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Nitrogen effects on plant species richness in herbaceous communities are more widespread and stronger than those of phosphorus

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ABSTRACT

Both nitrogen (N) and phosphorus (P) enrichment are known to impact plant diversity globally. Recent studies suggest that P enrichment may be as important, or even more important, as a driver of terrestrial plant species loss as N enrichment. However, the generality and relative contribution of these critical nutrients to species losses remains unclear. Here, we quantitatively compared effects of N, P and combined NP enrichment on species richness of natural and semi-natural herbaceous ecosystems across the world in a meta-analysis of 189 long-term nutrient addition experiments in the field. Our experiment-based approach shows that, across terrestrial and wetland ecosystems, N and NP enrichment had widespread and strong negative effects on plant species richness. N reduced plant species richness across experiments by on average 16% ($p < 0.001$), while P did not (on average 3%, NS). Combined NP enrichment also reduced species richness, by on average 16% ($p = 0.009$), with the dominant effect statistically attributed to N. N enrichment effects were greater in China than in Europe and America, which may be explained by background atmospheric N deposition rates and earlier species losses in Europe and America. P enrichment reduced species numbers only in the most species-rich communities and even increased species numbers at high latitudes. All nutrient enrichment combinations (N, P, NP) stimulated aboveground biomass production, and biomass-mediated mechanisms are likely to have contributed to reported species losses. Our findings demonstrate that for the protection of the world's herbaceous plant diversity, it is of the highest priority that N loads be drastically reduced.

1. Introduction

Over the past 4–5 decades, anthropogenic nitrogen (N) and phosphorus (P) enrichment have both emerged as fundamental drivers of profound changes in natural and semi-natural terrestrial, freshwater and coastal ecosystems (Millennium Ecosystem Assessment, 2005). Many studies have contributed to the firm scientific basis supporting that N enrichment leads to terrestrial species losses at the local, regional and global scale (Bobbink et al., 1998, 2010; Stevens et al., 2004, 2010; Suding et al., 2005; Gilliam, 2006; Cleland et al., 2008; De Schrijver et al., 2011; Hautier et al., 2014; Humbert et al., 2016), while other studies documented the correlation between P enrichment and low abundance of rare species in particular (Wassen et al., 2005; Fujita et al., 2014; Ceulemans et al., 2014). While ongoing enrichments of both N and P have been identified as important drivers of global vegetation changes and plant species losses, studies providing the scientific evidence almost always focused on one nutrient exclusively. Hence, although we have increasing knowledge about individual effects, we lack a comprehensive analysis quantitatively comparing N and P effects on a global scale in order to evaluate how general their effects are and how their effect sizes relate.

Worldwide anthropogenic N enrichment is caused by agricultural activities and fossil fuel combustion, together creating reactive N at an accelerating rate, most of which is at some point released into the biosphere (Galloway and Cowling, 2002; Galloway et al., 2008). Reactive N is highly mobile in the environment (N deposition, run-off, leaching), resulting in diffuse N enrichment of natural areas (Vitousek et al., 1997; Bobbink et al., 1998; Tilman et al., 2001; Galloway and Cowling, 2002), particularly in the world's growing economies where N additions are still on the rise (Millennium Ecosystem Assessment, 2005; Phoenix et al., 2006; Galloway et al., 2008). After its release into the environment, increasing N availability has the potential to increase net primary productivity (Elser et al., 2007; Stevens et al., 2015), which in turn may reduce the diversity of terrestrial vegetation through favoring common,

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fast-growing species adapted to high nutrient availability or simply through random loss of the least common species (Vitousek et al., 1997; Bobbink et al., 1998; Stevens et al., 2004; Soons and Ozinga, 2005; Suding et al., 2005; Hautier et al., 2009; Borer et al., 2014b). In addition, N may decrease diversity through factors such as soil acidification, ammonium toxicity, and increased susceptibility to secondary stress factors such as pests, drought and other disturbances (Vitousek et al., 1997; Bobbink et al., 1998; Gilliam, 2006; Kleijn et al., 2008).

In contrast, anthropogenic P enrichment has levelled off in many regions of the world due to legislation, technology-driven reductions of P in wastewater and lower agricultural application rates (Millennium Ecosystem Assessment, 2005). P enrichment of natural areas is less diffuse given the low mobility of P in soils, and mainly restricted to aquatic ecosystems and wetlands where it is more mobile (Reddy et al., 1999). Hence, P (along with N; Grimm et al., 2003; Conley et al., 2009) is an important driver of eutrophication in aquatic ecosystems, where it may lead to vascular plant species replacement, mass development of floating macrophytes and blooms of algae and cyanobacteria (Richardson, 1985; Reddy et al., 1999; Schindler, 2000; Smith, 2003). On the effects of P enrichment on terrestrial ecosystems much less is known. Terrestrial systems have been suggested to be just as often P-limited as N-limited (Elser et al., 2007) and thus equal effects of N and P enrichment in terms of increasing net primary productivity and corresponding loss of biodiversity may be expected. Moreover, recent studies recorded lower species richness on soils with very high P levels (Gilbert et al., 2009; Ceulemans et al., 2013, 2014) and within herbaceous ecosystems the number of endangered plant species tend to be highest under P-limited conditions (Fujita et al., 2014) or persist specifically under conditions of low P availability (Wassen et al., 2005), suggesting that P release into these herbaceous systems is correlated with rare species loss.

While these recent studies suggest that P enrichment may be as important, or even more important, as a driver of terrestrial plant species loss as N enrichment (e.g. Wassen et al., 2005; Ceulemans et al., 2013), it remains difficult to evaluate the contributions of N versus P. The focus of most nutrient-enrichment studies and recent meta-analyses has been solely on N (Suding et al., 2005; Clark et al., 2007; Clark and Tilman, 2008; Chalcraft et al., 2008; LeBauer and Treseder, 2008; De Schrijver et al., 2011; Humbert et al., 2016) and the effects of N and P enrichment on species richness have never been compared comprehensively across ecosystems, let alone on a global scale. Yet, as the sources, pathways and chemical properties of both nutrients differ greatly, this information is highly relevant for designing successful environmental management strategies.

