LETTER

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Grassland biodiversity bounces back from long-term nitrogen addition

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The negative effect of increasing atmospheric nitrogen (N) pollution on grassland biodiversity is now incontrovertible[1–3]. However, the recent introduction of cleaner technologies in the UK has led to reductions in the emissions of nitrogen oxides, with concomitant decreases in N deposition[4]. The degree to which grassland biodiversity can be expected to 'bounce back' in response to these improvements in air quality is uncertain, with a suggestion that long-term chronic N addition may lead to an alternative low biodiversity state[5]. Here we present evidence from the 160-year-old Park Grass Experiment at Rothamsted Research, UK[6], that shows a positive response of biodiversity to reducing N addition from either atmospheric pollution or fertilizers. The proportion of legumes, species richness and diversity increased across the experiment between 1991 and 2012 as both wet and dry N deposition declined. Plots that stopped receiving inorganic N fertilizer in 1989 recovered much of the diversity that had been lost, especially if limed. There was no evidence that chronic N addition has resulted in an alternative low biodiversity state on the Park Grass plots, except where there has been extreme acidification, although it is likely that the recovery of plant communities has been facilitated by the twice-yearly mowing and removal of biomass. This may also explain why a comparable response of plant communities to reduced N inputs has yet to be observed in the wider landscape.

Total emissions of oxidized plus reduced N from intensive agriculture and the burning of fossil fuels increased markedly from the middle of the twentieth century in industrialized nations[7]. There is strong evidence from comparisons of similar habitats along N deposition gradients[3,8] that these increases have led to declining biodiversity in semi-natural ecosystems through acidification and eutrophication. These 'space-for-time' studies assume that air pollution has only increased, and that the deposition gradient is representative of a unidirectional temporal shift in grassland biodiversity. Since the late 1980s, however, measures to reduce atmospheric pollution have successfully reduced UK emissions of NO_x by ~50% and of sulfur (S) by ~90% (ref. 9). Quantifying the potential recovery of biodiversity in response to reducing air pollution requires an alternative to the space-for-time approach, ideally monitoring long-term community dynamics on permanent plots[2]. In this context, the Park Grass Experiment at Rothamsted, which started in 1856, presents a unique opportunity to study shifts in biodiversity in response to environmental change both pre- and post-industrialization[6].

Park Grass consists of permanent plots with different fertilizer treatments that were established on a uniform pasture that was at least 100 years old in 1856. In the early 1900s, most plots were divided in two, and lime was applied to one half—designated the limed (L) or unlimed (U) sub-plots. In 1965, the limed sub-plots were further split into sub-plots 'a' and 'b', and the unlimed sub-plots were further divided into sub-plots 'c' and 'd'. Since this time, varying amounts of lime have periodically been added to maintain a target pH of 7, 6 and 5 for sub-plots a, b and c, respectively; sub-plot d is left unlimed (Extended Data Table 1). The liming treatments mean that the eutrophication effect of atmospheric N deposition on plant community dynamics can be quantified independently of soil pH (which also responds to changes in S deposition). Park Grass is in a semi-urban environment, close to a road and on the edge of the town of Harpenden, which act as local sources of atmospheric pollutants[4,10]. Local measurements of ammonium and nitrate deposited in rainfall show that they have both declined by a comparable amount since 1985, and reflect the current national downward trend in total N emissions (Fig. 1). Our measurements did not

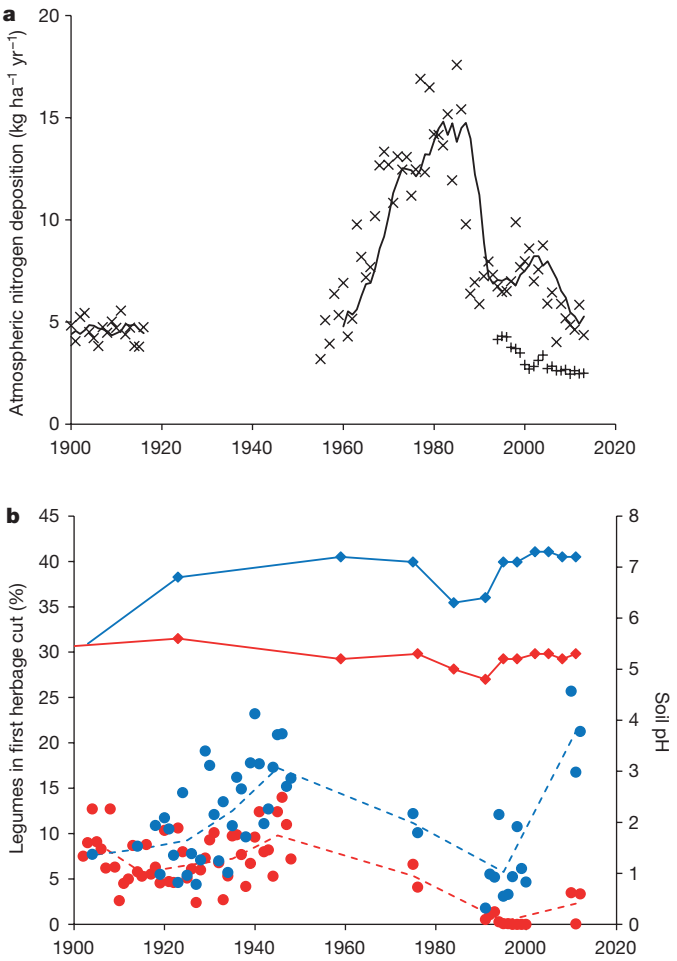

Figure 1 | Changes in atmospheric N deposition, pH and proportion of legumes on the limed and unlimed sub-plots of the Park Grass nil plot. **a**, Changes in wet (×) and dry (+) N deposition; line indicates moving 5-year average (the small increase in the early 2000s may be a legacy of a run of mild winters in the 1990s). **b**, Change in proportion of legumes (by dry weight) measured in the first herbage cut. Lines indicate the change in decadal average of percentage legumes on the limed plot (blue circles, blue dashed line), or unlimed plot (red circles, red dashed line). The plots had an average pH over the period of 7.0 when limed (blue diamond, blue continuous line) and 5.2 when unlimed (red diamond, red continuous line).

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Figure 2 | Change in N concentration measured on archived herbage samples taken from Park Grass sub-plot 3d between 1960 and 2012 that has never received any external inputs of lime or fertilizer. A split-line regression fitted to the data has an intersection point of 1980 (95% confidence limits, 1976 and 1988), with a significant decline after 1980 ($R^2 = 0.67$, $P < 0.001$ using least squares linear regression). During the sampling period, legumes never exceed 5% of total biomass, and the contribution of nitrogen fixation to N in the herbage is therefore expected to be minimal.

include all species of N, including ammonia; however, estimated total N deposition for grassland at this site in 2010–2012, including all N species, was ~21 kg ha^{-1} yr^{-1} (http://www.apis.ac.uk) compared with ~45 kg ha^{-1} yr^{-1} measured in 1996 (ref. 4). Analysis of the N concentration in archived herbage samples from the first hay cut from the plot that has never received any fertilizer inputs also showed a significant decline in percentage N since the 1980s (Fig. 2).

We analysed data on relative biomass of vascular plant species sampled on a range of sub-plots between 1903 and 2012, with a focus on both the 'nil plot' (plot 3), which has never received any fertilizers, and the 'transition plots' (Extended Data Table 2). The latter received 96 kg N ha^{-1} (plus P, K, Na and Mg), either as ammonium sulfate (plot 9) or sodium nitrate (plot 14) until 1989, when the plots were split. Since then, no further N was applied to one-half of the plots (now 9/1 and 14/1). The original treatment continued on the remaining halves (now plots 9/2 and 14/2). Generalized linear models (GLMs) and mixed models (GLMMs) were used to quantify the effect of changes in wet atmospheric N deposition (measured as either a 3- or 5-year moving average) on the proportion of plant functional groups, species richness and the exponent of the Shannon diversity index ($e^{H'}$). Temporal trends in relative species abundance and dissimilarity between plots were also analysed using multivariate methods.

