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Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply

BENOÎT O. L. DEMARS AND A. C. EDWARDS[1]
The Macaulay Institute, Craigiebuckler, Aberdeen, U.K.

SUMMARY

1. The elemental composition and stoichiometry of aquatic plants has often been suggested to reflect the nutrient enrichment of aquatic habitats. However, the relationship is often weak. Moreover, uncertainties remain in the relevance of laboratory derived critical plant tissue nutrient concentrations to maximum yield or growth rates in the field.
2. Aquatic vascular plants and bryophytes, overlying water and sediment samples were collected to test whether freshwater aquatic macrophytes: (i) show tissue nutrient deficiencies when growing in oligotrophic freshwater habitats, and (ii) have strict homeostatic stoichiometry.
3. Plant nutrient concentrations were significantly related to total inorganic nitrogen (or nitrate), total dissolved phosphorus and sediment total phosphorus. However, these relationships were weak. Virtually all the variance in plant tissue nutrient concentrations, however, could be explained by species (taxon) identity.
4. Critical tissue nutrient concentrations for 95% maximum yield or 95% maximum growth rate in aquatic angiosperms, determined from laboratory bioassays, suggested that nutrients should not limit yield in wild aquatic macrophytes. However, there were a substantial number of samples where potential growth rate limitation was possible, particularly due to phosphorus.
5. Strict C : N : P stoichiometric ratios were found for both vascular plants and bryophytes, suggesting little scope for plants as indicators of nutrient enrichment, but provide robust stoichiometric data for studies on ecosystem metabolism and nutrient cycling.

Keywords: aquatic plants, C : N : P stoichiometry, elemental composition, eutrophication

Introduction

Freshwater macrophytes rely upon the surrounding sediment and water to satisfy their nutrient requirements. The relationship between measured nutrient availability and the nutrient concentration of the plants is often weak, however (Dykyjová, 1979; Shardendu & Ambasht, 1991; Robach *et al.*, 1996; Thiébaut & Muller, 2003).

Critical nutrient threshold concentrations (i.e. the cellular nutrient concentration below which the growth rate or final yield of a species is impaired) have been defined for a number of freshwater angiosperms, using controlled laboratory-based experimental bioassays (Gerloff & Krombholz, 1966; Gerloff, 1975; Colman *et al.*, 1987). It is also clear from these bioassays, however, that freshwater angiosperms can accumulate nitrogen (N) and phosphorus (P) during times of nutrient sufficiency, often termed 'luxury consumption'.

Correspondence: Dr Benoît Demars, The Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, U.K.
E-mail: b.demars@macaulay.ac.uk
[1]Present address: Nether Backhill, Ardallie, By Peterhead, Aberdeenshire AB42 5BQ, U.K.

Measured plant tissue nutrient concentrations are usually above the yield-derived critical nutrient thresholds (Hutchinson, 1975, p. 356; Allenby, 1981). Therefore, it has been concluded that, under such conditions, the plants are not limited by the availability of nutrients (e.g. Kern-Hansen & Dawson, 1978; Haslam, 1987, p. 168–169). Such a conclusion is open to debate for two main reasons. Firstly, the critical nutrient thresholds will differ between species (Gerloff, 1975), and depend not only on the methods employed (Schmitt & Adams, 1981; Colman *et al.*, 1987) but also on the environmental conditions used (Madsen, Hahn & Johansen, 1998). Critical thresholds below which species growth rate becomes impaired are higher than critical thresholds below which the final standing biomass (=yield) of the bioassay is impaired (Colman *et al.*, 1987). Hence, there might have been no yield limitation but growth rate limitation (Madsen & Cedergreen, 2002). Secondly, relatively little sampling effort has been devoted to plants growing under conditions of low nutrient concentrations (e.g. NO_3-N < 100 μg L^{-1}, SRP-P < 10 μg L^{-1}), which might also help explain why the majority of plant tissue nutrient concentrations measured so far have been above critical thresholds.

Basic data on C : N : P stoichiometry can be used to express the net chemical reaction of vascular plant metabolism (Atkinson & Smith, 1983):

$$550CO_2 + 580H_2O + 30HNO_3 + H_3PO_4 \rightarrow (CH_2O)_{550}(NH_3)_{30}H_3PO_4 + 610O_2.$$

Using this stoichiometric relationship provides a way of estimating ecosystem N and P plant uptake from an estimation of ecosystem metabolism based on organic carbon. While significant stoichiometric differences were found to exist between vascular plants and algae (Atkinson & Smith, 1983; Duarte, 1992), no equivalent metabolic-based relationship has been suggested for bryophytes. Hence, if the stoichiometry of vascular plants and bryophytes turn out to be strictly homeostatic, this would suggest there is little scope for their use as bioindicators of nutrient limitation. However, even if this is true, they would still be informative in studies of ecosystem metabolism and nutrient cycling.

Here, we hypothesized that freshwater aquatic macrophytes growing in oligotrophic freshwater habitats do not show nutrient deficiencies based upon their tissue nutrient concentration, have strict homeostatic stoichiometry, and that their elemental composition is taxon-specific rather than environmentally induced by varying external nutrient concentrations. More specifically, we hypothesized that (i) freshwater aquatic plant N and P tissue concentrations are above a yield-derived and growth rate-derived critical nutrient threshold (except during periods of senescence), (ii) the range in plant N : P ratio is narrow, (iii) plant C : N and C : P ratios are inversely related to plant N and P concentrations, respectively (i.e. C : nutrient ≈ nutrient^{-1}), and (iv) plant N and P tissue concentration is under genetic, rather than phenotypic, control. These were tested by assembling a comprehensive set of data on wild plants, sediment and overlying water (at low, steady state flow and nutrient concentrations) across various surface water habitats and biophysical zones, located within an oligotrophic river basin.

Methods

River Spey catchment

The River Spey rises in the Monadhliath Mountains and flows 157 km north-eastwards to the North Sea. The catchment (*circa* 3000 km^2) is underlain by crystalline rocks (schists and gneisses) dating from the Precambrian and Cambrian periods with granite intrusions (Maizels, 1988). Much of the current landscape reflects the action of successive glaciations. Further information can be found in Jenkins (1988) and North East River Purification Board (1995).

Sampling area

The study area, a 10 × 10 km grid square ranging from 220 to 980 m altitude (Long 3°55′W, lat 57°07′N), with the Monadhliath Mountains to the north west and the Cairngorm Mountains to the south east, is characterized by contrasting geology, soil and land cover (Fig. 1). Human pressure on water quality is generally slight (Potter, 1988). Land use is varied, with more intensive agriculture in the SW–NE flood plain and extensive grazing and forestry on higher land. There are a few consented point sources of effluent in the area and other unidentified local sources, such as septic tank outflows. See Demars & Edwards

