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Distribution of aquatic plants in the Northern Vosges rivers: implications for biomonitoring and conservation

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ABSTRACT

1. The continual confusion over the definition of the term 'trophic status' has led to ambiguous demonstrations of the influence of alkalinity versus inorganic N and P on aquatic plant distribution.

2. Data from the Northern Vosges rivers (sandstone geology) were reinvestigated to test whether it was possible to separate the effect of (i) spatial isolation from surface water chemistry, and (ii) alkalinity from inorganic N and P on aquatic macrophyte distribution.

3. Alkalinity and pH exerted the strongest influence on plant distribution, soluble reactive phosphorus (SRP) and pCO_2 being subordinate. The effect of spatial isolation between catchments was not significant, which may indicate that aquatic plants dispersed well over the area. The effect of longitudinal connectivity on species distribution was strong, although largely confounded by the effects of water chemistry and possibly other physical factors not recorded. The partial effect of SRP (after removing the effect of pH or alkalinity) was still significant. However this was not the case for NH_4.

4. The floristic composition was more likely to indicate the role played by alkalinity than inorganic P, with inorganic N being further subordinate. However, the causality of the significant relationships needs to be investigated further. This study questioned the validity of current macrophyte biomonitoring tools striving to indicate the concentrations of inorganic N and P.

5. More work is needed to quantify the role of connected and isolated aquatic habitats in the region, in order to understand how to maintain the species pool and to ensure that recolonization rates compensate for the losses due to disturbances. It is not clear how the vegetation would respond to inorganic P enrichment (or control), based on the individual species response observed here, and river P uptake studies from other rivers. Future monitoring should also include measurements of physical degradation.

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INTRODUCTION

There has been continual confusion over the definition of the term ‘trophic status’. The effects of inorganic N and P on aquatic macrophyte distribution have been confounded in early studies with organic pollution (saproby; e.g. Carbiener, 1965). Later on, these effects were clearly separated (e.g. Carbiener, 1969). However, early field studies were unable to separate the two effects because the source of pollution came from untreated municipal sewage (e.g. Kohler *et al*., 1971, 1973). A further layer of confusion was introduced when the pragmatic decision was made to define ‘trophic status’ using inorganic N and P and alkalinity simultaneously (e.g. Newbold and Palmer, 1979). This combined definition underpins the ‘trophic ranking scores’ developed for aquatic plants either in standing (e.g. Palmer *et al*., 1992) or running waters (e.g. Holmes and Newbold, 1984; Newbold and Holmes, 1987). These plant scores were derived subjectively and this was fully acknowledged by the authors: ‘in the absence of knowledge about the levels of [nitrogen, phosphorus, calcium carbonate] is there any way in defining the trophic category . . .’ (Newbold and Palmer, 1979); ‘the report [. . .] subjectively evaluated the trophic status [. . .] based on the floral composition and a knowledge of the geology, there being no data available on the chemistry of the substrates or the water’ (Holmes and Newbold, 1984). In later studies, the ‘trophic rank scores’ or ‘mean trophic rank’ were still derived without inorganic N and P data, as acknowledged by the authors (Palmer *et al*., 1992; Holmes, 1996; Holmes *et al*., 1999a).

The Mean Trophic Rank (MTR; Holmes, 1996; Holmes *et al*., 1999a) was first tested in the River Welland catchment (Demars, 1996; Demars and Harper, 1998). Although significant relationships were found between the MTR and inorganic N and P, these were confounded by other factors such as stream size (Demars and Harper, 1998). Following on from the methodological proposals by Holmes (1996) and Holmes *et al*. (1999a), another project (Dawson *et al*., 1999) confirmed that the species ‘trophic’ scores were more or less aligned with the water inorganic N and P and so suggested no reason to modify the scores. Dawson *et al*. (1999) recommended, however, that further work should try to disentangle the role of inorganic N and P from other chemical, physical and spatial factors. They concluded that until then the MTR may reflect environmental pressures other than phosphate such as the influence of geology, hydro-geomorphology and plant dispersal as previously reported (e.g. Tansley, 1911; Butcher, 1927, 1933; Haslam, 1978; Holmes and Newbold, 1984).

Two systems of diagnosis based on aquatic macrophytes have so far been based on independent and objective approaches: the ‘Trophie-Index Macrophyten’ of Kohler and Schneider (2003) and the metric-based approach of Dodkins *et al*. (2005). The ‘Trophie-Index Macrophyten’ is simple and based on field measurements of inorganic N and P in the water and the sediment. However, like the MTR, it cannot distinguish the role of inorganic N and P from other environmental factors. The metric-based approach of Dodkins *et al*. (2005) is complicated and unconvincing: e.g. nitrate was identified as a discriminant factor, but although nitrate was significant, it only explained 1.9% of the variance in the species data and was partially correlated with conductivity. The selection of discriminant variables was also highly dependent on the order of selection of variables, hence different outcomes were possible but not explored.

