Title: Potential vulnerability of 348 herbaceous species to atmospheric deposition of nitrogen and sulfur in the U.S.

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Atmospheric nitrogen (N) and sulfur (S) pollution increased over much of the U.S. during the 20th century from fossil fuel combustion and industrial agriculture [1-4]. Despite recent declines [5,6], N and S deposition continue to affect many plant communities in the U.S. [7], although which individual species are at risk remains uncertain. We used species composition data from >14,000 survey sites across the contiguous U.S. to evaluate the association between N and S 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 N or S deposition somewhere in the contiguous U.S. (56% for N, 51% for S). Fifteen percent and 51% of species potentially decreased at all N and S deposition rates, respectively, suggesting thresholds below the minimum deposition they receive. Although more species potentially increase than decrease with N deposition, increasers tend to be introduced, and decreasers tend to be higher-value native species. More vulnerable species tend to be shorter with lower tissue N and Mg. These relationships constituted 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 U.S. and globally.

Atmospheric deposition of nitrogen (N) and sulfur (S) are two key drivers of plant biodiversity decline worldwide after habitat loss and climate change [8]. N deposition reduces biodiversity through several mechanisms [1], including soil acidification and subsequent foliar nutrient imbalances [9,10], increased pest pressures on nutrient-enriched foliage [11], and stimulating growth of opportunistic species allowing them to outcompete local neighbors through light limitation or other processes [12-14]. Sulfur deposition primarily reduces diversity by acidifying soils, again leading to base cation imbalances, as well as frost sensitivity and inhibition of germination [9,15,16].

In the U.S., levels of N and S deposition have declined after decades of successful air quality policies enacted under the Clean Air Act [4,5,17]. These amendments have reduced total N and S deposition in the eastern U.S. by an average of 23.7% and 56.9%, respectively, between 2000-2002 and 2013-2015 [5]. Nevertheless, N and S deposition both remain 5-10 times above pre-industrial levels (i.e., 0.4 kg N ha^{-1} yr^{-1}, 0.1 kg S ha^{-1} yr^{-1}, [17]) across most of the country, and N deposition trends are flat or increasing in many areas outside of the eastern U.S. [5,18]. Furthermore, the composition of N deposition is shifting from regulated forms (i.e., oxidized NO_x) to largely unregulated forms (i.e., reduced NH_x, except as a portion of particulate matter which is regulated) [5,19].

Current levels of both N and S deposition remain elevated above many known thresholds (termed "critical loads") for detrimental ecological effects [17,20-22], and likely will remain so in the near future [17,22]. To date, most critical loads have been developed for ecosystems or ecoregions rather than species (2, 16), although species-level estimates are beginning to emerge in Europe (20, 21). Simkin et al. (2016) compiled a database of herbaceous plant species composition across 15,136 plots in the contiguous U.S. [7]. 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 [7]. However, it was not reported which among the roughly 4000 species in that dataset are potentially vulnerable, where they occur, their conservation value, and whether any traits may be associated with sensitivity versus insensitivity.

Many of these species are too rare to confidently assess, but for those that remain we fill these critical knowledge gaps with a comprehensive analysis of the Simkin et al. (2016) database.

We found that 348 species had sufficient data to analyze according to our criteria. Of these, 70% (243 species) decreased with increasing N or S along some portion of the deposition gradient. For some of these species, however, even the best models don't explain much variation in the probability of occurrence (i.e., AUC < 0.7 or $R^2 < 0.1$) because species distributions are a complex function of many factors including but not exclusive to those evaluated here (e.g., historical land use, disturbance, ozone, grazing pressures, etc.). Thus, we focused on a subset of 198 species that we considered had "robust relationships" with the predictor variables included (i.e., AUC ≥ 0.7, $R^2 \geq 0.1$, and monotonically increasing, decreasing, or unimodal relationships with N; Table 1, S1, Figure S1). Results for all 348 species are in Table S2. Of these 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) (Figure 1a-f). The steepness of these relationships, and the N deposition associated with the highest species occurrence, also varied widely among species (Figure 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 (Figure 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 U.S. 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 [7,25] and modeling [26] studies, which show that low levels of N input acts to relieve nutrient limitation and enhance growth for many species [7,23]. 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 neighbors [12]. The average N-response was for a negative association around 10 kg N ha^{-1} yr^{-1} (Figure 1f), a common critical load from community-level research [25,27]. S deposition acidifies soils, explaining the large number of species that had negative associations with S [28]. The few species with positive associations with S deposition we hypothesize are acid tolerant species that benefitted from the loss of competitors, rather than evidence of a fertilization effect from S. S-limitation can occur, but such cases are rare in natural communities [23,29]. There is more evidence that a shift towards P-limitation may occur with high N deposition [30,31]. In agricultural settings, S-limitation can occur but only when N and P are abundant [32], which is likely not the case for our plots.

We then calculated N and S critical loads using partial derivatives of the best statistical model for each species (cf. Simkin et al. 2016 – Supplemental Table S2, SI). 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*) (Figure 2a). The intervals in Figure 2a represent spatial variation in the CL – not error associated with the mean. Such variation reflects how species can have lower or higher CLs in a particular location based on covarying factors (e.g., lower CLs in more acidic conditions). This has been reported elsewhere for habitats in Ireland [33], where the CL 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 strongly on its environmental context [34,35]. This is more realistic ecologically – for example, adding 2 kg of N to a strongly 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 like we did, which retains relationships with relevant covariates, allowing one to refine estimates of the critical load using local edaphic or climatic factors (Table S2; SI, eqn 1-4).

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 (Figure 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 SI), and more complex mathematical relationships (e.g., 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 ($Chi^2 = 28.04$, $P < 0.001$; Table S3). Short deposition gradients may be especially problematic analytically for species that only occur in the western U.S.

Many species-level critical loads reported here and elsewhere are below community-level critical loads (e.g., ~8-20 kg N ha^{-1} yr^{-1}, [1,7,33,36]). 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 (roughly 8 – 22 kg N ha^{-1}

yr^{-1}, [36]), but comparable to those from Ireland (roughly 2.8 – 19 kg N ha^{-1} yr^{-1}, [33]). This may be explained because most of the plots from the acid grassland study were from Great Britain and mainland Europe [37] where deposition rates are higher (8-35 kg N ha^{-1} yr^{-1}), as opposed to the U.S. and Ireland where N deposition was lower (2-20 kg N ha^{-1} yr^{-1}). The Irish study also found critical loads of a species could vary widely among different habitats, and bootstrapped intervals within a habitat were also often 2-6 kg N ha^{-1} yr^{-1} wide [33]. We compared our results with critical loads for 304 European species (24 from acid grasslands in [36] and 280 across many habitats in Ireland in [33]). There were only eight species in common between our study and those (Table S4, Figure S2) and only one that was present across all three (*Campanula rotundifolia*, Figure 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 5^{th}-95^{th} interval) compared well with estimates from Ireland (two habitats: 6.2 and 8.2 kg N ha^{-1} yr^{-1}), and all three estimates were lower than from European acid grasslands (13 kg N ha^{-1} yr^{-1}). The correspondence between our estimates and those from Ireland is encouraging since the methods were completely independent (i.e., 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.

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 species potentially at risk highly valued natives or are they common or non-native species? We used results compiled from many plant surveys across the U.S. based on "coefficients of conservatism" (C values: 0-10) assigned to individual plant species (C_i) based on their tolerance to human disturbance and the degree to which the species represent natural undisturbed habitats [38]. Higher

C-scores are associated with higher quality flora and habitats, with non-natives receiving score of zero. Natives range from 1-10 based on 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 (i.e., $C_i \geq 7$, $4 \leq C_i \leq 6$, respectively). There was a negative correlation between C-scores and the species average N critical loads ($r = -0.260$, $P = 0.001$), 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 moderate-to-high conservation value. These include *Muhlenbergia cuspidata*, *Lysimachia quadriflora*, and *Prosartes lanuginose*, all highly valued native species (average $C \geq 7.8$) of North America.

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 km x 12 km grid cell. Overall, more species were positively than negatively associated with N deposition. But, most eastern areas had significant fractions of decreasers (>15%, Figure 4a and 4c). Out of the 3,122 grid cells containing one or more of the focal 198 species, 75.8% had an exceedance for one or more species, and 24.3% had an exceedance of 50% or more unique species in the grid cell. 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 deposition areas, suggesting that fine scale processes affect local risk (e.g., differences in species composition, historical land use, the degree of nutrient limitation, and other stressors such as ozone that were not included [7,34]). Lower fractions of species at risk were estimated in the west, likely partly due to shorter N deposition gradients that did not make our threshold for assessment (see SI).

Hotspots of decreasers with S deposition occurred throughout the U.S., even in relatively low deposition areas in the west (Figure 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 generally harmed by S deposition. Another plausible explanation is S deposition was not allowed to have complex nonlinear patterns (e.g., 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 U.S. (see Figure 3), it is not possible to say how this translates to vulnerability across the entire range of each species.