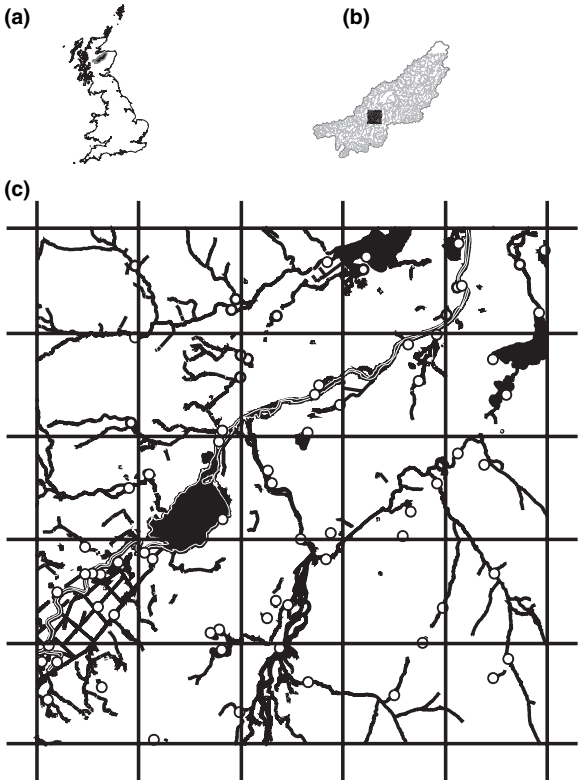

Fig. 1 (a) River Spey catchment within Great Britain, (b) 10 × 10 km grid square within the River Spey catchment, and (c) sampling sites within the 10 × 10 km grid square divided in 25 tetrads. Water flows mostly from SW (bottom left corner) to NE (top right corner). © Crown copyright 2006. All rights reserved Macaulay Institute GD27237X 2006.

(in press) for further details on the spatial heterogeneity of the surface water chemistry.

Sampling strategy

The 10 × 10 km square was divided into 25 smaller 2 × 2 km tetrads (Fig. 1). Ordnance Survey maps (1/25 000) were used to identify aquatic habitats within each tetrad (Demars & Edwards, in press). A representative sampling plan (stratified random survey) was devised (based on water body types) which resulted in a different number of sites being sampled per tetrad (Fig. 1). The number of sampling sites was constrained by resources to an average of three sites per tetrad. Sampling sites covered a wide range of surface water bodies (including running and standing waters and two bogs) and were situated in different biophysical zones based on solid geology, soils and land cover (including floodplain, glacial terraces and mountainous areas); see Demars & Edwards, in press.

Collection and analysis of samples

Water samples were collected during four periods in 2003, 22 February – 1 March; 1–7 June; 6–9 and 13–16 September; 23–27 November and 1–4 December. Sites were sampled under base flow conditions as far as possible. Thus nutrient concentrations reflected steady state concentrations. Nitrate plus nitrite (hereafter NO_3), ammonium (NH_4), soluble reactive phosphorus (SRP), total dissolved nitrogen (TDN) and phosphorus (TDP) were determined on filtered (Whatman GF/C) water samples following standard analytical procedures as detailed in Demars & Edwards, in press. The detection limits were 1 μg L^{-1} for NO_3-N, NH_4-N and SRP and 2.5 μg L^{-1} for TDN and TDP. Total soluble organic N (TON) was calculated as TDN minus total inorganic N (TIN = NO_3-N + NH_4-N). Molybdate unreactive P was calculated as TDP minus SRP. Three sediment samples (zero to 5–7 cm depth) per site (reflecting substratum heterogeneity) were collected during summer 2002 or 2003, using a plastic hand-scoop, then immediately passed through a 2 mm sieve and the <2 mm fraction stored in plastic containers. Samples were kept cool and in the dark while being transported to the laboratory, where they were oven-dried at 60 °C. Sub-samples of about 3 g of sediment per sample were subsequently milled to a fine powder which was used for the determination of total carbon (C) and nitrogen (N) using a CHN elemental analyser. Total P was determined after a sodium hydroxide fusion (Smith & Bain, 1982). The detection limits were 0.03 and 0.02% dry weight (DW) for N and C, respectively, and 4.5 mg kg^{-1} DW for Total P. Dry weight was determined on oven-dried samples at 105 °C prior to C, N and P analysis.

The total percentage cover and proportion of vascular plants, bryophytes and charophytes were assessed visually within the surveyed sites (area of ≈ 500 m^2). The most frequent/abundant plant taxa (three-12 taxa per site) were collected during July and August 2003 (listed in Appendix 1). Three to 10 samples were collected from different individuals for each species of vascular plants within a site. Mosses and liverworts were sampled from three isolated patches per site. Samples from emergent species were collected only when growing within the water body, i.e. not on the shore or streambank. Vascular plants were all identified to species, but mosses (except *Fontinalis antipyretica*, *F. squamosa* and *Sphagnum* sp.)

and liverworts were left unidentified. The anchoring system, degenerating leaves and inflorescences were removed, except for the isoetids where rooting material was included. Care was taken to clean the plant material gently with either the water from the site or water from a private water supply with extremely low concentrations of N and P (11 μg L^{-1} NH_4-N, 125 μg L^{-1} NO_3-N, 1 μg L^{-1} SRP). Moss and liverwort samples were cleaned shoot by shoot in a tray filled with water. Total C and N were determined with an elemental analyser by combustion. Total P was determined after 30 min digestion in 50% nitric acid at 120 °C. The recovery of P from hay reference material (CRM129, Community Bureau of reference) was 97 ± 1%. Nitric acid digestion was an efficient method for total P analysis for a wide range of other plant reference materials (see Havlin & Soltanpour, 1980; Huang & Schulte, 1985; Zarcinas, Cartwright & Spouncer, 1987; Batten *et al.*, 1992; Rodushkin, Ruth & Huhtasaari, 1999). The detection limit for P was 6 mg kg^{-1} dry weight.

Plant tissue critical nutrient thresholds

Two nutrient thresholds were considered using the data published by Gerloff (1975) based on yield limitation, and the correction suggested by Colman *et al.* (1987), to estimate growth rate limitation. The N and P critical nutrient concentrations for 95% maximum yield of the four species studied by Gerloff (1975) (*Myriophyllum spicatum* L., *Elodea nuttallii*, *Ceratophyllum demersum* L. and *Lemna minor* L.) were averaged to provide a common baseline for all vascular plant species. The critical nutrient concentrations for 95% maximum yield were N = 1.14 (±0.39 SD) and P = 0.10 (±0.03 SD)% DW, and for 95% maximum growth N = 1.82 (±0.62 SD) and P = 0.16 (±0.05 SD)% DW. The study by Gerloff (1975) was selected because it was the only one providing critical concentration values for N and P for several species, even though *E. nuttallii* was the only species recorded in the present study.

Data processing

Individual sites were only retained for statistical analysis when their datasets consisted of a minimum of three taxa, three sediment samples and four seasonal water samples. The resulting dataset consisted of 378 plant samples across 65 sites. This was further reduced to 344 plant samples in regression analysis quantifying the effect of taxon on plant tissue nutrient concentration. The latter dataset contained 33 taxa with a minimum of three different plant samples per taxa (Appendix 1).

Nutrient sediment concentrations were averaged for each site. Data for individual water samples were averaged for each location as autumn/winter, spring/summer and all-seasons. Data transformations were applied when necessary to reduce heteroscedasticity and increase the normality of the data.

Linear mixed model regression analyses were performed using the method of residual maximum likelihood (REML), with 'site' introduced as a random effect, to investigate the fixed effects of environmental nutrient concentrations, taxon, habitat types and biophysical zones. The REML function of Genstat 8 was run to perform the analyses using the average information algorithm (see Payne, 2005).

