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Impact of nitrogen deposition at the species level

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In Europe and, increasingly, the rest of the world, the key policy tool for the control of air pollution is the critical load, a level of pollution below which there are no known significant harmful effects on the environment. Critical loads are used to map sensitive regions and habitats, permit individual polluting activities, and frame international negotiations on transboundary air pollution. Despite their fundamental importance in environmental science and policy, there has been no systematic attempt to verify a critical load with field survey data. Here, we use a large dataset of European grasslands along a gradient of nitrogen (N) deposition to show statistically significant declines in the abundance of species from the lowest level of N deposition at which it is possible to identify a change. Approximately 60% of species change points occur at or below the range of the currently established critical load. If this result is found more widely, the underlying principle of no harm in pollution policy may need to be modified to one of informed decisions on how much harm is acceptable. Our results highlight the importance of protecting currently unpolluted areas from new pollution sources, because we cannot rule out ecological impacts from even relatively small increases in reactive N deposition.

plant ecology | Threshold Indicator Taxon Analysis | gradient survey

Since the 1980s, the key policy tool for the control of pollution in Europe has been the critical load (1). A critical load is defined as a "quantitative estimate of an 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" (1). Empirical critical load values are currently set for pollutants and habitats based primarily on pollution addition experiments and expert judgment, and they were most recently revised in 2010 (2). Critical loads have a central role in pollution management and are used for mapping pollution impacts, controlling and permitting individual pollution sources, and framing international negotiations on transboundary air pollution. They have recently been applied in the United States (3) and Canada (4), and they are under active consideration and development in many parts of the developing world (5).

Embedded in the critical load concept is the idea that it is possible to define a level of pollution that does not harm the natural environment. We test this concept for the deposition of reactive nitrogen (N). N deposition is recognized as one of the most serious pollution threats to global ecosystems (6), and it is ranked among the top five drivers of global biodiversity loss (7). Current critical loads for N deposition are primarily based on N addition experiments, which are valuable for identifying cause–effect relationships but have some limitations. Many experiments are located in regions with high ambient N deposition (often above the established critical load) (8) and use elevated N loads with few treatments over relatively short time periods. As a result, they are poorly suited to identifying the initial impacts of N deposition, vulnerable to treatment artifacts, and predisposed to short-term responses. Given these limits, there is a need to test experimentally derived critical load values with field survey data. Field surveys also have limitations, but the strengths of each approach complement the drawbacks of the other. Properly constructed and analyzed, survey data along pollution gradients provide a means of testing the efficacy of experimentally determined critical loads in the real world. If the experimentally derived critical load value adequately protects

plant communities, we would expect to find no evidence of harmful effects in sites receiving N deposition below this level.

We focus on European acid grasslands: a widespread semi-natural habitat in which gradient studies (9–12), time series analyses (13), and manipulation experiments (14) all show N deposition to be a primary driver of change in vegetation composition and diversity. The critical load for this habitat is currently set at 10–15 kg N ha^{-1} y^{-1}, with three indicators of critical load exceedance: an increase in graminoids, a decrease in total species richness, and a decline of typical species (the indicator that we focus on here) (2). We apply Threshold Indicator Taxon Analysis (TITAN) to identify the points along the N deposition gradient at which individual plant species change in abundance (15) and compare these values with the critical load. TITAN identifies for each species the point along the N deposition gradient at which any systematic difference in cover is maximized (the change point), whether the difference in cover on either side of this change point is statistically significant, and if so, the abruptness of the difference. We use strict criteria (99%) for both "purity" (the proportion of change-point response directions among bootstrap replicates that agree with the observed response) and "reliability" [the proportion of bootstrap change points that are significant at the chosen probability level (in our case, 0.05)]. This conservative approach provides a higher level of confidence in the reliability of change points, but at a lower level of confidence that all species that change are identified. We also applied TITAN to examine relationships between species abundance and climate to account for any multicollinearity between N deposition and climate.

