

Endangered plants persist under phosphorus limitation

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Nitrogen enrichment is widely thought to be responsible for the loss of plant species from temperate terrestrial ecosystems. This view is based on field surveys and controlled experiments showing that species richness correlates negatively with high productivity^{1,2} and nitrogen enrichment³. However, as the type of nutrient limitation has never been examined on a large geographical scale the causality of these relationships is uncertain. We investigated species richness in herbaceous terrestrial ecosystems, sampled along a transect through temperate Eurasia that represented a gradient of declining levels of atmospheric nitrogen deposition—from ~50 kg ha⁻¹ yr⁻¹ in western Europe to natural background values of less than 5 kg ha⁻¹ yr⁻¹ in Siberia⁴. Here we show that many more endangered plant species persist under phosphorus-limited than under nitrogen-limited conditions, and we conclude that enhanced phosphorus is more likely to be the cause of species loss than nitrogen enrichment. Our results highlight the need for a better understanding of the mechanisms of phosphorus enrichment, and for a stronger focus on conservation management to reduce phosphorus availability.

Alterations to the environment by humans have reduced the plant species diversity in many ecosystems, and in some cases these reductions have affected ecosystem functioning⁵. To counteract this loss of diversity, there is an urgent need to uncover the underlying mechanisms responsible. Studies of diversity–productivity patterns in Canadian and European terrestrial wetlands suggest that increased productivity is a major factor influencing species extinction^{1,2}. According to Grime's 'hump-backed' model, there is a critical level of productivity at which species richness reaches a peak, and above which it declines rapidly because all but a few fast-growing, tall species are unsuccessful in competing for light⁶. Low or moderate nutrient availability has been thought to be a mechanism that reduces the competitive advantage of fast-growing, tall species relative to smaller ones⁷.

Aquatic freshwater ecosystems are generally thought to be P-limited⁸; although in freshwater lakes, species may also be lost due to nitrate enrichment⁹. In contrast, most terrestrial ecosystems of the temperate zone are considered to be N-limited^{10,11}; therefore, N-enrichment is seen as a major cause of plant species loss in temperate grasslands³ and forests¹¹. Although productivity only increases with increasing availability of the limiting resource¹², the type of nutrient limitation has never been examined for a large number of sites.

Here we provide evidence that P- rather than N-enrichment is more important in the loss of plant species from some ecosystems. We investigated the plant species composition of 274 sites with herbaceous vegetation, ranging from terrestrial freshwater wetlands

(fens, bogs and fluvial marshes) to moist grasslands. The sites—all in temperate Eurasia (51–57° N)—were scattered along a west–east transect from the Netherlands/Belgium (5° E) through eastern Poland (23° E) to western Siberia (85° E). This transect also represents a gradient of declining levels of atmospheric nitrogen deposition, from high in the Netherlands and Belgium (40–60 kg N ha⁻¹ yr⁻¹), to much lower in Poland (5–10 kg N ha⁻¹ yr⁻¹), to very low in Siberia (<5 kg N ha⁻¹ yr⁻¹; ref. 4). For all sites we recorded species richness of vascular plants, the number of endangered species (using the Dutch Red List¹³; see Methods) and the above-ground standing crop of vegetation. The type of nutrient limitation was inferred by analysing plant material and calculating nutrient ratio values^{14,15}.

As already indicated, if species loss is the result of increased productivity, then we would expect the endangered species in our samples to occur mainly at sites of low productivity. In addition, if the main cause of higher productivity is enhanced N deposition, then we can expect two further patterns to emerge. In western Europe we expect endangered species to be restricted to P-limited ecosystems. This is because N-enrichment will have transformed formerly unproductive, N-limited ecosystems into either highly productive ecosystems or low-productive P-limited ecosystems. In Poland and Siberia (where N-deposition is low) we expect endangered species to occur at sites of low productivity, irrespective of the type of nutrient limitation.

Two well established relationships are confirmed from our data. Firstly, the species richness–productivity relationships show the classical hump pattern^{1,2,6}, with highest species richness at productivities of 200–600 g m⁻² (Fig. 1a). Secondly, the sites with intermediate tissue N:P ratios (6–20) are on average the most species rich¹⁶ (Fig. 1b). Examining the patterns of endangered species shows that some aspects of our results seem to support the above mentioned expectations: (1) endangered plant species only occur at low-productivity sites (biomass <~600 g m⁻²; Fig. 1c), (2) in the Netherlands/Belgium 70% of the endangered species occur at P-limited sites (Fig. 2a), and (3) in Poland and Siberia many endangered species are indifferent to the type of nutrient limitation (Fig. 2a). However, other findings are inconsistent with the initial hypotheses. For example, in Poland and Siberia endangered species are more frequent in P-limited sites than in N-limited sites (Fig. 2a), and in Poland this cannot be because P-limited sites are more frequent ($P < 0.05$, Fig. 2). Even more unexpectedly, most of the low-productivity sites in the Netherlands/Belgium are limited not by P but by N (Fig. 2b). Furthermore, maximum numbers of endangered species are higher under conditions of P- than N-limitation (indicated by the height of the red and blue 'peaks' in Fig. 1d, with the peak of the regression being at N:P

