

Mowing exacerbates the loss of ecosystem stability under nitrogen enrichment in a temperate grassland

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Summary

1. Global reactive nitrogen (N) is projected to further increase in the coming years. Previous studies have demonstrated that N enrichment weakens the temporal stability of the ecosystem and the primary productivity through decreased biodiversity and species asynchrony. Mowing is a globally common practise in grasslands; and infrequent mowing can maintain or increase plant diversity under N enrichment conditions. However, it is unclear how infrequent mowing affects ecosystem stability in the face of N enrichment.

2. By independently manipulating the frequency (twice vs. monthly additions per year) and rate (i.e. 0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m⁻² year⁻¹) of NH₄NO₃ inputs and mowing (unmown vs. mown) over 3 years (2011–2013) in a temperate grassland of northern China, we aimed to examine the interactive effects of N enrichment and mowing on ecosystem stability.

3. The results show that mowing maintained a positive relationship between species richness and ecosystem stability despite N addition, but that it exacerbated the negative effects of N addition on ecosystem stability. Mowing increased mean primary productivity and plant species richness, but it also increased the synchrony of population fluctuations and the variability of primary productivity under N enrichment, thereby contributing to a decline in the ecosystem stability.

4. Thus, our study reveals that infrequent mowing can buffer the negative effects of N enrichment on biodiversity to some extent and further increase the primary productivity, but it exacerbates the loss of ecosystem stability with N enrichment, thereby threatening local and/or semiarid regional food security.

Key-words: biodiversity, biomass harvest, clipping, community stability, hay, nitrogen addition, nitrogen addition frequency, productivity, steppe

Introduction

Nitrogen (N) fertilization is a common practise in the world's semi-natural and managed grasslands and provides important benefits to modern agriculture (Erisman *et al.* 2008; Gibson 2009). For example, fertilization of natural grasslands and pastures in Argentina, Brazil, China, the Netherlands, Russia, the United Kingdom, the United States of America, Uruguay, and other countries has significantly increased plant and animal production (Tilman 1987; Suttie, Reynolds & Batello 2005; Zhang *et al.* 2015). However, intentional (fertilizer application) or

unintentional (atmospheric deposition) reactive N enrichment is also one of the major causes of species extinction and changes in species composition in grassland ecosystems (Mountford, Lakhani & Kirkham 1993; Stevens *et al.* 2004; Clark & Tilman 2008; Socher *et al.* 2013; Zhang *et al.* 2014), and it threatens ecosystem stability (defined as the inverse of the coefficient of temporal variability of net primary productivity: Tilman & Downing 1994; Yang *et al.* 2012; Hautier *et al.* 2015; Zhang *et al.* 2016a). The production and consumption of N fertilizers has increased in most developing countries, such as Brazil and China, and at the global scale since the 1960s (Fig. 1a) (International Fertilizer Industry Association 2015). Because of the rising global human population and the lack of technologies to improve nitrogen utilization efficiency, the

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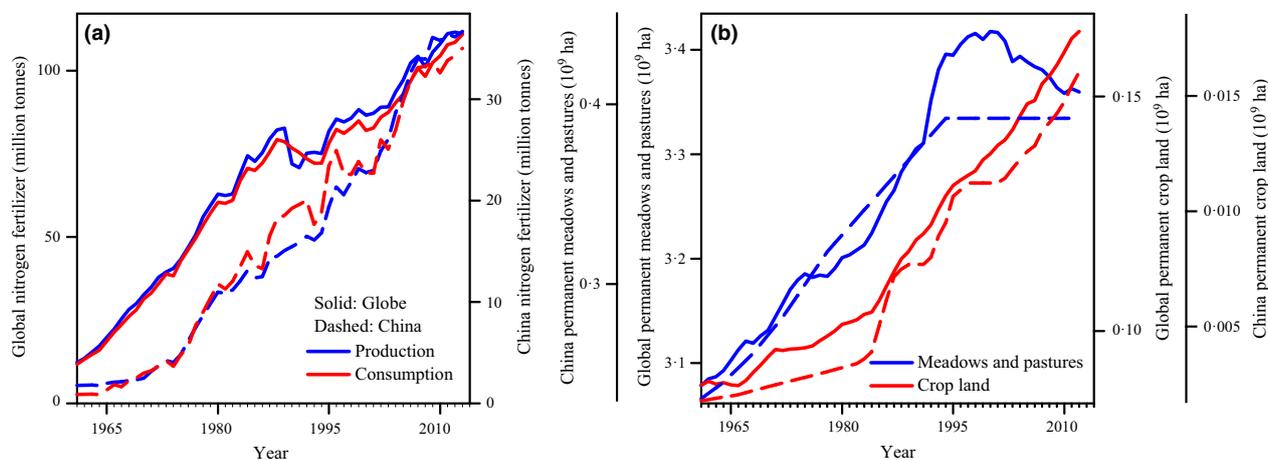


Fig. 1. Temporal trends in N fertilizer, permanent meadows and pastures, and permanent crop lands. (a) Temporal trends in the annual amount and consumption of N fertilizer for most nations around the world (globe) and for China during 1961–2013, indicating that global N deposition has increased in the past decades. (b) Temporal trends in the land area of permanent meadows and pastures or crops globally and for mainland China during 1961–2012. Solid and dashed lines correspond to the data at global scale and China national scale, respectively. Data in (a) and (b) were obtained from the International Fertilizer Industry Association (2015) and the FAOSTAT (2014), respectively.

application of reactive N is expected to further increase in the coming decades at a rate of 1.4% per year globally (The Food and Agriculture Organization of the United Nations 2015). Consequently, global atmospheric N deposition will continue to increase accordingly (Galloway *et al.* 2008).

