

LETTER

Low biodiversity state persists two decades after cessation of nutrient enrichment

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Abstract

Although nutrient enrichment frequently decreases biodiversity, it remains unclear whether such biodiversity losses are readily reversible, or are critical transitions between alternative low- and high-diversity stable states that could be difficult to reverse. Our 30-year grassland experiment shows that plant diversity decreased well below control levels after 10 years of chronic high rates (95–270 kg N ha⁻¹ year⁻¹) of nitrogen addition, and did not recover to control levels 20 years after nitrogen addition ceased. Furthermore, we found a hysteretic response of plant diversity to increases and subsequent decreases in soil nitrate concentrations. Our results suggest that chronic nutrient enrichment created an alternative low-diversity state that persisted despite decreases in soil nitrate after cessation of nitrogen addition, and despite supply of propagules from nearby high-diversity plots. Thus, the regime shifts between alternative stable states that have been reported for some nutrient-enriched aquatic ecosystems may also occur in grasslands.

Keywords

Alternative stable states, fertilisation, grasslands, hysteresis, nitrogen deposition, recovery, regime shift.

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INTRODUCTION

Human activities are causing gradual but chronic nutrient enrichment of many terrestrial, freshwater and marine ecosystems worldwide (Vitousek *et al.* 1997; Galloway *et al.* 2008; Rockström *et al.* 2009). Although such eutrophication can favour a few formerly rare species and cause local loss of biodiversity (Stevens 2004; Phoenix *et al.* 2006; Clark & Tilman 2008), its long-term environmental impacts hinge on whether these changes in composition and diversity are readily reversible. Theory predicts that gradual environmental changes may reduce the resilience of a high-diversity stable state in a competitive community, increasing the likelihood that the system will cross a critical threshold into a low-diversity self-reinforcing stable state (van Nes & Scheffer 2004; Downing *et al.* 2012). Although it is well known that chronic nitrogen (N) enrichment decreases terrestrial biodiversity (Stevens 2004; Phoenix *et al.* 2006; Clark & Tilman 2008), there have been no data of sufficient duration to determine if such diversity loss represents a difficult-to-reverse critical transition between alternative stable states (Carpenter *et al.* 1999; Scheffer *et al.* 2001; Scheffer & Carpenter 2003; van Nes & Scheffer 2004; Schröder *et al.* 2005; Scheffer 2009), or if changes in community composition and diversity are readily reversible, as predicted by simple resource competition theory (Tilman 1982). Distinguishing between these two possibilities could improve our understanding of the dynamics of natural systems, including the role that self-reinforcing feedbacks play in ecosystems (Schmitz 2004; Schröder *et al.* 2005). Improved understanding of ecosystem dynamics could also inform policies for minimising switches to undesirable alternative states and for maximising recovery (Carpenter *et al.* 1999).

Although field experiments can uniquely provide direct evidence for alternative stable states in natural systems (Connell & Sousa 1983; Scheffer & Carpenter 2003; Schmitz 2004; Schröder *et al.* 2005), few field experiments in natural ecosystems have been of sufficient duration or designed to adequately test for alternative stable states (Schröder *et al.* 2005). Much of the evidence for alternative stable states comes from theoretical modelling (Holling 1973; May 1977; van Nes & Scheffer 2004), observational (Hirota *et al.* 2011; Staver *et al.* 2011) and lab (Schröder *et al.* 2005; Drake & Griffen 2010) studies, and thus have a limited ability to test for alternative stable states in natural systems (Connell & Sousa 1983; Scheffer & Carpenter 2003; Schmitz 2004; Schröder *et al.* 2005). For example, observational studies are unable to distinguish between nonlinear changes in systems with a single attractor, or discontinuous changes in systems with multiple attractors. Field experiments can address these limitations of non-manipulative studies, but have rarely been conducted for a long enough time period to rigorously assess the stability of apparent alternative states (Schröder *et al.* 2005). Therefore, further experimental evidence is needed to determine whether few or many terrestrial ecosystems have alternative stable states (Scheffer & Carpenter 2003; Schmitz 2004; Schröder *et al.* 2005).

One experimental approach to test for alternative stable states consists of determining the extent to which systems recover to their former state, or persist in a new stable state, following a perturbation-induced shift (Schmitz 2004; Schröder *et al.* 2005). For example, Schmitz (2004) shifted grassland plant communities to an alternative state by removing predatory spiders. He subsequently re-introduced the predators and found that the plant community did not recover to the original state during the next 2–4 years. This lack of recovery sup-

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ports the hypothesis that predators control the structure and functioning of these grasslands (Schmitz 2003), and that disruption of this top-down control led to a critical transition between alternative stable states (Schmitz 2004; Schröder *et al.* 2005).

Here, we experimentally test for alternative stable states in competitive grassland plant communities that are bottom-up controlled by soil nutrients (Tilman 1987). Some theory predicts that alternative stable states could be common in competitive communities, and that gradual environmental changes could erode the resilience of a current state, enabling even a moderate stochastic event to shift the system to an alternative attractor (van Nes & Scheffer 2004; Scheffer 2009). Here, we first shifted grassland plant communities to an alternative low-diversity state by adding nutrients for 10 years, and then tested the extent to which cessation of nutrient enrichment led to recovery of grassland plant diversity during the next 20 years. Our experiment provides an exceptionally long test of the stability of the high- and low-diversity states (Connell & Sousa 1983; Schröder *et al.* 2005).

