LETTER

Environmental and plant community determinants of species loss following nitrogen enrichment

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Abstract

Global energy use and food production have increased nitrogen inputs to ecosystems worldwide, impacting plant community diversity, composition, and function. Previous studies show considerable variation across terrestrial herbaceous ecosystems in the magnitude of species loss following nitrogen (N) enrichment. What controls this variation remains unknown. We present results from 23 N-addition experiments across North America, representing a range of climatic, soil and plant community properties, to determine conditions that lead to greater diversity decline. Species loss in these communities ranged from 0 to 65% of control richness. Using hierarchical structural equation modelling, we found greater species loss in communities with a lower soil cation exchange capacity, colder regional temperature, and larger production increase following N addition, independent of initial species richness, plant productivity, and the relative abundance of most plant functional groups. Our results indicate sensitivity to N addition is co-determined by environmental conditions and production responsiveness, which overwhelm the effects of initial community structure and composition.

Keywords

Biogeochemistry, functional traits, meta-analysis, nitrogen, SEM, species loss.

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INTRODUCTION

Research dedicated to understanding the impacts of N enrichment on ecosystems is prompted by unresolved questions from both theoretical and applied ecology. Theory has long predicted a hump-shaped relationship between diversity and productivity (Grime 1979), although the patterns observed across natural fertility gradients vary widely and are often scale dependant (Gross *et al.* 2000; Mittelbach *et al.* 2001). In contrast, experimental increases in production via N addition have been shown to reduce diversity independent of initial productivity (Gough *et al.* 2000), suggesting that the effects of N addition on diversity may not mimic patterns of diversity across natural fertility gradients. From an applied standpoint, understanding how biotic and abiotic factors modulate the cascade of effects following N addition, including soil acidification, changes in plant tissue chemistry, and losses of diversity, remains a major goal (Galloway *et al.* 2003; Sutherland *et al.* 2006). N availability commonly limits production in temperate terrestrial ecosystems (Vitousek & Howarth 1991). This condition, combined with the recent

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doubling of N inputs globally over the past century from human activity (Galloway *et al.* 1995; Vitousek *et al.* 1997), highlight the importance of understanding the effects of N enrichment on plant community structure and function.

Comparative studies between and within ecosystems have demonstrated general patterns that underlie wide variation in response to N enrichment. In a meta-analysis of N addition studies across seven North American herbaceous ecosystems, Gough *et al.* (2000) found a general increase in productivity (+50%) and decrease in diversity (−30%) following N addition. However, there existed wide variation in the magnitude of productivity response (+10% to +125%) and diversity response (+8% to −65%) with analyses suggesting that there was nearly as much variation among communities within sites as among sites. Indeed, although N addition to calcareous grasslands in the Netherlands dramatically reduced species richness (Bobbink 1991), there was comparatively little impact of N addition on similar grasslands in the UK (Wilson *et al.* 1995; Carroll *et al.* 2003). Within ecosystems, one study comparing the responses of two alpine meadows in Colorado (Theodose & Bowman 1997) and another comparing 13 tundra communities in Alaska (Shaver & Chapin 1995) found that the identity of the limiting nutrient varied by community and by species within communities, greatly influencing the response to N addition. In contrast to this variation, experiments in three Minnesota successional grasslands (Tilman 1987) and in two Dutch calcareous grasslands (Bobbink 1991) found that N addition consistently reduced diversity and that the same few species came to dominate each field after fertilization (*Agropyron repens* and *Poa pratensis* in Minnesota; *Brachypodium pinnatum* in the Netherlands).

Why do some communities respond so strongly to N enrichment while others do not; and, why do some sites respond so consistently while others are highly variable? Although a few general predictions have been proposed (Bobbink *et al.* 1998), no comparative tests of these have been performed across ecosystems due to the large amount of physical, biological and biogeochemical data that is required. As a consequence, our understanding is commonly restricted to site-specific mechanisms, limiting our ability to develop a general predictive framework of the net effect of N addition on plant species richness.

In this paper, we focus on the question: do initial plant community properties (structure, productivity and composition), or the environmental context in which these communities occur (climate, soil and biogeochemistry), or some combination of these, explain the variation of community responses to fertilization? Climatic, soil and biogeochemical properties likely influence the potential for soil acidification and changes in N availability to the plant community following N-fertilization. Plant community properties subsequently influence the potential growth response to increased N availability, affecting whether and to what degree, competition may change. We hypothesize that plant species richness will be maximally reduced by N addition when five conditions are present: (H1) the primary limiting nutrient is N; (H2) the ambient production rates and availability of N are low; (H3) the potential for N-induced soil acidification is high; (H4) many species and/or functional groups sensitive to N addition are present; and (H5) the potential for secondary impacts from stress factors such as from increased herbivory or frost damage is high. Differences between sites in any of these conditions may explain the variation in response to N addition.

