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Phosphorus fertilization is eradicating the niche of northern Eurasia's threatened plant species

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The greater bioavailability of nitrogen (N), phosphorus (P) and potassium (K) in the Anthropocene has strongly impacted terrestrial plant communities. In northwest Europe, because high N deposition is considered the main driver of plant diversity loss, European Union (EU) legislation to reduce N deposition is expected to promote plant species recovery. However, this expectation is simplistic: it ignores the role of other macronutrients. Analysing the relationship between plant species pools and species stoichiometric niches along nutrient gradients across northern Eurasia's herbaceous ecosystems, we found that both absolute and relative P availability are more critical than N or K availability. This result is consistent with stoichiometric niche theory, and with findings from studies of hyperdiverse forests and shrublands at lower latitudes. We show that ecosystems with low absolute and relative P availability harbour a unique set of threatened species that have narrower nutrient-based niche widths than non-threatened species. Such ecosystems represent a conservation priority, but may be further threatened by latent effects of relative P enrichment arising from reduction of N availability without simultaneous reduction of P. The narrow focus of EU legislation on reducing N, but not P, may therefore inadvertently increase the threat to many of Europe's already threatened plant species. An EU Phosphate Directive is needed.

Since the onset of the Industrial Revolution, anthropogenic modification of global biogeochemical cycles has doubled the rate at which biologically available nitrogen (N) enters the terrestrial biosphere[1,2] and more than doubled phosphorus (P) inputs to the environment[2]. The resulting perturbations of ecosystems worldwide may exceed the planetary boundaries that define a safe operating space for humanity[3,4]. Together with potassium (K), N and P are the main macronutrients that limit plant growth in natural ecosystems; increased bioavailability of these three macronutrients has direct impacts on terrestrial plant community diversity[5–8].

Among the many different natural factors influencing macronutrient availability are climate, bedrock composition, soil type and disturbance history[9,10]. At the global scale there is a latitudinal gradient of increasing N/P ratios from temperate regions to the tropics[11]. This pattern is consistent with the notion that erosion, leaching and the prolonged absence of soil rejuvenation processes lead to P depletion in old, climatically buffered infertile landscapes[12,13]. Importantly, species of hyperdiverse forests and shrublands within these landscapes have evolved a suite of adaptations to P-impoverished conditions[13,14]. Studies of chronosequences comparing soils from the same parent material but of different age have shown that plant diversity increases with soil age, and thus with declining P availability[15–18].

The repeated glaciations during the Quaternary in northern Eurasia left exposed substantial amounts of P- and K-rich rocks and unweathered sediments[9], providing ecosystems with a steady supply of P and K. Because the N cycle is slow in temperate and boreal latitudes, here autochthonous N was poorly available and so the recent increases in N availability have had a dramatic impact[7]. Nitrogen enrichment is therefore considered a major cause of species loss in northwest European herbaceous ecosystems[19–21], in accordance with ecological theory describing how shifts from competition for soil resources to competition for light may trigger exclusion of species adapted to low nutrient supply[6,22]. Within the northern Eurasian context, one would then expect currently threatened species to be those adapted to low N availability and that, to conserve the nutrient-based niches of these species, anthropogenic N inputs should be reduced[19–21].

The current concept of resource limitation is more nuanced, however. The paradigm of single-nutrient limitation has expanded to accommodate concepts of co-limitation by multiple nutrient, and plant species are now thought to occupy stoichiometric nutrient niches[23–25]. At the plant community level, co-limitation of nutrients may emerge because different plant species are limited by a different nutrient[22,26,27]. Support for this supposition is provided by widespread observations of synergistic impacts of the addition of N, P and K on plant community productivity and diversity[23,28–31]. Although these observations imply the need to assess stoichiometric plant niches involving multiple nutrients, no such assessment has yet been undertaken for northern Eurasian ecosystems and hence it remains unclear how changes in the bioavailability of N, P and K may affect the persistence or disappearance of the nutrient-based niches of threatened plant species.

To better understand the potential consequences of changes in the bioavailability of N, P and K for terrestrial plant community diversity, we analysed a dataset of 673 plots in herbaceous ecosystems from eight countries in northern Eurasia (Methods). In these plots, species composition of vascular plants was recorded and N, P and K concentrations in above-ground vegetation were measured. The dataset includes 574 vascular plant species, 216 of which are threatened species on European Red Lists. For our analysis, we estimated the species pools for each plot based on co-occurrence patterns[32] (Methods). This estimation reflects the number of species that could potentially occur in a given plot, assuming no dispersal limitations. Using the estimated species pools, we calculated nutrient-based niche optima and niche widths of plant species and compared these with observed species occurrences. Plot species richness, as well as nutrient-based niche optima and widths, were also calculated from

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the observed species occurrence data. Comparisons between the two approaches are presented in Supplementary Discussion 1.

Results and discussion

Species pool sizes. We found that the species pool size remained relatively constant along the absolute N (range of the regression line (RRL), 15 species) and K gradients (RRL, 12 species), with little deviance explained (N, 7.1%; K, 4.2%) (purple symbols in Fig. 1a,e). However, the species pools showed strong association with absolute P availability (RRL, 44 species), with 31.6% of deviance explained (Fig. 1c). For low P availability, the species pool size was small (regression estimate, minimum of 24 species) but, with increasing P availability, it increased steeply towards values similar to those for the N and K pools (regression estimate, maximum 68 species). Above the critical threshold for P limitation of 1 mg g^{-1} (refs. [33,34]), the pool size remained constant. Similarly, the pool size decreased as nutrient ratios increased along the gradients of relative availability of N/P and K/P (Fig. 1g,k). Hence, vascular plant species richness in northern Eurasian herbaceous ecosystems seems to be relatively constant along nutrient gradients of N and K but is lower for ecosystems where absolute and relative availability of P are low. The same trends were present when using the observed species richness in plots rather than the estimated species pool size (Extended Data Figs. 1 and 2).

Surprisingly, the trend in diversity of threatened species only (red symbols in Fig. 1) along the P availability gradient differs strongly from that in total species pool size (Fig. 1c). The largest threatened species pools occur under low P availability (regression estimate, maximum 24 species). At absolute P availabilities below the threshold value of 1 mg g^{-1}, ~60% of all observed species are threatened; this falls to as low as ~5% at high P availabilities (Fig. 1d; the average proportion of threatened species in the total dataset was 37.6%). Similarly, when the relative availability of P is low (that is, high N/P ratio; Fig. 1g), species pools contain a large proportion of threatened species (Fig. 1h). Remarkably, the decrease in the proportion of threatened species relative to all species (Fig. 1d) is not linear but asymptotic, suggesting that at low P availability minor increases in P availability result in rapid loss of threatened species.

Nutrient niches. The patterns described above are supported by an analysis of the niches occupied by plant species along absolute and relative nutrient gradients (Fig. 2). Along absolute nutrient gradients (Fig. 2a,c,e), the largest proportion of species (40.3%) comprises those that have an optimum niche in P-limited conditions (Fig. 2c); fewer species have an optimum niche that is N or K limited (32.7% in N limitation and 10.3% in K limitation: Fig. 2c,e, respectively). The distribution of species optima along gradients of relative nutrient availability (Fig. 2g,i,k) shows that most species also have their optimum niche in communities where neither P nor K is limited relative to N (Fig. 2g,i). We found that hardly any species have their optimum in the K-limited range (12 and 1%; Fig. 2i,k, respectively). Focusing on relative N and P availability (Fig. 2g), it appears that only very few species (8.8 %) have their optimum niche at intermediate N/P ratios, whereas 58.5% have their optimum in the N-limited range and 32.7% in the P-limited range. Threatened species show a remarkably different distribution along these gradients (red bars in Fig. 2). Most threatened species have their optimum niche at low absolute P availability (Fig. 2c,d) and at high N/P (Fig. 2g,h) and high K/P ratios (Fig. 2k,l), so at low absolute and relative P. Moreover, niches of threatened species are significantly narrower than those of non-threatened species (Extended Data Fig. 3). The narrower niche width of threatened species for relative availabilities (N/P, N/K, K/P) shows that these species are more sensitive to changes in stoichiometric balances than non-threatened species. Importantly we found that, contrary to what would be expected if N enrichment were the main driver of species loss, these narrow niches of threatened species (Extended Data Fig. 3) occurred in the P-limited part of the gradient rather than in the N-limited part (Fig. 2g). All these results are in line with findings from previous studies suggesting that adaptation to low P availability can be considered a stress-tolerant strategy[28,35] that comes at the cost of reduced stoichiometric and biogeochemical plasticity[25,36,37]. This limited trait plasticity may confine species adapted to low P to environments where adaptation gives them a competitive advantage over other plant species[36,38–40]. Even in the relatively young and fertile landscapes of northern Eurasia this adaptation is important[41], given the substantial proportion of the species pool that has its niche optimum under P-limited conditions (Fig. 2g).

Stoichiometric niches. Although absolute P availability and N/P ratio were strongly correlated (Extended Data Fig. 4 and Supplementary Discussion 2), a previous study[24] showed that a unique part of the variation in species composition across northern Eurasia was explained by the N/P ratio independently from conventional explanatory variables: the N/P ratio was less influential than soil moisture or pH but more influential than productivity or the absolute availability of N and P. That finding highlights the importance of N/P stoichiometry for species composition. Moreover, that study appears to confirm predictions generated by mathematical plant competition models that plants competing for multiple nutrients have stochiometric niches and that species turnover responds to changes in relative nutrient availability[22].

Our analysis of a large, continental-scale dataset yields empirical evidence that plant community composition patterns can be explained by stoichiometric niche theory, thereby underlining the risk that species from the northwest European species pool will go extinct as a result of subtle changes in N/P stoichiometry and be replaced by commoner species—a widespread response to environmental change[42–44]. We therefore also investigated whether changing the stoichiometric balance between N and P to reflect current policies aimed at reducing atmospheric N deposition would affect species numbers (Fig. 3). We found that the largest number of species niches would occur at intermediate levels of N, suggesting that if N deposition in high-deposition areas were reduced, plant species diversity could indeed recover. However, if N availability were to fall from intermediate to low levels, the effect would be reversed because of a decrease in the number of stoichiometric niches. Lowering of P availability to almost 1 mg g^{-1} would benefit both non-threatened and threatened species, and this effect would be greatest at intermediate N availability (Fig. 3). Hence, our study adds to the growing body of evidence showing that nutrient ratios are an important driver of biodiversity impacts[4].

Implications for conservation management. In Europe, agriculture is the main driver increasing P availability in terrestrial ecosystems[4,45]. Although fertilizer use is plateauing[46], annual P fertilizer input for wheat in northwest Europe still exceeded 10–15 kg P ha^{-1} in 2000 and, after decades of heavy application, most soils in Europe are now saturated with P[45]. Assuming current legislation scenarios, N deposition in Europe is projected to have decreased by 10–20% by 2030 compared to 2000 (ref. [47]). This implies that the relative availability of P compared to N will increase—which, as we have shown, is bad news for many threatened species and is contrary to general expectations that policies aimed at reducing N emissions will allow the recovery of threatened species to recover[21]. European Union (EU) legislation, such as the 1991 EU Nitrate Directive and more recent efforts to reduce NO_x emissions from traffic and industry[7], have been demonstrated to effectively reduce the N load to the environment.

To prevent species loss in response to increases in relative P availability resulting from effective reduction in N availability, targeted reduction of soil P stocks should be included in conservation management. However, effectively removing the legacy of decades

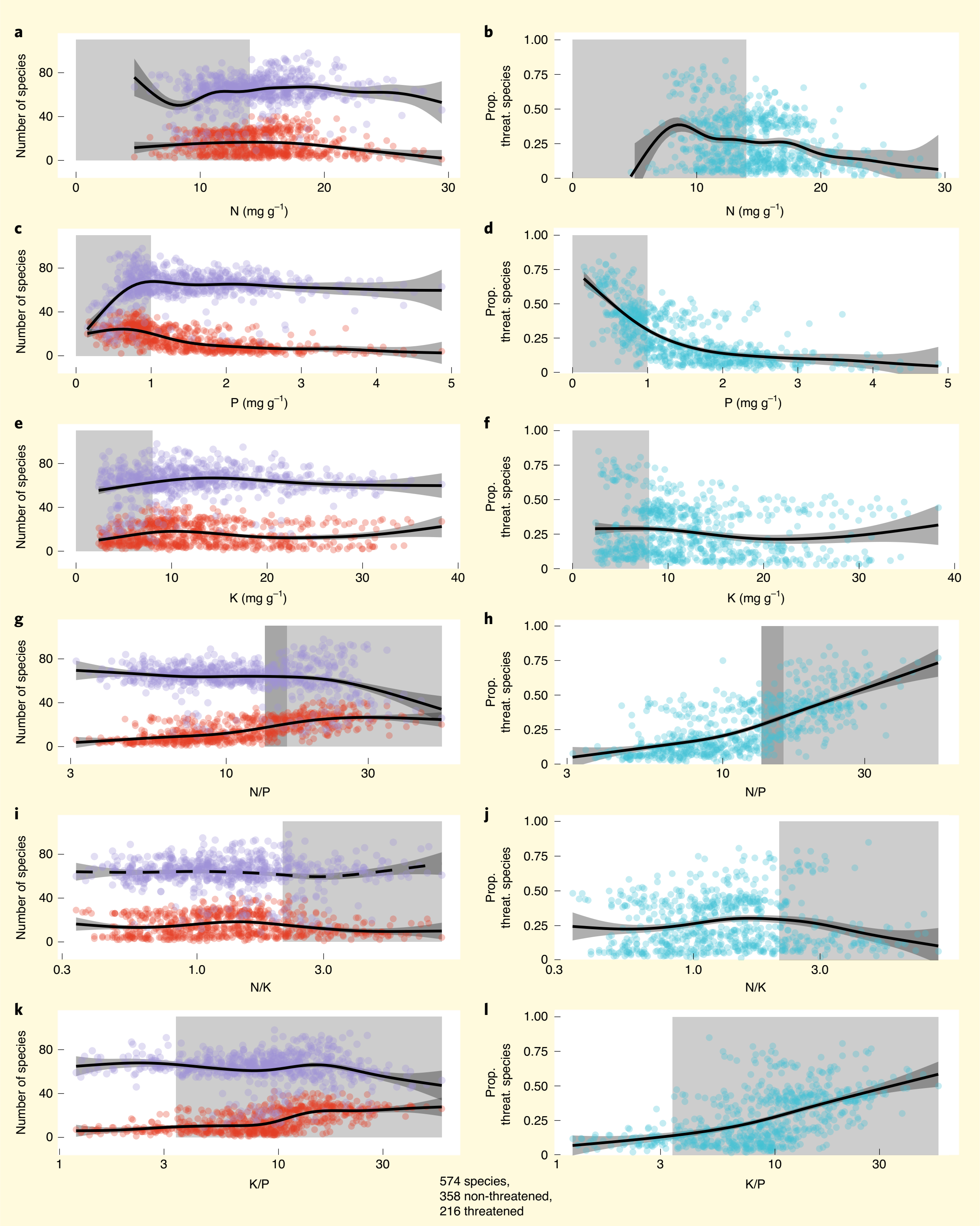

Fig. 1 | Species pool sizes for 673 herbaceous vegetation plots along nutrient gradients in northern Eurasia. a–l, Pool sizes were calculated for all species (purple) and all threatened species (red, left-hand panels) and the proportion (prop.) of threatened (threat.) species in the total pool (blue, right-hand panels). Pool sizes were calculated for each plot using co-occurrence patterns based on Beal´s smoothing transformation[32]. Regression lines are estimates obtained using generalized additive models; standard errors of model estimates are indicated as grey bands. Solid lines indicate significant smoothing factors; the dashed line indicates that the smoothing factor was not significant. Light grey rectangles indicate ranges of nutrient limitation as determined by the following thresholds: **a**,**b**, N limitation when N < 14 mg g^{-1}. **c**,**d**, P limitation when P < 1 mg g^{-1}. **e**,**f**, K limitation when K < 8 mg g^{-1}. **g**,**h**, P limitation relative to N when N/P > 16. Absence of clear limitation of either N or P is denoted by dark grey, indicating that 13.5 ≤ N/P ≤ 16 (N limitation relative to P when N/P < 13.5, no grey background). **i**,**j**, K limitation relative to N when N/K > 2.1 and N limitation relative to K when N/K ≤ 2.1 (no grey background). **k**,**l**, P limitation relative to K when K/P > 3.4 and K limitation relative to P when K/P ≤ 3.4 (no grey background). See Methods for definitions of nutrient limitations.

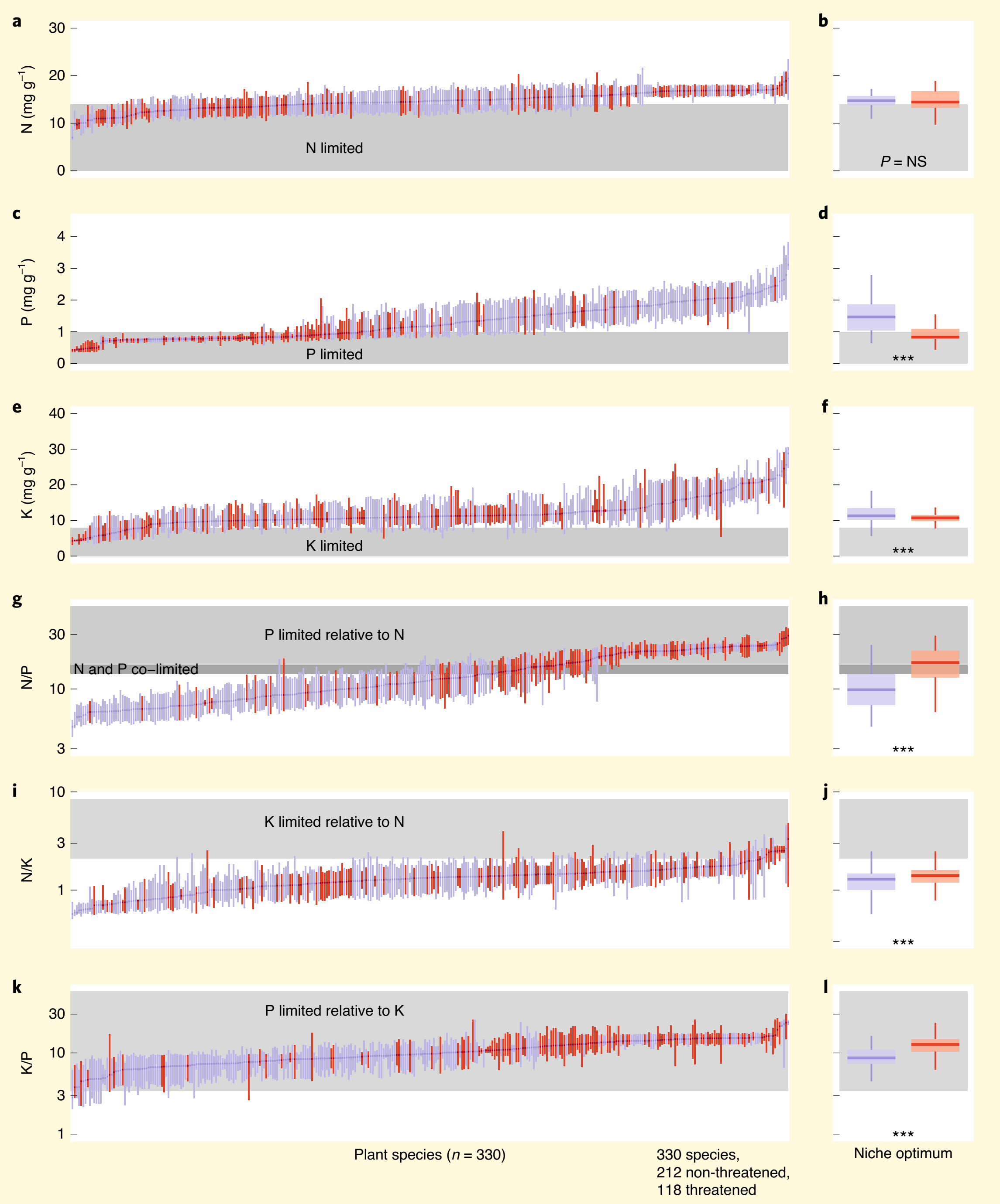

Fig. 2 | Estimated plant species niches. a,**c**,**e**,**g**,**i**,**k**, Estimated species niches, distinguishing between non-threatened (purple) and threatened species (red) and sorted by optimum along nutrient gradients. Each bar in the left-hand panels indicates one species. The species median is indicated by the lines in the middle of the bars. Species bars correspond to upper and lower quartiles. **b**,**d**,**f**,**h**,**j**,**l**, Boxplots indicating that threatened species had their niche optimum at significantly lower absolute P and K availability than non-threatened species, and at significantly higher N/P, N/K and K/P ratios (***$P \leq 0.001$). Boxplots in right-hand panels show median, upper and lower quartiles and whiskers indicate the upper and lower quartiles plus or minus 1.5× interquartile range. Niches were estimated by the species pool method. Species were included in the analyses if at least ten data points were obtained (yielding $n = 330$ species). Grey background indicates nutrient-limitation ranges (Fig. 1). Numbers of species with niche optimum in nutrient-limited conditions (light grey backgrounds) are for N (**a**, 108 species), P (**c**, 15 species), K (**e**, 34 species), P limited relative to N (**g**, 108 species), no clear limitation by either N or P (**g**, 29 species; dark grey background), K limited relative to N (**i**, 12 species) and P limited relative to K (**k**, 329 species). Species counts are based on the median of all plot nutrient values or ratios in which the given species occurred. NS, not significant.

of overfertilization with phosphates that have accumulated in the environment is a huge practical challenge[48]. Mining the soil P by harvesting and exporting above-ground vegetation and removing the biomass (hay removal) is applied as a nature management measure in many protected herbaceous ecosystems in western Europe, and is common practice in many protected wetlands[49–51]. According to nutrient budget calculations for western European herbaceous ecosystems, annual hay removal seems to result

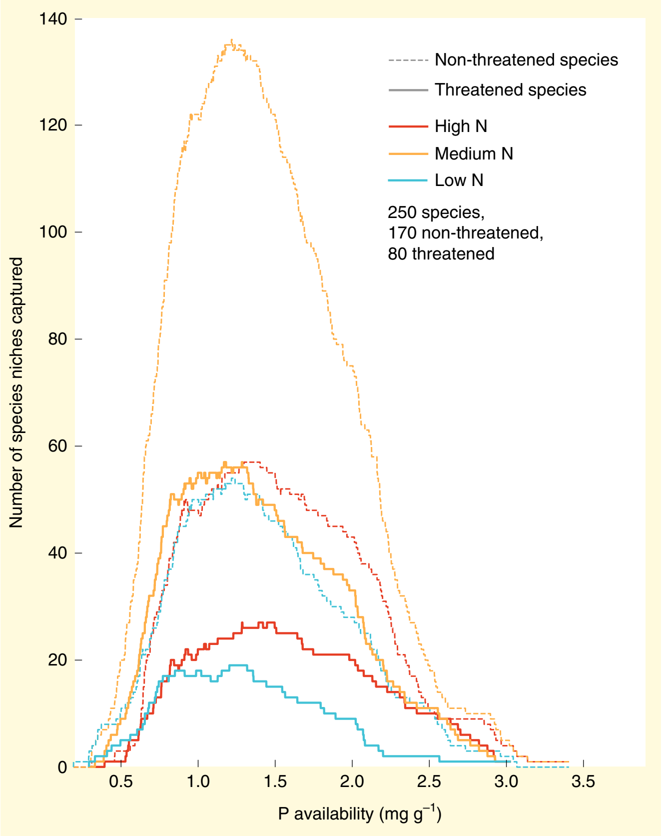

Fig. 3 | Number of species niches for various combinations of N and P. In this plot, three levels of N are considered (high, 18.2 mg g^{-1}; medium, 14.5 mg g^{-1}; low, 10.9 mg g^{-1}) along the entire P-availability gradient, demonstrating that N availability constrains the number of niches included. Considering non-threatened plant species (dashed lines), most niches are included at intermediate N levels, meaning that merely reducing N below these levels (that is, without simultaneously reducing P) would reduce the number of species niches included. When considering threatened species only (solid lines), this pattern is expressed even more clearly. Species niches are calculated for each nutrient as the unweighted mean of all plot values in which the given species occurred, ±1 s.d. (ref. [24])).

in greater net export of P and K from the ecosystem than of N, which would lead to P and/or K limitation in the long term[52,53]. Hence, although hay removal in nutrient-enriched areas may prevent increased vegetation productivity, it may also alter N/P/K stoichiometry, induce K limitation at drained sites and change vegetation structure and composition[50]. Budget calculations indicate that, in regions with low rates of atmospheric N deposition, haymaking need not induce a shift from N towards P and/or K limitation[54]. Re-wetting of drained areas risks initiating a strong P release, which may stimulate eutrophication rather than prevent it[55]. Manipulation of water levels so that they fluctuate in nature areas in which phosphates are mobilized under inundated and anoxic conditions and are flushed away in wet periods has only recently been applied experimentally as a measure to export P from wetlands[56]. However, drained meadows can be restored only if their topsoil is removed before they are re-wetted[57]. Given the major challenge of removing accumulated P, it is unfortunate that EU legislation has, to date, targeted only the reduction of P loads in surface waters and that no EU directive has targeted the agricultural use of P fertilizer[46]. Our results suggest that, to safeguard Europe's threatened terrestrial plant species from extinction, an EU Phosphate Directive is required.

Methods

Dataset. We used the plot dataset for vegetation recordings of ref. [24], which is a selection from the dataset of ref. [35]. The 673 plots were located at 48–63° N and span eight Eurasian countries/regions (Extended Data Fig. 6): the Netherlands (287 plots), Poland (155 plots), Germany (90 plots), Siberia (83 plots), Belgium (20 plots), Sweden (16 plots), Scotland (12 plots) and Belarus (10 plots). Plots were in herbaceous ecosystems and included grasslands, fens, bogs, marshes, reedlands, floodplains and dune grasslands, on a large variety of soils. Atmospheric N deposition in our study areas ranged between <5 and >45 kg N ha^{-1} yr^{-1} at the time of data collection.

In these plots, species compositions of vascular plants were recorded and N, P and K concentrations in above-ground vegetation were measured as a proxy for nutrient availability. We sampled herbaceous vegetation in plots of at least 0.06 m^2, from which we harvested above-ground vascular plant biomass by clipping vegetation to ground level at the peak of the growing season. We removed dead plant material (litter and senesced leaves), mosses and woody parts (the latter present only as dwarf shrubs in some samples). Because we compiled our dataset from several previously published separate subsets, the laboratory methods used differ slightly. Most plant material samples ($n = 583$) had been dried for at least 24 h at 70 °C (refs. [53,58–64]). The remaining samples ($n = 90$) had been dried for 48 h at 60 °C (refs. [65]). After drying, the plant samples were weighed and ground. Next, random subsamples were taken from the ground plant material. Most of these subsamples ($n = 630$) were digested according to Kjeldahl (as described in refs. [53,58–61,63,65,66]. The remaining samples ($n = 43$) were digested with hydrofluoric acid (HF) (as described in ref. [62]). In most samples ($n = 540$), the N concentration (as NH_4-N) of the digests was measured by the indophenol-blue method using a Skalar autoanalyser[53,58–61,63]. For the remaining samples ($n = 133$), N concentration was measured via dynamic flash combustion[62,64,65]. For most samples ($n = 421$), P and K concentrations were determined by an inductively coupled plasma technique based on optical emission spectroscopy[53,61–65]. For the remaining samples ($n = 252$), the P concentration (as H_2PO_4) was determined by the molybdenum-blue method using a Skalar autoanalyser[58–60]. In this latter subset of samples, K concentration was determined by flame spectroscopy.

The dataset contained 574 herbaceous plant species, of which 216 were threatened. Woody species were excluded from the analyses. We considered a species to be 'threatened' if it occurred on one or more of the Red Lists of the Netherlands, Germany, Poland, Sweden, the UK or Russia, based on their (negative) population trends[67]. This criterion corresponds to an aggregation of the International Union for Conservation of Nature (IUCN) criteria 'critically endangered', 'endangered' or 'vulnerable', and was used to account for potential differences between countries in national Red List criteria (Supplementary Discussion 3 provides further discussion). Although most countries considered in this study specifically apply IUCN criteria and categories to assess the conservation status of species, there were a few exceptions. The Netherlands uses a more detailed categorization, and generally considers longer timescales to assess trends than does the IUCN. The Red Lists of Poland and Russia are not clear about the exact criteria used to assign a species to a certain category, but the categories used are congruent with the IUCN criteria. By including only Red List categories based on negative population trends, our approach excluded species that are on a Red List solely because of their low population numbers within country borders and that may therefore be Red Listed only because they are on the fringe of their biogeographical distribution range. To ensure that our results would be robust to our decision to deem a species threatened if it occurred on only one national Red List, we repeated our analysis with a stricter criterion that a species was considered threatened only when it occurred on a minimum of two national Red Lists. That analysis revealed that our main results were the same when this stricter criterion was applied, indicating that our conclusions was not critically dependent on the criterion of single Red List occurrence. The results of this extra analysis are reported in Supplementary Discussion 3.

To determine nutrient limitations, we used critical values for absolute single-nutrient concentrations in above-ground biomass, as defined by refs. [68,69], yielding critical N, P and K values of 14, 1 and 8 mg g^{-1}, respectively. To distinguish relative nutrient limitation, we used the critical values as defined by ref. [61] based on N/P, N/K and K/P ratios in above-ground biomass. N- and P-limited sites were distinguished on the basis of N/P ratios. N/P ratios ≤13.5 indicate N limitation (relative to P) while ratios >16 indicate P limitation (relative to N); following ref. [61], we defined values between 13.5 and 16 as indicating no clear limitation by N or P. To distinguish relative K limitation, we used the critical N/K ratio of 2.1 (values >2.1 indicating K limitation relative to N) and the K/P ratio of 3.4 (values >3.4 indicating P limitation relative to K). Thresholds defining nutrient limitation on the basis of nutrient concentrations in above-ground biomass are indicative and are therefore more suitable for identification of broad patterns of variation in nutrient limitation across plant species rather than for determination of the nutrient limitation of a single species at a particular point in space and time[28,58,61,68,70,71]. In our database, 269 of the 673 plots (40%) met one or both of the criteria for P limitation. Within this subset of plots, 65% were indicated as being P limited based on both [P] < 1 mg g^{-1} and N/P > 16, 30% were indicated to be P limited based solely on [P] < 1 mg g^{-1} while only 5% were indicated to be P limited based solely on N/P > 16. This not only points to absolute P levels having an important role but also shows that, for some of the data, the effects of low absolute P cannot be fully disentangled from the effects of high N/P ratios (Extended Data Fig. 4 and Supplementary Discussion 2).

Species pool method. The species pool for each plot was calculated using co-occurrence patterns based on Beal´s smoothing transformation[32]. This transformation takes into account that a species mostly co-occurring with other species but absent in a given plot where the other species occur is probably part of that site's species pool[72]. Hence, for each species in each plot a probabilistic value for co-occurrence was calculated using Beal's smoothing index[73]. The value of this index is based on the presence of other species in the plot, with frequent species being assigned higher values than less common or rare species[32]. Species presences and absences from plots were then determined based on species-specific thresholds. The threshold for each species was determined using the lowest Beal´s value for the plot in which the species was actually present. All species attributed as being 'present' in the plot were then included in the species pool; all other species in the dataset were considered 'not included' in the species pool[32]. Comparison of the estimated species pools with observed species occurrences in each plot showed that the estimated species pools are robust and are not biased along the nutrient gradients (Extended Data Figs. 1 and 2 and Supplementary Discussion 1). This was confirmed by detrended correspondence analysis (DCA; Extended Data Fig. 5), which showed that the estimated species pools for most countries overlapped (except for some German sites) and resulted in species clusters similar to those identified by ref. [24] from the observed data (Supplementary Discussion 4). An alternative approach to assigning species' presences and absences to pools is the probabilistic approach, in which each species is assigned an occurrence probability for each plot calculated using dispersal, environmental suitability and biotic interactions based on species distribution modelling[74]. Although the pros and cons of these approaches are still being debated, the presence–absence approach may be better suited for plot data when environmental data and data on dispersal ability and biotic interactions are missing[32,75,76]. Ratio plots in Fig. 1 (right-hand panels) were calculated as the ratio between the species pool size for threatened species and all species, and represent the proportion of threatened species within the total species pool. Because we did not assume that a particular model would best predict the relationship between species pools and species pool ratios with nutrient values, we fitted generalized additive models with Gaussian distributions that fit smooth nonlinear functions to the nutrient values. We conducted all analyses in R v.3.4.4 (ref. [77]), using the vegan package[78] to calculate Beal´s smoothing index and the mgcv package to calculate generalized additive models[79].

Niche widths. Niche optima along the absolute and relative nutrient gradients were determined for species for which at least ten data points were available (yielding $n=330$ species, 118 of which were threatened), and were calculated as the median of all plot nutrient values or ratios in which the given species occurred. The niche width was calculated as the species variance along the nutrient values (± the average squared deviation from the mean). Comparison of the niche widths obtained with the species pool method with actual species observations (compare Fig. 2 with Extended Data Fig. 7 for observed species occurrences in ten or more plots) revealed that the estimated values are robust and not biased along the nutrient gradients (Supplementary Discussion 5).

Implications of N reduction for species niches captured. We counted the number of species niches that included each combination of N and P availability (measured as N and P concentrations in sampled biomass), a species' niche for each nutrient being quantified as the unweighted mean of all plot values in which the given species occurred ±1 s.d. (ref. [24]). Then, for three levels of N (high, $18.2\,\mathrm{mg\,g^{-1}}$; medium, $14.5\,\mathrm{mg\,g^{-1}}$; low. $10.9\,\mathrm{mg\,g^{-1}}$), the number of species niches included was plotted as a function of absolute P availability (Fig. 3). The levels of N were chosen based on the median N concentration of all species' times plot observations in the dataset ($n=11{,}782$), ±1 s.d. for high and low levels of N (approximately corresponding to the 85th and 15th percentiles, respectively.

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

Data availability

Source data underlying this manuscript can be accessed from the Yoda Data Repository of Utrecht University at https://doi.org/10.24416/UU01-I815KS. The data available include the plot data of nutrient concentrations and ratios and calculated species pools using Beal's smoothing index, as used in Fig. 1 and Extended Data Figs. 1 and 2 (https://geo.public.data.uu.nl/vault-npk-plants/Supplementary_Data_Wassen_et_al_2020_Nat_Ecol_Evol[1596797967]/original/plot_data_Fig_1&Ext_Data_Fig_1_2.csv), the species data of niches (median and variance of nutrient contents and nutrient ratios of species recorded in all plots of occurrence of that species) and threatened status of all species used in Fig. 2 and Extended Data Fig. 3 (estimates using the species pool method) (https://geo.public.data.uu.nl/vault-npk-plants/Supplementary_Data_Wassen_et_al_2020_Nat_Ecol_Evol[1596797967]/original/species_data_Fig_2&Ext_Data_Fig_3.csv), the number of niches captured in Fig. 3 (https://geo.public.data.uu.nl/vault-npk-plants/Supplementary_Data_Wassen_et_al_2020_Nat_Ecol_Evol[1596797967]/original/niche_number_data_Fig_3.csv), the species data (median and variance of nutrient contents and nutrient ratios of species recorded in all plots of occurrence of that species) and threatened status of all species used in Extended Data Figs. 7 and 8 (using observed species occurrences) (https://geo.public.data.uu.nl/vault-npk-plants/Supplementary_Data_Wassen_et_al_2020_Nat_Ecol_Evol[1596797967]/original/species_data_Ext_Data_Fig_7_8.csv) and the generalized addition model statistical parameters estimated from generalized linear mixed-effect models (Gaussian distribution) used in Fig. 1 (https://geo.public.data.uu.nl/vault-npk-plants/Supplementary_Data_Wassen_et_al_2020_Nat_Ecol_Evol[1596797967]/original/GAM_Parameter_Fig_1.csv).

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Author contributions

M.J.W. originally conceived the idea and wrote the drafts of the manuscript. J.S., J.v.D. and M.B.E. analysed the data and contributed to the writing.

Competing interests

The authors declare no competing interests.

Additional information

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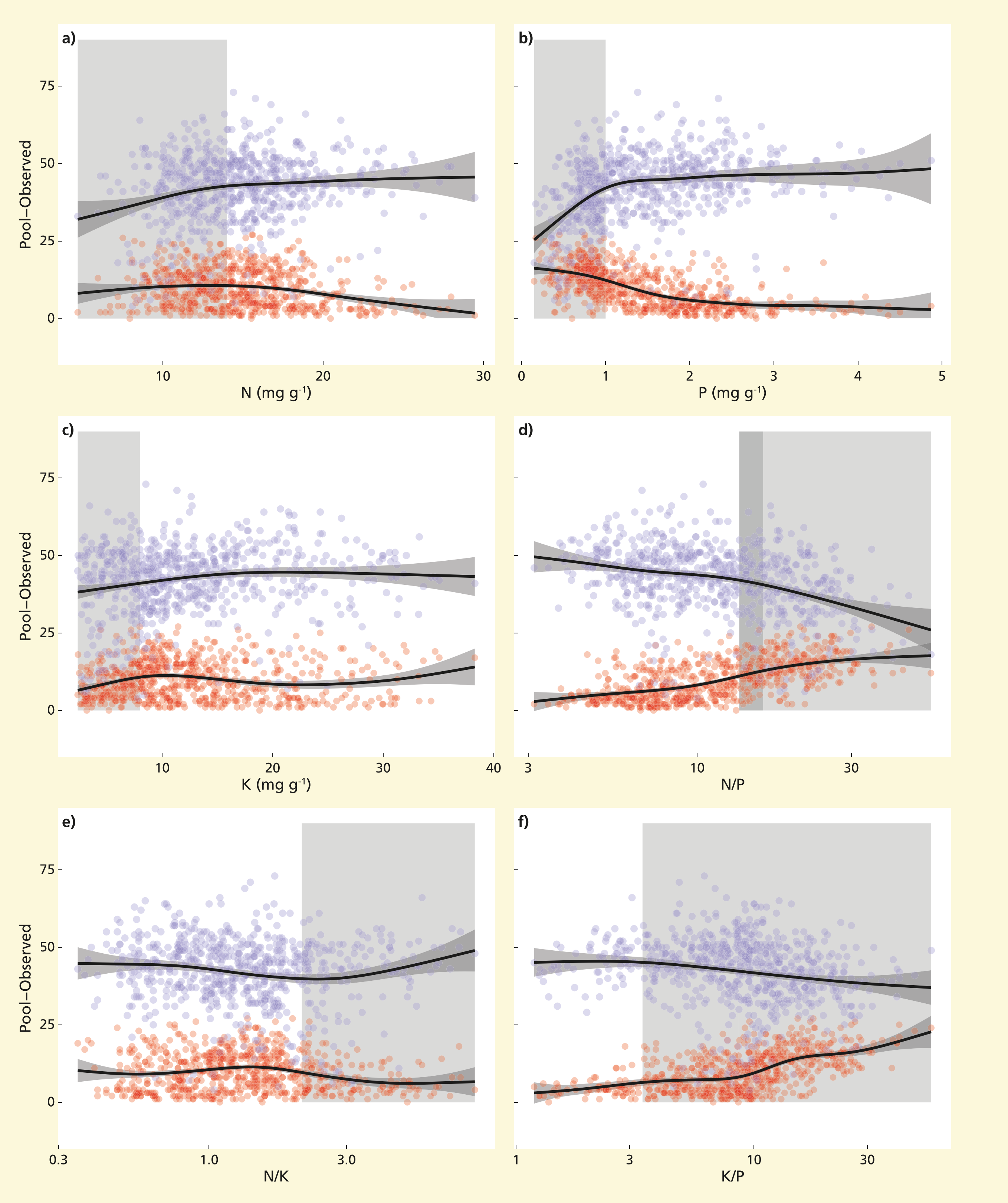
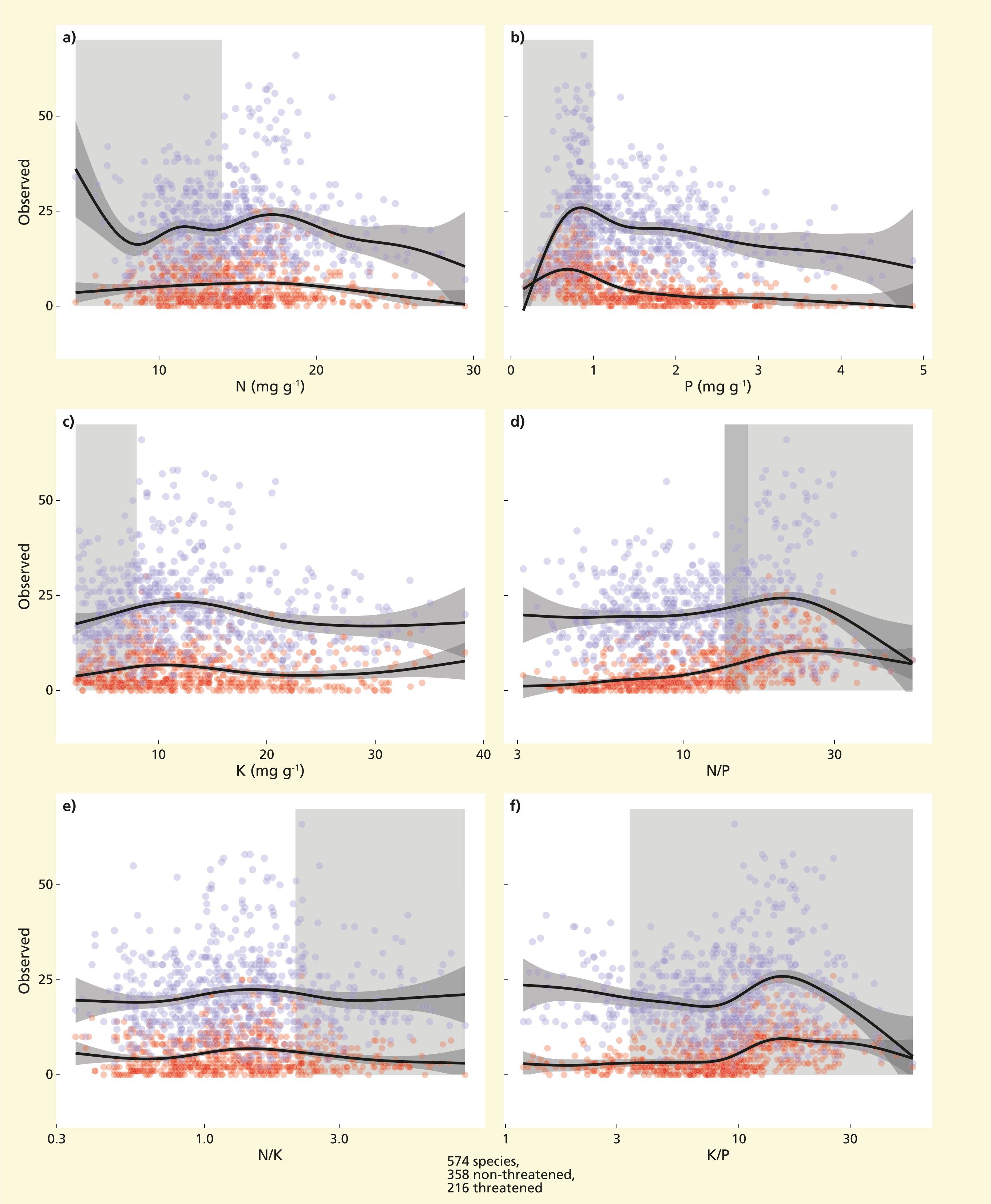
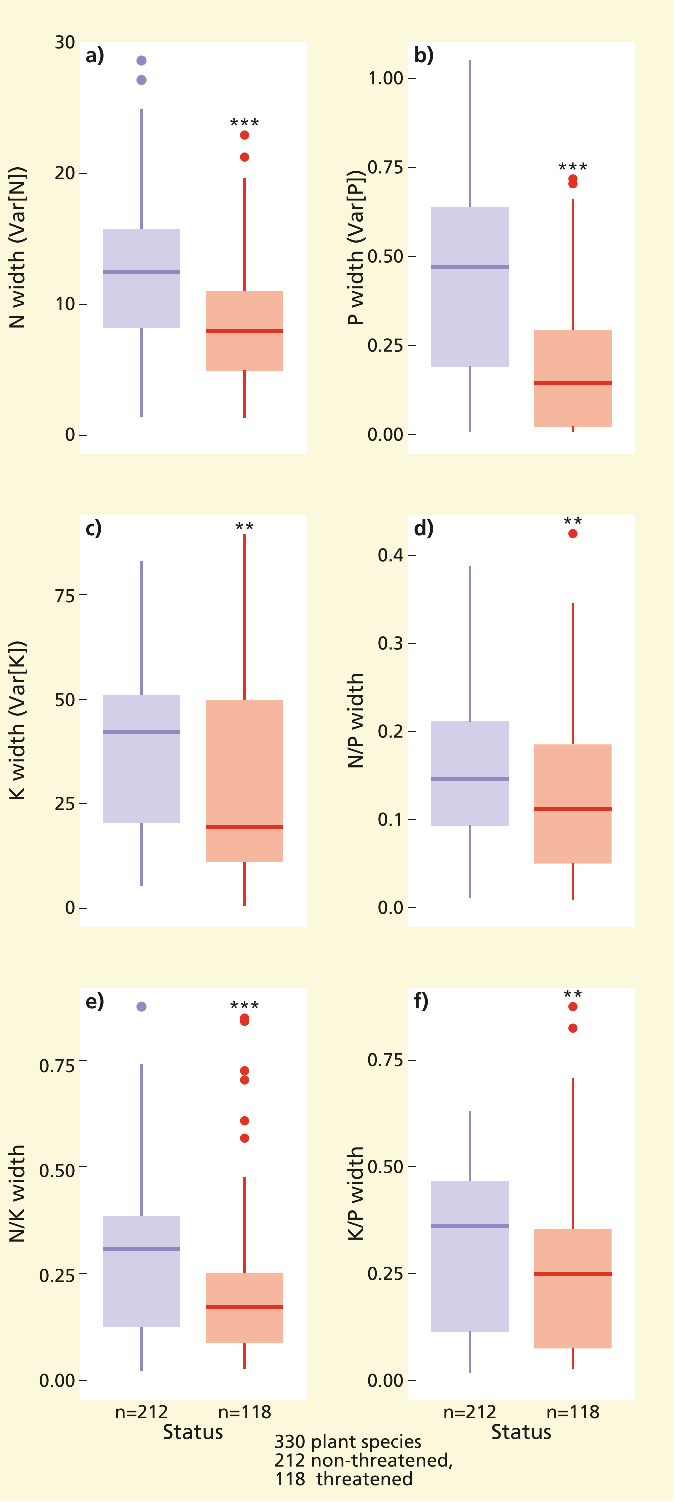


Extended Data Fig. 1 | Plots showing the difference between estimated species pool size and observed species number (pool minus observed) for each plot in the dataset, along all the absolute and relative nutrient gradients considered. Differences are shown for all species (purple) and all threatened species (red). Regression lines are estimates obtained using generalized additive models, with standard errors of model estimates indicated as grey bands. Solid lines indicate significant smoothing factors. Light grey rectangles indicate ranges of nutrient limitation derived from thresholds given in the literature: **a**, N limitation when N < 14 mg g^{-1}; **b**, P limitation when P < 1 mg g^{-1}; **c**, K limitation when K < 8 mg g^{-1}. **d**, N limitation relative to P occurs when N/P < 13.5; N and P co-limitation when 13.5 ≤ N/P ≤ 16 (dark grey); P limitation relative to N when N/P > 16 (grey); **e**, K limitation relative to N when N/K > 2.1; **f**, P limitation relative to K when K/P > 3.4 (see Methods for definitions of nutrient limitations).

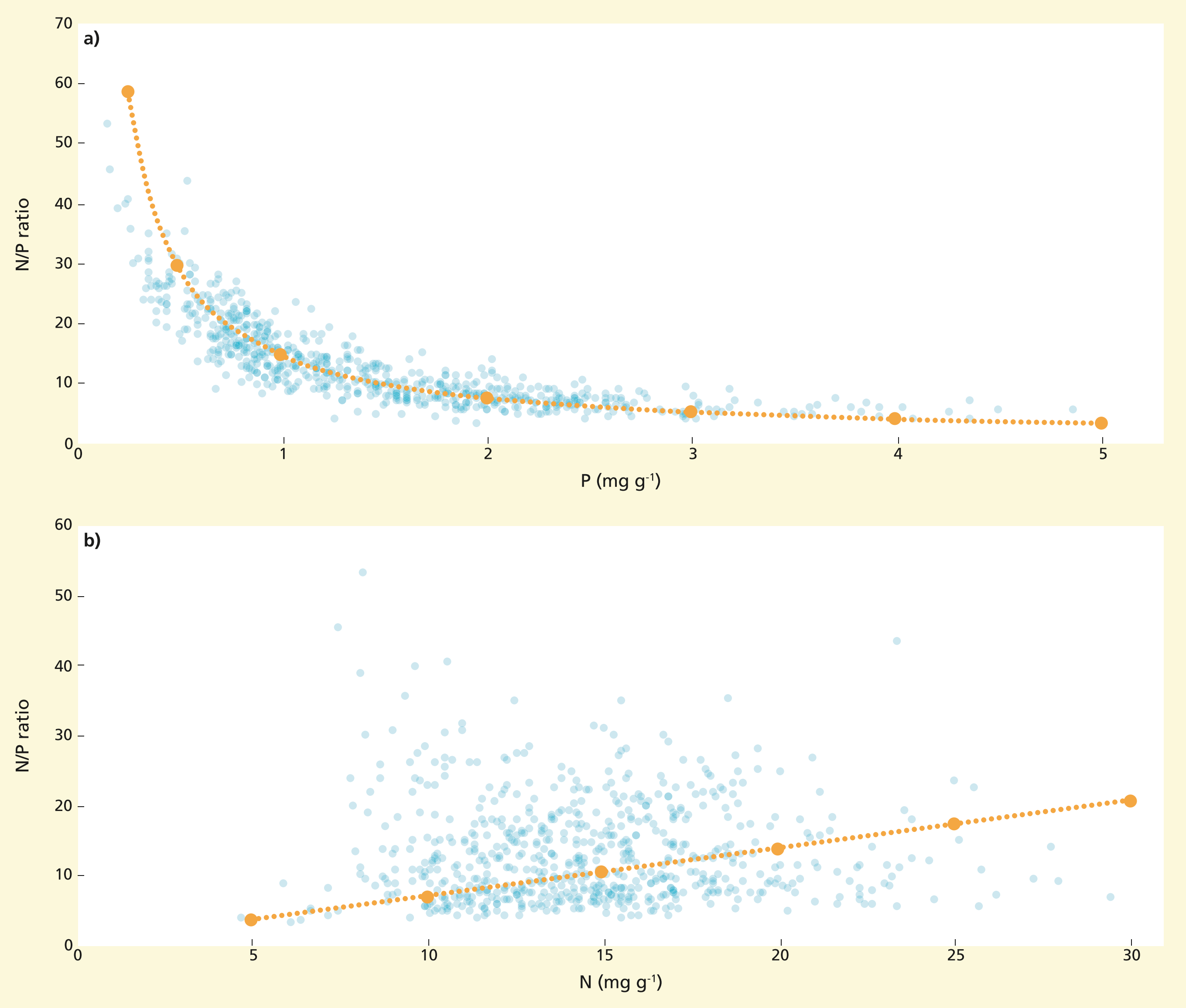


Extended Data Fig. 2 | See next page for caption.

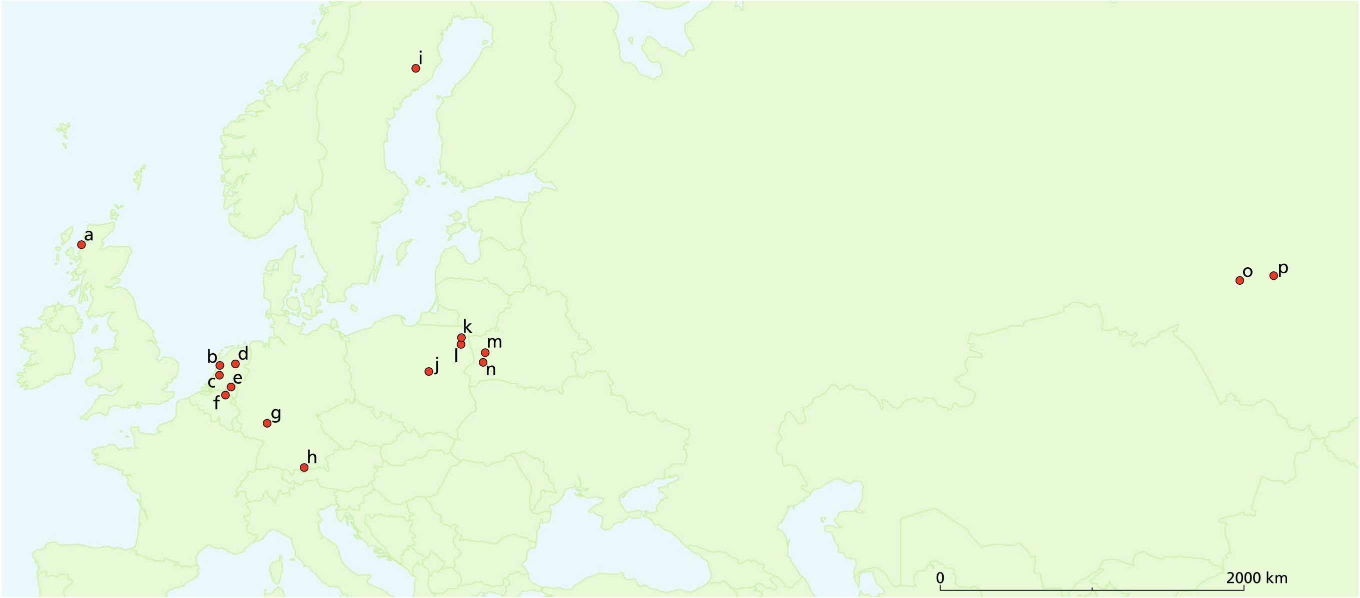
Extended Data Fig. 2 | Observed species numbers for 673 herbaceous vegetation plots in northern Eurasia, counting all species (purple) or all threatened species (red) for each plot along nutrient gradients. The figure follows the same layout as Fig. 1 of the main text and reveals the same species richness patterns across the absolute and relative nutrient gradients. Regression lines are estimates obtained using generalized additive models, with standard errors of model estimates indicated as grey bands. Solid lines indicate significant smoothing factors. Light grey rectangles indicate ranges of nutrient limitation derived from thresholds given in the literature: **a**, N limitation when $N < 14$ mg g^{-1}; **b**, P limitation when $P < 1$ mg g^{-1}; **c**, K limitation when $K < 8$ mg g^{-1}. **d**, N limitation relative to P occurs when $N/P < 13.5$; N and P co-limitation when $13.5 \leq N/P \leq 16$ (dark grey); P limitation relative to N when $N/P > 16$ (grey); **e**, K limitation relative to N when $N/K > 2.1$; **f**, P limitation relative to K when $K/P > 3.4$ (see Methods for definitions of nutrient limitations).



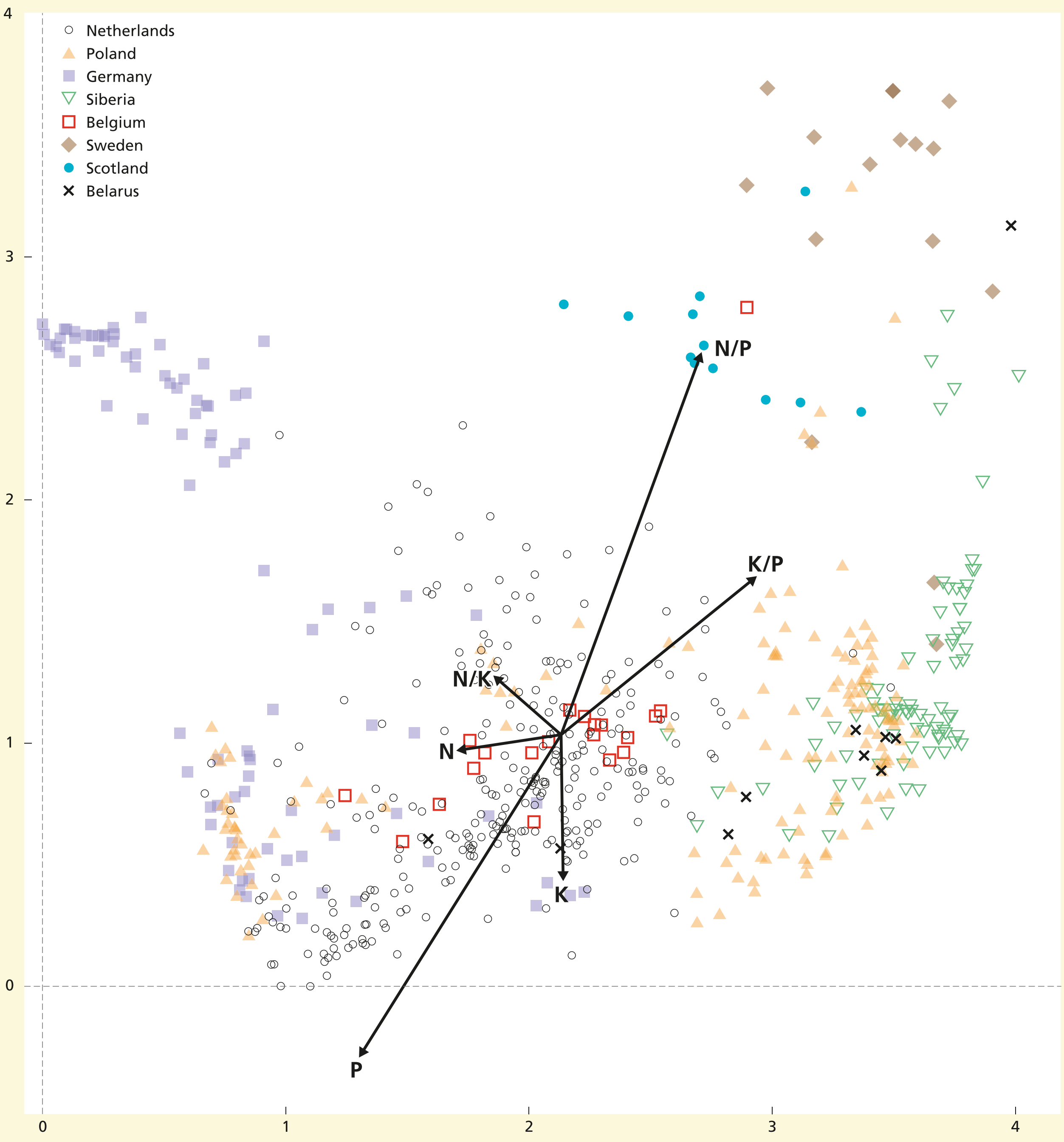
Extended Data Fig. 3 | Boxplots of niche widths of non-threatened (purple) and threatened species (red). Niche widths of threatened species are significantly smaller for all nutrient gradients (significance levels: **: $P \leq 0.01$; ***: $P \leq 0.001$). Boxplots show median, upper and lower quartiles and whiskers indicate the upper and lower quartiles plus or minus 1.5 times the interquartile range. Data points considered outliers are indicated by dots. Niche width was calculated using the variance of the species along the nutrient gradients (nutrient ratios were log-transformed). Species were included in the analyses if at least 10 data points were obtained (yielding n = 330 species).



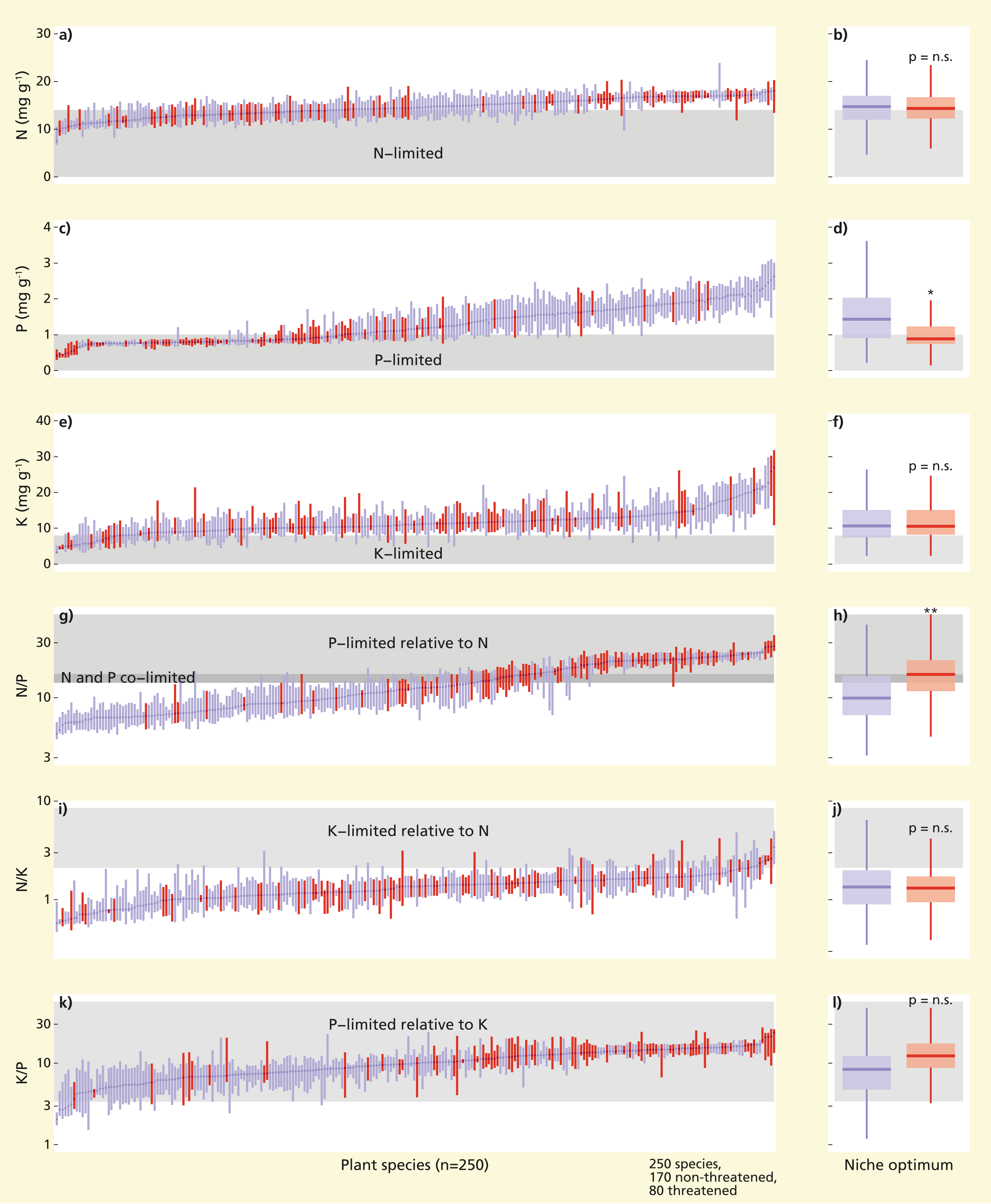
Extended Data Fig. 4 | N:P ratio of the aboveground biomass plotted against P concentration in mg g^{-1} dry weight (**a**) and N concentration in mg g^{-1} dry weight (**b**). The dotted line in **a**) indicates the N:P ratios expected if N:P is solely determined by the variation in P concentration (that is if N equals the average N of the full dataset) and the dotted line in **b**) indicates the N:P ratios expected if N:P is solely determined by the variation in N concentration (that is if P equals the average P of the full dataset).



Extended Data Fig. 5 | Map showing the 16 regions in which data was collected. The letters indicate the following regions: **a**, Poolewe, Scotland (n=12 plots); **b**, Noord-Holland, Netherlands (n = 125); **c**, Zuid-Holland, Netherlands (n = 60); **d**, Noordwest Overijssel, Netherlands (n = 48); **e**, Dommel catchment, Netherlands (n = 54); **f**, Zwarte Beek catchment, Belgium (n=20); **g**, Upper Rhine area, Germany (n=43); **h**, Bavarian Alpine foothills (n=47); **i**, Degerö Stormyr, Sweden (n=16); **j**, Kampinowska, Poland (n=38); **k**, Rospuda valley, Poland (n=41); **l**, Biebrza catchment, Poland (n=76); **m**, Neman valley downstream, Belarus (n=5); Neman valley upstream, Belarus (n=5); **o**, Ob valley, Siberia (n=51); **p**, Great Vasyugan mire, Siberia (n=32).

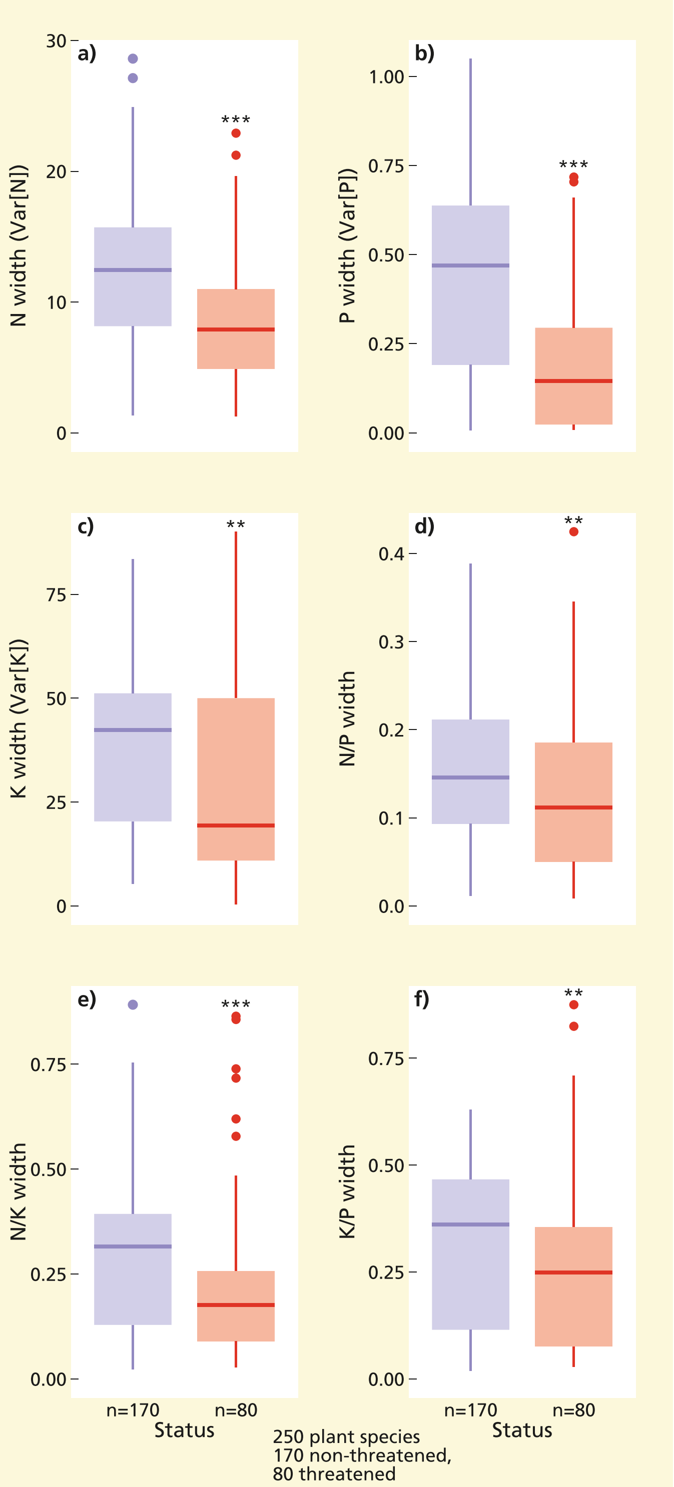


Extended Data Fig. 6 | Ordination diagram based on a Detrended Correspondence Analysis (DCA) of the species composition of the species pool (based on co-occurrence analysis) of 673 herbaceous vegetation plots in northern Eurasia. Scaling focused on inter-sample distances, so plots with similar species composition cluster together. Different symbols indicate different geographical locations (as indicated in the legend). The main nutrient gradients, as indicated by absolute and relative nutrient availabilities, were plotted as supplementary variables and are indicated by black arrows. The cumulative variance in species composition explained by the first 4 axes was 32.4%. Axis 1 explained 18.8% of the variance, axis 2 explained an additional 7.5%. The supplementary variables accounted for 15.6% of the variation in species composition.

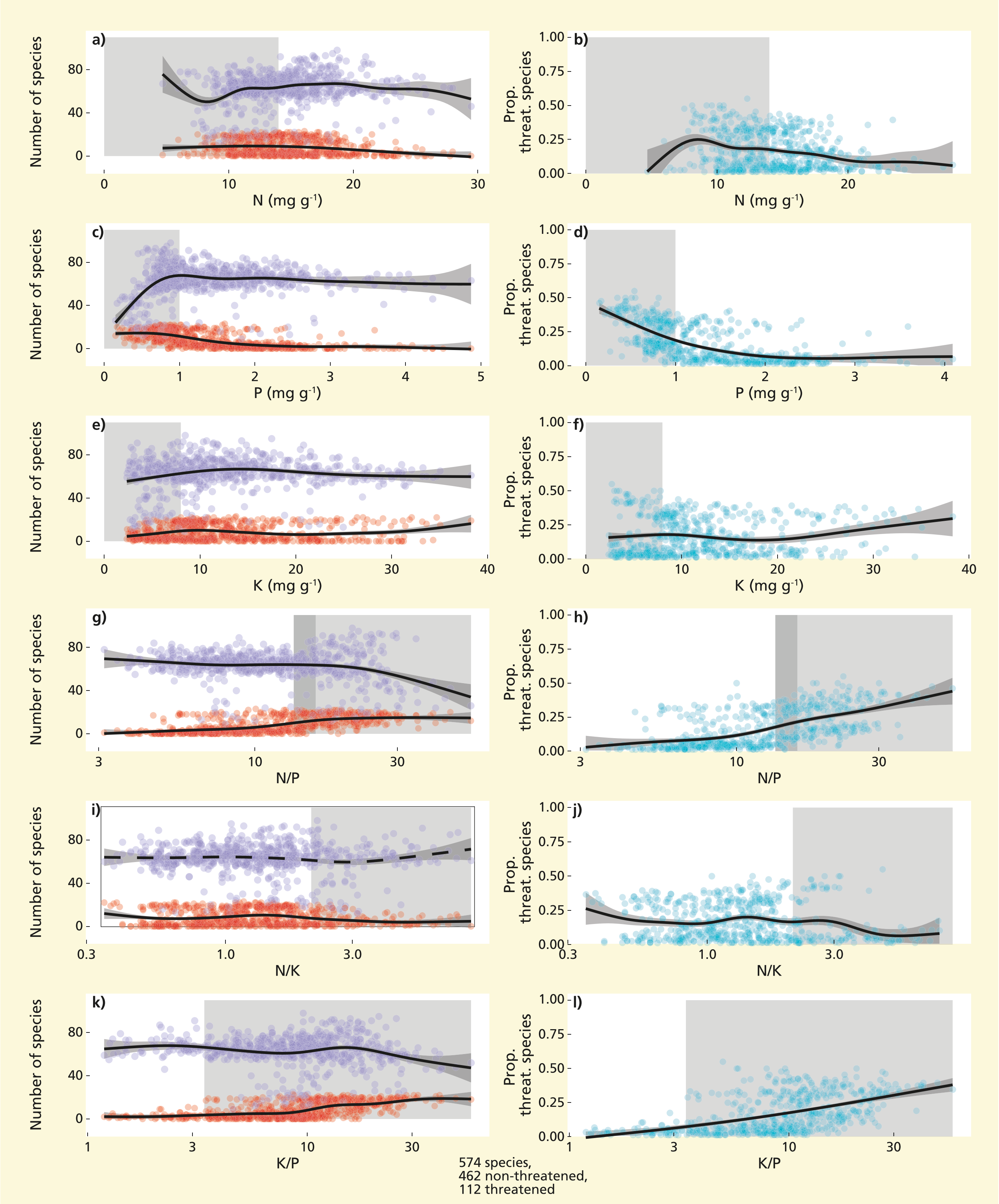


Extended Data Fig. 7 | See next page for caption.

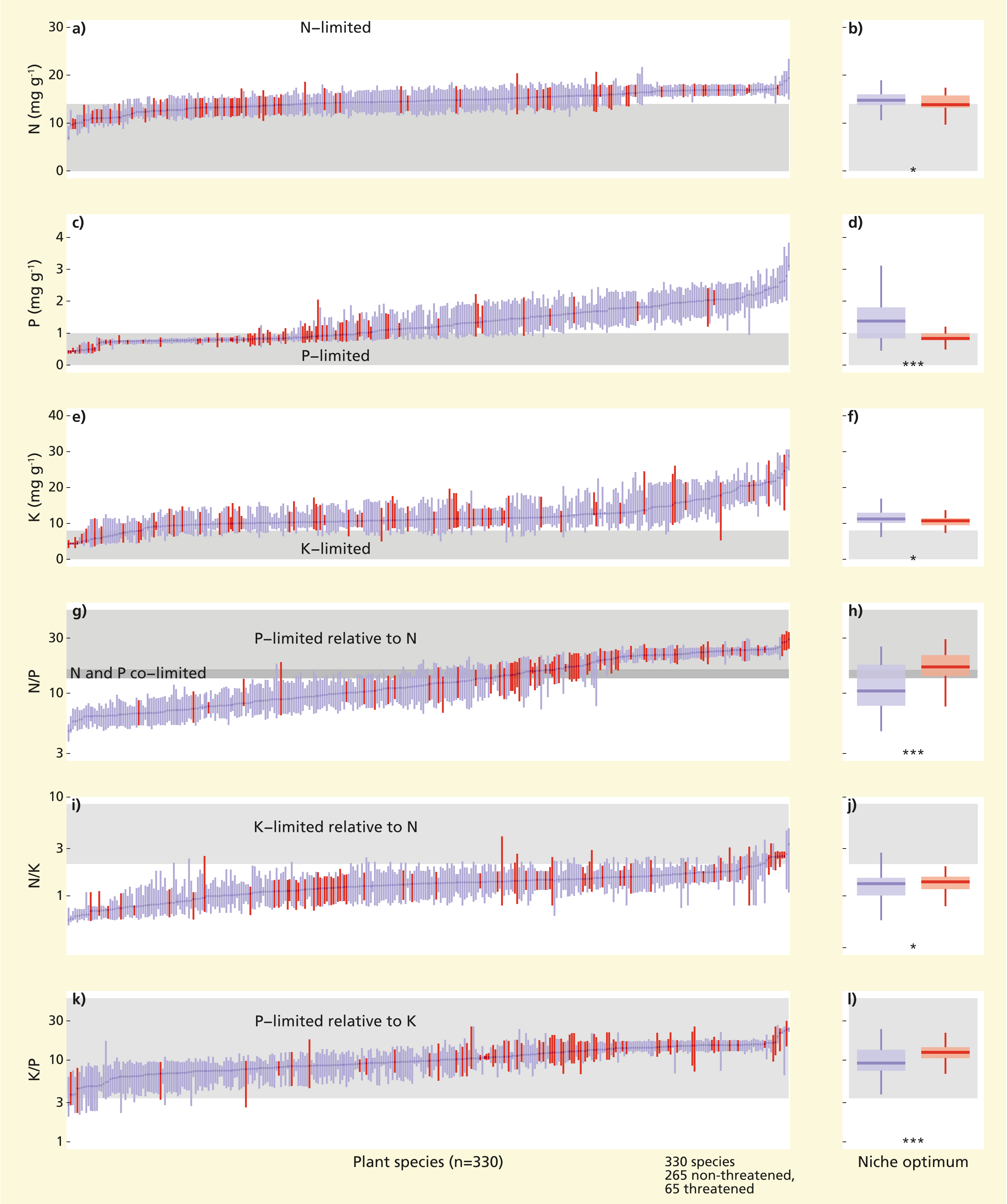
Extended Data Fig. 7 | Observed plant species occurrences along nutrient gradients, distinguishing between non-threatened (purple) and threatened species (red). Left-hand panels (**a**, **c**, **e**, **g**, **i** and **k**): each boxplot indicates one species. The species median is indicated by the line in the middle of the bars. Species bars correspond to the upper and lower quartiles. Right-hand panels (**b**, **d**, **f**, **h**, **j** and **l**): Boxplots indicate that threatened species had their niche optimum at significantly lower absolute P availability, and at significantly higher N:P ratios (significance level: n.s.: non-significant; *: $P \leq 0.05$; **: $P < 0.01$). Right-hand panels (**b**, **d**, **f**, **h, j** and **l**): boxplots show median, upper and lower quartiles and whiskers indicate the upper and lower quartiles plus or minus 1.5 times the interquartile range. Species were included in the analyses if they occurred in at least 10 plots (yielding $n = 250$ species). Grey backgrounds indicate nutrient limitation ranges. Numbers of species with their niche optimum in nutrient-limited conditions (light grey backgrounds): 97 for N limitation (**a**), 10 for P limitation (**c**) and 22 for K limitation (**e**). Species counts of niche optima were also conducted along relative nutrient gradients. Along the N/P gradient, 146 species had a niche optimum in the N-limited regime (**g**; no grey background), 85 species in the P-limited regime (**g**; grey background), and 19 species in the N and P co-limitation regime (**g**; dark grey background). Along the N/K gradient, 9 species had a niche optimum in the K-limited regime (**i**). Along the K/P gradient, 244 species had a niche optimum in the P-limited regime (**k**). Species counts are based on the median of all plot nutrient values or ratios in which the given species occurred. See Methods for definitions of nutrient limitations.



Extended Data Fig. 8 | Boxplots of niche widths of non-threatened (purple) and threatened species (red). Niche widths were calculated not by the species pool method described in the main text but from observed species occurrences in plots and have been used in the corresponding Extended Data Fig. 3. Similar to the results shown in Extended Data Fig. 3, niche widths of threatened species are significantly narrower for all nutrient gradients (significance levels: **: $P \leq 0.01$; ***: $P \leq 0.001$). Boxplots show median, upper and lower quartiles and whiskers indicate the upper and lower quartiles plus or minus 1.5 times the interquartile range. Data points considered outliers are indicated by dots. Niche width was calculated using the variance of the species along the nutrient gradients (nutrient ratios were log-transformed). Species were included in the analyses if they occurred in at least 10 plots of the dataset (yielding n = 250 species).



Extended Data Fig. 9 | Species pool sizes of all species and all threatened species (left-hand panel), and the proportion of threatened species in the total pool (right-hand panel), calculated using the criterion that all species occur on at least two national Red Lists. The criterion used was that all species occur on at least two national Red Lists, unlike Fig. 1 of the main text where we used the criterion that species occur on at least one national Red List.



Extended Data Fig. 10 | Estimated plant species niches, distinguishing between non-threatened (purple) and threatened species (red) and sorted by niche optimum along nutrient gradients using the criterion that all species occur on at least two national Red Lists. The criterion used was that all species occur on at least two national Red Lists, unlike Fig. 2 of the main text where we used the criterion that species occur on at least one national Red List. Left-hand panels: each bar indicates one species. The species median is indicated by the line in the middle of the bars. Species bars correspond to the upper and lower quartiles. Right-hand panels: Boxplots show median, upper and lower quartiles and whiskers indicate the upper and lower quartiles plus or minus 1.5 times the interquartile range. (significance levels: *: $P \leq 0.1$; ***: $P \leq 0.001$).

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Data collection	The computer codes used for data analyses mostly contain standard procedures, but specific packages used in R are listed in the manuscript. We also used Matlab for part our analyses and visualisation of the results, but these analyses could also be executed straightforwardly in alternative programs such as R or SPSS).
Data analysis	As noted above, we used R (R project, version 3.4.4, 2018) and Matlab (Mathworks, version 9.4, 2018). In addition, we used CANOCO for additional statistical analysis of the data (version 5.1, 2018).

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

Study description	This study analyzed plant community composition and nutrient niches in Eurasian grasslands, along an environmental gradient of nitrogen deposition.
Research sample	The dataset includes vegetation analysis of 673 plots, located in eight Eurasian countries or regions: Netherlands (287 plots), Poland (155 plots), Germany (90 plots), Siberia (83 plots), Belgium (20 plots), Scotland (12 plots), Sweden (16 plots) and Belarus (10 plots). Atmospheric N deposition in these study locations ranged between < 5 and > 45 kg N per ha per yr. In each plot the species composition was recorded, and aboveground biomass (current year's production) was harvested. Subsequently, the nitrogen, potassium and phosphorus content of of this biomass was determined in the laboratory.
Sampling strategy	The dataset has been collected over many years and by a large group of researchers (see also Fujita et al., Nature, 2014 and Roeling et al., Oecologia, 2018), with sampling locations being determined by study sites selected in ongoing, related research projects.
Data collection	The dataset has been collected over many years and by a large group of researchers (see also Fujita et al., Nature, 2014 and Roeling et al., Oecologia, 2018), with sampling locations being determined by study sites selected in ongoing, related research projects.
Timing and spatial scale	The 673 plots in the dataset ranged between 0.06m^2 and 25m^2 in size. Previous analysis on the majority of these plot (n=599) showed that the variation in size of these plots did not affect relationships between biodiversity and nutrient stoichiometry (Fujita et al., Nature, 2014), which forms also the focus of the current study. Plots were surveyed once, with the majority of plots being visited between 1990 and 2010.
Data exclusions	We used the same data selection criteria as described in Roeling et al. (Oecologia, 2018). Specifically, plots were excluded from the analysis if (a) more than 50% of the plot surface was covered with woody species; (b) K content of aboveground biomass was not measured, or (c) if the plots contained plants typical of saline (coastal) soils. In addition, all woody species were excluded from the dataset.
Reproducibility	As noted above, source data underlying this manuscript can be accessed from the Yoda Data Repository of Utrecht University at: https://doi.org/10.24416/UU01-I815KS. All data is freely available. The scripts used to generate the figures presented in the data are using standard commands implemented in widely used software programs.
Randomization	not applicable
Blinding	not applicable

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

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

Materials & experimental systems

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

Methods

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