The Wald test was used to test the significance of the fixed model terms (Payne, 2005). The Wald test has two parts: sequentially adding terms to a fixed model and dropping individual terms from a full fixed model. As the fixed effects are non-orthogonal, the statistics depend on the order in which the terms are specified in the fixed model. It is then necessary to fit the model terms in several different orders to judge which of the main effects should be retained. Hence, several tests were carried out permuting the order of the co-variables taxa, water and sediment chemistry. The order of the variables presented in the result section reflects well the output of other alternative tests. Habitat types and biophysical zones were always introduced after the nutrient chemistry and taxonomic factors, however, because it was not the main focus of this study. The effect of removing terms from the complete mixed model allows the assessment of the effects of a term after eliminating all the other fixed terms. This is particularly useful for seeing how the model might be simplified. For more details on the mixed model regression analyses, see Payne (2005).

An approximate regression coefficient was calculated as the square of the linear Pearson correlation coefficient between predicted and observed plant nutrient concentration. The regression analysis assumed that sites were independent entities. However, because many sites are related through the river

network, for instance, or are relatively aggregated (Fig. 1), the residuals of the regression analyses were plotted on variograms along four directions at 45 ° from each other in an attempt to detect any spatial autocorrelation.

Results

Distribution of aquatic plants

The maximum total percentage plant cover within a tetrad ranged from 1% to 90% (Fig. 2). Particularly low values occurred in the streams with unstable beds (Monadhliath Mountains to the north west and piedmont of the Cairngorm Mountains to the south east), intermediate values in the Cairngorm Mountains and highest coverage in the floodplain. The proportion of bryophytes to vascular plants was highest in the mountains (Fig. 3) while the proportion of charophytes was negligible at all sites.

Environmental nutrient conditions

Sediment C and N concentrations ranged between <0.1–52 and <0.03–2.55% DW, respectively, and were highly correlated (Fig. 4). Sediment P ranged between 0.01 and 0.2% DW and was less strongly correlated

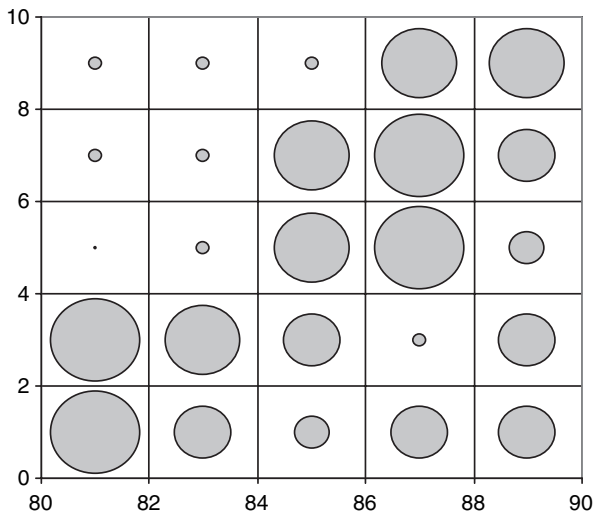

Fig. 2 Maximum total percentage of plant cover encountered at surveyed sites within each tetrad. The area of the discs is proportional to the percentage of total plant cover ranging from 1 to 90%. Monadhliath Mountains to the NW, Cairngorm Mountains to the SE.

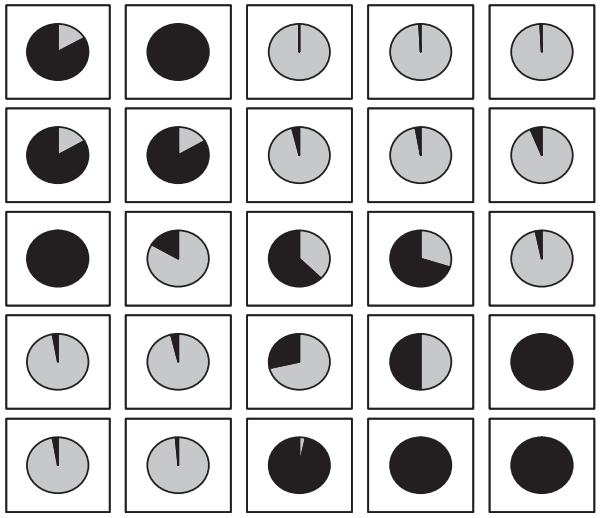

Fig. 3 Proportion of bryophytes (black) and vascular plants (grey) within each tetrad.

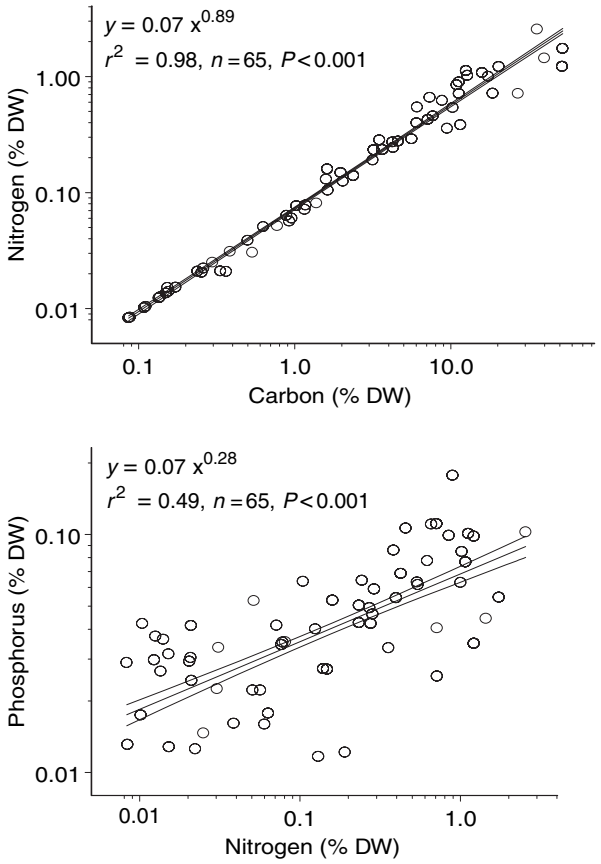

Fig. 4 Sediment carbon–nitrogen and nitrogen–phosphorus relationships. Power regression with 95% interval confidence, from samples collected from 65 sites.

with sediment N (Fig. 4). The median sediment C : N : P ratio was 56 : 4 : 1 by mass, but with wide uncertainties for the N : P ratio. On average, the within-site sediment coefficient of variation was 64%, 60% and 33% for C, N and P respectively. This variability is small compared to the lengths of the gradients: three, two and one orders of magnitude for C, N and P respectively.

Concentrations of TDN and TDP in the water column were generally dominated by soluble organic N and molybdate unreactive P forms. Concentrations of NH_4-N, NO_3-N and SRP were extremely small, with 50% of samples falling below 8, 5 and 1 μg L^{-1}, respectively, during spring and summer. The annual average range in NH_4-N, NO_3-N and TDP were within 3–600, 1–630 and 4–71 μg L^{-1} respectively (Fig. 5). Annual median TDN : TDP and TIN : SRP by mass were 14 : 1 and 23 : 1, while summer median values were 19 : 1 and 27 : 1, although these were rather variable.