On the nil plot, the proportion of legumes tracked changes in atmospheric N deposition, declining to low relative abundance at the end of the twentieth century before showing a degree of recovery over the recent sampling period (Fig. 1 and Extended Data Table 3). The addition of lime also increased the proportion of legumes and forbs at the expense of grasses. A decrease in pH was observed between 1985 and 1991 resulting from the deposition of S and N that was not always compensated for by the addition of lime[11] (Extended Data Table 1). It is likely that this contributed to some of the observed decline in the proportion of legumes at this time. However, in the recent sampling period (1991–2012), pH has largely remained constant while N deposition has continued to decline, and pH was not significantly correlated with wet N deposition in any of the models, allowing them to be treated as independent variables. Comparisons of species richness between historical sampling periods are confounded by the fact that the area sampled and protocol used has changed through time. However, as the Simpson diversity index has a low sensitivity to sample size, it can be used as an indication of temporal trends in species diversity on Park Grass (Fig. 3). A decline in diversity was observed on the nil sub-plots between the 1940s and 1990s—declines were steeper on the unlimed

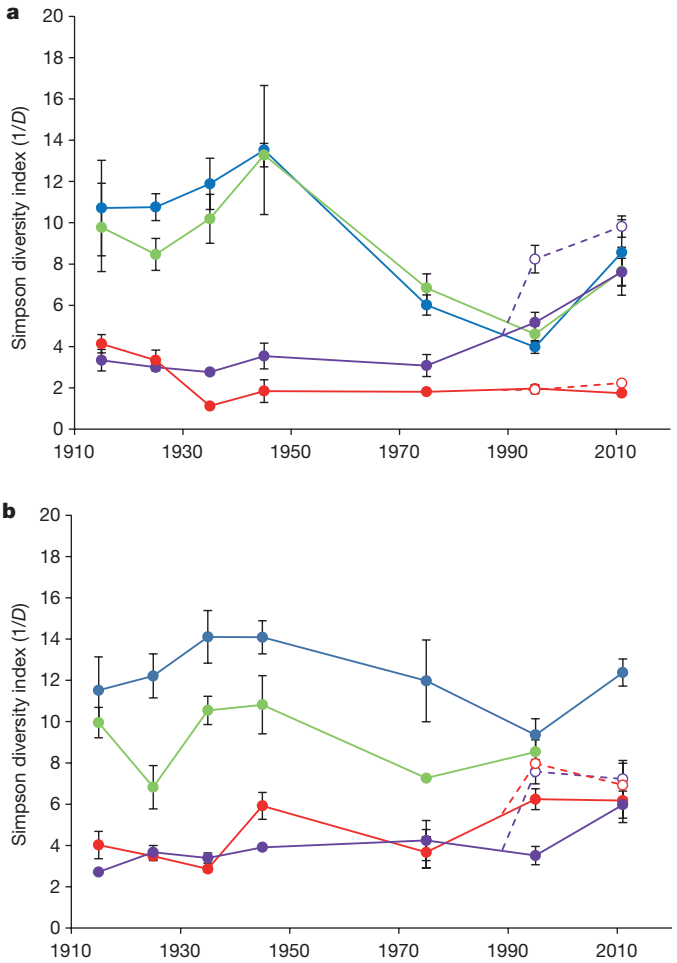

Figure 3 | Historical trends in Simpson's plant diversity index between 1910 and 2012 for unlimed and limed sub-plots. a, **b**, Results are from unlimed (**a**) and limed (**b**) sub-plots. Decadal mean and s.e.m. are presented for: plot 3 (no fertilizers; blue circles), plot 7 (PKNaMg; green circles), plot 9/2 (PKNaMg plus 96 kg N ha^{-1} applied as ammonium sulfate; red circles), plot 14/2 (PKNaMg plus 96 kg N ha^{-1} applied as sodium nitrate; purple circles), plot 9/1 (N withheld since 1989; red open circles, dashed line) and plot 14/1 (N withheld since 1989; purple open circles, dashed line). For the limed plots in **b**, the data post-1965 are from the 'a' sub-plots, as they are closest to the pH maintained on the limed half of the plots before they were split. The relatively high value for plot 14/1d compared to plot 3d and 7d in the 1990s can be explained by the temporary increase in diversity during the transition period. Plot 7a was not sampled between 2010 and 2012.

sub-plot because of the combined effect of eutrophication and acidification. The latest samples, taken since 2010, show diversity is recovering, although it is still at levels below those recorded in the 1930–1940s on the unlimed nil plot (Extended Data Table 3). An analysis across all the individual sub-plots sampled confirmed the positive effect of decreasing atmospheric deposition on plant species richness and diversity, as well as an increase in the proportion of legumes (Extended Data Table 4).

The expected increase in diversity and directional shift in species communities on the transition plots after the cessation of N fertilization in 1989 was observed, except on plot 9/1d, which continues to be constrained by very low soil pH (Figs 3 and 4). Plot 7, which receives the same amount of the other nutrients as plots 9/1 and 14/1 but has never had any N fertilizer additions, can be viewed as the plot towards which the transition plots should be moving. In the case of the b sub-plots, this appears to be the case, but plot 9/1d is only recovering very slowly from a low pH, and 14/1d appears to still have a community that is intermediate between 14/2 d and 7d. Over most of the recent sampling period, the plant community dynamics on plots 9/2 and

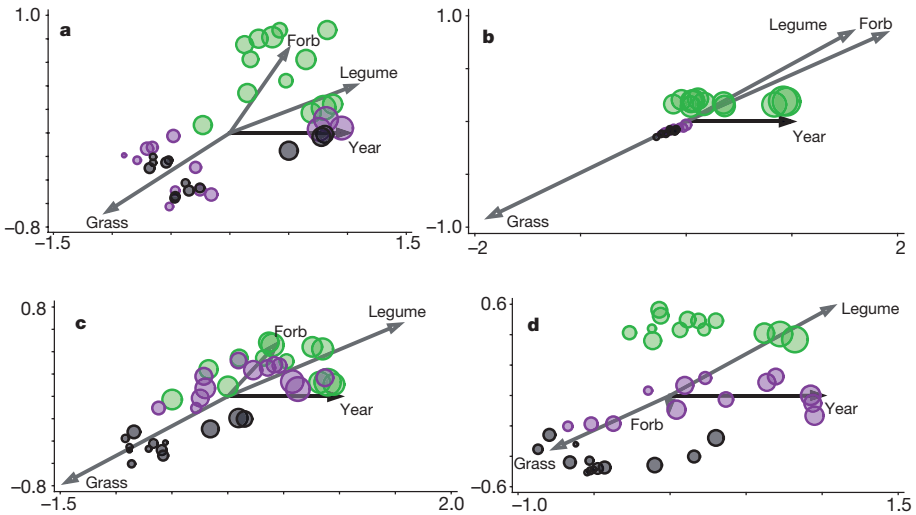

Figure 4 | Temporal trends in plant communities, between 1991 and 2012, on selected Park Grass plots. a–d, Redundancy analysis ordinations are presented for plot 9b (**a**), plot 9d (**b**), plot 14b (**c**) and plot 14d (**d**), including transition plots (purple circles) and plots that have continued to receive fertilizer N (grey circles). In each case, the samples from plot 7b or 7d have been included as having the species composition to which the transition plots are moving towards (green circles). Sub-plots a and c are excluded as they were not sampled on plot 7 in 2010–1012. The size of the symbols is proportional to the numbers of species in each sample, and the relative proportion of the plant functional groups have been projected as supplementary variables.