MATERIALS AND METHODS

Experimental design

Our long-term nitrogen (N) addition and cessation study has been underway for three decades in a successional grassland (field C at Cedar Creek Ecosystem Science Reserve), which was abandoned from agriculture in 1934, and disked before the start of the experiment, and remained unburned throughout the study (Tilman 1987; Wedin & Tilman 1996; Clark & Tilman 2008). Plots received annual wet N deposition of approximately $6 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (58% NH_4^+ , 42% NO_3^-) and fertiliser N (pelletised NH_4NO_3) at eight rates of N addition: 0, 10, 20, 34, 54, 95, 170 or $270 \text{ kg N ha}^{-1} \text{ year}^{-1}$ from 1982 to 2011 (Clark & Tilman 2008). To ensure primary limitation by N availability, plots also received P, K, Ca, Mg and trace metals, none of which are limiting (Tilman 1987). There were also unamended control plots that received no nutrients. Nutrient addition was ceased in a randomly selected half of the fertilised plots after 1991. The study includes a total of 54 plots, each 4 by 4 m, separated by 1 m buffers ([8 N-addition treatments \times 2 cessation treatments \times 3 replicates] + 6 totally unamended control plot replicates = 54 plots). We measured plant species number and biomass in each plot (10 by 300 cm strip) every year from 1982 to 1994, at least two of every three years from 1995 to 2004, and in 2008 and 2011. We measured nitrate concentration in soil cores (2.5 cm diameter, 15 cm deep) by 1 M KCl extraction and colorimetric analysis with an Alpkem autoanalyzer during 1985, 1989, 1994 and 2002. See previous publications (Tilman 1987; Wedin & Tilman 1996; Clark & Tilman 2008) for additional information.

Experimental approach

Much previous empirical evidence for alternative stable states comes from non-manipulative observational studies (e.g. analyses of frequency distributions (Hirota *et al.* 2011; Staver *et al.* 2011) or time series (Andersen *et al.* 2009)), and is open to alternative explanations (Connell & Sousa 1983; Schröder *et al.* 2005). Field experiments of sufficient replication and duration are uniquely able to test for alternative stable states in natural systems (Connell & Sousa 1983; Scheffer & Carpenter 2003; Schmitz 2004; Schröder *et al.* 2005).

An experimental approach for testing whether a system has alternative stable states involves testing for non-recovery (Schmitz 2004; Schröder *et al.* 2005) by: (1) disrupting a factor that controls ecosystem structure and functioning long enough to allow the system to reach an alternative state, (2) restoring the controlling factor and (3) observing whether the system persists in the alternative state or recovers to the original state after a sufficient amount of time (i.e. enough time for turnover of individuals). We disrupted soil nutrients by adding N long enough to shift the system to the low-diversity state dominated by a cool season grass species, *Elymus repens* (formerly *Agropyron repens*, *Elytrigia repens*). We then restored N input rates by cessation of N addition on a randomly chosen half of the plots that received each N addition. Finally, we tested for two decades whether the low-diversity state persisted, or was on a trajectory to recover its original high-diversity state. The duration of our recovery census time was five times longer than that of a previous study that also considered stability of perennial grassland plant communities (Schmitz 2004; Schröder *et al.* 2005).

Statistical analyses

All statistical tests were conducted in R 2.13.2 (www.r-project.org). To summarise temporal trends in plant species richness, plant species diversity and *E. repens* relative biomass, we fit loess regressions using the `geom_smooth` function in the `ggplot2` package (Wickham 2010). To test for a hysteretic response in *E. repens* relative biomass, we used the `glm` function in the `stats` package to fit a quasibinomial generalised linear model as a function of Cessation Treatment (continuous or cessation), Soil Nitrate and their interaction. The quasimaximum-likelihood version of this generalised linear model accounted for over- or under-dispersion in the data. To describe the nonlinear responses in plant species richness and *E. repens* relative biomass to rates of N addition (Fig. 3), we used the `glm` function in the `stats` package to fit quasipoisson (for species richness) and quasibinomial (for *E. repens* relative biomass) generalised linear models as a function of N addition rate. To test for a multimodal distribution of *E. repens* relative biomass (Fig. 5), we conducted a Dip test (Hartigan & Hartigan 1985) with the `dip.test` function in the `dip.test` package. We tested whether the observed lack of recovery in diversity at high N addition rates was simply due to low diversity in neighbouring plots in two ways. First, we tested whether there were significantly more species in the 26 exterior plots around the perimeter of the study area than in the 28 plots that were surrounded by other experimental plots, which potentially reduced opportunity for re-colonisation. Second, we tested whether the observed lack of recovery depended on neighbour plot richness (Hawthorne 2012), which was quantified as the mean number of species in all adjacent plots (including up to eight plots along the four edges and at the four corners of a plot completely surrounded by other plots). Movement of plants between plots might increase or decrease diversity in some plots, but would not be expected to affect any particular treatment more than others, due to randomisation of treatments to plots.

RESULTS

We found that plant diversity decreased well below control levels after one decade of high rates of N addition ($95\text{--}270 \text{ kg N ha}^{-1} \text{ year}^{-1}$), or three decades of medium rates of N

addition ($20\text{--}54 \text{ kg N ha}^{-1} \text{ year}^{-1}$) (Figs 1a, b and S1). Plant diversity remained well below control levels two decades after cessation of chronic high rates of N addition (solid blue lines, Fig. 1a, b). Plant diversity recovered to control levels after cessation of chronic medium rates of N addition (dashed blue lines, Fig. 1a, b) (Clark & Tilman 2008). However, plant diversity has continued to decline with continuous addition of medium rates of N addition, and, after

three decades, is now at levels as low as those from which diversity did not recover after cessation of chronic high rates of N addition (dashed red lines, Fig. 1a, b).

