nature plants

ARTICLES

https://doi.org/10.1038/s41477-019-0442-8

Potential vulnerability of 348 herbaceous species to atmospheric deposition of nitrogen and sulfur in the United States

Christopher M. Clark[1,18]*, Samuel M. Simkin[2,17,18], Edith B. Allen[3], William D. Bowman[2], Jayne Belnap[4], Matthew L. Brooks[5], Scott L. Collins[6], Linda H. Geiser[7], Frank S. Gilliam[8], Sarah E. Jovan[9], Linda H. Pardo[10], Bethany K. Schulz[11], Carly J. Stevens[12], Katharine N. Suding[13], Heather L. Throop[14,15] and Donald M. Waller[16]

Atmospheric nitrogen and sulfur pollution increased over much of the United States during the twentieth century from fossil fuel combustion and industrial agriculture. Despite recent declines, nitrogen and sulfur deposition continue to affect many plant communities in the United States, although which species are at risk remains uncertain. We used species composition data from >14,000 survey sites across the contiguous United States to evaluate the association between nitrogen and sulfur deposition and the probability of occurrence for 348 herbaceous species. We found that the probability of occurrence for 70% of species was negatively associated with nitrogen or sulfur deposition somewhere in the contiguous United States (56% for N, 51% for S). Of the species, 15% and 51% potentially decreased at all nitrogen and sulfur deposition rates, respectively, suggesting thresholds below the minimum deposition they receive. Although more species potentially increased than decreased with nitrogen deposition, increasers tended to be introduced and decreasers tended to be higher-value native species. More vulnerable species tended to be shorter with lower tissue nitrogen and magnesium. These relationships constitute predictive equations to estimate critical loads. These results demonstrate that many herbaceous species may be at risk from atmospheric deposition and can inform improvements to air quality policies in the United States and globally.

Atmospheric deposition of nitrogen (N) and sulfur (S) are two key drivers of biodiversity decline of plants worldwide after habitat loss and climate change[1]. Nitrogen deposition reduces biodiversity through several mechanisms[2], including soil acidification and subsequent foliar nutrient imbalances[3,4], increased pest pressures on nutrient-enriched foliage[5] and stimulating growth of opportunistic species allowing them to outcompete local neighbours through light limitation or other processes[6–8]. Sulfur deposition primarily reduces diversity by acidifying soils, leading to base cation imbalances, as well as frost sensitivity and inhibition of germination[3,9,10].

In the United States, levels of N and S deposition have declined after decades of successful air quality policies under the Clean Air Act[11–13]. These amendments have reduced total N and S deposition in the eastern United States by an average of 23.7 and 56.9%, respectively, between 2000–2002 and 2013–2015 (ref. [12]). Nevertheless, N and S deposition both remain five to ten times above estimates of pre-industrial levels ($0.4\,kg\,N\,ha^{-1}\,yr^{-1}$ and $0.1\,kg\,S\,ha^{-1}\,yr^{-1}$, ref. [13]) across most of the country. Nitrogen deposition trends are flat or increasing in many areas outside the eastern United States[12,14]. Furthermore, the composition of N deposition is shifting from regulated forms (oxidized NO_x) to mostly unregulated forms (reduced NH_x, except as a portion of particulate matter which is regulated)[12,15].

Current levels of both N and S deposition remain above many known thresholds (termed 'critical loads') for detrimental ecological effects[13,16–18] and probably will remain so in the near future[13,18]. To date, most critical loads have been developed for ecosystems or ecoregions rather than species[2,16,19], although species-level estimates are beginning to emerge in Europe[20,21]. Simkin et al.[22] compiled a database of herbaceous plant species composition across 15,136 plots in the contiguous United States[22]. Comparing this with the spatial gradient of N deposition they found that total richness had a unimodal association with N deposition (one that was steeper in more acidic soils and in grasslands compared with forests) and that decreases in total richness were potentially occurring in 24% of plots[22]. However, it was not reported which among the 4,000 species in that dataset are potentially vulnerable, where they occur, their conservation value and whether any physiological traits may be associated with sensitivity versus insensitivity. Many of these species are too rare to confidently assess but for those that remain we

[1]National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC, USA. [2]Department of Ecology and Evolutionary Biology and INSTAAR, University of Colorado, Boulder, CO, USA. [3]Department of Botany and Plant Sciences, University of California, Riverside, CA, USA. [4]Southwest Biological Science Center, US Geological Survey, Moab, UT, USA. [5]Western Ecological Research Center, US Geological Survey, Oakhurst, CA, USA. [6]Department of Biology, University of New Mexico, Albuquerque, NM, USA. [7]Washington Office—Water, Fish, Wildlife, Air & Rare Plants, USDA Forest Service, Washington, DC, USA. [8]Department of Biology, University of West Florida, Pensacola, FL, USA. [9]Forest Inventory and Analysis Program, USDA Forest Service, Portland, OR, USA. [10]Northern Research Station, USDA Forest Service, Burlington, VT, USA. [11]Forest Inventory and Analysis Program, USDA Forest Service, Anchorage, AK, USA. [12]Lancaster Environment Centre, Lancaster University, Lancaster, UK. [13]Department of Ecology & Evolutionary Biology, University of Colorado, Boulder, USA. [14]School of Earth and Space Exploration, Arizona State University, Tempe, AZ, USA. [15]School of Life Sciences, Arizona State University, Tempe, AZ, USA. [16]Department of Botany, University of Wisconsin, Madison, WI, USA. [17]Present address: National Ecological Observatory Network, Boulder, CO, USA. [18]These authors contributed equally: Christopher M. Clark, Samuel M. Simkin. *e-mail: clark.christopher@epa.gov

Table 1 | Summary of responses and vulnerability to N and S deposition

		S relationship			
		Decrease	None	Increase	Total
N relationship	Decrease	11[a] (6)	5[b] (3)	14[c] (7)	30 (15)
	None	5[b] (3)	15[d] (8)	1[e] (1)	21 (11)
	Increase	26[c] (13)	6[e] (3)	8[f] (4)	40 (20)
	Unimodal	81[c] (41)	6[c] (3)	20[c] (10)	107 (54)
	Total	123 (62)	32 (16)	43 (22)	198 (100)

The number of species out of the 198 (with percentages in parentheses) with robust results for N or S that monotonically decreased, showed no response, monotonically increased or had a unimodal relationship (N only) with N or S deposition. Species with 'U-shaped' N relationships (45 species) are omitted as not being ecologically realistic and species names in each category are in Supplementary Tables 1 and 2. Superscript letters represent levels of vulnerability: [a]high (decrease with both), [b]moderate (decrease with one and unaffected by the other), [c]conditional (either contrasting relationships or conditional on the rate of deposition) or [d]neutral (no relationship with either). [e]Species that partially benefit (increase with one and unaffected by the other) and [f]species that strongly benefit (increase with both) are also indicated.

fill these critical knowledge gaps with a comprehensive analysis of the Simkin et al.[22] database.

Results and discussion

Species responses to N and S deposition. We found that 348 species had sufficient data to analyse according to our criteria. Of these, 70% (243 species) were associated with decreasing probability of occurence with increasing N or S deposition along some portion of the deposition gradient. For some of these species, however, even the best models did not explain much variation in the probability of occurrence (area under curve (AUC) < 0.7 or coefficient of determination $R^2 < 0.1$) because species distributions are a complex function of many factors including but not exclusive to those evaluated here (for example, historical land use, disturbance, ozone, grazing pressures, and so on). We focused on a subset of 198 species that we considered had 'robust relationships' with the predictor variables included (AUC ≥ 0.7, $R^2 \geq 0.1$ and monotonically increasing, decreasing or unimodal relationships with N; Table 1, Supplementary Table 1 and Supplementary Fig. 1). Results for all 348 species are in Supplementary Table 2. Of the subset of 198 species, 54% had a unimodal relationship with N (107 species), 20% had a monotonically increasing relationship (40 species), 15% had a monotonically decreasing relationship (30 species) and 11% had no association with N deposition (21 species) (Fig. 1a–f). The steepness of these relationships and the N deposition associated with the highest species occurrence, also varied widely among species (Fig. 1). For S deposition, 62% had negative associations (123 species), whereas 22% had positive associations (43 species) and 16% had no association with S deposition (32 species). The steepness of these relationships also varied widely (Fig. 1g–i).

