

Low investment in sexual reproduction threatens plants adapted to phosphorus limitation

Yuki Fujita¹, Harry Olde Venterink², Peter M. van Bodegom³, Jacob C. Douma⁴, Gerrit W. Heil⁵, Norbert Hölzel⁶, Ewa Jabłońska⁷, Wiktor Kotowski⁷, Tomasz Okruszko⁸, Paweł Pawlikowski⁷, Peter C. de Ruiter⁹ & Martin J. Wassen¹⁰

Plant species diversity in Eurasian wetlands and grasslands depends not only on productivity but also on the relative availability of nutrients, particularly of nitrogen and phosphorus^{1–4}. Here we show that the impacts of nitrogen:phosphorus stoichiometry on plant species richness can be explained by selected plant life-history traits, notably by plant investments in growth versus reproduction. In 599 Eurasian sites with herbaceous vegetation we examined the relationship between the local nutrient conditions and community-mean life-history traits. We found that compared with plants in nitrogen-limited communities, plants in phosphorus-limited communities invest little in sexual reproduction (for example, less investment in seed, shorter flowering period, longer lifespan) and have conservative leaf economy traits (that is, a low specific leaf area and a high leaf dry-matter content). Endangered species were more frequent in phosphorus-limited ecosystems and they too invested little in sexual reproduction. The results provide new insight into how plant adaptations to nutrient conditions can drive the distribution of plant species in natural ecosystems and can account for the vulnerability of endangered species.

Species diversity is influenced both by overall nutrient availability and by nutrient stoichiometry—that is, how the ratio of available nutrients relates to their consumers' requirements⁵. In terrestrial plant communities the two nutrients that most frequently limit growth are nitrogen (N) and phosphorus (P)⁶. It has long been recognized that in sites that are N- or P-limited, the species assembly is different^{5,6}; this difference is also reflected in the occurrence of endangered species^{1–3,7,8}, most of

which are found in P-limited sites. The explanation of why these species are associated with P-limited sites may lie in their functional traits.

In general, fast-growing species dominate in nutrient-rich environments, whereas slow-growing species dominate in nutrient-poor conditions^{9,10}. With respect to the N:P stoichiometry, a number of studies indicate that low N:P-ratio environments favour fast-growing species with long roots, or species that fix N, whereas high N:P-ratio environments favour slow-growing species with specialized P uptake traits; for example, cluster roots, arbuscular mycorrhizae or high phosphatase activity^{3,11–16}. The association between fast-growing species and low N:P ratios is also consistent with the growth rate hypothesis¹⁷, which states that a fast growth rate is enabled by high investment in P-rich RNA, resulting in relatively high leaf P concentrations and concomitant low N:P ratios. However, these particular traits do not necessarily explain differences in total species richness along N:P availability gradients and, moreover, it seems possible that the selection for these traits may depend on environmental conditions other than the relative availability of N and P. We looked for an explanation based on inherent plant life-history traits, particularly investments in growth versus reproduction. We had access to a large comparative data set, which enabled us to separate the effects of overall nutrient availability from those of N:P stoichiometry across many species. In addition, by linking our traits analysis to the Red List statuses of the species involved (Red Lists of seven different countries), we were able to assess the mechanisms that might account for why certain species are more vulnerable for extinction than others.

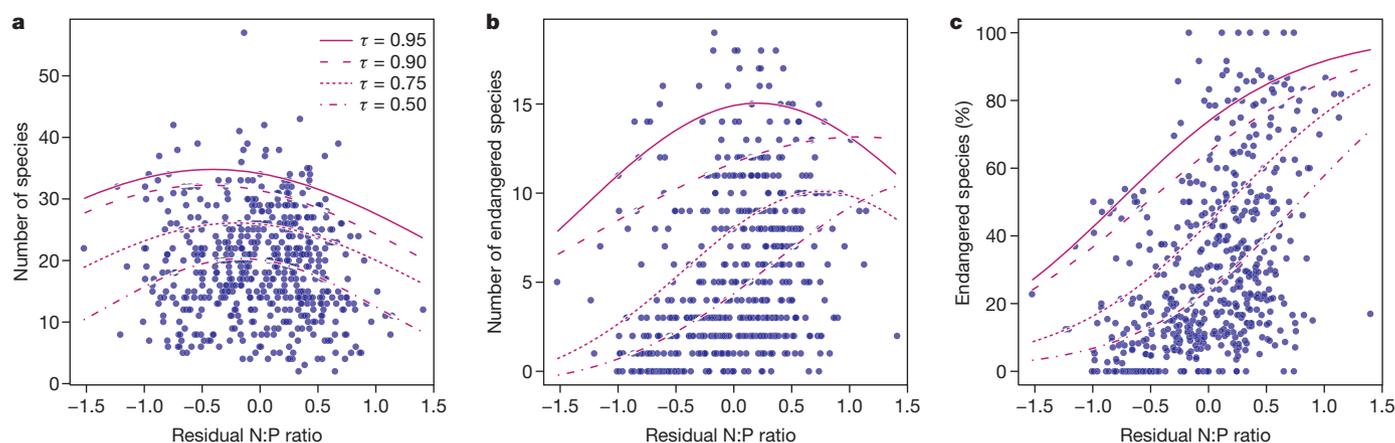


Figure 1 | Relationship between biodiversity indices of vascular plants and N:P ratio corrected for productivity effects. a–c, Tested biodiversity indices are the number of species (a), the number of endangered species (b), and the percentage of endangered species (c) (number of sites (n) = 539). N:P ratio was corrected for the confounding effects of productivity by using the residual

values of N:P ratio regressed by productivity (see Supplementary Discussion 1 and Extended Data Fig. 1). τ th quantile regression functions ($\tau = 0.50, 0.75, 0.90, 0.95$) are also shown. See Extended Data Fig. 2 for the 95% confidence intervals of the regression coefficients. The results were not biased by the selection of habitat types in our data set (Extended Data Fig. 3).

¹KWR Watercycle Research Institute, PO Box 1072, 3430 BB Nieuwegein, The Netherlands. ²Plant Biology and Nature Management, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium. ³Department of Ecological Science, VU University Amsterdam, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands. ⁴Centre for Crop Systems Analysis, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands. ⁵Faculty of Science, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands. ⁶Institute of Landscape Ecology, Faculty of Geosciences, University of Münster, Robert-Koch-Strasse 28, 48149 Münster, Germany. ⁷Department of Plant Ecology and Environmental Conservation, Institute of Botany, University of Warsaw, Al. Ujazdowskie 4, Warsaw, Poland. ⁸Warsaw University of Life Sciences, Faculty of Civil and Environmental Engineering, 02-722 Warsaw, Nowoursynowska 166, Poland. ⁹Biometris, Wageningen University, PO Box 100, 6700 AC Wageningen, The Netherlands. ¹⁰Environmental Sciences, Copernicus Institute of Sustainable Development, Faculty of Geosciences, Utrecht University, PO Box 80115, 3508 TC Utrecht, The Netherlands.