Foliar plant tissue nutrient concentration

Out of the 378 samples 268 were vascular plants, 105 bryophytes and five charophytes. Tissue nutrient concentrations (C, N, P) and stoichiometric ratios (C : N : P) were significantly different among these taxonomic groups (ANOVA tests, $P < 0.001$). Histograms of frequency distribution were plotted for vascular plants and bryophytes (Fig. 6) and showed that vascular plants had a greater C, N and P concentration but a narrower N : P by mass. The nutrient concentration data were generally only slightly skewed towards high concentrations (the right), except for the bryophyte C concentration, which included some lower values (skewing data to the left). For vascular plants, according to the nutrient thresholds defined in the Methods section, only 2% (0–8%) and 10% (1–19%) of the samples would indicate yield limitation, but 18% (3–43%) and 32% (12–54%) of the samples would indicate growth rate limitation, for N and P respectively (Fig. 6). As the average nutrient critical thresholds derived from Gerloff (1975) were based on hydrophytes (mostly submerged plants), vascular plants were subsequently divided into helophytes (emergent plants, $n = 85$) and hydrophytes ($n = 154$) using the dataset with 344 samples – Appendix 1. In hydrophytes, only <0.1% (0–2%) and 3% (0.1–10%) of the samples indicated yield limitation, and only 7% (2–30%) and 19% (10–42%) of the samples indicated growth rate limitation, for N and P respectively. The range in N : P ratio was narrow (Fig. 6), with 88% and 76% of individual samples falling within N : P ≈ 5–20 and N : P ≈ 10–25 (by mass) for vascular plants and bryophytes respectively. The critical C : N and C : P ratios (by mass) for aquatic angiosperms were C : N ≈ 39, C : P ≈ 443 and C : N ≈ 24, C : P ≈ 272 for yield and growth rate limitation, respectively, derived from the following power regression equations: C : N = 45.0 $N^{-1.03}$ and C : P = 40.4 $P^{-1.04}$. The C : N : P critical nutrient threshold (by mass) for aquatic angiosperms sampled

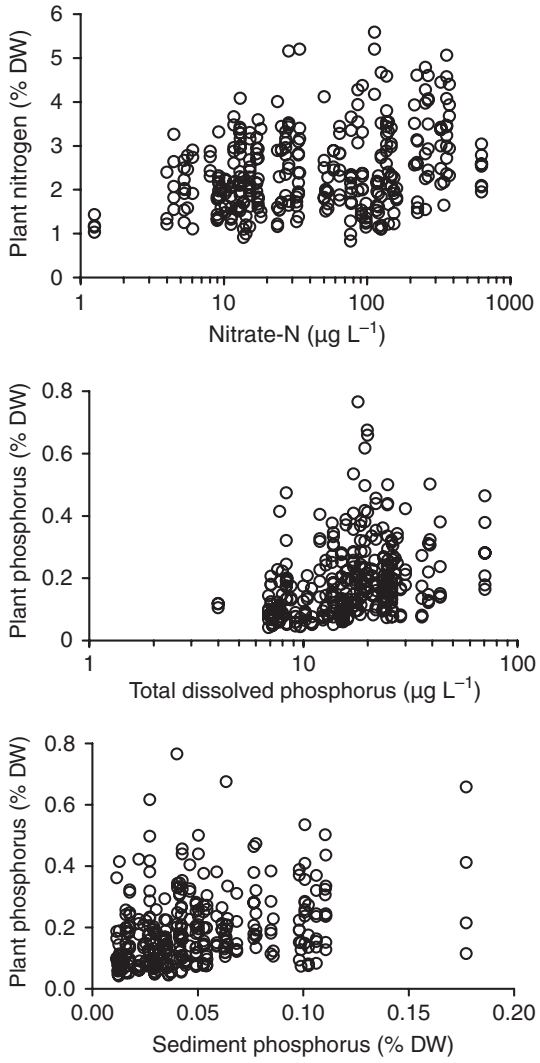

Fig. 5 Relationship between external nutrient concentrations and plant nutrient concentrations, from 344 samples across 65 sites and 33 taxa.

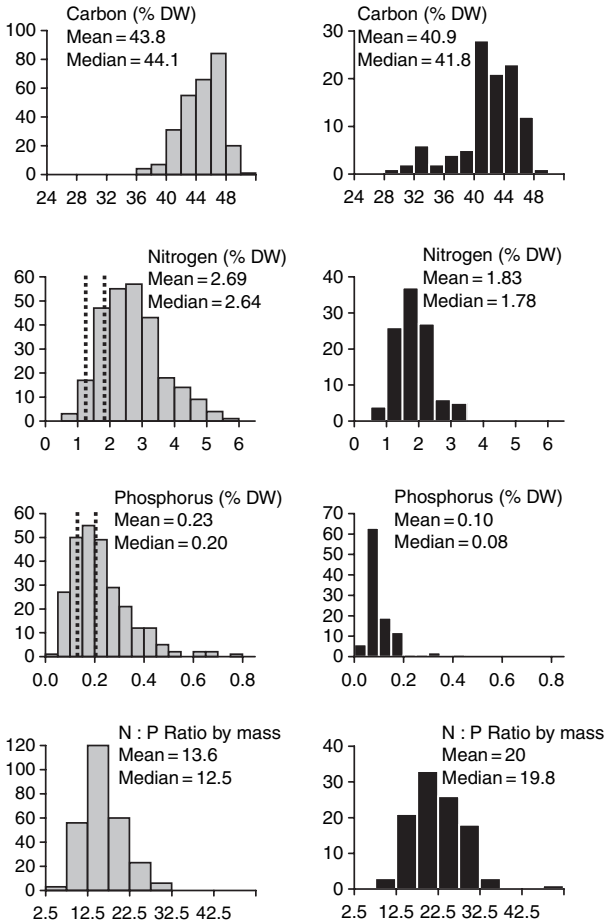

Fig. 6 Frequency distribution of plant nutrient concentrations in the vascular plants (grey) and bryophytes (black). The *y*-axis represent the number of samples. The *x*-axis represents % dry weight of nutrients or N : P ratio as labelled in the insets. The dotted line represents the yield- and growth-derived critical nutrient thresholds as determined by Gerloff (1975) and corrected by Colman *et al.* (1987) for growth rate.

in this survey was therefore 443 : 11 : 1 and 272 : 11 : 1 for yield and growth rate limitation respectively. The C : N and C : P ratios did not deviate from the expected inverse relationship with N and P, respectively (i.e. C : nutrient ≈ nutrient^{-1}), for both the vascular plants (see equations above) and the bryophytes (C : N = 41.1 N$^{-1.01}$ and C : P = 43.7 P$^{-0.97}$). Similarly C was generally not related to either N ($r = 0.12$, n.s. and $r = 0.23$, $P < 0.001$) or P ($r = 0.09$, n.s. and $r = 0.05$, n.s.) for vascular plants and bryophytes respectively. The Pearson correlation coefficients between plant tissue N and P were highly significant for vascular plants $r = 0.72$ ($P < 0.001$) and bryophytes $r = 0.63$ ($P < 0.001$).

Table 1 Linear mixed model to predict plant tissue N concentration: selection of fixed effects. The variables in bold were those selected for the final model (see Fig. 7)

		Wald statistics		Chi probability	
Fixed term	d.f.	Adding	Dropping	Adding	Dropping
Sediment N (SN)	1	3.59	1.07	0.058	0.300
Sediment P (SP)	1	16.73	1.79	<0.001	0.181
SN × SP	1	6.41	4.03	0.011	0.045
TIN†	**1**	**23.49**	**4.37**	**<0.001**	**0.037**
TDP†	1	5.65	4.89	0.018	0.027
TIN† × TDP†	1	0.01	0.02	0.940	0.881
Taxa	**32**	**472.82**	**462.88**	**<0.001**	**<0.001**
Habitat	5	3.57	2.59	0.612	0.763
Zone	7	6.34	6.34	0.501	0.501

†Annual average log transformed.