Biomass harvest (mowing) is one of the oldest and most widespread practises in grassland management because it produces hay, which can be stored for on-farm/agricultural use. Hay is also a sellable commodity as it is easy to transport and store (Suttie, Reynolds & Batello 2005; Mladkova *et al.* 2015). Over the past half century, cropland area increased significantly in the mainland of China and across the globe (Fig. 1b). The area of land in permanent meadows and pastures that is used for hay production increased from the 1960s to the 1990s but has remained stable during the past two decades (i.e. from 1995 to 2012) in the mainland of China (Fig. 1b) (FAOSTAT 2014). As mowing can increase light availability for small, subdominant plant species and thus affect their germination rates, it is now considered a crucial management strategy for restoring plant diversity under increased N deposition/fertilization scenarios (Collins *et al.* 1998; Storkey *et al.* 2015; Jones *et al.* 2017). Infrequent mowing after plant reproduction can reduce the negative effects of N enrichment on species richness (Socher *et al.* 2013; Jones *et al.* 2017), and thereby partly restore species richness (Collins *et al.* 1998; Härdtle *et al.* 2006; Stevens 2016). By contrast, however, intensive mowing can have negative effects on species richness (Blüthgen *et al.* 2012; Socher *et al.* 2012) because frequent mowing before flowering and/or seed release decreases seed production, seed bank diversity, and germination quality and quantity (Socher *et al.* 2012, 2013).

Biodiversity, in particular plant species richness, plays an important role in ecosystem stability by increasing the

asynchrony of species fluctuations (Hector *et al.* 2010; Loreau & de Mazancourt 2013; Hautier *et al.* 2015). The portfolio effect (Tilman, Lehman & Bristow 1998) is the phenomenological outcome of this asynchrony (Loreau 2010), and highlights the positive effect of biodiversity on ecosystem stability. As previous studies reported opposite impacts of N enrichment and mowing on species richness (Collins *et al.* 1998; Yang *et al.* 2012; Storkey *et al.* 2015; Jones *et al.* 2017), how the combination of mowing and N enrichment may affect plant species richness and ecosystem stability remains unclear.

In this study, we use a unique dataset of plant species richness (m^{-2}) and above-ground net primary productivity (ANPP; $g\ m^{-2}\ year^{-1}$) in a temperate grassland in China to examine the interactive effects of mowing and N enrichment on ecosystem stability. We hypothesized that (i) N enrichment would have negative effects on biodiversity, based on the results of previous studies (Stevens *et al.* 2004; Clark & Tilman 2008; Hautier, Niklaus & Hector 2009; Zhang *et al.* 2014), (ii) N enrichment would decrease ecosystem stability via a reduction in species richness and species asynchrony, and (iii) infrequent mowing, which increases species richness (Collins *et al.* 1998; Jones *et al.* 2017), would increase ecosystem stability, based on the positive relationship between species richness and ecosystem stability in the temperate grasslands studied (Yang *et al.* 2012; Zhang *et al.* 2016a).

Materials and methods

STUDY SITE

The field experiment was carried out at a grassland near the Inner Mongolia Grassland Ecosystem Research Station (116°14'E, 43°13'N), Inner Mongolia, China. A 50-ha field had

been fenced since 1999 to exclude large animals from grazing. The topography of the experimental area was flat, with an elevation range of 1255–1260 m. The mean annual temperature was 0.9 °C, with a mean monthly temperature that ranged from –21.3 °C in January to 19.7 °C in July for the period of 1983–2013. Mean annual precipitation was 348.5 mm, with 81.8% falling in from May to September (here after termed the growing season). Temperature and precipitation, in the growing seasons of 2011 to 2013, ranged from 15.1 to 16.0 °C and from 244.6 to 406.7 mm, respectively. The soil was classified as Haplic Calcisol based on the FAO soil classification system. The plant community was dominated by rhizomatous perennial C₃ grass, i.e. *Leymus chinensis* (Trin.) Tzvel, and perennial C₃ bunchgrass, i.e. *Stipa grandis* P. Smirn., which together accounted for more than 60% of the total above-ground biomass (Zhang *et al.* 2015). There were about 50 vascular plant species, averaging eight species per m² in the control plots across 2008–2013 (Zhang *et al.* 2014, 2016b). This ecosystem had received no fertilizer or mowing before this experiment. The ambient total (wet and dry) N deposition in this region was less than 1.5 g N m⁻² year⁻¹ in the 2000s (Zhu *et al.* 2015).

EXPERIMENTAL DESIGN

The experiment was established during September 2008, and followed a randomized complete block design that consisted of nine rates (i.e. 0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m⁻² year⁻¹) crossed with two frequencies (two times per year vs. monthly) (Zhang *et al.* 2014) of N addition and mowing (unmown vs. mown) (Zhang *et al.* 2013). The use of higher N addition rates served as a proxy for N fertilization activities and/or long-term extreme N enrichment in a temperate grassland ecosystem. The higher frequency (i.e. monthly: 12 times per year) of N addition was used to simulate atmospheric N deposition (Smith, Knapp & Collins 2009), which occurs continuously throughout the year (Aneja *et al.* 2001). During the growing season from May to October, fertilizer was weighed and mixed with purified water (9.0 L total for all treatments receiving water: either 9.0 L once in June or 1.5 L monthly from May to October), and sprinkled evenly using a sprayer to each plot to simulate wet N deposition. It was estimated that less than 1 mm of water was added to each plot annually, except the control plots, which had no water added. In winter (from November to April), NH₄NO₃ was mixed with sand (because of low amount of added NH₄NO₃ at low N rates in 12 N additions per year; 0.5 kg total for each treatment receiving sand: either 0.5 kg once in November or 0.08 kg monthly from November to April) and broadcast uniformly by hand. Sand was sieved through less than 1 mm in size, hydrochloric acid dipped, washed in purified water, and then heated at 120 °C for 24 h in an oven. To avoid otherwise potentially confounding effects, the plots received the same amount of water and sand, regardless of whether they received the high or low frequency of N addition treatment. An untreated control (control, with no added N, water, sand, or mowing) was used to detect the influence of water, sand addition, and mowing. In addition, a mowing only control (mowing control, with no added N, water, and sand) was included to detect the influence of added water and sand with mowing, which was also compared with the untreated control. Each plot was 8 × 8 m in area. Hence, there were 38 experimental treatments in total, with 10 replicate blocks for each treatment (i.e. 380 treatment plots in total). Plots were mown annually, in late August (after reproduction had finished for most species), simulating typical hay-cutting management in this region. Single mowing was performed with a mower at a 10-cm height. The harvested above-ground biomass was removed immediately after mowing.