Finally, we determined if simple predictive relationships existed between species traits and their potential sensitivity to N deposition. Such a relationship 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 were generally poor predictors (all $R^2<0.02$) of either the shape of the response or the CL (Table S5), although natives tended to have more negative relationships ($P=0.036$) and lower CLs ($P=0.028$) than introduced species, perennial species tended to have lower CLs 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 [39,40]. Although not inconsistent with ecological theory, these relationships were notably weak (e.g. not all natives decreased with N deposition and invasives increased), reinforcing the notion that these broad groups may be less helpful than we'd like in describing ecological responses. We found physiological traits were much more predictive of the critical load, and led to several predictive equations:

(1) $CL(N) = 6.20 + 7.32*LMgC + 0.06*VH$; $AdjR^2 = 0.36$; $P < 0.001$; $N=37$

(2): $CL(N) = 5.03 + 2.63*LNC$; $AdjR^2 = 0.22$; $P<0.001$; $N = 55$

(3): $CL(N) = 4.28 + 2.51*LNC + CS_i$; $AdjR^2 = 0.32$; $P < 0.001$; $N=55$,

The best *overall* model (equation 1) predicted the N critical load was a two-factor model with leaf magnesium content (LMgC, $P<0.001$) and vegetative height (VH, $P=0.06$). Leaf Mg is strongly associated with photosynthetic rates [41], while vegetative height influences access to light. Thus, species that were more potentially vulnerable had lower photosynthetic rates and were shorter-statured as reported in many other site-specific studies [13,20,24,39]. Leaf magnesium, however, is not commonly available for most species, and photosynthetic rates are also correlated with leaf N [42] (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 based on more widely available traits (i.e., 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 CLs 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 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 [13,39].

It is difficult to confidently assign causality to deposition in a gradient study such as ours [7,37]. 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 SI). Lower VIFs mean less of a change 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 (Table S6). Comparing the results for the 22 species with low multicollinearity (i.e., 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% vs. 15% for decreasers, 50% versus 54% for unimodal, Table S6). The same was true for species with decreasing relationships with S (Table S6). '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 (Table S6). Thus, results are likely robust for species that decrease with N or S and for species with unimodal N-relationships, but results for species that increase with S or show no change with N may be interpreted cautiously. Given the large numbers of species tested, we also tested our results for possible Type I errors using a Holm Bonferroni multiple comparisons adjustment [43], and found that 66% of species relationships with N remained significant after such an adjustment (see SI). Given decades of research documenting that climate, soil pH, and atmospheric deposition affect plant communities (2, 33, 50), we assume relationships that lost significance after adjustment are likely still ecologically valid.

Thus, even though a correlative study such as ours cannot confidently assign causality, the confluence of findings from controlled experimental manipulations [13,44-46], gradient studies such as ours [33,37,47], communities tracked through time as deposition changes [48,49], and dynamic modeling [26,50], all suggest that N and S deposition 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 U.S., strongly indicating widespread vulnerability to N and/or S deposition, and that species respond differently based on local environmental context. The wide range of thresholds even within a species strongly suggests that potential vulnerability is linked to local edaphic factors and atmospheric co-pollutants. This work can help inform the review of the U.S. Environmental Protection Agency's secondary standards for oxides of nitrogen, oxides of sulfur, and particulate matter[51] to identify species and regions of particular concern from these stressors to natural ecosystems.

Methods

Data assembly and species filtering

Simkin et al. (2016) compiled data from a variety of sources to develop a consolidated dataset that included plot level information for species composition (percent 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 [52], soil pH was from a combination of locally assessed empirical measurements and SSURGO [7], and N deposition was calculated as the sum of the 1985-2011 mean annual wet deposition interpolated from NADP plus 2002-2011 CMAQ modeled mean annual dry deposition [7]. Updated deposition estimates from the Total Deposition project (TDEP[53]) were not available at the time of Simkin et al. (2016), but Simkin et al. (2016) reported good correspondence between our estimate and TDEP (i.e., r2 = 0.89, TDEP(2000-2012) = SimkinNdep(1985-2011)*0.91 + 0.3, [7,53]). Total S deposition was calculated in the same manner as N deposition.

To filter plots and species to a subset to analyze, we restricted plots to those that were 100-700m^2 as was done in Simkin et al. (2016) 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 5 records overall, and sparse species that did not have at least 5 records or comprise 5% of records in at least one Alliance using the National Vegetation Classification system [54]. 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 15223 and species to 1027. 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 14041 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 inter annual variation in N deposition (often 2-3 kg ha^{-1} yr^{-1}, [55]) by roughly double to detect a spatial trend. See SI for more details.

Species analysis

We performed binomial generalized linear models (GLMs) 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, and Sdep*pH, and Ndep*Sdep. Rationale for individual terms is described in the SI. To prevent model overfitting, we required there to be at least 5 detections per model term (e.g., 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 AICc (Akaike Information Criterion) and AUC (Area Under ROC Curve) 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, [56]), 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 Variance Inflation Factors (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 [57] and a conservative cutoff of 3.0. A VIF of 10.0 and 3.0 mean that 1/10th and 1/3rd 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 [43].

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" [58]. Here we interpret the N deposition value above which the probability of occurrence potentially declines as an estimate of the critical load. We estimated the critical load using the same approach in Simkin et al. (2016), 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 depends on other covarying terms (e.g., lower under more acidic conditions or when S deposition is already high). See SI for further details.

Assessment of floristic quality

We used "coefficients of conservatism" (C-scores: 1-10) from various Floristic Quality Assessments (FQAs) conducted across the U.S. FQAs are plant surveys conducted by professional botanists to determine the quality of the flora in a particular area [38], 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 based on their tolerance to human disturbance and the degree to which the species represent natural undisturbed habitats [38]. Non-native species are assigned a score of zero, and natives are assigned a score from 1-10, with 10 being the highest conservation value. Freyman et al. (2016) compiled C-scores from 30 inventories across the country representing >100,000 species into an online tool called the Universal Floristic Quality Assessment (FQA) Calculator (https://universalfqa.org/about). We used this database to assess the C-scores for all 348 species analyzed in our study, averaging across inventories if the C-score for a species differed across inventories. We consider species with C-scores from 7-10 and 4-6 to be of “high” and “moderate” conservation value, respectively (see SI for more information and [59]).

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 (i.e., increase, decrease, flat, unimodal for N; increase, decrease, or flat for S) to six plant functional groups from the USDA PLANTS database (https://plants.usda.gov/): (1) functional group (2 levels: forb, graminoid), (2) taxonomic group (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 (i.e., to capture the potential for N-fixation). Second, we used ANOVA to assess whether the average CL for the focal 198 species differed among the same six plant functional groups above. Results are in Table S5. The highly imbalanced composition of the different subgroups limited our ability to examine combinations of characteristics (e.g., introduced grasses). Third, detailed trait information was available for a subset of 98 species 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 used trait information from one region (Wisconsin, Don Waller *pers comm*) rather than from different geographic locations (e.g., the TRY database, [60]) 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 (i.e., 6 plant functional groups above, 9 physiological traits, and the species C-score) as candidate predictors, to the average CL 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.

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Author contributions

C.M.C., S.M.S., E.B.A., W.D.B., J.B., and M.L.B. designed research; S.M.S. collected the data; S.M.S. and C.M.C. analyzed data; and 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.

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Figure Legends

Figure 1: Species response curves for nitrogen (177 species, a-f) and sulfur (166 species, g-i). 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 based on the N deposition where probability of occurrence was highest to improve readability (b: peak at 3.1-10 kg N/ha/yr, 39 species; c: peak at 10.1-12 kg N/ha/yr, 32 species; d: peak at 12.1-19 kg N/ha/yr, 36 species). 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 gray). 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.

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

Figure 3: Detailed example of species response. GLM results for *Campanula rotundifolia* (common name: harebell). Shown above are the marginal probabilities of occurrence

individually by term from the best model for N deposition (a), N x pH (b), N x S (c), S deposition (d), S x pH (e), soil pH (f), precipitation (g), and temperature (h). All terms P<0.01 (Table S4). Black lines in main effect plots are average response and red lines are 95^{th} CI. For interaction terms (b, c, e) the effect of the modifying term is shown as separate quartile lines (Q1-Q4). The best model is shown below the species name. Also shown is a photo of the species (i), a range map from the USDA (j, [61]), and a plot map from this study (k). *C. rotundafolia* is a northern latitude wildflower that grows in drier, low nutrient soils.[62] This species had a hump shaped relationship with N (average CL = 7.9 kg N ha^{-1} yr^{-1}; 10^{th}-90^{th} CL = 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 x S interaction, where the eutrophication effect was stronger (i.e., higher peak and lower N CL) if S deposition was low. The 10^{th}-90^{th} interval reported here is similar to that reported for *C. rotundafolia* in Ireland [33] and lower than that found in acid grasslands across Europe [36]. See Figure S1 for results for all 198 species.

Figure 4: Geographic variation in sensitivity to N and S deposition. Shown are the percent 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 within a 12 x 12 km grid cell and unique species were only counted once if they were potentially vulnerable anywhere in the cell. Note the color ramps are flipped between decreasers and increasers, with hotter colors denoting negative effects (i.e., more decreasers and fewer increasers, most species assessed were native).