Most species had a negative association at some level of N or S deposition received (Table 1). This suggests that many species may be threatened by N and/or S deposition in the United States. The most common joint response by far was a unimodal relationship with N and a decreasing relationship with S (41% or 81 species, Table 1). This agrees with ecological theory[23,24] as well as empirical[16,22] and modelling[25] studies, which show that low levels of N input can act to relieve nutrient limitation and enhance growth for many species[22,23]. Higher levels of N deposition reduce these benefits and can acidify and enrich soils with nutrients, progressively excluding species unable to tolerate or capitalize on the new conditions. The few species that decreased monotonically with N could be poor competitors in the community that persisted only in low N conditions. Greenhouse and field experiments demonstrate that such species may be out-competed due to light limitation brought on by growth of opportunistic neighbours[6]. The average N-response was for a negative association around 10 kg N ha^{-1} yr^{-1} (Fig. 1f), a common critical load from community-level research[16,26]. Sulfur deposition acidifies soils, explaining the large number of species that had negative associations with S (ref. [27]). The few species with positive associations with S deposition may be acid tolerant species that benefit from the loss of competitors, rather than evidence of a fertilization effect from S. Sulfur-limitation can occur but such cases are rare in natural communities[23,28]. There is more evidence that a shift towards phosphorus (P)-limitation may occur with high N deposition[29,30]. In agricultural settings, S-limitation can occur but only when N and P are abundant[31], which is probably not the case for our study plots.

Species-level critical loads. We then calculated N and S critical loads using partial derivatives of the best statistical model for each species (compare Simkin et al.[22]; Supplementary Information). Mean critical loads for N ranged from 3.2 kg N ha^{-1} yr^{-1} (*Cirsium arvense*) to 17.6 kg N ha^{-1} yr^{-1} (*Solidago canadensis*) (Fig. 2a). The intervals in Fig. 2a represent spatial variation in the critical loads, not error associated with the mean. Such variation reflects how species can have lower or higher critical loads in a particular location on the basis of covarying factors (for example, lower critical loads in more acidic conditions). This has been reported for habitats in Ireland[21], where the critical load for a species may vary widely across habitats.

The wide variability for species-level N-critical loads across a species' range demonstrates that vulnerability for any given species depends on its environmental context[32,33]. This is more realistic ecologically than a single value for a species; for example, adding 2 kg of N to a very N-limited site elicits a different response than would occur at a more fertile site. This wide variation, however, also cautions against using any single critical load for most species. Instead, this supports using the partial derivative from multivariate models as we did, which retains relationships with relevant covariates, allowing us to refine estimates of the critical load using local edaphic or climatic factors (Supplementary Table 2; equations (1)–(4) in the Supplementary Methods).

Average critical loads could not be defined for species that monotonically increased or decreased because thresholds (if present) are outside the range of the observed data (Fig. 2b). For these species there is no observed threshold in the probability of occurrence and thus a critical load cannot be quantified. This limitation is partly explained by the range of observational data for each species and partly by our approach. Only monotonic relationships with S were allowed for ecological and statistical reasons (see Supplementary Information) and more complex mathematical relationships (for example, sigmoid) were not explored, which may have revealed critical loads for some species. Supplemental analysis revealed that species receiving a minimum N deposition greater than 4 kg N ha^{-1} yr^{-1} were less likely to have unimodal and more likely to have decreasing relationships ($Chi^2 = 28.04$, $P < 0.001$; Supplementary Table 3). Short deposition gradients may be especially problematic analytically for species that only occur in the western United States.

Many species-level critical loads reported here and elsewhere are below community-level critical loads (for example, ~8–20 kg N ha^{-1} yr^{-1}; refs. [2,20–22]). This is expected given that community-level critical loads are essentially averages over sensitive and insensitive species. Many species critical loads reported here are lower than those from acid grasslands across Europe (around 8–22 kg N ha^{-1} yr^{-1}; ref. [20]) but comparable to those from Ireland (~2.8–19 kg N ha^{-1} yr^{-1}; ref. [21]). This may be explained because most of the plots from the acid grassland study were from the United Kingdom and mainland Europe[34] where deposition rates are higher (8–35 kg N ha^{-1} yr^{-1}), as opposed to the United States and Ireland where N deposition included lower levels (2–20 kg N ha^{-1} yr^{-1}). The Irish study also