14/2, which continued to receive N, unexpectedly showed a temporal trend that was largely parallel with the transition plots (Extended Data Figs 1 and 2 and Extended Data Tables 5 and 6). This suggests that the effect of withholding N fertilizer became apparent within the first few years of the treatment change, and since then all the plots have been responding to the same underlying environmental trend. A comparison of multivariate analyses using either year or ±N fertilizer as the explanatory variable showed that the species that responded to withholding N on the transition plots were very similar to those driving the temporal trends in plant communities observed on the wider experiment (Extended Data Fig. 3). In particular, abundance of the legume species, *Trifolium pratense* and *Lathyrus pratensis*, and the forbs, *Plantago lanceolata* and *Ranunculus acris*, all increased significantly when N fertilizer was withheld and also across the whole experiment with time. This was confirmed from the analysis at the sub-plot level for plots 3, 9 and 14 (Extended Data Table 7).

The positive responses of plant diversity to decreasing atmospheric deposition and N fertilizer inputs on Park Grass shows that grasslands have the capacity to recover from the negative effect of eutrophication, particularly where the confounding effect of decreasing pH had been removed by applying lime. The fact that legumes showed the strongest temporal response (coinciding with measured reductions in the N concentration of the cut herbage on the nil plot) supports the view that reducing N deposition was a causal factor of the observed community dynamics. However, only wet N deposition was included in our models, and we were unable to quantify the contributions from dry deposition and other species of N over a sufficient time period, including ammonia and nitric acid. Sulfur emissions have also declined since the 1980s, and although the liming treatments mean that the indirect effect of changing S deposition on soil pH can be treated independently of the eutrophication effect of N deposition, we cannot fully discount the direct nutritional effect of S. However, the plots included in the analysis with K, Na and Mg as part of the fertilizer treatment also receive up to 122 kg S ha^{-1} yr^{-1}, meaning they are unlikely to be limited by S.

The continuity of the experimental treatments on Park Grass, together with the measurement of atmospheric chemistry and plant community data on the same local scale, avoids some of the problems associated with attributing large-scale ecological changes observed in national vegetation surveys to anthropogenic drivers[12]. This may partly explain why a clear signal of a recovery of plant diversity from eutrophication has yet to be detected in the wider landscape[13,14]. However, it is also the case that the magnitude of local scale reductions in N deposition we observed at Rothamsted are not yet reflected at the national scale to the same degree[9]. Interpreting changes on the Park Grass Experiment more widely in the context of comparisons with other grassland studies and its relevance to the wider landscape must also take into account the specific management context. The twice-yearly mowing and removal of biomass on Park Grass may explain the relatively rapid transient dynamics observed on the experiment when compared to equivalent studies in systems with less disturbance, leading to the accumulation of litter[5], or dominated by slower growing, woody species[15,16]. In addition, the close proximity of plots with differing plant communities means that limitation of propagules is not likely to be as important a constraint in the recovery of the communities as may be the case for larger scale grassland restoration[17]. Despite these considerations, the Park Grass Experiment remains a unique indicator of the effects of environmental change and an important part of the evidence base for assessing the biological effects of changes in management or policy on the wider environment.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.S. (jonathan.storkey@rothamsted.ac.uk).

METHODS

Description of Park Grass Experiment and vegetation sampling protocol. The Park Grass Experiment was established on old grassland at Rothamsted in 1856 to examine the effects of different mineral fertilizers and organic manures on productivity of permanent pasture cut for hay[6]. The experiment is located on a moderately well-drained silty clay loam overlying clay-with-flints, a chronic or vertic Luvisol according to the FAO classification. The soil pH was slightly acidic when the experiment began (5.4–5.6), and the nutrient status was poor. The original vegetation of Park Grass was classified as dicotyledon-rich *Cynosurus cristatus–Centaurea nigra* grassland; one of the mesotrophic grassland communities in the British National Vegetation Classification system[18]. Treatments imposed in 1856–1865 included controls (nil, no fertilizer or manure), and various combinations of P, K, S, Mg and Na, with N applied as either sodium nitrate or ammonium salts. Each plot (ranging from 75 to 634 m^2) now consists of plant communities adapted to the fertilizer treatments naturally assembled from the local species pool. Farmyard manure was applied to two plots but was discontinued after 8 years, because when applied annually to the surface in large amounts it did not decompose quickly and had adverse effects on the sward. Farmyard manure, applied every 4 years, was re-introduced on three plots in 1905.

The experiment consists of 20 main plots. The plots are cut in mid-June and made into hay. For 19 years, the re-growth was grazed by sheep penned on individual plots, but since 1875 a second cut, usually carted green, has been taken in place of grazing. The plots were originally cut by scythe, then by horse-drawn and then tractor-drawn mowers. Yields were originally estimated by weighing the produce, either of hay (first harvest) or green crop (second harvest), and dry matter was determined from the whole plot. Since 1960, yields of dry matter have been estimated from strips cut with a forage harvester. However, for the first cut the remainder of the plot is still mown and made into hay, continuing earlier management and ensuring the return of seed. For the second cut, the whole plot is cut with a forage harvester. A small amount of lime, 4 t $CaCO_3$ ha^{-1}, was added to all plots in the late 1880s. Most plots were divided in two in 1903 or 1920 to introduce a test of regular liming on one half. In 1965, they were further divided into four sub-plots (a–d). The a, b and c sub-plots now receive lime every 3 years, if necessary, sufficient to maintain a target soil pH of 7, 6 and 5, respectively. The d sub-plots are unlimed. In 1989, the plots receiving 96 kg N ha^{-1} were split, and nitrogen fertilizer withheld from half of the plots to investigate the ability of the plant communities to recover from chronic nitrogen addition.

Vegetation surveys have been carried out on Park Grass on more than 30 occasions since the experiment began[19]. The original botanical sampling protocol was to take handfuls of cut herbage at regular intervals from every swath after the scythe or cutting machine. Each sample was then sub-sampled until a weight of approximately 12–20 lb (5.4–9.1 kg) was obtained. For the samples taken between 1973 and 1976, samples were cut by hand every two to three paces along ten transects on the larger plots and six on the smaller plots. Approximately 600 g of material was analysed from each sub-plot. Between 1991 and 2012, above-ground biomass from six randomly located 50 × 25 cm quadrats was sampled from all sub-plots using a standard protocol. The herbage was cut with scissors to ground level in early June, immediately before harvesting the first hay crop. The plant material was taken back to the laboratory where it was sorted into species. Samples were oven-dried at 80 °C for about 24 h, after which dry mass was determined for each species. Data from the six quadrats were aggregated to provide an estimate of species richness for each plot in each year.

Monitoring of atmospheric nitrogen deposition: wet 'bulk' deposition. The methods of collection and the amount of data available have varied over time. Initial precipitation data (1853–1968) were collected using a rain gauge with a surface area of one-thousandth of an acre (7 ft 3.12 in. × 6 ft, or 4.04 m^2). The gauge being constructed at ground level of lead supported by wood over a brick lined cellar housing four collection tanks from which a sample of rain water was taken. From 1969 to 1986, precipitation was collected in what is described as a 'simple funnel-and-bottle bulk gauge'[20]. In the latter years, 1986 to present, precipitation has been collected in a bulk rain water collector of a design described previously[21]. All these collection methods took place within the Rothamsted meteorological enclosure, which is located approximately 817 m east-northeast of the Park Grass Experiment. The amounts of nitrate-N and ammonium-N, in mg l^{-1} in solution, were then determined. The amount of nitrogen in kg ha^{-1} deposited by wet deposition is determined by the formula: kg ha^{-1} = (mg l^{-1} × R)/(A × 10^5), in which R is the amount of rain water collected in mm, and A is the surface area of collector funnel in m^2. The total amount of nitrogen was then calculated for each year.