Table 1: Summary of responses and vulnerability to N and S deposition. Shown are the number of species out of the 198 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. Shadings represent qualitative levels of vulnerability: high (red - decrease with both), moderate (orange - decrease with one and unaffected by the other), conditional (yellow - either contrasting relationships or conditional on the rate of deposition), or neutral (grey - no relationship with either). Species that partially benefit (light green - increase with one and unaffected by the other), or strongly benefit (dark green, increase with both) are also indicated. Species with “U-shaped” N relationships (45 species) are omitted as not ecologically realistic, and species names in each category are in Supplemental Table S1 and S2.

		S relationship			
		Decrease	**None**	**Increase**	**Total**
N relationship	**Decrease**	11 (6%)	5 (3%)	14 (7%)	*30 (15%)*
	None	5 (3%)	15 (8%)	1 (1%)	*21 (11%)*
	Increase	26 (13%)	6 (3%)	8 (4%)	*40 (20%)*
	Unimodal	81 (41%)	6 (3%)	20 (10%)	*107 (54%)*
	Total	*123 (62%)*	*32 (16%)*	*43 (22%)*	*198 (100%)*

Figure 1

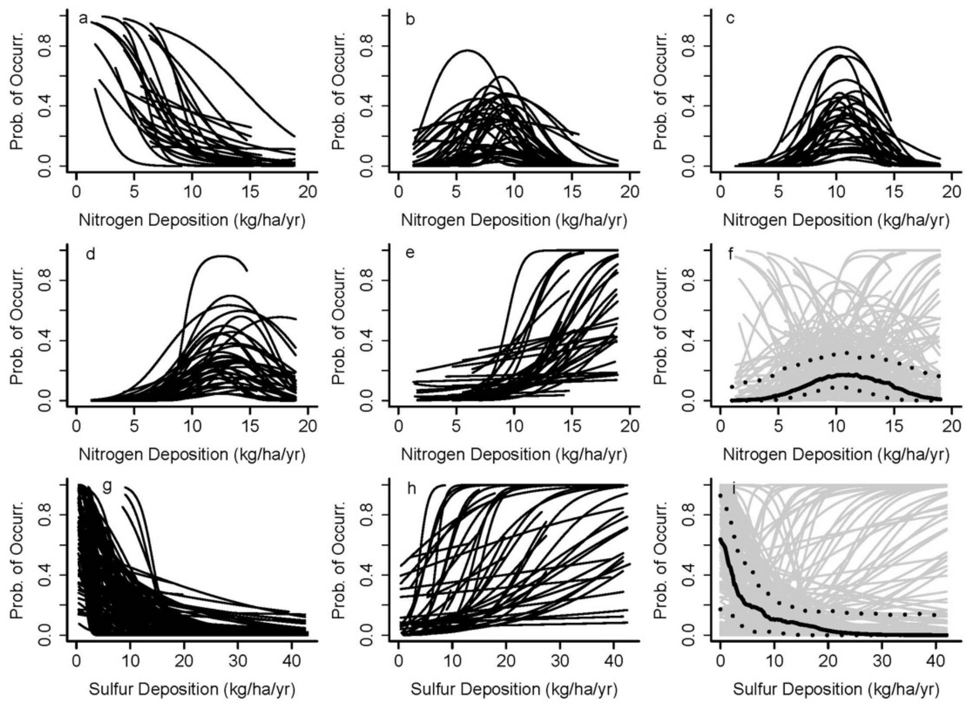

a
b
c
d
e
f
g
h
i
Prob. of Occurr.
Nitrogen Deposition (kg/ha/yr)
Sulfur Deposition (kg/ha/yr)

Figure 2

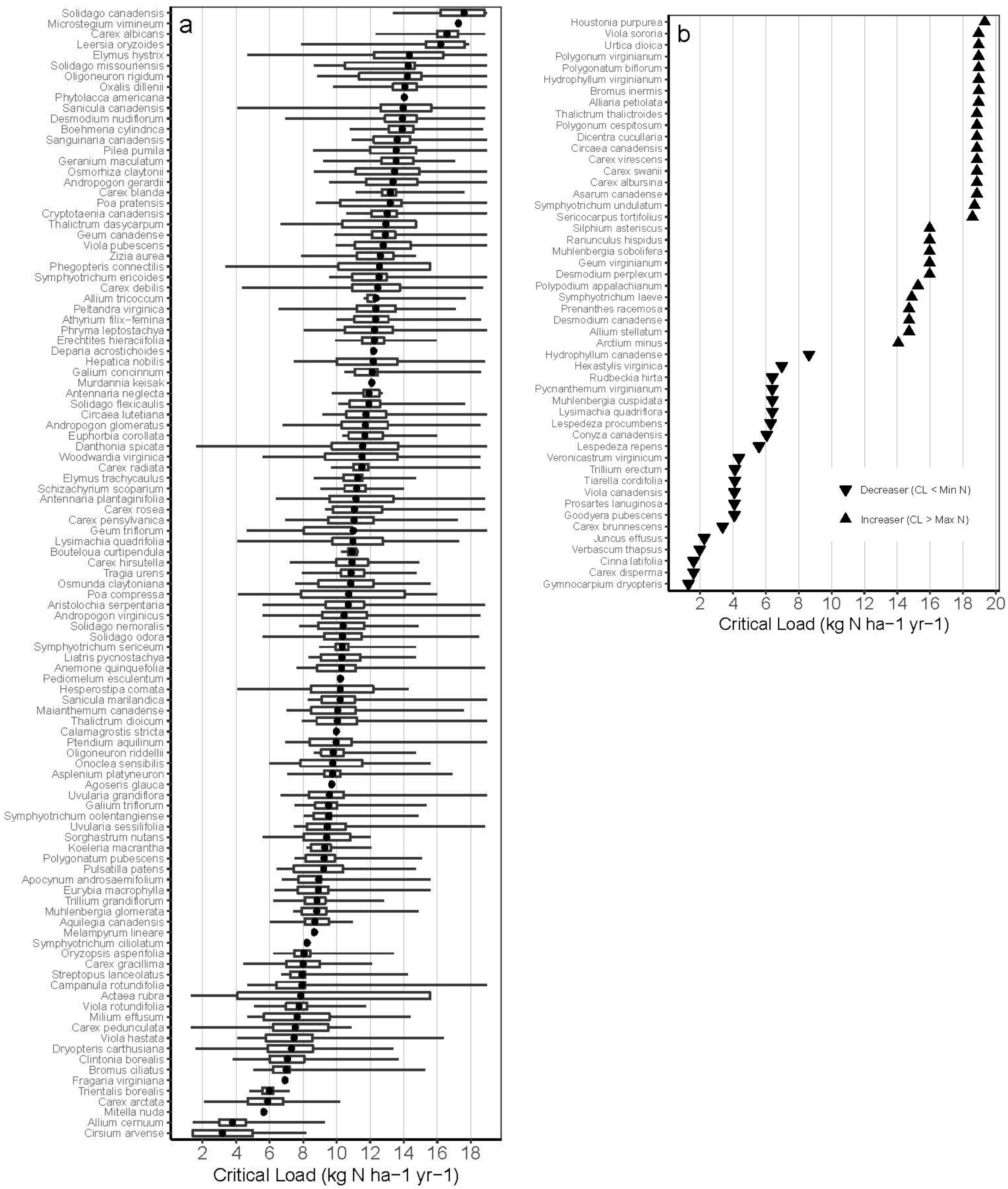
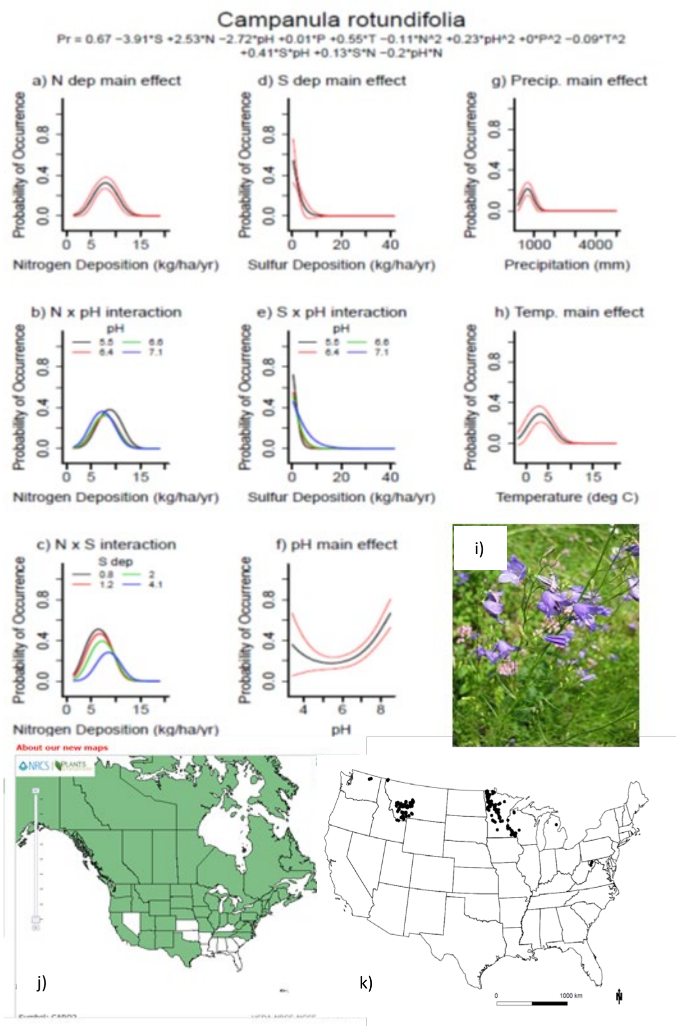
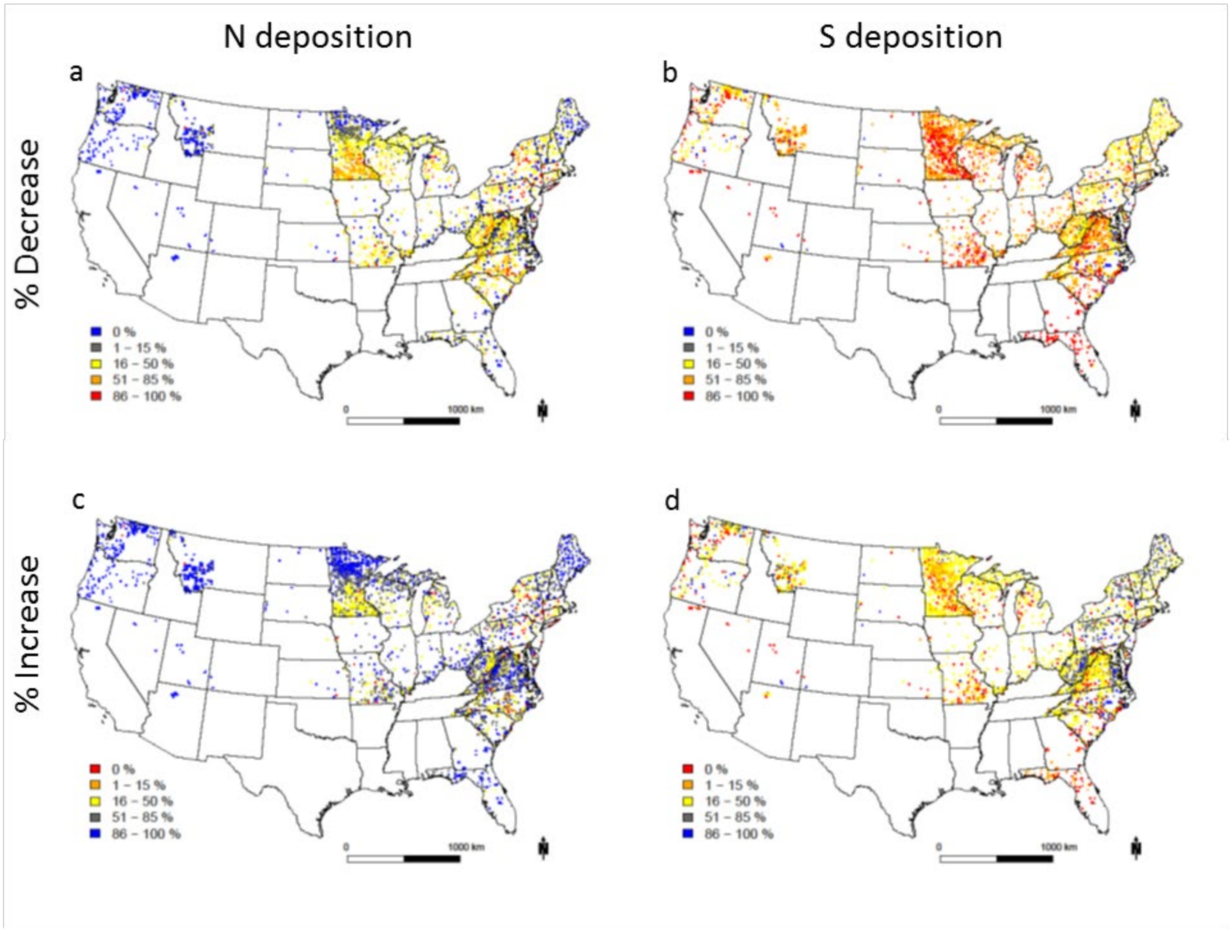

