Nitrogen enrichment weakens ecosystem stability through decreased species asynchrony and population stability in a temperate grassland

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Abstract

Biodiversity generally promotes ecosystem stability. To assess whether the diversity–stability relationship observed under ambient nitrogen (N) conditions still holds under N enriched conditions, we designed a 6-year field experiment to test whether the magnitude and frequency of N enrichment affects ecosystem stability and its relationship with species diversity in a temperate grassland. Results of this experiment showed that the frequency of N addition had no effect on either the temporal stability of ecosystem and population or the relationship between diversity and stability. Nitrogen addition decreased ecosystem stability significantly through decreases in species asynchrony and population stability. Species richness was positively associated with ecosystem stability, but no significant relationship between diversity and the residuals of ecosystem stability was detected after controlling for the effects of the magnitude of N addition, suggesting collinearity between the effects of N addition and species richness on ecosystem stability, with the former prevailing over the latter. Both population stability and the residuals of population stability after controlling for the effects of the magnitude of N addition were positively associated with ecosystem stability, indicating that the stabilizing effects of component populations were still present after N enrichment. Our study supports the theory predicting that the effects of environmental factors on ecosystem functioning are stronger than those of biodiversity. Understanding such mechanisms is important and urgent to protect biodiversity in mediating ecosystem functioning and services in the face of global changes.

Keywords: asynchrony, biodiversity, community stability, complementarity, insurance hypothesis, nitrogen addition frequency, nitrogen deposition, portfolio effect, species stability

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Introduction

Scientists have long been interested in whether species diversity is associated with ecosystem stability (May, 1974; McNaughton, 1977; Loreau, 2010; Tilman & Snell-Rood, 2014; Hautier *et al.*, 2015). A huge body of recent research on this topic has focused on the relationship between species diversity and ecosystem temporal stability (Jiang & Pu, 2009; Loreau, 2010; Tilman *et al.*, 2014), and suggested a number of underlying mechanisms for this relationship, such as the insurance effect (species asynchrony or compensation) (Yachi & Loreau, 1999; Bai *et al.*, 2004; Loreau & de Mazancourt, 2013), overyielding (Hector *et al.*, 2010), the portfolio effect (statistical averaging)

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(Tilman *et al.*, 1998), and the selection effect (Loreau & Hector, 2001). Rapidly increasing human population (Gerland *et al.*, 2014) and consumption (Westhoek *et al.*, 2014) have generated overexploitation of the remaining natural and managed ecosystems, resulting in high rates of species extinction and ecosystem simplification (Vitousek *et al.*, 1997; Rands *et al.*, 2010). These rapid changes call for an urgent understanding of how ecosystem structure, functioning, and services will be altered following realistic biodiversity loss (Cardinale *et al.*, 2012; Balvanera *et al.*, 2014; Isbell *et al.*, 2015).

Nitrogen (N) enrichment by both atmospheric deposition (Stevens *et al.*, 2004) and fertilization (Bobbink *et al.*, 2010) resulting from human activities is a serious threat to biodiversity, with consequences for ecosystem functioning and services (Vitousek *et al.*, 1997; Isbell *et al.*, 2011; Stevens *et al.*, 2015). Nitrogen

enrichment generally reduces plant species diversity but increases primary production (Hooper *et al.*, 2012). Theoretically, nutrition enrichment may desta-bilize the ecosystem (Rosenzweig, 1971). Whether the relationship between biodiversity and ecosystem sta-bility observed under ambient N conditions in both natural and artificial ecosystems is still present under N enriched conditions is an important but unresolved question. Many previous reports on diversity–stability relationships in controlled N addition experiments (e.g. Tilman & Downing, 1994; Grman *et al.*, 2010; Song & Yu, 2015) did not carefully separate the speci-fic effect of diversity on stability from the confound-ing effects of N availability on diversity and stability (Huston, 1997; Loreau, 1998; Wardle *et al.*, 2000; Lepš, 2004; Duffy, 2009). Using diversity as a covariate or testing its effect on the residuals of ecosystem stability after controlling the effects of N treatment are ways to explore the effect of diversity on stability with real-istic losses of species under N enrichment (Huston, 1997; Yang *et al.*, 2012).

Moreover, the impacts of enriched N on ecosystem properties might be dependent not only on the magni-tude but also on the frequency of N inputs (Zhang *et al.*, 2014a,b). Atmospheric N deposition occurs as many frequent events, each of which adding a relatively small dose of reactive N to the ecosystem (Aneja *et al.*, 2001; Smith *et al.*, 2009). In contrast, in many previous studies, reactive N is usually added during the growing season with only a few additions per year (e.g. Tilman & Downing, 1994; Grman *et al.*, 2010; Yang *et al.*, 2012; Hautier *et al.*, 2014; Song & Yu, 2015). Thus, results from previous controlled N experi-mental studies might lead to a potentially biased assessment for the effects of atmospheric N deposition on species diversity and ecosystem stability, due to the excessively large and infrequent pulses of N added (Smith *et al.*, 2009). For example, species richness decreased less steeply at higher frequency of N addi-tion in a steppe (Zhang *et al.*, 2014b), suggesting that previous N addition studies may overestimate the rate of biodiversity loss following atmospheric N deposi-tion. The response of ecosystem stability may also depend on the frequency of N deposition, as indicated by the contrasting effects of fertilizers with different releasing rates on the temporal stability of the same ecosystem (Yang *et al.*, 2011; Wang *et al.*, 2013). Under-standing the effects of N addition frequency on ecosys-tem stability and on the diversity–stability relationship would help accurately assess its impacts on ecosystem functioning and services under enriched N scenarios (e.g. N fertilization, atmospheric N deposition). However, the effect of the frequency of N additions on ecosystem stability and on the diversity–stability relationship has not been tested so far.

