2 times year^{-1}) frequency of the N addition only in certain years. Moreover, averaged over 3 years, suppression of total species gain overrode that of total species loss in determining species richness and consequently the population dynamics of the plant community that continued to receive N.

TABLE 2 Results of repeated-measure mixed model ANOVA of N addition frequency (F), N addition amount (N), month (M), year (Y) and their interactions on the monthly species gain rate (G_p), species loss rate (L_p), species turnover rate (T_p) and community species similarity (SJ) during the growing season (June–August) in 2018–2020. Values in bold denote significance at $p = 0.05$

		G_p		L_p		T_p		SJ	
Fixed effect	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
N	1528	53.549	**<0.0001**	57.188	**<0.0001**	62.463	**<0.0001**	89.560	**<0.0001**
F	1528	2.450	0.118	7.041	**0.008**	4.714	**0.030**	7.050	**0.008**
M	21,056	74.847	**<0.0001**	53.833	**<0.0001**	34.997	**<0.0001**	10.490	**<0.0001**
Y	2528	10.915	**<0.0001**	4.514	**0.011**	10.215	**<0.0001**	19.470	**<0.0001**
N×F	1528	4.992	**0.026**	0.223	0.637	3.398	0.066	9.260	**0.003**
N×M	21,056	8.376	**0.0002**	11.733	**<0.0001**	12.063	**<0.0001**	13.220	**<0.0001**
F×M	21,056	1.902	0.150	2.294	0.101	3.493	**0.031**	1.720	0.179
N×Y	2528	0.643	0.526	0.760	0.468	0.991	0.372	0.320	0.730
F×Y	2528	2.837	0.060	2.710	0.068	3.913	**0.021**	3.730	**0.025**
M×Y	41,056	24.048	**<0.0001**	2.431	**0.046**	20.051	**<0.0001**	4.650	**0.001**
N×F×M	21,056	0.338	0.713	0.354	0.702	0.709	0.492	0.630	0.533
N×F×Y	2528	0.740	0.478	0.224	0.800	0.354	0.702	2.040	0.132
N×M×Y	41,056	5.629	**0.0002**	0.961	0.428	4.604	**0.001**	4.750	**0.001**
F×M×Y	41,056	2.881	**0.022**	1.851	0.117	1.836	0.120	1.080	0.366
N×F×M×Y	41,056	4.708	**0.001**	0.293	0.883	4.655	**0.001**	5.320	**0.0003**

4.1 | Effects of N addition amount on species gain and species loss in the community

After a decade N enrichment, species richness drastically decreased with increasing N addition amount in comparison to the baseline species richness prior to N treatment (Figure S7), which was apparent even in May, which is the early stage of the growing season. Throughout the entire growing season, species richness varied significantly in different months among treatments, possibly due to effects of N treatment, phenology of plants and environmental fluctuations (Cleland et al., 2007; Norghauer & Newbery, 2010; Veenendaal et al., 1996). To explore the underlying mechanisms of biodiversity loss in this grassland ecosystem, we investigated the seasonal changes of the species gain and loss for three consecutive years. We found that N addition significantly reduced species gain, which agrees with results from several previous germination studies on N addition (Roem et al., 2002; Zhong et al., 2019). The decrease in species gain with increasing N addition amounts may have been caused by a combination of several factors. First, N-induced changes in soil chemical conditions (pH and inorganic N concentrations) could mediate the response of species gain to N enrichment (Figure S8). These changes included a decrease in soil pH and increases in soil inorganic N concentrations that can inhibit seed germination (Boudell & Stromberg, 2015; Roem et al., 2002). Second, seed sources were limited, mainly due to reduced production of seeds by plants (Basto et al., 2015), increased microbial degradation of seeds with the decreasing soil C:N ratio (Davis, 2007) and aggravated toxicity of soil metal ions to seeds (Salemaa & Uotila, 2001). It should be noted that the timing of the field experiment is important in examining the effects of N addition on plant community dynamics. Our measurements were started after a decade of N addition treatment, but the response of species gain to N addition may have started much earlier in the experiment, particularly under high amounts of N addition. For example, decreased soil pH and increased soil inorganic N concentrations under high amounts of N addition may have inhibited seed germination and survival of seedlings much earlier than when we began this study. From the same experiment, Zhang et al. (2014) found that species richness gradually declined from 2008 to 2014 with increasing amounts of N addition, mainly due to acidification of soils and increased NH_4^+ toxicity.

Species loss also decreased with the increasing N. This is consistent with our prediction, but it differs from the results of other previous studies. For example, Hautier et al. (2009) found that species loss was greater due to amplified competition for light, resulting in reduction in species richness in grasslands (Hautier et al., 2009). However, we showed that species loss was small in the N addition treatments. Since only a few species were gained at high N, there was a potential for only a few species to be lost. The decrease in species gain and species loss further led to the decrease in species turnover rate with increasing of N addition amounts. This also explains the temporal increase in species similarity of community with increasing N addition amounts (Milbau et al., 2017). It has been suggested that the species turnover rate in response to N addition would increase in the steppe community (Xu et al., 2012). However, the study by Xu et al. (2012) was focused on variations of interannual, and not the intra-annual, species turnover. Moreover, their study was short term in duration. Other studies showed that long nutrient enrichment resulted in delayed species replacement during succession (Carson & Barrett, 1988; McLendon & Redente, 1991), but most of such findings were based on theoretical models (Shugart & Hett, 1973)

