

LETTERS

Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands

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Rates of atmospheric deposition of biologically active nitrogen (N) are two to seven times the pre-industrial rates in many developed nations because of combustion of fossil fuels and agricultural fertilization^{1,2}. They are expected to increase similarly over the next 50 years in industrializing nations of Asia and South America². Although the environmental impacts of high rates of nitrogen addition have been well studied^{3–8}, this is not so for the lower, chronic rates that characterize much of the globe. Here we present results of the first multi-decadal experiment to examine the impacts of chronic, experimental nitrogen addition as low as 10 kg N ha⁻¹ yr⁻¹ above ambient atmospheric nitrogen deposition (6 kg N ha⁻¹ yr⁻¹ at our site). This total input rate is comparable to terrestrial nitrogen deposition in many industrialized nations². We found that this chronic low-level nitrogen addition rate reduced plant species numbers by 17% relative to controls receiving ambient N deposition. Moreover, species numbers were reduced more per unit of added nitrogen at lower addition rates, suggesting that chronic but low-level nitrogen deposition may have a greater impact on diversity than previously thought. A second experiment showed that a decade after cessation of nitrogen addition, relative plant species number, although not species abundances, had recovered, demonstrating that some effects of nitrogen addition are reversible.

Biologically available nitrogen is the major limiting nutrient structuring most temperate terrestrial ecosystems, influencing ecosystem diversity, species composition and functioning^{9,10}. Combustion of fossil fuels and modern agriculture have increased atmospheric nitrogen deposition from pre-industrial levels of approximately 1–3 kg ha⁻¹ yr⁻¹, to 7 kg ha⁻¹ yr⁻¹ over central and eastern USA, 17 kg ha⁻¹ yr⁻¹ over central Europe, and to as much as 100 kg ha⁻¹ yr⁻¹ over parts of the Netherlands^{1,2,11,12}. Experiments in many ecosystems demonstrate that nitrogen addition at rates of 25 kg ha⁻¹ yr⁻¹ or more reduce plant species numbers and change ecosystem composition and functioning^{3–8}. However, the short- and long-term ecological impacts of, and potential to recover from, the chronically elevated but lower rates of nitrogen deposition that characterize much of the Earth's land surface remain unclear.

Short-term mesocosm experiments¹³, observational research along geographic nitrogen deposition gradients¹⁴ and nutrient mass balance modelling¹⁵ suggest that there may be ecosystem-specific rates of nitrogen deposition, termed N_{crit} or critical nitrogen load, below which there are no negative ecological impacts¹⁶. Alternatively, effects of chronic nitrogen deposition at rates higher than pre-industrial levels may accumulate through time and eventually cause ecological impacts similar to those observed in shorter-term experiments that add nitrogen at higher rates^{3–8}. For instance, recent observational studies of UK grasslands suggest plant species numbers may have decreased as a result of elevated nitrogen deposition^{12,17}, and

show that species numbers correlate negatively with nitrogen deposition rates ranging from 5 to 35 kg N ha⁻¹ yr⁻¹, with no clear evidence of a threshold¹². The few studies of the dynamics of plant diversity recovery after reductions of nitrogen input have had divergent results^{18–21}; however, none have involved addition of nitrogen for as long and at such low rates as have our plots before cessation of treatment.

Here we use a 23-year field experiment to test these alternative hypotheses by determining the effects of chronic low-level rates of nitrogen addition, and its cessation, on numbers of grassland plant species. Our nitrogen-addition experiment was performed in two Minnesota prairie-like successional grasslands and in a native savanna grassland, each originally dominated by a species-rich mixture of native C₄ grasses and forbs²² (Data access is available through the Cedar Creek Long Term Ecological Research Site website at <http://www.cedarcreek.umn.edu/>). Plots received annual wet nitrogen deposition of approximately 6 kg ha⁻¹ yr⁻¹ (58% NH₄, 42% NO₃) and fertilizer nitrogen at 0, 10, 20, 34, 54 or 95 kg ha⁻¹ yr⁻¹ from 1982 to 2004. There were six replicates in each successional grassland and five replicates in the native savanna grassland (total 102 plots)²². To ensure primary limitation by nitrogen availability, all plots also received P, K, Ca, Mg and trace metals, none of which are limiting²². An additional site adjacent to one successional grassland was initially disk ploughed and had 36 plots receiving the same six nitrogen addition treatments as above ($n = 6$) for a decade (1982–1991). By that time, plant composition and diversity of the disk-ploughed nitrogen addition treatments had converged with those of the adjacent undisked treatments²³. From 1991 onwards, all treatments were stopped for a randomly selected half of the six replicates per treatment in this additional site to observe recovery ($n = 3$). We measured plant species number and biomass in each plot every year from 1982 to 1994, and at least two of every three years from 1995 to 2004. Because climatic variation and other factors also affected plant species numbers²⁴, we calculated relative species numbers by dividing the number of plant species observed in a plot in a given year by the mean number of plant species observed in control plots for that year and field.