We sought to include a comprehensive set of variables, from a wide range of herbaceous ecosystems, to predict the contributions of these five conditions in regulating the magnitude of species loss following N addition. The identity of the limiting nutrient (N, P, K, water, etc.) would greatly impact the effect of added N (H1), and is influenced by many biotic and abiotic factors including parent material, climate, soil biogeochemistry, and the nutrient demands of species within the community (Chapin *et al.* 2002). Although N limitation is often primary or co-occurring in temperate regions (Vitousek & Howarth 1991; Wassen *et al.* 2005), water, phosphorous, or light respectively may be limiting or co-limiting in arid, acidic (or alkaline), or highly productive sites (Newman 1973; Chapin *et al.* 2002; Wassen *et al.* 2005). Ambient production rates of available N would influence the relative effect-size of a given N-treatment (H2), and can be estimated by assessing net N mineralization rates (Chapin *et al.* 1986; Falkengren-Grerup 1998). Soil cation exchange capacity (CEC), initial pH, and regional precipitation patterns influence the retention of nutrients in the soil matrix, affecting the availability of added N (H2). The presence (H1) and severity (H2) of N limitation can also be estimated by the magnitude of plant production increase following N addition (Chapin *et al.* 1986). Soil CEC and initial pH would also influence the magnitude of acidification and the final pH of the soil following N addition (H3), which could impact the plant community through changes in nutrient availability and the mobilization of the toxic mineral aluminium (Roem & Berendse 2000; Falkengren-Grerup & Diekmann 2003). The presence of species (Bobbink & Willems 1993; Tilman 1993; Falkengren-Grerup 1998) or functional groups (Diekmann & Falkengren-Grerup 2002; Suding *et al.* 2005) sensitive to N addition likely modulate many responses to N addition (H4). Species that are short-statured, leguminous, locally rare, and capable of tolerating low nutrient availabilities are expected to be especially susceptible to loss via competitive exclusion following N-induced increases in production (Oksanen 1996; Aerts & Chapin 2000; Craine *et al.* 2002; Suding *et al.* 2005). Finally, N addition can lead to changes in phenology

(Cleland *et al.* 2006), and stimulate increases in shoot length, tissue N content, and aboveground production (Bobbink *et al.* 1998; Falkengren-Grerup 1998; Bret-Harte *et al.* 2001). These changes may subsequently lead to deleterious feedbacks involving secondary factors (H5), such as from increased herbivory, frost damage, and changes in the timing of species interactions (Bobbink *et al.* 1998; Cleland *et al.* 2006).

To test the contribution of these five hypotheses in explaining the observed variation in response to N addition, we compiled a database including 23 experiments across eight herbaceous communities in North America (Fig. 1). Our analyses were guided by an *a priori* model of how various predictors relate to each other and to community responses to N addition, using structural equation modelling (SEM) as a means of disentangling these factors. Predictors were classified into four broad categories: climate, soil and biogeochemistry, initial plant community, and experimental methodology. The primary responses of interest were the proportional increase in production and the proportional reduction of species richness following N fertilization.
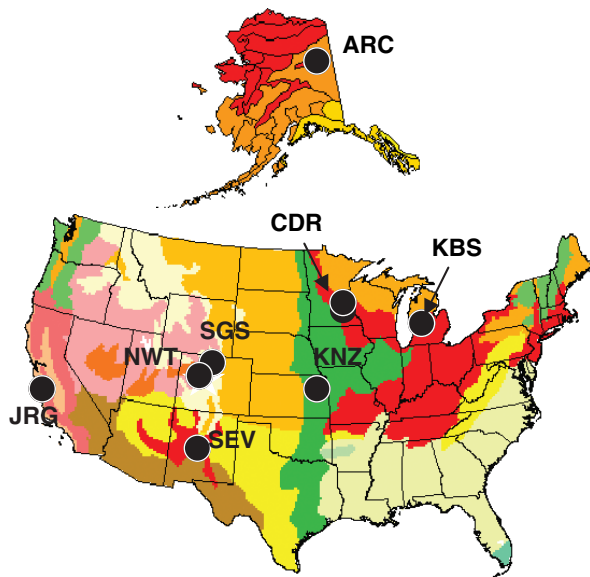

Figure 1 Location of the 23 nitrogen enrichment experiments from eight sites included in this study. Sites represent major herbaceous ecosystems in temperate North America (ARC: Toolik Lake, AK, arctic tundra, $n = 5$; CDR: Cedar Creek Natural History Area, MN, sand prairie abandoned agricultural fields and native savannah, $n = 3$; JRG: Jasper Ridge Biological Preserve, CA, annual grassland, $n = 1$; KBS: Kellogg Biological Station, MI, abandoned agricultural field, $n = 1$; KNZ: Konza Prairie, KS, tallgrass prairie, $n = 8$; NWT: Niwot Ridge, CO, alpine tundra, $n = 2$; SEV: Sevilleta National Wildlife Refuge, NM, arid scrub-grassland, $n = 1$; and SGS: Central Plains Experimental Range, CO, shortgrass steppe, $n = 2$. The number of communities within each site is specified above (n). Map delineations are Holdrige life zones.

MATERIALS AND METHODS

Data collection

For this study, we modified a data set from a previous meta-analysis (Suding *et al.* 2005) that described the production and richness responses following N addition to 34 experiments across nine herbaceous ecosystems of North America. In this data set, species ($n = 967$) were characterized by functional group and relative abundance in unfertilized and fertilized treatment plots. We modified this data set by (1) adding several soil and climatic variables from the literature or from unpublished data to characterize each particular community (described below), (2) excluding communities within sites for which these data were not available, and (3) adding data from a fertilization experiment conducted at the Sevilleta National Wildlife Refuge, NM. For all responses, we compared the mean unfertilized (no N added) and fertilized (approx. 100 kg N ha^{-1} year^{-1} added) treatments from each experiment (see Table S1 in Appendix S1 of the Supplementary Material for experimental details).