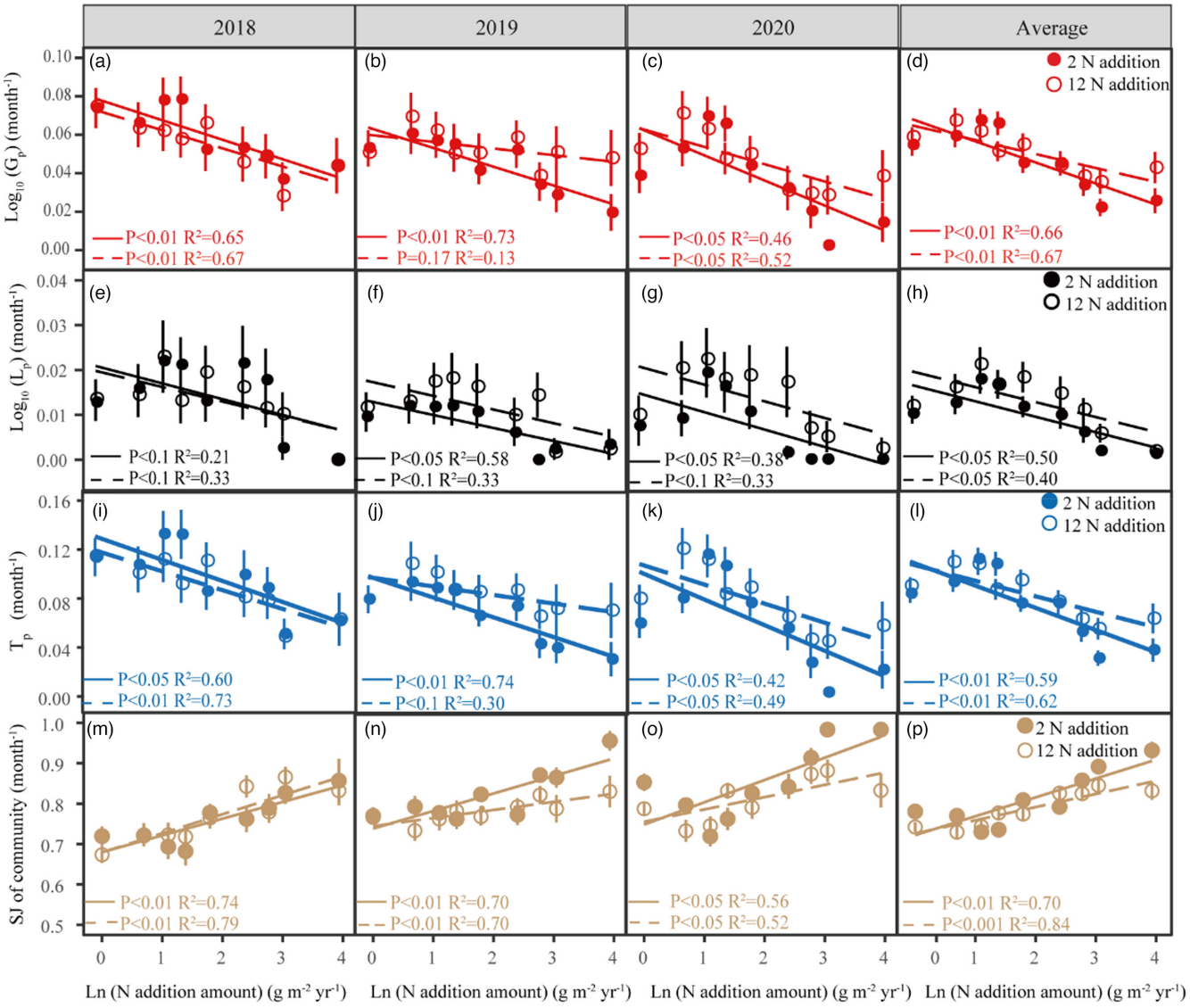


FIGURE 4 Effects of amount and frequency of N addition on monthly species gain rate (G_p, a–d), species loss rate (L_p, e–h), species turnover rate (T_p, i–l) and species similarity of community (SJ, m–p) on average during the growing season (June–August) from 2018 to 2020. Solid circles and solid lines denote 2 N addition year^{-1}, open circles and dashed lines denote 12 N addition year^{-1}. Data are means of 30 replications of each treatment ± SE. In 2019, N addition amount* N addition frequency = 4.81* (species gain rate), N addition amount* N addition frequency = 7.36** (species similarity); in 2020, N addition amount* N addition frequency = 7.38** (species similarity); species similarity on average from 2018 to 2020, N addition amount* N addition frequency = 12.14**. There was no significant interaction for N addition amount and N addition frequency in Figure 3a–m.

or field studies without N addition (Inouye et al., 1987). Our results provided robust experimental evidence showing that the trade-off between species gain and loss is a major regulator of the dynamics of species turnover under N enrichment.

4.2 | Effects of N addition frequency on species turnover in the community

We show for the first time that species turnover in the community during the growing season can depend not only on the amount of N added, but also on the frequency of N addition. Infrequent N addition suppressed species gain more than frequent N addition only in the early growing season. Infrequent N addition, which is usually implemented during the growing season, will result in higher cation toxicity than frequent N addition involving multiple small N additions with some occurring during the non-growing season. As for frequent N additions, more N loss through ammonia volatilization, leaching, denitrification and microbial immobilization will weaken the N effect on community dynamics (Wang et al., 2019). Another reason for differences between frequencies of N addition may be related to the seed source. We conducted a survey of plant sexual reproduction in the above-ground community in 2019 and found that more species reproduced in the frequent than infrequent N addition treatments,