a
Solidago canadensis
Microstegium vimineum
Carex albicans
Leersia oryzoides
Elymus hystrix
Solidago missouriensis
Oligoneuron rigidum
Oxalis dillenii
Phytolacca americana
Sanicula canadensis
Desmodium nudiflorum
Boehmeria cylindrica
Sanguinaria canadensis
Pilea pumila
Geranium maculatum
Osmorhiza claytonii
Andropogon gerardii
Carex blanda
Poa pratensis
Cryptotaenia canadensis
Thalictrum dasycarpum
Geum canadense
Viola pubescens
Zizia aurea
Phegopteris connectilis
Symphyotrichum ericoides
Carex debilis
Allium tricoccum
Peltandra virginica
Athyrium filix−femina
Phryma leptostachya
Erechtites hieraciifolia
Deparia acrostichoides
Hepatica nobilis
Galium concinnum
Murdannia keisak
Antennaria neglecta
Solidago flexicaulis
Circaea lutetiana
Andropogon glomeratus
Euphorbia corollata
Danthonia spicata
Woodwardia virginica
Carex radiata
Elymus trachycaulus
Schizachyrium scoparium
Antennaria plantaginifolia
Carex rosea
Carex pensylvanica
Geum triflorum
Lysimachia quadrifolia
Bouteloua curtipendula
Carex hirsutella
Tragia urens
Osmunda claytoniana
Poa compressa
Aristolochia serpentaria
Andropogon virginicus
Solidago nemoralis
Solidago odora
Symphyotrichum sericeum
Liatris pycnostachya
Anemone quinquefolia
Pediomelum esculentum
Hesperostipa comata
Sanicula marilandica
Maianthemum canadense
Thalictrum dioicum
Calamagrostis stricta
Pteridium aquilinum
Oligoneuron riddellii
Onoclea sensibilis
Asplenium platyneuron
Agoseris glauca
Uvularia grandiflora
Galium triflorum
Symphyotrichum oolentangiense
Uvularia sessilifolia
Sorghastrum nutans
Koeleria macrantha
Polygonatum pubescens
Pulsatilla patens
Apocynum androsaemifolium
Eurybia macrophylla
Trillium grandiflorum
Muhlenbergia glomerata
Aquilegia canadensis
Melampyrum lineare
Symphyotrichum ciliolatum
Oryzopsis asperifolia
Carex gracillima
Streptopus lanceolatus
Campanula rotundifolia
Actaea rubra
Viola rotundifolia
Milium effusum
Carex pedunculata
Viola hastata
Dryopteris carthusiana
Clintonia borealis
Bromus ciliatus
Fragaria virginiana
Trientalis borealis
Carex arctata
Mitella nuda
Allium cernuum
Cirsium arvense
2 4 6 8 10 12 14 16 18
Critical Load (kg N ha−1 yr−1)
b
Houstonia purpurea
Viola sororia
Urtica dioica
Polygonum virginianum
Polygonatum biflorum
Hydrophyllum virginianum
Bromus inermis
Alliaria petiolata
Thalictrum thalictroides
Polygonum cespitosum
Dicentra cucullaria
Circaea canadensis
Carex virescens
Carex swanii
Carex albursina
Asarum canadense
Symphyotrichum undulatum
Sericocarpus tortifolius
Silphium asteriscus
Ranunculus hispidus
Muhlenbergia sobolifera
Geum virginianum
Desmodium perplexum
Polypodium appalachianum
Symphyotrichum laeve
Prenanthes racemosa
Desmodium canadense
Allium stellatum
Arctium minus
Hydrophyllum canadense
Hexastylis virginica
Rudbeckia hirta
Pycnanthemum virginianum
Muhlenbergia cuspidata
Lysimachia quadriflora
Lespedeza procumbens
Conyza canadensis
Lespedeza repens
Veronicastrum virginicum
Trillium erectum
Tiarella cordifolia
Viola canadensis
Prosartes lanuginosa
Goodyera pubescens
Carex brunnescens
Juncus effusus
Verbascum thapsus
Cinna latifolia
Carex disperma
Gymnocarpium dryopteris
Decreaser (CL < Min N)
Increaser (CL > Max N)
2 4 6 8 10 12 14 16 18 20
Critical Load (kg N ha−1 yr−1)

Figure 3



Campanula rotundifolia
Pr = 0.67 −3.91*S +2.53*N −2.72*pH +0.01*P +0.55*T −0.11*N^2 +0.23*pH^2 +0*P^2 −0.09*T^2
+0.41*S*pH +0.13*S*N −0.2*pH*N
a) N dep main effect
d) S dep main effect
g) Precip. main effect
b) N x pH interaction
e) S x pH interaction
h) Temp. main effect
c) N x S interaction
f) pH main effect
Probability of Occurrence
Nitrogen Deposition (kg/ha/yr)
Sulfur Deposition (kg/ha/yr)
Precipitation (mm)
Temperature (deg C)
pH
S dep
i)
j)
k)
About our new maps
1000 km

Figure 4


N deposition
S deposition
a
b
c
d
% Decrease
% Increase
0 %
1 – 15 %
16 – 50 %
51 – 85 %
86 – 100 %
0
1000 km
N