PLANT DATA COLLECTION

The ANPP of the community was estimated from peak above-ground plant biomass, which is an acceptable approximation for ANPP in this region as above-ground plant tissues die during the winter season. Because of a lack of labourers in 2009 and in 2010, plant above-ground biomass was sampled only from 2011 to 2013. This was conducted between 10 and 15 August using a 0.5 m × 2 m rectangle, which was randomly placed in each plot without a spatial overlap of quadrats amongst years and at least 50 cm inside the border of each plot to avoid edge effects. All living vascular plants were sorted according to species, oven-dried at 65 °C for 48 h to a constant weight, and then weighed. Species richness (number of plant species per m²) was recorded in the same quadrat in which above-ground biomass was measured. Hence, there were about 12 540 plant above-ground biomass measurements within 1140 plots in the study.

SHANNON-DIVERSITY (*H'*)

H' was measured as

$$-\sum_{i=1}^N \left(\frac{b_i}{\sum_{i=1}^N b_i} \times 100 \right) \times \ln \left(\frac{b_i}{\sum_{i=1}^N b_i} \times 100 \right)$$

where *N* is species richness, and *b_i* is the ANPP of species *i* in the community.

EVENNESS

Evenness was calculated as (1/*D*)/*N*, where *N* is species richness, and *D* is Simpson's dominance index (Smith & Wilson 1996). *D* was calculated as

$$\sum_{i=1}^N \left(\frac{b_i}{\sum_{i=1}^N b_i} \right)^2$$

where *b_i* is the ANPP of species *i* in the community.

ECOSYSTEM STABILITY

The temporal ecosystem stability in each plot was defined as μ/σ (Lehman & Tilman 2000), where μ and σ are the inter-annual mean and standard deviation of the ecosystem ANPP, respectively.

SPECIES ASYNCHRONY

Species asynchrony was quantified as

$$1 - \frac{\sigma^2}{\left(\sum_{i=1}^N \sigma_{b_i} \right)^2}$$

where σ² is the temporal variance in ecosystem ANPP, and σ_{*b_i*} is the temporal standard deviation in the ANPP of species *i* in a community with *N* species over years (Loreau & de Mazancourt 2008). This measure of species asynchrony ranges between 0 (perfect synchrony) and 1 (perfect asynchrony) (Loreau & de Mazancourt 2008).

THE MEAN-VARIANCE SCALING RELATIONSHIP (TAYLOR'S POWER LAW)

This relationship has the form σ_{*i*}² = *cm^z*, where σ_{*i*}² is the variance in the ANPP of species *i* in a community, *c* is a constant, *m* is the average (mean) of species ANPP, and *z* is the scaling coefficient (Tilman, Lehman & Bristow 1998).

STATISTICAL ANALYSES

Ecosystem stability, ecosystem mean (μ), and ecosystem variance (σ^2) were natural log transformed to ensure normality and homogeneity before the analyses. A two-way analysis of covariance (ANCOVA) was used to explore the effects of the frequency and the rate of N addition, mowing, and their interactions on species richness, the temporal stability of ecosystem ANPP, ecosystem mean (μ), ecosystem variance (σ^2), species asynchrony, evenness, and Shannon-diversity (H'), using the rate of N addition as a continuous variable (Table 1).

As N enrichment impacted on both plant species richness and ecosystem stability simultaneously (Table 1), it is likely to hide the specific effects of diversity on stability (Huston 1997; Loreau

1998). For testing the effects of N addition and mowing (treatments) on diversity-dependent variables, ANCOVAs were performed (Yang *et al.* 2012; Zhang *et al.* 2016a). Firstly, a three-way ANCOVA was used to test the effects of species richness, the rate and frequency of N addition, mowing, and their interactions on ecosystem stability, ecosystem mean (μ), ecosystem variance (σ^2), species asynchrony, evenness, and H' , using the rate of N addition as a continuous variable (Table 2). Secondly, because there were no interactive effects of treatment \times species richness on ecosystem stability, species asynchrony, evenness, and H' (Table 2), two-way ANCOVA was employed, using the frequency of N addition and mowing as fixed variables, species richness as a covariate, and the rate of N addition as a continuous

Table 1. Effects of treatments and their interactions on variables

	d.f.	Species richness		Ecosystem stability		Ecosystem μ		Ecosystem σ^2		Asynchrony		Evenness		H'	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>N</i>	1355	216.4	<0.001	75.5	<0.001	1138.0	<0.001	243.7	<0.001	18.5	<0.001	17.2	<0.001	120.5	<0.001
<i>F</i>	1355	3.5	0.064	3.7	0.056	0.3	0.594	2.9	0.088	2.3	0.133	0.6	0.426	2.1	0.145
<i>M</i>	1355	107.0	<0.001	4.1	0.044	3.4	0.066	5.4	0.021	7.4	0.007	14.7	<0.001	33.7	<0.001
<i>F</i> \times <i>M</i>	1355	1.7	0.196	0.2	0.686	1.6	0.209	0.4	0.510	0.1	0.805	0.8	0.386	0.3	0.606

Results of the two-way ANCOVA for the effects of the rate (*N*) and the frequency (*F*) of N addition, mowing (*M*), and their interactions on species richness, the temporal stability of ecosystem above-ground net primary productivity, ecosystem mean (μ), ecosystem variance (σ^2), species asynchrony, evenness, and Shannon-diversity (H'), using the rate of N addition as a continuous variable. d.f., degrees of freedom. *F*- and *P*-values are given.