The lack of recovery following cessation of chronic high rates of N addition was not simply due to low diversity in neighbouring plots. First, although the experiment is surrounded by a field containing a high diversity of native perennials, there was no significant difference between the number of plant species observed in the exterior plots that bordered this high-diversity vegetation and the interior plots in 2011 ($t = 0.294$; $P = 0.77$). Second, there was no significant difference between the number of species observed in plots that received high rates of N addition for 30 years or plots that received high rates of N addition for 10 years and then no N addition for the next 20 years (Cessation Treatment main effect: $F_{1,14} = 2.05$, $P = 0.17$), regardless of how many species were in adjacent neighbouring plots (Cessation Treatment \times Neighbour Richness interaction: $F_{1,14} = 1.20$, $P = 0.59$), even though plot richness marginally significantly increased with increasing neighbour plot richness (Neighbour Richness main effect: $F_{1,14} = 3.59$, $P = 0.079$). Thus, the nearby presence of high-diversity vegetation that added propagules to plots for 20 years did not allow them to recover their original diversity after N cessation, suggesting that the low-diversity state was stable.

Plant diversity exhibited a discontinuous, hysteretic response to increases and subsequent decreases in soil nitrate concentrations (Fig. 2). Soil nitrate decreased after cessation of N addition in 1991 (Figs 2a and 3); however, this recovery of soil conditions did not

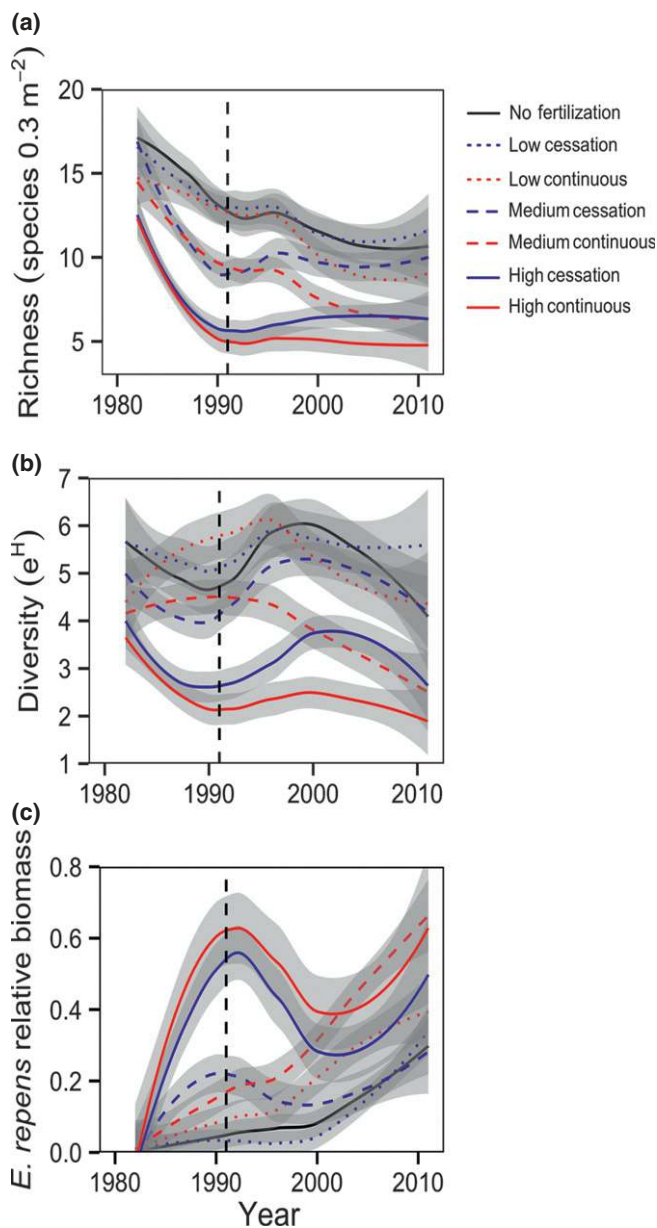


Figure 1 Nutrient enrichment decreased local plant species richness (a) and diversity (b), and increased *Elymus repens* relative biomass (c). These effects persisted two decades after cessation of high rates of N addition. Temporal trends are summarised by loess regressions; error bands indicate 95% confidence intervals. Plots received: (1) no fertilisation for three decades (black line); (2) continuous fertilisation for three decades (red lines); or (3) fertilisation for one decade, followed by cessation of fertilisation for two decades (blue lines). Fertilisation rates: low (0, 10); medium (20, 34, 54); high (95, 170, 270) $\text{kg N ha}^{-1} \text{ year}^{-1}$, plus non-N nutrients; unfertilised plots received no N or non-N nutrients.

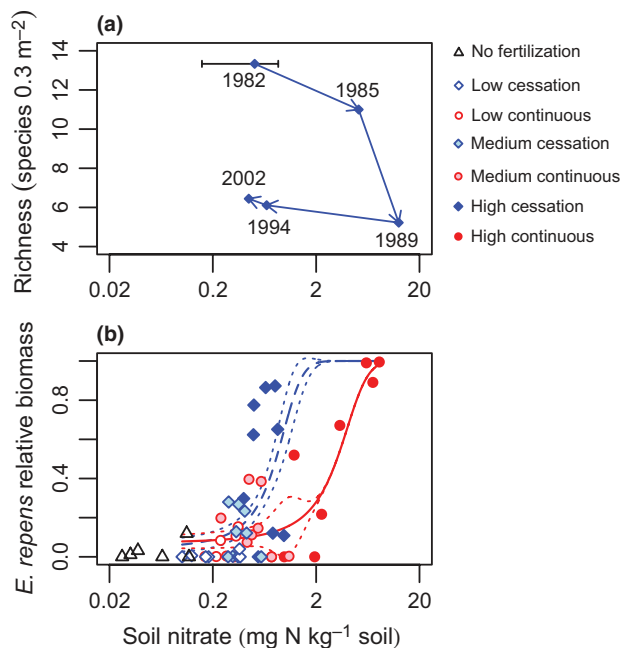


Figure 2 Species richness (a) and *Elymus repens* relative biomass (b) exhibited a discontinuous, hysteretic response to forward and backward changes in soil nitrate. Panel (a) shows mean temporal trends for the plots that experienced cessation of high rates of N addition in 1991. Soil nitrate was not measured during 1982, so we show its mean value (error bars indicate 1 SEM) observed in all control plots during all four years that soil nitrate was measured. Panel (b) shows all plot-level values observed during 1994, three years after cessation of N addition in half of the plots (blue symbols and lines). See Fig. 1 for fertilisation rates. The x-axis is on \log_{10} scale. Dotted lines in panel (b) show 1 SEM.