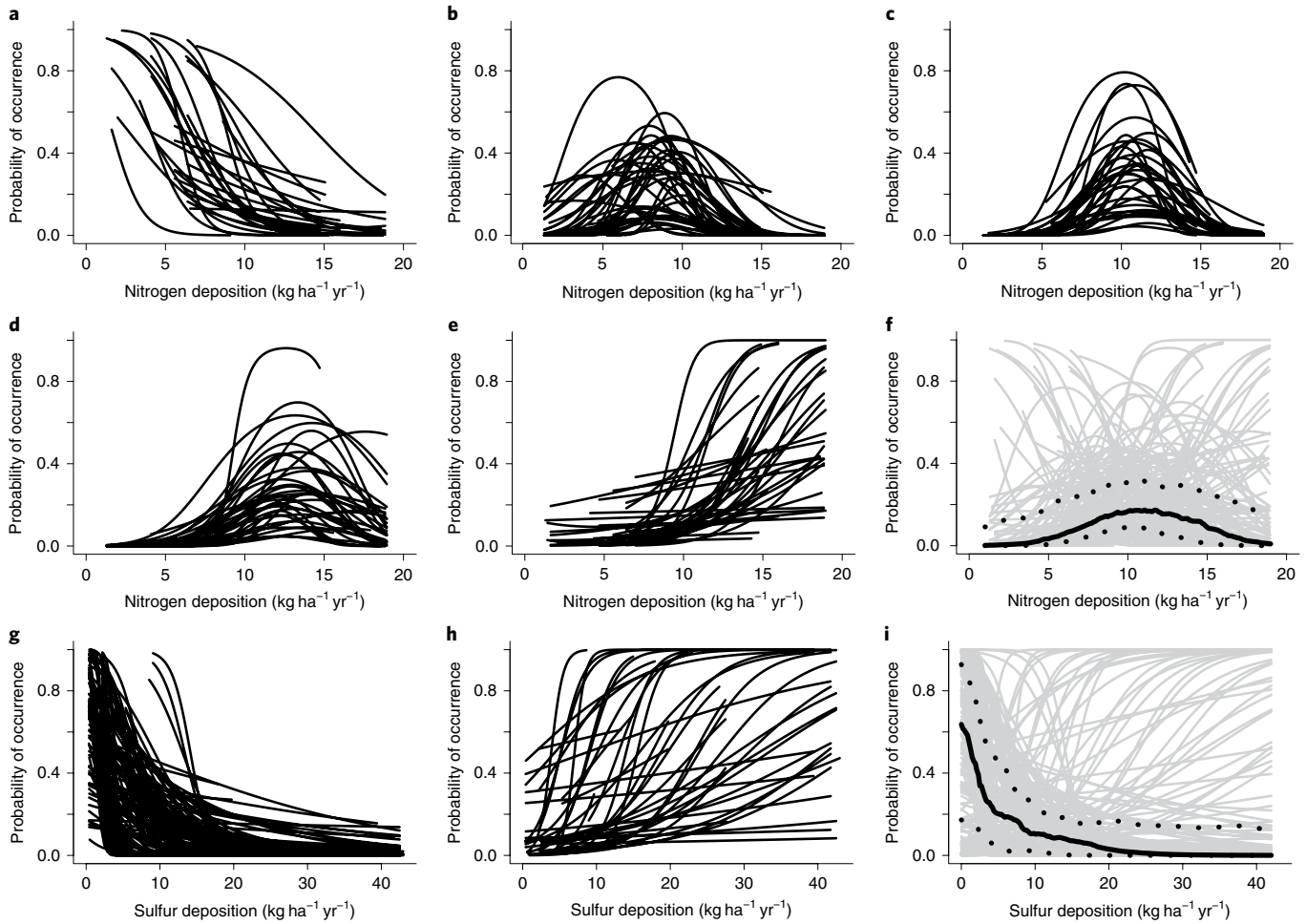


Fig. 1 | Species response curves for nitrogen (177 species) and sulfur (166 species). a–e, For N, response types are decreasing (**a**, 30 species), unimodal (**b–d**, 107 species) or increasing (**e**, 40 species). Species with unimodal relationships are split into three panels to improve readability on the basis of the N deposition where probability of occurrence was highest (**b**, peak at 3.1–10 kg N ha^{-1} yr^{-1}, 39 species; **c**, peak at 10.1–12 kg N ha^{-1} yr^{-1}, 32 species; **d**, peak at 12.1–19 kg N ha^{-1} yr^{-1}, 36 species). **g**,**h**, For S, response types are decreasing (**g**, 123 species) or increasing with S deposition (**h**, 43 species). The average response across all species is shown for N (**f**) and S (**i**) as a solid black line and the 25th and 75th percentiles are shown in dotted black lines (individual species curves from panels **a–e** and **g**,**h** are shown in grey). Other factors are evaluated at the species-level average. Species with no relationship (21 and 37 species for N and S, respectively) or a U-shaped relationship with N (45 species) are not shown.

found critical loads of a species could vary widely among different habitats and bootstrapped intervals in a habitat were also often 2–6 kg N ha^{-1} yr^{-1} wide[21]. We compared our results with critical loads for 304 European species (24 from acid grasslands in ref. [20] and 280 across many habitats in Ireland in ref. [21]). There were only eight species in common between our study and those (Supplementary Table 4, Supplementary Fig. 2) and only one that was present across all three (*Campanula rotundifolia*, Fig. 3). The critical load for *C. rotundifolia* reported here (7.9 kg N ha^{-1} yr^{-1} average, 5.7–14.8 kg N ha^{-1} yr^{-1} for 5th–95th interval) compared well with estimates from Ireland (two habitats: 6.2 and 8.2 kg N ha^{-1} yr^{-1}; from ref. [21]) and all three estimates were lower than from European acid grasslands (13 kg N ha^{-1} yr^{-1}; from ref. [20]). The correspondence between our estimates and those from Ireland is encouraging since the methods were completely independent (TITAN analysis versus partial derivatives), suggesting both approaches are capturing similar ecological relationships. One advantage of our approach is the predictive equation that retains the associations among moderating factors. One advantage of the TITAN approach is that it is not restricted to any particular mathematical form.

Floristic quality of vulnerable species. We next assessed the floristic quality of species positively and negatively associated with N and S deposition. We were primarily concerned with the following question: are the species that are potentially at risk highly valued natives or are they common or non-native species? We used results compiled from many plant surveys across the United States on the basis of 'coefficients of conservatism' (C values: 0–10) assigned to individual plant species (C_i) on the basis of their tolerance to human disturbance and the degree to which the species represent natural undisturbed habitats[35]. Higher C-scores are associated with higher quality flora and habitats, with non-natives receiving a score of zero. Natives range from 1 to 10 on the basis of their tolerance to disturbance (higher C-score for lower tolerance). Of the 137 species that were associated negatively with N along some portion of the gradient, roughly 84% were highly or moderately valued (that is, $C_i \geq 7$, $4 \leq C_i \leq 6$, respectively). There was a negative correlation between C-scores and the species average N-critical loads (Pearson's coefficient of correlation, $r = -0.260$, $P = 0.001$), indicating that species of higher conservation value had lower critical loads. Of the 123 species that were associated negatively with S deposition, ~82% were of

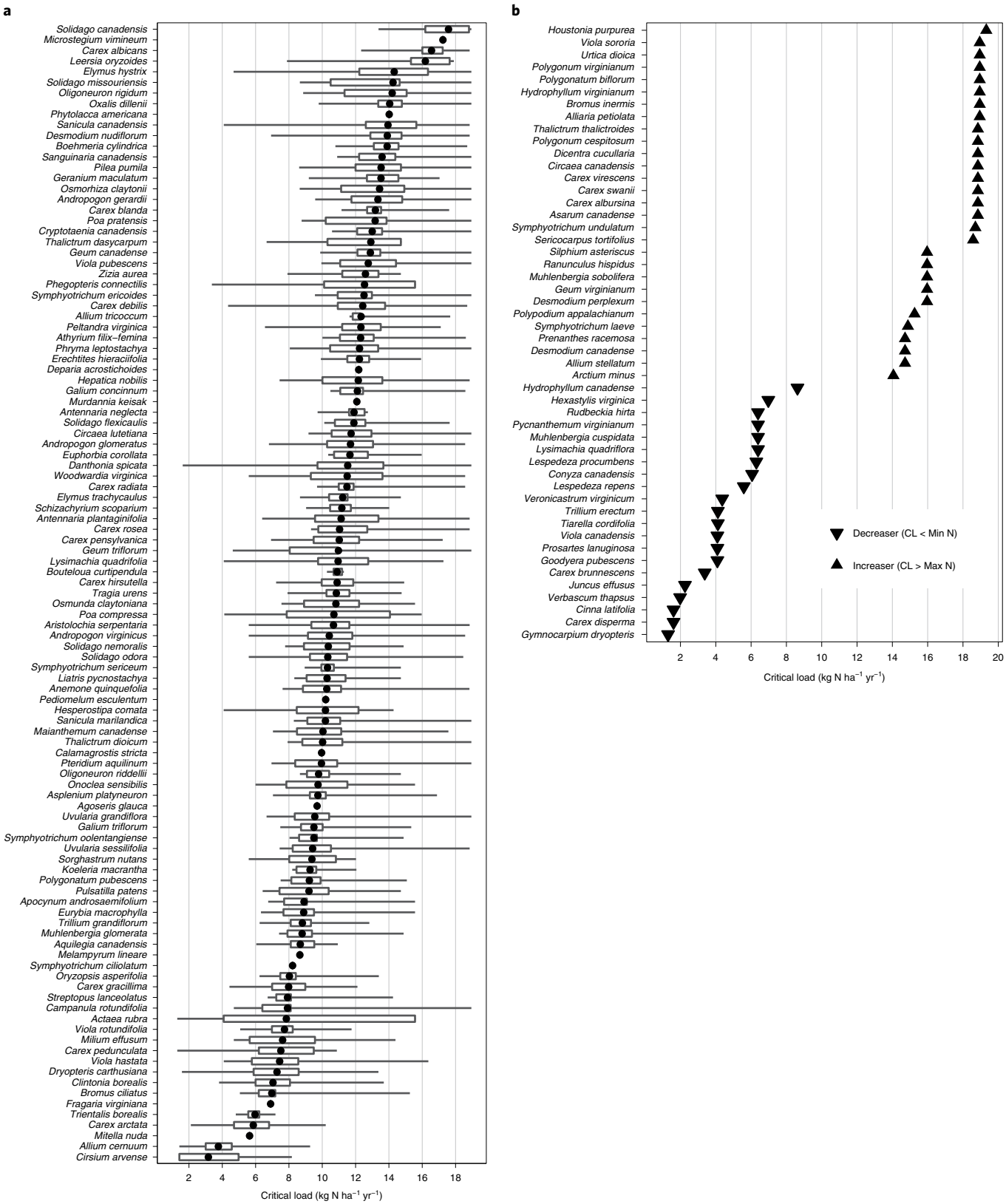


Fig. 2 | Spatial variation in species-level nitrogen critical loads. a,**b**, Nitrogen critical loads (CL) for 107 species with a unimodal-shaped relationship (**a**) and 50 species with a monotonic relationship (**b**) that either decreased (▼) or increased (▲) with N deposition. In **a**, the mean (circle), minimum and maximum (bars) and 25th to 75th percentile range (box) represent spatial variation (not error) in the critical load on the basis of covarying factors that affect sensitivity (more sensitive species have lower critical loads). In **b**, only point estimates are shown because the critical load for decreasers is below the minimum N deposition and the critical load for increasers is above the maximum (how far outside this range is not known). The 20 species with a 'see-saw' relationship are not shown because the average critical load is not meaningful.

moderate-to-high conservation value. These include *Muhlenbergia cuspidata*, *Lysimachia quadriflora* and *Prosartes lanuginosa*, all highly valued native species (average $C \geq 7.8$) of North America.