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ratio 21). Moreover, the percentage of endangered species in the vegetation clearly increases with increasing P-limitation (Fig. 1f). Hence, endangered plant species appear better able to persist in P-limited sites than in sites with other types of nutrient limitation.

Three different mechanisms may explain the observed pattern: (1) there is a wider variation in adaptations to low P-availability (for example, phosphatase exudation, soil acidification, cluster roots or mycorrhiza) than to low N-availability (for example, symbiotic N₂-fixation or organic N-uptake). This may have resulted in a larger pool of species adapted to low P-availability; (2) low productive systems that used to be N-limited have become P-limited because of N-enrichment; or (3) human impact has impaired P-limited ecosystems more strongly than N-limited ecosystems, leading to a larger loss of species adapted to low P-availability.

The first explanation is unlikely because if species pools differed between N-limited and P-limited sites, then the total species richness would be expected to differ, which is not the case (Fig. 1a, b). The second explanation must also be discarded because in the Netherlands and Belgium (where N deposition is high) N-limited ecosystems with low productivity are more common than P-limited ecosystems (Fig. 2b). The third explanation seems the most plausible, because in western Europe various human impacts have enhanced P-availability in wet and moist ecosystems. Increased groundwater

extraction for drinking water and industry have diminished the discharge of calcium- and iron-rich groundwater into wetlands and consequently this has reduced the binding of P^{17,18}. Together with this effect, P-enrichment of surface waters¹⁹ and internal eutrophication (through for instance increased drying-wetting dynamics²⁰ or sulphate pollution²¹) of the soil have also contributed to enhanced P-availability. On a global scale, it has been estimated that human intervention in the biogeochemical P cycle has increased the magnitude of P fluxes by 400%, which is far more than for carbon, nitrogen and sulphur²². We conclude that, in spite of severely enhanced atmospheric N-deposition in Western Europe⁴, P-enrichment has been more important than N-enrichment for species loss from wet and moist herbaceous ecosystems. P-enrichment may have caused productivity increase and species loss through competitive exclusion, or a shift from P- to N-limitation, to the disadvantage of species adapted to low P availability. In a discussion of whether species richness can ever be raised by nutrient addition, Güsewell and colleagues¹⁶ suggested that addition of P may increase the total