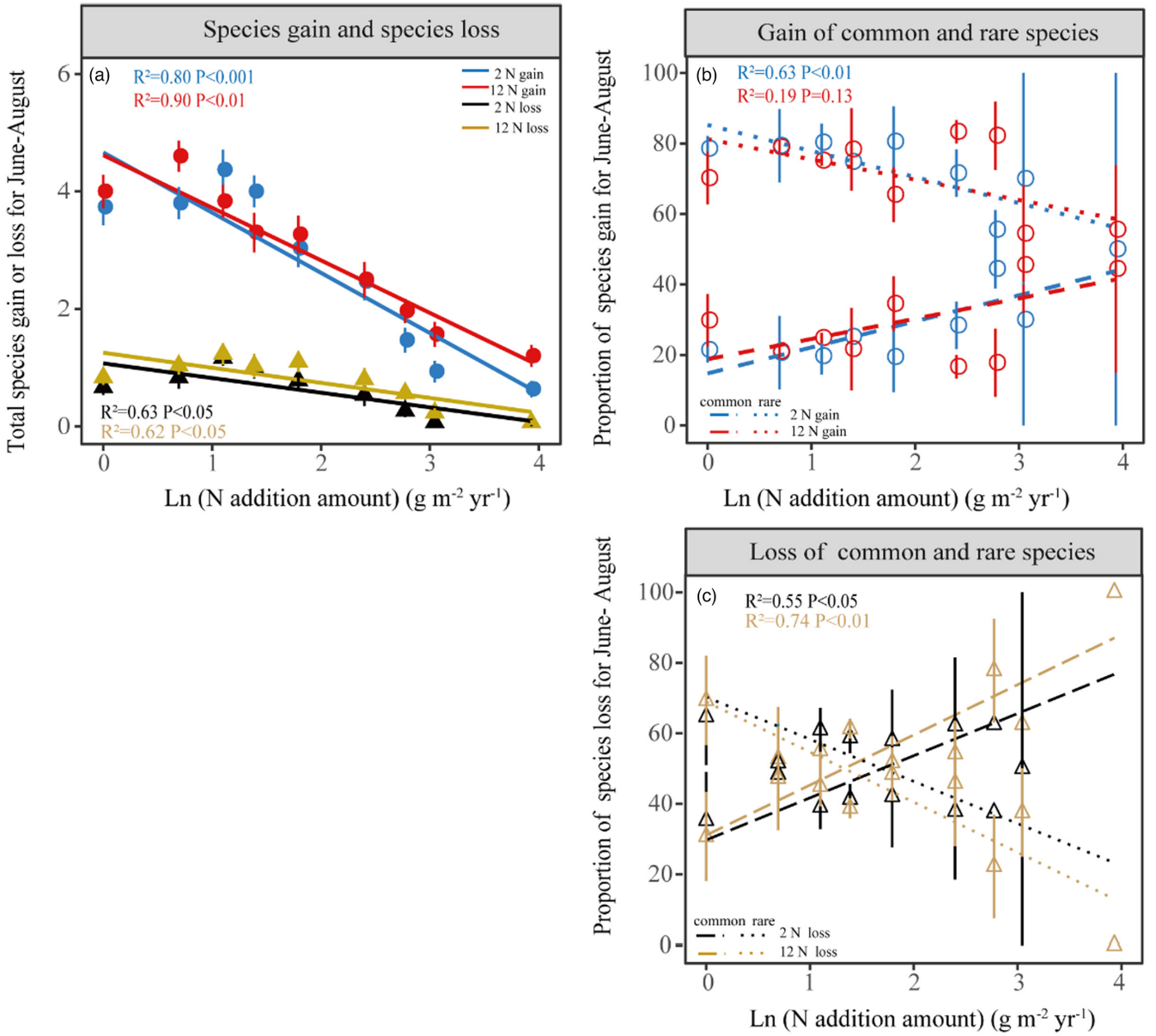


FIGURE 5 Effects of amount and frequency of N addition on total species gain (TS_{gain}) and total species loss (TS_{loss}) during the growing season (June–August) averaged across 2018–2020 (a). Proportion of gain of common and rare species (b) and proportion of loss of common and rare species averaged a year in 2018–2020 (c). Circles denote species gain, solid blue circles 2 N additions $year^{-1}$ and solid red circles 12 N addition $year^{-1}$. Triangles denote species loss. Solid black triangles indicate 2 N additions $year^{-1}$ and solid brown triangles 12 N addition $year^{-1}$. Long dash lines denote common species and short dash lines denote rare species. Open circles denote the proportion of species gain, open triangles denote the proportion of species loss.

especially at high N addition (Figure S9). This difference in seed production may result in higher species richness of the soil seed bank under high-frequency than under low-frequency N addition (Basto et al., 2015), which, in turn, affects subsequent species turnover. For the same reason, high species loss under high N addition amount and high frequency possibly can be explained by high mortality of new seedlings, which are intolerant to immediate nitrogen applications. Our findings suggested that previous fertilization studies involving pulsed (infrequent) N additions might have overestimated the negative effects of N deposition on species turnover in the real world (Foster & Gross, 1998; Kitajima & Tilman, 1996).

The frequency of N addition affects not only species gain and species loss, but also the rates of species gain, loss and turnover and species similarity of the community. There were higher rates of species gain, loss and turnover and lower species similarity of community at high than at low frequency of N addition in 2019 and 2020, but the frequency of N addition did not affect these indicators in 2018. This difference between years may be related to the interannual precipitation fluctuations. There was more precipitation during the growing season in 2019 and 2020 than in 2018 (Figure S2). High precipitation could alleviate the effects of soil moisture limitation on species gain (Michael Anderson, 2008). In years with insufficient

TABLE 3 Results of repeated-measure mixed model ANOVA of N addition frequency (F), N addition amount (N), year (Y) and their interactions on total species gain (TS_{gain}) and species loss (TS_{loss}), the proportion of common species gained (PSC_{gain}), the proportion of rare species gained (PSR_{gain}), the proportion of common species lost (PSC_{loss}), the proportion of rare species lost (PSR_{loss}) during the entire growing season (June–August) in 2018–2020. Values in bold denote significance at $p = 0.05$