Supplemental Information

Contents:

Supplemental Methods:

Data assembly. We compiled data from a variety of sources to develop a consolidated dataset that included plot level information for species composition, temperature, precipitation, soil pH, and N and S deposition. Vegetation data came from multiple sources and used the same initial site filters as Simkin et al. (2016). Using the same sources and approach as Simkin et al. (2016), we overlaid the coordinates of each vegetation sampling site onto our modeled deposition and climate raster data to extract total N deposition (wet plus dry), S deposition (wet plus dry), mean annual precipitation, mean annual temperature, and soil pH. Total N deposition was calculated as the sum of the 1985-2011 mean annual wet deposition interpolated from NADP plus 2002-2011 CMAQ modeled mean annual dry deposition [7]. The mismatch in years between wet and dry N deposition was because of a desire to capture the long-term deposition experienced at a site (which includes the 1980s and 1990s), and the fact that earlier years for dry deposition nationally from CMAQ are not available. Comparisons with other years (e.g., most recent 5 years) and data sources (e.g., TDEP), was conducted in the original Simkin et al. (2016) effort and compared well (see SI in Simkin et al. 2016). Simkin et al. (2016) reported our estimated total N deposition correlated well with TDEP (i.e., r2 = 0.89, TDEP(2000-2012) = SimkinNdep(1985-2011)*0.91 + 0.3, [7,53]). Total S deposition was calculated in the same manner as N deposition. Mean annual temperature and precipitation were extracted from the 1981-2010 PRISM climate normals [52]. Soil pH was measured on-site or extracted from SSURGO [63] if local field samples were not available. For further details see Simkin et al. (2016). To determine the potential range of a species, we calculated the number of detections and non-detections of each species in each community alliance following the National Vegetation Classification System [54]. Because many species are not nationally distributed, including the entire database would have included absences beyond the known range of the species. Thus, alliances where a given species was almost always absent (see detailed criteria below) were excluded for that species.

Data processing. The 16,395 unique sites and 4,730 "species" in the initial dataset, were filtered to identify a robust subset of species for further analyses. First, as in Simkin et al. (2016) we restricted plots to those that were 100-700m^2 to reduce effects of species-area relationships, reducing the number of plots to 15,980 and species to 4,334. Of particular note, many of the California sites were excluded from this analysis because of the smaller plot sizes (15-68 m^2) that introduced complications with plot area. Second, we removed species that were only identified to genus or classified in broad categories (e.g., "Forb"), reducing the number of plots to 15,946 and species to 3,945. Some of these were included in Simkin et al. (2016) because that effort focused on total species richness, and if there was an additional species that was only identifiable by functional group, that would be included in Simkin et al. (2016) but excluded here. Third, because rare species have low sample sizes, the modeled response to climate or deposition is less likely to be reliable, so these were excluded from the analysis. We excluded rare species using two methods, by removing (a) species with fewer than 5 records overall, and (b) species that did not have at least 5 records or comprise 5% of records in at least one Alliance. The Alliance condition is present because with presence/absence analyses such as this one where most species are not nationally distributed, we needed to identify the "core community" from which to draw the absences. This third filtering step reduced the number of plots to 15,223 and species to 1,027. Most of the reduction was from very rare or sparse species, with 1,781 species having fewer than 5 observations, and the remaining not having enough observations in any Alliance to assign a core community. We then required that each species span an N deposition gradient of at least 7 kg N ha^{-1} yr^{-1} to increase the chances of detecting a response, reducing the number of plots to 14,041 and species to 348 (Figure S3). The choice of using a 7 kg N ha^{-1} yr^{-1} gradient was arbitrary, but balanced conflicting objectives to focus on species where we were likely to see a pattern (encouraging a long gradient), but that included a large number of species (encouraging a short gradient). Given that inter annual variation in N deposition can be 2-3 kg N ha^{-1} yr^{-1} [55] we felt that the gradient should at least double what is experienced across years at any given site (i.e., 4-6 kg N ha^{-1} yr^{-1}). Shorter gradients may be sufficient in less polluted sites in the west, but we wanted to apply a common approach throughout the contiguous U.S. We did not consider the range of S deposition in the filtering process; however, N and S deposition are often correlated and so a similar span for S deposition resulted (range $\geq$ 5.9 kg S ha^{-1} yr^{-1} for all species). We recognize that there are many insights that remain to be found with the rarer and sparsely distributed species, and/or examining shorter deposition gradients, but feel that beginning with a species set with robust data is the appropriate place to start.

Data Analysis. We performed binomial generalized linear models (GLMs) separately for each species on presences and absences from the set of Alliances that were considered its core community (described above). GLMs extend the linear regression framework to variables that are not normally distributed; GLMs are commonly used to model binary data, such as presence absence data. 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, and Sdep*pH, and Ndep*Sdep. Quadratic terms (same as "unimodal") for Ndep, P, T, and pH were included to allow for positive and negative effects for Ndep, and to capture the possibility for species to have an "optimum" precipitation, temperature, and soil pH for their presence. The three interactions were selected to allow for the Ndep and Sdep effects to vary with pH (Ndep*pH, Sdep*pH) and for Ndep and Sdep effects to depend on the other (Ndep*Sdep).

Additional terms in a full second-order model were considered (e.g., Ndep*T, Ndep*P, T*P, Sdep^2) but not included because of high multicollinearity, a lack of theory supporting such interactions, and/or our focus on the effects of atmospheric deposition of N. To prevent model overfitting, we required there to be at least 5 detections per model term. If this condition was not met, then the model complexity was reduced until the condition was met (e.g., if there were only 15 presences then the most complex model for that species would have three terms). Most of the species requiring model simplification did not have "robust relationships" described below. We compared all remaining models using AICc (Akaike Information Criterion) to estimate model quality relative to other models and AUC (Area Under ROC Curve) to summarize the accuracy of the model. ROC is the receiver operating characteristic that shows the ability of a quantitative diagnostic test to classify subjects correctly as the decision threshold is varied). We selected the best model by first examining all models with an AICc within 2.0 of the best model, and then selecting the model with highest AUC.

For some of the 348 analyzed species, even the best model as assessed by AICc and AUC is not very predictive with the set of predictors we evaluated and responses of these models are not further summarized. Specifically, model quality was determined to be "robust" if three criteria were all met: (1) AUC > 0.7, (2) an estimated McFadden's $R^2 > 0.1$, and (3) the nitrogen response was not "U" shaped. There were 243 species that met conditions #1 and #2. Of these, there were 45 species with "U" shaped N responses and thus 198 that constituted the species with "robust relationships" for which we report N and S results.

We used two methods to assess multicollinearity between Ndep, Sdep and other variables for each species. These were: (1) correlations of N and S deposition with other variables (precipitation, temperature, and pH), and (2) variance inflation factors (VIFs) for N and for S.

VIFs measure the amount of multicollinearity in a set of multiple regression variables. For best models that contained N or S deposition, VIFs were calculated by regressing N or S deposition against the other main effect terms included in the best model (e.g., N dep ~ intercept + S dep + precip + temp + pH), and using the R^2 from that model in the equation for VIF (i.e., VIF = $1/(1-R^2)$). For species with a correlation between N or S deposition and any other variable that is greater than 0.4-0.6, and/or with a VIF of 3.0 or more, the causal effect of atmospheric deposition may be considered in question but not determined (i.e., it could be a spurious correlation, or it could just be a correlation). Many textbooks recommend a conventional VIF cutoff of 10.0 [57], but we also considered a VIF cutoff of 3.0 to be more conservative. We found that 167 and 98 species met the conventional and conservative VIF criteria for N deposition, respectively, and 61 and 29 species met the corresponding criteria for S deposition. Twenty-two species met the conservative criteria for both N and S (Table S6).

Given the large number of species tested, we checked our N results using a multiple comparisons approach. Specifically, we used the Holm-Bonferroni correction, which adjusts for family-wise error rates [43]. We had to check results separately for each relationship type (i.e., linear, hump, saddle), given that different P-values are ranked and compared for different relationship types. Specifically, the linear N terms are compared for increasing and decreasing relationships, the quadratic N terms are compared for hump-shaped relationships, and the N-interaction terms are compared for saddle species (e.g., N*pH, for a species whose N response depended on soil pH). For hump-shaped species, we found that of the 94 species with conventionally significant quadratic N terms (i.e., $P \leq 0.05$), 62 remained significant after adjusting for multiple comparisons (see Table S2). There were 13 species for which the quadratic N term was not significant at $P = 0.05$, and thus multiple comparisons was not performed. However, it is important to note that we are using an information theoretic approach based on AIC to select the best overall model; thus, even though the quadratic N term was not conventionally significant (i.e., $P = 0.05$), it was in the best overall model and thus included in our assessment. For species with a linear relationship with N, we found that of the 30 species with a linear N term significant at $P \leq 0.05$, 17 remained significant after adjusting for multiple comparisons. For species with a see-saw relationship with N, we found that 16 of 20 relationships remained significant after adjusting for multiple comparisons.