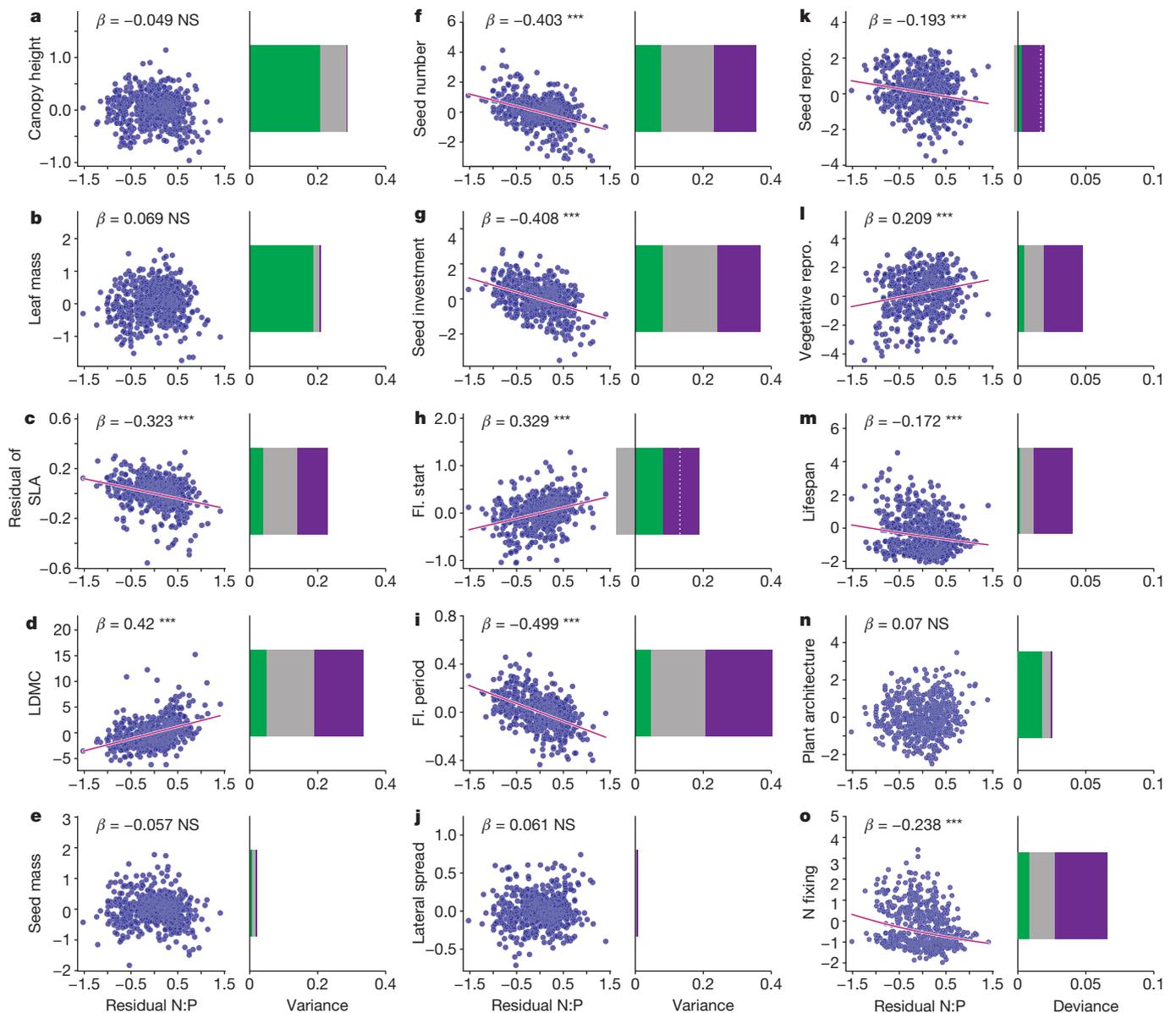


Figure 2 | Relationship between N:P ratios and community-mean trait values of herbaceous vascular plant species, after removing confounding effects of productivity. The residual values of N:P ratio (x axis) and community-mean traits (y axis; Extended Data Fig. 4) regressed by productivity were used (see Supplementary Discussion 1 and Extended Data Fig. 1). **a–o**, The 15 traits tested are canopy height (**a**, number of sites (n) = 530), leaf mass (**b**, n = 525), specific leaf area (**c**, n = 529), leaf dry matter content (**d**, n = 525), seed mass (**e**, n = 533), seed number per shoot (**f**, n = 524), seed investment (**g**, n = 523), month flowering started (**h**, n = 528), flowering period (**i**, n = 528), lateral spread (**j**, n = 526), reproduction by seeds (**k**, n = 528), vegetative reproduction (**l**, n = 528), lifespan (**m**, n = 531), plant architecture (**n**, n = 533), and N fixation (**o**, n = 502). Extended Data Table 1

The data set consisted of 599 field plots in herbaceous ecosystems in Eurasia, with data on plant species composition, aboveground productivity of vascular plants as a proxy for overall nutrient availability, and the N:P:potassium (K) ratio in the aboveground plant biomass as a proxy for nutrient stoichiometry (See Supplementary Discussion 1). Sites considered to be K-limited (n = 60, based on N:K and K:P ratios¹) were excluded from further analysis. Across the remaining part of the data set (all N- and/or P- (co-)limited, n = 539), species richness of vascular plants is highest at intermediate N:P ratios (N and P co-limitation; Fig. 1a), whereas the numbers of endangered species tend to be highest at higher N:P ratios (Fig. 1b) and the percentage of endangered species

gives abbreviations and trait units. Linear regression models are shown, plus their standardized coefficient values (β) and two-tailed P values of the coefficients (*** P < 0.001; NS, not significant). Bar graphs show fraction of variance (for continuous traits) or deviance (for binary traits) of community-mean trait values explained by productivity and N:P ratio. Variances in community-mean trait values are separated into unique effects of productivity (green) and N:P ratio (purple), and shared effects of productivity and N:P ratio (grey). Negative shared effects indicate that the trait is suppressed by interaction between productivity and N:P ratio. For these traits, total variance explained (dotted lines) is smaller than the sum of unique effects of N:P ratio and productivity.

rises as N:P ratios increase towards P limitation (Fig. 1c). For 446 herbaceous plant species occurring in the 539 plots, we retrieved 15 life-history plant traits related to nutrient acquisition, growth and reproductive strategy (see Extended Data Table 1). After accounting for potentially confounding effects of productivity on N:P ratio and community-mean trait values (see Extended Data Fig. 1), we found that plots with a high N:P ratio were significantly (P < 0.001) associated with the occurrence of species with a small investment in sexual reproduction (such as low seed number (Fig. 2f) and seed investment (Fig. 2g), late start of flowering (Fig. 2h), short flowering period (Fig. 2i), vegetative reproduction rather than reproduction by seeds (Fig. 2k, l), perennials rather than

annuals or biennials (Fig. 2m)) and less association with N fixers (Fig. 2o). Species occurring at high N:P ratio plots also had leaf traits characteristic of slow-growing species, such as low specific leaf area (SLA) (Fig. 2c) and high leaf dry-matter content (LDMC) (Fig. 2d). Some traits (such as canopy height, leaf mass and plant architecture (monocot or eudicot)) correlated strongly ($P < 0.001$) with productivity but not ($P > 0.05$) with N:P ratio, as shown by a dominant contribution of productivity to their explained variance (Fig. 2a, b, n). The relationships between N:P ratio and particular plant traits also became apparent when species were classified into ‘strategies’ according to a previous paper¹⁸; that is, the N:P ratios correlated positively with abundance of ‘stress tolerators’ ($P < 0.001$), correlated negatively with abundance of ‘ruderalers’ ($P < 0.001$), and were not significantly correlated with abundance of ‘competitors’ ($P > 0.05$) (Fig. 3). Furthermore, a principal component analysis (PCA) indicated that the trade-off between seed and vegetative reproduction was strongly correlated ($P < 0.001$) with N:P ratio, with the unique effect of N:P ratio being stronger (21% of variation explained) than that of productivity (9% of variation explained), a finding that confirmed the robustness of our analysis (See Supplementary Discussion 2).