Regional and species vulnerability across the contiguous United States. To determine spatial patterns of vulnerability to N and S deposition, we calculated the percentage of species that were positively or negatively associated with local deposition in each $12 \times 12\,km^2$ grid cell. Overall, more species were positively than negatively associated with N deposition. But, most eastern areas had significant fractions of decreasers (>15%; Fig. 4a,c). Out of the 3,122 grid cells containing one or more of the focal 198 species, 75.8% had an exceedance for N deposition for one or more species and 24.3% had an exceedance of 50% or more unique species in the grid cell (Fig. 4c). Hotspots of negative associations with N deposition included southern Minnesota, eastern West Virginia and scattered grid cells in the northeast, mid-Atlantic and midwest. There was wide variation in the fraction of species potentially at risk even in high N deposition areas, suggesting that fine-scale processes affect local risk (for example, differences in species composition, historical land use, the degree of nutrient limitation and other stressors such as ozone that were not included[22,32]). Lower fractions of species at risk were estimated in the west, probably partly due to shorter N deposition gradients that did not make our threshold for assessment (see Supplementary Information).

Hotspots of decreasers with S deposition occurred throughout the United States, even in relatively low deposition areas in the west (Fig. 4b). Our leading explanation for this is the dominant mechanism for N is through eutrophication while the dominant effect for S is through acidification—thus, species and communities may benefit from low levels of N deposition which transitions to harm at higher levels, while species and communities are primarily harmed by S deposition. Another plausible explanation is that S deposition was not allowed to have complex non-linear patterns (for example, sigmoid, unimodal) that would facilitate a flat or positive response transitioning to a negative response. Notably, we found higher fractions of increasers (>50%) with S in historically highly polluted sites like West Virginia, which could be indicative of a local community that has already shifted towards acid-tolerant species.

Of the 198 species with robust responses, critical loads were exceeded at more than half the observed sites for 17% (34 species) and 55% (108 species) for N and S, respectively. Because these plots are not a random sample across the conterminous United States (see Fig. 3), it is not possible to say how this translates to vulnerability across the entire range of each species.

Do functional traits predict vulnerability? Finally, we explored whether simple predictive relationships existed between species traits and their potential sensitivity to N deposition. Such relationships would yield a predictive tool for decision makers to apply to species lacking plot occurrence data across a deposition gradient. We found that simple plant functional groups (for example, functional group (FG), cotlyedon status (CS), and so on) were generally poor predictors (all $R^2 < 0.02$) of either the shape of the response or the critical load (Supplementary Table 5), although natives tended to have more negative relationships ($P = 0.036$) and lower critical loads ($P = 0.028$) than introduced species, perennial species tended to have lower critical loads than non-perennials ($P = 0.046$) and legumes tended to have more decreasers ($P = 0.104$). These broad trends are in line with ecological theory, where native and perennial species tend to have traits focused on N-retention and slower growth and legumes rely partly or wholly on fixing atmospheric N, both strategies that may be more susceptible to competitive exclusion from opportunistic non-native or annual species[36,37]. Although consistent with ecological theory, these relationships were notably weak (for example, not all natives decreased with N deposition and not all invasives increased), reinforcing the notion that these broad groups may be less helpful than we'd like in describing ecological responses. Detailed information was available for nine traits: leaf nitrogen content (LNC), leaf carbon content (LCC), specific leaf area (SLA), vegetative height, (VH), leaf lignin content (LLC), leaf phosphorus content (LPC), leaf calcium content (LCaC), leaf potassium content (LKC) and leaf magnesium content (LMgC). We found physiological traits were much more predictive of the critical load and led to several predictive equations:

$$\mathrm{CL(N)} = 6.20 + 7.32 \times \mathrm{LMgC} + 0.06 \times \mathrm{VH};\ \mathrm{Adj}R^2 = 0.36;\ P < 0.001;\ n = 37 \quad (1)$$

$$\mathrm{CL(N)} = 5.03 + 2.63 \times \mathrm{LNC};\ \mathrm{Adj}R^2 = 0.22;\ P < 0.001;\ n = 55 \quad (2)$$

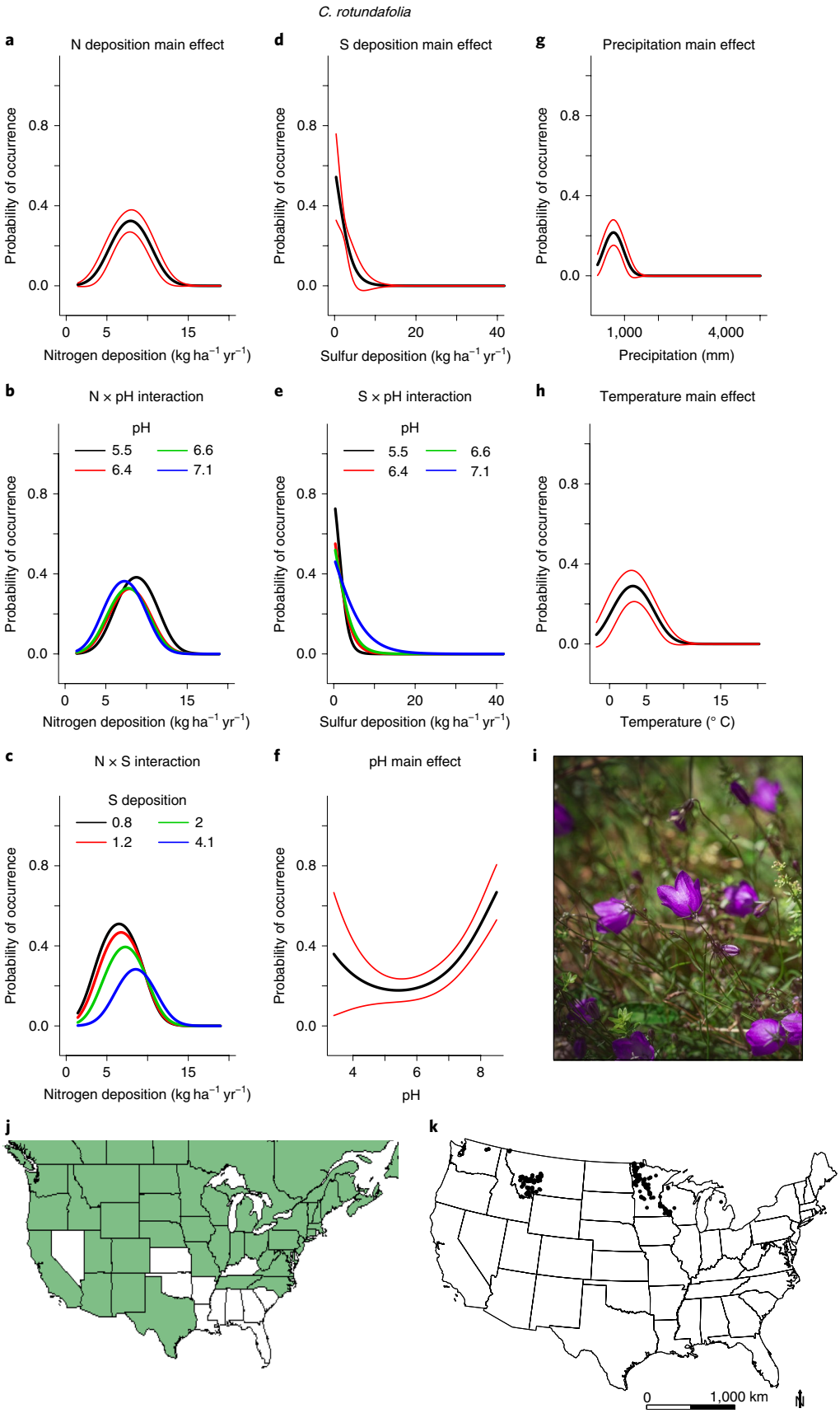
$$\mathrm{CL(N)} = 4.28 + 2.51 \times \mathrm{LNC} + \mathrm{CS}_i;\ \mathrm{Adj}R^2 = 0.32;\ P < 0.001;\ n = 55 \quad (3)$$