Response definition and calculation

We used species richness (number of species per unit area) as our measure of species diversity (Gough *et al.* 2000). Following standard meta-analytic approaches (Hedges *et al.* 1999; Osenberg *et al.* 1999), we calculated the community-level species richness response as the natural log of the ratio of the average species richness in the fertilization treatment divided by the average species richness in the unfertilized treatment. Values of 0 indicate no change in species richness due to fertilization, negative numbers correspond to a decline in species richness, and positive numbers correspond to an increase in species richness. We refer to this log-transformed proportional species richness as 'richness response'. Plot level data was not available for many predictors examined; thus, we treated every community within a site as a single replicate N addition study for a particular combination of climatic, soil, and plant community conditions ($n = 23$).

Predictor selection and classification

Predictors were selected based on (1) a hypothesized mechanistic relationship with the richness response to fertilization and (2) availability of comparable data. Predictors were broadly classified as proximate, intermediate or distal based on expected causal proximity to the final richness response (Fig. 2). These classifications were not coded in the statistical analysis except indirectly through the development of model structure. Distal predictors included regional-scale climatic differences between sites. These macro-variables, along with historical constraints, greatly
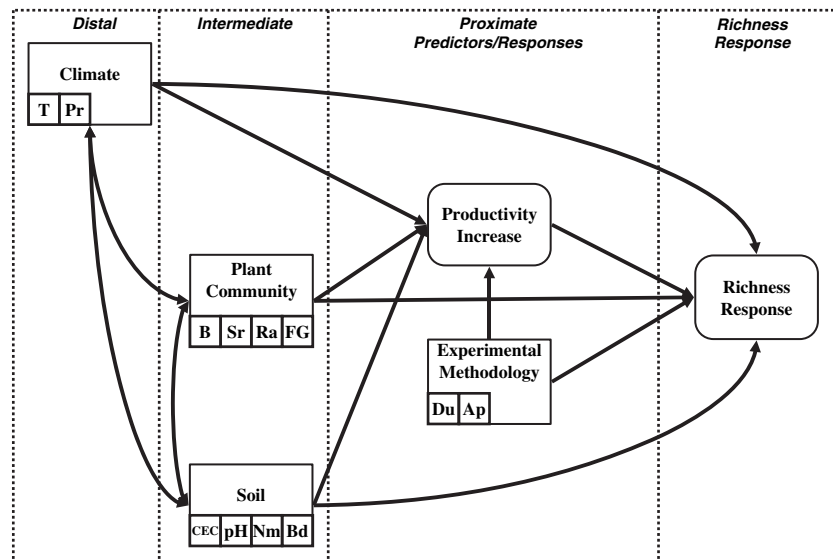
Figure 2 Initial construct model relating proximate, intermediate and distal predictors to the final richness response to N fertilization. Proximity categories are used for conceptual purposes only and do not affect the statistical model. Unidirectional arrows represent causal pathways (i.e. regressions) while bidirectional arrows represent unanalysed relationships (i.e. correlations). Distal predictors include regional climatic properties (T, temperature; Pr, precipitation). Intermediate predictors include plant community properties (B, standing biomass; Sr, species richness; Ra, proportion of species that were rare; FG, relative abundance of different functional groups), and soil/biogeochemical properties (CEC, soil cation exchange capacity; pH; Nm, monthly net N mineralization averaged over the growing season; Bd, bulk density). All intermediate properties are based on average conditions in control plots. Proximate predictors include the relative increase in standing aboveground biomass following fertilization and specific experimental conditions (Du, duration of study; Ap, application rate of fertilizer).

influence site-to-site variation in soil properties and plant community structure and composition (Zobel 1992). Intermediate predictors were composed of community-scale properties of the soil and the initial plant community, represented by average conditions in control plots. Proximate predictors included site-specific experimental properties and the increase in production following fertilization.

Distal predictors (n = 2)
Climatic predictors included a temperature index (estimated as USDA Hardiness factor, http://www.usna.usda.gov/Hardzone/ushzmap.html) and precipitation. Plant hardiness zones (categories 1–11) are based on the average annual minimum temperature from 1974–1986. Precipitation was estimated as the mean annual precipitation over 10 years or more from individual site monitoring programmes (mm year^{-1}).

Intermediate predictors (n = 19)
All intermediate predictors were estimated using average conditions in control plots. Soil/biogeochemical properties of a community included soil CEC (cmol kg^{-1}), soil pH, net N mineralization rate averaged over the growing season (g N m^{-2} season^{-1}), and soil bulk density (g cm^{-3}). Plant community properties included site productivity (estimated

by standing aboveground biomass per square meter), species richness (estimated as the number of species per square metre), the proportion of the total number of species that were rare in controls (rare defined as having relative abundance $\leq 1\%$), and the relative abundance of species with different functional traits. We selected six different functional groupings with two states each to include in our analyses following Suding *et al.* (2005). First, graminoid species were grouped by C_3 or C_4 photosynthetic pathway. Second, forb species were classified as to whether or not they were associated with an N-fixing symbiont. Third, life history was categorized as either annual or perennial (biennial species were considered to be short-lived perennials). Fourth, species were classified according to their height relative to the canopy (bottom third, middle third and upper third) in control plots for each experiment. Because there were few differences between responses of species in the middle and upper third of the canopy, those categories were subsequently combined. Fifth, species were classified based on whether they were clonal or non-clonal (no vegetative spread). Last, we classified species based on whether they were native to North America or non-native based on information in the U.S. Department of Agriculture plants database (http://plants.usda.gov). Further details are provided in Appendix S1.