We found three contrasting but not mutually exclusive relationships between nutrient availability or stoichiometry and life-history plant traits. First, overall nutrient availability—but not N:P stoichiometry—is related to traits involved in competition for light (for example, plant size, ‘competitor’ strategy). Second, both N:P stoichiometry and overall nutrient availability are related to leaf economy traits (for example, specific leaf area, leaf dry-matter content). Previous studies have shown that leaf economy traits are related to overall nutrient availability^{17,19}, but our results show that fast-growing species also have an affinity for low N:P ratio environments, independently of the overall nutrient availability effect. Third, N:P stoichiometry is related to investment in sexual reproduction almost independently of overall nutrient availability; that

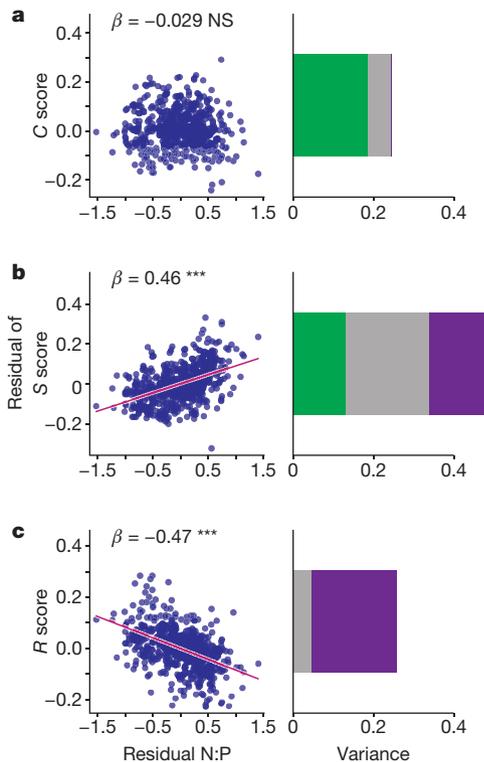


Figure 3 | Relationship between N:P ratios and community-mean values for C, S and R scores of CSR strategy after removing confounding effects of productivity. a, C (competitor) scores. b, S (stress tolerator) scores. c, R (ruderal) scores. Confounding effects of productivity were removed using the residual values of N:P ratio (x axis) and community-mean CSR scores (y axis) regressed by productivity. Number of sites (n) = 528; see Fig. 2 legend for figure specifications.

is, high N:P conditions (P limitation) correlated with low investment in sexual reproduction. Such low investment restricts P losses, since reproductive organs are P-rich²⁰. Classical allocation studies have shown that plants can invest up to 50 to 60% of all acquired P in sexual reproduction, and that this percentage is generally higher for P than for N and other elements^{21,22}. Impaired investment in sexual reproduction under P limitation is also supported by experimental data²³. An alternative strategy for a plant is to produce few seeds in order to maintain a high P concentration per seed, which is an important factor for successful recruitment in P-impooverished soils²⁴.

Our study clearly shows that endangered species have different suites of functional traits than non-endangered species (permutational multivariate analysis of variance (PERMANOVA), $F_{1,281} = 2.67$, $P < 0.05$). Compared with non-endangered species, they have a lower canopy height, less investment in sexual reproduction (fewer seeds and smaller seed mass per individual), a shorter flowering period and a later starting time of flowering, and are perennials rather than annuals (all differences significant at least at the $P < 0.05$ level) (Fig. 4). The lower reliance of endangered species on seed reproduction is also seen in the PCA axis scores (Supplementary Discussion 2), and confirms the findings of previous

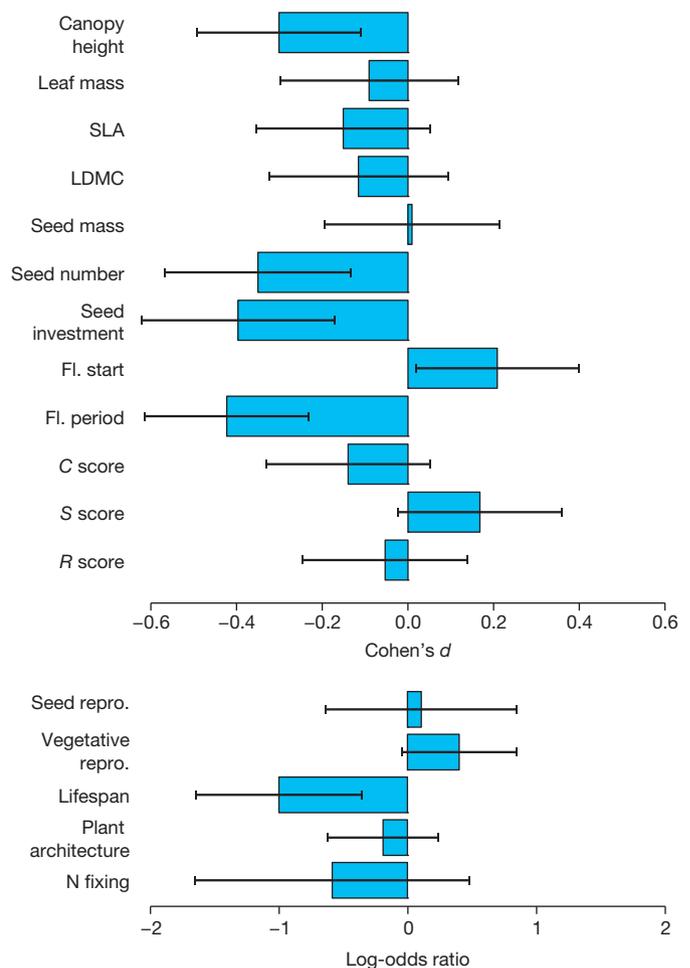


Figure 4 | Difference in trait values between endangered and non-endangered herbaceous vascular plant species. The difference was expressed with Cohen’s d for continuous traits, and with log-odds ratios for binary traits. The number of endangered versus non-endangered species examined are (from top to bottom); 150/267, 123/237, 128/246, 121/239, 126/243, 113/226, 101/223, 153/265, 153/265, 149/259, 149/259, 149/259, 149/253, 149/253, 155/268, 152/281 and 157/289. Positive values of Cohen’s d or log-odds ratios indicate that endangered species have higher trait values. 95% confidence intervals are shown as whiskers. Lateral spread, for which effect size could not be calculated, was not significantly different between endangered and non-endangered species (two-tailed Mann–Whitney U-test, $P > 0.05$).

studies comparing common with endangered or rare species (for example, a shorter flowering period²⁵, smaller seed mass²⁶ and poorer dispersal ability^{27,28}).