Monitoring of atmospheric nitrogen deposition: dry deposition. Nitrogen deposition in the form of NO_2 was collected passively using diffusion samplers over an exposure period of 2 weeks. The samplers are made up of a 30-μl aliquot of 20% triethanolamine/water absorbent sandwiched between two stainless steel meshes housed in a coloured thermoplastic rubber cap. Into this cap, a 70-mm-long × 11-mm-diameter acrylic tube is inserted with a protective white thermoplastic rubber cap on the opposite end. These samplers were then placed, in sets of three, at locations around the edge of the Park Grass Experiment at a height of 1.5 m above ground and the protective cap removed. After 2 weeks, samplers were sealed using a protective cap and collected. They were then extracted into 2 ml 18.2 MΩ RO (reverse osmosis) water and analysed for nitrite N (NO_2-N) by continuous colourimetric flow analysis. The resulting levels of NO_2-N in μg N m^{-3} were then averaged over the year and converted to kg N ha^{-1} by multiplying by the deposition velocity for managed grassland at Rothamsted (0.3751 mm s^{-1})[22].

Analysis of herbage samples for nitrogen concentration. Representative sub-samples of plant material from the archived hay or herbage samples on sub-plot 3d were dried at 40 °C for 48 h, ball-milled to a homogenous fine powder, dried again at 60 °C for 24 h and analysed with an elemental analyser (NA 1110; Carlo Erba) interfaced (ConFlo II, Finnigan MAT) to a continuous-flow isotope ratio mass spectrometer (Delta Plus; Finnigan MAT), EA-CF-IRMS. After every tenth sample, a solid internal laboratory standard (SILS) with similar C/N ratio as the respective sample material (fine ground wheat flour) was run as a control. The precision (s.d.) for sample repeats was better than 0.04%. Samples taken from herbage cut between 1960 and 2012 were analysed, before this date, the herbage sampling protocol differed with material dried *in situ*, which is affected by disintegration losses in the hay making process.

Statistics. For the plots where all the sub-plots were sampled (3, 9 and 14), all sub-sets regression using GLMs was used to identify the model that explained the maximum variability in species richness, $e^{H'}$ and the relative proportion of functional groups using only independent explanatory variables with $P < 0.05$. The following explanatory variables were included: pH, wet atmospheric nitrogen deposition (included both as a 3- and 5-year moving average), total rainfall in the previous growing season (March–August) and rainfall in current growing season (March–May)—rainfall has been found to explain short-term variability in community composition significantly[23]. For the proportion of the different functional groups (legumes, grasses and 'other'), a binomial distribution with a logit link function was used to allow for the variability in the total first cut biomass to be accounted for. The proportion of each functional group was analysed separately. A normal distribution with an identity link was used for species richness and $e^{H'}$ except for the acid plots with a high frequency of low species counts, in which a Poisson distribution with a log link was used. As opposed to a step-wise approach, all sub-sets regression analyses included all possible combinations of explanatory variables, using the adjusted R^2 and Mallows' C_p as criteria for comparing models.

For the nil treatment (plot 3), that has never received any fertilizers, data were available on relative proportion of functional groups that covered an important historical period from 1903 in which wet nitrogen deposition and pH were also measured on the experiment. This whole data set ($n = 70$) was therefore included in the models. Although less frequent data were available on species richness and diversity, changes in the area sampled and sorting effort meant that a comparison of data over the whole historical period would not have been valid. However, between 1991 and 2012, a standard area and sampling protocol was used. This time period coincided with the reductions in atmospheric nitrogen deposition observed on the experiment and was, therefore, used to quantify responses of species richness and $e^{H'}$ ($n = 13$) across all the plots.

For the analysis of the data from all sub-plots sampled across a range of fertilizer treatments, a GLMM was used with sub-plot and year input as random factors. Fixed effects were input in the same order to a model previously fitted to explain variance in species richness between fertilizer treatments[24]: pH, nitrogen addition (three levels; +48, +96 or +144 kg N ha^{-1}) and ±phosphorus before inputting N deposition as a final continuous explanatory variable as either a 3- or a 5-year moving average. Two further fixed effects were initially included, ±potassium and whether the plot was a transition plot, but neither significantly explained any additional variance in any of the diversity metrics. Plot 13 was the only farmyard manure plot in the data set and was not included in this analysis. Both the GLMs and the GLMMs were run using the software GenStat[25].

The temporal shifts in plant communities was analysed at the species level using multivariate approaches. To investigate any directional response of the transition plots following the cessation of nitrogen fertilization, the Bray–Curtis dissimilarity index was calculated using the first sample date, 1991, as a reference point and regression models fitted to the data using year as the explanatory variable. This was also done for the plots that continued to receive nitrogen fertilizer with the expectation that these plots would increasingly diverge from the transition plots with time. Nonlinear regression with groups was used to quantify differences in the responses of the Bray–Curtis index to time of the transition plots and those that had continued to receive nitrogen. Redundancy analysis, using rainfall in the current growing season as a covariate, was used to identify community shifts over time, using GLMs to identify species that responded significantly to the first ordination axis,

constrained by year. This was done for each of the sub-plots separately for plots 3, 9/1, 9/2, 14/1 and 14/2. Finally, the species responding to withholding N fertilizer were compared with those driving the temporal responses using two additional partial canonical correspondence analyses. First, data from 1991–2012 for plots 9 and 14 were analysed with year input as a categorical covariate and ±nitrogen as the explanatory variable. Second, the data from all the sub-plots sampled from 1991–2012 were analysed, excluding the transition plots (9/1 and 14/1) with plot input as a covariate, and year as a continuous explanatory variable. Only species that were recorded at least three times at the level of the sub-plot were included in the analysis. The software, Canoco 5, was used for all the multivariate analyses[26].