Proximate predictors/responses (n = 3)

A key proximate predictor that may precede losses in diversity is the degree of production change following N fertilization. We calculated the productivity response as the natural log of the ratio of productivity in the fertilization treatment divided by productivity in the control (unfertilized) treatment, with values of 0 indicating no change in response to fertilization. In our model, we treated the degree of production increase following fertilization as both a response of the community and a proximate predictor of the richness response. The other proximate predictors were the site-specific experimental conditions: experiment duration (years), and the rate of N addition (kg N ha^{-1} year^{-1}). Experiments were selected that had added approximately 100 kg N ha^{-1} year^{-1}, although there was small variation around this value (Table S1).

Structural equation modelling

Structural equation modelling provides a framework for handling correlations between predictors that are related to a common response. SEM is based on a simultaneous solution procedure, where the residual effects of predictors are estimated (partial regressions) once common causes from intercorrelations have been statistically controlled. This allows appropriate tests of multivariate hypotheses, such as the relationship between many interacting predictors and the richness response to fertilization. We developed an *a priori* structural equation model to represent hypothesized dependencies between predictor and response variables based on expected causal relationships discussed above (Fig. 2). We used standard SEM procedures (Grace 2006) to evaluate this model using M *plus* (version 4.1). We examined all bivariate relationships for signs of nonlinearities as well as patterns in the residuals for heteroscedasticity. Log transformations were sufficient to linearize relationships involving richness response and soil CEC. All other relationships were approximately linear. We substituted mean values where missing entries were encountered. We treated the relationships between climate, soil, and plant community properties as associative (represented by double-headed arrows) rather than dependent (represented by directional arrows) because of limited sampling across biogeographic regions. Because the 23 individual communities were nested within eight geographic sites, a hierarchical model was used along with 'robust' maximum likelihood estimation procedures (the Yuan-Bentler T_2* test). This test procedure is based on adjustment for the hierarchical nature of the data and accommodates both non-normality and non-independence of observations (Muthén & Satorra 1995). Predictors that did not contribute to an understanding of either productivity increase or richness response were eliminated from the final model. We evaluated the significance of

contribution for individual variables based on z-values and single-degree-of-freedom chi-square tests of model fit. The chi-square difference tests were adjusted (using a scaling correction factor provided by M *plus*) to accommodate for the fact that the difference between two robust chi-square values does not follow a chi-square distribution without adjustment. The stability of final model results was confirmed using bootstrapping procedures (Bollen & Stine 1992).

RESULTS

General responses to fertilization across sites

Site differences in response to fertilization depended on whether we considered species richness or community productivity. Sites differed in their proportional loss of species ($F = 3.248$; d.f. = 4, 15; $P = 0.042$, Fig. 3a), losing between 1 and 48% of initial species richness. However, changes in productivity did not vary significantly across sites
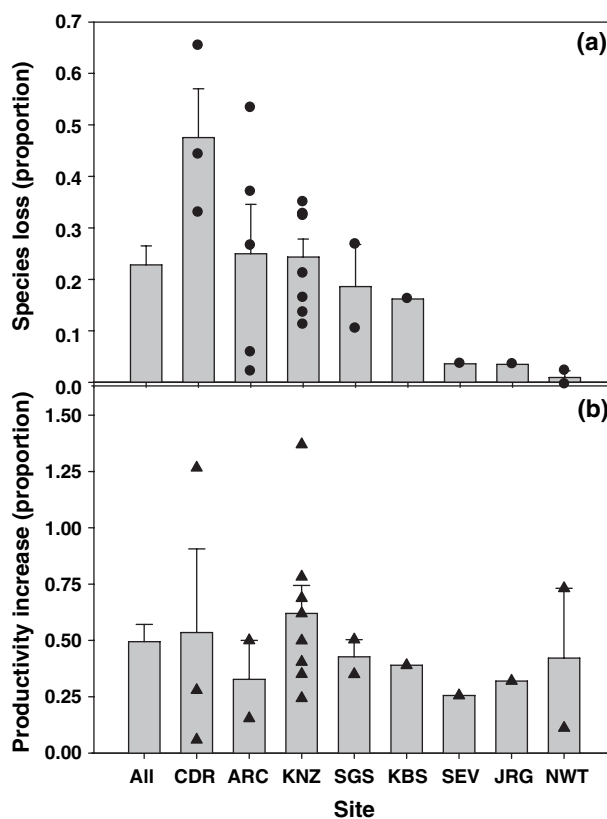

Figure 3 Impacts of fertilization at each site. Shown below are (a) the proportional losses of diversity and (b) the proportional increase in productivity in each site averaged over individual communities (standard error bars included where appropriate). Sites are ordered from highest to lowest richness response to fertilization.