Endangered species occur more frequently under P-limited conditions (high N:P ratio environments) than can be explained by chance, as shown for temperate regions in our data set and in a previously studied much more limited data set² as well as for a tropical region⁸. Our trait analysis provides two possible explanations for the frequent occurrence of endangered species in P-limited conditions. The first is that endangered species are often small and are therefore poor competitors for light. Small size is a major disadvantage when growing in productive sites, but on poorly productive sites, which are associated with a high plant N:P ratio (see Supplementary Discussion 1), they face little competition. Second, the relatively low investment of endangered species in sexual reproduction is characteristic of plant species under P limitation (high N:P ratio environments). Thus, both increased productivity of ecosystems and changed N:P stoichiometry potentially threaten the survival of such species; moreover, their low dispersal capacity makes them vulnerable to such threats. The idea that endangered species are vulnerable for changes in the relative availabilities of N and P is supported by a global study showing that species with a narrow geographical range (that is, those more likely to become endangered) have higher leaf N:P ratios than those with a wide range²⁹. The exact mechanisms and potentially interacting processes that may explain why species vulnerable for extinction occur on P-limited sites need to be tested further. However, it is likely that large-scale P enrichment of herbaceous ecosystems that boosts productivity and ends P limitation causes species adapted to P limitation to be more vulnerable to extinction. Moreover, the low investment in sexual reproduction of these species, which is a beneficial trait in P-poor environments, is a drawback for their dispersal ability. N fertilization will probably not promote survival of endangered species, as there are a number of mechanisms for increasing P uptake from diverse forms of P in soil (for example, root exudates, mycorrhizae) under N-rich conditions³⁰, and therefore P limitation might not be enhanced by N enrichment. Instead, to better protect endangered species, we should aim to preserve P-limited and poorly productive sites. Given that these sites are already scarce and scattered, that landscapes are increasingly human-influenced and urbanized, and that endangered species have less sexual reproduction (and so are disadvantaged in long-distance dispersal), it is clear that these species' vulnerability for extinction is acute.

METHODS SUMMARY

Species composition of vascular plants, their aboveground biomass (as a proxy for site productivity), and N:P:K ratio in the biomass (as a proxy for relative availability of N, P and K for plants) were recorded in 599 plots in herbaceous ecosystems. The selected ecosystems range from wet to moist conditions and include grasslands, fens, bogs, marshes, reedland, floodplains and dune slacks in nine countries in Eurasia. As N:P stoichiometry is our focus, K-limited plots (N:K ratio >2.1 and K:P ratio <3.41; $n = 60$) were excluded from further analysis. Of the total 491 vascular plant species recorded, 172 endangered species were identified from the combined Red Lists of seven of the countries. We examined the effects of N:P ratio on biodiversity indices (number of species, number and percentage of endangered species) and community-mean values of 15 functional traits and Grime's CSR (competitor, stress tolerator, ruderal) strategy of 446 herbaceous species retrieved from trait databases (see Extended Data Table 1). In all analyses, the confounding effects of productivity on the variables of interest were statistically removed (Extended Data Fig. 1). For the effects of N:P ratio on biodiversity indices, quantile regression analysis was carried out between N:P ratios (corrected for productivity) and biodiversity indices for the 0.50 to 0.95 quantiles. The impact of N:P ratio on community-mean trait values (both corrected for productivity) was tested using path analysis, and the relative contributions of productivity and N:P ratio to community-mean trait values were quantified by partitioning the explained variance of traits to unique and shared effects of productivity and N:P ratio. Furthermore, differences between endangered and non-endangered species in terms of their functional trait composition were examined with a PERMANOVA. In addition, for each of 15 traits and CSR strategy, the differences between endangered and non-endangered species were examined by Cohen's d for continuous traits and log-odds ratio for binary traits.

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

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Supplementary Information is available in the online version of the paper.

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METHODS

Plot selection. We collected vegetation data on 599 plots from herbaceous ecosystems, including the 276 sites studied in ref. 2. The sample consists of grasslands, fens, bogs, marshes, reed beds and dune-slack vegetation. All plots are non-brackish, with moist to wet conditions, thus impacts of drought and salinity on species richness were avoided. Only 19 plots are lightly fertilized ($\leq 100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and 102 plots are exposed to periodic river flooding. All plots are dominated by herbaceous species (>50% cover). The plots were selected to span a wide geographical range of Eurasian countries: The Netherlands (255 plots), Poland (153), Russia (82), Germany (43), Belgium (20), Iceland (17), Sweden (10), Scotland (10) and Belarus (9). These plots encompass most of the west and central European lowlands. The flora in the country-wise partial data sets we collected is similar: on average, 86% (and a minimum of 70%) of the species recorded in any country had also been recorded in the other countries. For each plot, aboveground standing biomass of vascular plants was harvested at the peak of the growing season; that is, from June to August. The harvested area ranged from 0.06 m^2 to 1 m^2 . The content of N, P and K in the biomass was analysed after Kjeldahl digestion³¹. Composition of vascular plant species was recorded in or around the harvested area in plots of 0.06 to 25 m^2 . The different plot sizes did not affect the relationships between biodiversity and N:P stoichiometry (see Supplementary Discussion 3).

Functional characteristics of species. Per species, we quantified those functional traits available in trait databases^{32–38} for most of the recorded plant species that are known to be important for the growth or reproduction strategies of herbaceous plant species³⁹ (See Extended Data Table 1 for an overview of traits, units and sources). We excluded woody species (45 species out of the total 491 species) from the trait analysis, as most woody species recorded in our plots were seedlings and therefore the trait values available in databases (which are for adult individuals) are not relevant. The selected traits were those related to competition for light (canopy height, leaf mass), leaf economy traits (specific leaf area (SLA), leaf dry-matter content (LDMC)), seed traits (seed mass, number of seeds per individual, seed investment (that is, seed mass per individual, calculated as seed mass times number of seeds per individual)), phenology traits (starting month of flowering, duration of flowering period), reproduction strategy traits (lateral spread, type of reproduction, plant lifespan), a plant architecture trait (that is, eudicots or monocots), and a nutrient acquisition trait (N fixation) (Extended Data Table 1). We note that seed investment is a trait that may be biased by the size of the plant. However, even when we corrected seed investment roughly for plant size, the relationship between N:P ratio and this trait did not change (Supplementary Discussion 4). Duration of flowering period (expressed in months) and starting month of flowering (ranging from January to August, and thus coded 1 to 8) were treated as continuous variables. All continuous traits except LDMC and starting month of flowering were log-transformed to adjust the right-skewed frequency distributions. Type of reproduction, expressed on an ordinal scale with five classes, was converted into two binary variables (reproduction by seeds and vegetative reproduction) to be used in subsequent analyses. Additionally, the CSR strategy¹⁸ was attributed to each species by using seven traits (canopy height, LDMC, leaf mass, SLA, flowering period, starting month of flowering, and lateral spread) according to a method described previously⁴⁰. When this method could not be applied because trait information was incomplete, the CSR classification in BioFlor³⁶ was used. Ultimately, CSR classes were defined for 408 out of 446 herbaceous species. For each species, scores for each primary component (C, competitor; S, stress tolerator; R, ruderal) were assigned from its proportional contributions (for example, C scores are 1 for the strategy 'C', 0.5 for 'CS', 0.33 for 'CSR' and 0.75 for 'C/CR'⁴¹). In addition, we used a principal component analysis (PCA) to identify the major axes of variation in multiple functional traits (see Supplementary Discussion 2).

For each plot, community-mean values (unweighted for the abundance of species) of continuous traits were calculated as an indicator of the mean response of the plant community to the site conditions. We treated the community-mean values of lateral spread, an ordinal trait, in the same way as those of continuous traits, because they were approximately normally distributed. For binary traits, the number of species with 1s and 0s were counted per plot. Plots with fewer than three species with a valid trait value, and plots in which less than 50% of occurring herbaceous species had a valid trait value, were omitted from the analysis. The omitted plots ranged from 6 to 16 plots (average 10.7 plots) of the total (539 plots).