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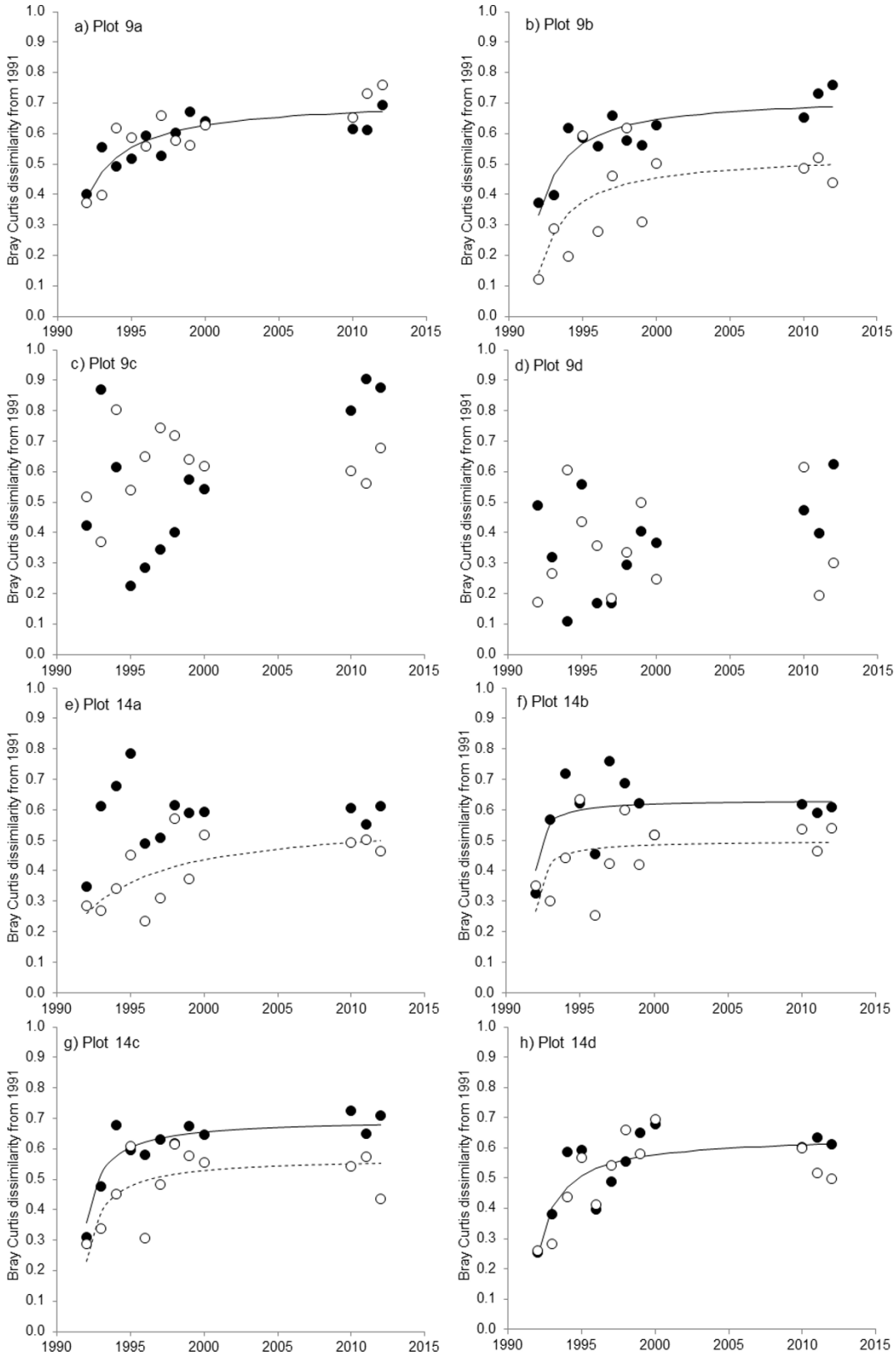
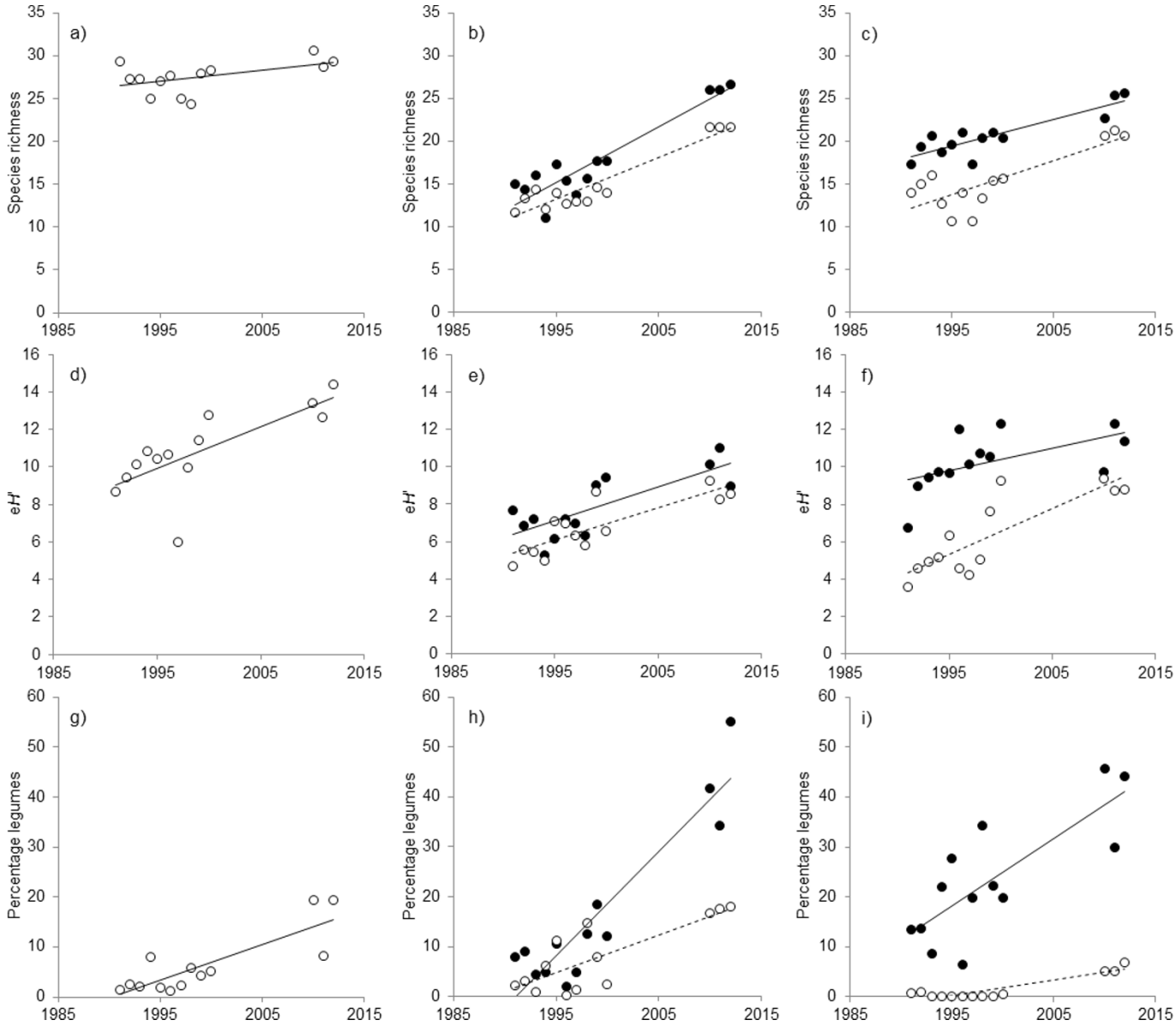
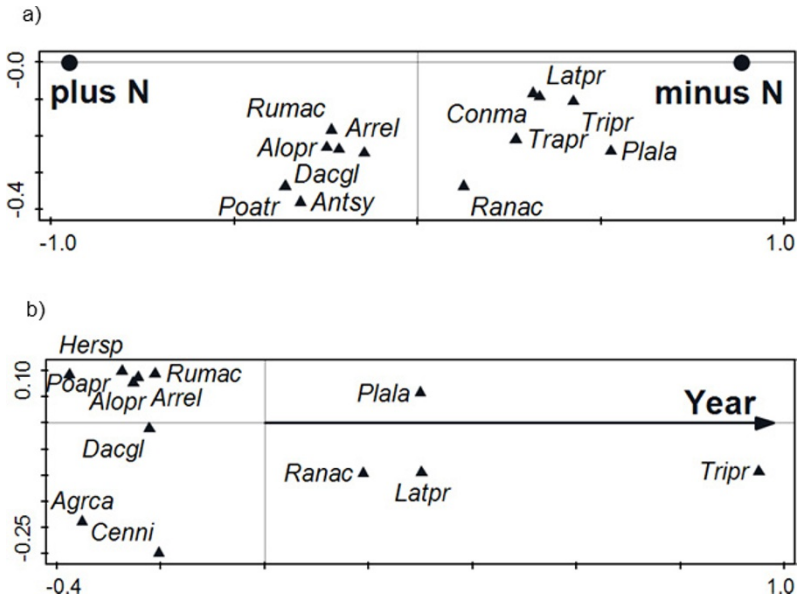


Extended Data Figure 1 | The Bray–Curtis dissimilarity index. a–h, The response of Bray–Curtis dissimilarity for all sub-plots on plot 9 (**a**–**d**) and plot 14 (**e**–**h**). Community data from 1992–2012 have been compared to samples taken in 1991 for the transition plots (filled circles) and plots that continue to receive inorganic N fertilizer (open circles). Lines indicate a significant fit for a rectangular hyperbola function; where separate lines have been fitted in a single panel, a significant difference in the asymptote of the responses was observed.