Table 2 Linear mixed model to predict plant tissue P concentration: selection of fixed effects. The variables in bold were those selected for the final model (see Fig. 7)

		Wald statistics		Chi probability	
Fixed term	d.f.	Adding	Dropping	Adding	Dropping
Sediment P (SP)	**1**	**46.91**	**12.79**	**<0.001**	**<0.001**
Sediment N (SN)	1	1.60	0.12	0.206	0.725
SP × SN	1	2.47	2.07	0.116	0.150
TDP†	**1**	**26.10**	**14.04**	**<0.001**	**<0.001**
TIN†	1	1.43	0.25	0.232	0.615
TDP† × TIN†	1	0.31	3.33	0.576	0.068
Taxa	**32**	**279.70**	**252.72**	**<0.001**	**<0.001**
Habitat	5	6.18	5.88	0.289	0.318
Zone	7	11.26	11.26	0.128	0.128

†Annual average log transformed.

Sources of variability in aquatic plant tissue nutrient concentration

The most parsimonious linear mixed models relating external nutrient concentrations and foliar tissue nutrient concentration involved only TIN (or NO_3), TDP and sediment total P (see Tables 1 & 2). There were no additional effects of habitat and biophysical zone. There were also some significant effects of sediment P, sediment N (depending on sediment P) and TDP on macrophyte tissue N concentration. These effects were small, however, and their variance was confounded by other stronger effects. These fixed effects were not significant when the order of the fixed effects was changed. The Wald statistics (Tables 1 & 2), as well as the actual data in Fig. 5, suggested that the external nutrient variables, although highly

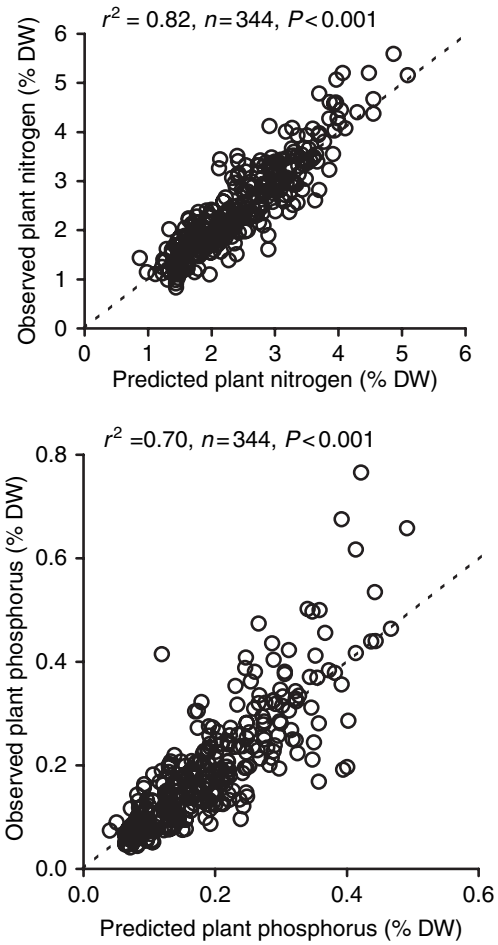

Fig. 7 Predicted plant tissue nutrient concentrations against observed values. Predictions were based on linear mixed model regression analyses using water and sediment N and P concentrations and 33 taxa (fixed effects, see Tables 1 & 2) as predictors, across 65 sites (random effect). The dotted line represents the expected 1 : 1 relationship.

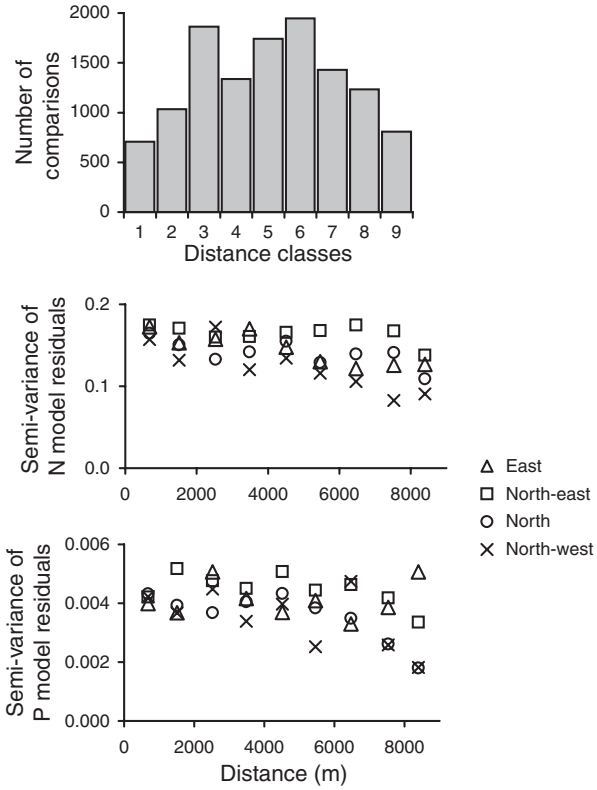

Fig. 8 Variograms of the plant nutrient concentration residuals of the regression analyses (see Fig. 7) run in four directions.

significant, were weak (due to the large degree of scatter). The phenotypic effect was therefore weak. The taxonomic effect, however, was very strong and explained virtually all the variance in the final models (see Tables 1 & 2). Plant tissue N concentration was best predicted by the fixed effects annual average logarithmic TIN (or NO_3-N) and taxon ($r^2 = 0.82$; Fig. 7). Plant tissue P was best predicted by annual average logarithmic TDP, total P in the sediment and taxon ($r^2 = 0.70$; Fig. 7). The autumn–winter average data for water chemistry gave very similar but weaker results than the annual average data. The effect of the spring–summer average data of the water chemistry was not significant. There did not seem to be a substantial spatial component left in the residuals of these regressions, as shown by variograms (Fig. 8).

Discussion

Methodological considerations

Variability in cellular nutrient concentration measured within species could result from a combination of analytical error, variability with age, physiological stage of development, the type of plant organ sampled, species growth form and, finally, the chemical environment (Dykyjová, 1979). In the present study, care was taken to minimize these errors: analytical limits of detection were well below the lowest concentrations recorded, and only young foliar tissue was sampled during the summer period. At least during the summer, the isoetids generally have, the same tissue nutrient (N, P) concentration in their stem/root and foliar tissue (e.g. Moeller, 1978; Boston & Adams, 1987; Christensen & Sand-Jensen, 1998). Sampling the whole plant resulted in the collection of a smaller number of individuals. Different growth

forms of the same species were sometimes present due to the drying out of temporary pools during the summer. This is probably not a large source of variability, however (e.g. Nielsen & Sand-Jensen, 1997). A more critical point was the choice of the sediment analysis. Logistical issues during large-scale field surveys reduce the opportunity for an immediate analysis of bioavailable nutrient fractions in sediment samples and, as a result, the choice was to measure total nutrient concentrations (e.g. Demars & Harper, 2002). Part of the explanation for the poor relationship between sediment total N and P, and internal plant N and P may be because we had no good predictor of bioavailable nutrient concentration. However, the species differ in their anatomical and physiological ability to take up sediment N and P (especially as some are rootless), and so it is unlikely that one analytical method would be ideal for predicting what constitutes bioavailable P for all plant species. For example, isoetids have access to P from the solid-phase fraction via mycorrhizal infections (Wigand *et al.*, 1998).