Results

All species showing high-significance change points have lower cover in sites with greater N deposition. Change points are identified from the lowest N deposition at which it is possible to identify any change (7 kg N ha^{-1} y^{-1}) (Fig. 1). The species change points predominately are at the lower end of the N deposition gradient: one-third occur below the lower boundary of the currently established critical load for this habitat, and a similar number is within the critical load range. Independent evidence of sensitivity to N deposition exists for many of these species, such as *Hypericum pulchrum* (11), *Plantago lanceolata* (16, 17), *Hylocomium splendens* (11, 18, 19), *Achillea millefolium* (20), and *Succisa pratensis* (21, 22). Most of the species that decline in cover are forbs, and this finding agrees with studies showing this functional group to be particularly sensitive to N deposition (12). Above 25 kg N ha^{-1} y^{-1}, there are very few species change points, suggesting that sensitive species have already been
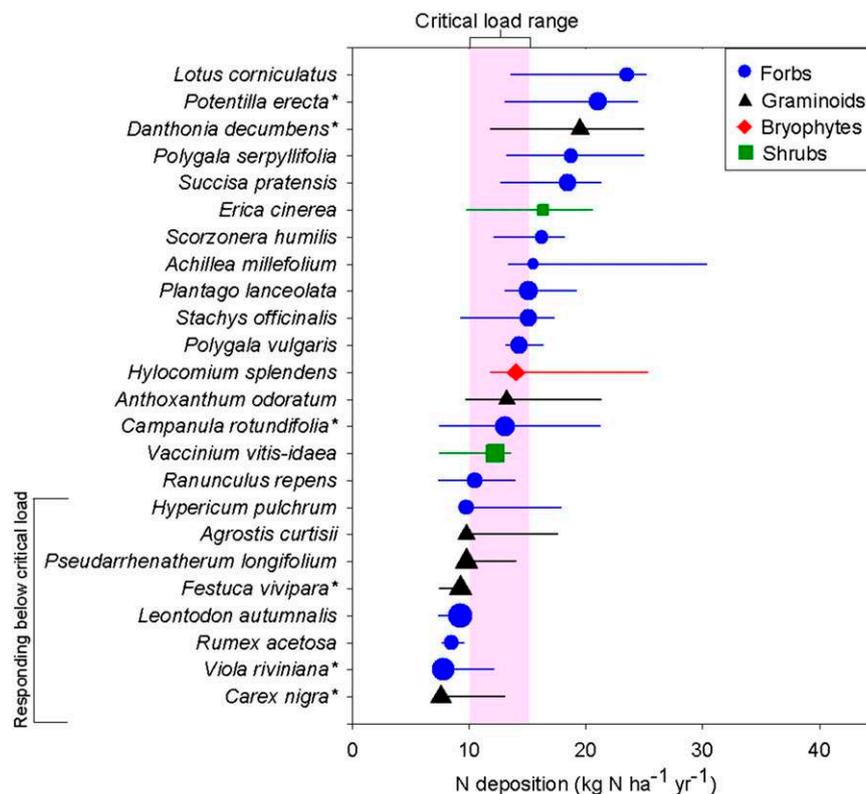
Fig. 1. Species change points (purity > 99%, $P < 0.05$ in >99% bootstraps) showing 5% and 95% bootstrap percentiles; symbols are sized in proportion to z score. *Species that also show a change point at the equivalent position on the precipitation gradient.

impacted and that changes in remaining species are comparatively minor. Change points strongly converge at an N deposition of 14.2 kg N ha^{-1} y^{-1} (Fig. 2), indicating a community-level ecological threshold (15). These results suggest that the impacts of N deposition on sensitive species typical of acid grasslands begin at very low deposition levels and are highly nonlinear, with most change occurring below the upper limit of the critical load.

Our key results showing individual species change points below the current critical load and a community-level threshold around 14.2 kg N ha^{-1} y^{-1} seem quite robust. Excluding all species showing significant change points at corresponding positions on the precipitation gradient, all data from any one sampling region, taxa with more than 75% of occurrences in a sampling region, or the most extreme three instead of five of the candidate change points (shifting the minimum deposition to 6 kg N ha^{-1} y^{-1}) displaces the community threshold by no more than 1.5 kg N ha^{-1} y^{-1}, and in all cases, species respond from the lowest levels of N deposition detectable. We, therefore, have confidence in the general robustness of our results to potential confounding factors such as regional biogeography and climate. Relaxing our criteria for purity and reliability to 95% instead of 99% identifies almost twice as many species, but does not alter the proportion of approximately 60% of species change points occurring at or below the range of the critical load.

Discussion

Based on this detailed analysis of a large targeted survey, we cannot confirm the existence of a level of N pollution at which impacts on plants are not detectable. Individual species decline in abundance from the lowest level of N deposition at which any change could be identified by our analysis (6–7 kg N ha^{-1} y^{-1}). We are not aware of any long-term acid grassland field experiment with a total treatment (including ambient) of less than 10 kg

N ha^{-1} y^{-1}. Because gradient studies cannot identify a deposition level at which there is no decline of typical species, and N addition experiments have limited ability to identify impacts at low levels of pollution or at a fine scale, we conclude that a true critical load for acid grasslands, if it exists, is too low to detect with existing data and methods. However, we do find a convergence of many species change points at an N deposition of 14.2 kg N ha^{-1} y^{-1} (Fig. 2), indicating a community-level ecological threshold (15). This threshold coincides with the upper boundary of the critical load, suggesting that, rather than the onset of change, the experimentally observed changes on which the critical load is based represent the point at which the community shifts to a more pollution-tolerant assemblage (23).

Our analysis identifies many more changes in the community below the critical load than above it. Thus, a long-term incremental increase in N deposition would have a greater impact in a site with a current loading of 10 kg N ha^{-1} y^{-1} than a site with a current loading of 30 kg N ha^{-1} y^{-1}. This finding highlights the importance of protecting currently unpolluted areas from new pollution sources: we cannot rule out ecological impacts from even relatively small increases in reactive N deposition in these areas. It also suggests that less overall ecological damage may result from new sources of reactive N in current high-N deposition regions, where a shift to a more pollution-tolerant community may have already occurred, rather than regions that currently receive low levels of pollution and are sensitive to even small increases in N deposition. Effective pollution control policy should focus on avoiding all new pollution sources in currently unpolluted regions.