Extended Data Figure 2 | Change in time over the recent sampling period (1991–2012) of species richness, $e^{H'}$, and percentage legumes in the first herbage cut. a–i, Plot 3 (**a**, **d**, **g**), plot 9 (filled circles, 9/1; open circles, 9/2) (**b**, **e**, **h**) and plot 14 (filled circles, 14/1; open circles, 14/2) (**c**, **f**, **i**). The averages of sub-plots a, b and c are presented; sub-plot d was excluded to avoid the confounding effect of very low pH on plot 9/1d allowing a direct comparison between treatments at the main plot level.



Extended Data Figure 3 | Comparison of the effect of decreasing atmospheric N inputs and the cessation of N fertilization on plant communities. a, Partial canonical correspondence analysis (CCA) of the effect of withholding nitrogen fertilizer on plant communities on the transition plots 9/1 and 14/1 compared to the plots that continued to receive N, 9/2 and 14/2. Data from all sub-plots during the modern day sampling period (1991–2012) were used, and year was included as a categorical covariate. **b**, Partial CCA of the temporal response of plant communities on all sub-plots sampled during the modern day period, 1991–2012, excluding the transition plots 9/1 and 14/1, with year entered as a continuous variable and plot as a covariate. In both ordination plots, species were only included if they were in the top 20 species ranked by their weighting in the CCA and had a *P* value indicating their association with the constrained axis of <0.1. *Agrca*, *Agrostis capillaris*; *Alopr*, *Alopecurus pratensis*; *Antsy*, *Anthriscus sylvestris*; *Arrel*, *Arrhenatherum elatius*; *Conma*, *Conopodium majus*; *Dacgl*, *Dactylis glomerata*; *Hersp*, *Heracleum sphondylium*; *Latpr*, *Lathyrus pratensis*; *Plala*, *Plantago lanceolata*; *Poapr*, *Poa pratensis*; *Poatr*, *Poa trivialis*; *Ranac*, *Racunculus acris*; *Rumac*, *Rumex acetosa*; *Trapr*, *Tragopogon pratense*; *Tripr*, *Trifolium pratense*.

Extended Data Table 1 | pH (in water) measured on soil cores, 0–23 cm, on seven occasions between 1991 and 2012

Plot	Treatment	Nov-91	Feb-95	Mar-98	Mar-02	Mar-05	Mar-08	Mar-11
1b	N_1	6.4	7.0	7.1	7.3	7.2	7.1	6.9
1d		3.6	4.2	4.0	4.1	4.0	3.8	4.0
2/2b	Nil	6.5	6.9	6.9	7.4	7.5	7.3	7.1
2/2b		4.8	5.0	5.2	5.1	5.2	5.1	5.1
3a	Nil	6.4	7.1	7.1	7.3	7.3	7.2	7.2
3b		6.4	6.5	6.5	6.3	6.4	6.1	6.3
3c		5.0	5.4	5.3	5.2	5.1	4.9	5.2
3d		4.8	5.2	5.2	5.3	5.3	5.2	5.3
7b	PKNaMg	6.2	6.0	5.9	5.8	6.0	6.1	6.2
7d		4.8	5.0	4.7	4.9	4.9	4.9	4.9
9/1a	(N_2)PKNaMg	5.7	6.5	6.5	6.9	7.0	7.1	7.1
9/1b		5.2	5.9	6.1	6.4	6.1	6.1	6.4
9/1c		4.4	4.6	4.8	5.3	5.0	4.9	5.2
9/1d		3.7	4.1	4.0	4.1	4.0	4.0	4.1
9/2a	N_2PKNaMg	6.2	6.8	6.8	7.1	7.2	7.1	7.1
9/2b		5.4	6.2	6.2	6.4	6.0	6.3	6.2
9/2c		4.4	5.3	4.8	4.8	5.4	4.8	5.1
9/2d		3.6	3.9	3.6	3.6	3.6	3.4	3.7
10b	N_2PNaMg	5.4	5.5	5.9	6.4	6.7	6.2	6.3
10d		3.4	3.8	3.7	3.7	3.7	3.5	3.7
11/1b	N_3PKNaMg	5.4	6.4	6.1	6.2	6.4	6.0	6.4
11/1d		3.2	3.7	3.5	3.6	3.5	3.4	3.6
11/2b	N_3PKNaMgSi	5.2	5.9	5.6	6.3	6.5	6.1	6.1
11/2d		3.4	3.9	3.7	3.6	3.6	3.4	3.6
13/2b	FYM / PM	6.3	6.1	6.2	5.9	6.0	5.9	6.1
13/2d		4.6	5.1	5.1	5.1	5.2	5.2	5.0
14/1a	(N^*_2)PKNaMg	6.6	6.9	6.8	7.0	6.9	7.0	6.9
14/1b		6.6	6.3	6.1	6.0	5.7	6.2	6.0
14/1c		5.8	5.8	5.6	5.5	5.4	5.3	5.3
14/1d		5.6	5.8	5.6	5.6	5.6	5.6	5.4
14/2a	N^*_2PKNaMg	6.8	6.8	6.9	7.0	7.0	7.0	7.0
14/2b		6.8	6.4	6.5	6.3	6.3	6.3	6.2
14/2c		6.0	6.1	6.1	6.0	6.1	6.0	5.9
14/2d		5.8	5.7	6.1	6.0	6.1	6.1	6.0
17b	N^*_1	6.7	6.5	6.6	6.2	6.4	6.2	6.3
17d		5.6	5.8	5.8	5.7	6.0	5.8	5.7

Treatments: N_1, N_2 and N_3: ammonium sulfate supplying 48, 96 and 144 kg N and 55, 110 and 165 kg S ha^{-1}; N^*_1 and N^*_2: sodium nitrate supplying 48 and 96 kg N and 78 and 157 kg Na ha^{-1}; (N_2), (N^*_2): N last applied 1989; P: triple superphosphate supplying 35 kg P ha^{-1}; K: potassium sulfate supplying 225 kg K and 99 kg S ha^{-1}; Na: sodium nitrate supplying 15 kg Na and 10 kg Na ha^{-1}; Mg: magnesium sulfate (Epsom salts) supplying 10 kg Mg and 13 kg S ha^{-1}; Si: water-soluble sodium silicate supplying 135 kg Si and 63 kg Na ha^{-1}; FYM: 35 t farmyard manure ha^{-1} supplying ~240 kg N, 45 kg P, 350 kg K, 25 kg Na, 25 kg Mg, 40 kg S, 135 kg Ca ha^{-1}; PM: pelleted poultry manure (replaced fishmeal in 2003) supplying ~65 kg N ha^{-1}. Sub-plots a, b and c receive differential amounts of lime, if needed, every 3 years to maintain soil pH at 7, 6 and 5, respectively; sub-plot d receives no lime.