It is also worth mentioning that we assert that adjusting for multiple-comparisons is a useful cross-check, but not one that changes our findings. All the terms included in the models are known to affect species abundances; thus, the relationships found are likely not a result of spurious correlations brought on by multiple comparisons of unrelated factors. Furthermore, the information-theoretic approach used here (as opposed to null-hypothesis testing approach) assumes that the best model is among those being tested. Thus, for completeness we include all P-values for all terms in Table S2. Most relationships retained conventional statistical significance under null-hypothesis testing – for example, of the 107 species with a hump shaped relationship, 94 had a P-value for the N^2 term that was less than 0.05.

Calculating species critical loads

To calculate the critical load for a given species, we followed the same approach in Simkin et al. (2016), 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. We briefly summarize here the approach using N deposition and a linear model as an example (eqn. 1-3).

$$(1)\ Y = \beta_0 + \beta_1 N + \beta_2 pH + \beta_3 T + \beta_4 P + \beta_5 S + \beta_6 N^2 + \beta_7 pH^2 + \beta_8 T^2 + \beta_9 P^2 + \cdots$$

$$\beta_{10} N * pH + \beta_{11} N * S + \beta_{12} S * pH$$

$$(2)\ \frac{\partial Y}{\partial N} = \beta_1 + 2\beta_6 N + \beta_{10} pH + \beta_{11} S$$

$$(3)\ \beta_1 + 2\beta_6 N + \beta_{10} pH + \beta_{11} S < 0$$

The best statistical model for any given species describes how various factors (i.e., Ndep, Sdep, T, P, pH) affect the occurrence of that species (eqn. 1). The partial derivative with respect to Ndep of the best statistical model describes how the probability of occurrence for a species changes with N deposition (eqn. 2). Setting that expression to less than zero (eqn. 3) changes the meaning of the equation to now describe the conditions under which the probability of occurrence decreases. Any combination of variables that satisfies the inequality in eqn. 3 indicates conditions where the probability of occurrence is decreasing. For species with a significant negative quadratic N term (i.e., a "unimodal" shaped species, $\beta_6 < 0$), solving for N, gives the value of N deposition above which the probability of occurrence declines for a species, which we interpret as an expression for the critical load (eqn. 4).

$$(4)\ CL(N) = N > \frac{\beta_1 + \beta_{10} pH + \beta_{11} S}{-2\beta_6}$$

For species without a significant negative quadratic N term (i.e., $\beta_6 = 0$), then eq. 4 does not apply, but equation 3 can still be used to find combinations of variables that satisfy the inequality. In these cases, the relationship with N is either monotonically increasing or decreasing depending usually on the sign of the β_1 term. In these cases, the CL is assigned as in Pardo et al. 2010 as less than the minimum N dep experienced (for monotonic decreasing relationships), greater than the maximum N dep experienced (for monotonic increasing relationships), or as “NA” (for flat relationships). For species that have a non-flat relationship with N dep (or S dep), a species is considered to be “decreasing” if N dep is above the critical load, and “increasing” if N dep is below the critical load. In rare cases, species with monotonic relationships may flip directions (e.g., from decreasing to increasing, or vice versa), depending on the sign and magnitude of the interaction terms with N (β_{10} and β_{11}). In these cases, the relationship can be a “see-saw” where the species either increases, or decreases (21 species for N, 37 species for S, 48 total [10 had both N and S see-saw relationships]). For these species, there was generally a dominant relationship (i.e., either increasing or decreasing), which is how these species were categorized in Table 1 (but see Supplemental Table 2 for full information). Furthermore, for see-saw species the distribution of the CL is bimodal, either being below the minimum (if it is decreasing) or above the maximum (if it is increasing). Thus, we did not calculate an “average CL” for these see-saw species as they are misleading, albeit mathematically calculable.

Assessment of conservation value

Floristic quality assessments (FQAs) are conducted by professional botanists to determine the quality of the local flora in a particular area, often at the behest of a state or federal agency (e.g., US Forest Service and the Fish and Wildlife Service) for environmental assessments, planning, or permit reviews). They are based on “coefficients of conservatism” (C values: 1-10) assigned to individual plant species based on their tolerance to human disturbance and the degree to which the species represent natural undisturbed habitats [38]. The most conservative species (C values >7) are typically found under long unchanged conditions similar to those under which such species and communities evolved. In contrast, the least conservative species (C values <3) tend to be widely distributed and adapted to many conditions including higher levels of anthropogenic disturbance that usually eliminate more conservative species. We used the online database from [59] to assess the C-scores for all 348 species analyzed in our study (Table S2). We compared the species in our dataset with all inventories in the FQA Calculator. Since different species can have different values (i.e., C-scores) in different regions, we averaged the C-scores across regions. The negative correlation between C-score and the average CL may be spurious, since C-scores for species found in disturbed habitats tend to be lower. That being said, the botanists conducting FQAs are often discerning whether a site is "perturbed" or not on the basis of severe perturbation (e.g., bulldozing, toxic spills), not more subtle perturbation from atmospheric deposition [59].

Examining whether traits predict vulnerability

We used two overall approaches to explore how plant functional groups and physiological traits are associated with sensitivity to N deposition: (1) univariate analyses comparing how six broad plant functional groups (PFGs) are related to either the shape of the relationship (i.e., categorical Contingency Analysis for four shapes: increase, decrease, flat, unimodal) or the average CL (i.e., ANOVA relating the same six groups to the average CL), and (2) general linear models (GLMs) to examine how the relationship between these six plant functional groups plus an additional 10 physiological and quantitative traits related to the average CL. For the first approach, we used the USDA PLANTS database (https://plants.usda.gov/) to collect basic information on eight species characteristics for the 198 species with robust results: (1) functional group (2 levels: forb, graminoid), (2) taxonomic group (monocot, dicot, fern), (3) federal noxious status (listed, not listed), (4) invasive (yes, no), (5) threatened and endangered (listed, not listed), (6) life history (perennial, annual, biennial, mixed), (7) native status (native, non-native), and (8) whether the species was in the Fabaceae family or not (i.e., to capture the potential for N-fixation). There were no threatened and endangered species or federal noxious species among our filtered set of species; thus, these categories were removed from the analysis. We then analyzed differences among levels within each grouping using Contingency Analyses (Pearson's and Likelihood), and differences between mean CLs differed among levels using simple ANOVA. Results are shown in Table S3. The highly imbalanced composition of the different subgroups limited our ability to detect more significant results and examine combinations of characteristics (e.g., introduced graminoids). These findings underscore two conclusions: (1) natives ($P = 0.036$), perennials ($P = 0.046$) and marginally legumes ($P = 0.104$) appear to be more vulnerable to increasing N deposition that other PFGs, and (2) broad functional and taxonomic classifications are too coarse to capture trends, and more detailed trait-based characteristics may be better able to predict responses.

For the second approach, we examined the detailed trait information available from an intensive study in Wisconsin (Don Waller, *unpublished data*). We considered using global databases of trait values (e.g., TRY[60]), but decided that trait information specific to the Wisconsin survey sites was more relevant than trait information from many different geographic locations. Trait information from one region preserves the distribution of traits *among* species, which is important for this type of analysis. We hypothesized that species with lower critical loads would have traits associated with slower growth and/or were shorter-ruderal species (e.g., lower LNC, LPC, LMgC, SLA, VH; higher LCC, LLC). We then ran all possible linear models with 16 traits (i.e., the 6 plant functional groups, 9 physiological traits, and the C=score) as candidate predictors, and compared these with the average CL from each species (N=98). We compared models with AICc and explored many different competing model structures.

Table S1: Identity of the 198 species from Table 1 with different combinations of N and S responses (first row in each cell repeats the number of species from Table 1). Vulnerability is color coded for each set of species as in Table 1.

	S relationship		
N relationship	**Decrease**	**None**	**Increase**
Decrease	11	5	14
	Bouteloua gracilis	*Carex brunnescens*	*Carex disperma*
	Carex tetanica	*Hexastylis virginica*	*Cinna latifolia*
	Dichanthelium ovale	*Muhlenbergia cuspidata*	*Conyza Canadensis*
	Eryngium yuccifolium	*Verbascum thapsus*	*Epifagus virginiana*
	Goodyera pubescens	*Veronicastrum virginicum*	*Eupatorium compositifolium*
	Lespedeza procumbens		*Gymnocarpium dryopteris*
	Lespedeza repens		*Hydrophyllum canadense*
	Lysimachia quadriflora		*Juncus effuses*
	Rudbeckia hirta		*Prosartes lanuginose*
	Spartina pectinata		*Pycnanthemum virginianum*
	Stylosanthes biflora		*Scutellaria elliptica*
			Tiarella cordifolia
			Trillium erectum
			Viola Canadensis
None	5	15	1
	Echinacea angustifolia	*Carex communis*	*Saururus cernuus*
	Euthamia graminifolia	*Carex laxiculmis*	
	Helianthus giganteus	*Chenopodium album*	
	Helianthus grosseserratus	*Cirsium vulgare*	
	Juncus arcticus	*Dactylis glomerata*	
		Dryopteris cristata	
		Elymus virginicus	
		Poa cuspidata	
		Salvia lyrata	
		Solidago bicolor	
		Symphyotrichum lanceolatum	
		Symphyotrichum patens	
		Trillium sessile	
		Veronica hederifolia	