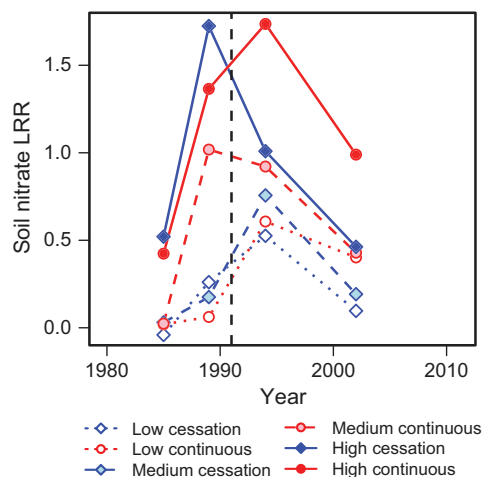


Figure 3 Temporal trends in soil nitrate by N addition treatment. Soil nitrate \log_{10} response ratio (LRR) quickly decreased after N cessation (i.e. after 1991, vertical dashed line) in plots that received high rates of N addition for one decade, and then received no nutrient additions for two decades (solid blue line). Soil nitrate was measured 4 times: 1985, 1989, 1994 and 2002. LRR was quantified as the \log_{10} ratio of the mean value observed in fertilised plots (e.g. continuous N addition at a high rate: solid red line) to the mean value observed in control plots. Values greater than zero indicate that N addition increased soil nitrate above control levels. See Fig. 1 for N addition rates.

lead to recovery of plant diversity (Figs 1a, b and 2a). Species richness decreased as soil nitrate increased between 1985 and 1989, but species richness did not recover as soil nitrate decreased after cessation of high rates of N addition (Fig. 2a).

Plots that had the fewest species were often dominated by *E. repens* (Figs 4 and S2a), and plots dominated by this species often had large amounts of litter (Fig. S2b). Mean native species richness, averaged across years within plots, decreased as mean litter increased (Pearson's $r = -0.878$, $P < 2 \times 10^{-16}$). *E. repens* dominance exhibited a discontinuous, hysteretic response to the increase and subsequent decrease in soil nitrate concentration across treatments in 1994 (Soil Nitrate main effect: $F_{1,46} = 32.307$, $P = 9.815 \times 10^{-7}$; Soil Nitrate \times Cessation Treatment interaction: $F_{1,44} = 6.099$, $P = 0.01747$) (Fig. 2b). Furthermore, *E. repens* was often either very rare or nearly completely dominant, and was less often observed at intermediate dominance (Figs 5 and 6). Consistent with theoretical predictions (Fig. 6a, b), unfertilised control plots often remained near a diverse native state (Fig. 6c), while plots continuously receiving high rates of N addition often shifted to a low-diversity state where *E. repens* was dominant (Fig. 6d). Plots that received high rates of N addition exhibited rapid transitions in *E. repens* relative biomass, while plots that received low rates of N addition often resisted invasion by *E. repens* (Fig. S3).

DISCUSSION

Our experiment provides a long-term test of the stability of the high-diversity state and of the low-diversity state induced by N addition. Plant diversity remained well below control levels two decades after cessation of high rates of N addition. Moreover, the post-cessation temporal dynamics of species richness and species diversity suggest that recovery, if it is occurring, is extremely slow (Figs 1 and 2a). The lack of recovery after two decades, and the

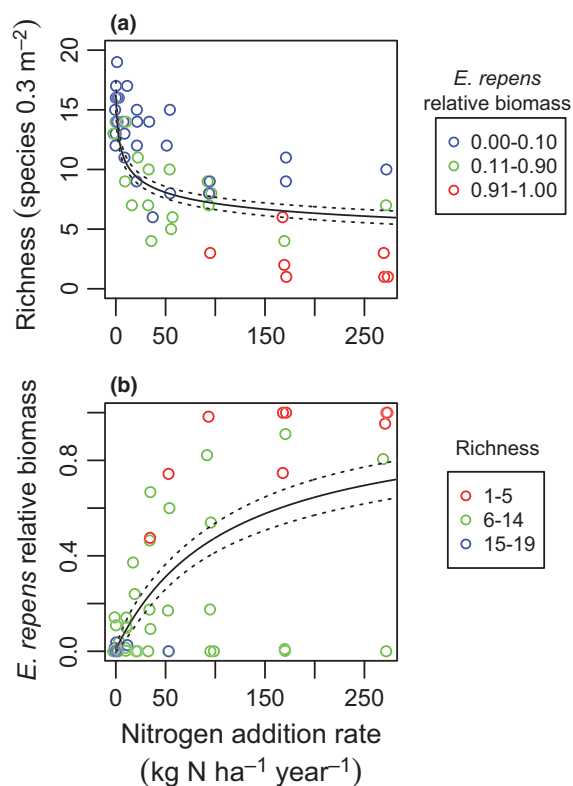


Figure 4 Effects of N addition rate on species richness (a) and *Elymus repens* relative biomass (b). Dominance (relative biomass) flipped from very low to very high values, especially at high rates of N addition. *E. repens* dominated the plots with few species. Plot-level values observed during 1991, after one decade of continuous N addition, are shown in both panels. After this time, fertilisation was ceased in three of the six replicate plots at each rate of N addition. Points are jittered on the x-axis for clarity. Dotted lines show 1 SEM.