		TS_{gain}		TS_{loss}		PSC_{gain}		PSR_{gain}		PSC_{loss}		PSR_{loss}	
Fixed effect	**df**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
N	1176	166.914	**<0.0001**	74.104	**<0.0001**	2.756	0.099	100.442	**<0.0001**	22.577	**<0.0001**	50.383	**<0.0001**
F	1176	1.593	0.209	5. 0.320	**0.022**	5.007	**0.027**	1.681	0.197	9.776	**0.002**	0.020	0.889
Y	2352	15.886	**<0.0001**	1.329	0.266	14.145	**<0.0001**	0.780	0.450	0.175	0.839	2.890	0.057
N×F	1176	1.534	0.217	0.287	0.593	4.493	**0.035**	1.068	0.303	0.065	0.800	0.051	0.822
N×Y	2352	0.094	0.910	0.054	0.948	3.500	**0.031**	0.740	0.478	0.128	0.880	0.529	0.589
F×Y	2352	3.378	**0.035**	2.907	0.056	2.199	0.112	2.909	0.056	0.196	0.822	1.098	0.335
N×F×Y	2352	0.054	0.948	0.445	0.641	5.582	**0.004**	1.487	0.227	0.194	0.824	0.362	0.697

precipitation, germination of seeds is hampered by low soil water, resulting in no difference under different frequency of N addition. Previous studies also suggested that turnover of species was co-limited by both N and water in temperate grasslands in Northern China (Xu et al., 2012). Our findings imply that precipitation may mediate the temporal responses of species to amount of N addition.

4.3 | The relative importance of total species gain and total species loss on species richness in the community

Our findings suggested that total species gain, rather than total species loss, was the dominant factor accounting for the reduction of species richness at high N addition amounts. This finding is consistent with the conclusion of other researchers who reported that the suppression of seed germination (Kitajima & Tilman, 1996) or impediment of emergence of seedlings (James et al., 2011), rather than plant survivorship, was the primary mechanism underlying decreased species richness in high-N plots. Decreased total species gain may be related to the decrease in proportion of species gain for the N-sensitive rare species under high N addition amounts (Lan & Bai, 2012; Su et al., 2021; Tian et al., 2016). This is congruous with the niche dimensionality hypothesis in that addition of a limiting resource (N) could reduce the niche dimension, leading to a decrease in species gain (Harpole & Tilman, 2007). In our experiment, stem density of *L. chinensis* was increased dramatically, especially at the high N addition amount treatment (Figure S10). *L. chinensis*, the dominant species in Eurasian steppe, is a clonal plant that can still reproduce via clonal growth under high nutrient availability (Bai et al., 2008; Fischer & Van Kleunen, 2001; Sun et al., 2001). It will monopolize the space and available resources (Prati & Schmid, 2000), making it difficult for other plant species, especially rare ones, to colonize.

The resource competition hypothesis and the mal-adaptation to soil toxicity hypothesis suggest that survival failure of the species that are already present could explain the decline in species diversity (Hautier et al., 2009; van den Berg et al., 2005). However, the applicability of these two hypotheses may depend on the duration of experiment. Ecosystems may respond differently to short-term and long-term stresses (Eisenhauer et al., 2012). In short-term N addition experiments (N addition <3 year), N-sensitive species may fail to become established due to light competition and/or underground resource competition among plants and thus be eliminated (DeMalach et al., 2017; Niu et al., 2008; Salemaa & Uotila, 2001; van den Berg et al., 2005). In this case, the competition hypothesis holds true. However, in long-term N addition experiments (>10 years), N-sensitive species may have disappeared in the early years of the experiment (Phoenix et al., 2012), particularly at extremely high levels of N addition. For example, about 50% of the species were already lost in 2013 (5 years after N treatment in our experiment) at the highest N addition amount (50 g N m^{-2} $year^{-1}$) compared to the control (Zhang et al., 2014). Up to the time when we began this study, this number increased to about 60% (Figure S7).

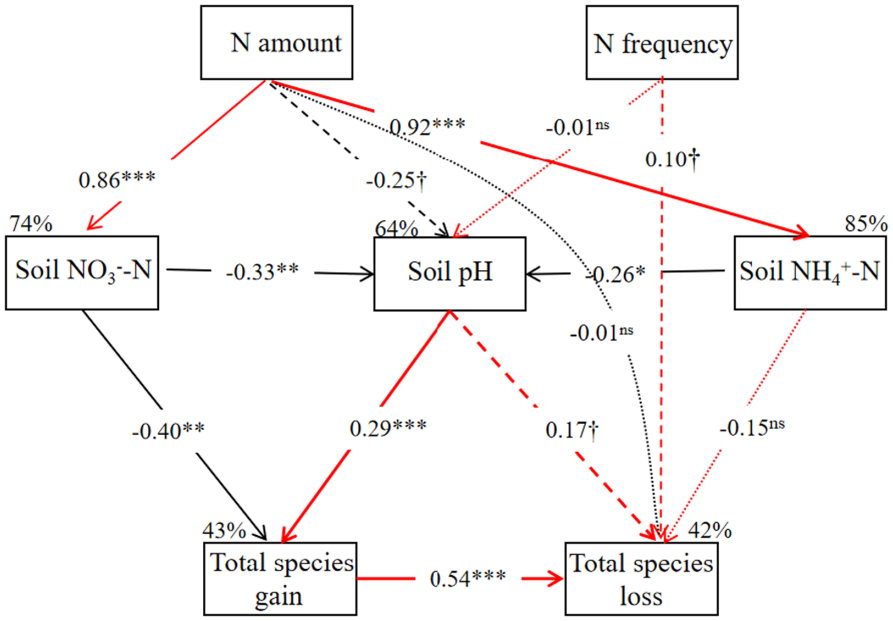


FIGURE 6 Pathway analysis showing that total species gain and total species loss response to amount and frequency of N addition directly or mediated by soil factors (soil pH, soil NO_3^--N and soil NH_4^+-N) indirectly per year on average (2018 and 2020). The model fit the data well: $\chi^2 = 7.37$, df = 9, $p = 0.60$, root mean square error of approximation (RMSEA) < 0.05, Akaike information criteria (AIC) = 56.00 values associated with arrows mean standardized path coefficients (equivalent to correlation coefficients). The red line represents a positive correlation and the black line represents a negative correlation. Solid arrows indicate significant relationships ($p < 0.05$), and dashed arrows indicate marginally significant relationships ($p < 0.1$); dotted arrows indicate non-significant relationships ($p > 0.1$). †, * **and ***: Statistically significant at $p < 0.1$, 0.05, 0.01 and 0.001. Percentages associated with response variables represent the variance explained by the model (R^2).