We here quantify the effects of experimental N, P and combined NP additions on herbaceous plant species richness in terrestrial and wetland ecosystems around the world. Regarding species diversity, species richness is a conservative estimate. It only addresses total species numbers and hence ignores reductions in species abundances and losses of (rare or characteristic) species that are replaced by (more common) other species. Yet, it is the quantitative measure that is most widely available, allowing for comparisons between a wide range of studies and should be seen as critical first step towards understanding the differences and similarities between N and P enrichment effects. We carried out this analysis using the most accurate data available, by compiling results on changes in species richness from long-term nutrient enrichment experiments in the field. As a null-hypothesis for comparison between the two nutrients, we hypothesized that long-term nutrient enrichment results in species losses and that increasing levels of nutrient enrichment result in increasing species losses, for both N and P. We also quantified the response of aboveground plant biomass to nutrient enrichment, to test whether nutrients were limiting plant production across the experiments and to help identify possible mechanisms explaining changes in plant species richness. Here, we hypothesized that both nutrients increase above-ground plant biomass and that this biomass increase correlates with species loss.

2. Methods

We performed a meta-analysis combining the results of 189 field nutrient enrichment experiments in natural and semi-natural, herbaceous terrestrial and wetland ecosystems, from 51 different sites, derived from >42 independent studies on N and/or P enrichment effects on species richness and aboveground biomass. Nutrient addition studies from peer-reviewed publications, book chapters, nationally published papers, reports of institutes and from Long Term Ecological Research (LTER) sites were included in our compilation. Studies were collected by systematic literature search in the ISI Web of Knowledge database (final search date 3-Oct-2016), using the following search terms in any combination: nitrogen, phosphorus, N, P, addition, fertilization, experiment, effect, species richness, vegetation. The resulting set of papers was amended with studies from personal literature collections and studies cited in any of the papers, and through contacts with experts in the field.

From this compilation, only studies meeting strict criteria were selected for our analyses. We only included studies with yearly N, P or NP application treatments in combination with unfertilized control treatments, with a duration of at least three growing seasons (resulting in studies with yearly additions of N and P ranging from 0.375 to 60 g N m^{-2} and from 1 to 15 g P m^{-2}). We excluded experiments in which any other nutrients or trace elements were added in combination with N, P or NP. Experiments with additional supply of calcium or replacement fertilization after mowing were also excluded. Furthermore, we only used studies that included quantitative data on vascular plant species richness (number of vascular plant species per plot) or full lists of species. Although we searched the literature extensively, studies that met our selection criteria were limited to temperate to arctic regions in the Northern hemisphere (latitudes 19.4 to 78.9 N, longitudes −159.6 to 141.7 E), except for one at −15.9 S. A list of all studies included in our analysis is given in the Online supporting information (Table S1).

We began by testing whether changes in plant species richness at the end of each experiment were associated to the total cumulative N or P addition during the experiment. Numbers of plant species per plot were obtained from the publications, calculated from presented species tables or gained through personal contact. To avoid pseudoreplication or over-representation of experiments, we averaged experimental data per treatment per investigated site and only included data from the final time step if time series of data were available. In our analyses, species richness values per experiment were weighted by the number of replicates. We calculated the response ratio as the mean species number in the treatment plots divided by the mean species number in the control plots (S_N/S_C, S_P/S_C or S_{NP}/S_C). The response ratio measures the proportional change in treatment plots relative to reference plots. It is simple and straightforward, for example a ratio of 0.75 indicates 25% fewer species in the treatment than in the control while a ratio of 1.25 indicates 25% more species in the treatment than in the control. Analyses of log10-transformed response ratios gave similar results (Table S2). We here report untransformed response ratios as they are symmetric in their evaluation of species added and species lost, and more intuitive to interpret.

Next, we tested whether changes in plant species richness were associated with other biotic and abiotic predictors. Per experiment we noted the additions of N or P (g m^{-2} $year^{-1}$) applied, as well as the total (cumulative) N or P enrichment during the experiment, the duration of the study, the number of replicates, the average amount of aboveground dry weight biomass (g m^{-2}) in the control and treatment plots, the plot size, geographical location (latitude and longitude) and ecosystem type. We also analyzed the response in biomass of the vegetation to nutrient enrichment, to evaluate whether the nutrients supplied were limiting plant production and to help identify possible mechanisms across studies included in our analyses. For biomass

responses we calculated response ratios as described above (B_N/B_C, B_P/B_C or B_{NP}/B_C).

Before analyses, we explored whether the range of conditions under which the response data were collected was similar for experiments on N, P and NP. We tested for differences between these groups regarding nutrient additions (annually and cumulative), number of replicates, duration of the study, average number of species in the control, average biomass in the control, plot size and geographical location (latitude and longitude) using one-way ANOVAs.

In our meta-analysis we tested if the values of the species richness and biomass response ratios at the end of each experiment were significantly different from 1 using weighted one-sample *t*-tests (test value 1). We used Pearson correlations to test for correlations between the species richness and biomass ratios. We related the species richness response ratios to the log-transformed cumulative (total) N or P addition during the experiment, as we assumed the response to level off at very large nutrient loads. We first used weighted linear regression and then used weighted stepwise multiple regression allowing for inclusion of effects of duration of the study, average number of species in the control, average biomass in the control, biomass response ratio, plot size and geographical location (latitude and longitude). In our final (reported) analyses, we did not include the average biomass in the control and biomass response ratios, because these were not selected as explanatory variables in any of the stepwise regression models but greatly reduced the number of analyzed cases. We compared regression slopes between nutrients using unstandardized regression coefficients (as all nutrient additions are in g m^{-2}; Table 3), but compared effects of different explaining variables (measured in different units) in the multiple regressions using standardized regression coefficients (Table 4). Statistical analyses were carried out in R version 3.2.5 (2016 The R Foundation for Statistical Computing).

3. Results

Clearly more nutrient addition experiments have been carried out for N (>41 studies, 131 experiments) than for P (17 studies, 31 experiments). Even fewer experiments combined N and P (15 studies, 27 experiments), and in these experiments the levels of both additions often correlated, i.e. treatments with higher N additions also had higher P additions (although N.S. across studies; Pearson's $r = 0.286$, $p = 0.147$). The conditions under which studies of all types of nutrient additions were carried out did not differ significantly between any of the nutrient combinations (N, P or NP; Table 1), except for control species richness: experiments using P additions were carried out in significantly richer plots than for N or NP.