Chronic experimental nitrogen addition reduced relative plant species number compared with controls, even at the lowest treatment addition rate (10 kg N ha⁻¹ yr⁻¹). Plot averages for 2002–2004 showed that chronic nitrogen addition significantly ($P < 0.05$) reduced relative species number at all treatment rates, including a 17% loss at the lowest rate of nitrogen addition (Fig. 1a; we define nitrogen input as the sum of experimental nitrogen addition and regional wet nitrogen deposition). In contrast, analysis for 1983–1985 (second to fourth years of the experiment) showed lower overall loss rates and no significant plant species loss at our lowest treatment rate (Fig. 1b). This suggests that effects of low nitrogen addition rates

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take years to occur, and short-term studies may underestimate the effects of low-level but chronic nitrogen addition on the loss of plant species (compare Fig. 1b with Fig. 1a).

The fitted curve of Fig. 1a shows a greater proportional loss of species, per unit of nitrogen added, at lower rates of nitrogen addition. Specifically, proportional species loss in 2002–2004 was better fit by a logarithmic than by a linear function of the rate of nitrogen addition (differences in Akaike's information criterion, $\Delta\text{AIC} = 6.5$, Supplementary Information). The slope of the fitted curve is greater at lower nitrogen addition rates; thus, lower nitrogen addition rates cause a larger loss of relative plant species number per unit of nitrogen added. This nonlinear relation may result from greater efficiency of nitrogen capture, use and retention by the C_4 -dominated, species-rich plant community present at lower rates of nitrogen addition, and greater leaching loss of nitrogen by the C_3 -dominated, species-poor plant community that became dominant at higher rates of nitrogen addition⁵. In contrast, for 1983–1985, logarithmic and linear fits were indistinguishable ($\Delta\text{AIC} = 0.52$, Supplementary Information).

How well might loss of species at high rates of nitrogen addition predict actual long-term loss from chronic nitrogen addition at low