Critical nutrient concentrations for freshwater aquatic angiosperms

The minimum concentrations in freshwater angiosperms that permit growth are predicted to be P ≈ 0.08% and N ≈ 0.6% DW (Hutchinson, 1975, from Gerloff & Krombholz's, 1966 data). These are close to the critical nutrient concentrations for maximum yield limitation (P ≈ 0.13%, N ≈ 1.3% DW in Gerloff & Krombholz's, 1966 data). A nutrient cellular concentration slightly below these critical thresholds may lead to a disproportionate impairment of growth. This is due to the logarithmic response of plant tissue nutrient concentrations to a linear increase in external nutrient concentrations. It also means that a slight difference in critical nutrient ratio between species could potentially bias strongly the interpretation placed upon nutrient limitation.

The median nutrient concentrations in the angiosperms investigated here were P ≈ 0.20% and N ≈ 2.64%, which is much higher than the critical nutrient threshold for yield limitation but approaches the critical nutrient threshold for growth rate limitation. The range of concentrations, P ≈ 0.05–0.77% and N ≈ 0.76–5.59%, showed that some individual samples were below the critical thresholds as well as the minimum concentrations that permit growth, which seems unlikely. The lowest concentrations were measured in emergent species, such as *Carex rostrata* and *Schoenoplectus lacustris*.

Further analyses (using the 344 samples) showed that helophytes had lower average (±95% confidence interval) nutrient concentrations N = 2.11 (±0.14) and P = 0.15 (±0.01)% DW than hydrophytes N = 2.99 (±0.14) and P = 0.26 (±0.02)% DW. These differences cannot be attributed to differences in the average carbon concentration: helophytes C = 44.5 (±0.6) and hydrophytes C = 43.3 (±0.4). The taxonomic effect was not confounded by access to different pools of resources i.e. water only for mosses, sediment only for helophytes, sediment and water for hydrophytes, because the phenotypic effects (N and P sediment and water concentrations) were very weak (see Tables 1 & 2). This weak response of plant nutrient concentration to external environmental nutrient concentrations was confirmed further by examining single species response (B. O. L. Demars, unpublished). This makes it more likely that species from different functional or taxonomic groups have different critical and minimum tissue nutrient concentration. It certainly suggests that critical nutrient thresholds should be used with caution, preferentially as species-specific indicators (Gerloff, 1975).

Overall, the results suggest that there may be no aquatic hydrophytes with tissue nutrient deficiency impairing yield, as hypothesized in the introduction, but a substantial proportion of the samples showed potential growth rate limitation, particularly due to P as hypothesized by Demars & Edwards, in press. This may explain the presence of many species of isoetids (slow growing, perennial species, with a high root : shoot ratio) in this study area. Growth rate limitation may prevent fast growing species from taking over.

Stoichiometric homeostasis in freshwater aquatic plants

Although there were relatively wide ranges in tissue N and P concentrations, the N : P ratio was narrow and N–P relationships were strong and highly significant, as generally shown previously for a range of taxa (e.g. Garten, 1976; Atkinson & Smith, 1983; Duarte, 1992). This is not surprising as N and P play a critical role in plant cells: N is mostly found in nucleic acids, amino acids and proteins and P in

nucleic acids and adenosine triphosphate (the basis for enzyme synthesis and the intracellular energy transfer system). Carbon was not related to N or P, showing that the structural role of C was independent from the biochemical roles played by N and P. Interestingly, plant C : N and C : P relationships were strictly inversely related to plant N and P, respectively, for both vascular plants and bryophytes, as first suggested by Duarte (1992) for freshwater angiosperms with a more limited range of species. This was not the case for other aquatic plant groups, however (Duarte, 1992). In all, the amount of structural C in the vascular plants and bryophytes reported here was independent of the N and P concentration.

Environmental effects and response to nutrient enrichment

The hypothesis that external nutrient concentration had no effect on the foliar tissue nutrient concentration was rejected. However, the effect was weak compared with the taxonomic influence. This is apparent when comparing the raw data presented in Fig. 5 and the linear mixed model prediction including taxon as a fixed effect (Tables 1 & 2; Fig. 7). There was a very wide range of total C, N and P in the sediment, and soluble inorganic N and TDP in the water. For example, although external NO_3 concentration ranged over almost three orders of magnitude between sites, the range in internal tissue N concentration was narrower (one order of magnitude), 0.82–5.59% DW. As more than half of the summer water samples had extremely low soluble inorganic N and P concentrations, there was probably too little variation in the dataset to find any significant relationships. Therefore, the only significant relationships were based on annually (or autumn–winter) averaged data. The least variability in the data was associated with SRP (because many samples were below the detection limit), which is probably why TDP was found to be a better predictor of plant tissue P concentration. However, the proportion of organic N and molybdate unreactive P available for uptake by aquatic macrophyte is not known.

Sediment and water P together had a significant effect. The effect of sediment N, however, was not significant. This may suggest that these are different sources of nutrients. The much weaker or insignificant effect of NH_4 was surprising, however, because the energetic cost of uptake and processing of NH_4 is less than NO_3. One limitation of our empirical approach is that our conclusions depend on the range of internal and external nutrient concentration in the dataset. We plan to extend our dataset using published data to make the test more robust.

Differences between bryophytes and vascular plants

The lower N and P foliar tissue concentration and higher N : P ratio in bryophytes, compared with vascular plants, have also been reported for wetlands. For example, Bedford, Walbridge & Aldous (1999) reported N, P and N : P of 0.65 ± 0.06% DW, 0.04 ± 0.01% DW and 16.2 ± 1 (by mass), respectively, for bryophytes. Aquatic bryophytes have comparatively higher N, P and N : P than wetland bryophytes. Thus, either aquatic bryophytes are subject to less nutrient limitations or other factors are more important and reflect the result of different selective pressures.

While bioassays linking growth rate (or yield) to N, P and N : P exist for submerged freshwater angiosperms, none has been reported for bryophytes, except *Sphagnum* spp. These lower N, P and higher N : P ratios cannot be interpreted as evidence of strict nutrient limitation. This is more likely to be attributed primarily to taxonomic differences. Indeed the variability (range) in N and P foliar tissue concentration of *Sphagnum* spp. is still well below the median of other growth forms (Aerts, Wallen & Malmer, 1992; Aerts, Verhoeven & Whigham, 1999). The N : P ratio (by mass) in *Sphagnum* spp. (from hummocks and lawns) can vary from 10–40 along a gradient of atmospheric N deposition and nutrient critical thresholds for *Sphagnum* shoot tips were N ≈ 1.2% DW and N : P ≈ 34 (by mass) (Bragazza *et al.*, 2004).