Our meta-analysis shows that N and NP enrichment generally reduced plant species richness, but that P enrichment had much smaller effects. Specifically, across all sole-N experiments, N enrichment significantly reduced plant species richness by an average of 16% compared with the control (Table 2, Fig. 1A). This effect became increasingly negative with increasing cumulative N addition (Fig. 1A, Table 3). Across all sole-P experiments, we did not find a significant effect of P enrichment on plant species richness (Table 2, Fig. 1B, Table 3). Across NP experiments, plant species richness was reduced significantly by an average of 16% (Table 2, Fig. 1C, D). This effect became increasingly negative with increasing cumulative N or P additions when both nutrients were analyzed separately (Fig. 1C, D, Table 3), but analysis on both nutrients together showed that changes in plant species richness were attributed exclusively to N, with no additional role of P (Table 3). Further analysis revealed that the effect of P addition on plant species richness in the NP treatments was curvilinear, and was better explained by a quadratic regression than by linear regression (weighted quadratic regression analysis, $df = 24$, $R^2 = 0.558$, $F = 15.12$, $p < 0.001$). For all nutrient combinations (N, P or NP) there were also a few sites at which plant species richness increased following low cumulative nutrient addition (Fig. 1).

All nutrient combinations (N, P and NP) generally increased plant aboveground biomass, suggesting that nutrients were limiting plant biomass production at all sites (Table 2, Fig. 2). Specifically, across all sole-N experiments, N enrichment significantly increased plant biomass by an average of 62% (Table 2, Fig. 2A). This effect became increasingly positive with increasing cumulative N addition (Fig. 2A, Table 3). P addition had the smallest effect, increasing biomass by on average 33% (Table 2, Fig. 2B) and no significant relation between cumulative P addition and biomass increase (Fig. 2B, Table 3). NP addition had the strongest effect, increasing biomass by on average 172% (Table 2, Fig. 1C, D), but there was only a significant relation between cumulative N addition and biomass increase and none for P (Fig. 2C, D, Table 3). Changes in plant species richness and changes in plant aboveground biomass in response to nutrient addition were significantly, negatively, correlated for N and NP, but not for P (Fig. S1).

Multiple linear regression analyses exploring the effects of nutrient enrichment in relation to other potentially explaining variables showed that S_N/S_C was also significantly negatively related to longitude (Table 4, Fig. S3A). In effect, these analyses showed that species richness decreased more strongly in China (longitudes 112.16 to 122.35) compared to Europe (longitudes −1.8 to 41.7) and the Americas (longitudes −159.61 to −75.67). Interestingly, biomass increases in response to N

Table 2
Results of weighted one-sample *t*-tests comparing the species richness and biomass response ratios to a test value 1. Reported are number of weighted degrees of freedom (df), mean ratio, standard error (SE) and level of significance (*p*).

Response ratio	df	Mean	SE	*p*
S_N/S_C	130.7	0.84	0.02	<**0.001**
S_P/S_C	27.7	0.97	0.02	0.228
S_{NP}/S_C	24.9	0.84	0.06	**0.009**
B_N/B_C	64.5	1.62	0.07	<**0.001**
B_P/B_C	16.2	1.33	0.11	**0.008**
B_{NP}/B_C	15.0	2.72	0.30	<**0.001**

Table 1
Results of one-way ANOVAs comparing the experimental conditions between the three studied nutrient combinations (N, P or NP addition). Reported are degrees of freedom (df), mean per treatment (±s.d.) and significance of the difference test (*p*). Significant differences (Tukey HSD) between nutrient combinations are indicated by indices A and B.

Variable	df	N	P	NP	*p*
Annual nutrient load (g m^{-2} $year^{-1}$)	N: 156 P: 56	10.7 ± 10.8	5.4 ± 3.7	N: 13.3 ± 17.2 P: 5.2 ± 4.0	N: 0.310 P: 0.880
Total (cumulative) nutrient load (g m^{-2})	N: 156 P: 56	130 ± 299	62 ± 109	N: 100 ± 201 P: 50 ± 99	N: 0.600 P: 0.425
Duration (years)	186	13.9 ± 32.0	12.6 ± 26.0	11.3 ± 27.4	0.907
Replicates (number)	186	4.6 ± 2.7	4.2 ± 1.2	4.3 ± 1.4	0.584
S_C (species/plot)	185	$16.0 \pm 10.6^{(A)}$	$21.8 \pm 14.2^{(B)}$	$18.9 \pm 15.6^{(A,B)}$	**0.047**
B_C (kg m^{-2} $plot^{-1}$)	94	0.357 ± 0.54	0.481 ± 0.87	0.278 ± 0.15	0.567
Plot size species richness (m^2)	176	2.6 ± 7.7	6.2 ± 15	6.2 ± 16	0.119
Plot size biomass (m^2)	89	2.1 ± 5.4	3.6 ± 9.3	1.5 ± 2.1	0.533
Latitude (decimal)	186	45.1 ± 12.0	44.9 ± 16.5	45.8 ± 18.0	0.963
Longitude (decimal)	186	20.3 ± 84.7	−12.0 ± 78.9	12.5 ± 62.2	0.139