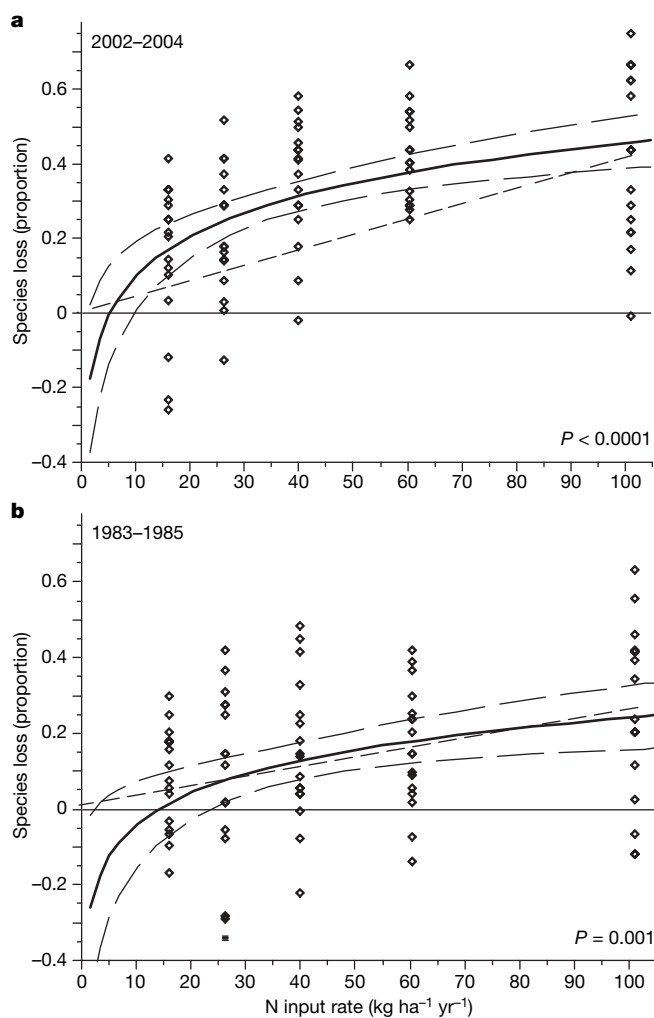


Figure 1 | Proportional species loss versus nitrogen input rate for (a) 2002–2004 and (b) 1983–1985. Shown are plot averages for each field over the three-year period fitted to a logarithmic curve excluding controls (95% confidence curves included). P values correspond to the significance of the nitrogen input term ($N_{\text{input}} = \text{experimental N addition} + \text{atmospheric N deposition}$) in a model of the proportional loss of species regressed on the natural logarithm of the nitrogen input rate, Field, and their interaction (Supplementary Information). Dashed lines correspond to linear interpolation between the mean effect at the highest nitrogen addition rate and controls.

rates? Consider the dashed line from the origin (zero proportional species loss in control plots) to the mean proportional species loss with $95 \text{ kg ha}^{-1} \text{ yr}^{-1}$ of nitrogen added (Fig. 1). For the long-term data (2002–2004), this line shows that the highest nitrogen addition rate underestimated the observed proportion of species lost at the lowest nitrogen addition rate by 60% (predicting 6.7% as opposed to 17% loss). This suggests that long-term studies using high rates of nitrogen addition may poorly predict, and even underestimate, the impact of chronic low rates of nitrogen deposition.

Repeated-measures multivariate analyses of variance (MANOVAs) over sequential three-year intervals (for example 1983–1985, 1985–1987, etc.) highlight the number of years of treatment, at each treatment rate, resulting in a loss of relative species numbers. There were significant ($P \leq 0.01$) and consistent reductions of relative species numbers after three, five, five, seven and nine years, respectively, for experimental addition of 95, 54, 34, 20 and $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ above the ambient rate of nitrogen deposition, corresponding to total nitrogen inputs (experimental addition plus regional wet deposition) of 303, 300, 200, 182 and 144 kg ha^{-1} (Fig. 2, and Supplementary Table 1). Thus, longer periods resulted in detection of loss of plant species relative to controls for progressively lower rates of nitrogen addition. Absolute species numbers in treated plots diverged from controls similarly to relative species numbers (Supplementary Fig. 1).

Because relative species number was reduced at all rates of nitrogen input, N_{crit} must be lower than our lowest input rate. To estimate N_{crit} , we extrapolated our fitted curve and its confidence intervals (Fig. 1a), which predicted N_{crit} as $5.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ with a 95% inverse prediction interval of $1.3\text{--}9.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. This estimate should be interpreted with care, as we lack data at sufficiently low input rates to estimate N_{crit} definitively. However, our work demonstrates that N_{crit} is below our lowest nitrogen input rate, and suggests that it may be lower than the $10\text{--}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ previously estimated²⁵ for similar European ecosystems. Other approaches such as shelters to remove ambient deposition would refine this estimate of N_{crit} .

The decline in plant species number observed in 2002–2004 at our lower chronic rates of nitrogen addition (rates of $34 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and lower) was mainly from loss of rare species, where rare species are defined as those with a relative abundance of less than 1% in control plots on average across the entire experimental period (Supplementary Information). The number of rare species was reduced by nitrogen addition (N_{add}), differed among fields (Field)

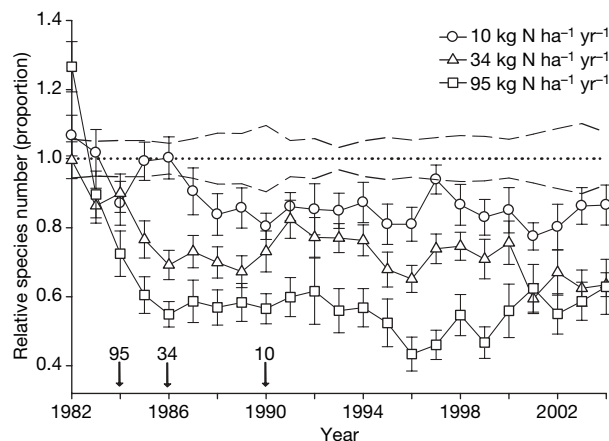


Figure 2 | Relative species number versus time. The treatment-specific average annual relative species numbers (\pm one s.e.m.) through time averaged over the three fields are shown. Dashed lines correspond to annual standard errors in control plots, and arrows indicate the year of first significant ($P < 0.01$) detection of relative species loss for a particular nitrogen addition treatment rate using MANOVA over three-year intervals (middle year highlighted). For clarity, only three of five nitrogen addition treatments are shown.