($F = 0.293$; d.f. $= 4, 12$; $P = 0.877$, Fig. 3b), with productivity increasing by an average of 50% across sites. Three sites were excluded from the above analyses because only one experiment was performed at these sites; this lack of replication within sites precluded our ability to test for significant differences among sites in their productivity and richness responses to N addition. There was a wide range of responses within sites, both for richness and for productivity, though formal tests of differences in variation were not appropriate due to small and unequal sample sizes.

Bivariate relationships

Many of the variables examined in this study were correlated with one another, making this dataset particularly well suited for SEM (Table 1). Increased loss of species (more negative richness response) was directly correlated with larger increases in production ($r = -0.50$), lower soil CEC ($r = 0.48$), and longer running experiments ($r = -0.57$). Warmer sites received more precipitation ($r = 0.60$), were correlated with communities that were more productive ($r = 0.62$), and had soils with a higher pH ($r = 0.53$), and a higher bulk density ($r = 0.55$). Warmer sites were not related to initial richness ($r = -0.04$), although they did have a higher proportion of species that were rare ($r = 0.47$). Communities with a higher proportion of rare species also received more precipitation ($r = 0.53$), and had less acidic soils ($r = 0.64$). The only variables correlated with initial species richness in our data set were soil CEC ($r = -0.52$) and bulk

density ($r = 0.58$). In addition, communities abundant in C_4 species were associated with warmer ($r = 0.51$), wetter ($r = 0.44$), more productive ($r = 0.44$) sites, with a higher proportion of rare species ($r = 0.59$), and with soils that were less acidic ($r = 0.52$). All correlations mentioned above were significant at least at $P < 0.05$.

SEM results

Once non-significant variables and paths were removed, a good fit between data and model was obtained ($\chi^2 = 2.838$, d.f. $= 3$, $P = 0.42$, $R^2 = 0.56$, Fig. 4). (Note that in SEM analyses good model fit is indicated by a non-significant parameter estimate, in this case the χ^2 parameter, demonstrating good agreement between observed and model covariance matrices.) All terms in the final model were individually significant at least at $P < 0.01$. Perturbation analysis indicated that the accepted model is stable against the inclusion of any deleted variables and robust statistics indicated little influence from non-normality.

The final model shows that across a wide range of climatic, soil and plant community conditions, relatively few properties contributed uniquely to predict the variation in species loss following N enrichment (Fig. 4). Of the six climatic and soil properties examined, only temperature and soil CEC explained community responses, with colder temperatures and a lower soil CEC predisposing a site to greater species loss following fertilization. Many of the initial plant community conditions did not explain either the

Table 1 Bivariate correlations between variables. Shown below are the correlations between temperature (Temp), precipitation, (Precip), bulk density (Bd), pH, net N mineralization (N min), soil cation exchange capacity (natural log transformed, Ln CEC), standing biomass production (Prod), species richness per m^2 (Sr), proportion of species that were rare (Rarity; rare is defined as relative abundance < 1%), proportion of the biomass made up by C4 graminoids (C4 RelAb), duration of experiment (Duration), experimental rate of N fertilization (Fert), standing biomass increase following fertilization (Prod Inc), and richness response following fertilization (RR)

	Temp	Precip	Bd	pH	N min	Ln CEC	Prod	Sr	Rarity	C4 RelAb	Duration	Fert	Prod Inc	RR
Temp	1													
Precip	0.60**	1												
Bd	0.55**	0.42*	1											
pH	0.53**	0.2	0.35	1										
Nmin	0.25	−0.16	0.24	0.41	1									
LnCEC	−0.19	−0.07	−0.62**	−0.04	−0.35	1								
Prod	0.62**	0.73***	0.05	0.26	−0.11	0.01	1							
Sr	−0.04	0.11	0.58**	0.10	0.35	−0.52*	−0.13	1						
Rarity	0.47*	0.53*	0.25	0.64**	0.11	0.16	0.34	−0.04	1					
C4 RelAb	0.51*	0.44*	0.20	0.52*	0.31	−0.17	0.44*	−0.16	0.59**	1				
Duration	−0.3	−0.21	0.03	−0.31	0.24	−0.48*	−0.26	0.40	−0.14	−0.10	1			
Fert	−0.23	0.12	−0.34	−0.14	0.12	0.20	0.16	−0.11	0.08	−0.16	0.34	1		
Prod Inc	0.05	0.29	−0.02	−0.12	−0.17	0.05	0.11	−0.11	0.40	0.44	0.22	0.11	1	
RR	0.27	0.05	0.09	0.39	0.08	0.48*	0.06	−0.07	0.25	−0.18	−0.57**	−0.07	−0.50*	1