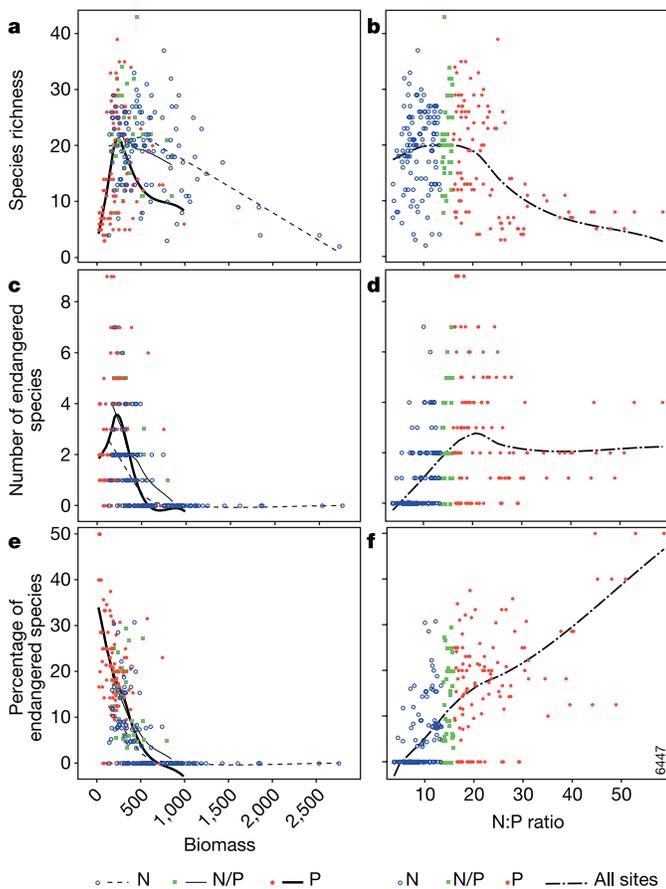


Figure 1 | Species richness affected by nutrient limitation in herbaceous ecosystems in Eurasia. a–f, The total number of vascular plant species (a and b), endangered species among them (c and d) and endangered species as percentage of all vascular species (e and f) are plotted against above-ground biomass (in g m⁻²) of vascular plants (a, c, e), and N:P ratio in above-ground vascular plant material (b, d, f). Blue circles are N-limited sites, red filled circles are P-limited sites and green filled squares are N/P co-limited sites. Trends were analysed using LOWESS regression (span 2/3; degree 1). For f we also performed a linear regression ($y = 0.77x - 0.94$; $R^2 = 0.40$; $P < 0.001$).

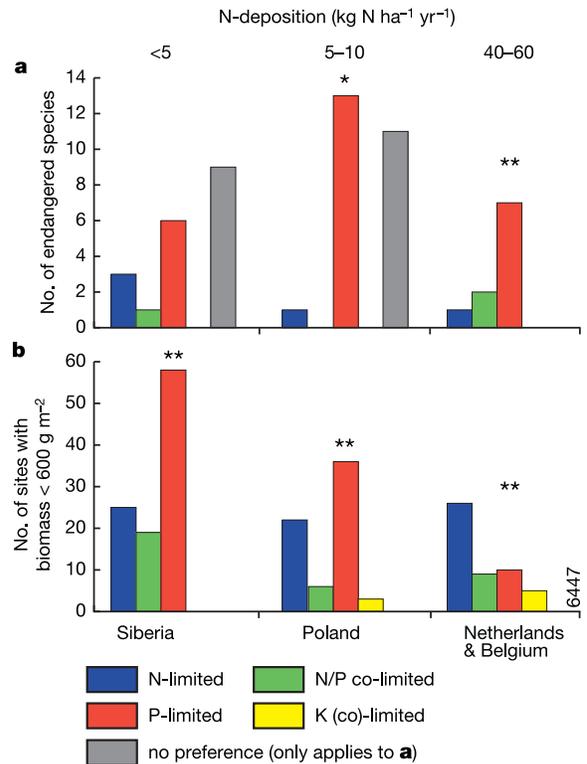


Figure 2 | Frequency distributions of endangered species and sites with low productivity. a, b, Frequency distributions of endangered species with preference for a certain type of nutrient limitation per region (a), as well as frequency distribution of sites with biomass < 600 g m⁻², that is, the biomass range where endangered species occur (b). Preference of species α for example, P-limitation means that species α occurred only at P-limited sites or it occurred at least three times more often at P-limited sites than at sites limited by another nutrient (N, N/P or K). Species not showing preference according to this definition were labelled as no preference. In b, the observed distributions all differ significantly from an equal frequency distribution among the various types of nutrient limitation (χ^2 test; two asterisks indicates $P < 0.001$). In a, we tested whether the observed distribution of endangered species among N-, N/P-, P- and K-limited sites differs significantly from the frequency patterns shown in b (that is, distributions that could be expected on the basis of frequency of occurrence of N-, N/P-, P- and K-limited sites assuming endangered species had equal probabilities for occurring on N-, N/P-, P- and K-limited sites ($P = 0.25$)). For the Polish and Dutch/Belgian sites, the observed distribution differs significantly from expected distributions, for the Siberian sites it does not (χ^2 test; one asterisk indicates $P < 0.05$; two asterisks indicate $P < 0.001$).

species richness of P-limited wetlands, but the authors warned that this would tend to promote common species at the expense of rare ones. Our result fully supports their expectation; regardless of patterns in total species richness, both the absolute number and the proportion of endangered species in the vegetation appeared to be greatest in P-limited wetlands.

Our findings suggest that P-limitation in terrestrial systems may be more widespread than generally acknowledged²³, and imply that the conservation of endangered species requires the preservation and restoration of P-limited ecosystems. Conservation managers often attempt to enhance the plant species diversity of an ecosystem by reducing its nutrient capital, and thereby its productivity. Although these attempts are sometimes successful²⁴, productivity reduction often does not lead to an increase in species richness^{25,26} because conditions of P-limitation are not restored. For example, re-establishment of endangered species on former agricultural fields generally fails²⁷ because of the large P-pool accumulated in the soil over decades of fertilization²⁸. In general, policies biased towards reducing nitrogen enrichment (for example, the European Union Directive on Nitrate) are unlikely to provide adequate protection for the majority of endangered species in herbaceous ecosystems. A multivariate consideration of anthropogenic impacts on the water cycle, the biogeochemical cycles (of C, N, P and S) and soil acidity is needed. This requires a systems approach²² that includes the assessment of the effect of changes in ecological stoichiometry on biodiversity.