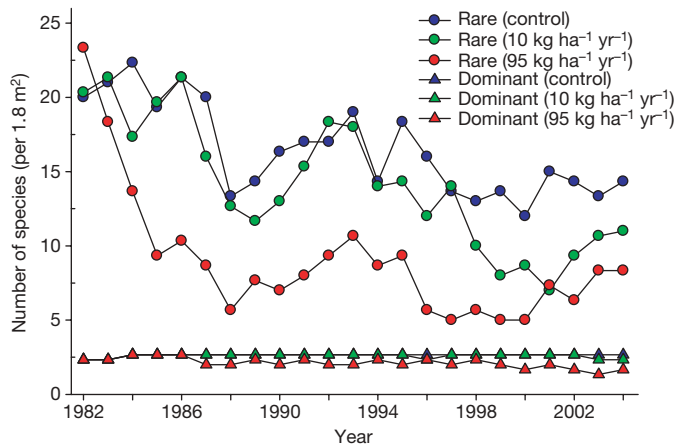


Figure 3 | Losses of rare versus dominant species. Dynamics of the numbers of rare and dominant species, expressed as the total numbers of such species across all replicates of a treatment in a field (see Methods). The average number across all fields of rare and dominant species in the controls (no added nitrogen) and in the lowest and the highest nitrogen addition treatments is shown. For clarity, intermediate nitrogen treatments and subordinate species are not shown, but demonstrated intermediate results.

and decreased through time (Year) (Main effects model $F_{4,259} = 77.150$, $P < 0.0001$; N_{add} , $F_{1,259} = 105.090$, $P < 0.0001$; Year, $F_{1,259} = 180.712$, $P < 0.0001$; Field, $F_{2,259} = 11.398$, $P < 0.0001$; $P \leq 0.05$ for Tukey's honestly significant difference (HSD)-adjusted contrasts between controls and each nitrogen addition rate of $34 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and lower). Although all abundance classes were negatively impacted by nitrogen addition (Supplementary Information), there was a larger proportional and absolute decline in rare species numbers compared with other abundance classes (Fig. 3). For example, the average number of rare species (2002–2004) was lower at the treatment rate of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ compared with controls by 3.7 species or 26% (10.3 compared with 14 species), subordinate species by 1.4 species or 16% (7.8 compared with 9.2 species) and dominant species by 0.2 species or 8% (2.4 compared with 2.6 species). These trends in species losses were repeated at higher treatment rates (Supplementary Information). Logistic regressions of the presence or absence of individual species in each plot on the nitrogen addition rate (for rates of $34 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and lower) identified nine native perennial forb and grass species that were especially susceptible to loss after nitrogen addition (Supplementary Table 2: *Achillea millefolium*, *Asclepias tuberosa*, *Hieracium longipilum*, *Liatrus aspera*, *Panicum oligosanthes*, *Physalis virginiana*, *Schizachyrium scoparium*, *Solidago nemoralis* and *Viola pedatifida*).

In 1992 we ceased fertilizer treatment to observe recovery dynamics in an additional set of plots. Relative species numbers increased after cessation and converged with controls after 13 years (Fig. 4, and Supplementary Information). Rare species, disproportionately lost with nitrogen addition, recovered in relative numbers but not in absolute numbers over the 13 years of nitrogen cessation (Supplementary Information). In contrast, species composition showed few signs of recovery. Of the 15 most common species in the field from 1991 to 2004, comprising 95% of the total plant production over this period, only three responded to nitrogen cessation (Supplementary Table 3). *Schizachyrium scoparium* stopped declining and *Agropyron repens* stopped increasing in relative abundance, on average, in plots not receiving nitrogen. The sedge *Carex* was generally increasing with nitrogen cessation except at the highest prior rate of nitrogen addition ($95 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), where it was relatively unchanged (*Carex* individuals are not identified to species, but generally were *C. pensylvanica* or *C. muehlenbergii*²²). Other studies of responses after nitrogen cessation have shown that plant populations^{18–21} tend to recover more slowly than do plant tissue chemistry²¹, soil pH²⁶ and nitrate leaching²¹.