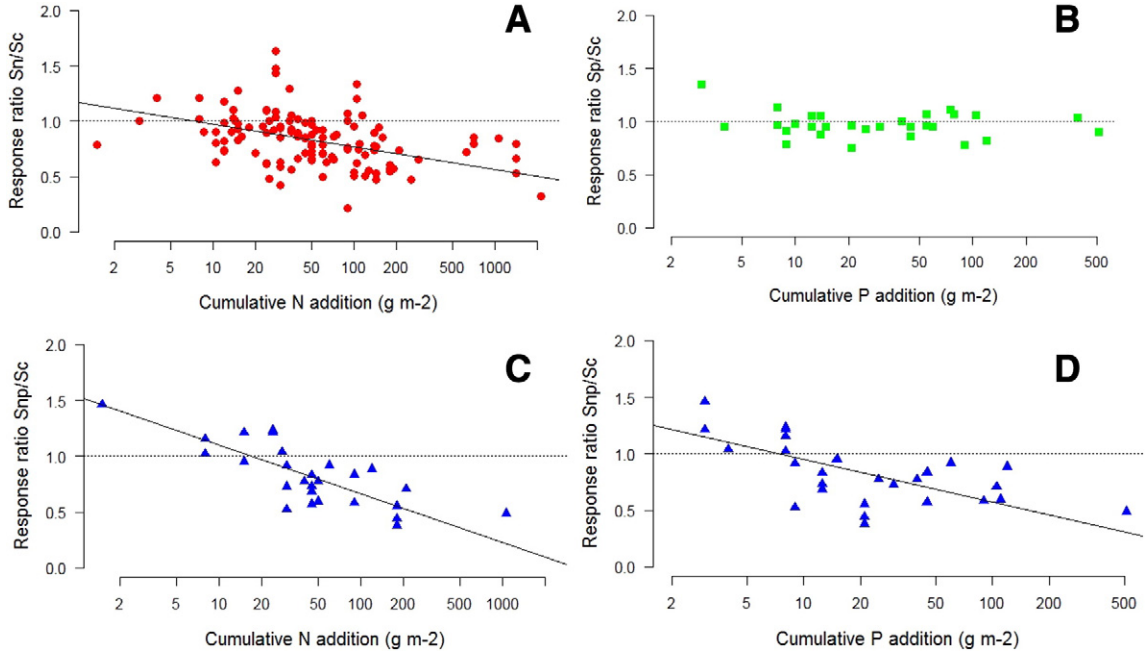

Fig. 1. Effects of N, P and NP enrichment on plant species richness. Response variables S_N/S_C, S_P/S_C and S_{NP}/S_C below 1 indicate species loss following nutrient enrichment; values above 1 indicate an increase in species number following enrichment. (A) N enrichment reduced plant species richness in general, and increasing total experimental N addition reduced species richness more. (B) P enrichment did not affect plant species richness in general, and there was no relation with increasing total experimental P addition. (C, D) NP enrichment reduced plant species richness in general, and increasing total experimental N and P addition both reduced species richness more. Note log-scale on x-axes.

addition were also larger in China (Fig. S3B), in correspondence with higher reported atmospheric background N deposition rates in China (Fig. S3C).

Changes in species richness resulting from P enrichment were less pronounced than for N. In the multiple regression analysis they were only related to latitude (Table 4), with fewer species losses (and even gains) at greater latitudes. No other variables contributed to explaining changes in species numbers in the stepwise multiple regression analysis, although control species richness S_C (when tested separately) did relate to S_P/S_C significantly, with slightly higher species losses in the richest sites (Fig. S2B).

Changes in species richness resulting from NP enrichment were primarily negatively associated with cumulative N addition (Table 4). Additional variation was explained by cumulative P addition, which was included in the regression model when duration and plot size were also included (Table 4). Small but significant contributions to the regression model were reported for duration and plot size. S_{NP}/S_C was also significantly negatively related to S_C (suggesting more species losses in the richest sites; Fig. S2C) when tested separately.

Table 3
Results of weighted linear regression analyses relating the species richness and biomass response ratios to experimental N and P additions. For S_{NP}/S_C also the result of a stepwise multiple regression on experimental N and P addition is included. Dependent and independent variables are followed by weighted degrees of freedom (df) and regression statistics R^2, unstandardized regression coefficient (*rc*) and level of significance (*p*).

Dependent	Independent	df	R^2	*Rc*	*F*	*p*
S_N/S_C	log(total N load)	125	0.172	−0.205	25.49	<**0.001**
S_P/S_C	log(total P load)	29	0.074	−0.071	2.32	0.139
S_{NP}/S_C	log(total N load)	25	0.654	−0.438	47.33	<**0.001**
S_{NP}/S_C	log(total P load)	25	0.427	−0.376	18.65	<**0.001**
S_{NP}/S_C	log(total N load)	25	0.654	−0.438	47.33	<**0.001**
	log(total P load)		Excluded			
B_N/B_C	log(total N load)	57	0.121	0.421	7.86	**0.007**
B_P/B_C	log(total P load)	16	0.002	−0.046	0.03	0.871
B_{NP}/B_C	log(total N load)	15	0.314	1.954	6.88	**0.019**
B_{NP}/B_C	log(total P load)	15	0.001	−0.088	0.01	0.923

4. Discussion

Our meta-analysis including 189 long-term nutrient addition experiments clearly shows that N enrichment generally reduced plant species richness in herbaceous terrestrial and wetland ecosystems. This result is concordant with previous meta-analyses on the effects of N enrichment on plant species richness (Suding et al., 2005; Clark et al., 2007; De Schrijver et al., 2011). Although several studies suggest that P enrichment may be as important as, or even more important than, N enrichment in reducing terrestrial plant species richness (Wassen et al., 2005; Gilbert et al., 2009; Ceulemans et al., 2013; Fujita et al., 2014), we find no support for such a general, negative effect resulting from P enrichment. Even though the number of experiments meeting our strict selection criteria is considerably lower for P than for N, the range of conditions across all P experiments is so similar to that across all N experiments that we consider inclusion of more P experiments unlikely to change this result.

NP enrichment further revealed the overriding effect of N. Enrichment with both N and P significantly and greatly reduced plant species richness, but statistical analyses attributed this effect primarily to N, most likely because the effect of P levelled off with increasing cumulative P addition. Alternatively, NP co-limitation may occur as has been found in 28% of studies across a wider range of ecosystems (including marine and freshwater aquatic systems; Harpole et al., 2011). However, evidence for co-limitation from our data is inconclusive as there is a clear trend of increasing species loss with increasing NP addition, but there is only a significant relation between productivity and N addition – and none with P (although productivity is indeed always increased; Fig. 2). Future studies could more thoroughly manipulate N and P in order to avoid their correlation and unravel the exact contributions of N and P in combined nutrient addition experiments, as is being done in the globally coordinated nutrient network 'NutNet' experiments (Borer et al., 2014a). Yet, so far the existing evidence suggest that P