Endangered species. We compiled a list of endangered species by combining the regional Red Lists of the Netherlands⁴², Germany⁴³, Poland⁴⁴, Sweden⁴⁵, UK⁴⁶, Iceland⁴⁷ and the Novosibirsk region in Siberia⁴⁸. The Red List of Belgium was not included, because the Belgian plots were near the border with the Netherlands and their flora was comparable with the flora in the Dutch plots. We also did not use the list of Belarus, because the number of plots in this country was small ($n = 9$) and the flora in these plots overlapped with those of Poland. The Red List status of a species reflects both the decline of the habitat in the region and the susceptibility of the species to the changing environment. Some species are Red

Listed in one country but not in others, because in those countries their habitat is not deteriorating. For our analysis, only the susceptibility of an endangered species is relevant, not the region-specific habitat deterioration, because we are interested in the mechanisms whereby species become endangered (that is, their functional traits). Therefore, we identified a species as 'endangered' if it is on at least one regional Red List (meaning that this species has fragile characteristics that are susceptible to environmental changes), and applied this new list to all plots. In this way we corrected for habitat loss, which is largely region-specific. Note that we included the categories which refer to actual decline of the species ('critically endangered', 'endangered' and 'vulnerable' species), but not the category which refers to the scarcity of the species ('rare'). In this way, we excluded species which are always rare irrespective of environmental change. Of our 491 vascular plant species, we identified 172 endangered species (157 herbaceous and 15 non-herbaceous species). For each plot, we counted number and percentage of endangered species. The latter is the number of endangered species divided by the total number of species ($\times 100$) per plot.

Data analysis approach. We are interested in the effects of nutrient stoichiometry (that is, the ratio between N and P availability for plants) on species diversity and on prevailing functional traits of species in plant communities, irrespective of confounding effects of overall nutrient availability. We use N:P ratios of aboveground plant biomass as a proxy for nutrient stoichiometry (see Supplementary Discussion 1 for a justification of using plant N:P ratio), and site productivity (that is, aboveground biomass of vascular plants) as a proxy for overall nutrient availability. As the consideration of N:P ratio is relevant only when a plot is limited or co-limited by N or P, we excluded K-limited plots ($n = 60$) from the analysis. We considered a plot as K-limited if the N:K ratio was more than 2.1 and the K:P ratio was less than 3.4 (ref. 1).

We propose the following relationships between plant N:P ratio, site productivity, species diversity and community-mean species traits (see also Extended Data Fig. 1). Plant N:P ratio and site productivity are related through a scaling law proposed by the growth rate hypothesis^{29,49} (arrow *a* in Extended Data Fig. 1): plants grown in fertile environments (these tend to be fast-growing species) exhibit low biomass N:P ratios because of the high amount of P-rich RNA needed for rapid division of cells^{50,51} (see Supplementary Discussion 1 for more details). We are aware of the opposite direction of effect too (that is, N:P ratio influencing site productivity), particularly at extreme values of N:P ratios, where a deficiency of N or P limits site productivity, but we consider this effect to be minor. Furthermore, we posit that both species diversity and community-mean trait values are influenced by site productivity and N:P ratio. However, there is an intrinsic difference between how these drivers affect species richness and how they affect community-mean traits. Site productivity and N:P ratio 'filter' the community-mean traits (arrows *e* and *d*, respectively, in Extended Data Fig. 1b), acting on the mean of the community-mean trait values. In contrast, site productivity and N:P ratio 'limit' species diversity (arrows *c* and *b*, respectively, in Extended Data Fig. 1a), acting on the upper values of species diversity. Given the different nature of the relationships, we employed two sets of methods to eliminate potential confounding factors from our analysis. All analyses were performed in R⁵².

Effects of N:P ratio on species diversity. A relationship between a dependent variable (species diversity) and one or more limiting factors (site productivity and N:P ratio) can be tested with quantile regression analysis. Unlike conventional regression, which considers solely changes in the mean of the response variable, quantile regression excludes the effect of unmeasured limiting factors⁵³. It is therefore a powerful method to analyse the change in the potential species diversity as a function of the limiting factor; that is, N:P ratio, only.

We used three indices to assess species diversity: number of species (y_1), number of endangered species (y_2), and percentage of endangered species over total number of species (y_3 , which equals to $100 \cdot y_2 / y_1$). For the number of species and number of endangered species, we assumed a quadratic effect of N:P ratio, since previous studies suggested that on an N:P ratio gradient there is an optimum biodiversity rather than a continuously increasing biodiversity². Both response variables were log-transformed (that is, $\ln(y_1)$ and $\ln(y_2 + 1)$) to correct the right-skewed frequency distributions. For the percentage of endangered species, a logistic quantile regression analysis was applied to restrict the prediction to between 0 and 100. Here we used 'empirical logits'⁵⁴, $\ln((y_2 + 0.5)/(y_1 - y_2 + 0.5))$, instead of normal logits, $\ln(y_2/(y_1 - y_2))$, to enable computation of logits when a plot has 0% or 100% of endangered species. Effects of N:P ratio on species diversity indices (arrow *b* in Extended Data Fig. 1a), irrespective of the confounding effects of site productivity on N:P ratio (arrow *a* in Extended Data Fig. 1a), were examined by using 'the residual values of N:P ratio versus productivity' (obtained from a linear regression model on a log-log scale) as an explanatory variable.

For each diversity index, the τ th quantile regression function $B_{(\tau)}$ was determined, indicating the approximately τ th proportion of the observations found to be below $B_{(\tau)}$ (ref. 55). As we are interested in the upper boundary of the relationship

when N:P ratio actively limits biodiversity, we examined high values of τ only ($\tau > 0.50$). To evaluate the precision of the obtained model, 95% confidence intervals of the coefficients were computed with the rank inversion method⁵⁵ (Extended Data Fig. 2). These analyses were performed with the R package 'quantreg'⁵⁵.

The analyses were run for the complete data set. A control run was carried out on the data set excluding two dominant habitat types (fens and bogs). This tested whether our analysis results could have been biased by habitats that may be severely deteriorated (for example, fens and bogs in western Europe with desiccation problems) or that have intrinsically high or low N:P ratios (for example, bogs tend to have high N:P ratios because this rainwater-fed ecosystem receives relatively high N supply through atmospheric deposition but poor P supply) (Extended Data Fig. 3). We did not find evidence for such bias.

We also tested whether other factors potentially related to P limitation (for example, soil acidity, soil moisture) confound the association between N:P ratio and richness of endangered species (Supplementary Discussion 5), but this was not the case.

Effects of N:P ratio on community-mean traits. Our aim was twofold: to examine, first, the significance of N:P ratios in affecting community-mean trait values; and second, the explained variation in community-mean trait values by N:P ratios in addition to and in interaction with productivity.

First, we tested the association between N:P ratio and community-mean traits in the proposed relationships between N:P ratio, productivity and community-mean traits (Extended Data Fig. 1b) using concepts of path modelling⁵⁶. Note that we proposed the causal model as depicted in Extended Data Fig. 1b to be true, even though causality as such cannot be tested in this model because the hypothesized model is 'saturated' (that is, all the possible interconnections are specified⁵⁷). However, it is still possible to refute the existence of a relationship between N:P ratio and community-mean trait values (arrow d in Extended Data Fig. 1b) by testing the significance of this association while statistically holding productivity constant⁵⁶. More concretely, the association between N:P ratio and community-mean values of a trait was examined from the relationship between the residuals of community-mean trait values regressed by productivity ($res_{tr,p}$) and the residuals of N:P ratio regressed by productivity ($res_{NP,p}$). When the regression coefficient of $res_{tr,p}$ regressed by $res_{NP,p}$ was not significantly different from zero, we concluded that there was no significant association between N:P ratio and the community-mean trait. $res_{tr,p}$ were derived from logistic models with negative binomial distribution for the binary traits, and from linear models for continuous traits, using the generalized linear model (GLM) (Extended Data Fig. 4). To test the relationship between $res_{tr,p}$ and $res_{NP,p}$ we assumed linear regression models (after square-root-transformation of $res_{tr,p}$ for lifespan and log-transformation of $res_{tr,p}$ for N fixation). The residuals of the linear regression models did not deviate from a normal distribution ($P > 0.05$ with Kolmogorov–Smirnov test except for SLA ($P = 0.004$), lateral spread ($P = 0.046$) and N fixation ($P = 0.01$); for these three traits we also checked the $res_{tr,p} - res_{NP,p}$ relationship with Spearman's correlation analysis: our conclusions were identical). To visualize the effects of N:P ratio on community-mean traits (Fig. 2 and Fig. 3), we have shown $res_{tr,p}$ against $res_{NP,p}$ and the significance and strength of this relationship (β , the standardized regression coefficient). In a path analysis context, β corresponds to a relationship between N:P ratio and community-mean traits for which direction of the causality is not prescribed. The significance levels of β s and the path coefficients (which prescribe the direction of causality) were nearly identical (results not shown), showing that our conclusion on the existence of association between community-mean traits and N:P ratio is robust against the assumed direction of the causality.