The best overall model (equation (1)) predicted that the N-critical load (CL(N)) was a two-factor model with LMgC ($P < 0.001$) and VH ($P = 0.06$). Leaf Mg is strongly associated with photosynthetic rates because it is a key element of chlorophyll[38], while VH influences access to light. Thus, species that were more potentially vulnerable (for example, lower CLs) had lower photosynthetic rates and were shorter-statured as reported in many other site-specific studies[7,16,24,36]. Leaf Mg, however, is a trait not commonly available for most species and photosynthetic rates are also correlated with leaf N (ref. [39]) (LNC and LMgC were highly correlated in our study: $r = 0.57$, $P = 0.001$). To develop an operational equation for wider use we examined relationships on the basis of more widely available traits (LNC, SLA and the six categorical traits). We found that LNC was also highly predictive (equation (2)) and adding a factor for cotyledon status (monocot, dicot, fern; CS_i) improved the model further (equation (3), CL(N) = +1.7, +0.7 and −2.8 for dicot, monocot and ferns respectively) with no significant interaction in slope ($P = 0.36$). Nitrogen critial loads from the three equations were also correlated (all $r > 0.65$) and generally within ±1 and ±2 kg N ha^{-1} yr^{-1} of one another (for 56 and 80% of species, respectively). This is the first instance we know of reporting a predictive equation for critical loads of individual plant species.

It is important to note our assessment of 348 species represents only about 10% of the species in the initial dataset and it is unknown whether species that were not assessed are more or less vulnerable to N or S deposition. Most species were excluded on the basis of rarity (3,643 had fewer than 50 presences) but many also had deposition

Fig. 3 | Detailed example of species response. Generalized linear model results for *C. rotundifolia* (common name: harebell). **a**–**h**, Marginal probabilities of occurrence individually by term from the best model for N deposition (**a**), N × pH (**b**), N × S (**c**), S deposition (**d**), S × pH (**e**), soil pH (**f**), precipitation (**g**) and temperature (**h**). All terms: $P < 0.01$ (Supplementary Table 2. Black lines in main effect plots are average response and red lines are 95th confidence intervals. For interaction terms (**b**,**c**,**e**), the effect of the modifying term is shown as separate quartile lines (Q1–Q4). **i**–**k**, Also shown is a photo of the species (**i**), a range map from the USDA (**j**, ref. [58]) and a plot map from this study (**k**). *C. rotundafolia* is a northern latitude wildflower that grows in drier, low-nutrient soils[59]. This species had a hump-shaped relationship with N (average critical load = 7.9 kg N ha^{-1} yr^{-1}; 10th–90th percentile critical load = 5.9–10.6 kg N ha^{-1} yr^{-1}) and a negative relationship with S. Interactions were statistically significant with little effect on marginal relationships, except for the N × S interaction, where the N effect was stronger if S deposition was low. The 10th–90th percentile reported here is similar to that reported for *C. rotundafolia* in Ireland[21] and lower than that found in acid grasslands across Europe[20]. See Supplementary Fig. 1 for results for all 198 species.

gradients that we considered too short relative to interannual variation to assess (3,433 had N deposition gradients < 7 kg N ha^{-1} yr^{-1}). However, evidence from N fertilization experiments suggests that rarer species are more likely to be lost with N addition[7,36].



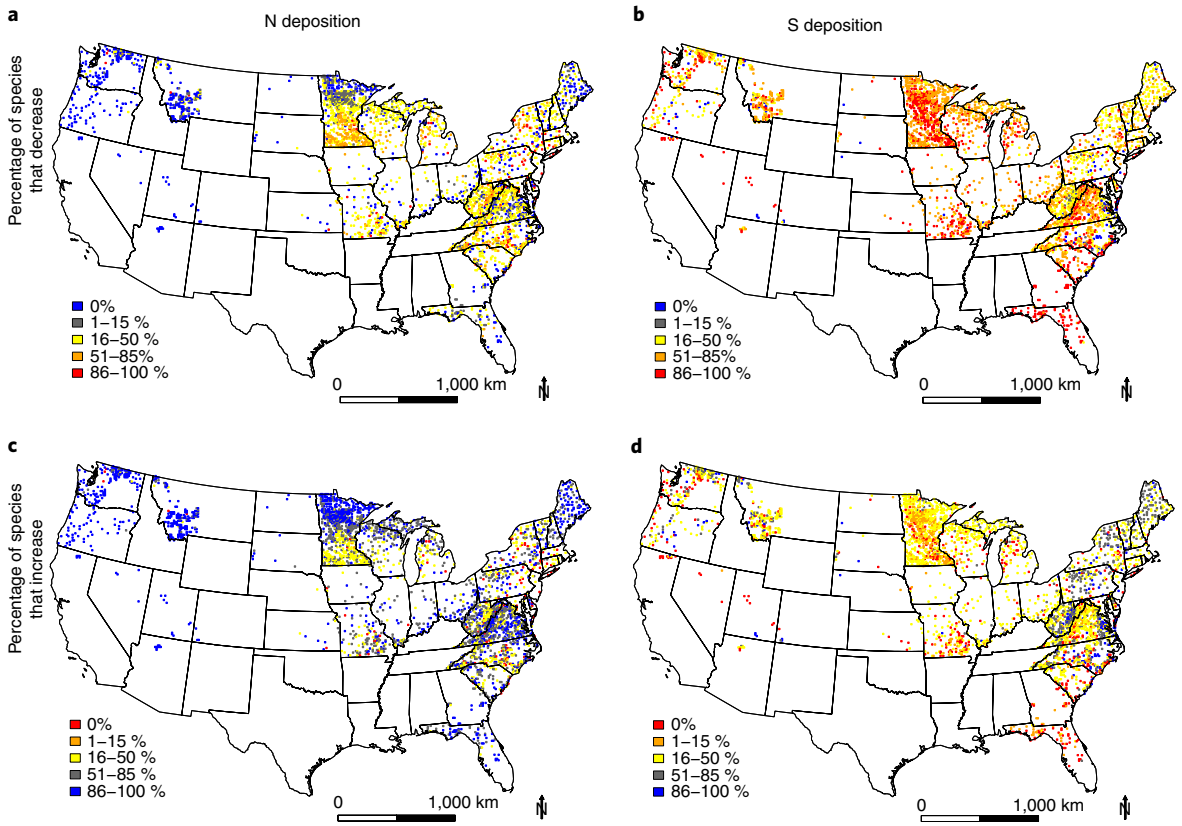


Fig. 4 | Geographic variation in sensitivity to N and S deposition. a–d, Percentage of species that decrease (**a**) or increase (**c**) in probability of occurrence with increasing N deposition and decrease (**b**) or increase (**d**) with increasing S deposition. Plots were aggregated in a 12 × 12 km^2 grid cell and unique species were only counted once if they were potentially vulnerable anywhere in the cell. Note the colour ramps are flipped between decreasers and increasers, with hotter colours denoting negative effects (more decreasers and fewer increasers, most species assessed were native).