Extended Data Table 2 | Species richness and $e^{H'}$ observed in a total sample area of 0.75 m^2 averaged over three biomass samples taken in 1991–1993 and 2010–2012

Plot	Treatment	Species number (s.e.m.)		eH' (s.e.m.)	
		1991-93	2010-12	1991-93	2010-12
1b	N_1	22(1.5)	22(0.3)	8.4(0.42)	8.8(1.13)
1d		4(0.6)	4(0.3)	1.6(0.23)	2.3(0.19)
2/2b	Nil	27(0.9)	33(3.2)	12.4(0.48)	15(1.19)
2/2d		24(1.2)	26(0.6)	5.8(0.64)	9.9(0.7)
3a	Nil	30(1.2)	31(1.2)	11.4(0.58)	15.7(0.33)
3b		29(0)	30(1.7)	10.6(0.85)	15.1(0.22)
3c		25(3.1)	28(1.2)	6.2(0.42)	9.7(1.21)
3d		28(2.7)	27(0.9)	7.3(0.43)	11.2(1.5)
7b	PKNaMg	22(0.9)	25(1.7)	10.3(0.58)	8.9(1.31)
7d		20(0.3)	26(1.5)	7.9(0.38)	10.5(0.91)
9/1a	(N_2)PKNaMg	20(1.3)	26(0.9)	11(0.46)	11.1(0.51)
9/1b		15(1.2)	26(0.3)	8.1(0.69)	7.3(0.79)
9/1c		10(0.8)	27(0.3)	2.6(0.66)	11.7(0.79)
9/1d		3(0.3)	6(1.2)	1.5(0.07)	2.4(0.17)
9/2a	N_2PKNaMg	16(1.5)	21(0.6)	7(0.22)	9.3(0.64)
9/2b		13(0.9)	22(0.3)	4.8(0.24)	8.4(0.46)
9/2c		10(0.9)	22(0.3)	3.9(0.76)	8.4(0.39)
9/2d		3(0.6)	3(0)	1.5(0.2)	1.9(0.03)
10b	N_2PNaMg	9(0.7)	15(1)	5(0.86)	5.5(0.37)
10d		2(0.3)	3(0.3)	1.1(0.05)	1.5(0.4)
11/1b	N_3PKNaMg	12(1)	14(0.3)	4.2(0.4)	6.5(0.47)
11/1d		1(0.3)	1(0)	1(0.01)	1(0)
11/2b	N_3PKNaMgSi	8(0)	14(0.6)	3.9(0.26)	4.8(0.24)
11/2d		1(0.3)	3(0.3)	1(0)	1.3(0.11)
13/2b	FYM / PM	21(0.3)	25(1.5)	9.7(0.76)	11.6(0.59)
13/2d		20(1.2)	25(0.9)	7.7(0.34)	10.7(0.51)
14/1a	(N^*_2)PKNaMg	20(1.2)	25(0.6)	8.7(0.38)	10(0.89)
14/1b		20(1.5)	23(1.5)	8.7(1.34)	10.8(0.78)
14/1c		18(1)	26(1.5)	7.8(0.84)	12.6(1.46)
14/1d		18(0.9)	22(0.6)	8.6(0.08)	12.4(0.33)
14/2a	N^*_2PKNaMg	17(1.2)	22(1.2)	4.3(0.26)	9(0.43)
14/2b		14(1.5)	20(0.6)	4.6(0.42)	8.5(0.62)
14/2c		14(1.7)	20(1.2)	4.2(0.6)	9.4(0.08)
14/2d		16(0.7)	19(0.9)	5.6(0.52)	9.8(0.31)
17b	N^*_1	23(3)	27(2.4)	8.2(1.28)	10.8(1.66)
17d		25(1.9)	29(0.9)	10.1(0.52)	10.8(0.46)

Sub-plots a and b have been receiving lime since the early 1900s while lime has only been added to sub-plot c since 1965, which also shows the largest increases in species richness over the modern day sampling period, possibly reflecting a continuing slow recovery from low soil pH. Treatments: N_1, N_2 and N3: ammonium sulfate supplying 48, 96 and 144 kg N and 55, 110 and 165 kg S ha^{-1}; N^*_1, N^*_2: sodium nitrate supplying 48 and 96 kg N and 78 and 157 kg Na ha^{-1}; (N_2), (N^*_2): N last applied 1989; P: triple superphosphate supplying 35 kg P ha^{-1}; K: potassium sulfate supplying 225 kg K and 99 kg S ha^{-1}; Na: sodium nitrate supplying 15 kg Na and 10 kg S ha^{-1}; Mg: magnesium sulfate (Epsom salts) supplying 10 kg Mg and 13 kg S ha^{-1}; Si: water-soluble sodium silicate supplying 135 kg Si and 63 kg Na ha^{-1}; FYM: 35 t farmyard manure ha^{-1} supplying ~240 kg N, 45 kg P, 350 kg K, 25 kg Na, 25 kg Mg, 40 kg S, 135 kg Ca ha^{-1}; PM: pelleted poultry manure (replaced fishmeal in 2003) supplying ~65 kg N ha^{-1}. Sub-plots a, b and c receive differential amounts of lime, if needed, every 3 years to maintain soil pH 7, 6 and 5 respectively; sub-plot d receives no lime.

Extended Data Table 3 | GLMs fitted to relative biomass data from the Park Grass nil plot, which has never received any external inputs of lime or fertilizer

Response variable	Explanatory variable	Estimate (s.e.)	*t* statistic	Degrees of freedom	*F* probability
Species number$_{1991\text{-}2012}$	Soil pH Atm N$_{3\ year}$	3.2(0.55) -1.2(0.45)	5.75*** -2.68*	49	<0.001
$eH_{1991\text{-}2012}$	Soil pH Atm N$_{5\ year}$	3.2(0.40) -1.2(0.32)	8.01*** -3.69***	49	<0.001
Legumes$_{1903\text{-}2012}$	Soil pH Atm N$_{5\ year}$	0.74(0.140) -0.48(0.089)	5.34*** -5.37***	67	<0.001
Grass$_{1903\text{-}2012}$	Soil pH Atm N$_{5\ year}$	-0.56(0.078) 0.14(0.041)	-7.04*** 3.30**	67	<0.001
Other$_{1903\text{-}2012}$	Soil pH	0.40(-0.069)	5.83***	69	<0.001

A normal distribution with an identity link was used for species number and $e^{H'}$ and a binomial distribution with a logit link for the proportion of legume, grass and other (non-leguminous forbs), each analysed separately (over-dispersion was corrected for by estimating the dispersion parameter and using the *F* statistic to test for significance). For plant functional groups, data were available from 1903 but, because of changes in the sampling protocol, only data from the recent sampling period (1991–2012) were analysed for species richness and $e^{H'}$.

Extended Data Table 4 | Effect of declining N deposition (measured either as a 3- or 5-year moving average), pH and N or P fertilizers on metrics of plant diversity

Response variable	Explanatory variable	Estimate (s.e.)	*F* statistic	Degrees of freedom	*P* value
Species number	pH	0.20 (0.035)	44.2	366	**<0.001**
	+N:48kgha^{-1}*	-0.92 (0.346)	11.5	27	**<0.001**
	96kgha^{-1}	-0.47 (0.210)			
	144kgha^{-1}	-1.28 (0.467)			
	+Phosphorous	ns	2.6	26	0.120
	N Deposition$_{3\ year}$	-0.09 (0.028)	9.8	11	**0.010**
eH'	pH	0.18 (0.044)	28.3	134	**<0.001**
	+N:48kgha^{-1}	-0.71 (0.291)	11.3	23	**<0.001**
	96kgha^{-1}	-0.47 (0.174)			
	144kgha^{-1}	-1.14 (0.389)			
	+Phosphorous	ns	0.4	21	0.514
	N Deposition$_{5\ year}$	-0.08 (0.033)	6.9	11	**0.023**
Proportion of Legumes	pH	0.55 (0.151)	13.9	298	**<0.001**
	+N:48kgha^{-1}	-2.87 (1.739)	6.8	43	**<0.001**
	96kgha^{-1}	-2.33 (0.625)			
	144kgha^{-1}	-5.08 (2.304)			
	+Phosphorous	2.23 (0.672)	11.1	23	**0.003**
	N Deposition$_{5\ year}$	-0.55 (0.185)	8.8	10	**0.014**
Proportion of Grass	pH	-0.85 (0.088)	110.4	115	**<0.001**
	+N:48kgha^{-1}	1.07 (0.265)	31.7	24	**<0.001**
	96kgha^{-1}	1.63 (0.489)			
	144kgha^{-1}	3.04 (0.265)			
	+Phosphorous	ns	0.0	19	0.997
	N Deposition$_{5\ year}$	0.27 (0.122)	4.8	11	**0.050**

*Effect size of additional N fertiliser expressed in relation to plots receiving no added nitrogen.