Second, to examine the explained variation in community-mean trait values by N:P ratios in addition to and in interaction with productivity, we partitioned the variation in community-mean trait values into values explained by unique effects of N:P ratio and productivity and their shared effects. We did so by sequentially calculating the measures of goodness-of-fit (G) of regression models⁵⁸. In this way, the relative contribution of N:P ratio and productivity on community-mean trait values can be quantified irrespective of the underlying multicollinearity among three variables⁵⁹. As a measure of G , we used log likelihood for logistic models and R^2 for linear models. Unique effects of N:P ratio were calculated as an increase in G in the model of community-mean traits regressed by productivity and N:P ratio ($G(X_1, X_2)$) compared to that regressed by productivity only ($G(X_2)$). Unique effects of productivity were calculated identically, as an increase in $G(X_1, X_2)$ compared to $G(X_1)$. Shared effects of productivity and N:P ratio were calculated as: $G(X_1) + G(X_2) - G(X_1, X_2)$.

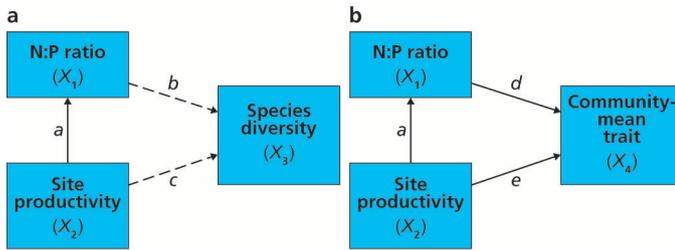
For some traits, shared effects were negative, indicating that the trait is suppressed by interaction between productivity and N:P ratio.

Traits of endangered species versus non-endangered species. The difference in a suite of functional traits (11 traits including all the continuous- and ordinal-scale traits) between endangered and non-endangered species was tested with PERMANOVA⁶⁰ using the R package 'vegan'⁶¹ for 283 herbaceous species (for which full data were

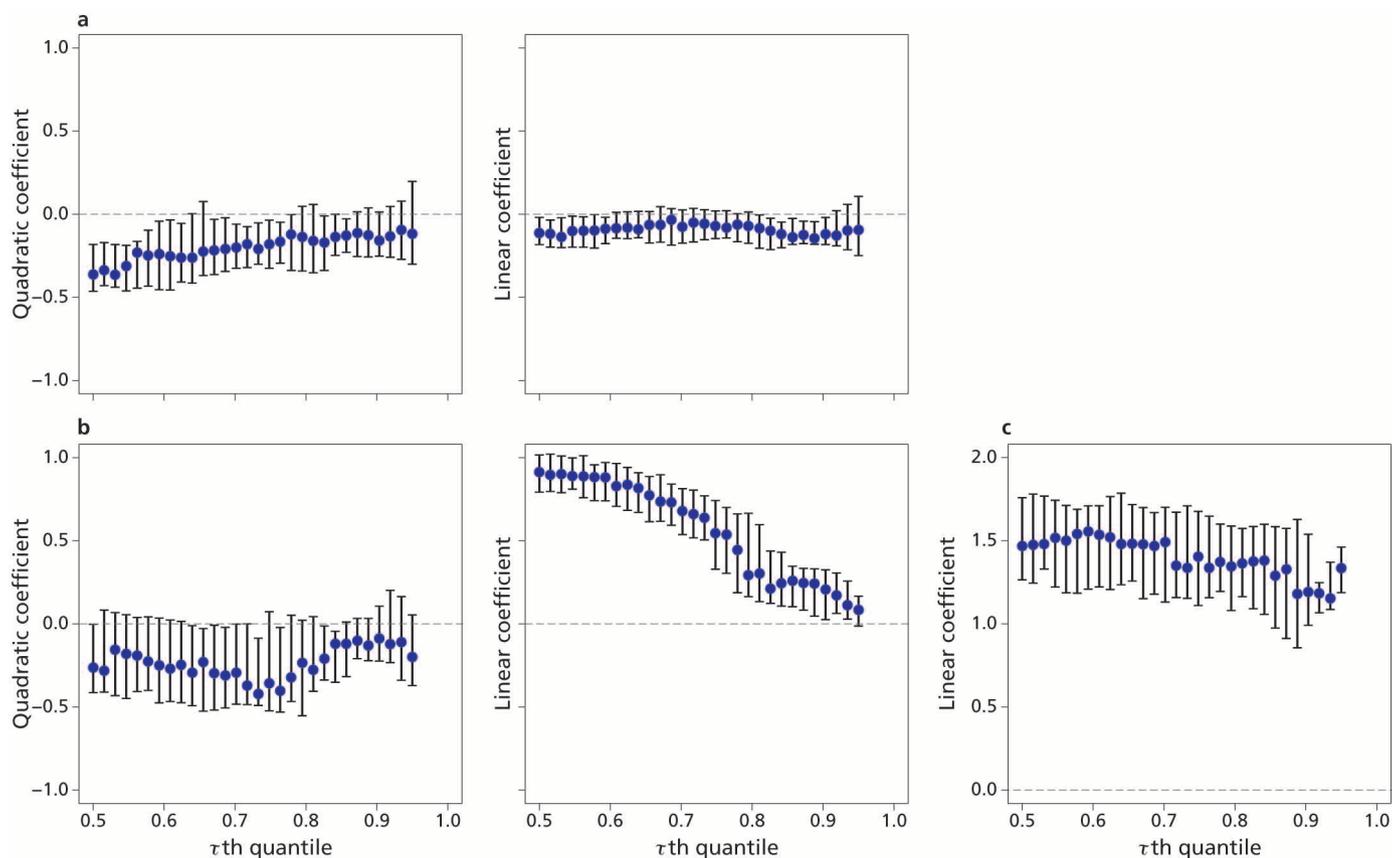
available for the 11 traits). The distance matrices were calculated based on Gower's distance. The relative difference in each functional trait between endangered and non-endangered species was then quantified by calculating the effect size. Positive values for the effect size mean that endangered species have higher trait values than non-endangered species. For continuous traits, we used Cohen's d as effect size measure. Canopy height, leaf mass, SLA, seed mass, number of seeds, seed investment, and flowering period were log-transformed before the analysis. The equations of Cohen's d and its 95% confidence intervals⁶² are shown in Supplementary equation (1). For traits that were only marginally normally distributed (flowering start, flowering period, C, S, R scores; $P < 0.05$ with Kolmogorov–Smirnov test), the difference between endangered and non-endangered species was also tested with the Mann–Whitney U-test; the conclusions were identical. For binary traits, the effect size was examined by the log-odds ratio. The equations of log-odds ratio and its 95% confidence intervals⁶⁴ are shown in Supplementary equation (2). Note that the scales of Cohen's d and log-odds ratio are different, so the effect size of continuous and binary traits cannot be compared. For ordinary traits, effect size cannot be calculated. For these traits, the Mann–Whitney U-test was used to test the difference between endangered and non-endangered species.