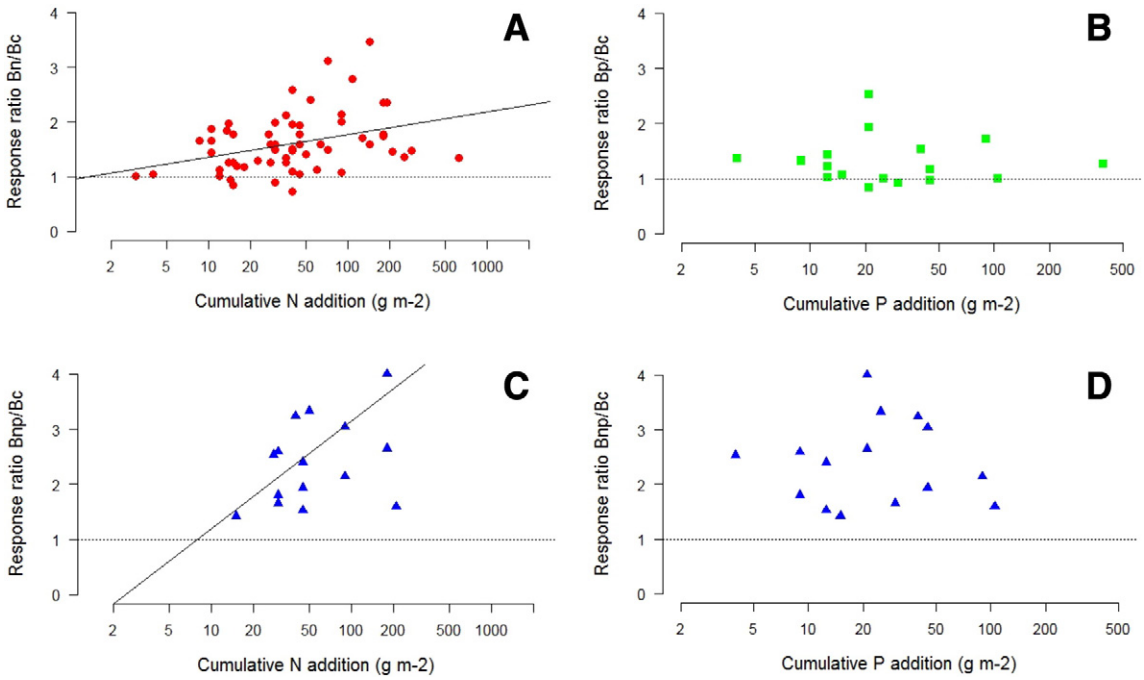

Fig. 2. Effects of N, P and NP enrichment on plant aboveground biomass. Response variables B_N/B_C, B_P/B_C and B_{NP}/B_C below 1 indicate a reduction in biomass following nutrient enrichment; values above 1 indicate an increase in biomass following enrichment. (A) N enrichment increased biomass in general, and increasing total experimental N addition increased biomass more. (B) P enrichment increased biomass in general, but there was no relation with increasing total experimental P addition. (C, D) NP enrichment increased biomass in general, and increasing total experimental N addition increased biomass more, but there was no relation with increasing total experimental P addition. Note log-scale on x-axes.

plays an important role in species losses only in combination with N, since sole P additions appeared to have little effect.

Aboveground biomass increased in almost all enrichment experiments. This result is concordant with Elser et al. (2007) and Fay et al. (2015) that terrestrial ecosystems are often limited by both N and P. The significant correlations between changes in biomass and changes in species richness in response to N and NP enrichment suggest that the negative effect of N enrichment on species richness is mediated through increased net primary productivity (NPP) and resulting competition for light (Hautier et al., 2009, Borer et al., 2014b) or random losses of less abundant species (Suding et al., 2005, Yang et al., 2015). Other important process related to N enrichment that can lead to species loss are soil acidification (Van den Berg et al., 2005; Horswill et al., 2008; Maskell et al., 2010) and resulting mobility of soil Al^{2+} or Mn^{2+} (De Graaf et al., 2009; Tian et al., 2016). N addition can have negative effects through both eutrophication and acidification, which can significantly and independently contribute to species loss (Grace, 2001; Dupré et al., 2010; McClean et al., 2011). Unfortunately, studies from our dataset rarely reported on pH changes throughout the experimental period. The few studies that did, however, almost all reported a decrease in soil pH after N addition and a concomitant reduction in species richness (Paschke et al., 2000; Crawley et al., 2005; Zeng et al., 2010; Pierik et al., 2011; Fang et al., 2012; Tian et al., 2016). Still, this does not exclude other mechanisms such as altered plant-soil relationships, increased sensitivity to pests (Gilliam, 2006; Bobbink et al., 2010; Smits et al., 2010) or even reduced dispersal capacity (Soons et al., 2004). This multitude of alternative pathways through which N enrichment may reduce species numbers provides a potential explanation of the greater effects of N enrichment in experiments across the world.

More species were lost following experimental N additions in China compared to Europe and the Americas. This may be explained by the contemporary atmospheric N (background) deposition rates. Atmospheric N deposition has been documented as a critical factor in reducing terrestrial plant species richness (Gilliam, 2006; Gilliam et al., 2016; McClean et al., 2011; Van den Berg et al., 2011; Dupré et al., 2010; Bobbink et al., 2010). The reported background deposition rates in China (8–33 kg N ha^{-1} $year^{-1}$) during the experimental periods are of relatively recent origin, and will have severely increased the cumulative N addition in this area, stimulating biomass production and species loss. In contrast, reported background deposition rates during many experiments in Europe and the Americas were either lower than in China, or had been high for some time before the start of the experiments (so

Table 4
Results of weighted stepwise multiple linear regression analyses relating the species richness response ratios to experimental N and P additions, duration of the experiment, control species richness S_C, plot size, latitude and longitude. Dependent and independent variables are followed by weighted degrees of freedom (df) and regression statistics R^2, standardized regression coefficient (*rc*) and level of significance (*p*).

Dependent	Independent	df	R^2	Standardized *rc*	*F*	*p*
S_N/S_C	log(total N load)	115	0.238	−0.433	18.75	<**0.001**
	Longitude			−0.237		**0.003**
	S_C			Excluded		
	Plot size			Excluded		
	Duration			Excluded		
	Latitude			Excluded		
S_P/S_C	Latitude	28	0.246	0.509	9.31	**0.005**
	Duration			Excluded		
	log(total P load)			Excluded		
	S_C			Excluded		
	Plot size			Excluded		
	Longitude			Excluded		
S_{NP}/S_C	log(total N load)	21	0.862	−0.597	32.78	<**0.001**
	log(total P load)			−0.563		<**0.001**
	Duration			0.410		**0.001**
	Plot size			0.323		**0.003**
	Longitude			Excluded		
	Latitude			Excluded		
	S_C			Excluded		

that part of the biomass and species richness changes had already taken place). In future analyses, more detailed metrics involving biotic responses to background deposition rates (such as moss tissue N enrichment or mineral N leaching) could be used to better evaluate the effects of past and current background deposition per ecosystem (Rowe et al., 2017).

For P enrichment, species losses were smaller at higher latitudes, even shifting towards species gains at the highest latitudes. At high latitudes, shorter growing seasons, colder temperatures, low mineralization rates and relatively low atmospheric N deposition rates (<3 kg N ha^{-1} $year^{-1}$) limit plant growth (Bobbink et al., 2010). There, (moderate) nutrient enrichment may alleviate stress and result in species gains.

For both P and NP enrichment, negative effects were greater in more species-rich vegetation types. Higher species losses in species-rich vegetation types may have both a biological and a stochastic component, as in more species-rich vegetation there are simply more species to be lost, either through biological mechanisms (competition for light or space) or statistics (random loss) (Suding et al., 2005). Functional trait-based mechanisms are also likely to play a role. Analysis of the LTER sites (of which data are included in our meta-study) showed that species loss was not random, but trait-specific. The identities of the species that disappeared from these sites were community-specific (Gough et al., 2000; Suding et al., 2005), with a varying degree of predictability from the local biotic and abiotic conditions (Pennings et al., 2005; Clark et al., 2007).