GLMMs were fitted to the data from all sub-plots sampled in the modern day period (1991–2012), with the exception of the one plot that receives organic manures, with plot and year input as random factors. A Poisson distribution with a log link was used for species richness and $e^{H'}$ and a binomial distribution with a logit link for the proportion of legumes and grasses, each analysed separately. Where necessary, over-dispersion was corrected for by estimating the dispersion parameter and using the *F* statistic to calculate *P*. Two additional variables, ±potassium and whether the data were from a transition plot, did not explain any variance in the response variables in any of the models.

Extended Data Table 5 | GLMs fitted to relative biomass data from the Park Grass plot 9 (N_2PKNaMg)

Plot 9/1					
Response variable	Explanatory variable	Estimate (s.e.)	*t* statistic	Degrees of freedom	*F* probability
Species number	Soil pH	3.5(0.74)	4.74***	36	<0.001
	Atm $N_{3\ year}$	-3.2(0.60)	-5.31***		
eH'	Soil pH	2.6(0.47)	5.56***	37	<0.001
Legumes	Atm $N_{5\ year}$	-0.94(0.130)	-7.19***	37	<0.001
Grass	Soil pH	-0.66(0.165)	-3.99***	36	<0.001
	Atm $N_{3\ year}$	0.58(0.109)	5.30***		
Other	Soil pH	0.54(0.145)	3.69***	37	<0.001

Plot 9/2					
Response variable	Explanatory variable	Estimate (s.e.)	*t* statistic	Degrees of freedom	*F* probability
Species number	Soil pH	1.7(0.48)	3.63***	36	<0.001
	Atm $N_{5\ year}$	-2.9(0.38)	-7.69***		
eH'	Soil pH	1.3(0.28)	4.77***	36	<0.001
	Atm $N_{5\ year}$	-0.8(0.22)	-3.62***		
Legumes	Atm $N_{5\ year}$	-0.73(0.174)	-4.16***	37	<0.001
Grass	Soil pH	-0.37(0.124)	-3.02**	36	<0.001
	Atm $N_{5\ year}$	0.30(0.094)	3.20**		
Other	Soil pH	0.74(0.121)	6.12***	36	<0.001
	$Rain_{t-1}$	0.002(0.0008)	2.22*		

On plot 9/1, N was last applied as ammonium sulfate in 1989. Sub-plot d with very low pH and species diversity was excluded from the analysis. All data are for the recent sampling period, 1991–2012. A normal distribution with an identity link was used for species number and $e^{H'}$ and a binomial distribution with a logit link for the proportion of legume, grass and other (non-leguminous forbs), each analysed separately (over-dispersion was corrected for by estimating the dispersion parameter and using the *F* statistic to test for significance).

Extended Data Table 6 | GLMs fitted to relative biomass data from the Park Grass plot 14, (N*$_2$PKNaMg)

Plot 14/1 Response variable	Explanatory variable	Estimate (s.e.)	*t* statistic	Degrees of freedom	*F* probability
Species number	Atm $N_{5\,year}$	-2.0(0.29)	-6.70^{**}	50	<0.001
eH'	Soil pH Atm $N_{5\,year}$	-1.0(0.45) -0.8(0.24)	-2.21^{*} -3.41^{**}	49	<0.001
Legumes	Atm $N_{5\,year}$	-0.47(0.098)	-4.84^{**}	50	<0.001
Grass	Atm $N_{5\,year}$	0.26(0.075)	3.46^{**}	50	<0.001
Other	$Rain_{t-1}$	0.003(0.0005)	5.44^{**}	50	<0.001
Plot 14/2					
Response variable	**Explanatory variable**	**Estimate (s.e.)**	***t* statistic**	**Degrees of freedom**	***F* probability**
Species number	Atm $N_{5\,year}$	-2.1(0.40)	-5.41^{**}	50	<0.001
eH'	Soil pH Atm $N_{5\,year}$	-1.5(0.57) -1.3(0.23)	-2.61^{*} -5.51^{**}	49	<0.001
Legumes	Atm $N_{3\,year}$	-1.50(0.225)	-6.65^{**}	50	<0.001
Grass	Atm $N_{5\,year}$	0.31(0.073)	4.20^{**}	50	<0.001
Other	Atm $N_{5\,year}$ $Rain_{t-1}$	-0.35(0.076) 0.003(0.0007)	-4.59^{**} 4.08^{**}	49	<0.001

On plot 14/1, N was last applied as sodium nitrate in 1989. All data are for the recent sampling period, 1991–2012. A normal distribution with an identity link was used for species number and $e^{H'}$ and a binomial distribution with a logit link for the proportion of legume, grass and other (non-leguminous forbs), each analysed separately (over-dispersion was corrected for by estimating the dispersion parameter and using the *F* statistic to test for significance).

Extended Data Table 7 | Significant responses of individual species to year at the level of the sub-plot

	3a	3b	3c	3d	9/1a	9/2a	9/1b	9/2b	9/1c	9/2c	9/1d	9/2d	14/1a	14/2a	14/1b	14/2b	14/1c	14/2c	14/1d	14/2d
% Variance explained	20.6	20.5	24.8	25.3	28.8	40.9	40.9	46.0	52.4	29.1	44.4	8	23.1	33.1	20.6	25.9	31.7	26.5	36.9	23.6
P-value	0.006	0.002	0.003	0.003	0.004	0.001	0.001	0.001	0.001	0.003	0.001	ns	0.004	0.003	0.005	0.003	0.001	0.001	0.001	0.004
Monocots																				
Agrostis capillaris	-*										+**						+*		+*	
Alopecurus pratensis									+*											
Anthoxanthum odoratum																	+*			
Arrhenatherum elatius									+***	+*										-*
Briza media		+**	+**	+***																
Bromus hordeaceus					+*	+***	+***	+**	+*	+*									+*	
Carex caryophyllea	+*	+**	+*	+**																
Carex flacca	+***		+*																	
Dactylis glomerata				-**					+**	+*										-*
Festuca rubra										+*							+**			
Helictotrichon pubescens					+**				+*											
Holcus lanatus												-**	+***	+***		+***		+***		
Lolium perenne					+***			+***						+***					+***	+**
Luzula campestris			+***	+*																
Poa pratensis									+*											
Poa trivialis		+*			+*	+**	+*	+**	+***	+**									+**	
Forbs																				
Achillea millefolium				+***	+***				+***						+*				+*	
Ajuga reptans			+*	+**																
Anthriscus sylvestris						+***	+***			+**										
Cerastium fontanum								+**	+*				+**					+*		+*
Conopodium majus	-*								+*	+***										
Heracleum sphondylium													+**	+**					-**	
Leontodon autumnalis			+**																	
Leontodon hispidus					+**	+*	+*									+*				
Pilosella officinarum			+**	+**																
Pimpinella saxifraga	+*	+**	+*															+**		
Potentilla reptans				+*																
Plantago lanceolata					+***	+***	+***	+*	+***	+***				+***	+*	+***	+*	+*		+**
Ranunculus acris					+**	+**	+**	+***	+***					+*		+*		+*		+*
Rumex acetosa																			+***	
Taraxacum officinale agg.										+**							-*		-**	
Tragopogon pratensis			+**					+*	+*	+**										
Legumes																				
Lathyrus pratensis		+*			+*	+*	+*	+*	+*						+***					
Lotus corniculatus	+**	+**	+*																	
Trifolium pratense		+*	+*	+*	+*	+***	+***	+***	+***					+**	+**	+***	+**	+*	+**	+***
Trifolium repens	+***								+*				+***	+*			+***	+**	+**	

GLMs were used to quantify species responses and precipitation before the first cut included as a covariate for all plots.