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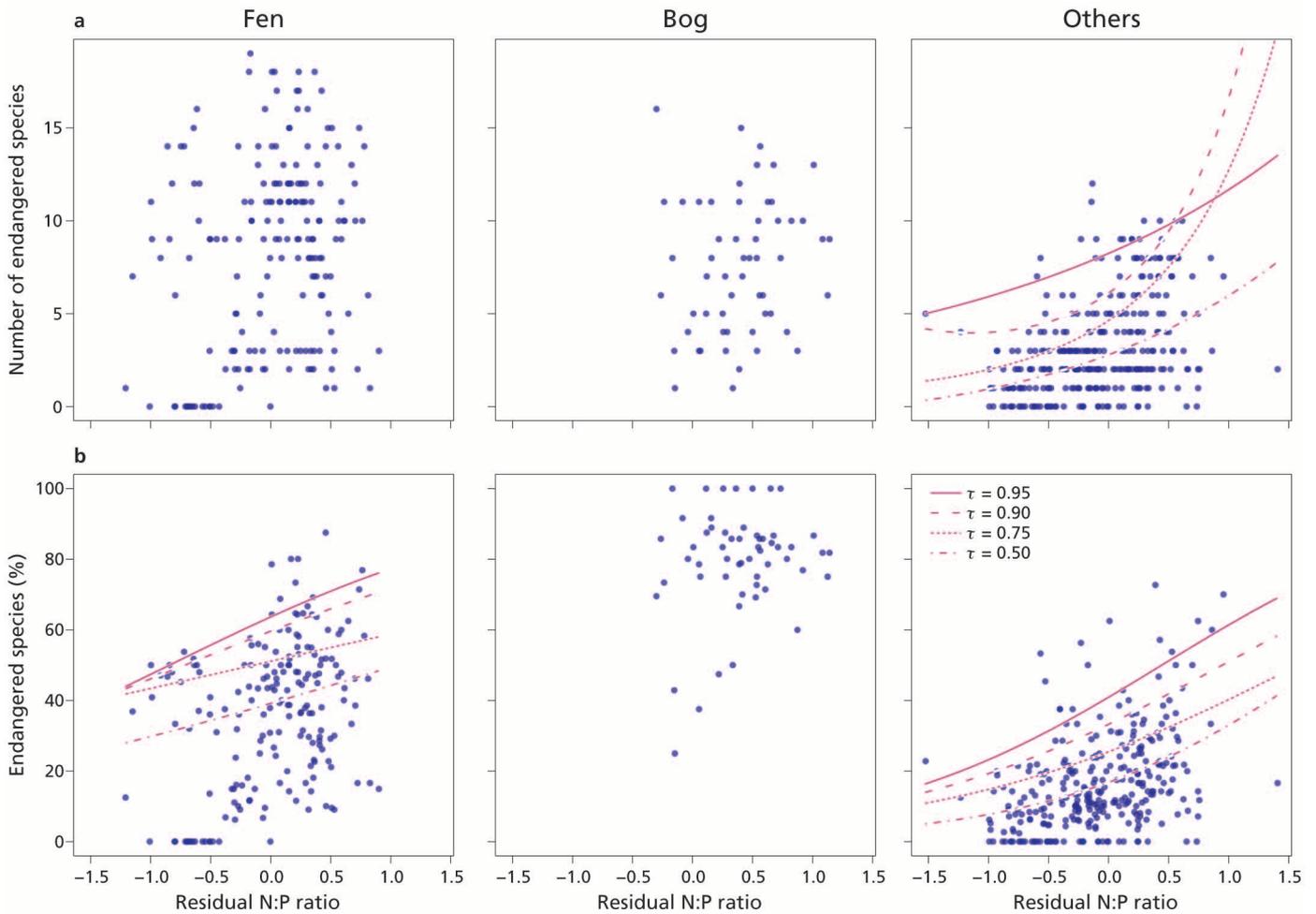


Extended Data Figure 1 | Data analysis approach. **a, b,** Schematic proposed relationships between site productivity (that is, aboveground biomass of vascular plants; X_2), N:P ratio in aboveground plant biomass (X_1), and species diversity (**a**; X_3) or community-mean traits (**b**; X_4). Solid arrows are relationships in which the explanatory variable is constrained by the response variable (direct causality); dashed arrows are relationships in which upper bound of the explanatory variable is constrained by the response variable (limitation). Arrow *a* represents the pattern predicted by the growth rate hypothesis (see Supplementary Discussion 1 for details). The effect of N:P ratio on species diversity (arrow *b*) was tested by quantile regression analysis (thus treating arrow *c* as another limiting factor) with the residual values of X_1 versus X_2 as an explanatory variable (thus removing the effect illustrated by arrow *a*). The effect of N:P ratio on a community-mean trait (arrow *d*) was tested by comparing the residual values of X_1 versus X_2 (thus removing the effect illustrated by arrow *a*) with the residual values of X_4 versus X_2 (thus removing the effect illustrated by arrow *e*), using concepts of path analysis.