Causality and multicollinearity. It is difficult to confidently assign causality to deposition in a gradient study such as ours[22,34]. We addressed this by assessing correlations among predictor variables individually for each species and summarizing these as variance inflation factors (VIFs) for nitrogen (VIF-N) and sulfur (VIF-S) (see Supplementary Information). Lower VIFs mean less of a chance for spurious correlations to affect results. There were larger correlation concerns with S than N, with fewer species under the conventional or conservative cutoffs for S as opposed to N (Supplementary Table 6). Comparing the results for the 22 species with low multicollinearity (both VIFs < 3) with the full set of 198 species yielded several insights. The proportion of species with decreasing and unimodal relationships with N was nearly identical between the two sets of species (14% versus 15% for decreasers, 50% versus 54% for unimodal; Supplementary Table 6). The same was true for species with decreasing relationships with S (Supplementary Table 6). However, in the set of species with low VIFs we found no species that increased with S and no species that showed no change with N (Supplementary Table 6). Thus, results are probably robust for species that decrease with N or S and for species with unimodal N-relationships. However, results for species that increase with S or show no change with N may be interpreted with caution. Given the large numbers of species tested, we also tested our results for possible Type I errors using a Holm–Bonferroni multiple comparisons adjustment[40] and found that 66% of species relationships with N remained significant after such an adjustment (see Supplementary Information). Given decades of research documenting that climate, soil pH and atmospheric deposition affect plant communities, we assume relationships that lost significance after adjustment are probably still ecologically valid.

Conclusions and policy implications. Even though a correlative study such as ours cannot definitively assign causality, the confluence of findings from controlled experimental manipulations[7,41–43], gradient studies such as ours[21,34,44], communities tracked through time as deposition changes[45,46] and dynamic modelling[25,47], all suggest that N and S deposition can alter plant community composition. We found that 70% of the 348 species assessed and 85% of the 198 species that had a robust relationship, were negatively associated with N and/or S somewhere in the contiguous United States. Our results are unprecedented at this scale and in numbers of species assessed in the United States, strongly indicating widespread vulnerability to N and/or S deposition and that species respond differently on the basis of local environmental context. The wide range of thresholds within species suggests that potential vulnerability is linked to local edaphic factors and atmospheric co-pollutants. This work can help inform the review of the US Environmental Protection Agency (EPA) secondary standards for oxides of nitrogen, oxides of sulfur and particulate matter[48] to identify species and regions of particular concern from these stressors.

Methods

Data assembly and species filtering. Simkin et al.[22] compiled data from a variety of sources to develop a consolidated dataset that included plot level information for species composition (percentage abundance), temperature, precipitation, soil pH and N deposition for 15,136 plots nationwide. All variables were selected to represent long-term conditions at a site. Temperature and precipitation were 30-year normals from PRISM (ref. [49]), soil pH was from a combination of locally assessed empirical measurements and SSURGO (ref. [22]) and N deposition was calculated as the sum of the 1985–2011 mean annual wet deposition interpolated from the National Atmospheric Deposition Program (NADP) plus 2002–2011 CMAQ modelled mean annual dry deposition[22]. Updated deposition estimates from the Total Deposition project (TDEP, ref. [50]) were not available at the time but

Simkin et al.[22] reported good correspondence between our estimate and TDEP ($r^2 = 0.89$, TDEP(2000–2012) = SimkinNdep(1985–2011) × 0.91 + 0.3; refs. [22,50]). Total S deposition was calculated in the same manner as N deposition.

To filter plots and species to a subset to analyse, we restricted plots to those that were 100–700 m^2 as was done in Simkin et al.[22] to reduce effects of species–area relationships and removed all taxonomic groups that were only identified to genus or functional group. We excluded rare species by removing species with fewer than five records overall and sparse species that did not have at least five records or comprise 5% of records in at least one Alliance using the National Vegetation Classification system[51]. The second condition is needed because in a presence/absence dataset such as ours, we needed to identify the 'core community' from which to draw the absences. This filtering reduced the number of plots to 15,223 and species to 1,027. We then required that each species span an N deposition gradient of at least 7 kg ha^{-1} yr^{-1}, reducing the number of plots to 14,041 and species to 348. The choice of a 7 kg ha^{-1} yr^{-1} gradient was arbitrary but was guided by the assumption that the spatial gradient of deposition should exceed interannual variation in N deposition (often 2–3 kg ha^{-1} yr^{-1}; ref. [52]) by about double to detect a spatial trend. See Supplementary Information for more details.

Species analysis. We performed binomial generalized linear models separately for each species on presences and absences from the set of Alliances that were considered its core community. We ran all possible models using 12 candidate terms: N deposition (Ndep), S deposition (Sdep), precipitation (*P*), temperature (*T*), soil pH (pH), $Ndep^2$, P^2, T^2, pH^2, Ndep × pH, Sdep × pH and Ndep × Sdep. Rationale for individual terms is described in the Supplementary Information. To prevent model overfitting, we required there to be at least five detections per model term (for example, for the full model with all 12 predictors plus the intercept, the species was required to have 65 observations). We compared all remaining models using Akaike Information Criterion corrected for small sample size (AICc) and AUC and selected the best model as the one that optimized both AICc and AUC. We did this by first examining all models with an AICc within 2.0 of the best overall model (which are considered statistically indistinguishable, ref. [53]) and then selecting the model with highest AUC. We assessed bivariate correlations among predictors using Pearson's correlations between N or S and all other factors and multivariate correlations among predictors using VIFs between N or S and all other main effects in the best model. We interpret our results using a conventional cutoff for VIF of 10.0 (ref. [54]) and a conservative cutoff of 3.0. A VIF of 10.0 and 3.0 mean that one-tenth and one-third of the information, respectively, in the predictor is uncorrelated with other predictors. Given the large number of species assessed, we checked for multiple comparisons using a Holm–Bonferroni adjustment[40].

Critical loads estimation. Critical loads are formally defined as 'quantitative estimates of exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge'[55]. Here we interpret the N deposition value above which the estimated probability of occurrence begins to decline as an estimate of the critical load. We estimated the critical load using the same approach in Simkin et al.[22] by taking the partial derivative of the best statistical model with respect to N and to S deposition and solving for N or S deposition. Using this approach, the critical load can be an expression, where the deposition value associated with the CL depends on other covarying terms (for example, lower under more acidic conditions or when S deposition is already high). See Supplementary Information for further details.

Assessment of floristic quality. We used *C*-scores (1–10) from various Floristic Quality Assessments (FQAs) conducted across the United States. FQAs are plant surveys conducted by professional botanists to determine the quality of the flora in a particular area[35], usually as part of the process of applying for a state or federal permit. *C*-scores are assigned to individual plant species by professional botanists on the basis of their tolerance to human disturbance and the degree to which the species represent natural undisturbed habitats[35]. Non-native species are assigned a score of zero and natives are assigned a score from 1–10, with ten being the highest conservation value. Freyman et al.[56] compiled *C*-scores from 30 inventories across the country representing >100,000 species into an online tool called the Universal Floristic Quality Assessment Calculator (https://universalfqa.org/about). We used this database to assess the *C*-scores for all 348 species analysed in our study, averaging across inventories if the C-score for a species differed across inventories. We consider species with *C*-scores 7–10 and 4–6 to be of 'high' and 'moderate' conservation value, respectively (see Supplementary Information and ref. [56]).

Relating plant traits to critical loads. We ran three analyses to relate plant traits to critical loads. First, using the focal 198 species, we used contingency analyses to relate the shape of the relationship (categorical response; increase, decrease, flat, unimodal for N; increase, decrease or flat for S) separately to six plant functional groups from the USDA PLANTS database (https://plants.usda.gov/): (1) functional group (forb, graminoid), (2) cotyledon status (monocot, dicot, fern), (3) invasive (yes, no), (4) life history (perennial, non-perennial), (5) native status (native, non-native) and (6) whether the species was in the Fabaceae family or not (to capture the potential for N-fixation). Second, we used analysis of variance (ANOVA) to assess whether the average critical load for the focal 198 species differed among the same six plant functional groups. Results are in Supplementary Table 5. The highly imbalanced composition of the different combinations of groups limited our ability to examine combinations of characteristics (for example, introduced grasses). Third, detailed trait information was available for a subset of 98 species for nine traits: LNC, LCC, SLA, VH, LLC, LPC, LCaC, LKC and LMgC. We used trait information from one region (Wisconsin, Don Waller personal communication) rather than from different geographic locations (for example, the TRY database[57]) to limit the degree to which geographic variation in trait values could confound variation among species. We ran all possible linear models relating 16 traits (six plant functional groups above, nine physiological traits and the species *C*-score) as candidate predictors, to the average critical load from each species. We compared models with AICc and explored many different competing model structures. Not all combinations of traits were available for all models, explaining the differences in sample sizes.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets generated during and/or analysed during the current study are available in the EPA Environmental Dataset Gateway repository (https://edg.epa.gov/metadata/catalog/main/home.page) at https://doi.org/10.23719/1500914.