Based on a long-term experiment that included nine rates (from 0 to 50 g N m^{-2} yr^{-1}) crossed with two frequencies (two inputs per year or monthly) of N addition in the form of ammonium nitrate (NH$_4$NO$_3$) in a temperate steppe, we aim to evaluate the effects of the rate and frequency of N addition on ecosystem stability and on the relationship between biodiversity and ecosystem stability. Based on our previous studies showing that a higher fre-quency of N addition had less negative effects on species richness (Zhang *et al.*, 2014b) and species colonization (Zhang *et al.*, 2015b) in the same experi-mental setup, we hypothesized that (i) the amount of N addition would reduce stability, (ii) a lower frequency of N addition would result in a smaller temporal stability at both the population and ecosys-tem levels, and (iii) frequent N addition would weaken the effects of the amount of N addition on the relationship between species richness and ecosys-tem stability. To our knowledge, this is the first study that jointly considers the effects of the fre-quency and rate of N addition on ecosystem stability and on the diversity–stability relationship with realis-tic species extinctions under N enrichment.

Materials and methods

Study site

The field experiment was carried out in a temperate steppe near the Inner Mongolia Grassland Ecosystem Research Sta-tion (IMGERS; 116°14′E, 43°13′N), which is located in the Xilin River Basin, Inner Mongolia Autonomous Region, China. A 50 ha natural grassland has been fenced since 1999 to exclude large animal grazing. The topography of the experimental area is relatively flat, with an elevation ranging from 1255 to 1260 m. Long-term (1980–2013) mean annual temperature was 0.9 °C, with mean monthly temperatures ranging from −21.4 °C in January to 19.7 °C in July. Long-term mean annual precipitation was 351.4 mm, with approximately 72.8% falling during the growing season (May to August). During the growing seasons from 2009 to 2013, precipitation varied more than twofold, from 167.2 to 343.5 mm, and mean monthly temperature ranged from 16.2 to 22.7 °C. The soil is classified as Calcic-Orthic Aridisol by the U.S. soil classifica-tion system. The plant community contained about 60 species. The mean plant community peak aboveground biomass was 176.34 g m^{-2} yr^{-1}, ranging from 68.49 to 342.3 g m^{-2} yr^{-1}. *Stipa grandis* and *Leymus chinensis* together accounted for >60% of the total peak aboveground biomass in the community. The annual ambient atmosphere N deposition was <1.0 g N m^{-2} in this region (Zhu *et al.*, 2015). No fertilizer was received prior to this experiment.

Experimental design

The experiment was established in September 2008, following a randomized complete block design (Zhang *et al.*, 2014b). There were nine rates (0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m^{-2} yr^{-1}) crossed with two frequencies (2 times yr^{-1}, 2 N additions yr^{-1} vs. monthly, 12 N additions yr^{-1}) of N addition. There was also a control treatment (without additions of N, water, or sand) to detect the effects of water and sand additions (see next paragraph) by comparing responses between the control and 0 N addition plots. Each plot is 8 m × 8 m. Hence, there were 19 experimental treatments in total, all present in each of the 10 blocks (10 replicates).

NH$_4$NO$_3$ (>99%) addition started on September 1, 2008 and continued on the first day of each month thereafter for the high-frequency treatment (12 N additions yr^{-1}), and started on November 1, 2008 and continued on the first day of June and November thereafter for the low-frequency treatment (2 N additions yr^{-1}). Thus, total annual N loadings between the two treatments were equal after the monthly N addition in August. 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 <1 mm of water was added to each plot annually, except for the control plots, which had no water added. Control plots received no water whereas water was added to 0 N plots. In winter (from November to April), NH$_4$NO$_3$ was mixed with sand (mixture of NH$_4$NO$_3$ and sand ensured an even distribution of additional N in every plot; 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 spread uniformly by hand. Sand was sieved to <1 mm, dipped in hydrochloric acid, washed in purified water, and then oven-dried at 120 °C for 24 h. To avoid otherwise potentially confounding effects, plots received the same amount of water and sand, regardless of whether they received the high- or low-frequencies of N addition treatment.

Field sampling

Aboveground net primary productivity (ANPP) of the community was estimated annually from peak aboveground plant biomass, which is an acceptable approximation for ANPP in this region as aboveground plant tissues die during the winter season (Bai *et al.*, 2004). Aboveground biomass was sampled each year from 2008–2013 between 10 and 15 August using a 0.5 m × 2 m quadrat (Zhang *et al.*, 2015a), which was randomly placed in each plot without spatial overlap of quadrats among years and at least 50 cm inside the border of each plot to avoid edge effects. All living vascular plants were sorted to species, oven-dried at 65 °C for 48 h to a constant weight, and then weighed. Plant species richness (number of species) was recorded in the same strip of aboveground biomass measured per year (Zhang *et al.*, 2014b).

Statistical analyses

Community ANPP and species richness were not significantly different prior to treatment (i.e. year 2008) between the plots assigned to the various treatments (All $P > 0.1$).

Ecosystem/species temporal stability in each plot was defined as μ/σ (Lehman & Tilman, 2000), where μ is the temporal mean of ecosystem/species ANPP and σ is its standard deviation across the years 2009–2013. To ensure normality and homogeneity, ecosystem stability, species stability, ecosystem μ and ecosystem σ were ln transformed before analyses. Population stability was defined as the average of species stability across all species in the community.

Community-wide species asynchrony (Loreau & de Mazancourt, 2008) was quantified as $1 - \varphi_b = 1 - \sigma_{b_T}^2 / \left(\sum_{i=1}^{S} \sigma_{b_i} \right)^2$, where $\sigma_{b_T}^2$ is the variance of ecosystem ANPP and σ_{b_i} is the standard deviation of ANPP of species i in a community with S species over the years 2009–2013. This measure of species asynchrony ranges between 0 (perfect synchrony) and 1 (perfect asynchrony). A significant increase in community stability with species asynchrony suggests that species asynchrony contributes to stability (Loreau & de Mazancourt, 2013).