Phosphatase activity in bryophytes has been suggested as a potential indicator of nutrient deficiency and been shown in general to be negatively related to P tissue concentration and positively related to N : P (Christmas & Whitton, 1998a,b; Turner *et al.*, 2001, 2003). It suggested no nutrient limitation when either P > 0.3% DW or N : P > 9 : 1, which is respectively above (P) and below (N : P) the range of data observed in the present study. It is very doubtful, however, that all samples from the present survey fall in the category of nutrient deficiency, even though organic N and molybdate unreactive P fractions of the

overlaying water generally dominated the total dissolved N and P (Demars & Edwards, in press). The validity of these bioassays can be questioned (cf. Turner *et al*., 2001). Similar bioassays were tried on vascular plants and it showed that taxonomic variability was much more important than the degree of nutrient enrichment (e.g. Melzer, 1980; Cedergreen & Madsen, 2003).

The molar C : N : P ratios (based on median nutrient concentrations) in vascular plants was 570 : 29 : 1, similar to values previous reported (e.g. 550 : 30 : 1, Atkinson & Smith, 1983), but bryophytes were markedly different 1350 : 49 : 1 from vascular plants. This is also very different from the molar C : N : P ratios for planktonic (106 : 16 : 1, Redfield, 1958) and benthic algae (158 : 18 : 1, Kahlert, 1998). As the stoichiometric ratios in bryophytes are strict and different from other taxonomic groups, a useful, general net chemical reaction for bryophyte metabolism has the form:

$$1350CO_2 + 1399H_2O + 49HNO_3 + H_3PO_4 \rightarrow (CH_2O)_{1350}(NH_3)_{49}H_3PO_4 + 1448O_2.$$

In conclusion, the null hypothesis that external nutrient concentration had no effect on the foliar tissue nutrient concentration was rejected. Although highly significant, these relationships were weak due to the large degree of variability. The phenotypic effect was therefore slight. The taxonomic effect (mostly at the species level), however, was very strong and alone explained virtually all the variance in the final models. This was further illustrated by the remarkable stoichiometric differences between vascular plants and bryophytes.

Based on nutrient critical thresholds, there was no yield limitation in wild aquatic macrophytes. However, there were a substantial number of samples indicating growth rate limitation, particularly due to phosphorus. Critical nutrient thresholds should therefore be used with caution, if at all, preferably as species specific indicators. The implication for bioindication is that it might be better to use deviation from the median, geometric mean (for N and P plant tissue concentration) or mean (N : P ratio) rather than critical nutrient thresholds, and to interpret the results with a knowledge of taxonomic effects.

Nitrogen and P tissue concentrations were highly correlated, with a median N : P ratio of 13 : 1 and 20 : 1 for vascular plants and bryophytes respectively. C : N and C : P ratios were strictly inversely related to N and P, respectively, for both vascular plants and bryophytes (i.e. C : nutrient $\approx$ nutrient^{-1}), suggesting that C, and, N and P plant metabolism were not coupled. The stoichiometry of aquatic macrophytes is therefore rather strict, and while it does not lend itself to the biological assessment of nutrient enrichment, it provides a robust approach for studies on ecosystem metabolism and nutrient cycling.

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References

Aerts R., Wallen B. & Malmer N. (1992) Growth-limiting nutrients in *Sphagnum*-dominated bogs subject to low and high atmospheric nitrogen supply. *Journal of Ecology*, **80**, 131–140.

Aerts R., Verhoeven J.T.A. & Whigham D.F. (1999) Plant-mediated controls on nutrient cycling in temperate fens and bogs. *Ecology*, **80**, 2170–2181.

Allenby K.G. (1981) Some analyses of aquatic plants and their waters. *Hydrobiologia*, **77**, 177–189.

Atkinson M.J. & Smith S.V. (1983) C:N:P ratios of benthic marine plants. *Limnology and Oceanography*, **28**, 568–574.

Batten G.D., Dowling V., Short C. & Blakeney A.B. (1992) Mineral-content of shoots of Australian rice crops. *Communications in Soil Science and Plant Analysis*, **23**, 1195–1208.

Bedford B.L., Walbridge M.R. & Aldous A. (1999) Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology*, **80**, 2151–2169.

Boston H.L. & Adams M.S. (1987) Productivity, growth and photosynthesis of two small 'isoetid' plants, *Littorella uniflora* and *Isoetes macrospora*. *Journal of Ecology*, **75**, 333–350.

Bragazza L., Tahvainainen T., Kutnar L. *et al.* (2004) Nutritional constraints in ombrotrophic *Sphagnum* plants under increasing atmospheric nitrogen deposition in Europe. *New Phytologist*, **163**, 609–616.

Cedergreen N. & Madsen T.V. (2003) Nitrate reductase activity in roots and shoots of aquatic macrophytes. *Aquatic Botany*, **76**, 203–212.

Christensen K.K. & Sand-Jensen K. (1998) Precipitated iron and manganese plaques restrict root uptake of phosphorus in *Lobelia dortmanna*. *Canadian Journal of Botany*, **776**, 2158–2163.

Christmas M. & Whitton B.A. (1998a) Phosphorus and aquatic bryophytes in the Swale-Ouse river system, north-east England. 1. Relationship between ambient phosphate, internal N:P ratio and surface phosphatase activity. *The Science of the Total Environment*, **210/211**, 389–399.

Christmas M. & Whitton B.A. (1998b) Phosphorus and aquatic bryophytes in the Swale-Ouse river system, north-east England. 2. Phosphomonoesterase and phosphodiesterase activities of *Fontinalis antipyretica*. *The Science of the Total Environment*, **210/211**, 401–409.

Colman J.A., Sorsa K., Hoffmann J.P., Smith C.S. & Andrews J.H. (1987) Yield-derived and photosynthesis-derived critical concentrations of tissue phosphorus and their significance for growth of Eurasian Water Milfoil, *Myriophyllum-Spicatum* L. *Aquatic Botany*, **29**, 111–122.

Demars B.O.L. & Edwards A.C. (in press) A seasonal survey of surface water habitats within the River Spey basin, Scotland: major nutrient properties. *Aquatic Conservation: Marine and Freshwater Ecosystems*, doi: 10.1002/aqc.797/.

Demars B.O.L. & Harper D.M. (2002) Phosphorus in a lowland calcareous river basin: a multiscale approach to understanding human impacts. *International Journal of Ecohydrology and Hydrobiology*, **2**, 149–164.

Duarte C.M. (1992) Nutrient concentration of aquatic plants: patterns across species. *Limnology and Oceanography*, **37**, 882–889.

Dykyjová D. (1979) Selective uptake of mineral ions and their concentration factors in aquatic higher plants. *Folia Geobotanica et Phytotaxonomica (Praha)*, **14**, 267–325.

Garten C.T. (1976) Correlations between concentrations of elements in plants. *Nature*, **261**, 686–688.

Gerloff G.C. (1975) *Nutritional Ecology of Nuisance Aquatic Plants*. EPA 660-3-75-027, University of Wisconsin, Madison.

Gerloff G.C. & Krombholz P.H. (1966) Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnology and Oceanography*, **11**, 529–537.

Haslam S.M. (1987) *River Plants of Western Europe*. Cambridge University Press, Cambridge.

Havlin J.L. & Soltanpour P.N. (1980) A nitric-acid plant-tissue digest method for use with inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis*, **11**, 969–980.