Table 2. Effects of species richness, treatment, and their interactions

	d.f.	Ecosystem stability		Ecosystem μ		Ecosystem σ^2		Asynchrony		Evenness		H'	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>N</i>	1276	42.5	<0.001	609.3	<0.001	138.6	<0.001	7.8	0.006	0.7	0.405	4.8	0.029
<i>F</i>	1276	2.6	0.110	1.0	0.317	1.8	0.182	5.0	0.026	0.2	0.690	0.9	0.334
<i>M</i>	1276	5.4	0.021	3.2	0.076	7.0	0.009	8.9	0.003	1.5	0.226	0.4	0.515
<i>R</i>	26 276	2.3	0.001	1.8	0.011	2.6	<0.001	0.8	0.739	2.3	0.001	7.1	<0.001
<i>F</i> \times <i>M</i>	1276	0.1	0.781	1.2	0.265	0.0	0.980	0.0	0.881	0.8	0.373	0.7	0.397
<i>F</i> \times <i>R</i>	22 276	0.9	0.660	0.8	0.671	0.8	0.706	1.3	0.166	1.1	0.327	1.3	0.145
<i>M</i> \times <i>R</i>	18 276	1.5	0.074	2.0	0.009	1.8	0.021	1.0	0.439	1.0	0.436	1.3	0.191
<i>F</i> \times <i>M</i> \times <i>R</i>	13 276	1.4	0.152	0.6	0.848	1.5	0.114	1.0	0.462	1.3	0.200	1.6	0.073

Results of the three-way ANCOVA (with the treatment \times species richness interaction term) for the effects of the rate (*N*) and the frequency (*F*) of N addition, mowing (*M*), species richness (*R*), and their interactions on the temporal stability of ecosystem above-ground net primary productivity, ecosystem mean (μ), ecosystem variance (σ^2), species asynchrony, evenness, and Shannon-diversity (H'), using the rate of N addition as a continuous variable. d.f., degrees of freedom. *F*- and *P*-values are given.

Table 3. Effects of treatments and their interactions on diversity-dependent variables

	d.f.	Ecosystem stability		Asynchrony		Evenness		H'	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>R</i>	1354	0.7	0.394	0.8	0.370	30.7	<0.001	169.2	<0.001
<i>N</i>	1354	40.0	<0.001	8.1	0.005	0.0	0.991	6.3	0.013
<i>F</i>	1354	3.3	0.069	2.0	0.159	0.1	0.779	0.2	0.627
<i>M</i>	1354	4.8	0.030	7.9	0.005	0.7	0.409	0.0	0.933
<i>F</i> \times <i>M</i>	1354	0.2	0.645	0.1	0.759	0.3	0.603	0.1	0.789

Results of two-way ANCOVA (without treatment \times species richness interaction term) for the effects of species richness (*R*), the rate (*N*) and the frequency (*F*) of N addition, mowing (*M*), and their interactions on ecosystem stability, species asynchrony, evenness, and H' , using species richness as a covariate and the rate of N addition as a continuous variable. d.f., degrees of freedom. *F*- and *P*-values are given.

variable (Table 3). Because evenness was affected by neither the rate of N addition nor mowing (Table 1; all $P > 0.4$), evenness was excluded from further regression analyses. In addition, because the frequency of N addition had no effect on the above-mentioned variables (Tables 2 and 3; all $P > 0.05$), values were combined in further analyses (i.e. 20 replicates for each rate of N addition under either unmown or mown treatment). Two-way ANCOVA was employed to test whether mowing affected the slopes as a function of the rate of N addition (species richness or H'). In these mentioned two-way ANCOVAs, Type I was used and F -test was the interaction effect of mowing (H') and variables (N rates, species richness, species asynchrony, ecosystem stability, etc.).

Structural equation modelling (SEM) was employed to estimate the effects of N addition and annual mowing (biomass removing) via the alterations in species richness and species asynchrony, on ecosystem stability. Data were fitted to the model using the maximum likelihood estimation method. Adequacy of the model was determined using a chi-squared test, root square mean errors of approximation (RMSEA), and Akaike information criteria (AIC). Adequate model fits are indicated by a non-significant chi-squared test ($P > 0.05$), low RMSEA (< 0.08), and AIC (Grace 2006).

AMOS 22.0 (Amos Development Co., Greene, ME, USA) was used for the SEM analysis. All statistical analyses were carried out using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

EFFECTS OF N ENRICHMENT

Irrespective of mowing, ecosystem stability (Fig. 2a; $F_{1,358} = 74.5$, $P < 0.0001$; $R^2 = 0.17$), species richness (Fig. 2b; $F_{1,358} = 165.8$, $P < 0.0001$; $R^2 = 0.32$), Shannon-diversity (H' ; Fig. 3a; $F_{1,358} = 110.3$, $P < 0.0001$; $R^2 = 0.24$), and species asynchrony (Fig. 2c; $F_{1,358} = 18.2$, $P < 0.0001$; $R^2 = 0.05$) were all significantly reduced by increasing the rate of N addition, whereas ecosystem mean ANPP (Fig. 3b; $F_{1,358} = 1130.8$, $P < 0.0001$; $R^2 = 0.76$) and ecosystem variance (Fig. 3c; $F_{1,358} = 239.8$, $P < 0.0001$; $R^2 = 0.40$) increased significantly. Ecosystem mean ANPP and ecosystem variance were positively correlated (Fig. 4a; $F_{1,358} = 299.9$, $P < 0.0001$; $R^2 = 0.46$). The rate of increase of the ecosystem mean ANPP with increasing the rate of N addition was slower than that of the ecosystem variance (i.e. smaller slope; $F_{1,716} = 138.8$, $P < 0.0001$).