We also examined the effect of the mean-variance scaling relationship (Taylor's power law) on ecosystem stability. This relationship has the form $\sigma^2 = cm^z$, where σ^2 is the variance in species ANPP, c is a constant, m is the average species ANPP, and z is the scaling coefficient (Tilman *et al.*, 1998). When $2 > z > 1$, diversity is expected to enhance ecosystem stability (Tilman *et al.*, 1998). We also used each species' residual from Taylor's power law to estimate its relative stability at each N addition rate. A larger negative residual indicated that a species was relatively more stable (Grman *et al.*, 2010). The relationship between dominance and stability was calculated from Pearson's correlation coefficient between species relative abundances and these residuals. A negative value of Pearson's correlation coefficient suggested a positive effect of dominance on stability (Grman *et al.*, 2010).

Two-way analysis of variance (ANOVA) was used to test the effects of the frequency and rate of N addition on ecosystem stability, population stability, ecosystem μ, ecosystem σ, and species asynchrony across the years 2009–2013 (Table S1).

As N enrichment impacted on both plant species richness and stability simultaneously, it is likely to hide the specific effects of diversity on stability (Huston, 1997; Loreau, 1998; Wardle *et al.*, 2000; Duffy, 2009). Therefore, ANCOVAS were employed to test treatment and species richness effects on ecosystem stability, population stability, ecosystem μ, ecosystem σ, and species asynchrony. Because there were no interactive effects of treatments × species richness on these factors (Table S2), ANCOVAS were employed, in which the rate and the frequency of N addition were the fixed variables and species richness was the covariate (Table 1). As the frequency of N addition and the interaction between the rate and frequency of N addition showed no effects on the above-mentioned variables (Tables 1, S1, S2; all $P > 0.1$), values of the N frequency addition were combined in further analyses (20 replicates for each N addition rate). Nitrogen-induced changes in species

Table 1 Results of analysis of covariance (ANCOVA) (without the treatment × species richness interaction term) for the effects of average community species richness (SR), the frequency (F) and rate (N) of nitrogen addition, and their interactions on the ecosystem temporal stability, ecosystem mean (μ) of aboveground net primary productivity, ecosystem standard deviation (σ), population stability, and species asynchrony. Degrees of freedom (df), *F*-test values and *P*-values are given

	df	Ecosystem stability		Ecosystem μ		Ecosystem σ		Population stability		Asynchrony	
		F	P	F	P	F	P	F	P	F	P
SR	1,161	0.0	0.859	7.4	0.007	1.0	0.325	0.0	0.889	0.0	0.893
F	1,161	0.0	0.852	2.0	0.162	0.2	0.665	0.0	0.876	1.3	0.250
N	8,161	16.5	<0.001	38.4	<0.001	34.7	<0.001	5.0	<0.001	3.3	0.002
F × N	8,161	0.8	0.590	1.4	0.185	0.7	0.733	0.6	0.768	0.5	0.827

richness, stability, species asynchrony, ecosystem μ, and ecosystem σ were calculated as the difference between N addition and unmanipulated plots. Residuals from regressions of variables with N addition (or species richness) were used to explore the remaining effects of diversity (or N addition). Path analysis was further conducted to evaluate the relationships between N addition rate, species richness, species asynchrony, population stability, and ecosystem stability. All statistics were performed through SPSS software package (SPSS 18.0 for windows, SPSS Inc., Chicago, IL, USA).

Results

Temporal stability and species asynchrony

The frequency of N addition had no effect on ecosystem stability, population stability, ecosystem μ, ecosystem σ, and species asynchrony (Table S1, all $P > 0.05$). Regardless of the frequency of N addition, ecosystem stability (Fig. 1a; $F_{1,178} = 152.3$, $P < 0.0001$; $R^2 = 0.46$), species richness (Fig. 1b; $F_{1,178} = 88.2$, $P < 0.0001$; $R^2 = 0.33$), population stability (Fig. 1c; $F_{1,178} = 53.3$, $P < 0.0001$; $R^2 = 0.23$), and species asynchrony (Fig. 2a; $F_{1,178} = 7.5$, $P < 0.01$; $R^2 = 0.04$) significantly decreased with increasing N addition rates, whereas ecosystem μ (Fig. 2b; $F_{1,178} = 471.0$, $P < 0.0001$; $R^2 = 0.73$) and ecosystem σ (Fig. 2b; $F_{1,178} = 361.4$, $P < 0.0001$; $R^2 = 0.67$) significantly increased. As expected, ecosystem μ and σ were positively correlated (Fig. 2c; $F_{1,178} = 662.5$, $P < 0.0001$; $R^2 = 0.79$). The rate of increase in ecosystem μ with increasing N addition rate was slower than that of ecosystem σ (i.e. smaller slope; $F_{1,356} = 83.6$, $P < 0.0001$), indicating that increasing rates of N addition weakened ecosystem stability via a decrease in the mean-to-variance ratio.

Log-transformed values of the variance of species ANPP were positively correlated to log-transformed values of its mean (Fig. 3a; All $P < 0.0001$). The scaling coefficient z of the mean-variance scaling relationship was 1.669 and 1.594 for N-enriched and unmanipulated

plots, respectively (Fig. 3a). Ecosystems with N addition had a greater scaling coefficient z compared with unmanipulated plots (Fig. 3a; $F_{1,2192} = 14.7$ $P < 0.0001$). Coefficient z increased with the N addition rate (Fig. 3b; $F_{1,7} = 69.4$, $P < 0.0001$; $R^2 = 0.91$). Species dominance (relative ANPP in the community) and species relative temporal stability (residuals from Taylor's power law) were negatively correlated (Fig. 3c) and the slopes of the regressions between species dominance and relative stability were marginally significantly increased by the increases in N addition rates (Fig. 3d; $F_{1,7} = 4.3$, $P < 0.08$; $R^2 = 0.38$), indicating that increases in the rate of N addition decreased the effects of dominant species on species stability.