Huang C.Y.L. & Schulte E.E. (1985) Digestion of plant-tissue for analysis by ICP emission-spectroscopy. *Communications in Soil Science and Plant Analysis*, **16**, 943–958.

Hutchinson G.E. (1975) *A Treatise on Limnology. Volume III. Limnological Botany*. John Wiley and Sons, New York.

Jenkins D. (1988) *Land Use in the River Spey Catchment*. Aberdeen Centre for Land Use, Aberdeen.

Kahlert M. (1998) C:N:P ratios of freshwater benthic algae. *Archiv für Hydrobiologie, Advances in Limnology*, **51**, 105–114.

Kern-Hansen U. & Dawson F.H. (1978) The standing crop of aquatic plants of lowland streams in Denmark and the inter-relationships of nutrients in plant, sediment and water. In: *Proceedings of the European Weed Research Council. 5th International Symposium on Aquatic Weeds*, pp. 143–150. European Weed Research Society, Amsterdam.

Madsen T.V. & Cedergreen N. (2002) Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology*, **47**, 283–291.

Madsen T.V., Hahn P. & Johansen J. (1998) Effects of inorganic carbon supply on the nitrogen requirement of two submerged macrophytes, *Elodea canadensis* and *Callitriche cophocarpa*. *Aquatic Botany*, **62**, 95–106.

Maizels J.K. (1988) The physical environment. Geology and geomorphology of the Spey Valley. In: *Land Use in the River Spey Catchment* (Ed. D. Jenkins), pp. 29–35. Aberdeen Centre for Land Use, Aberdeen.

Melzer A. (1980) Ökophysiologische Aspekte der N-Ernährung submerser Wasserpflanzen. *Verhandlungen der Gesellschaft für Ökologie*, **8**, 357–363.

Moeller R.E. (1978) Seasonal changes in biomass, tissue chemistry, and net production of the evergreen hydrophyte, *Lobelia dortmanna*. *Canadian Journal of Botany*, **56**, 1425–1433.

Nielsen S.L. & Sand-Jensen K. (1997) Growth rates and morphological adaptations of aquatic and terrestrial forms of amphibious Littorella uniflora (L.) Aschers. *Plant Ecology*, **129**, 135–140.

North East River Purification Board (1995) *River Spey Catchment Review*. NERPB, Aberdeen.

Payne R.W. [Ed] (2005) *The guide to Genstat Release 8*. Part. 2. Statistics VSN International, Oxford.

Potter R. (1988) The socio-economic characteristics of the Spey valley. In: *Land Use in the River Spey Catchment* (Ed. D. Jenkins), pp. 12–19. Aberdeen Centre for Land Use, Aberdeen.

Redfield A.C. (1958) The biological control of chemical factors in the environment. *American Scientist*, **46**, 205–221.

Robach F., Merlin S., Rolland T. & Trémolières M. (1996) Assimilation et stockage du phosphore par les macrophytes aquatiques bioindicateurs du niveau trophique de l'eau. *Ecologie*, **27**, 203–214.

Rodushkin I., Ruth T. & Huhtasaari A. (1999) Comparison of two digestion methods for elemental determinations in plant material by ICP techniques. *Analytica Chimica Acta*, **378**, 191–200.

Schmitt M.R. & Adams M.S. (1981) Dependence of rates of apparent photosynthesis on tissue phosphorus concentrations in Myriophyllum spicatum L. *Aquatic Botany*, **11**, 379–387.

Shardendu & Ambasht R.S. (1991) Relationship of nutrients in water with biomass and nutrient accumulation of submerged macrophytes of a tropical wetland. *New Phytologist*, **117**, 493–500.

Smith B.F.L. & Bain D.C. (1982) A sodium hydroxide fusion method for the determination of total phosphate in soils. *Communications in Soil Science and Plant Analysis*, **13**, 185–190.

Thiébaut G. & Muller S. (2003) Linking phosphorus pools of water, sediment and macrophytes in running waters. *Annales de Limnologie*, **39**, 307–316.

Turner B.L., Baxter R., Ellwood N.T.W. & Whitton B.A. (2001) Characterization of the phosphatase activities of mosses in relation to their environment. *Plant, Cell & Environment*, **24**, 1165–1176.

Turner B.L., Baxter R., Ellwood N.T.W. & Whitton B.A. (2003) Seasonal phosphatase activities of mosses from Upper Teesdale, northern England. *Journal of Bryology*, **25**, 189–200.

Wigand C., Andersen F.Ø., Christensen K.K., Holmer M. & Jensen H.S. (1998) Endomycorrhizae of isoetids along a biogeochemical gradient. *Limnology and Oceanography*, **43**, 508–515.

Zarcinas B.A., Cartwright B. & Spouncer L.R. (1987) Nitric-acid digestion and multielement analysis of plant-material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis*, **18**, 131–146.

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Appendix 1 List of taxa with number of samples (*n*)

Taxa	*n*	Taxa	*n*
Hydrophytes		*Sparganium emersum* Rehmann	2
Agrostis stolonifera L.	2	*Sparganium natans* L.	3
Apium inundatum (L.) Rchb. f.	1	*Subularia aquatica* L.	1
Callitriche hamulata Kütz. ex W.D.J. Koch	8	*Utricularia vulgaris* L. / *australis* R. Br.	3
Callitriche stagnalis Scop.	3	*Utricularia intermedia* Hayne *sensu lato*	2
Eleogiton fluitans (L.) Link	2	*Veronica beccabunga* L.	1
Elodea canadensis Michx.	9	Helophytes	
Elodea nuttallii (Planch.) H. St. John	1	*Caltha palustris* L.	1
Glyceria fluitans (L.) R. Br.	2	*Carex* sp L.	1
Grass	1	*Carex aquatilis* Wahlenb.	6
Hippuris vulgaris L.	5	*Carex rostrata* Stokes	29
Hydrocotyle vulgaris L.	1	*Carex vesicaria* L.	5
Isoetes lacustris L.	4	*Cicuta virosa* L.	2
Juncus bulbosus L.	11	*Eleocharis palustris* (L.) Roem. & Schult.	6
Littorella uniflora (L.) Asch.	11	*Equisetum fluviatile* L.	12
Lobelia dortmanna L.	5	*Menyanthes trifoliata* L.	10
Montia fontana L.	2	*Mimulus guttatus* DC.	2
Myosotis scorpoides L.	1	*Phalaris arundinacea* L.	1
Myriophyllum alterniflorum DC.	13	*Phragmites australis* (Cav.) Trin. ex Steud.	4
Nitella flexilis† (L.) Agardh	5	*Potentilla palustris* (L.) Scop.	4
Nuphar pumila (Timm) DC.	3	*Schoenoplectus lacustris* (L.) Palla	2
Nymphaea alba L.	16	*Sparganium erectum* L.	9
Potamogeton alpinus Balb.	2	Bryophytes	
Potamogeton natans L.	18	*Fontinalis antipyretica* Hedw.	10
Potamogeton obtusifolius Mert. & W.D.J. Koch	6	*Fontinalis squamosa* Hedw.	14

Appendix 1 (*Continued*)

Taxa	*n*	Taxa	*n*
Potamogeton polygonifolius Pourr.	12	Liverwort	44
Potamogeton pusillus L.	1	Moss	29
Ranunculus flammula L.	6	*Sphagnum sp* L.	8
Sparganium angustifolium Michx.	13		

†Charophyte.