As a final point, it is important to realize that our investigation on the effects of nutrient enrichments on species numbers, probably substantially underestimates effects on biodiversity. Species losses are usually preceded by years of reducing abundances of dwindling species and our response variables do not capture replacement of rare and characteristic species by common ones. Hence, effects on biodiversity are expected to be (much) larger than effects on species numbers alone. Could it be that the effect of P on total species richness is small, because its main effect is species turnover instead of species loss? This would be in accordance with findings that a significant number of rare (and threatened) plant species specifically persist under conditions of low soil P availability (Olde Venterink et al., 2003; Wassen et al., 2005; Fujita et al., 2014). Our current analysis does not provide the answer to this question, but such a difference in effects of N versus P (species loss versus species turnover) suggests that both nutrients affect ecosystems via very different mechanisms, and we consider it an important issue to address.

Our assessment raises serious concerns for global biodiversity conservation, which focuses particularly on protection of the most species-rich sites. We show how ongoing N enrichment reduces species-richness of plant communities, but these effects also cascade to associated animal communities, e.g. butterflies (Wallis de Vries and Van Swaay, 2017), and recovery rates after cessation of enrichment are slow (Stevens, 2016). Worldwide N addition has increased fivefold since 1960 and is still rising, especially in growing economies like in Southeast Asia (Millennium Ecosystem Assessment, 2005; Phoenix et al., 2006; Galloway et al., 2008) and biodiversity hotspots are also predicted to receive significant N loading in the future (Phoenix et al., 2006; Bleeker et al., 2011). Consequently, ongoing N pollution poses a significant and very serious threat to terrestrial plant species richness that cannot be prevented by local field management alone (McClean et al., 2011; Van den Berg et al., 2011; Verhoeven et al., 2008; Jones et al., 2017). This implies that for the protection of the world's herbaceous plant diversity, it is of the highest – and immediate – priority that N loads be drastically reduced.

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Supplementary data

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References

Bleeker, A., Hicks, W.K., Dentener, E., Galloway, J., Erisman, J.W., 2011. N deposition as a threat to the world's protected areas under the Convention on Biological Diversity. Environ. Pollut. 159, 2280–2288.
Bobbink, R., Hornung, M., Roelofs, J.G.M., 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. J. Ecol. 86, 717–738.
Bobbink, R., Hicks, K., Galloway, J., et al., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20, 30–59.
Borer, E.T., Harpole, W.S., Adler, P.B., et al., 2014a. Finding generality in ecology: a model for globally distributed experiments. Methods Ecol. Evol. 5, 65–73.
Borer, E.T., Seabloom, E.W., Gruner, D.S., et al., 2014b. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508, 517–520.
Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2013. Plant species loss from European semi-natural grasslands following nutrient enrichment – is it nitrogen or is it phosphorus? Glob. Ecol. Biogeogr. 22, 73–82.
Ceulemans, T., Stevens, C.J., Duchateau, L., et al., 2014. Soil phosphorus constrains biodiversity across European grasslands. Glob. Chang. Biol. 20, 3814–3822.
Chalcraft, D.R., Cox, S.B., Clark, C., et al., 2008. Scale-dependent responses of plant biodiversity to nitrogen enrichment. Ecology 89, 2165–2171.
Clark, C.M., Tilman, D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451, 712–715.
Clark, C.M., Cleland, E.E., Collins, S.L., et al., 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. Ecol. Lett. 10, 596–607.
Cleland, E.E., Clark, C.M., Collins, S.L., et al., 2008. Species responses to nitrogen fertilization in herbaceous plant communities, and associated species traits. Ecological archives E089-070. Ecology 89 (1175).
Conley, D.J., Paerl, H.W., Howarth, R.W., et al., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 323, 1014–1015.
Crawley, M.J., Johnston, A.E., Silvertown, J., et al., 2005. Determinants of species richness in the park grass experiment. Am. Nat. 165, 179–192.
De Graaf, M.C.C., Bobbink, R., Smits, N.A.C., Van Diggelen, R., Roelofs, J.G.M., 2009. Biodiversity, vegetation gradients and key biogeochemical processes in the heathland landscape. Biol. Conserv. 142, 2191–2201.
De Schrijver, A., De Frenne, P., Ampoorter, E., et al., 2011. Cumulative nitrogen input drives species loss in terrestrial ecosystems. Glob. Ecol. Biogeogr. 20, 803–816.
Dupré, C., Stevens, C.J., Ranke, T., et al., 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. Glob. Chang. Biol. 16, 344–357.
Elser, J.J., Bracken, M.E.S., Cleland, E.E., et al., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142.
Fang, Y., Xun, F., Bai, W., Zhang, W., Li, L., 2012. Long-term nitrogen addition leads to loss of species richness due to litter accumulation and soil acidification in a temperate steppe. PLoS One 7, e47369.
Fay, P.A., Prober, S.M., Harpole, W.S., et al., 2015. Grassland productivity limited by multiple nutrients. Nature Plants 1, 15080.
Fujita, Y., Olde Venterink, H., Bodegom, P.M., et al., 2014. Low investment in sexual reproduction threatens plants adapted to phosphorus limitation. Nature 505, 82–86.
Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31, 64–71.
Galloway, J.N., Townsend, A.R., Erisman, J.W., et al., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Gilbert, J., Gowing, D., Wallace, H., 2009. Available soil phosphorus in semi-natural grasslands: assessment methods and community tolerances. Biol. Conserv. 142, 1074–1083.
Gilliam, F.S., 2006. Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. J. Ecol. 94, 1176–1191.
Gilliam, F.S., Welch, N.T., Phillips, A.H., et al., 2016. Twenty-five-year response of the herbaceous layer of a temperate hardwood forest to elevated nitrogen deposition. ECOSPHERE 7, e01250.
Gough, L., Osenberg, C.W., Gross, K.L., Collins, S.L., 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos 89, 428–439.
Grace, J.B., 2001. The roles of community biomass and species pools in the regulation of plant diversity. Oikos 92, 193–207.
Grimm, N.B., Gergel, S.E., McDowell, W.H., et al., 2003. Merging aquatic and terrestrial perspectives of nutrient biogeochemistry. Oecologia 137, 485–501.