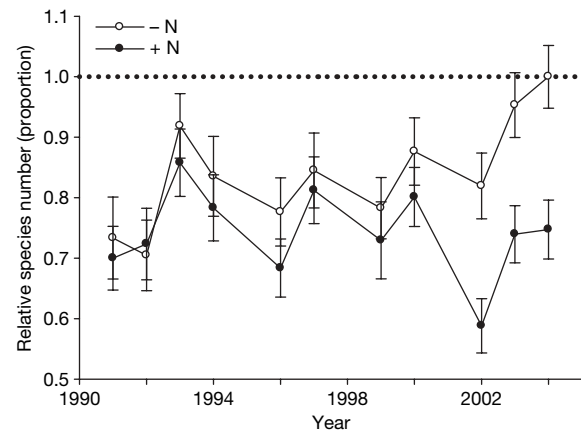


Figure 4 | Recovery of relative species number after cessation of nitrogen addition. Relative species number of all plots that continued to receive nitrogen (+N) and of those plots for which nitrogen addition ceased from 1991 and on (–N) as the average across all nitrogen addition levels each year (\pm s.e.m.). There were no significant interactions between the rate of nitrogen addition and either year or the cessation treatment (Supplementary Information).

Much of the industrialized world currently receives nitrogen deposition at rates of $5\text{--}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, with other regions expected to follow suit during industrial development over the next 50 years². Our experiments demonstrate that grassland ecosystems on low-nitrogen soils were sensitive to chronic nitrogen inputs at low rates, and were capable of recovering some community properties within a decade after cessation of nitrogen addition. Because our plots are relatively small and frequently adjacent to plots of higher diversity, our observed rate of recovery is likely faster than would occur after reduction in regional nitrogen deposition. Determination of the generality of our results will require chronic low-level nitrogen addition experiments in various ecosystems. We suggest, however, that two aspects may prove general: first, that there are larger effects over the long term per unit of nitrogen if deposited at lower rates; and second, that many ecosystems that currently receive chronic nitrogen deposition at low rates, but elevated above pre-industrial levels, may be experiencing slow but chronic loss of biological diversity.

METHODS

Experimental design and data. We only included the two later successional prairie-like grassland fields (fields B and C, abandoned from agriculture in 1957 and 1934, respectively) and the prairie opening in native savannah field (field D, never cultivated) in the analyses because the youngest field (field A, abandoned from agriculture in 1968) was relatively species poor and dominated by exotic invasive grasses at the beginning of the experiment. The initially disclipped experiment was in field C only. Nitrogen was added as pelletized NH_4NO_3 . Other treatments not included in this study include two higher nitrogen addition rates (170 and $270 \text{ kg ha}^{-1} \text{ yr}^{-1}$), which were excluded to restrict our comparisons to nitrogen deposition rates actually experienced globally, and one treatment with no nutrients added ('unamended'). We compare nitrogen addition plots with controls; however, similar results were obtained if we compared nitrogen addition plots with unamended plots. Because non-nitrogen nutrients were added and soil pH was controlled through addition of base cations, our study addresses the eutrophication effects of nitrogen addition while controlling for soil acidification, likely underestimating the total long-term impact of nitrogen deposition on species numbers²⁷. We estimated the mean nitrogen deposition across the central and eastern USA by using all years of available data for sites from these regions in the EPA CASTNET program for total inorganic (wet plus dry) nitrogen deposition (<http://www.epa.gov/castnet/>). We determined the composition of local wet deposition from the on-site National Atmospheric Deposition Program monitoring station (Site MN01; <http://nadp.sws.uiuc.edu/>). Data for dry nitrogen deposition were unavailable at our site; however, examination of the three nearest sites in the EPA CASTNET program (sites PRK134, STK138 and VOY413) suggested that wet deposition is 72–84% of total nitrogen deposition, and is thus a reasonable proxy for total nitrogen deposition. See prior publications for additional information^{5,22}.

Estimation of loss of relative species numbers for 2002–2004 and 1983–1985.