DIVERSITY AND DIVERSITY-STABILITY RELATIONSHIP

Mowing significantly increased both species richness and H' (Table 1). Species richness was positively associated with the ecosystem stability (Fig. 5a; $F_{1,358} = 20.0$, $P < 0.0001$) and species asynchrony (Fig. 5b; $F_{1,358} = 4.1$, $P = 0.0431$), whilst species asynchrony was positively related to ecosystem stability (Fig. 4b; $F_{1,358} = 440.8$, $P < 0.0001$; $R^2 = 0.55$). Species richness was negatively correlated with ecosystem mean ANPP (Fig. 5c; $F_{1,358} = 126.7$, $P < 0.0001$; $R^2 = 0.26$) and ecosystem variance (Fig. 5d; $F_{1,358} = 53.6$, $P < 0.0001$; $R^2 = 0.13$). The results were similar when using H' instead of species richness. We also found that ecosystem stability, species richness, and

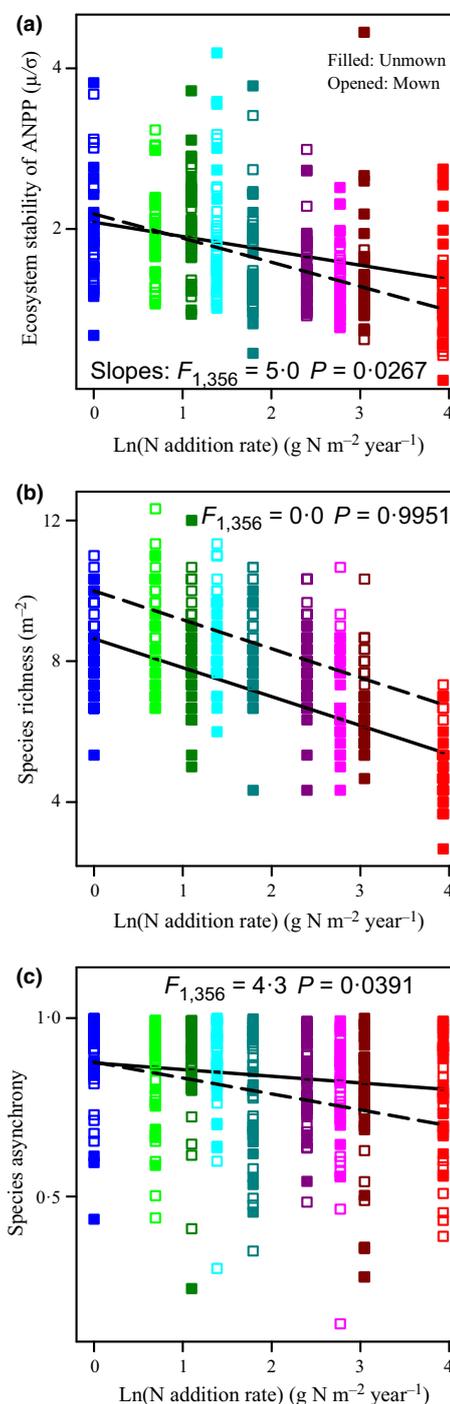


Fig. 2. Effects of N and mowing on ecosystem stability, species richness, and species asynchrony. Changes in (a) the temporal stability of ecosystem above-ground net primary productivity (ANPP), (b) species richness (number of species per m^2), and (c) species asynchrony according to the rate of N addition (different colours; $g N m^{-2} year^{-1}$) and mowing (filled = unmown, opened = annual mown). Solid and dashed lines correspond to unmown and mowing treatments, respectively. F - and P -values are given to demonstrate the effects of mowing on the slopes.

species asynchrony were positively correlated (Figs 4b and 5a,b), whereas N addition reduced ecosystem stability, species richness, and species asynchrony (Fig. 2a–c).

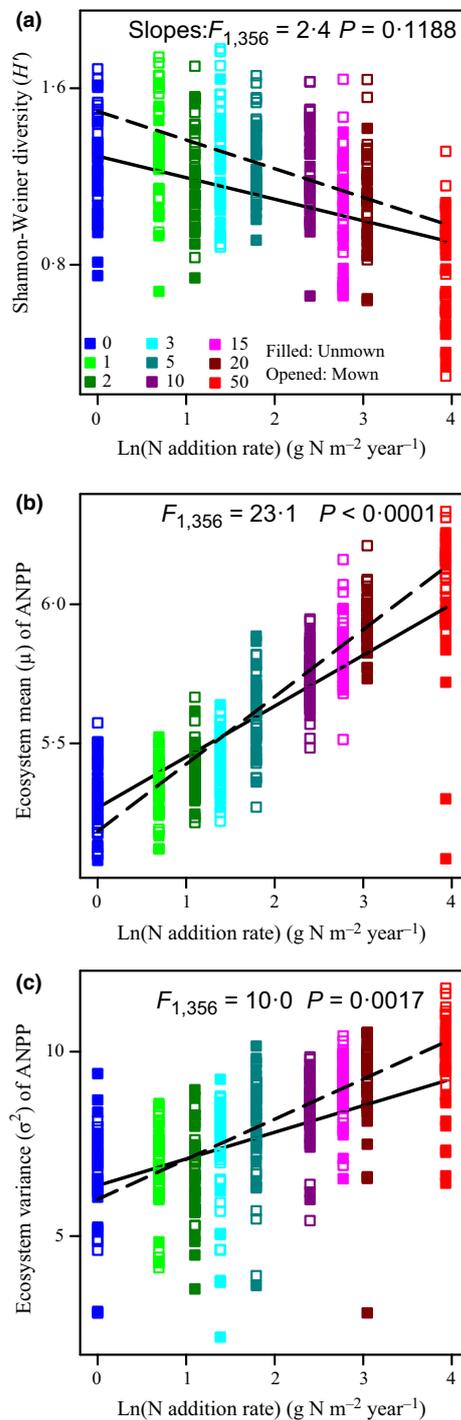


Fig. 3. Effects of N and mowing on diversity, ecosystem mean, and ecosystem variance. Changes in (a) Shannon-diversity index (H'), (b) ecosystem mean above-ground net primary productivity (ANPP) (μ), and (c) ecosystem variance (σ^2). F - and P -values are given to demonstrate the effects of mowing on the slopes. See symbols in Fig. 2.