We found that the community consisted of a few common species (*Leymus chinensis*, *Stipa capillata* and *Agropyron cristatum*) at high N addition amounts. The common species are nitrophilous and can tolerate low soil pH and cation toxicity (Guo et al., 2014; Tulloss & Cadenasso, 2016). Consequently, these species were successful in their establishment. In other words, with continuous N addition, the non-tolerant species will be filtered out, leaving only very few N-tolerant species in the community. This suggests that the maladaptation to soil toxicity hypothesis may be applied to explain the biodiversity loss due to reduced species gain in ecosystems receiving long-term N enrichment.

5 | CONCLUSIONS

In the 10th to 12th year of experimental N addition in an Inner Mongolian steppe ecosystem, both plant species gain and species loss decreased with increasing amounts of N addition, leading to low rates of species turnover at high N levels. The few species observed in the high N treatments were likely tolerant of high N levels and thus were unlikely to be lost, whereas it is likely that species losses under high N were more frequent in early years of the experiment. Our findings imply that the amelioration of soil properties such as alleviation of soil acidity may be a strategy to facilitate the restoration of species-poor grassland ecosystems induced by high load of N deposition.

AUTHORS' CONTRIBUTIONS

M.Z., H.X.Z. and X.G.H. planned and designed the research; M.Z. and J.J.Y. performed the experiments, conducted the field and laboratory work and managed the research site; M.Z. performed the modelling work and analysed the output data; M.Z., H.X.Z. and X.G.H. wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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CONFLICT OF INTEREST

The authors have no competing interest.

DATA AVAILABILITY STATEMENT

Data are available on the Dryad Digital Repository https://doi.org/10.5061/dryad.41ns1rngg (Zhao et al., 2022).

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REFERENCES

Abedi, M., Bartelheimer, M., & Poschlod, P. (2013). Aluminium toxic effects on seedling root survival affect plant composition along soil reaction gradients—a case study in dry sandy grasslands. *Journal of Vegetation Science*, *24*, 1074–1085. https://doi.org/10.1111/jvs.12016

Anderson, K. J. (2007). Temporal patterns in rates of community change during succession. *The American Naturalist*, *169*, 780–793. https://doi.org/10.1086/516653

Asner, G. P., Townsend, A. R., Riley, W. J., Matson, P. A., Neff, J. C., & Cleveland, C. C. (2001). Physical and biogeochemical controls over terrestrial ecosystem responses to nitrogen deposition. *Biogeochemistry*, *54*, 1–39. https://doi.org/10.1023/a:1010653913530

Avolio, M. L., Forrestel, E. J., Chang, C. C., La Pierre, K. J., Burghardt, K. T., & Smith, M. D. (2019). Demystifying dominant species. *New Phytologist*, *223*, 1106–1126. https://doi.org/10.1111/nph.15789

Avolio, M. L., La Pierre, K. J., Houseman, G. R., Koerner, S. E., Grman, E., Isbell, F., Johnson, D. S., & Wilcox, K. R. (2015). A framework for quantifying the magnitude and variability of community responses to global change drivers. *Ecosphere*, *6*, 280. https://doi.org/10.1890/ES15-00317.1

Bai, W. M., Wang, Z. W., Chen, Q. S., Zhang, W. H., & Li, L. H. (2008). Spatial and temporal effects of nitrogen addition on root life span of *Leymus chinensisin* a typical steppe of Inner Mongolia. *Functional Ecology*, *22*, 583–591. https://doi.org/10.1111/j.1365-2435.2008.01403.x

Bai, Y. F., Wu, J. G., Clark, C. M., Naeem, S., Pan, Q. M., Huang, J. H., Zhang, L. X., & Han, X. G. (2010). Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: Evidence from inner Mongolia grasslands. *Global Change Biology*, *16*, 358–372. https://doi.org/10.1111/j.1365-2486.2009.01950.x

Baillie, I. C. (2001). Soil survey staff 1999, soil taxonomy: A basic system of soil classification for making and interpreting soil surveys, 2nd edition. Agricultural handbook 436, natural resources conservation service, USDA, Washington DC, USA, pp. 869. *Soil Use and Management*, *17*, 57–60. https://doi.org/10.1111/j.1475-2743.2001.tb00008.x

Basto, S., Thompson, K., Phoenix, G., Sloan, V., Leake, J., & Rees, M. (2015). Long-term nitrogen deposition depletes grassland seed banks. *Nature Communications*, *6*, 6185. https://doi.org/10.1038/ncomms7185

Beierkuhnlein, C., Thiel, D., Jentsch, A., Willner, E., & Kreyling, J. (2011). Ecotypes of European grass species respond differently to warming and extreme drought. *Journal of Ecology*, *99*, 703–713. https://doi.org/10.1111/j.1365-2745.2011.01809.x

Boudell, J. A., & Stromberg, J. C. (2015). Impact of nitrate enrichment on wetland and dryland seed germination and early seedling development. *Journal of Vegetation Science*, *26*, 452–463. https://doi.org/10.1111/jvs.12258