Population stability was positively correlated with ecosystem stability (Fig. 4a; $F_{1,178} = 63.2$, $P < 0.0001$; $R^2 = 0.26$). The residuals (from Fig. 1c) of population stability after controlling for the effects of the magnitude of N addition was also positively associated with ecosystem stability (Fig. 4b; $F_{1,178} = 8.4$, $P < 0.01$; $R^2 = 0.05$). These suggest that N enrichment did not alter the stabilizing effects of component populations on ecosystem stability.

Both species asynchrony (Fig. 4c; $F_{1,178} = 60.0$, $P < 0.0001$; $R^2 = 0.25$) and the residuals (from Fig. 2a) of species asynchrony after controlling for N addition rates were positively correlated with ecosystem stability (Fig. 4d; $F_{1,178} = 31.2$, $P < 0.0001$; $R^2 = 0.15$). N-induced changes in species asynchrony were also positively associated with changes in ecosystem stability (Fig. S1a; $F_{1,178} = 44.2$, $P < 0.0001$; $R^2 = 0.22$). This indicates that species asynchrony enhanced ecosystem stability under both unmanipulated and N enriched conditions. Residuals (from Fig. 2a) of species asynchrony were not correlated with ecosystem μ (Fig. 4e; $F_{1,178} = 1.3$, $P > 0.1$; $R^2 = 0.01$) and were negatively correlated with ecosystem σ (Fig. 4e; $F_{1,178} = 15.4$, $P < 0.0001$; $R^2 = 0.08$), suggesting that the stabilizing effects of species asynchrony on ecosystem occurred through decreases in ecosystem variance.
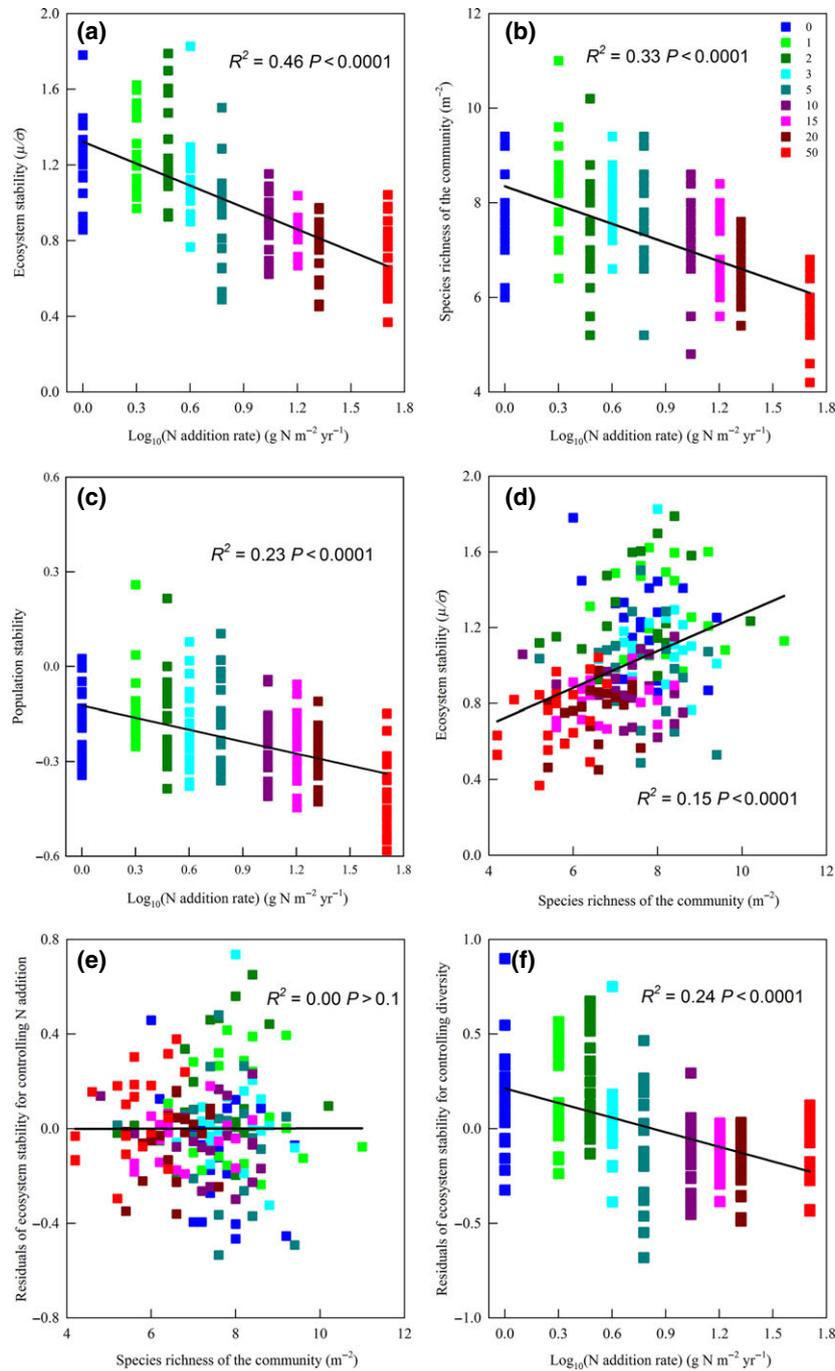
Fig. 1 Ecosystem temporal stability and species richness. (a) Ecosystem temporal stability of aboveground net primary productivity (ANPP), (b) species richness (number m^{-2}), and (c) population stability decreased with increases of N addition rate. (d) Ecosystem stability and (e) residuals (from Fig. 1a) of ecosystem stability as a function of the average species richness of the community. (f), Residuals (from Fig. 1d) of ecosystem stability decreased with increases of N addition rate. Colors corresponded to increasing N addition rate (g N m^{-2} yr^{-1}).