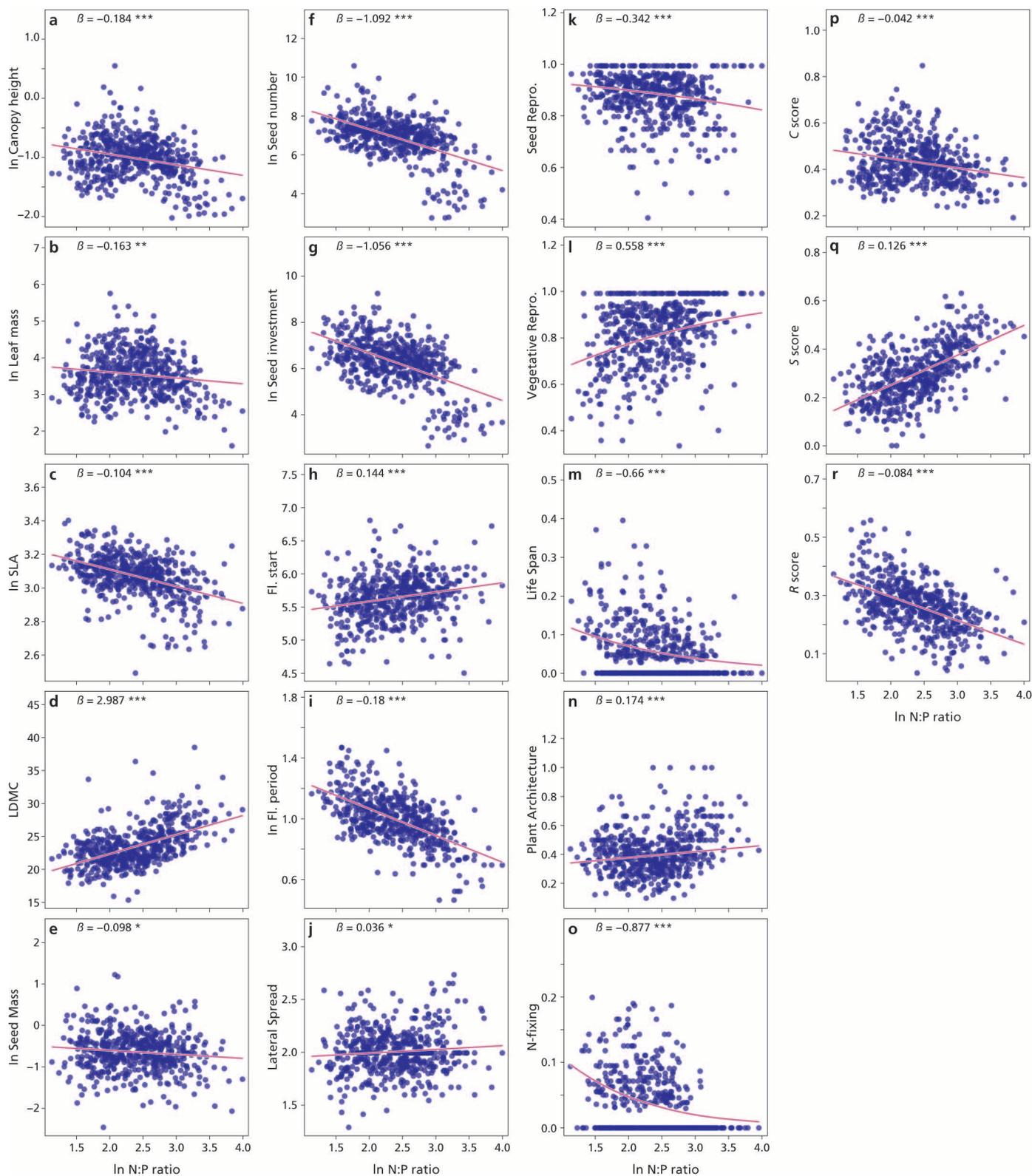
Extended Data Figure 2 | Ninety-five per cent confidence intervals of the quantile regression coefficients. a–c, Estimates (dots) and 95% confidence intervals (bars) of quadratic and linear coefficients (b_2 and b_1 , respectively) of quantile regression models are shown for the number of vascular plant species (a), the number of endangered species (b), and the percentage of endangered species (c) regressed by N:P ratio corrected for productivity effects. The fitted models were (y_1): $\ln(y_1) = b_0 + b_1x + b_2x^2$ for number of species;

(y_2): $\ln(y_2 + 1) = b_0 + b_1x + b_2x^2$ for number of endangered species; and ($y_3 = 100*y_2/y_1$): $\ln((y_2 + 0.5)/(y_1 - y_2 + 0.5)) = b_0 + b_1x$ for percentage of endangered species, where x is the residuals of plant N:P ratio regressed by productivity. Models were examined for 50% ($\tau = 0.50$) to 95% ($\tau = 0.95$) quantiles. See Fig. 1 for the shape of the quantile regression models for $\tau = 0.50, 0.75, 0.90, 0.95$.



Extended Data Figure 3 | Effects of habitat types on relationships between residual N:P ratio and biodiversity indices. Relationships between N:P ratio corrected for productivity effects and the number of endangered species (a) and percentage of endangered species (b) are shown for different habitat types (left, 187 fens; middle, 56 bogs; and right, 296 other habitat types). Linear, rather than quadratic, quantile regression models were applied because for most quantiles the quadratic coefficients did not differ significantly from zero. τ th linear quantile regression models ($\tau = 0.50, 0.75, 0.90, 0.95$) are shown only

when the 95% confidence intervals of the linear coefficients of the regression models were above or below zero for the majority of the quantiles. Number and percentage of endangered species increased concomitantly with increasing N:P ratio (corrected for productivity) even in plots that are not fens and bogs, indicating that our findings on the relationship between N:P ratio and endangered species were not an artefact resulting from the stratified sampling of habitat types.



Extended Data Figure 4 | Relationships between community-mean trait values and plant N:P ratio. a–r, The tested traits are canopy height (a, number of sites ($n = 530$)), leaf mass (b, $n = 525$), specific leaf area (c, $n = 529$), leaf dry-matter content (d, $n = 525$), seed mass (e, $n = 533$), seed number per shoot (f, $n = 524$), seed investment (g, $n = 523$), starting month of flowering (h, $n = 528$), flowering period (i, $n = 528$), lateral spread (j, $n = 526$), reproduction by seeds (k, $n = 528$), vegetative reproduction (l, $n = 528$), life span (m, $n = 531$), plant architecture (n, $n = 533$), N fixation (o, $n = 502$), C score (p, $n = 528$), S score (q, $n = 528$) and R score (r, $n = 528$). See Extended

Data Table 1 for abbreviations and units of the traits. Canopy height, leaf mass, specific leaf area, seed mass, number of seeds, seed investment, and flowering period were log-transformed before the calculation of community-mean values. For binary traits, plot mean values were shown as a fraction of species with 1s over total species (that is, sum of 1s and 0s) to allow graphical presentation. Standardized regression coefficients (β) of community-mean trait regressed by N:P ratio using GLM and their two-tailed p -values ($***P < 0.001$, $**P < 0.01$, $*P < 0.05$) are shown.

Extended Data Table 1 | List of functional traits of herbaceous vascular plant species. 14 species functional traits were retrieved from trait databases, and 2 binary traits ('Reproduction by seeds' and 'Vegetative reproduction') were derived from an ordinal trait ('Type of reproduction'). For regression analyses of community-mean trait values (Fig. 2) and for effect-size calculation (Fig. 4), we did not use 'Type of reproduction' but the two derived binary traits instead. The traits were retrieved for 446 herbaceous vascular plant species. Canopy height, leaf mass, SLA, seed mass, number of seeds, seed investment, and flowering period were log-transformed before all analyses.

Trait	Scale and Unit	% of species with a trait value	Source
Canopy height	Continuous; m	93	32, 33
Leaf mass	Continuous; mg	81	33
Specific leaf area (SLA)	Continuous; mm ² /mg	84	33
Leaf dry matter content (LDMC)	Continuous; %	81	33
Seed mass	Continuous; mg per seed	83	33, 36, 38
Number of seeds	Continuous; number per shoot	76	33
Seed investment	Continuous; mg per shoot	73	Seed mass x Number of seeds
Starting month of flowering	Continuous*†; month	94	32, 36
Duration of flowering period	Continuous*; month	94	32, 36
Lateral spread	Ordinal‡; 0: annuals, 1: <0.01m, 2: 0.01-0.25m, 3: >0.25m	85	34, 35
Type of reproduction §	Ordinal; 1: s, 0.75: ssv, 0.5: sv, 0.25: svv, 0: v	90	36
Reproduction by seeds	Binary; 1: yes (s/ssv/sv), 0: no or seldom (svv/v)	90	Derived from 'Type of reproduction'
Vegetative reproduction	Binary; 1: yes (v/vvs/sv), 0: no or seldom (ssv/s)	90	Derived from 'Type of reproduction'
Life span	Binary; 1: annual or biennial, 0: Perennial	95	32, 33, 36
Plant architecture	Binary; 1: monocot, 0: eudicot	97	32
N-fixation	Binary; 1: nodulated legume, 0: others	100	37

* Month is strictly speaking an ordinal scale, but treated here as a continuous scale.

† Range from January to August, thus coded as 1 to 8.

‡ Community-mean values of lateral spread were treated as continuous variables, as lateral spread does not deviate from normal distribution.

§ Expressed as the relative dependency on seed reproduction. The original categories in the database are: s (by seeds), ssv (mostly by seeds), sv (both by seeds and vegetatively), vvs (mostly vegetatively), v (vegetatively).