Boyer, K. E., & Zedler, J. B. (1999). Nitrogen addition could shift plant community composition in a restored California salt marsh. *Restoration Ecology*, *7*, 74–85. https://doi.org/10.1046/j.1526-100X.1999.07109.x

Broadbent, A., Stevens, C. J., Peltzer, D. A., Ostle, N. J., & Orwin, K. H. (2018). Belowground competition drives invasive plant impact on native species regardless of nitrogen availability. *Oecologia*, *186*, 577–587. https://doi.org/10.1007/s00442-017-4039-5

Carson, W. P., & Barrett, G. W. (1988). Succession in old-field plant communities: Effects of contrasting types of nutrient enrichment. *Ecology*, *69*, 984–994. https://doi.org/10.2307/1941253

Clark, C. M., & Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, *451*, 712–715. https://doi.org/10.1038/nature06503

Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A., & Schwartz, M. D. (2007). Shifting plant phenology in response to global change. *Trends in Ecology & Evolution*, *22*, 357–365. https://doi.org/10.1016/j.tree.2007.04.003

Davis, A. S. (2007). Nitrogen fertilizer and crop residue effects on seed mortality and germination of eight annual weed species. *Weed Science*, *55*, 123–128. https://doi.org/10.1614/WS-06-133.1

deGraaf, M. C. C., Bobbink, R., Roelofs, J. G. M., & Verbeek, P. J. M. (1998). Differential effects of ammonium and nitrate on three heathland species. *Plant Ecology*, *135*, 185–196. https://doi.org/10.1023/A:1009717613380

DeMalach, N., Zaady, E., & Kadmon, R. (2017). Light asymmetry explains the effect of nutrient enrichment on grassland diversity. *Ecology Letters*, *20*, 60–69. https://doi.org/10.1111/ele.12706

Ding, J. X., Kong, D. L., Zhang, Z. L., Cai, Q., Xiao, J., Liu, Q., & Yin, H. J. (2020). Climate and soil nutrients differentially drive multidimensional fine root traits in ectomycorrhizal-dominated alpine coniferous forests. *Journal of Ecology*, *108*, 2544–2556. https://doi.org/10.1111/1365-2745.13407

Dorland, E., Bobbink, R., Messelink, J. H., & Verhoeven, J. T. A. (2003). Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology*, *40*, 804–814. https://doi.org/10.1046/j.1365-2664.2003.00845.x

Eisenhauer, N., Reich, P. B., & Scheu, S. (2012). Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology*, *13*, 571–578. https://doi.org/10.1016/j.baae.2012.09.002

Elser, J. J., Andersen, T., Baron, J. S., Bergstrom, A. K., Jansson, M., Kyle, M., & Hessen, D. O. (2009). Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. *Science*, *326*, 835–837. https://doi.org/10.1126/science.1176199

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geoscience*, *1*, 636–639. https://doi.org/10.1038/ngeo325

Fischer, M., & Van Kleunen, M. (2001). On the evolution of clonal plant life histories. *Evolutionary Ecology*, *15*, 565–582. https://doi.org/10.1023/A:1016013721469

Foster, B. L., & Gross, K. L. (1998). Species richness in a successional grassland: Effects of nitrogen enrichment and plant litter. *Ecology*, *79*, 2593–2602. https://doi.org/10.2307/176503

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., & Vöosmarty, C. J. (2004). Nitrogen cycles: Past, present, and future. *Biogeochemistry*, *70*, 153–226. https://doi.org/10.1007/s10533-004-0370-0

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., & Sutton, M. A. (2008). Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science*, *320*, 889–892. https://doi.org/10.1126/science.1136674

Giese, M., Brueck, H., Gao, Y. Z., Lin, S., Steffens, M., Kögel-Knabner, I., & Han, X. G. (2013). N balance and cycling of Inner Mongolia typical steppe: A comprehensive case study of grazing effects. *Ecological Monographs*, *83*, 195–219. https://doi.org/10.1890/12-0114.1

Guo, Q., Meng, L., Mao, P. C., & Tian, X. X. (2014). An assessment of *Agropyron cristatum* tolerance to cadmium contaminated soil.

Biologia Plantarum, *58*, 174–178. https://doi.org/10.1007/s10535-013-0359-4

Harpole, W. S., Sullivan, L. L., Lind, E. M., Firn, J., Adler, P. B., Borer, E. T., Chase, J., Fay, P. A., Hautier, Y., Hillebrand, H., MacDougall, A. S., Seabloom, E. W., Williams, R., Bakker, J. D., Cadotte, M. W., Chaneton, E. J., Chu, C., Cleland, E. E., D'Antonio, C., … Wragg, P. D. (2016). Addition of multiple limiting resources reduces grassland diversity. *Nature*, *537*, 93–96. https://doi.org/10.1038/nature19324/

Harpole, W. S., & Tilman, D. (2007). Grassland species loss resulting from reduced niche dimension. *Nature*, *446*, 791–793. https://doi.org/10.1038/nature05684

Hautier, Y., Niklaus, P. A., & Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science*, *324*, 636–638. https://doi.org/10.1126/science.1169640

Hautier, Y., Seabloom, E. W., Borer, E. T., Adler, P. B., Harpole, W. S., Hillebrand, H., Lind, E. M., MacDougall, A. S., Stevens, C. J., Bakker, J. D., Buckley, Y. M., Chu, C., Collins, S. L., Daleo, P., Damschen, E. I., Davies, K. F., Fay, P. A., Firn, J., Gruner, D. S., … Hector, A. (2014). Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, *508*, 521–525. https://doi.org/10.1038/nature13014