Diversity–stability relationship

The frequency of N addition did not alter the diversity–stability relationship (Table 1; $F_{1,161} = 0.0$, $P > 0.1$). Species richness was positively correlated with ecosystem stability (Fig. 1d; $F_{1,178} = 32.4$, $P < 0.0001$; $R^2 = 0.15$) and the loss of diversity after N addition led to a reduction of ecosystem stability (Fig. S1b; $F_{1,158} = 36.1$, $P < 0.0001$; $R^2 = 0.19$), suggesting that species richness promotes ecosystem stability.
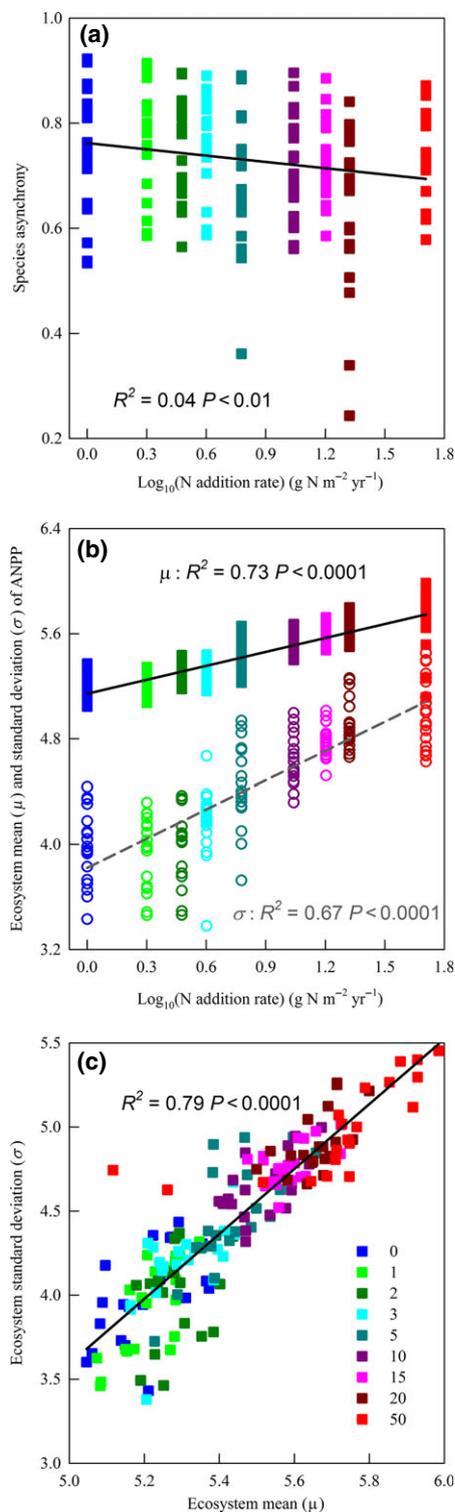
Fig. 2 Effects of N enrichment on species asynchrony, ecosystem mean and its standard deviation, and mean-variance scaling relationship. (a) Changes of species asynchrony with increases of N addition rate, respectively. (b) Ecosystem mean (μ; squares) and its standard deviation (σ; cycles) of ANPP increased with increases of N addition rate, respectively. (c) Relationship between ecosystem μ and ecosystem σ.

But species richness was not related to the residuals (from Fig. 1a) of ecosystem stability after controlling for the effects of N addition (Fig. 1e; $F_{1,178} = 0.0$, $P > 0.1$; $R^2 = 0.00$). In contrast, the residuals (from Fig. 1d) of ecosystem stability after controlling for diversity were still reduced by increases in N addition rates (Fig. 1f; $F_{1,178} = 57.1$, $P < 0.0001$; $R^2 = 0.24$), indicating the effect of N addition on ecosystem stability was stronger than that of diversity.

Species richness was negatively correlated with both ecosystem μ (Fig. 4f; $F_{1,178} = 89.0$, $P < 0.01$; $R^2 = 0.33$) and ecosystem σ (Fig. 4f; $F_{1,178} = 63.2$, $P < 0.0001$; $R^2 = 0.26$). The negative slope between species richness and ecosystem μ, however, was greater than that between species richness and ecosystem σ (Fig. 4f; $F_{1,356} = 12.3$, $P < 0.001$), suggesting that diversity enhanced stability through a decrease in ecosystem μ and an increase in the mean-to-variance ratio under N addition.

The loss of diversity after N addition led to an increase in ecosystem μ (Fig. S1c; $F_{1,158} = 86.3$, $P < 0.0001$; $R^2 = 0.35$) and ecosystem σ (Fig. S1c; $F_{1,158} = 61.2$, $P < 0.0001$; $R^2 = 0.30$). The slope of changes in ecosystem μ was greater than that of changes in ecosystem σ (Fig. S1c; $F_{1,316} = 14.1$ $P < 0.0001$), again indicating that the reduced stability due to N-induced decreases in species richness was caused by a decrease in the mean-to-variance ratio.

Path analysis showed that N addition was the main factor determining ecosystem stability (Fig. 5). There were also significant indirect effects of N addition on ecosystem stability through increases in species asynchrony and population stability (Fig. 5), but no indirect effect of N addition on stability through diversity was detected.