All predictor values are based on control conditions. Asterisks indicate the strength of linear relationship in a pairwise comparison (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
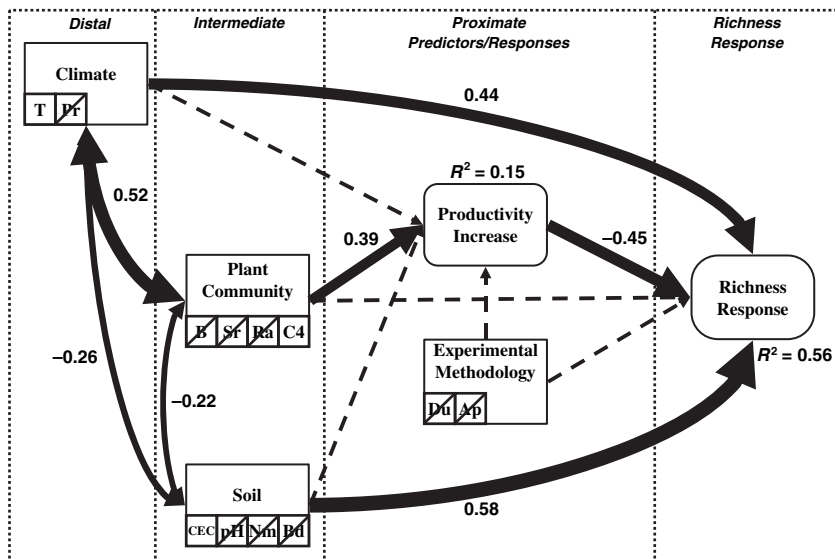
Figure 4 Final model results ($\chi^2 = 2.838$, d.f. = 3, $P = 0.416$). Numbers correspond to partial regression coefficients for unidirectional arrows and correlations for bidirectional arrows. Arrow sizes approximate the strength of relationship (non-significant pathways are dashed). Predictors are crossed out if not included in the final model. Also shown is the amount of variance explained (R^2) in the productivity increase, and the richness response.

decline in species richness or the increase in production with fertilization. An exception was the relative abundance of C_4 species: higher abundance of C_4 species in controls predicted a larger productivity increase following fertilization, and therefore indirectly predicted larger proportional species loss (from Fig. 4; $0.39 \times -0.45 = -0.18$).

The magnitudes of the standardized path coefficients (Fig. 4) provide information regarding the sensitivity of responses to individual predictors while controlling for the effects of all other predictors (Grace 2006). These results indicate that the loss of species following N addition was most sensitive to soil CEC, while being equally sensitive (though of opposing sign) to temperature and productivity increase.

Overall, our model results (Fig. 4) indicate that greater species loss was associated with (1) lower soil CEC, (2) colder regional temperatures, and (3) larger production increases following fertilization; additionally, (4) neither the rate nor the duration of N addition predicted the decline in diversity within the range of experimental methodologies explored (60–120 kg N ha^{-1} year^{-1} and 4–18 years respectively), and (5) with the exception of C_4 abundance, few climatic, soil, or plant community properties directly predicted the increase in production following fertilization ($R^2 = 0.15$).

DISCUSSION

We hypothesized that plant species richness would be maximally reduced by N addition when five conditions were present: (H1) the primary limiting nutrient was N; (H2) the ambient production rates of available N were low; (H3) the potential for N-induced soil acidification was high; (H4) the

many species and/or functional groups sensitive to N addition were present; and (H5) the potential for impacts from secondary stress factors was high (e.g. frost and herbivory). Any differences between communities in these conditions could explain the variation in response to N addition. These five conditions were captured, in part, by lower soil CEC (higher potential for soil acidification and loss of nutrient cations), lower regional temperature (higher potential for low N production rates and for damage from secondary stress factors such as frost), higher abundance of C_4 graminoids (potentially sensitive functional group), and a larger production increase following N addition (reflecting stronger N limitation). Individually, none of these predictors explained > 25% of the variation in richness response, while collectively they explained 56%. Thus, our results provide evidence for potential acidification (H3), secondary stress factors such as frost (H5), and the presence (H1) and strength (H2) of N limitation in regulating the magnitude of N-induced species loss. We did not find support for specific plant community properties (H4), including the presence of certain functional groups, ambient plant productivity, or community structure, as having a direct influence on species loss following N addition across herbaceous ecosystems.

Soil CEC was the strongest contributor to our multivariate explanation of richness response, with lower soil CEC predicting larger losses in richness. This relationship likely represents the dual effect of CEC in affecting sensitivity to soil acidification and the capacity for nutrient cation retention (Bobbink *et al.* 1998), which have been found to strongly impact species richness in observational (Roem & Berendse 2000) and experimental (Roem *et al.* 2002) studies. Soil CEC measures the buffering capacity of the soil to cations (whether acid cations like ammonium, base cations

like the essential nutrients calcium and magnesium, or aluminium, which is toxic). When N is added as ammonium, it may be nitrified by the microbial community, releasing protons into the soil which can displace nutrient base cations from the soil cation exchange complex (Ca, Mg), subsequently leading to leaching of important micronutrients, increased mobility of aluminium, and changes in the nutrient balance of plant tissue (Aber *et al.* 1998). Indeed, Roem *et al.* (2002) found that increased acidity, more than increased N availability, was responsible for the decrease in species number and rates of germination in a Dutch heathland study. In our study, all 23 experiments added N as both NH_4 and NO_3, with the exception of Jasper Ridge [added $Ca(NO_3)_2$], which experienced one of the weakest responses to fertilization (3.5% reduction in species richness, Fig. 3a). Although data on pH in fertilized plots were not available for all sites, proportional species loss tended to be greatest in sites where the control plots were already more acidic ($r = 0.39$, $P = 0.067$). However, we found soil CEC, representing the potential for acidification, to be a better predictor of species loss. Acidic sites are likely to be comprised by species adapted to acidic soils, and similar adaptation would be expected for calcareous sites; thus, it is the potential for changes in pH, more than its initial state, that predicts greater species loss with N addition.