In addition, log-transformed values of the variance of species ANPP were positively correlated with log-transformed values of its mean (Fig. 5e). The scaling coefficient z of the mean-variance scaling relationship was 1.640 and 1.622 for unmown and mown plots in combination with N

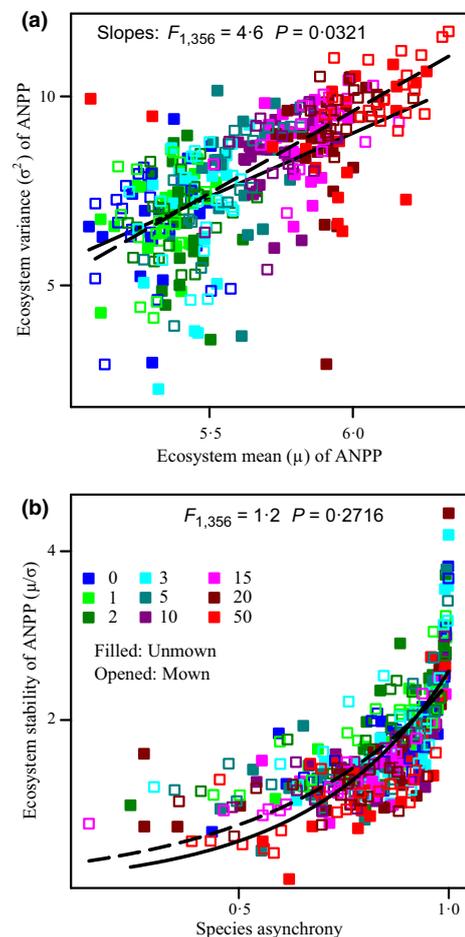


Fig. 4. Mowing accelerated the changes in ecosystem variance with N enrichment. (a) Ecosystem mean above-ground net primary productivity (ANPP) (μ) was positively associated with ecosystem variance (σ^2). The slope in mown treatment was greater, suggesting that mowing decreased ecosystem stability via increases in the variance-to-mean ratio under N enrichment conditions. (b) Species asynchrony promoted ecosystem stability, indicating that lower species asynchrony was likely to have contributed to the reduced ecosystem stability under mowing after N enrichment. F - and P -values are given to demonstrate the effects of mowing on the slopes. See symbols in Fig. 2.

enrichment, respectively (Fig. 5e). There was a similar scaling coefficient z between mown and unmown plots (Fig. 5e; $F_{1,4001} = 1.4$, $P = 0.2334$). Moreover, coefficient z increased with the rate of N addition (Fig. 5f; both $P < 0.05$, $R^2 > 0.60$) and had similar slopes between mown and unmown plots (Fig. 5f; $F_{1,14} = 0.8$, $P = 0.3772$). As the portfolio effect theory requires that the scaling coefficient of this mean-variance scaling relationship should be between one and two, our study highlights the portfolio effect of biodiversity on promoting ecosystem stability.

EFFECTS OF MOWING ON ECOSYSTEM STABILITY

Under N enrichment conditions, mowing significantly promoted the slope of the ecosystem mean ANPP with the increasing rate of N addition (Fig. 3b; $F_{1,356} = 23.1$,