Harpole, W.S., Ngai, J.T., Cleland, E.E., et al., 2011. Nutrient co-limitation of primary producer communities. Ecol. Lett. 14, 852–862.
Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636–638.
Hautier, Y., Seabloom, E.W., Borer, E.T., et al., 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature 508, 521–525.
Horswill, P., O'Sullivan, O., Phoenix, G.K., Lee, J.A., Leake, J.R., 2008. Base cation depletion, eutrophication and acidification of species-rich grasslands in response to long-term simulated nitrogen deposition. Environ. Pollut. 155, 336–349.
Humbert, J.-Y., Dwyer, J.M., Andrey, A., Arlettaz, R., 2016. Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review. Glob. Chang. Biol. 22, 110–120.
Jones, L., Stevens, C., Rowe, E.C., et al., 2017. Can on-site management mitigate nitrogen deposition impacts in non-wooded habitats? Biol. Conserv. 212, 464–475.
Kleijn, D., Bekker, R.M., Bobbink, R., De Graaf, M.C.C., Roelofs, J.G.M., 2008. Search for key biogeochemical factors affecting plant species persistence in heathland and acidic grasslands: a comparison of common and rare species. J. Appl. Ecol. 45, 680–687.
LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379.
Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K., Stevens, C.J., 2010. Nitrogen deposition causes widespread loss of species richness in British habitats. Glob. Chang. Biol. 16, 671–679.
McClean, C.J., Van den Berg, L.J.L., Ashmore, M.R., Preston, C.D., 2011. Atmospheric nitrogen deposition explains patterns of plant species loss. Glob. Chang. Biol. 17, 2882–2892.
Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute, Washington, DC.
Olde Venterink, H., Wassen, M.J., Verkroost, A.W.M., De Ruiter, P.C., 2003. Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. Ecology 84, 2191–2199.
Paschke, M.W., McLendon, T., Redente, E.F., 2000. Nitrogen availability and old-field succession in a shortgrass steppe. Ecosystems 3, 144–158.
Pennings, S.C., Clark, C.M., Cleland, E.E., et al., 2005. Do individual plant species show predictable responses to nitrogen addition across multiple experiments? Oikos 110, 547–555.
Phoenix, G.K., Hicks, W.K., Cinderby, S., et al., 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Glob. Chang. Biol. 12, 470–476.
Pierik, M., van Ruijven, J., Bezemer, T.M., Geerts, R.H.E.M., Berendse, F., 2011. Recovery of plant species richness during long-term fertilization of a species-rich grassland. Ecology 92, 1393–1398.
Reddy, K.R., Kadlec, R.H., Flaig, E., Gale, P.M., 1999. Phosphorus retention in streams and wetlands: a review. Crit. Rev. Environ. Sci. Technol. 29, 83–146.
Richardson, C.J., 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. Science 228, 1424–1427.
Rowe, E.C., Jones, L., Dise, N.B., et al., 2017. Metrics for evaluating the ecological benefits of decreased nitrogen deposition. Biol. Conserv. 212, 454–463.
Schindler, D.W., 2000. Evolution of phosphorus limitation in lakes. Science 195, 260–262.
Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems - a global problem. Environ. Sci. Pollut. Res. 10, 126–139.
Smits, N.A.C., Bobbink, R., Laanbroek, H.J., Paalman, A.J., Hefting, M.M., 2010. Repression of potential nitrification activities by matgrass sward species. Plant Soil 337, 435–445.
Soons, M.B., Ozinga, W.A., 2005. How important is long-distance seed dispersal for the regional survival of plant species? Divers. Distrib. 11, 165–172.
Soons, M.B., Nathan, R., Katul, G.G., 2004. Human effects on long-distance wind dispersal and colonization by grassland plants. Ecology 85, 3069–3079.
Stevens, C.J., 2016. How long do ecosystems take to recover from atmospheric nitrogen deposition? Biol. Conserv. 200, 160–167.
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.
Stevens, C.J., Dupre, C., Dorland, E., et al., 2010. Nitrogen deposition threatens species richness of grasslands across Europe. Environ. Pollut. 158, 2940–2945.
Stevens, C.J., Lind, E.M., Hautier, Y., et al., 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. Ecology 96, 459–1465.
Suding, K.N., Collins, S.L., Gough, L., et al., 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proc. Natl. Acad. Sci. U. S. A. 102, 4387–4392.
Tian, Q., Liu, N., Bai, W., et al., 2016. A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe. Ecology 97, 65–74.
Tilman, D., Fargione, J., Wolff, B., et al., 2001. Forecasting agriculturally driven global environmental change. Science 292, 281–284.
Van den Berg, L.J.L., Dorland, E., Vergeer, P., Hart, M.A.C., Bobbink, R., Roelofs, J.G.M., 2005. Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. New Phytol. 166, 551–564.
Van den Berg, L.J.L., Vergeer, P., Rich, T.C.G., Smart, S.M., Guest, D., Ashmore, M.R., 2011. Direct and indirect effects of nitrogen deposition on species composition change in calcareous grasslands. Glob. Chang. Biol. 17, 1871–1883.
Verhoeven, J.T.A., Soons, M.B., Janssen, R., Omtzigt, N., 2008. An operational landscape unit approach for identifying key landscape connections in wetland restoration. J. Appl. Ecol. 45, 1496–1503.
Vitousek, P.M., Aber, J.D., Howarth, R.W., et al., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.
Wallis de Vries, M.F., Van Swaay, C.A.M., 2017. A nitrogen index to track changes in butterfly species assemblages under nitrogen deposition. Biol. Conserv. 212, 448–453.
Wassen, M.J., Venterink, H.O., Lapshina, E.D., Tanneberger, F., 2005. Endangered plants persist under phosphorus limitation. Nature 437, 547–550.
Yang ZL, Hautier Y, Borer ET, Zhang CH, Du GZ. 2015. Abundance- and functional-based mechanisms of plant diversity loss with fertilization in the presence and absence of herbivores. Oecologia 179:261–270.
Zeng, D., Li, L., Fahey, T., Yu, Z., Fan, Z., Chen, F., 2010. Effects of Nitrogen Addition on Vegetation and Ecosystem Carbon in a Semi-arid Grassland. Springer, The Netherlands.