Discussion

In our long-term multi-level, 2-frequency N addition experiment that mimicked a realistic biodiversity loss scenario, we found that the effects of N addition were stronger than those of diversity on the temporal stability of ANPP. To our knowledge, this work is the first to test the simultaneous effects of the frequency and rate of N addition on the temporal stability of ecosystems and populations and on the diversity–stability relationship. Our results showed that N enrichment decreased ecosystem temporal stability, mainly through its negative impacts on the asynchronous growth between species and on the stabilizing effects of component populations. In contrast, the frequency of N addition did not alter the temporal stability of ecosystem and population and the diversity–stability relationship.
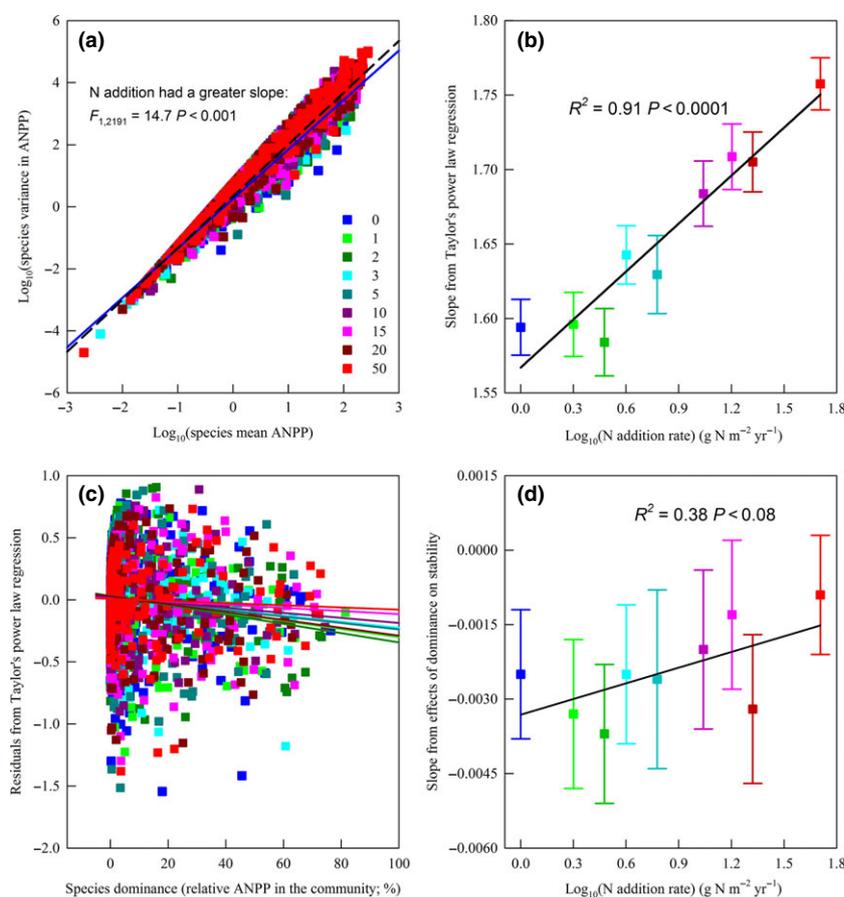
Fig. 3 Mean-variance scaling relationship and species dominance effects. (a) Coefficient (z) of mean-variance scaling relationships in unmanipulated (solid line) and N addition (dashed line) plots. (b) Slope (z) from Taylor's power law regression increased with increases of N addition rate. (c) The relationship between species dominance (relative ANPP in the community) and species relative temporal stability (residuals from Taylor's power law regression). (d) Effects of the rate of N addition on the slope (from effects of species dominance on stability in Fig. 3c).

Effects of the frequency of N addition on ecosystem stability

Neither ecosystem temporal stability nor the diversity–stability relationship was altered by the frequency of N addition. Ecosystem temporal stability was quantified by the ratio between the mean and standard deviation of ANPP (Lehman & Tilman, 2000), both of which did not vary between the two frequencies of N addition (Table S1). Our results showed that the effects of the frequency of N addition were much smaller than those of the rate of N addition. The stronger species loss at lower frequency of N addition did not affect ANPP (Zhang *et al.*, 2015a) because of the negligible biomass production of species lost (generally <5% of the total aboveground biomass). Previous studies found that the ecosystem stability was determined by dominant species rather than by rare ones, at least in the short term (Smith & Knapp, 2003; Polley *et al.*, 2007; Sasaki & Lauenroth, 2011). Moreover, species asynchrony, which

promotes ecosystem stability, did not differ between the two frequencies of N addition. Together, these results suggest that it is the total dosage, rather than the frequency of N enrichment, that affects ecosystem stability and the diversity–stability relationship. Because of the short duration of this study (5 years), the long-term effects of the frequency of N addition still need to be tested.