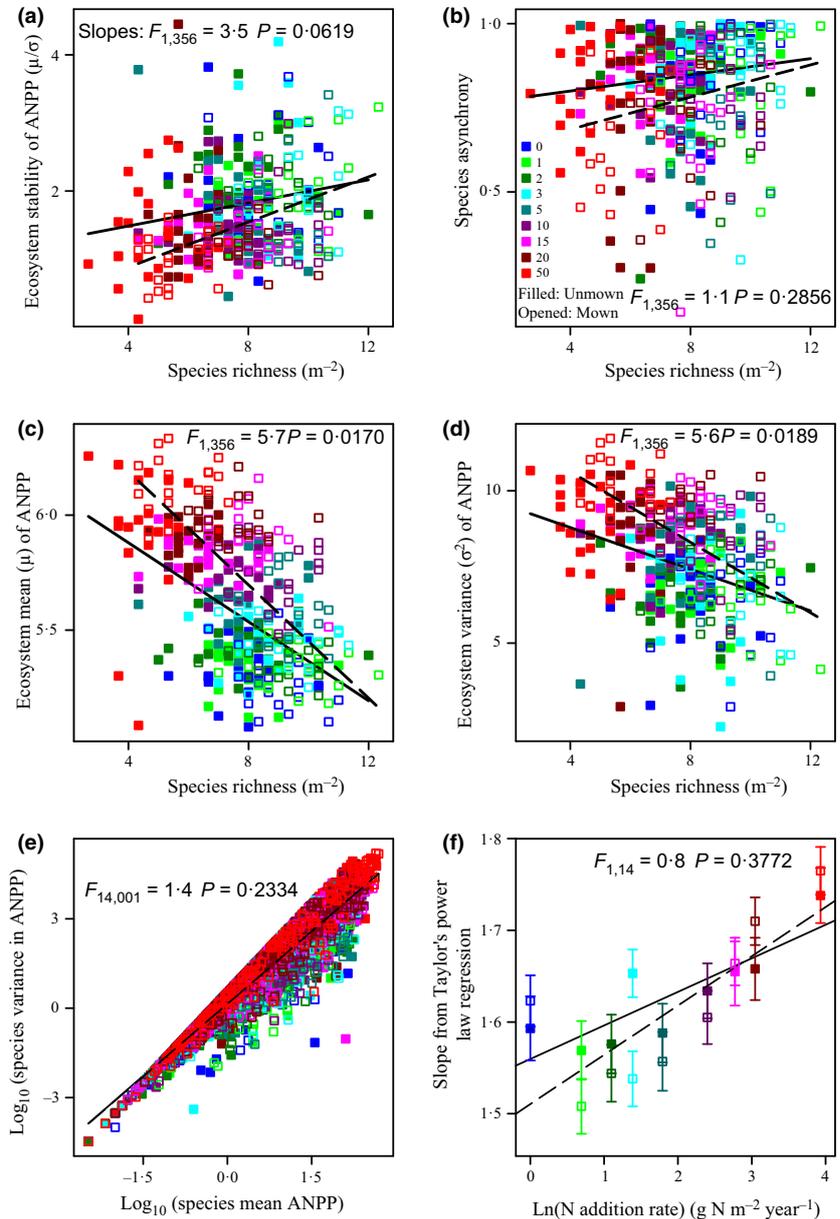


Fig. 5. Biodiversity effects on ecosystem stability. (a) Species richness was positively associated with ecosystem stability. (b) Species richness was positively associated with species asynchrony. Species richness was negatively associated with (c) ecosystem mean above-ground net primary productivity (ANPP) (μ) and (d) ecosystem variance (σ^2). (e), Mean-variance scaling relationships. (f), Slope (z) from Taylor's power law regression increased with increases in the rate of N addition. F - and P -values are given to demonstrate the effects of mowing on the slopes. Note: mowing did not alter the slopes of the relationship between Shannon–Weiner diversity (H') and ecosystem stability too ($F_{1,356} = 3.6$, $P = 0.0575$; Type I, the interaction effect of mowing $\times H'$). See symbols in Fig. 2.

$P < 0.0001$), revealing that mowing could stimulate the average of ecosystem primary productivity across years. Unexpectedly, mowing exacerbated the negative effects of N enrichment on ecosystem stability (Table 3) and species asynchrony (Table 3). By comparisons of slopes, the data showed that mowing significantly lowered the slopes of ecosystem stability (smaller slope; Fig. 2a; $F_{1,356} = 5.0$, $P = 0.0267$; Type I, the interaction effects of mowing \times the rate of N addition) and species asynchrony (Fig. 2c; $F_{1,356} = 4.3$, $P = 0.0391$; Type I, the interaction effects of mowing \times the rate of N addition) vs. increasing the rate of N addition, but significantly increased the slope of ecosystem mean ANPP (Fig. 5b; $F_{1,356} = 23.1$, $P < 0.0001$) and ecosystem variance (Fig. 5c; $F_{1,356} = 10.0$, $P = 0.0017$).

Specifically, because mowing increased the slope between the ecosystem mean ANPP and ecosystem variance (Fig. 4a; $F_{1,356} = 4.6$, $P = 0.0321$; Type I, the

interaction effects of mowing \times ecosystem mean ANPP), under N enrichment conditions, mowing accelerated the decreases in ecosystem stability by enhancing the variance-to-mean ratio. Moreover, mowing, under N enrichment conditions, caused a greater significant reduction in species asynchrony (Fig. 2c) that would result in ecosystem stability decrease (Fig. 4b), but did not alter the positive effects of species asynchrony on ecosystem stability (similar slopes; Fig. 4b; $F_{1,356} = 1.2$, $P = 0.2716$; Type I, the interaction effects of mowing \times species asynchrony). These analyses suggest that mowing exacerbated the negative effect of N enrichment on ecosystem stability, not through changes in the plant diversity but rather because of the combination of increased ecosystem variance and species synchrony.

The final model fitted the data well: $\chi^2 = 0.293$, d.f. = 2, $P = 0.864$; RMSEA = 0.000; AIC = 26.293

(Fig. 6). From the results of the SEM (Fig. 6), it showed that N addition had significantly directly reduced species richness (standardized effect size: -0.56 ; $P < 0.001$), species asynchrony (standardized effect size: -0.18 ; $P = 0.005$), and ecosystem stability (standardized effect size: -0.28 ; $P < 0.001$). Mowing significantly directly promoted species richness (standardized effect size: 0.40 ; $P < 0.001$) whereas was significantly directly and negatively associated with species asynchrony (standardized effect size: -0.17 ; $P = 0.004$). In total, N addition had negative impacts on species richness (standardized effect size: -0.56), species asynchrony (standardized effect size: -0.22), and ecosystem stability (standardized effect size: -0.42); mowing had positive influences on species richness (Fig. 6; standardized effect size: 0.40) but had negative effects on species asynchrony (standardized effect size: -0.14), and ecosystem stability (standardized effect size: -0.08). Overall, results from the SEM revealed that mowing could have beneficial effects on species richness but would exacerbate the negative effect of N enrichment on ecosystem stability via decreased species asynchrony.

Discussion

In this study, we found that N enrichment had positive effects on ecosystem ANPP and negative effects on species richness, species asynchrony, and ecosystem stability, consistent with our predictions. However, contrary to our hypothesis, mowing decreased ecosystem stability under N addition because it increased species synchrony.