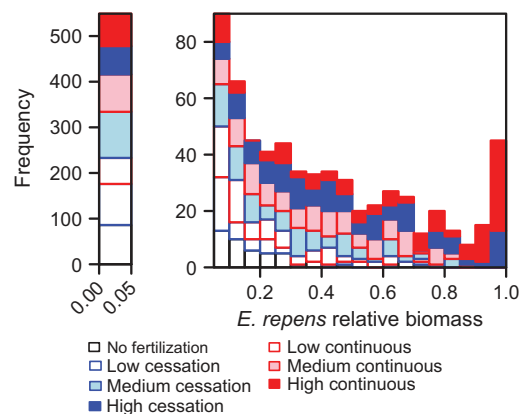


Figure 5 *Elymus repens* was often either rare or nearly completely dominant. Plot-level observations within each year are shown. For clarity, one bar (0–5%) is shown separately with a different y-axis. See (Fig. 1) for fertilisation rates. A Dip test rejected the null hypothesis of unimodal distribution ($D = 0.0173$, $P = 0.0169$).

absence of dynamics suggesting an eventual approach to a state of recovered diversity (Figs 1a, b and 2a), is consistent with the low-diversity state being stable, and thus with this system having two

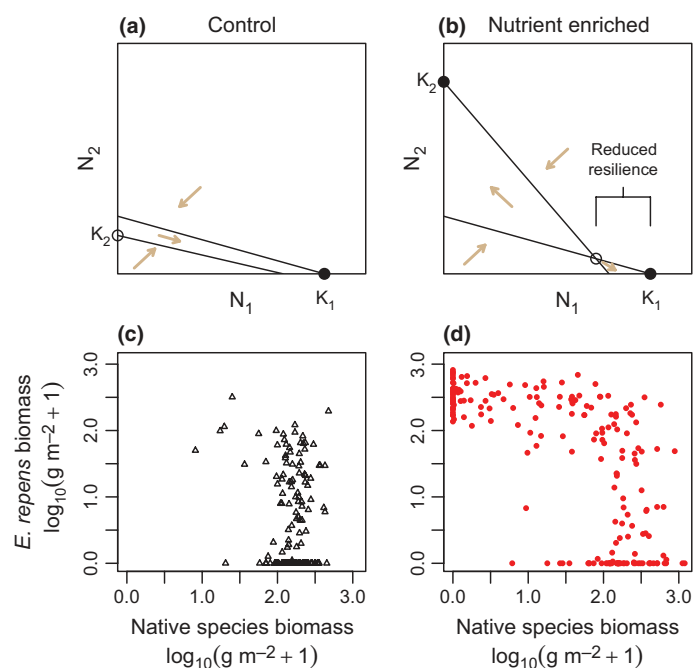


Figure 6 (a, b) Theory extending the Lotka-Volterra competition model predicts that a perturbation can erode the resilience of one stable state (K_1) by promoting some species (N_2) over others (N_1). For example, nutrient enrichment may increase K_2 more than K_1 , enabling stochastic events to shift the system from K_1 to K_2 . Lines represent zero net growth isoclines, open or closed circles, respectively, represent unstable or stable equilibria, and N_i and K_i are, respectively, the abundance and carrying capacity of species (or group of species) i . Tan arrows show the qualitative direction of change in the system within each region of state space. (c, d) Our empirical results were consistent with this theoretical prediction. Symbols match Fig. 2. Each point is one plot within one year.

alternative stable states (Connell & Sousa 1983; Schröder *et al.* 2005). However, it is not possible to reject the hypothesis that diversity may be slowly recovering, which might occur if the N that likely accumulated in biomass, litter and/or soil from a decade of N addition is not retained, but were to be slowly lost from this system. Longer term tests will thus be needed to fully understand the dynamics of these communities. In contrast, management decisions may not require distinguishing between lack of recovery and extremely slow recovery because additional restoration strategies may be desirable in either case.

Results from several other studies are also consistent with the possibility that nutrient enrichment creates a persistent low-diversity state in terrestrial ecosystems. In the Netherlands, for instance, nutrient enrichment has likely led to decreased plant diversity by promoting the dominance of *Molina caerulea* in wet heathlands, *Deschampsia flexuosa* in dry heathlands, *Brachypodium pinnatum* in calcareous grasslands, *Calamagrostis epigejos* in dunes, *Elymus athericus* in salt marshes and *Holcus mollis* in woodlands (Aerts & Berendse 1988; Bakker & Berendse 1999). Restoration of the original high-diversity state has proved difficult due to both abiotic constraints, such as persistent acidification, and biotic constraints, such as dispersal limitation (Bakker & Berendse 1999). In addition, in a sub-alpine grassland in the Czech Republic, species composition remained stable for more than six decades following cessation of nutrient enrichment after at least two centuries of fertilisation (Se-

melová *et al.* 2008). Interestingly, in this case fertilisation appears to have shifted the plant community from a low-diversity state where one species, *Nardus stricta*, was dominant, to an alternative state where two species, *Deschampsia cespitosa* and *Avenella flexuosa*, were co-dominant (Semelová *et al.* 2008).

Our results suggest that recovery in plant diversity following cessation of high rates of chronic N enrichment will either require either a long period of time (i.e. hysteretic changes) or perhaps will not be possible simply by reducing N input (i.e. irreversible changes) (Carpenter *et al.* 1999). Plant diversity exhibited a discontinuous, hysteretic response to increases and subsequent decreases in soil nitrate concentrations. This indicates that the lack of recovery in plant diversity was not simply due to a lack of recovery in soil nitrate. Diversity remained low, and *E. repens* dominance remained high, as soil nitrate decreased after cessation of N addition, rather than recovering to the levels observed at comparable values of soil nitrate in plots that had not yet shifted to the low-diversity state (Fig. 2). Such a hysteretic pattern might not indicate alternative stable states if the rate of change in the response of the system is slow compared to the rate of change in the control factor (Scheffer & Carpenter 2003). Note, however, that we found rapid shifts in species richness and *E. repens* dominance between the two potential stable states when we considered plot-level temporal trends. Species richness increased by as much as 10 species year⁻¹ and decreased by as much as 12 species year⁻¹, within plots. *E. repens* dominance increased by as much as 98.9% year⁻¹, and decreased by as much as 93.7% year⁻¹, within plots (Fig. S3). This suggests that the observed hysteresis in plant diversity may not simply be due to inherently slow population dynamics that could arise from slow vegetative growth of perennial plants.