Effects of the rate of N addition on ecosystem stability

By contrast, the rate of N addition had a significant negative effect on ecosystem stability. As temporal stability is defined as the ratio of the temporal mean of ANPP to its standard deviation, a decrease in ecosystem stability may be caused by a decrease in the mean, an increase in the standard deviation, or both. In agreement with Hautier *et al.* (2014), we found that the decrease in ecosystem stability due to N enrichment resulted from an increase in the mean and a larger increase in the
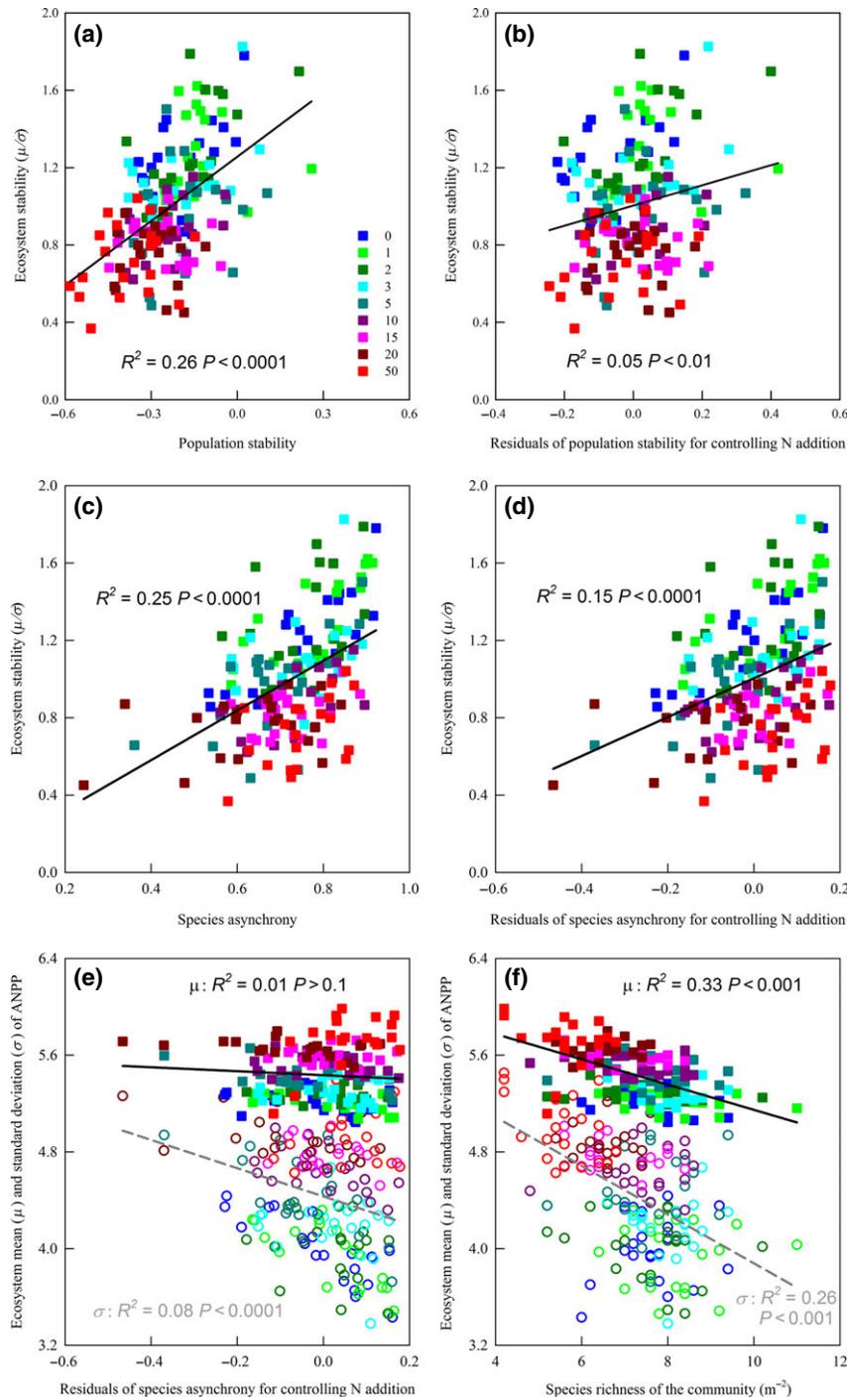
Fig. 4 Possible mechanisms of stabilizing ecosystem. (a) Relationship between population stability and ecosystem stability. (b) Residuals (from Fig. 1c) of population stability were positively associated with ecosystem stability. (c) Species asynchrony was positively associated with ecosystem stability. (d) Residuals (from Fig. 2a) of species asynchrony was positively associated with ecosystem stability. (e) Residuals (from Fig. 2a) of species asynchrony associated with ecosystem mean (μ; squares) and ecosystem standard deviation (σ; cycles). (f) Relationship between species richness and ecosystem μ (squares) and ecosystem σ (cycles), respectively.

standard deviation. We also found that species dominance affected ecosystem stability as there was a negative relationship between species relative ANPP and species relative stability. This result is consistent with the findings of Grman *et al.* (2010), who reported that dominant species were more stable than
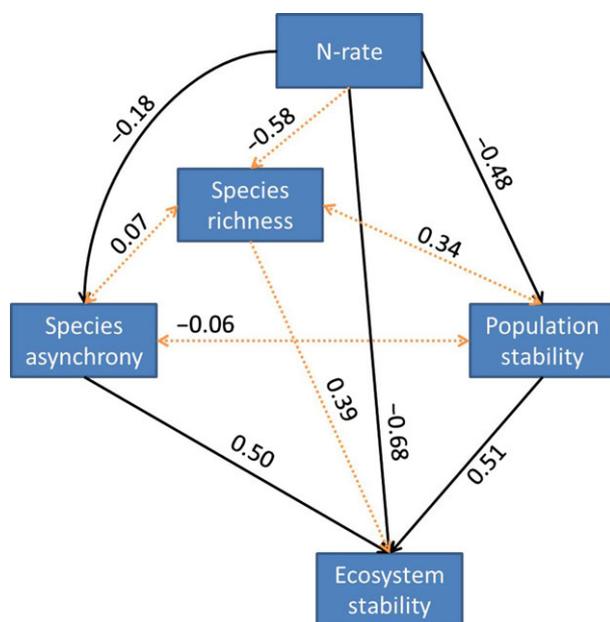
Fig. 5 Path chart illustrating the effect of N addition, species asynchrony, and population stability related to ecosystem temporal stability. Correlation coefficients are labeled. Solid and dotted lines are significant ($P < 0.05$) and non-significant ($P > 0.05$) paths, respectively.

subordinate species under both ambient and enriched N conditions in southwestern Michigan, USA. In our study, the slopes of these regressions were marginally significantly increased by increased N addition rates. This indicates that increases in the rate of N addition decreased the effects of dominant species on species stability and thus reduced average population stability. Our results are consistent with most previous N addition studies (Tilman, 1996; Yang *et al.*, 2012), but in sharp contrast with a few studies that reported either a negative effect (Yang *et al.*, 2011) or no effect (Lepš, 2004; Grman *et al.*, 2010) on ecosystem stability. For example, Yang *et al.* (2011) reported a positive effect of N addition on ecosystem stability in an alpine grassland, mainly via increasing the abundance of a stable dominant species through a selection effect.