Critical loads have been highly successful in reversing the acidification of European ecosystems, and we support the continuation and expansion of effects-based pollution policy. In addition, one practical application of critical loads is to rank the relative sensitivity of different habitats for making decisions on
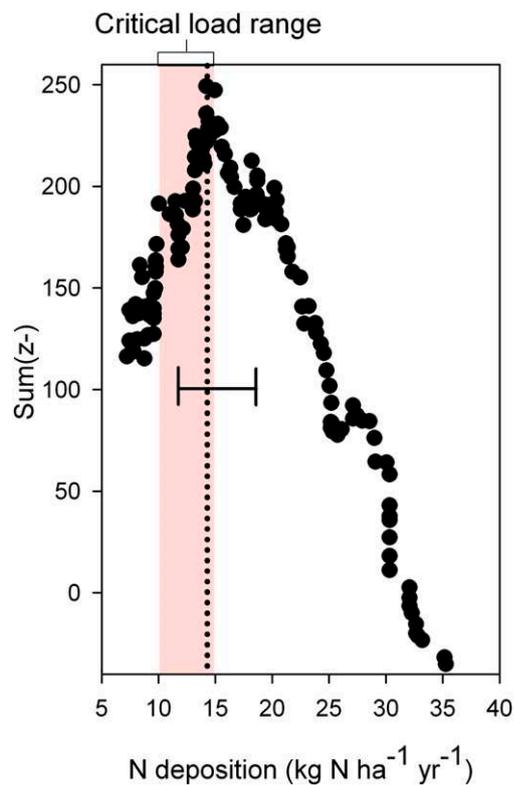
Fig. 2. Community change for species reduced in abundance [sum (z−)] showing critical load, inferred community threshold (dotted line at 14.2 kg N ha⁻¹ y⁻¹), and 5–95% bootstrap percentile range.

which may be most affected by new emissions sources. Our results do not challenge the value of critical loads for identifying pollution levels associated with undesirable biogeochemical change (such as leaching of reactive N to downstream ecosystems) or assessing relative harm to ecological communities. However, by introducing a quantitative way of evaluating individual species change, our study suggests that, for this community, the only way to have no harm is to have no pollution. If the results from this study are found more widely, the central no-harm concept of critical loads may have to be replaced with a sliding scale of harm, in which informed decisions must be made on which elements in an environment should be protected and which elements may have to be compromised.

Methods

One hundred fifty-three unimproved and undisturbed acid grasslands (*Violion caninae* association) were surveyed across the Atlantic fringe of northwest Europe (United Kingdom, Ireland, France, Belgium, The Netherlands, Germany, Norway, Sweden, and Denmark), with cover of all species estimated in five 2×2-m quadrats (9, 10). In total, 155 species were identified from sites that fulfilled our criteria: a typical 2×2-m quadrat contained 15–25 species in the less-polluted sites and 7–12 species in the more-polluted sites (9). For each site, estimates of N deposition were produced using the EMEP (European Monitoring and Evaluation Programme)-based IDEM (Integrated Deposition Model) model or appropriate national models (9, 10). The N deposition range is 2.4–43.4 kg N ha⁻¹ y⁻¹ (mean = 18.6 kg N ha⁻¹ y⁻¹); most of this gradient is evenly sampled, with the exception of a gap between 2.4 and 5.1 kg N ha⁻¹ y⁻¹.

Underlying the TITAN approach is the Indicator Value (IndVal) (24) method for the identification of taxa that typify groups of an a priori sample classification. A taxon with a high IndVal score will have a high concentration of abundances and high fidelity to a single group. A taxon with a maximal IndVal score of 100% would be found in all samples of a group and only in that group. In TITAN, IndVal scores are calculated for all species for all possible change points along the environmental gradient, with permutation tests to assess the uncertainty in these scores. Permuted IndVal scores are standardized as z scores and summed for positive [sum(z+)] and negative [sum(z−)] responses for each possible change point. Sum(z) peaks highlight values of the environmental variable around which many taxa exhibit strong directional changes in abundance representing community thresholds. Uncertainty is assessed by bootstrapping; quantiles of the bootstrapped maxima provide a guide to the abruptness of the response (15). Change points will be identified with both abrupt (threshold-like) and more gradual species responses, but the latter will have broader confidence intervals. For each species, TITAN also returns measures of purity (proportion of bootstrap replicates matching group assignment in the original data) and reliability (proportion of bootstrap replicates with maximum IndVal reaching a specified *P* value). TITAN has been shown to be effective at identifying known species and community thresholds in real and simulated data (15).

We applied TITAN to the mean Domin (25) score of the five quadrats for each species. TITAN was implemented in R (26) with 250 IndVal permutations and 500 bootstrap replicates. We excluded both rare species found in five or fewer sites and the five lowest and five highest candidate change points, because the partitioned sample size is considered too small to confidently identify differences (15). Because N deposition is correlated with mean annual precipitation (but not temperature) across these sites, we also applied TITAN to precipitation data.

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