References

Bakker, J.P., 1989. Nature Management by Grazing and Cutting. Kluwer Academic Publishers, Dordrecht.
Beltman, B., Willems, J.H., Gusewell, S., 2007. Flood events overrule fertiliser effects on biomass production and species richness in riverine grasslands. J. Veg. Sci. 18, 625–634.
Berlin, G.A.I., 1998. Semi-natural Meadows in Southern Sweden - Changes Over Time and the Relationship Between Nitrogen Supply and Management. Lund University, Lund, Sweden.
Bobbink, R., 1991. Effects of nutrient enrichment in Dutch chalk grassland. J. Appl. Ecol. 28, 28–41.
Bonanomi, G., Caporaso, S., Allegrezza, M., 2006. Short-term effects of nitrogen enrichment, litter removal and cutting on a Mediterranean grassland. Acta Oecol. 30, 419–425.
Bowman, W.D., Murgel, J., Blett, T., Porter, E., 2012. Nitrogen critical loads for alpine vegetation and soils in Rocky Mountain National Park. J. Environ. Manag. 103, 165–171.
Brancaleoni, L., Gerdol, R., 2014. Habitat-dependent interactive effects of a heatwave and experimental fertilization on the vegetation of an alpine mire. J. Veg. Sci. 25, 427–438.
Bustamante, M.M.C., de Brito, D.Q., Kozovits, A.R., et al., 2012. Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (cerrado). Plant Ecol. 213, 795–808.
Calvo, L., Alonso, I., Marcos, E., De Luis, E., 2007. Effects of cutting and nitrogen deposition on biodiversity in Cantabrian heathlands. Appl. Veg. Sci. 10, 43–52.
Cusell, C., Kooijman, A., Lamers, L.P.M., 2014. Nitrogen or phosphorus limitation in rich fens? – edaphic differences explain contrasting results in vegetation development after fertilization. Plant Soil 384, 153–168.
Day, F.P., Conn, C., Crawford, E., Stevenson, M., 2004. Long-term effects of nitrogen fertilization on plant community structure on a coastal barrier island dune chronosequence. J. Coast. Res. 20, 722–730.
Faust, C., Storm, C., Schwabe, A., 2012. Shifts in plant community structure of a threatened sandy grassland over a 9-yr period under experimentally induced nutrient regimes: is there a lag phase? J. Veg. Sci. 23, 372–386.
Gough, L., Hobbie, S.E., 2003. Responses of moist non-acidic arctic tundra to altered environment: productivity, biomass, and species richness. Oikos 103, 204–216.
Jacquemyn, H., Brys, R., Hermy, M., 2003. Short-term effects of different management regimes on the response of calcareous grassland vegetation to increased nitrogen. Biol. Conserv. 111, 137–147.
Li, K., Liu, X., Song, L., et al., 2015. Response of alpine grassland to elevated nitrogen deposition and water supply in China. Oecologia 177, 65–72.
Lu, X., Mo, J., Gilliam, F.S., Zhou, G., Fang, Y., 2010. Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest. Glob. Chang. Biol. 16, 2688–2700.
Lu, X., Mo, J., Gilliam, F.S., et al., 2011. Effects of experimental nitrogen additions on plant diversity in tropical forests of contrasting disturbance regimes in southern China. Environ. Pollut. 159, 2228–2235.
Lüdi, W., 1959. Versuche zur Alpweideverbesserung auf der Schynigen Platte bei Interlaken. Beil Jahresber des Vereins Alpengarten Schynige Platte, 8S.
Madan, N., Deacon, L.J., Robinson, C.H., 2007. Greater nitrogen and/or phosphorus availability increase plant species' cover and diversity at a High Arctic polar semidesert. Polar Biol. 30, 559–570.
Nilsson, M.C., Wardle, D.A., Zackrisson, O., Jaderlund, A., 2002. Effects of alleviation of ecological stresses on an alpine tundra community over an eight-year period. Oikos 97, 3–17.
Nishimura, A., Tsuyuzaki, S., 2015. Plant responses to nitrogen fertilization differ between post-mined and original peatlands. Folia Geobot. 50, 107–121.
Onipchenko, V.G., Makarov, M.I., Akhmetzhanoca, A.A., et al., 2012. Alpine plant functional group responses to fertilizer addition depend on abiotic regime and community composition. Plant Soil 357, 103–115.
Ostertag, R., Verville, J.H., 2002. Fertilization With Nitrogen and Phosphorus Increases Abundance of Non-native Species in Hawaiian Montane Forests. Springer, Netherlands.
Song, M.H., Yu, F.H., 2015. Reduced compensatory effects explain the nitrogen-mediated reduction in stability of an alpine meadow on the Tibetan Plateau. New Phytol. 207, 70–77.
Song, L., Bao, X., Liu, X., et al., 2011. Nitrogen enrichment enhances the dominance of grasses over forbs in a temperate steppe ecosystem. Biogeosciences 8, 2341–2350.
Song, L., Bao, X.M., Liu, X.J., Zhang, F.S., 2012. Impact of nitrogen addition on plant community in a semi-arid temperate steppe in China. J. Arid. Land 4, 3–10.
Soudzilovskaia, N.A., Onipchenko, V.G., 2005. Experimental investigation of fertilization and irrigation effects on an alpine heath, northwestern Caucasus, Russia. Arctic, Antarctic and Alpine. Research 37, 602–610.
Sun, X., Yu, K., Shugart, H.H., Wang, G., 2016. Species richness loss after nutrient addition as affected by N:C ratios and phytohormone GA3 contents in an alpine meadow community. J. Plant Ecol. 9, 201–211.
Verhoeven, J.T.A., Beltman, B., Dorland, E., Robat, S.A., Bobbink, R., 2011. Differential effects of ammonium and nitrate deposition on fen phanerogams and bryophytes. Appl. Veg. Sci. 14, 149–157.
Vitousek, P.M., Farrington, H., 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37, 63–75.

Wardle, D.A., Gundale, M.J., Jäderlund, A., Nilsson, M.C., 2013. Decoupled long-term effects of nutrient enrichment on aboveground properties in subalpine tundra. Ecology 94, 904–919.
Xu, Z., Wan, S., Ren, H., et al., 2012. Effects of water and nitrogen addition on species turnover in temperate grasslands in northern China. PLoS One 7, e39762.
Yang, H., Yang, L., Wu, M., Zhang, Z., Li, L., Wan, S., 2011. Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. Glob. Chang. Biol. 17, 2936–2944.
Yang, H., Jiang, L., Li, L., Li, A., Wu, M., Wan, S., 2012. Diversity-dependent stability under mowing and nutrient addition: evidence from a 7-year grassland experiment. Ecol. Lett. 15, 619–626.
Yu, L., Song, X.L., Zhao, J.N., et al., 2015. Responses of plant diversity and primary productivity to nutrient addition in a *Stipa baicalensis* grassland, China. J. Integr. Agric. 14, 2099–2108.
Zhang, T., Guo, R., Gao, S., Guo, J., Sun, W., 2015. Responses of plant community composition and biomass production to warming and nitrogen deposition in a temperate meadow ecosystem. PLoS One 10 (4), e0123160.