Received: 3 October 2018; Accepted: 9 May 2019;
Published online: 1 July 2019

References

1. Sala, O. E. et al. Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774 (2000).
2. Bobbink, R. et al. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* **20**, 30–59 (2010).
3. Sullivan, T. J. et al. Effects of acidic deposition and soil acidification on sugar maple trees in the Adirondack Mountains, New York. *Environ. Sci. Technol.* **47**, 12687–12694 (2013).
4. Bowman, W. D., Cleveland, C. C., Halada, L., Hresko, J. & Baron, J. S. Negative impact of nitrogen deposition on soil buffering capacity. *Nat. Geosci.* **1**, 767–770 (2008).
5. Throop, H. L. & Lerdau, M. T. Effects of nitrogen deposition on insect herbivory: implications for community and ecosystem processes. *Ecosystems* **7**, 109–133 (2004).
6. Hautier, Y., Niklaus, P. A. & Hector, A. Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**, 636–638 (2009).
7. Clark, C. M. & Tilman, D. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* **451**, 712–715 (2008).
8. Gilliam, F. S. et al. Twenty-five-year response of the herbaceous layer of a temperate hardwood forest to elevated nitrogen deposition. *Ecosphere* **7**, e01250 (2016).
9. Driscoll, C. T. et al. Nitrogen pollution in the northeastern United States: sources, effects, and management options. *Bioscience* **53**, 357–374 (2003).
10. Schaberg, P. G. et al. Effects of chronic N fertilization on foliar membranes, cold tolerance, and carbon storage in montane red spruce. *Can. J. For. Res.* **32**, 1351–1359 (2002).
11. Burns, D. A., Lynch, J. A., Cosby, B. J., Fenn, M. E. & Baron, J. S. *National Acid Precipitation Assessment Program Report to Congress 2011: An Integrated Assessment* (US EPA Clean Air Markets Division, 2011).
12. *Total Deposition 2015 Annual Map Summary* NADP Data Report 2016-02 (National Atmospheric Deposition Program, 2016).
13. Clark, C. M. et al. Atmospheric deposition and exceedances of critical loads from 1800–2025 for the conterminous United States. *Ecol. Appl.* **28**, 978–1002 (2018).
14. Houlton, B. Z. et al. Intentional versus unintentional nitrogen use in the United States: trends, efficiency and implications. *Biogeochemistry* **114**, 11–23 (2013).
15. Li, Y. et al. Increasing importance of deposition of reduced nitrogen in the United States. *Proc. Natl Acad. Sci. USA* **113**, 5874–5879 (2016).
16. Pardo, L. H. et al. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecol. Appl.* **21**, 3049–3082 (2011).
17. Baron, J. S., Driscoll, C. T., Stoddard, J. L. & Richer, E. E. Empirical critical loads of atmospheric nitrogen deposition for nutrient enrichment and acidification of sensitive US lakes. *BioScience* **61**, 602–613 (2011).
18. Ellis, R. A. et al. Present and future nitrogen deposition to national parks in the United States: critical load exceedances. *Atmos. Chem. Phys.* **13**, 9083–9095 (2013).
19. Bobbink, R. & Hettelingh, J.-P. (eds.) *Proc. of an Expert Workshop: Review and revision of empirical critical loads and dose-response relationships* (Netherlands National Institute for Public Health and the Environment, 2011).

20. Payne, R. J., Dise, N. B., Stevens, C. J., Gowing, D. J. & Partners, B. Impact of nitrogen deposition at the species level. *Proc. Natl Acad. Sci. USA* **110**, 984–987 (2013).
21. Wilkins, K., Aherne, J. & Bleasdale, A. Vegetation community change points suggest that critical loads of nutrient nitrogen may be too high. *Atmos. Environ.* **146**, 324–331 (2016).
22. Simkin, S. M. et al. Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. *Proc. Natl Acad. Sci. USA* **113**, 4086–4091 (2016).
23. Vitousek, P. M. & Howarth, R. W. Nitrogen limitation on land and in the sea—how can it occur?. *Biogeochemistry* **13**, 87–115 (1991).
24. Tilman, D. Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos* **58**, 3–15 (1990).
25. Belyazid, S. et al. A dynamic modelling approach for estimating critical loads of nitrogen based on plant community changes under a changing climate. *Environ. Pollut.* **159**, 789–801 (2011).
26. Bobbink, R., M. Ashmore, S. Braun, S., Flückiger, W. and Van den Wyngaert, I. J. J. *Empirical Nitrogen Critical Loads for Natural and Semi-natural Ecosystems: 2002 Update* 40–170 (SAEFL, 2003).
27. Driscoll, C. T. et al. Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. *BioScience* **51**, 180–198 (2001).
28. Garrison, M. T., Moore, J. A., Shaw, T. M. & Mika, P. G. Foliar nutrient and tree growth response of mixed-conifer stands to three fertilization treatments in northeast Oregon and north central Washington. *Ecol. Manag.* **132**, 183–198 (2000).
29. Crowley, K. et al. Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? *Ecosystems* **15**, 940–957 (2012).
30. Goswami, S. et al. Phosphorus limitation of aboveground production in northern hardwood forests. *Ecology* **99**, 438–449 (2018).
31. Kovar, J. L. & Karlen, D. L. In *Proc. 19th World Congress of Soil Science; Soil Solutions for a Changing World* (eds Gilkes, R. & Rakongkep, N.) 122–155 (International Union of Soil Sciences, 2010).
32. Clark, C. M. et al. Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecol. Lett.* **10**, 596–607 (2007).
33. Perring, M. P. et al. Understanding context dependency in the response of forest understorey plant communities to nitrogen deposition. *Environ. Pollut.* **242**, 1787–1799 (2018).
34. Stevens, C. J. et al. Nitrogen deposition threatens species richness of grasslands across Europe. *Environ. Pollut.* **158**, 2940–2945 (2010).
35. Swink, F. & Wilhelm, G. *Plants of the Chicago Region* 4th edn (Indiana Academy of Science, 1994).
36. Suding, K. N. et al. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc. Natl Acad. Sci. USA* **102**, 4387–4392 (2005).
37. Aerts, R. & Chapin III, F. S. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv. Ecol. Res.* **30**, 1–67 (1999).
38. Kirkby, E. A. in *Marschner's Mineral Nutrition of Higher Plants* Foreword v–vi (Academic Press, 2012).
39. Wright, I. J. et al. The worldwide leaf economics spectrum. *Nature* **428**, 821–827 (2004).
40. Holm, S. A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70 1979).
41. Mountford, J. O., Lakhani, K. H. & Kirkham, F. W. Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. *J. Appl. Ecol.* **30**, 321–332 (1993).
42. Bai, Y. et al. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. *Glob. Change Biol.* **16**, 358–372 (2010).
43. Silvertown, J. et al. The Park Grass Experiment 1856-2006: Its contribution to ecology. *J. Ecol.* **94**, 801–814 (2006).
44. Maskell, L. C., Smart, S. M., Bullock, J. M., Thompson, K. & Stevens, C. J. Nitrogen deposition causes widespread loss of species richness in British habitats. *Glob. Change Biol.* **16**, 671–679 (2010).
45. Smart, S. M. et al. Large-scale changes in the abundance of common higher plant species across Britain between 1978, 1990 and 1998 as a consequence of human activity: Tests of hypothesised changes in trait representation. *Biol. Conserv.* **124**, 355–371 (2005).
46. Dupre, C. et al. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Glob. Change Biol.* **16**, 344–357 (2010).
47. De Vries, W. et al. Use of dynamic soil–vegetation models to assess impacts of nitrogen deposition on plant species composition: an overview. *Ecol. Appl.* **20**, 60–79 (2010).
48. *Integrated Science Assessment for Oxides of Nitrogen, Oxides of Sulfur and Particulate Matter: Ecological Criteria* EPA/600/R-16/372 (EPA, 2017).
49. Daly, C. et al. Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. *Int. J. Climatol.* **28**, 2031–2064 (2008).
50. Schwede, D. & Lear, G. A novel hybrid approach for estimating total deposition in the United States. *Atmos. Environ.* **92**, 207–220 (2014).
51. Grossman D. H., et al. *International Classification of Ecological Communities: Terrestrial Vegetation of the United States* The National Vegetation Classification System: Development, Status, and Applications Vol. 1 (The Nature Conservancy, 1998).
52. *National Atmospheric Deposition Program* (NADP, accessed October 2018); http://nadp.sws.uiuc.edu/.
53. Burnham, K. A. D. A. *Model Selection and Multimodel inference* (Springer, 2002).
54. O'brien, R. M. A caution regarding rules of thumb for variance inflation factors. *Qual. Quant.* **41**, 673–690 (2007).
55. Nilsson, J. A. G. P. E. *Critical Loads for Sulfur and Nitrogen* Report 1988:15 (Nordic Council of Ministers, 1988).
56. Freyman, W. A., Masters, L. A. & Packard, S. The Universal Floristic Quality Assessment (FQA) Calculator: an online tool for ecological assessment and monitoring. *Methods Ecol. Evol.* **7**, 380–383 (2016).
57. Kattge, J. et al. TRY—a global database of plant traits. *Glob. Change Biol.* **17**, 2905–2935 (2011).
58. *PLANTS Database* (US Department of Agriculture, accessed 10 June 2018); http://plants.usda.gov
59. Stevens, C. J., Wilson, J. & McAllister, H. A. Biological flora of the British Isles: *Campanula rotundifolia*. *J. Ecol.* **100**, 821–839 (2012).