To determine the magnitude of relative species loss from each rate of nitrogen addition, we regressed the relative number of species on the rate of nitrogen input. To see if these relations changed through time, we conducted the same analyses early in the experiment (1983–1985) and two decades later (2002–2004). The proportional loss of species relative to controls was calculated as one minus the ratio of the plot average plant species number for the specified period (1983–1985 or 2002–2004) divided by the average number of species in the control treatment in that field over the same period. Analyses over the entire nitrogen addition gradient ($0\text{--}95\text{ kg ha}^{-1}\text{ yr}^{-1}$) required natural log transformation to meet assumptions of linearity. Control plots were excluded from the regressions to allow the x intercept to vary more freely. We found an increase in proportional species loss with chronic nitrogen inputs for the 2002–2004 period (Fig. 1a; proportional species loss = $-0.26 + 0.16 \times \log(\text{nitrogen input rate})$; $\log(\text{nitrogen input rate})$, $F_{1,83} = 25.204$, $P < 0.0001$). Plot averages over 1983–1985 show a less dramatic reduction (Fig. 1b; proportional species loss = $-0.33 + 0.12 \times \log(\text{nitrogen input rate})$; $\log(\text{nitrogen input rate})$, $F_{1,83} = 10.549$, $P = 0.002$). Over either period, 'Field' was a significant predictor of species loss; and there were no higher-order interactions (Supplementary Information). Linear versus logarithmic models of the nitrogen input rate were compared using differences in Akaike's information criterion (ΔAIC).

MANOVA of temporal trends. To determine the amount of cumulative nitrogen added, at each rate of application, required to significantly reduce diversity, we used repeated measures MANOVA over three-year sequential periods beginning with 1983 (1983–1985, 1985–1987 and on to 2002–2004), comparing the average number of species between controls and each treatment level over the specified period. We began analyses with 1983 to correct for a one-year transient increase in diversity after nitrogen addition²¹. Inclusion of the first year of data did not qualitatively change the results. MANOVAs were run separately for each three-year period, and used the natural log of the nitrogen input rate and Field as independent variables (Supplementary Table 1). Annual tests for differences between controls and nitrogen treatments were also examined, though these were often non-significant at lower rates of nitrogen addition likely because of small differences at lower nitrogen addition rates and high annual variability²⁸. In analyses using the entire 22-year dataset (1983–2004), all nitrogen addition levels had proportionally fewer relative numbers of species on average than controls at $P < 0.0001$. All within-subject tests (those including Time) for multivariate significance throughout this study are based on the Geisser-Greenhouse adjusted F -statistic. Between-subject tests (those not including Time) are based on exact F -tests.

Loss risk for species at low nitrogen addition rates. We used two methods to assess loss risk of species at low rates (rates of $34\text{ kg N ha}^{-1}\text{ yr}^{-1}$ and lower) of nitrogen addition: (1) multiple regressions on the number of species in different abundance classes; and (2) logistic regressions on the presence or absence of individual species. Analyses over these low treatment rates did not require log transformations to meet model assumptions. For the first method, we classified each species as rare, subordinate or dominant based on an average relative abundance in control treatment of less than 1%, 1–10% and greater than 10%, respectively, over the entire experimental period (1982–2004), and then added up the number of unique species present across all replicate plots of a given treatment level in each field for each year. We then ran multiple regression analyses comparing the number of species in each abundance class with the nitrogen addition rate (N_{add} ; rates of $34\text{ kg N ha}^{-1}\text{ yr}^{-1}$ and lower), Field, Year (1983–2004) and all higher interactions (Supplementary Information). Poisson distributions were assumed for subordinate and dominant abundance classes owing to violation of normality assumption (Supplementary Information). For the second method, a species was counted as present if it was found in a plot for any one of the three years from 2002–2004, and absent only if not found for all three years (we tested for year effects and none were significant). Logistic regressions were run separately for species within fields. Tests for significance are based on likelihood ratio χ^2 tests.

Received 10 July; accepted 29 November 2007.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank C. Stevens, J. Galloway, S. Collins, K. Suding, J. Hille Ris Lambers, and J. Fargione for their comments on the manuscript, and T. Mielke and the summer interns at Cedar Creek for their work. This project was supported by the Long-Term Ecological Research Network funded by the National Science Foundation.

Author Contributions D.T. envisioned, set up and managed the long-term experiment. C.C. performed the analysis and wrote much of the paper. Both authors discussed the results, and developed and commented on the manuscript.

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