Hautier, Y., Zhang, P. F., Loreau, M., Wilcox, K. R., Seabloom, E. W., Borer, E. T., Byrnes, J. E. K., Koerner, S. E., Komatsu, K. J., Lefcheck, J. S., Hector, A., Adler, P. B., Alberti, J., Arnillas, C. A., Bakker, J. D., Brudvig, L. A., Bugalho, M. N., Cadotte, M., Caldeira, M. C., … Wang, S. P. (2020). General destabilizing effects of eutrophication on grassland productivity at multiple spatial scales. *Nature Communications*, *11*, 5375. https://doi.org/10.1038/s41467-020-19252-4

Inouye, R. S., Huntly, N. J., Tilman, D., Tester, J. R., Stillwell, M., & Zinnel, K. C. (1987). Old-field succession on a Minnesota sand plain. *Ecology*, *68*, 12–26. https://doi.org/10.2307/1938801

IPCC. (2022). *Climate change 2022: Impacts, adaptation and vulnerability*. IPCC WGII sixth assessment report, 185–230.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., & Loreau, M. (2011). High plant diversity is needed to maintain ecosystem services. *Nature*, *477*, 199–202. https://doi.org/10.1038/nature10282

Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S., & Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 11911–11916. https://doi.org/10.1073/pnas.1310880110

James, J. J., Svejcar, T. J., & Rinella, M. J. (2011). Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology*, *48*, 961–969. https://doi.org/10.1111/j.1365-2664.2011.02009.x

Kang, L., Han, X. G., Zhang, Z. B., & Sun, O. J. (2007). Grassland ecosystems in China: Review of current knowledge and research advancement. *Philosophical Transactions of the Royal Society of London*, *362*, 997–1008. https://doi.org/10.1098/rstb.2007.2029

Kitajima, K., & Tilman, D. (1996). Seed banks and seedling establishment on an experimental productivity gradient. *Oikos*, *76*, 381–391. https://doi.org/10.2307/3546210

Lan, Z. C., & Bai, Y. F. (2012). Testing mechanisms of N-enrichment-induced species loss in a semiarid Inner Mongolia grassland: Critical thresholds and implications for long-term ecosystem responses. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *367*, 3125–3134. https://doi.org/10.1098/rstb.2011.0352

McLendon, T., & Redente, E. (1991). Nitrogen and phosphorus effects on secondary succession dynamics on a semi-arid sagebrush site. *Ecology*, *72*, 2016–2024. https://doi.org/10.2307/1941556

Michael Anderson, T. (2008). Plant compositional change over time increases with rainfall in Serengeti grasslands. *Oikos*, *117*, 675–682. https://doi.org/10.1111/j.0030-1299.2008.16516.x

Milbau, A., Vandeplas, N., Kockelbergh, F., & Nijs, I. (2017). Both seed germination and seedling mortality increase with experimental warming and fertilization in a subarctic tundra. *AoB Plants*, *9*, plx040. https://doi.org/10.1093/aobpla/plx040

Niu, K. C., Luo, Y. J., Choler, P., & Du, G. Z. (2008). The role of biomass allocation strategy in diversity loss due to fertilization. *Basic and Applied Ecology*, *9*, 485–493. https://doi.org/10.1016/j.baae.2007.06.015

Norghauer, J. M., & Newbery, D. M. (2010). Recruitment limitation after mast-seeding in two african rain forest trees. *Ecology*, *91*, 2303–2312. https://doi.org/10.1890/09-0071.1

Pato, J., & Obeso, J. R. (2013). Effects of clipping and N fertilization on insect herbivory and infestation by pathogenic fungi on bilberry. *Basic and Applied Ecology*, *14*, 347–356. https://doi.org/10.1016/j.baae.2013.02.005

Phoenix, G. K., Emmett, B. A., Britton, A. J., Caporn, S. J. M., Dise, N. B., Helliwell, R., & Power, S. A. (2012). Impacts of atmospheric nitrogen deposition: Responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology*, *18*, 1197–1215. https://doi.org/10.1111/j.1365-2486.2011.02590.x

Prati, D., & Schmid, B. (2000). Genetic differentiation of life-history traits within populations of the clonal plant *Ranunculus reptans*. *Oikos*, *90*, 442–456. https://doi.org/10.1034/j.1600-0706.2000.900303.x

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rajaniemi, T. K., Allison, V. J., & Goldberg, D. E. (2003). Root competition can cause a decline in diversity with increased productivity. *Journal of Ecology*, *91*, 407–416. https://doi.org/10.1046/j.1365-2745.2003.00768.x

Real, R., & Vargas, J. (1996). The probabilistic basis of jaccard's index of similarity. *Systematic Biology*, *45*, 380–385. https://doi.org/10.1093/sysbio/45.3.380

Roem, W. J., Klees, H., & Berendse, F. (2002). Effects of nutrient addition and acidification on plant species diversity and seed germination in heathland. *Journal of Applied Ecology*, *39*, 937–948. https://doi.org/10.1046/j.1365-2664.2002.00768.x

Sala, O. E., Stuart Chapin, F., III, Armesto, J. J., Berlow, E., Bloomfield, J., & Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *Science*, *287*, 1770–1774. https://doi.org/10.1126/science.287.5459.1770

Salemaa, M., & Uotila, T. (2001). Seed bank composition and seedling survivalin forest soil polluted with heavy metals. *Basic and Applied Ecology*, *2*, 251–263. https://doi.org/10.1078/1439-1791-00055

Seabloom, E. W., Adler, P. B., Alberti, J., Biederman, L., Buckley, Y. M., Cadotte, M. W., & Borer, E. T. (2021). Increasing effects of chronic nutrient enrichment on plant diversity loss and ecosystem productivity over time. *Ecology*, *102*, 11. https://doi.org/10.1002/ecy.3218

Shugart, H. H., & Hett, J. M. (1973). Succession: Similarities of species turnover rates. *Science*, *180*, 1379–1381. https://doi.org/10.1126/science.180.4093.1379