Soils with a low CEC, in addition to being more sensitive to acidification, are more sensitive to nutrient cation loss following N addition (Aber *et al.* 1998). Ammonium cycling through soils with a low CEC, all other conditions being equal, presumably has a shorter residence time, resulting in lower N availability. Selection would subsequently favour species adapted to these low N conditions, capable of efficient N uptake at low concentrations (Aerts & Chapin 2000), usage of alternate N sources (Kielland 1994; Schimel & Bennett 2004), and with long nutrient retention times (Aerts & Chapin 2000), and not favour species adapted to high N levels. Addition of a large amount of N (e.g. 100 kg ha^{-1} year^{-1}) alters these selection pressures, having a greater impact on these N-poor soils, and enhancing the potential for competitive exclusion by species that were previously not favoured. Thus, we speculate that the combination of increased acidity, aluminium mobility, and loss of nutrient cations following N addition in soils with low buffering capacity may (1) directly impact species already near their physiological limits of acid tolerance and nutrient demands, and (2) increase the potential for competitive exclusion through shifts in selection pressure towards species able to capitalize on these novel conditions.

Regional temperature was also found to be a unique and important predictor of richness response. Lower temperature predisposed a site to greater reduction in species richness perhaps through increased secondary stress from frost damage (Bobbink *et al.* 1998), or perhaps because of lower production rates of available N at colder temperatures (Aerts & Chapin 2000). Indeed, temperature and net N mineralization rates were positively correlated ($r = 0.25$), though this association was not significant. Addition of high levels of N can increase damage to shrubs from low-temperature desiccation (Carroll *et al.* 1999), probably through increases in shoot extension. Earlier spring bud-break (Gordon *et al.* 1999) and shifts in flower phenology (Cleland *et al.* 2006) can also be induced by N addition, possibly increasing the likelihood of damage from late frosts and changes in competitive interactions. At high temperatures, water limitation is more likely to restrict plant growth, possibly reducing the impact of N addition. While we did not find precipitation to be a strong predictor of species loss, the effects of water limitation are apparent in the arid scrub-grassland (SEV) and short-grass steppe (SGS) sites. The SEV site is primarily water limited (Noy-Meir 1973; Pennington & Collins 2007), and responded weakly to N addition (3.6% reduction in species richness, Fig. 3a). In the two SGS communities, water plus nitrogen amendment amplified production increase and diversity decrease by 44% and 158%, respectively, over the addition of fertilizer alone. Thus, increasing temperatures may describe a gradient of N-addition effects, shifting from increased potential frost damage and decreased N production rates at low temperatures, to decreased likelihood of primary limitation by N at high temperatures.

Finally, a strong direct relationship was found between species loss and production increase following fertilization. This may reflect, at least in part, the effects of biomass increase on intensified competitive exclusion. Increased competitive exclusion could occur through a number of proposed mechanisms (Rajaniemi 2003), including increased light competition by taller neighbours (Newman 1973; Wilson & Tilman 1991), a dilution effect of larger individuals that are fewer in number in a fixed plot size (Oksanen 1996), the inhibitive effects of increased litter (Foster & Gross 1998), or a shift in the composition of the soil microbial community (Johnson 1993; Johnson *et al.* 2003). Interactions between species are important in predicting the outcome of competition under elevated N (Wedin & Tilman 1993; Falkengren-Grerup 1998; Pennings *et al.* 2005), and subsequently, in predicting the impact of N addition on species richness at the community-scale. However, we were unable to address these dynamics due to the scale of our analysis and lack of comparable data across sites.

The relationship between production increase and richness decline was independent of most climatic, soil, and plant community parameters investigated. Only the abundance of C_4 species was retained in the final model, accounting for 15% of the variation in production response.

Communities abundant in C_4 species experienced large increases in production, even though C_4 species themselves may have decreased in abundance (Tilman 1987), leading ultimately to larger decreases in richness. The increase in production in all 23 of these communities following N addition suggests initial N-limitation. However, the wide variation in production response among communities (+6% to +137%) was left largely unexplained, possibly resulting from differential limitation by other nutrients such as water or phosphorous. Indeed, the variation found in response to N addition in alpine meadows (Theodose & Bowman 1997), arctic tundra (Shaver & Chapin 1995), and calcareous heathlands (Bobbink 1991; Wilson *et al.* 1995), have been suggested to result from differential limitation of N vs. P. We expected some of the soil predictors examined would describe relative N-limitation across sites (esp. N mineralization); however, we found no such relationships. Several metrics that were not available for these communities may better reveal relative N limitation. Koerselman & Meuleman (1996) found that community tissue N : P accurately predicted the limiting nutrient in 39 of 40 cases examined (N limitation for N : P < 14, P limitation for N : P > 16, and N, P, or co-limitation in between). In addition, Diekmann & Falkengren-Grerup (2002) found that many life history traits poorly predicted species responses to elevated N, and instead developed 'attribute syndromes' to predict suites of traits favoured with N addition. Incorporation of other biogeochemical or trait data such as these might help explain the remaining variance in our model.