Our results are consistent with theoretical predictions that multiple attractors can occur in communities with strong competition between groups of species, and that environmental changes can erode the resilience of a current state, shifting the system to a persistent alternative attractor (van Nes & Scheffer 2004; Scheffer 2009) (Fig. 6a, b). Consistent with this prediction, unfertilised control plots often remained near the diverse native state (Fig. 6c), while plots continuously receiving high rates of N addition often shifted to the low-diversity state where *E. repens* was dominant (Fig. 6d). Results from nearby experiment are consistent with the hypothesis that the unfertilised control state has only a single stable equilibrium where native species are dominant (Wedin & Tilman 1993), as drawn in Fig. 6a. Results from another nearby experiment are consistent with the hypothesis that nutrient enrichment increased the carrying capacity (i.e. monoculture biomass) of *E. repens* more than it increased the carrying capacity of many native species (Reich *et al.* 2001), which could shift the zero net growth isoclines as shown in Fig. 6a, b. Thus, it appears that nutrient enrichment could erode the resilience of the diverse native-dominated state, allowing stochastic events to subsequently shift the system to the low-diversity state that is induced by N addition and dominated by *E. repens*. The long-term persistence of this new low-diversity state may depend on the extent to which the added N is recycled and retained in the system (Carpenter *et al.* 1999).

The stability of the low-diversity state appears to depend on nutrient enrichment and litter accumulation. In the absence of nutrient enrichment, native perennial grasses are superior competitors on the sandy, low N soils of this site, and rapidly displace both *E. repens* and *Poa pratensis* (Tilman & Wedin 1991; Wedin & Tilman

1993). This displacement was consistently observed across experimental treatments including when plots were planted with various initial seed ratios, when seed of one species were added to established monocultures of the other, and when barriers between adjacent monocultures were removed, allowing vegetative invasion (Tilman & Wedin 1991; Wedin & Tilman 1993). These results suggest that the low-diversity state is unstable in the absence of nutrient enrichment (Fig. 6a, b).

Nutrient enrichment shifted the community to an alternative low-diversity persistent state by promoting the invasion of *E. repens*, which displaced most native perennial plant species and then self-perpetuated its dominance by its accumulated litter. This accumulation of litter disproportionately suppresses native species that evolved with more frequent fires, which reduce litter (Knapp & Seastedt 1986). In a nearby experiment, litter removal promoted recovery of species richness to some extent (Clark & Tilman 2010). There is also some evidence that *E. repens* is allelopathic (Weston *et al.* 1987), which could provide another positive feedback by which this species self-perpetuates its dominance. Seed additions might help restore the community to the high-diversity state (Clark & Tilman 2010) by perturbing the system from the low- to the high-diversity basin of attraction.

The results of this replicated, long-term experiment offer strong empirical evidence that chronic nutrient enrichment can lead to a competitively persistent, and perhaps truly stable state of low biodiversity. Together with previous results (Schmitz 2004), this suggests that disruption of top-down or bottom-up control of grasslands can shift the system to a low-diversity stable state. Two caveats remain. First, *E. repens* did not indefinitely completely dominate plots (Figs 1c and S3). Instead, another exotic grass, *P. pratensis*, has invaded alongside *E. repens* and increased in relative abundance after cessation of N addition (Fig. S4). Thus, if the low-diversity stable state persists in future years, then we suspect that it will consist of these two species, possibly cycling in co-dominance, rather than simply consisting of a monoculture of *E. repens*. As such, it may be more appropriate to use terms such as 'regimes' and 'attractors' when referring to the low-diversity community (Scheffer & Carpenter 2003). Second, some unfertilised control plots that were surrounded by non-control plots were eventually invaded by *E. repens* (Fig. S3). Thus, propagule supply from neighbouring plots may overwhelm the effects of local resources on invasion, analogous to the previous finding that propagule supply can overwhelm the effects of local diversity on invasion (Levine 2000). Also note, however, that several unfertilised control plots resisted invasion by *E. repens* throughout the study (Fig. S3), even though they inevitably received some *E. repens* propagules during and after the initial disturbance (Inouye & Tilman 1988). This suggests that the unfertilised and non-invaded state was itself highly persistent and also perhaps locally stable. Although exotic species invasions are often thought to be alternative stable states, there is rarely evidence that the uninvaded state is locally stable (Scheffer 2009), as we found here.

Will anthropogenic N deposition lead to widespread shifts in grassland plant communities to persistent low-diversity states? Results from this and other studies suggest that grassland plant communities can recover to some extent following up to two decades of chronic low to medium rates of N addition (Spiegelberger *et al.* 2006; Královec *et al.* 2009; Clark & Tilman 2010). It remains unclear, however, whether diversity will recover after more than two decades of chronic N deposition because plant diversity seems

to respond to the total cumulative amount of N added (Fig. 1a) (Clark & Tilman 2008). We found that plant diversity decreased well below control levels after one decade of high rates of N addition, or three decades of medium rates of N addition. This suggests that chronic atmospheric N deposition (at low to medium rates of N addition) for several decades may decrease grassland plant diversity as much as fertiliser application (at high rates of N addition) for a single decade.