METHODS

Sampling protocol. We sampled 150 plots as described in ref. 15. In 2001, 2002 and 2003 we sampled 124 additional plots of 10 m² following the same method. The data set contains terrestrial wetlands (for example, river marginal floodplains and marshes, wet grasslands, fens, bogs and fen meadows) as well as moist grasslands. Within each plot we randomly sampled a subplot of 0.16 m² of which we harvested above-ground standing crop at the height of the growing season (July). We separated mosses and vascular plants and determined dry weight and contents of N, P and K in plant material (Kjeldahl destruction). We used dry weight of above-ground biomass of vascular plants as an estimate of primary production (see refs 15 and 29).

Determining the type of nutrient limitation. To determine the type of nutrient limitation we used a method based on critical values of N:P, N:K and K:P ratios in above-ground plant material derived from literature reviews of fertilization experiments^{14,15}. N- and P-limited sites were distinguished on the basis of N:P ratios with N:P ratios >16 indicating P-limitation, N:P ratios <13.5 indicating N-limitation, and between 13.5 and 16 indicating N/P co-limitation¹⁴. This N/P co-limitation should be interpreted as true co-limitation by N and P together, or at least as no clear single limitation by N or P. For distinguishing K (co)-limited samples we used the critical N:K ratio of 2.1 and the critical K:P ratio of 3.4 (ref. 15). Only 11 sites were K (co)-limited (see Supplementary Table 1). These sites are excluded from Fig. 1.

The data set presented here includes different types of herbaceous vegetation, some herb-rich and others dominated by grasses. As grasses might have intrinsically higher N:P ratios than herbs¹⁴, differences in dominance of these plant groups could have affected average N:P ratios of a site. To evaluate whether dominance by either grasses or herbs has an influence on N:P ratios and the occurrence of endangered species we divided the data into three parts: (1) sites in which grasses accounted for more than half of the plant cover; (2) sites in which herbs accounted for more than half of the plant cover, and (3) sites in which neither grasses nor herbs accounted for more than half of the plant cover (excluding the 11 K-limited sites; $n = 263$). We tested whether N:P ratios differed significantly among these three groups (Tukey test after one-way analysis of variance, ANOVA). The majority of sites was neither dominated by grasses nor by herbs and only 9% of the sites in the biomass range where endangered species occur (<600 g m⁻²) were dominated by grasses (see Supplementary Table 2). The grass:herb ratio does affect N:P ratio, with grass-dominated sites having a significantly lower N:P ratio. However, in the subset where endangered species occur the N:P ratio of the sites dominated by grasses did not differ significantly from that of the sites dominated by herbs. Additional information and discussion concerning the method for assessing nutrient limitation by means of N:P ratios is given in the Supplementary Discussion.

Plant species. We analysed the similarity between the list of species recorded by us in Poland and Russia and the Dutch flora. Eighty seven percent of the species of the Polish sites, and 75% of the species of the west Siberian sites belong to the

Dutch flora. From the Red List of endangered species of the Netherlands¹³, we only used 'actually threatened species', that is, species that have disappeared in at least 25% of the map units (1 unit is 25 km²) between 1970 and 1990. The majority of the endangered species of the Netherlands also occur on red lists of other western and central European countries; that is, 100%, 80%, 71%, 67%, 63% and 27% of the species observed by us which are on the Dutch Red List (see Supplementary Table 1) also occur on the Red Lists of Flanders, Germany, Czech Republic, Switzerland, Luxembourg and Poland, respectively.

Other factors affecting diversity-productivity patterns. Species diversity in the data set is affected by stress factors such as acidity and inundation which operate through sizes of regional species pools³⁰ (see Supplementary Fig. 2). However, these factors cannot explain our main result, more endangered species at P-limited sites. The type of nutrient limitation was not related to acidity, and apart from some sites (riparian marshes deeply flooded in spring) nor to the maximum inundation depth. Other stress factors like salinity and drought stress did not play a role in our data set.