Greater ecosystem stability is generally associated with stronger species asynchrony in global grasslands under both natural and N enrichment conditions (Hector *et al.*, 2010; Hautier *et al.*, 2014). Our findings provide robust evidence for a positive relationship between species asynchrony and both ecosystem temporal stability and the residuals of ecosystem stability after controlling the effects of N addition, indicating that such relationship is not altered by enhanced N inputs. Species asynchrony promotes ecosystem stability via compensating growth between species or plant functional groups (Bai *et al.*, 2004; Song & Yu, 2015). In

the same experimental setup, our previous results showed that the decreased ANPP of some species can be compensated by an increased ANPP of other species (Zhang *et al.*, 2015a). For example, the decreased ANPP of four species, *Cleistogenes squarrosa*, *Koeleria cristata*, *Carex korshinskyi*, and *Allium tenuissimum* by N enrichment was be compensated by the increases in ANPP of other three species, *L. chinensis*, *Agropyon cristatum*, and *Chenopodium glaucum*. Thus, our results support the stabilizing effect of temporal complementarity between species on ecosystem stability (Loreau & de Mazancourt, 2013).

We also found a mean-variance scaling relationship consistent with a positive effect of biodiversity on ecosystem stability ($1 < z < 2$), in agreement with previous studies (Lepš, 2004; Grman *et al.*, 2010; Song & Yu, 2015). The scaling coefficient of this relationship was significantly greater in N enrichment plots ($z = 1.669$) than that in ambient N plots ($z = 1.594$), as previously found by Lepš (2004). This suggests that dominant species, i.e. species that have a high mean biomass, are more variable in N enriched conditions, thereby resulting in a greater z value. In the same experimental set-up, Zhang *et al.* (2015a) reported that the ANPP of dominant species was more strongly altered, either positively or negatively, than that of rare species by increased N addition rates. Thus, dominant species might be more sensitive to environmental fluctuations under N enrichment. However, the detailed mechanisms that generate this increased variability of dominant species are still unclear. Further work is needed to test these mechanisms thoroughly. Based on a neutral model, Loreau (2010) predicted that the scaling coefficient z should be related to community-wide species synchrony, φ_b, through the equation $z = 2 + \ln \varphi_b / \ln S$, where S is species richness. Thus, this scaling coefficient should be negatively correlated with species asynchrony. Our experimental results support this prediction ($F_{2,177} = 850.0$, $P < 0.0001$; $R^2 = 0.91$), which means that this relationship exists not only under the neutral assumption but also in real communities.

Diversity–stability relationship and its underlying mechanisms under N enrichment

In agreement with previous studies (Tilman, 1996; Yang *et al.*, 2012; Hautier *et al.*, 2015), we found a positive relationship between biodiversity and ecosystem temporal stability after N addition. This positive diversity–stability relationship, however, was no longer detected after controlling for the effects of N addition in our study, thus challenging a recent study on diversity–stability relationships in Minnesota grasslands

(Hautier *et al.*, 2015). Hautier *et al.* (2015) suggested that biodiversity affects ecosystem temporal stability independently of various anthropogenic environmental drivers, including N addition, water addition, CO_2 enriched, fire and herbivory exclusion, and water addition, at the Cedar Creek Ecosystem Science Reserve. Here, we recalculated the residuals of changes in the response ratio of ecosystem temporal stability as a function of the changes in the response ratio of species richness after controlling for the effects of N enrichment using the data in Hautier *et al.* (2015). We found that these two variables were not significantly correlated ($F_{1,6} = 0.2$, $P > 0.1$, see Fig. S2). Thus, their results do not provide strong evidence that the effect of N addition on ecosystem stability is mediated by changes in diversity, and are in line with ours. But we found that the R^2 of the regression between N addition rates and ecosystem stability largely decreased from 0.46 to 0.24 when the effect of species richness on ecosystem stability was controlled for, which suggests that species richness might promote ecosystem stability despite its relatively weak effect. Our results supports Loreau's (1998) theoretical prediction that diversity effects on ecosystem functioning do exist but that they should generally be weaker than, and masked by, the effects of environmental factors. Thus, the positive effect of diversity on ecosystem stability is likely to be masked by the stronger effect of N addition, particularly when large doses of N fertilizers are added once or when low doses were added by the experiments lasted for a long time.

In conclusion, the negative effect of N addition on ecosystem stability was independent of the frequency of N addition in our 5 year field experiment. Our study demonstrates that N enrichment weakens ecosystem stability and the diversity–stability relationship in a temperate grassland. It also suggests that there might be strong collinearity between the effects of N addition and those of species richness on ecosystem stability, with the former prevailing over the latter. Our work illustrates that reactive N inputs tend to weaken the insurance effects and species temporal complementarity through reduced species asynchrony and reduced stabilizing effects of component populations.

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Supporting Information

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

Table S1. Results of two-way ANOVA for the effects of the frequency and rate of nitrogen addition and their interactions on the ecosystem temporal stability, ecosystem mean, ecosystem standard deviation, population stability, and species asynchrony.

Table S2. Results of three-way ANOVA (with the treatment × species richness interaction term) for the effects of the frequency and rate of nitrogen addition, species richness, and their interactions on the ecosystem temporal stability, ecosystem mean, ecosystem standard deviation, population stability, and species asynchrony.

Figure S1. Nitrogen-induced changes in ecosystem temporal stability, species asynchrony, species richness, and ecosystem mean and its standard deviation.

Figure S2. Relationship between ecosystem stability, species richness and N addition rate using the data in Hautier *et al.* (2015).