Stevens, C. J., Dise, N. B., Mountford, J. O., & Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science*, *303*, 1876–1879. https://doi.org/10.1126/science.1094678

Stevens, M. H. H., Bunker, D. E., Schnitzer, S. A., & Carson, W. P. (2004). Establishment limitation reduces species recruitment and species richness as soil resources rise. *Journal of Ecology*, *92*, 339–347. https://doi.org/10.1111/j.0022-0477.2004.00866.x

Su, Y., Ma, X. F., Le, J. J., Li, K. H., Han, W. X., & Liu, X. J. (2021). Decoupling of nitrogen and phosphorus in dominant grass species in response to long-term nitrogen addition in an alpine grassland in central Asia. *Plant Ecology*, *222*, 261–274. https://doi.org/10.1007/s11258-020-01103-3

Sun, S. C., Gao, X. M., & Cai, Y. L. (2001). Variations in sexual and asexual reproduction of *Scirpus mariqueter* along an elevational gradient. *Ecological Research*, *16*, 263–274. https://doi.org/10.1046/j.1440-1703.2001.00395.x

Sutton, M. A., & Bleeker, A. (2013). The shape of nitrogen to come. *Nature*, *494*, 435–437. https://doi.org/10.1038/nature11954

Sutton, M. A., Oenema, O., Erisman, J. W., Leip, A., vanGrinsven, H., & Winiwarter, W. (2011). Too much of a good thing. *Nature*, *472*, 159–161. https://doi.org/10.1038/472159a

Tian, Q. Y., Liu, N. N., Bai, W. M., Li, L. H., Chen, J. Q., Reich, P. B., & Zhang, W. H. (2016). A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe. *Ecology*, *97*, 65–74. https://doi.org/10.1890/15-0917.1

Tulloss, E. M., & Cadenasso, M. L. (2016). Using realistic nitrogen deposition levels to test the impact of deposition relative to other interacting factors on the germination and establishment of grasses in the California oak savanna. *Plant Ecology*, *217*, 43–55. https://doi.org/10.1007/s11258-015-0558-5

van denBerg, L. J., Dorland, E., Vergeer, P., Hart, M. A., Bobbink, R., & Roelofs, J. G. (2005). Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. *New Phytologist*, *166*, 551–564. https://doi.org/10.1111/j.1469-8137.2005.01338.x

Veenendaal, E. M., Ernst, W. H. O., & Modise, G. S. (1996). Effect of seasonal rainfall pattern on seedling emergence and establishment of grasses in a savanna in south-eastern Botswana. *Journal of Arid Environments*, *32*, 305–317. https://doi.org/10.1006/jare.1996.0025

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinge, W. H., & Tilman, D. G. (1997). Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications*, *7*, 737–750. https://doi.org/10.2307/2269431

Wang, J., Gao, Y. Z., Zhang, Y. H., Yang, J., Smith, M. D., Knapp, A. K., Eissenstat, D. M., & Han, X. G. (2019). Asymmetry in above- and belowground productivity responses to N addition in a semi-arid temperate steppe. *Global Change Biology*, *25*, 2958–2969. https://doi.org/10.1111/gcb.14719

Widdig, M., Heintz-Buschart, A., Schleuss, P. M., Guhr, A., Borer, E. T., Seabloom, E. W., & Spohn, M. (2020). Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biology and Biochemistry*, *151*, 108041. https://doi.org/10.1016/j.soilbio.2020.108041

Wilkinson, L. (2011). ggplot2: Elegant graphics for data analysis by Wickham, H. *Biometrics*, *67*, 671–679. https://doi.org/10.1111/j.1541-0420.2011.01616.x

Xu, Z. W., Wan, S. P., Ren, H. Y., Han, X. G., Li, M. H., Cheng, W. X., & Jiang, Y. (2012). Effects of water and nitrogen addition on species turnover in temperate grasslands in northern China. *PLoS ONE*, *7*, e39762. https://doi.org/10.1371/journal.pone.0039762

Zhang, Y. H., Feng, J. C., Loreau, M., He, N. P., Han, X. G., & Jiang, L. (2019). Nitrogen addition does not reduce the role of spatial asynchrony in stabilising grassland communities. *Ecology Letters*, *22*(4), 563–571. https://doi.org/10.1111/ele.13212

Zhang, Y. H., Loreau, M., Lu, X. T., He, N. P., Zhang, G. M., & Han, X. G. (2016). Nitrogen enrichment weakens ecosystem stability through decreased species asynchrony and population stability in a temperate grassland. *Global Change Biology*, *22*, 1445–1455. https://doi.org/10.1111/gcb.13140

Zhang, Y. H., Lu, X. T., Isbell, F., Stevens, C., Xu, H., He, N. P., & Han, X. G. (2014). Rapid plant species loss at high rates and at low frequency of N addition in temperate steppe. *Global Change Biology*, *20*, 3520–3529. https://doi.org/10.1111/gcb.12611

Zhang, Y. H., Stevens, C. J., Lü, X. T., He, N. P., Huang, J. H., & Han, X. G. (2016). Fewer new species colonize at low frequency N addition in a temperate grassland. *Functional Ecology*, *30*, 1247–1256. https://doi.org/10.1111/1365-2435.12585

Zhao, M., Zhang, H. X., Baskin, C., Wei, C. Z., Yang, J. J., Zhang, Y. H., Jiang, Y., Jiang, L., & Han, X. G. (2022). Intra-annual species gain overrides species loss in determining species richness in a typical steppe ecosystem after a decade of nitrogen enrichment. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.41ns1rngg

Zhong, M., Miao, Y., Han, S., & Wang, D. (2019). Nitrogen addition decreases seed germination in a temperate steppe. *Ecology and Evolution*, *9*, 8441–8449. https://doi.org/10.1002/ece3.5151

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