		Zigadenus elegans	
Increase	26	6	8
	Agrimonia rostellata	*Carex albursina*	*Arctium minus*
	Alliaria petiolata	*Carex swanii*	*Asarum canadense*
	Allium stellatum	*Carex virescens*	*Polygonum cespitosum*
	Bromus inermis	*Desmodium canadense*	*Polygonum virginianum*
	Cerastium arvense	*Ranunculus hispidus*	*Prenanthes racemose*
	Circaea canadensis	*Silphium asteriscus*	*Solidago caesia*
	Desmodium perplexum		*Sporobolus cryptandrus*
	Dicentra cucullaria		*Woodwardia areolate*
	Elymus canadensis		
	Geum virginianum		
	Glycyrrhiza lepidota		
	Houstonia purpurea		
	Hydrophyllum virginianum		
	Muhlenbergia sobolifera		
	Polygonatum biflorum		
	Polypodium appalachianum		
	Ranunculus recurvatus		
	Sanicula odorata		
	Sericocarpus tortifolius		
	Silphium compositum		
	Symphyotrichum laeve		
	Symphyotrichum undulatum		
	Thalictrum thalictroides		
	Urtica dioica		
	Viola sororia		
	Zizia aptera		
Unimodal	81	6	20
	Actaea rubra	*Antennaria neglecta*	*Allium cernuum*
	Agoseris glauca	*Bouteloua curtipendula*	*Boehmeria cylindrical*
	Allium tricoccum	*Carex hirsutella*	*Carex arctata*
	Andropogon gerardii	*Cirsium arvense*	*Carex debilis*
	Andropogon glomeratus	*Deparia acrostichoides*	*Dryopteris carthusiana*
	Andropogon virginicus	*Woodwardia virginica*	*Fragaria virginiana*
	Anemone quinquefolia		*Koeleria macrantha*
	Antennaria plantaginifolia		*Leersia oryzoides*
	Apocynum androsaemifolium		*Melampyrum lineare*
	Aquilegia canadensis		*Microstegium vimineum*
	Aristolochia serpentaria		*Mitella nuda*
	Asplenium platyneuron		*Murdannia keisak*

	Athyrium filix-femina		*Peltandra virginica*
	Bromus ciliatus		*Phytolacca americana*
	Calamagrostis stricta		*Pilea pumila*
	Campanula rotundifolia		*Solidago Canadensis*
	Carex albicans		*Solidago nemoralis*
	Carex blanda		*Trientalis borealis*
	Carex gracillima		*Viola hastate*
	Carex pedunculata		*Viola rotundifolia*
	Carex pensylvanica		
	Carex radiata		
	Carex rosea		
	Circaea lutetiana		
	Clintonia borealis		
	Cryptotaenia canadensis		
	Danthonia spicata		
	Desmodium nudiflorum		
	Elymus hystrix		
	Elymus trachycaulus		
	Erechtites hieraciifolia		
	Euphorbia corollata		
	Eurybia macrophylla		
	Galium concinnum		
	Galium triflorum		
	Geranium maculatum		
	Geum canadense		
	Geum triflorum		
	Hepatica nobilis		
	Hesperostipa comata		
	Liatris pycnostachya		
	Lysimachia quadrifolia		
	Maianthemum canadense		
	Milium effusum		
	Muhlenbergia glomerata		
	Oligoneuron riddellii		
	Oligoneuron rigidum		
	Onoclea sensibilis		
	Oryzopsis asperifolia		
	Osmorhiza claytonii		
	Osmunda claytoniana		
	Oxalis dillenii		
	Pediomelum esculentum		

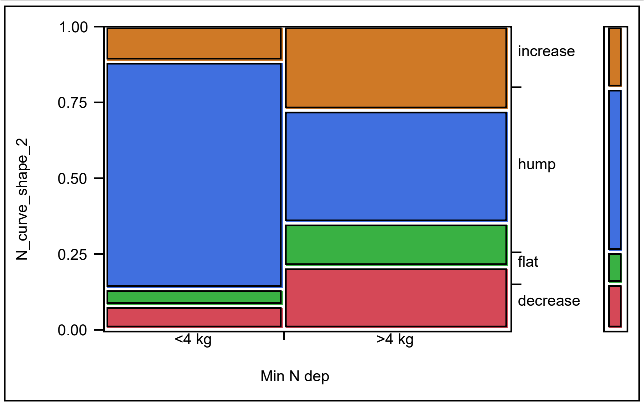
	Phegopteris connectilis		
	Phryma leptostachya		
	Poa compressa		
	Poa pratensis		
	Polygonatum pubescens		
	Pteridium aquilinum		
	Pulsatilla patens		
	Sanguinaria canadensis		
	Sanicula canadensis		
	Sanicula marilandica		
	Schizachyrium scoparium		
	Solidago flexicaulis		
	Solidago missouriensis		
	Solidago odora		
	Sorghastrum nutans		
	Streptopus lanceolatus		
	Symphyotrichum ciliolatum		
	Symphyotrichum ericoides		
	Symphyotrichum oolentangiense		
	Symphyotrichum sericeum		
	Thalictrum dasycarpum		
	Thalictrum dioicum		
	Tragia urens		
	Trillium grandiflorum		
	Uvularia grandiflora		
	Uvularia sessilifolia		
	Viola pubescens		
	Zizia aurea		

Table S2: (separate file, full db of results with metadata embedded)

Table S3: Contingency Analysis on whether the minimum N deposition affected the shape of the curve that was reported. We found a strong effect, where if the minimum N deposition was > 4 kg N ha^{-1} yr^{-1}, the species was more likely to have a decreasing relationship and less likely to have a unimodal relationship than if the minimum N deposition was < 4 kg ha^{-1} yr^{-1} (P=0.0001).

Contingency Analysis of N_curve_shape_2 By Min N dep

Mosaic Plot



Contingency Table

N_curve_shape_2

Min N dep	Count / Total % / Col % / Row % / Cell Chi^2	decrease	flat	hump	increase	
<4 kg	Count	7	5	66	10	88
	Total %	3.54	2.53	33.33	5.05	44.44
	Col %	23.33	23.81	61.68	25.00	
	Row %	7.95	5.68	75.00	11.36	
	Cell Chi^2	3.0083	2.0119	7.1537	3.4028	
>4 kg	Count	23	16	41	30	110
	Total %	11.62	8.08	20.71	15.15	55.56
	Col %	76.67	76.19	38.32	75.00	
	Row %	20.91	14.55	37.27	27.27	
	Cell Chi^2	2.4067	1.6095	5.7229	2.7222	
	Count	30	21	107	40	198
	Total %	15.15	10.61	54.04	20.20	

Tests

N	DF	-LogLike	RSquare (U)
198	3	14.481423	0.0620

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	28.963	<.0001 *
Pearson	28.038	<.0001 *

Table S4: Comparison of our average N critical loads (Ave N CL) and 5th – 95th intervals (Quant 05_N_CL and Quant 95_N_CL) with the center point from Europe estimated using TITAN (TITAN.cp, [64]) and the 5th – 95th bootstrapped intervals (TITAN.5th and TITAN.95th, respectively). The symbol is the first letter of the genus and species, followed by a 4-digit code to classify the Annex I Habitat (36). All European data are from [33], except Cr-6230 which is from [36]. Averages and intervals could not be calculated for *Equisetum arvense* and *Prunella vulgaris* in our study because of the curve shape ("N_curve_shape").

Species	Symbol	TITAN.cp	TITAN.5th	TITAN.95th	Ave N CL	Quant 05_N_CL	Quant 95_N_CL	N_curve shape
Athyrium filix-femina	Af-91A0	11.21	7	12.9	12.30	10.45	14.54	hump
Athyrium filix-femina	Af-91E0	15.28	13	15.5	12.30	10.45	14.54	hump
Campanula rotundifolia	Cr-5130	6.11	2.8	6.11	7.91	5.70	14.80	hump
Campanula rotundifolia	Cr-6210	8.26	5.7	8.26	7.91	5.70	14.80	hump
Equisetum arvense	Ea-6410	6	3.8	8.5				saddle/decrease
Koeleria macrantha	Km-5130	2.9	2.8	5.9	9.27	8.45	10.49	hump
Koeleria macrantha	Km-6210	7.16	6.3	8.1	9.27	8.45	10.49	hump
Poa pratensis	Pp-6410	6.88	4.1	7	13.18	9.71	18.95	hump
Prunella vulgaris	Pv-6210	5.98	5.98	6.5				none
Pteridium aquilinum	Pa-91A0	8.78	8	14.3	9.95	7.76	13.01	hump
Trifolium repens	Tr-6410	4.28	4.28	9.5	14.31	14.31	14.28	hump
Trifolium repens	Tr-5130	4.62	4.62	5.2	14.31	14.31	14.28	hump
Trifolium repens	Tr-6230	6.45	4	7.5	14.31	14.31	14.28	hump
Trifolium repens	Tr-6210	8.46	7.3	8.5	14.31	14.31	14.28	hump
Campanula rotundifolia	Cr-6230	13	8	21.5	7.91	5.70	14.80	hump

Table S5: Table of statistical results of how different species Plant Functional Groups affected vulnerability to N. Shown are the results for two responses: (1) curve shape (number of species in Decrease [D], Unimodal [U], Increase [I], None [N] categories) using contingency tables and Pearson's Chi^2 test (results the same with nominal regression and Likelihood ratio tests), and (2) the average CL among groups (ANOVA). Bold cells are significant at $P<0.05$. Species with a see-saw relationship were categorized according to the dominant relationship for the curve shape analysis, and were excluded for the CL analysis (because the average CL is not meaningful for these species, see SI).