Acknowledgements

We thank J. T. Smith, S. Leduc and J. Compton for commenting on earlier versions of this manuscript, as well as the NADP Critical Loads of Atmospheric Deposition (CLAD) Working Group that has provided helpful discussions. We also thank B. Cade at the USGS for his statistical advice throughout the project, and R. Payne, K. Wilkins and C. Stevens for sharing their TITAN data for cross comparisons. The original work underpinning this project (Simkin et al.[22]) was supported under the USGS Powell Center Working Group 'Evidence for shifts in plant species diversity along N deposition gradients: a first synthesis for the United States', EPA Contract no. EP-12-H-000491, National Park Service grant no. P13AC00407 and USGS grant no. G14AC00028. Continuation of this research represented here was supported under the EPA Office of Research and Development Safe and Healthy Communities (no. SHC 4.61.4). US-NSF Dimensions of Biodiversity award DEB-1046355 (DMW) supported collecting the functional trait data. J.B. was supported by the USGS Ecosystems and Land Change Science programs. The USGS supports the conclusions of research conducted by their employees and peer reviews and approves all of their products consistent with USGS Fundamental Science Practices. The views expressed in this manuscript are those of the authors and do not necessarily reflect the views or policies of the US EPA or the USDA Forest Service. Mention of trade names or commercial products is for descriptive purposes only and does not constitute endorsement or recommendation for use by the United States Government.

Author contributions

C.M.C., S.M.S., E.B.A., W.D.B., J.B. and M.L.B. designed the research. S.M.S. and D.M.W. collected the data. S.M.S. and C.M.C. analysed the data. C.M.C., S.M.S., E.B.A., W.D.B., J.B., M.L.B., S.L.C., L.H.G., F.S.G., S.E.J., L.H.P., B.K.S., C.J.S., K.N.S., H.L.T. and D.M.W. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41477-019-0442-8.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to C.M.C.

Peer review information: *Nature Plants* thanks Nancy Dise, Han van Dobben and Stuart Weiss for their contribution to the peer review of this work.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019

nature research

Corresponding author(s): Christopher M. Clark

Last updated by author(s): Jan 18, 2019

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No primary data was collected in this effort. We used existing datasets from many sources described in this publication and in Simkin et al. PNAS (2016). We accessed this data in tabular forms using Microsoft Excel, Microsoft Access, and R.
Data analysis	R 3.5.1 and JMP version 10.0.0 were used in data analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Upon acceptance for publication, this data will be made available through the Environmental Protection Agency's (EPA's) Environmental Data Gateway (https:/edg.epa.gov/). It is EPA policy to not make the data publicly available until acceptance.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This is a study of the effects of nitrogen and sulfur deposition on individual herbaceous plant species across the U.S., using an existing plot dataset compiled from many different sources and totaling ~14,000 plots non-randomly distributed across the contiguous U.S. There is no network of random plots across the country for herb species in the U.S., so this is the best option available for such an assessment in the U.S.
Research sample	A research sample is a plot. Inside each plot researchers assessed the relative abundance of species. Relative abundance was converted to whether a species was present (1) or not (0), and thus the sample is the whether the species occurred or not in a given plot. Plots were restricted to those sampled after 1989 to align with our deposition data, and which were between 100 m^2 and 700 m^2, to remove species-area effects (as was done in Simkin et al. 2016). As mentioned above, these plots are not a random sample, they are a compilation of available datasets across the U.S. There is no network of random plots across the country for herb species in the U.S., so this is the best option available for such an assessment in the U.S. That being said, it is a large sample (~14,000 plots) and so we assume that the plots are representative of herbaceous plant communities across the U.S. with the appropriate caveats mentioned in the text. There are 14 individual datasets, that are extensively described in the original publication (i.e. Simkin et al. PNAS 2016). This is a re-analysis of that dataset - the original publication focused on species richness, this manuscript focuses on individual species.
Sampling strategy	This is the largest available dataset for the U.S. and is the result of an extensive effort described in Simkin et al. (2016), where the PIs sent an email to ECOLOG, and contacted individual PIs at various universities, state agencies, and other organizations, over a period of two years, to compile all available data on herb species in the U.S.
Data collection	No data were collected in this effort. Source data collection varied across the 14 datasets, as described in Simkin et al. (2016), but always comprised of field crews that went into the field and estimated the relative abundance of species in a plot using either visual estimation with reference cards, or some other locally determined means.
Timing and spatial scale	Plot data were collected from 1990-2013. We excluded plots that were outside of the 100-700 m^2 range to remove the effect of area on our assessment.
Data exclusions	We excluded plots that were smaller than 100 m^2 and larger than 700 m^2 to remove the effect of area, and excluded plots sampled before 1990 to better align with the deposition and climate data. We also excluded plots where plants were not identified to species.
Reproducibility	There was no experiment conducted, so no reproducibility was assessed. This is a gradient assessment, where the probability of occurrence for a species is compared across a deposition gradient.
Randomization	Not relevant as described above. This is a sample of all known data, not a random sample because no random sample exists for the U.S. and we wanted to sample as much of the country as was possible. Nonetheless, we included six covariates (soil pH (pH), mean annual temperature (T), mean annual precipitation (P), pH^2, T^2, and P^2) in the analysis, and assessed bivariate correlations among all predictors and deposition (N and S), and multivariate correlations using Variance Inflation Factors.
Blinding	Not relevant.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Unknown, there were no field collections directly conducted, we are using existing datasets. I'm not sure whether to answer the field work question as "Yes" or "No." There was no primary collection of data, but we used datasets that were developed through field work. These answers are assuming "Yes" for completeness.
Location	Latitude and longitude of all ~14,000 plots will be available at the EPA's EDG site following acceptance. These are also already available in the data dryad from the original publication (Simkin et al. 2016).
Access and import/export	NA
Disturbance	NA

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging