Nutrient limitation and botanical diversity in wetlands: can fertilisation raise species richness?

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The 'resource balance hypothesis' proposes that the species richness of grassland vegetation is potentially highest when the N:P ratio of plant tissues is 10–15 (co-limitation), so that species richness could be raised by fertilisation with N or P at sites with lower or higher N:P ratios, respectively. Here we use data from field surveys in Swiss, Dutch and American fens or wet grasslands to analyse what changes in N:P ratios might produce noticeable changes in species richness. Plant species numbers, above-ground biomass, tissue N and P concentrations and soil pH were recorded in plots of 0.06–4 m^2. In each data set, plots with intermediate tissue N:P ratios (6–20) were on average most species-rich, but N:P ratios explained only 5–37% of the variation in species richness. Moreover, these effects were partially confounded with those of vegetation biomass and/or soil pH. The unique effects of N:P ratios (excluding those shared with biomass and pH) explained 11–17% of variation in species richness. The relationship between species richness and N:P ratios was asymmetric: plots with high N:P ratios were more species-poor than those with low N:P ratios. This was paralleled by a smaller species pool size at high N:P ratios (estimated from species numbers in multiple records), suggesting that fewer species are adapted to P-limited conditions than to N-limited conditions. According to these data, species richness in wetlands may possibly be raised by P-fertilisation when the initial N:P ratio of the vegetation is well above 20, but this option is not recommended for nature conservation as it might promote common species at the expense of rare ones.

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Of the numerous hypotheses proposed to explain variation in plant species richness, some have stressed the role of nutrient limitation (Tilman and Pacala 1993, Braak-hekke and Hooftman 1999). These hypotheses are based on the general assumption that species richness is promoted by mechanisms that prevent a few dominant species from excluding all others (Palmer 1994). Low or moderate nutrient availability has been thought to be one of these mechanisms, as it reduces the competitive advantage of fast growing, tall species relative to smaller ones (Taylor et al. 1990). Furthermore, the number of limiting resources has been considered important: if several resources together limit the biomass production of a vegetation, individual species may be limited by different resources and therefore unable to exclude each other (Palmer 1994, Tilman 1997). Co-limitation by several nutrient elements occurs when their relative supply is balanced, i.e. similar to the relative requirements of plants (Koerselman and Meuleman 1996, Sinclair et al. 1997). Under these conditions, interspecific

differences in the acquisition or utilisation of particular elements as well as small-scale variation in resource availability may cause co-existing species to be limited by different nutrients (Tilman et al. 1999, Mamolos and Veresoglou 2000). The 'resource balance hypothesis' (Braakhekke and Hooftman 1999) therefore predicts that species richness is promoted by a balanced supply of potentially limiting nutrients.

In temperate moist grasslands and wetlands, nitrogen (N) and phosphorus (P) are the main growth limiting resources (Bedford et al. 1999, Olde Venterink et al. 2003). For these vegetation types, the 'resource balance hypothesis' suggests that species richness should be greatest when the availability of N and P is balanced (Braakhekke and Hooftman 1999); fertilisation experiments have indicated this to be the case when the N:P ratio (ratio of N to P concentration) in the biomass of the vegetation (all species pooled) is about 15 (Güsewell and Koerselman 2002, Tessier and Raynal 2003). The 'resource balance hypothesis' was supported by survey work in Dutch grasslands, in which the most species-rich plots had intermediate (balanced) N:P ratios (Braakhekke and Hooftman 1999). Based on these results and on the aforementioned assumptions, Braakhekke and Hooftman (1999) suggested that in vegetation with high or low N:P ratios, fertilisation to create a more balanced supply of N and P (N:P ratios closer to 15) could increase species richness, at least if biomass production remains sufficiently low so that light does not become the single limiting resource.

Recently, Aerts et al. (2003) tested this suggestion using long term fertilisation experiments in two wet grasslands. They hypothesised that N fertilisation would increase species richness in a riverine grassland with biomass N:P ratio of 6, whereas P-fertilisation would do so in a peat grassland with N:P ratio of 16. However, the expected effects did not occur even though biomass production increased only little after fertilisation. Aerts et al. (2003) concluded that "patterns found in correlative studies of the relation between plant diversity and soil and vegetation N:P ratio can not be translated into successful experimental manipulations to enhance biodiversity". They explained their result with colonization limitation, proposing that diaspores of potential new species could not reach and colonize the fertilised plots to compensate for species that were displaced as a result of increased nutrient supply.

While this explanation is plausible given the recognized importance of colonization limitation for the species richness of grassland communities (Bakker et al. 1996, Lord and Lee 2001, Tofts and Silvertown 2002), there still remains the question whether a more balanced nutrient supply would raise species diversity over time at sites with unlimited propagule availability. There are at least three reasons to doubt this. First, there was no pronounced peak in species diversity at a N:P ratio of 15 in the data set of Braakhekke and Hooftman (1999). Rather, potential species diversity was maximal over a relatively broad range of N:P ratios and only decreased towards extreme values. If this pattern is general, only vegetation with very high or low N:P ratio might become more species-rich after fertilisation. Second, empirical relationships between N:P ratios and species diversity are not necessarily causal ones: they might reflect co-variation between N:P ratios and other factors that actually determine the species diversity, such as biomass production or pH (Seastedt and Vaccaro 2001). Third, the resource balance hypothesis (Braakhekke and Hooftman 1999) assumes that the species richness of a plant community is mainly determined by species coexistence or competitive exclusion. This view has been challenged by the species pool hypothesis, which emphasises the role of evolutionary and historical factors (Taylor et al. 1990, Zobel 1992, 1997, 2001, Wisheu and Keddy 1996). The species pool of wetlands is mainly related to water level, salinity and pH, and these factors might be more important for species diversity than competitive interactions (Gough et al. 1994, Pärtel 2002, Cornwell and Grubb 2003). It could be that the species pool of wetlands also depends on the N:P ratio, i.e. more species might be adapted to a certain range of N:P ratios than to others (Willby et al. 2001, Güsewell 2004). If so, however, species pool size might be maximal at high or low N:P ratios, and not necessarily at intermediate ones.

Here we use four data sets from field surveys in fens and wet grasslands to examine three questions. First, is there a (unimodal) relationship between vegetation N:P ratios and small-scale species richness in wetlands? How strong is this relationship? Second, is the (statistical) influence of N:P ratios on species richness confounded with the effects of biomass and soil pH, two factors that often correlate with species richness in wetlands (Bedford et al. 1999, Olde Venterink et al. 2001, 2003)? Third, is there evidence that species richness–N:P ratio relationships reflect variation in the size of species pools? Finally, we re-examine the conclusions of Aerts et al. (2003) in the light of our results.

Methods

Data source

The four data sets analysed here have been compiled largely from published field surveys (below), so that data collection will be explained only very briefly.

The first data set (CH) is based on surveys in 17 fen meadows in the region of Zurich, Switzerland (Brülisauer and Klötzli 1998, Güsewell and Edwards 1999, Bollens and Ramseier 2001). The meadows are part of lakeside wetlands (hydroseres) or of wetlands fed by seepage and rainwater on impermeable ground. Soils

are waterlogged or flooded from late autumn to spring but fairly dry in summer (Brülisauer and Klötzli 1998). All sites are mown annually in autumn and include several vegetation types (phytosociological alliances *Molinion*, *Filipendulion*, *Caricion davallianae* and *Magnocaricion*) owing to differences in water level and productivity. Within each site, the vegetation was surveyed along transects perpendicular to the main vegetation gradient, with 5–20 m distance between neighbouring plots, depending on the steepness of the vegetation gradient. The vascular plant species composition was recorded in June or July 1995–1999 in a total of 125 plots of 4 m^2, and the above-ground biomass was harvested in 0.5-m^2 subplots in July or August (biomass changed little between these two months). After drying, the biomass was ground and analysed for N and P concentrations (see original publications for analytical details). The soil pH was only determined for 42 plots by Brülisauer and Klötzli (1998) in a 1:2.5 suspension of fresh soil in 0.01 M CaCl$_2$.

The second data set (NL) is based on a vegetation study in "De Leemputten" nature reserve in the central part of the Netherlands (Roem and Berendse 2000). This vegetation includes heathlands and wet or dry grasslands, which developed on aeolian sandy deposits as a result of pasturing and exploitation of the underlying loam; grasslands are currently mown for conservation every year in August. Here we include data from 46 quadrats (25 × 25 cm^2) in wet grasslands. The plant species composition of each quadrat was recorded in August 1995, after which the above ground biomass was harvested, dried, weighed and analysed for N and P concentrations. The soil pH was measured after shaking 20 g fresh soil with 50 ml demineralized water (Roem and Berendse 2000).

The third data set (USA) is based on unpublished survey work of the authors (KMB and BLB) in 16 *Carex*-dominated, herbaceous fens within the glacial Lake Ontario drainage basin of New York State, north eastern USA. At each site, five 1-m^2 plots were randomly established within the zone of fen vegetation, and their plant species composition was recorded in July-early August 1998 or 1999. The above-ground biomass was harvested in 40 × 40 cm^2 subplots, dried, weighed and ground. The N concentration was determined by combustion on an elemental (CHN) analyser. The P concentration was determined by nitric-perchloric acid digestion and analysis via ICP. Pore water pH was measured in situ using hand-dug 15 cm deep soil pits.

The fourth data set (EUR) consists in a database of N and P concentrations in European wetland plants compiled by Güsewell and Koerselman (2002). The database includes 1248 records from 126 vascular plant species sampled at their natural field sites in non-forested European freshwater wetlands, such as fens,

bogs, wet grasslands, dune slacks or wet heaths (n = 1–102 per species). Sampling sites were managed (mown or grazed) or unmanaged but not fertilised. All nutrient concentrations were determined between June and August in the above-ground biomass of individual plant species.

Data analysis

The dependence of small scale species richness (number of species per plot) on biomass N:P ratios was analysed with second order polynomial regression, so that linear and unimodal relationships could be fitted. Species numbers were square-root transformed, and N:P ratios log-transformed. The latter was done even if there were few plots with low N:P ratios because N:P ratios are usually log-normally distributed (Güsewell and Koerselman 2002) and because the log-transformation gave N-limited and P-limited sites a more similar influence on the regressions (independent variables do not have to be normally distributed in regression analysis). The log-transformed N:P ratios were further centred by their means within each data set to remove the correlation between linear and quadratic terms, so that their significance could be tested independently. Similar regressions were also calculated using either biomass or pH as an independent variable to compare their effects to those of N:P ratios.

To examine whether the effects of N:P ratios on species richness were independent of those of biomass and pH, or confounded with them, we first tested the relationships between these variables with polynomial regressions. The three explanatory variables were indeed interrelated, which implies that part of the variation in species richness cannot be attributed unequivocally to one of them (Graham 2003). We therefore used sequential multiple regression to partial out the fraction of variation in species richness that was attributable to unique and shared effects of the three explanatory variables, respectively (Whittaker 1984, Graham 2003).

Unique (partial) effects are those which are not accounted for by any other explanatory variable. The fraction of variation in species richness accounted for by the unique effect of explanatory variable x was given by

$$\{SS(x|zt) + SS(x^2|zt)\}/SS_{total}$$

where $SS(x|zt)$ and $SS(x^2|zt)$ represent the sequential sums of squares of the linear and quadratic terms of x, respectively, when x enters the regression model after the two other variables z and t, and SS_{total} is the total (model+residual) variation in species richness.

Shared (confounded) effects are due to the co-variation of two or more explanatory variables. For the two variables x and z, variation accounted for by their shared

effects (but not by the third variable t) was given by

$$\{SS(x|t) + SS(x^2|t) - SS(x|zt) - SS(x^2|zt)\}/SS_{total}$$

where $SS(x|t)$ and $SS(x^2|t)$ represent the sequential sums of squares of the linear and quadratic terms of x, when x enters the regression model after t but before z. Note that the same result is obtained by calculating

$$\{SS(z|t) + SS(z^2|t) - SS(z|xt) - SS(z^2|xt)\}/SS_{total}$$

The resulting term is positive if unique and shared effects of the two variables act in the same direction, and negative if they act to compensate each other (Whittaker 1984). Variation due to effects shared by the three variables was given by the total explained variation (R square) minus the sum of the unique effects minus the sum of the pairwise shared effects.

Finally, the total effect of a variable x, i.e. the sum of its unique and shared effects, was given by

$$\{SS(x) + SS(x^2)\}/SS_{total}$$

where $SS(x)$ and $SS(x^2)$ represent the sequential sums of squares of the linear and quadratic terms of x when x enters the regression model first. Calculations were performed with the statistical software JMP, version 3.2 (SAS Institute 1989–2000).

To assess whether the size of the species pool of herbaceous wetlands is related to the biomass N:P ratio, we used the CH and EUR data sets, which included the largest numbers of records, and followed a procedure similar to that of Wisheu and Keddy (1996). With the CH data, relevés were grouped in classes with equal ranges of biomass N:P ratios, i.e. 0.2 on a log scale, so that most classes would include ≥15 plots. We allowed neighbouring classes to overlap by shifting their N:P ratio ranges in steps of 0.05 in order to avoid an undue influence of the (arbitrary) choice of class boundaries. Five times for each class (only once if n <15), we randomly drew ten relevés and determined the total number of species included. With the EUR data, we grouped records in the same N:P ratio classes (range of 0.2 on a log scale, shifted by 0.05) and determined five times for each class (only once if n <40) the total number of species included in a randomly drawn subset of 30 records. Subsets included 30 records here because a record corresponds to a single species, and not a relevé with many species. In both cases, means and SD of the calculated 'species pools' were plotted against class means of N:P ratios. No regression line was fitted given the overlap (lack of independence) of neighbouring classes (Wisheu and Keddy 1996). The 'species pools' calculated in this way were doubtless smaller than the true regional species pools, but given the random selection of relevés included in calculations, we consider likely that our results reflect the trends in species pool size.

Results

Small scale species richness was significantly related to the biomass N:P ratio of the vegetation in all three data sets (Fig. 1a–c). The relationship was weakly unimodal for the NL and CH data sets (significant quadratic term in the polynomial regression) but linear negative for the USA. Within each data set, the most species-rich plots had lower N:P ratios than the least species-rich ones. Thus, the N:P ratios of the most species-rich plots (upper 10% of each data set) ranged from 6.0 to 15.0 (CH), 11.3 to 20.7 (NL) and 11.4 to 17.5 (USA), whereas the N:P ratios of the least species-rich plots (lower 10% of each data set) ranged from 9.4 to 26 (CH), 19.7 to 35.5 (NL), 15.7 to 23.5 (USA).

Small scale species richness was also significantly related to the above ground biomass of the vegetation in the CH and USA data sets, with unimodal and decreasing relationships, respectively (Fig. 1d, f). In the NL data set, biomass varied little and was unrelated to species richness (Fig. 1e). Finally, species richness increased with increasing pH in the NL and USA data sets (Fig. 1h, i) but not in the CH data set (Fig. 1g).

Explanatory variables partly depended on each other (Fig. 2). Relationships between N:P ratios and biomass were negative in the CH data set (Fig. 2a) but unimodal in the two other data sets (Fig. 2b, c). Relationships between N:P ratios and pH were negative in the CH data set (Fig. 2d), unimodal with a decreasing overall trend in the NL data set (Fig. 2e) and not significant in the US data set (Fig. 2f).

The role of N:P ratios as predictors of species richness differed among the three data sets. In the CH data, unique effects of N:P ratios accounted for most of the explained variation (17%) and were hardly confounded with those of biomass. In the NL data, most variation was explained jointly by N:P ratios and pH (26%), but their unique effects were also relatively important. In the USA data, most variation was explained by the unique effects of biomass (25%), and further 11–12% by those of N:P ratios and pH; shared effects of two or three variables were partly opposite. Although the unique effects of N:P ratios were similar in the three data sets (11–17%), their total effects (unique+shared) differed considerably, ranging from 5% to 37% (Table 1).

Species pool size, as approximated by the number of species per 10 relevés (Fig. 3a) or per 30 records (Fig. 3b), changed little up to biomass N:P ratios around 15, but it declined considerably towards higher N:P ratios. The decrease was more pronounced in the EUR data set (Fig. 3b) because the latter included higher N:P ratios than the CH data set (Fig. 3a). In contrast, the frequency distribution of N:P ratios within each data set was rather symmetric (histograms in Fig. 3), meaning that sampling intensity was similar for N- and P-limited vegetation.
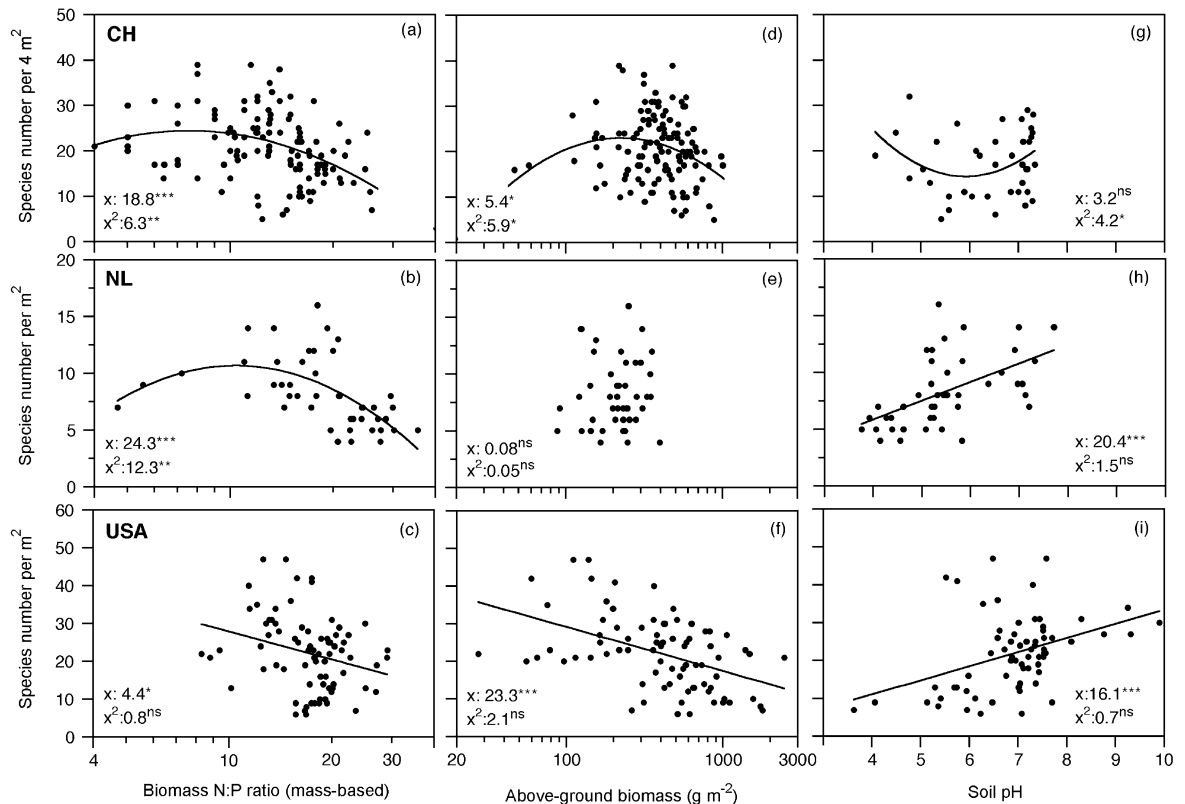
Fig. 1. Species richness of herbaceous wetland vegetation in relation to (a–c) the biomass N:P ratio (log scale), (d–f) the above-ground biomass (log scale), and (g–i) soil pH, determined for three sets of plots in (a, d, g) Switzerland, (b, e, h) the Netherlands, and (c, f, i) the north-eastern USA (source of data in text). Relationships were modelled with second-order polynomial regression; F-ratios and significance levels are indicated within each graph for the linear (x) and quadratic (x^2) terms. Regression lines are shown if significant. Species richness was square-root transformed for the regression calculations but is represented on a linear scale in the graphs for convenience.

Discussion

Type and significance of N:P ratio-species richness relationships

Biomass N:P ratios were significantly related to small scale species richness in all three data sets investigated here, and in every case the type of relationship was consistent with the unimodal relationship predicted by the resource balance hypothesis (Braakhekke and Hooftman 1999): species richness was maximal at intermediate N:P ratio, decreased slightly towards lower N:P ratios, and decreased more towards higher N:P ratios. The USA data included no plots with N:P <8 and only three plots with N:P <10; therefore, the montonic decrease in species richness towards higher N:P ratios found with this data set is still consistent with the hypothesis: a unimodal relationship might have been obtained across the full range of N:P ratios.

Although N:P ratios were significantly related to species richness, they only accounted for 11–17% of the variation in species richness on their own ('unique effects'), and a broad range of species richness

was possible at any N:P ratio. With reference to the experiment of Aerts et al. (2003), our results mean that shifts in biomass N:P ratios from initial values of 6 or of 16 towards intermediate values can hardly be expected to raise species richness: on average, variation in N:P ratios has virtually no effect across this range.

Vegetation with high N:P ratios was less species-rich than vegetation with low N:P ratios in all three data sets. This difference was not predicted by the resource balance hypothesis (Braakhekke and Hooftman 1999), but it is consistent with results of other surveys, which also found decreasing species richness with increasing N:P ratio (Seastedt and Vaccaro 2001, Schaffers 2002). Furthermore, species diversity correlated positively with P availability in salt marshes (Theodose and Roths 1999), which probably also implied a negative correlation with N:P ratios. In a recent survey of tundra vegetation, there was no significant relationship (Van der Welle et al. 2003), but only few plots in that survey had high N:P ratios, and the only one with N:P >20 did have a low species richness. Low species richness at high N:P ratios thus seems to be a rather common pattern.
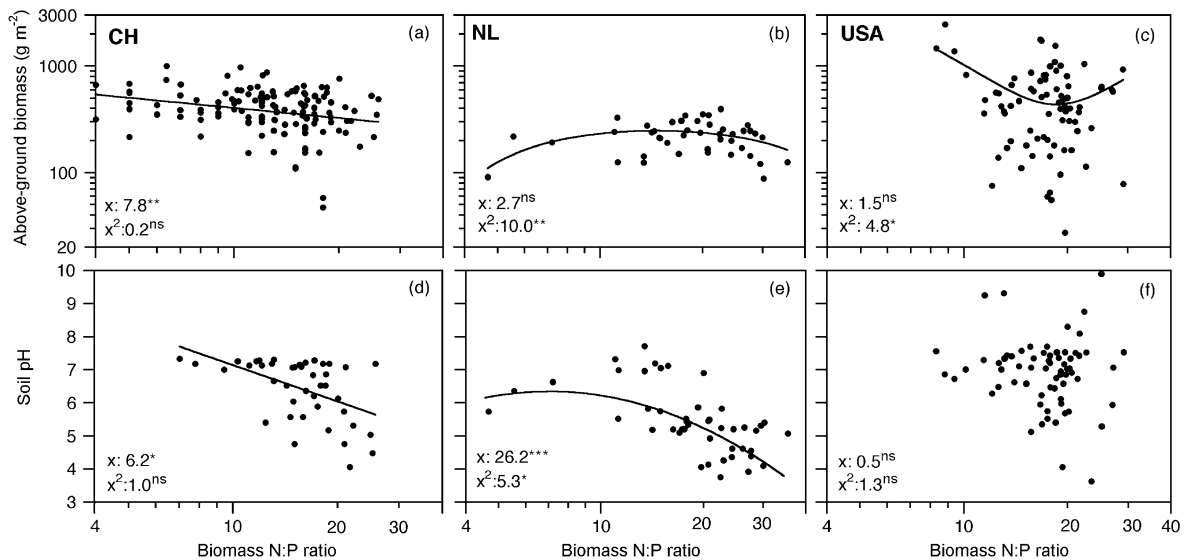
Fig. 2. Relationships between the biomass N:P ratio of herbaceous wetland vegetation (log scale) and (a–c) the above-ground biomass (log scale), and (d–f) soil pH, determined for three sets of plots in (a, d, g) Switzerland, (b, e, h) the Netherlands, and (c, f, i) the north eastern USA. Relationships were modelled with second-order polynomial regression; F-ratios and significance levels are indicated for the linear (x) and quadratic (x^2) terms.

If interspecific competition determined species richness in our surveys, our results would suggest that competition was most intense at high N:P ratios. This would contrast with the hypothesis that interspecific competition should be less intense – and thus, species coexistence facilitated – when growth is limited by a resource that is hardly mobile in soil, such as P (Huston and De Angelis 1994, Olde Venterink et al. 2003). Short term competition experiments with various graminoids indeed showed that for a given pair of species, inter-specific competition was weaker at high than at low N:P supply ratio (Güsewell and Olde Venterink, unpubl.). In the field, however, the situation differs in that vegetation composition changes along N:P ratio gradients (Willby et al. 2001, Güsewell 2004). Clonal graminoids tend to dominate in wetlands with high N:P ratios and to become more dominant after N fertilisation (De Kroon and Bobbink 1997, Pauli et al. 2002, Gough and Hobbie 2003, Güsewell et al. 2003). Species richness in grasslands and wetlands is often negatively related to the dominance of graminoids (Willems and van Nieuwstadt 1996, Theodose and Roths 1999). This might help explain the overall decline in species richness towards high N:P ratios.

Low P availability has sometimes been considered a prerequisite for high species richness (Janssens et al. 1998). Our data indicate that this does not hold for herbaceous wetlands: vegetation with high N:P ratios consistently had low P concentrations (Fig. 4b) and not high N concentrations (Fig. 4a), yet species numbers declined at high N:P ratios. Wetlands may be an exception within temperate vegetation because of the extremely high N:P ratios reached in some of them (Tomassen et al. 2004). P deficiency might therefore be a far more severe constraint in wetlands than in other vegetation types. There are indeed reports of P fertilisation increasing species richness in wetlands, but the effect was only transitional in the experiment of Roem et al. (2002), and it reduced the fraction of typical fen species in the experiment of Pauli et al. (2002). More generally, P-limited wetlands are particularly rich in rare and threatened species (Olde Venterink et al. 2001, 2003), which may outweigh a reduced species richness from a conservational point of view and make P fertilisation an unsuitable conservation tool.

Table 1. Partitioning of the variation in small scale species richness among the unique and the shared effects of tissue N:P ratios, above ground biomass, and soil pH, respectively, based on sequential regression including linear and quadratic terms for each variable. All fractions are given as percentages of total variation (explained+residual). See Methods for calculations.

Source of variation	CH	NL	USA
N:P ratio	17.18	11.48	11.49
Biomass	9.19	1.57	25.57
pH	*	10.09	12.25
N:P and biomass	−3.41	1.98	1.75
N:P and pH	*	26.51	5.01
Biomass and pH	*	−0.18	6.77
N:P and biomass and pH	*	−2.85	−12.94
N:P ratio total (unique+shared)	13.77	37.12	5.31
Total explained	22.96	48.61	49.89
Residual	77.04	51.39	50.11

* Because pH data were only available for a subset of the plots, they were not included in this analysis

Low N availability and/or N limitation also seem to reduce species richness according to the few records with extremely low N:P ratios included in our data sets (Fig. 1, 3). However, as very low N:P ratios are always associated with high P concentrations (Fig. 4), N fertilisation hardly increases the N:P ratio of such vegetation towards more balanced N:P ratios (van Duren et al. 1997, Aerts et al. 2003), so that no effect of N fertilisation on species richness would be expected according to Fig. 1.

Confounding between effects of N:P ratios and other factors

We hypothesised that the effects of N:P ratios might be confounded with those of biomass or pH. This expectation was confirmed only by the NL data set, where the effects of N:P ratios on species richness were largely confounded with those of pH. In this data set, base-rich plots (pH ≥ 7) had intermediate N:P ratios, whereas acidic plots (pH ≤ 5) had low N:P ratios. Since there is generally a positive relationship between pH and species richness in temperate vegetation (Bedford et al. 1999, Olde Venterink et al. 2001, Pärtel 2002, Chytry et al. 2003), a base-rich soil rather than balanced nutrient supply may well have been the main reason why vegetation with intermediate N:P ratios was most species-rich. A relationship between pH and N:P ratio might also have been expected in the two other datasets given the effect of pH on the chemical form (and thus, availability) of N and P in soil, but no relationship was found. Wetlands are often dominated by Cyperaceae species able to acquire N and P from compounds that are not available to all plants (Perez-Corona et al. 1996, Lipson and Näsholm 2001), and they have an efficient internal nutrient cycling; these properties may have made the N:P ratio of the vegetation unrelated to the soil pH, which in turn made the effects of N:P ratios and pH on species richness independent of each other.

There was no confounding between the effects of N:P ratios and of biomass on species richness because these
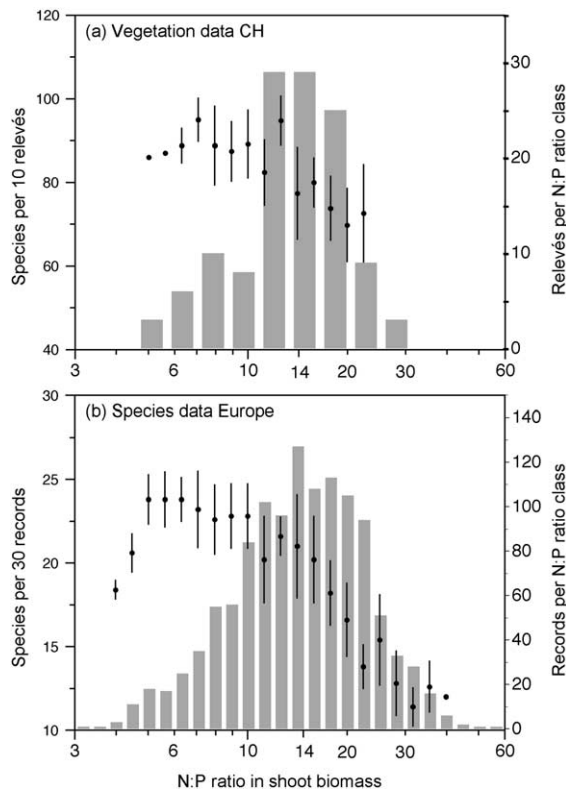

Fig. 3. Total number of plant species ('species pools') included in (a) ten vegetation relevés in Swiss wet meadows and (b) 30 species records in European wetlands in relation to the biomass N:P ratio of the vegetation. Species numbers (symbols, means $\pm$ SD) were calculated by randomly drawing five times ten relevés or thirty records from all relevés or records whose biomass N:P ratios lie within a range of 0.2 (log scale), shifting class boundaries by 0.05 between neighbouring classes. The frequency distribution of N:P ratios within each data set is shown by histograms.
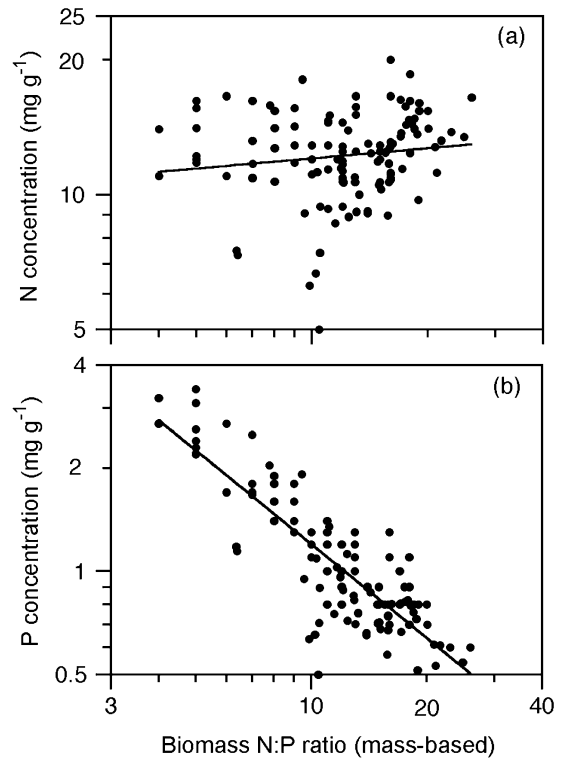

Fig. 4. Relationships between the biomass N:P ratio of herbaceous wetland vegetation and (a) its N concentration, and (b) its P concentration based on data from fen meadows in Switzerland (relationships were similar for the other three data sets included in Fig. 1–3). All variables are represented on a log scale.

two factors were hardly related (Fig. 2). The weak negative relationship found in the CH data set had no implications for species richness because biomass and species richness were unrelated in the CH data set.

Another factor not considered here but whose effect might potentially be confounded with that of N:P ratios is potassium (K) limitation (van Duren et al. 1997, Roem and Berendse 2000). Geerts and Oomes (2000) found high N:P ratios to be associated with K limitation in wet grasslands and proposed that both together reduced species richness. We did not include K concentrations in our data analysis because no data were available for the CH and EUR data sets. Thus, although our study did not reveal a consistent confounding between the effects of N:P ratios and other factors on species diversity, this problem should be considered before relationships between N:P ratios and species richness are assumed to be causal.

Species pools in relation to N:P ratios

The decline of our regional species pool estimates with increasing N:P ratio suggests that P-limited wetlands recruit their species composition from smaller species pools than N-limited wetlands. This might indicate that regional processes contribute to the observed relationships (but see Huston 1999, Herben 2000). It has been proposed that the size of regional species pools reflects past opportunities for speciation: fewer species would have evolved if a type of habitat was rare (Taylor et al. 1990, Zobel 1992, Schamp et al. 2002, Ewald 2003). In temperate regions of the northern hemisphere, P limitation has been relatively rare at an evolutionary time scale (Vitousek and Howarth 1991, Reich and Oleksyn 2004), so that there was less opportunity for diversification among species adapted to P limitation. The frequent occurrence of vegetation with high N:P ratios in our data probably results from increased atmospheric N deposition, and partly from management (Verhoeven et al. 1996, Willems and van Nieuwstadt 1996).

If the relationships shown in Fig. 3 have an evolutionary background, they should not exist in regions with old, acid-weathered soils, where plants have evolved under P-limited conditions (Handreck 1997). Alternatively, if relationships in Fig. 3 reflect the influence of other factors correlated with N:P ratios (above), they might be independent of historical nutrient limitation. Similar data from several regions of the world would help to understand why species pool size decreased with increasing N:P ratios in our data sets.

Conclusions

Can species richness be raised by fertilisation? The data presented here suggest that this might potentially be the case in northern temperate wetlands if the N:P ratio of the vegetation is well above 20: in such cases, P fertilisation would increase the size of the species pool. New species might therefore colonise the wetland after fertilisation provided that dispersal is not restricted (Aerts et al. 2003) and that other factors, such as pH, are also favourable. However, this option will usually be inappropriate for nature conservation as it will tend to promote common species at the expense of rare ones (De Kroon and Bobbink 1997, Olde Venterink et al. 2001). For N:P ratios below 20, N or P fertilisation can hardly be expected to increase the size of the species pool and is therefore unlikely to raise species richness. The experimental results of Aerts et al. (2003) are therefore not in conflict with patterns found in correlative studies, but in full agreement with them. Our data further point to the potential importance of species pools (Zobel 1997) and stoichiometric constraints (Sterner and Elser 2002) in determining the effects of (anthropogenic) changes in nutrient availability on species diversity (Bobbink et al. 1998).

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