The two sites which lost the largest proportion of species (ARC and CDR) often exhibited large increases in production by one or a few species. The deciduous shrub *Betula nana* increased dramatically in some arctic sites, effectively overtopping the extant community, leading to strong light limitation and reducing the richness of moss, lichen and evergreen shrubs (Chapin *et al.* 1995). Similarly, the C_3 grass, *A. repens*, increased dramatically with N addition at CDR, driving an increase in total production and causing a decline in the extant community of forbs and C_4 grasses (Tilman 1987; Wedin & Tilman 1996). Both *B. nana* and *A. repens*, relative to other species within their communities, have traits well adapted to establishing and maintaining dominance following increases in nutrient availability, including capacities for rapid growth and tall stature, increased shoot biomass and ramet density, and the build-up of litter (Wedin & Tilman 1996; Bret-Harte *et al.* 2001; Shaver *et al.* 2001; Reich *et al.* 2003). However, these opportunistic species did not always dominate following fertilization to communities in which they were present. *Betula nana* did not dramatically increase in one Alaskan (this study, moist non-acidic tundra) and several Swedish tundra sites (van Wijk *et al.* 2004); and, though *A. repens* increased in abundance dramatically at CDR, it increased only slightly at KBS. This variation could

be caused by limitation by non-N nutrients, recruitment limitation, transient dynamics, or local interactions between these species and the extant community. Indeed, Pennings *et al.* (2005) found that the response to N fertilization of 14 of 20 herbaceous species that occurred across several experimental communities was contingent on local community biotic or abiotic interactions. These findings demonstrate that increased species dominance following N addition is partially dependant upon certain environmental or community conditions, and cannot be predicted by species presence alone.

We recognize a number of important factors that limit the interpretations of our results. A considerable proportion of the variation in richness response (44%) and a large proportion of the variation in production response (85%) were left unexplained, even though we examined a wide range of predictors. First, few of our soil, climate, or initial plant community predictors captured the presence and strength of N limitation within a community. Only the production increase following N addition, considered both a response and a predictor in our study, captured these conditions. Incorporation of plant tissue N : P (Koerselman & Meuleman 1996) or nitrification ratios (Diekmann & Falkengren-Grerup 2002) may help remedy this shortcoming. Second, many known impacts of N addition, such as increased aluminium mobility and rates of herbivory, were left unexplored as comparable data were not available. Third, although efforts were made to collect data derived from identical methodologies to characterize communities, such data could not be found for many predictors (esp. N mineralization, Appendix S1). Indeed, while 17 of 23 experiments in this study used intact soil cores incubated in the field over a month or more to estimate net N mineralization, the remaining six make up a sizeable fraction of the dataset (26%), and the comparability of these estimates may be suspect (Raison *et al.* 1987). This shortcoming highlights the need for a concerted effort in the development and implementation of common methodologies in ecological research, critical in accurately predicting responses to global change across regions. Fourth, limitations of functional group classification schemes have been discussed (Lavorel *et al.* 1997), and may be especially problematic when different functional groups are wholly absent from different sites. Quantitative traits such as specific leaf area and growth rate might better explain community dynamics, including why *B. nana* and *A. repens*, though different in classical functional groupings, had similar impacts on their local community following N addition. Use of alternate functional classification schemes, including 'attribute syndromes' (N_{dev}, Diekmann & Falkengren-Grerup 2002), functional nitrogen index for species (Diekmann & Falkengren-Grerup 1998), or Ellenberg's N (Ellenberg *et al.* 1992) values, might

provide a more integrated framework for species classification. Finally, it is clear that addition of pelletized N at the high and infrequent dosages typical of fertilization studies is important in understanding ecosystem responses to N enrichment; but, that caution must be exercised when extrapolating these results to predict the effects of lower N input rates typical of N deposition over much of the globe (Galloway *et al.* 2004). Indeed, future experiments should focus on lower and frequent dosages of dissolved N, coupled with rain shelters to remove ambient deposition, to more confidently predict the impacts of increased N deposition from human activity.

With the above limitations noted, our findings predict that plant species richness is maximally reduced by N addition when soil CEC and regional temperatures are low, C_4 graminoids are abundant, and there is a large production increase following fertilization. Sites are most susceptible when all these factors align. Though not wholly captured by functional trait groupings used in our study, it is likely that the number of species with traits optimal for excluding others following N addition (rapid growth, tall stature, etc.) will be few in number in communities where temperatures, N production rates, and N retention capacities are low. Thus, novel conditions created by N addition may select for novel traits, disfavouring the majority of species and enhancing the potential for competitive exclusion.

There is strong evidence that we are already suffering losses of terrestrial plant biodiversity from current rates of N deposition to temperate ecosystems (Stevens *et al.* 2004). N deposition rates are expected to increase globally over the next 50 years (Galloway *et al.* 2004), largely in tropical areas close to biodiversity hotspots (Phoenix *et al.* 2006). Though generally considered P limited (Matson *et al.* 1999), the combination of low soil CEC in tropical sites, and reduced availability of P following soil acidification, caution against the generalization that N addition will not impact P-limited sites. Future increases in our understanding of the broad scale variation in response to N addition will require additional experimental manipulations using lower rates of N addition and comparable methodologies across a wider range of habitats. A challenge to understanding the factors that influence community responses to N fertilization is that many of the potentially important predictors are expected to be correlated. Multivariate modelling offers one approach to successfully disentangle some of these associations, enabling us to expand the generality of single-location experiments to examine continental-wide patterns of N induced species loss.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 Description of experiments and predictor characterization

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1461-0248.2007.01053.x

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