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- Moore, D. R. J., Keddy, P. A., Gaudet, C. L. & Wisheu, I. C. Conservation of wetlands: do infertile wetlands deserve a higher priority? *Biol. Conserv.* **47**, 203–217 (1989).
- Wheeler, B. D. & Shaw, S. C. Above-ground crop mass and species richness of the principal types of herbaceous rich-fen vegetation of lowland England and Wales. *J. Ecol.* **79**, 285–301 (1991).
- Stevens, C. J., Dise, N. B., Mountford, J. O. & Gowing, D. J. Impact of nitrogen deposition on the species richness of grasslands. *Science* **303**, 1876–1879 (2004).
- Holland, E. A., Dentener, F. J., Braswell, B. H. & Sulzman, J. M. Contemporary and pre-industrial global reactive nitrogen budgets. *Biogeochemistry* **46**, 7–43 (1999).
- Naeem, S., Thompson, J., Lawler, S. P., Lawton, J. H. & Woodfin, R. M. Declining biodiversity can alter the performance of ecosystems. *Nature* **368**, 734–737 (1994).
- Grime, J. P. *Plant Strategies and Vegetation Processes* (Wiley, Chichester, 1979).
- Taylor, D. R., Aarssen, L. W. & Loehle, C. On the relationship between r/K selection and environmental carrying capacity: a new habitat template for plant life history strategies. *Oikos* **58**, 239–250 (1990).
- Schlesinger, W. H. *Biogeochemistry: An Analysis of Global Change* (Academic, San Diego, 1997).
- Gilles, J. G. Nitrogen study fertilizes fears of pollution. *Nature* **433**, 791 (2005).
- Vitousek, P. M. & Howarth, R. W. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**, 87–115 (1991).
- Sala, O. E. *et al.* Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774 (2000).
- Chapin, F. S. I., Vitousek, P. M. & van Cleve, K. The nature of nutrient limitation in plant communities. *Am. Nat.* **127**, 48–58 (1986).
- van der Meijden, R., van Duuren, L., Weeda, E. J. & Plate, C. L. Standaardlijst van de Nederlandse flora 1990. *Gorteria* **17**, 75–127 (1991).
- Güsewell, S. & Koerselman, W. Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspect. Plant Ecol. Evol. Syst.* **5**, 37–61 (2002).
- Olde Venterink, H., Wassen, M. J., Verkroost, A. W. M. & de Ruiter, P. C. Species richness-productivity patterns differ between N-, P- and K-limited wetlands. *Ecology* **84**, 2191–2199 (2003).
- Güsewell, S., Bailey, K. B., Roem, W. J. & Bedford, B. L. Nutrient limitation and botanical diversity in wetlands: can fertilization raise species richness? *Oikos* **109**, 71–80 (2005).
- Wassen, M. J., van Diggelen, R., Wolejko, L. & Verhoeven, J. T. A. A comparison of fens in natural and artificial landscapes. *Vegetatio* **126**, 5–26 (1996).
- Richardson, C. J. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science* **228**, 1424–1427 (1985).
- Carpenter, S. R. *et al.* Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **8**, 559–568 (1998).
- Turner, B. L. & Haygarth, P. M. Phosphorus solubilization in rewetted soils. *Nature* **411**, 258 (2001).
- Lamers, L. P. M., Tomassen, H. B. M. & Roelofs, J. G. M. Sulphate induced eutrophication and phytotoxicity in freshwater wetland. *Environ. Sci. Technol.* **32**, 199–205 (1998).
- Falkowski, P. *et al.* The global carbon cycle: a test of our knowledge of Earth as a system. *Science* **290**, 291–296 (2000).
- Elser, J. J. *et al.* Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**, 578–580 (2000).
- Bakker, J. P. *Nature Management by Grazing and Cutting* (Kluwer Academic, Dordrecht, 1989).
- Berendse, F., Oomes, M. J. M., Altena, H. J. & Elberse, W. T. Experiments on the restoration of species-rich meadows in the Netherlands. *Biol. Conserv.* **62**, 59–65 (1992).
- Jansen, A. J. M. & Roelofs, J. G. M. Restoration of *Cirsio-Molinietum* wet meadows by sod cutting. *Ecol. Eng.* **7**, 279–298 (1996).

27. Bakker, J. P. & Berendse, F. Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends Ecol. Evol.* **14**, 63–68 (1999).
28. Gough, M. W. & Marris, R. H. A comparison of soil fertility between semi-natural and agricultural plant communities: implications for the creation of species-rich grassland on abandoned agricultural land. *Biol. Conserv.* **51**, 83–96 (1990).
29. Hector, A. *et al.* Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123–1127 (1999).
30. Grace, J. B. The factors controlling species density in herbaceous plant communities: an assessment. *Perspect. Plant Ecol. Evol. Syst.* **2**, 1–28 (1999).

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