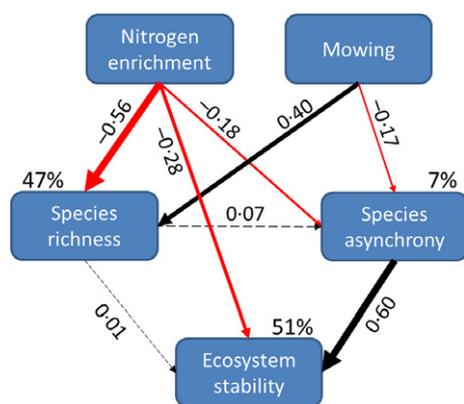


Fig. 6. Effects of N addition and annual mowing on species richness, species asynchrony, and ecosystem stability. The final structural equation modelling (SEM) fitted the data well: $\chi^2 = 0.293$, d.f. = 2, $P = 0.920$; root square mean errors of approximation < 0.001 ; AIC = 26.293. Numbers adjacent to arrows are standardized path coefficients, and width of the arrows indicates the strength of the relationship. Dashed and solid arrows indicate $P > 0.1$ and $P < 0.01$, respectively. Black and red arrows indicate positive and negative relationship, respectively. Percentages close to endogenous variables indicate the variance explained by the model (R^2). The SEM revealed that annual mowing exacerbated the negative effect of N enrichment on ecosystem stability through a decrease in species asynchrony.

In line with previous studies (Hautier *et al.* 2015; Zhang *et al.* 2016a), both the mean and variability of primary productivity increased with the rate of N addition irrespective of mowing, whereas species diversity (species richness and Shannon–Weiner diversity), ecosystem stability, and species asynchrony were all significantly reduced under elevated N. The reasons for the apparent decrease in ecosystem stability following N addition had already been discussed in our previous study (see Zhang *et al.* 2016a). In this study, we found that the increasing rate of N addition significantly reduced species richness, Shannon–Weiner diversity, and species asynchrony, thereby associated with decreasing ecosystem stability. Moreover, based on the portfolio effect that when $2 > z > 1$, diversity is expected to enhance ecosystem stability (Tilman, Lehman & Bristow 1998). In our study, the z was between one and two and increased with the increasing rate of N addition, consistent with theory (Tilman, Lehman & Bristow 1998) and previous reports (Grman *et al.* 2010; Zhang *et al.* 2016a).

Under N enrichment conditions, mowing significantly increased species richness, Shannon–Weiner diversity and the ecosystem mean ANPP, but it decreased ecosystem stability and species asynchrony. Interestingly, the study revealed that mowing with N enrichment could stimulate the average of ecosystem ANPP across years in this grassland which was not similar with previous study (Yuan *et al.* 2004; Niu *et al.* 2010). On the other hand, in line with previous studies (Collins *et al.* 1998; Yang *et al.* 2012; Storkey *et al.* 2015; Jones *et al.* 2017), we can conclude that annual infrequent mowing, which was conducted after the plants' reproductive phase, could partially offset the negative effects of N enrichment on plant diversity. We also found that mowing did not alter the coefficient z of the mean-variance scaling relationship under N enrichment conditions. These results suggest that, regardless of N addition, mowing can still maintain the buffering effect of biodiversity on ecosystem stability.

Unexpectedly, however, mowing exacerbated the negative effects of N enrichment on the temporal stability of ecosystem ANPP. Mowing increased the variance-to-mean ratio of ANPP under N enrichment, thereby contributing to a loss of ecosystem stability. Moreover, mowing resulted in a significantly stronger reduction in species asynchrony with N addition although it did not alter the positive effects of species asynchrony on ecosystem stability. Thus, mowing exacerbated the negative effect of N enrichment on ecosystem stability by further decreasing species asynchrony. Decreased species asynchrony because of mowing may have been caused by nutrient imbalance, ground surface warming, or increased light availability. Mowing can increase nutrient loss (such as N, phosphate, potassium, etc.) from the soil, causing nutrient imbalance (Giese *et al.* 2013; Liu *et al.* 2015), and thereby stronger competition between plants for nutrients after mowing. Mowing or biomass removal may also increase soil surface temperatures (Wan, Luo & Wallace 2002) by increasing ground irradiance, resulting in an increased

synchronization of responses amongst species to climatic conditions during the growing season (Shestakova *et al.* 2016). Theoretically, interspecific competition can destabilize aggregate ecosystem properties (Loreau & de Mazancourt 2013). Nutrients and light are important resources for plant growth in temperate grasslands, and because mowing causes a redistribution of the available nutrients and light, it might increase interspecific competition, and thus reduce species asynchrony and ecosystem stability. As the global climate is expected to become warmer in the coming decades, species responses to climatic conditions might become more synchronous, thus decreasing the stability and predictability of ecosystem ANPP. In contrast, mowing did not alter the effect of N addition on the loss of species diversity, nor did it affect the positive relationship between species richness and ecosystem stability. Overall, the negative effect of mowing on ecosystem stability under N enrichment was not caused by differences in plant diversity, but was a result of a combination of increased ecosystem variance and species synchrony.

In conclusion, N enrichment can increase ecosystem productivity on average, but it has negative effects on species richness, species asynchrony, and ecosystem stability. Mowing can promote ecosystem mean productivity under N enrichment and be beneficial for species richness, but it tends to decrease ecosystem stability via a decrease in species asynchrony. Importantly, we found that even under N enrichment, mowing can maintain a positive relationship between species richness and ecosystem stability, highlighting the fact that maintaining biodiversity is vital for supporting ecosystem functioning and providing ecosystem services.

Authors' contributions

Y.Z. and X.H. designed the research. Y.Z. collected data. Y.Z. and M.L. performed the analysis. Y.Z., M.L., N.H., G.Z., and X.H. wrote the article. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data deposited in the Dryad Digital Repository: <https://dx.doi.org/10.5061/dryad.sq15g> (Zhang *et al.* 2017).

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