		Response: Curve Shape					**Response: CL**	
Characteristic	**Levels**	**D**	**U**	**I**	**N**	**Pearson**	**Mean**	**ANOVA**
Functional group	Graminoid	9	30	7	6	0.565	10.9	0.607
	Forb	21	77	33	15		11.3	
Native status	Native	29	102	34	17	**0.036**	11.0	**0.028**
	Introduced	1	5	6	4		13.9	
Cotyledon status	Monocot	12	41	9	8	0.479	10.5	0.136
	Dicot	17	57	29	12		11.8	
	Fern	1	9	2	1		10.2	
Invasive status	Invasive	4	14	7	6	0.332	11.6	0.635
	Non-invasive	26	93	33	15		11.2	
N-fixation	N-fixer	3	2	3	0	0.104	11.1	0.947
	Non-N-fixer	27	105	37	21		11.2	
Life history	Perennial	26	102	35	18	0.204	11.0	**0.046**
	Annual/Biennial/Mixed	4	5	5	3		13.5	

Table S6: The percent of species with different relationships either from the set of 22 species with low VIFs (both N and S VIFs < 3.0) or from the 198 species without accounting for VIF (numbers of species in parentheses).

		Low VIFs	Any VIF
N relationship	**Decrease**	14% (3)	15% (30)
	None	0% (0)	11% (21)
	Increase	36% (8)	20% (40)
	Unimodal	50% (11)	54% (107)
S relationship	**Decrease**	64% (14)	62% (123)
	None	36% (8)	22% (43)
	Increase	0% (0)	16% (32)

Figure S1: Relationships for all 198 species with robust results (see separate file). Shown are the marginal probabilities of occurrence individually by term from the best model for N deposition (a), N x pH (b), N x S (c), S deposition (d), S x pH (e), soil pH (f), precipitation (g), and temperature (h). Black lines in main effect plots are average response and red lines are +/- 1.96 standard deviations for that term. For interaction terms (b, c, e) the effect of the modifying term is shown as separate quartile lines (Q1-Q4). All terms not in a plot are held at their average for that species. The best model is shown below the species name, and summary diagnostics for each species are in the lower right, which include the average N critical load, AUC, R^2, number of observations, VIF N and S, and the bivariate correlation between N and S. Other bivariate correlations, estimated values and significance of all terms (among other information) are in Table S2.

Figure S2: Comparison of our average N critical loads (x-axis) with those from Europe estimated using TITAN (y-axis, [64]). There were only eight species in common (15 in common for species in multiple European habitats) between our 348 species and studies from European acid grasslands [36] and several Irish habitats [33]: *Athyrium filix-femina* (Af), *Campanula rotundifolia* (Cr), *Equisetum arvense* (Ea, not shown, saddle relationship with N in our study), *Koeleria macrantha* (Kr), *Poa pratensis* (Pp), *Prunella vulgaris* (not shown, no relationship with N in our study), *Pteridium aquilinum* (Pa), and *Trifolium repens* (Tr). After the species symbol are the codes for the habitat types (5130: *Juniperus communis* formations on heaths or calcareous grasslands; 6210: Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*), 6230: Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe); 91A0: Old sessile oak woods with *Ilex* and *Blechnum* in the British Isles). Reference 1:1 line (solid), +/- 1 (dashed), and +/- 2 (dotted) kg ha^{-1} yr^{-1} shown. Whiskers are for 5th –95th intervals (horizontal, our study; vertical, TITAN analyses from [33] and [36], Table S4)

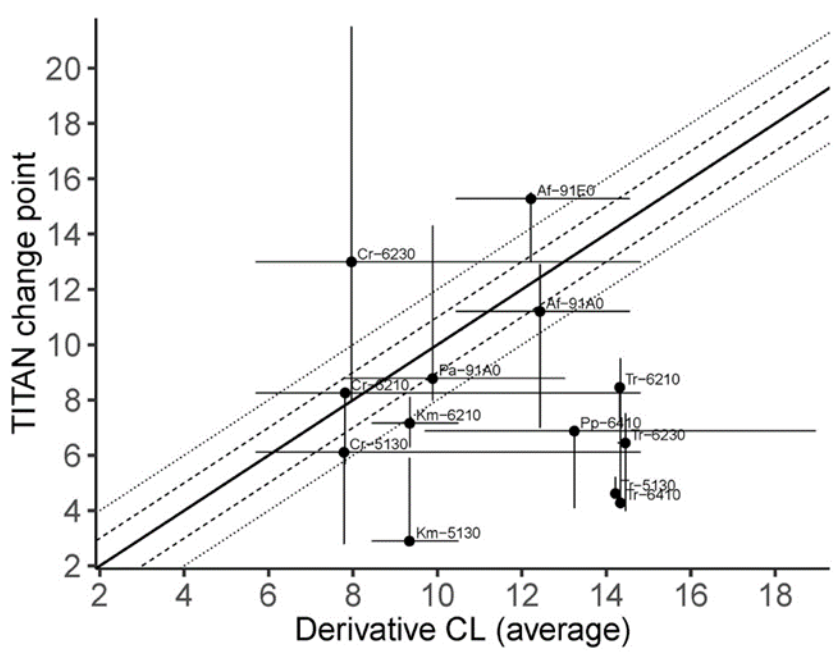


Figure S3: Map of the 14,041 plots analyzed in this study.

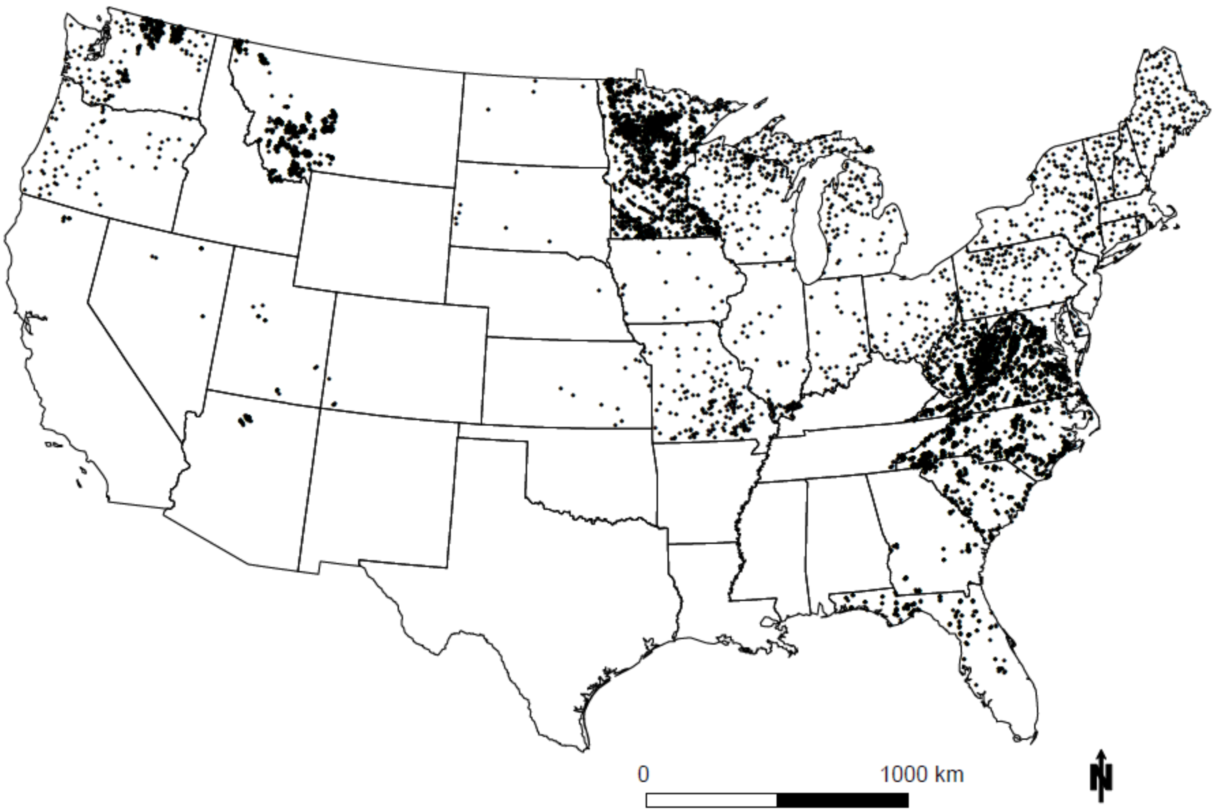


Figure S4: The number of species affected by N deposition (left) or S deposition (right) across the U.S. Shown are the number of unique species in a 12 x 12 km grid cell that decreased with N deposition (a), increased (b), the difference between these (c; $N_{decrease}$ - $N_{increase;}$ positive numbers indicate more species decreasing than increasing.). The same relationships for S deposition are in d-f.