Further study will also be needed to determine whether nutrient enrichment commonly leads to a shift from a high-diversity stable state to a low-diversity stable state in other ecosystems. This critical transition may be rare or only occur after extended periods when N deposition rates are low (Clark & Tilman 2008; Galloway *et al.* 2008). If this critical transition has not yet occurred in many places, then reducing current rates of anthropogenic N deposition may help prevent it. Alternatively, if this critical transition has already occurred in many grasslands, such as those where N enrichment and exotic plant invasions are associated with biodiversity declines (Vitousek *et al.* 1997; Stevens 2004; Galloway *et al.* 2008; Hejda *et al.* 2009; Isbell & Wilsey 2011; Wilsey *et al.* 2011), then recovery may be slow (Královec *et al.* 2009) or require active management (Clark & Tilman 2010). Further study is needed to determine the consequences of this critical transition for ecosystem functioning and services, which often depend on biodiversity (Tilman *et al.* 2001; Balvanera *et al.* 2006; Isbell *et al.* 2011). Higher resolution time series are needed to determine whether early warning indicators may (Scheffer *et al.* 2009; Drake & Griffen 2010) or may not (Hastings & Wysham 2010) be useful for predicting this critical transition.

Our results suggest that the unassisted recovery of biodiversity following cessation of nutrient enrichment in low-diversity grasslands that have undergone this critical transition will require several decades at a minimum, and might not be possible simply by reducing N inputs. Restoration strategies could help speed up this recovery process, but it is rarely possible to quickly and fully restore biodiversity and ecosystem services in degraded ecosystems (Benayas *et al.* 2009). Given these results and uncertainties, policies that reduce anthropogenic N deposition could help prevent critical transitions from high- to low-diversity grasslands that may be difficult to reverse.

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AUTHORSHIP

DT designed and conducted the experiment. FI analysed the data and wrote the first draft of the manuscript. All authors contributed to revisions.

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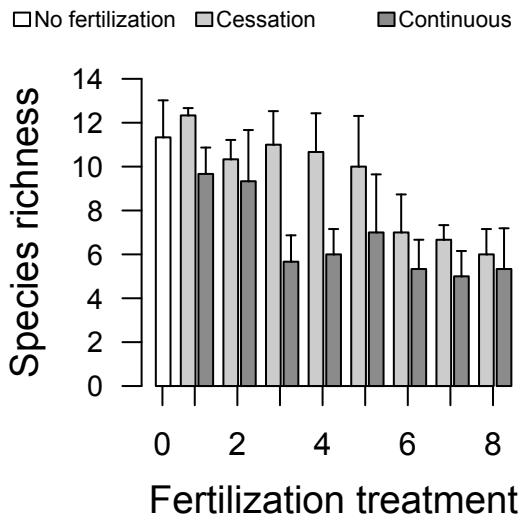


Figure S1 Species richness by N addition treatment during 2011 in plots that had received: (i) no fertilization for three decades; (ii) continuous fertilization for three decades; or (iii) fertilization for one decade, followed by cessation of fertilization for two decades. Error bars indicate 1 s.e.m. Fertilization rates: 0, 10, 20, 34, 54, 95, 170, and 270 kg N ha⁻¹ yr⁻¹ plus non-N nutrients for treatments 1-8, respectively. Treatment 0 included no nutrient additions (i.e., no N or non-N nutrients). In other figures, for clarity, treatments were grouped according to low (treatments 1 and 2), medium (treatments 3, 4, and 5), or high (treatments 6, 7, and 8) rates of N addition.

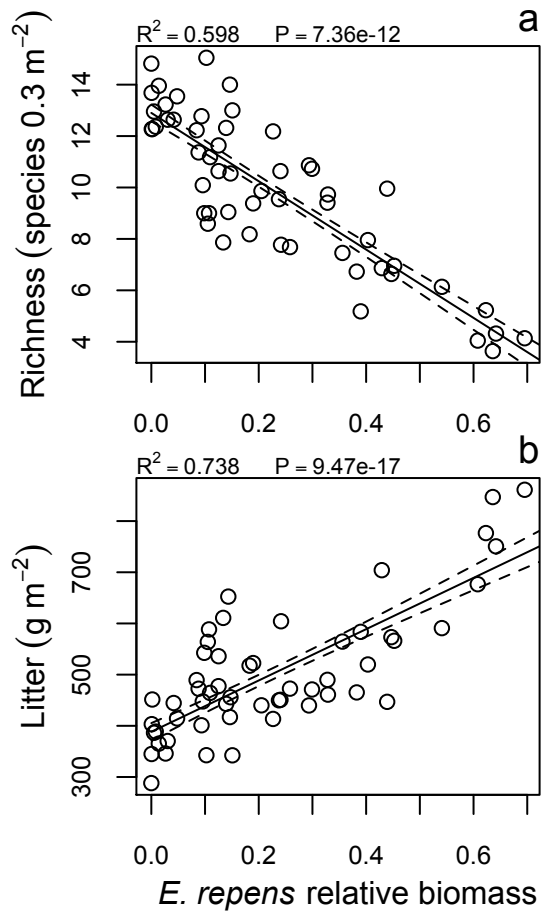


Figure S2 Plots dominated by *Elymus repens* often had (a) few species and (b) large amounts of litter. Mean values, averaged across all years, are shown for each plot. Dashed lines show 1 s.e.m.

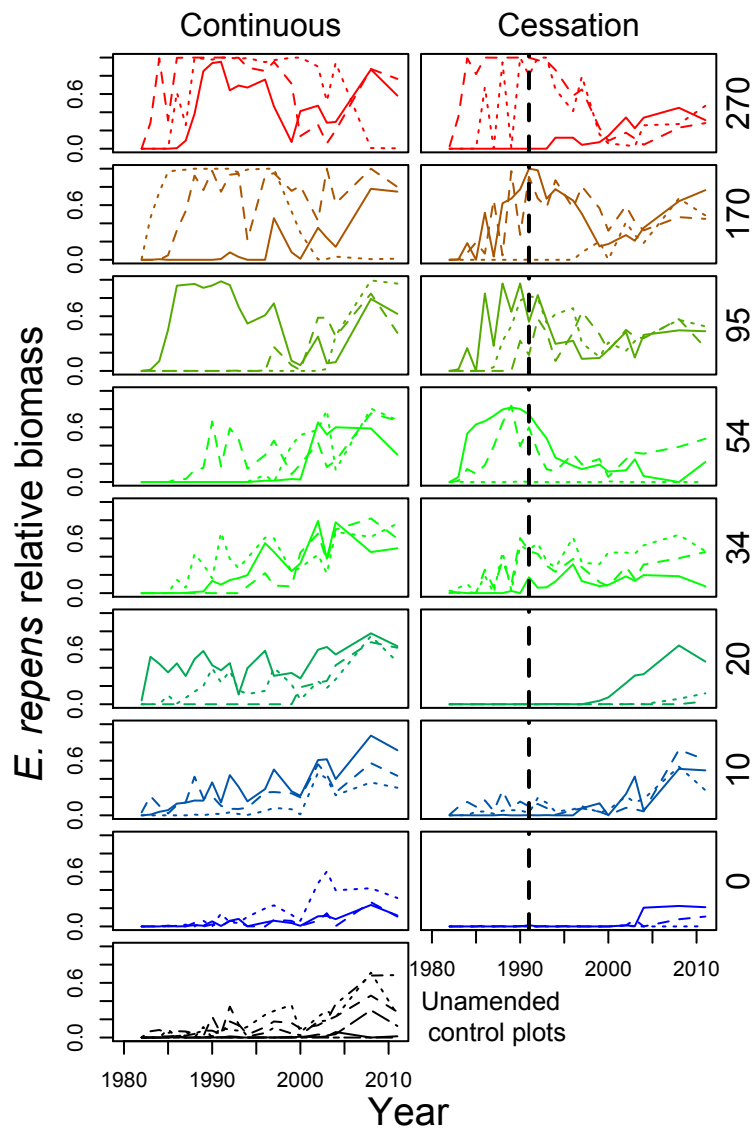


Figure S3 Plot-level temporal trends in *Elymus repens* relative biomass from high (red, top) to low (blue, bottom) rates of N addition. Numbers on the right (0-270) indicate rate of N addition in kg N ha⁻¹ yr⁻¹. Plots were either continuously fertilized for three decades (left) or fertilized for one decade and then unfertilized for two decades (right). Dashed vertical line indicates year (1991) cessation treatment began. Each line is one plot.

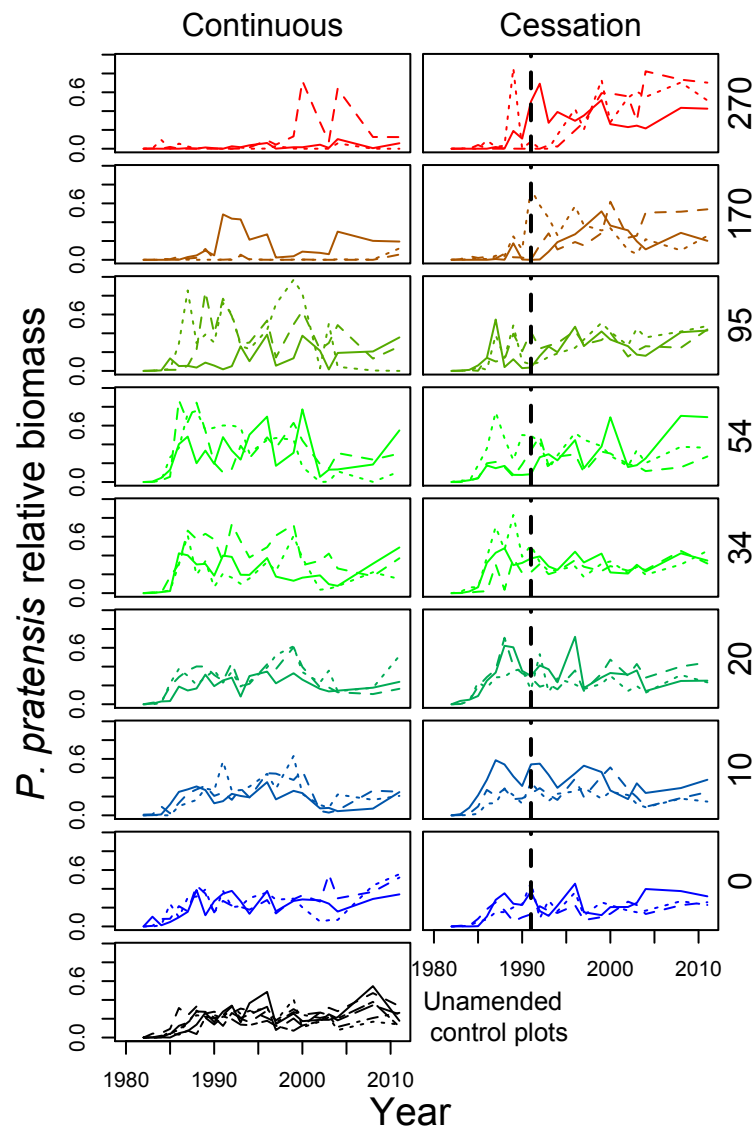


Figure S4 Plot-level temporal trends in *Poa pratensis* relative biomass from high (red, top) to low (blue, bottom) rates of N addition. Numbers on the right (0-270) indicate rate of N addition in $\text{kg N ha}^{-1} \text{ yr}^{-1}$. Plots were either continuously fertilized for three decades (left) or fertilized for one decade and then unfertilized for two decades (right). Dashed vertical line indicates year (1991) cessation treatment began. Each line is one plot.