So far, no studies have demonstrated unambiguously the effects of inorganic N and P on plant distribution, because the relationships were either not significant (Demars and Harper, 2005) or confounded by other factors (Wiegleb, 1984) such as changes in carbon availability (Carbiener *et al*., 1990), conductivity (Dawson and Szoszkiewicz, 1999) or pH-alkalinity (Thiébaut and Muller, 1999), and other pollution such as untreated municipal sewage effluent (Kohler *et al*., 1973). Most relationships were also established using indirect analyses where it is not possible to separate the effect of one factor while controlling for another (e.g. Robach *et al*., 1996; Demars and Harper, 1998; Thiébaut and Muller, 1999). The effect of spatial isolation (e.g. Haury, 1995) also plays a crucial role, owing to constraints on plant dispersal (Boedeltje, 2005; Demars and Harper, 2005). It is therefore important to re-examine the data

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collected so far with more powerful analytical tools and to take into account the structure of the river network as well as local habitat conditions.

'Consideration of confounding factors is essential if biological effects are to be related to specific water quality parameters with any certainty' (Mainstone *et al.*, 1994: 47). 'Clearly the effect of phosphorus may appear minimal when comparing communities from widely different habitats, but if the range of environmental conditions is restricted to a particular river type, within which can be found a wide range of ambient phosphorus concentrations (from background level to excessively enriched) the importance of phosphorus is likely to be paramount' (Mainstone *et al.*, 1994: 69). Following on these influential quotes, the data collected by Thiébaut (1997) in rivers draining a sandstone solid geology were reanalysed to (i) separate statistically the effects of local environmental conditions and spatial isolation on species distribution, (ii) test whether the effect of pH and inorganic carbon (HCO_3, CO_2) can be separated from soluble inorganic N and P, and if so, (iii) test whether some species respond better than others to one or other environmental pressure, and (iv) investigate the implications for biomonitoring and conservation.

MATERIAL AND METHODS

The Biosphere Reserve of the Northern Vosges

The Northern Vosges natural park (France) was designated as a Biosphere Reserve in 1988 under the Man and the Biosphere UNESCO programme. More recently (1998) it merged with the Pfälzerwald Natural Park (Germany) to form a transboundary Biosphere Reserve. These Biosphere Reserves aim to promote conservation (landscape, ecosystems, species and genetic variation), development (economic, human), knowledge and communication (research, monitoring, environmental education and training). Protected species living in the Northern Vosges include lynx (*Lynx lynx*), peregrine falcon (*Falco peregrinus*) and bog arum (*Calla palustris*). The streams of the Northern Vosges Biosphere Reserve drain hills (200–580 m altitude) covered by beech, oak and pine tree forests. The streams are also protected by the Habitats Directive (92-43/EEC), notably for their populations of water-crowfoot (*Ranunculus*), fish (*Lampetra planeri*, *Taurulus bubalis*), a dragonfly (*Ophiogomphus cecilia*), and a mollusc (*Unio crassus*). There are 11 types of habitat of European interest along the Northern Vosges streams, including aquatic habitats such as the *Potamogeton polygonifolius* community within the headwaters, and fragments of natural alluvial forest.

Data collection

Survey work was limited to rivers shallow enough to wade into (i.e. <1.2 m). The sites were investigated four times per year (January, June, August, October) from 1993 to 1995, under stable flow conditions. During each visit, the vegetation surveys were conducted following the Braun-Blanquet method where the abundance–dominance of each species is recorded in six classes (Braun-Blanquet, 1932), over a homogeneous 50-m stretch of river. Only species submerged for more than 85% of the year were recorded (see Holmes, 1983). Samples were taken back to the laboratory if field identification was inconclusive. Species names follow Smith (1992, 1996) for bryophytes and Lambinon *et al.* (1992) for vascular plants. *Sphagnum* and filamentous algae were only identified to the level of genus.

On each of the sampling dates, 500 mL of water was collected in midstream and analysed immediately upon return to the laboratory (within 24 h of collection). Alkalinity was determined by Gran's titration (NFT 90-035, AFNOR 1990). Conductivity ($\mu S\,cm^{-1}$ at 25°C) and pH were measured using a combined glass electrode. Soluble reactive phosphorus (SRP) and ammonium (NH_4^+-N) were analysed, using

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spectrophotometry (single reagent ascorbic acid technique for phosphorus, NFT 90-023, and indophenol technique for ammonia, NFT 90-015, AFNOR 1990). Main cations (Ca, Mg, Na, K) were analysed using atomic absorption spectrophotometry. Sulphate, chloride, nitrite (NO_2-N) and nitrate (NO_3-N) were determined in the laboratory by ion chromatography. Total aluminium was determined by inductively coupled plasma spectrometry after acidification with HNO_3.

The surface-water chemistry of the present study reflects low flow conditions because the individual values were averaged and because the low sampling frequency misses high flow events. The diurnal variability of pH in poorly buffered water can be large owing to the photosynthetic acitivity of the plants. However, in the Northern Vosges, the abundance of aquatic plants is often very low and the re-aeration rate may prevent large relative (day/night) pCO_2 depletion (see Fritz *et al.*, 1984).

Data processing

Thiébaut (1997) surveyed 58 sites in the Northern Vosges. The 48 sites situated on sandstone geology were selected for the current study. One acidified site ($pH = 4.4$) was subsequently removed on the grounds that it was a clear outlier. Hence the data analyses were based on 47 sites (Figure 1). Missing data, for

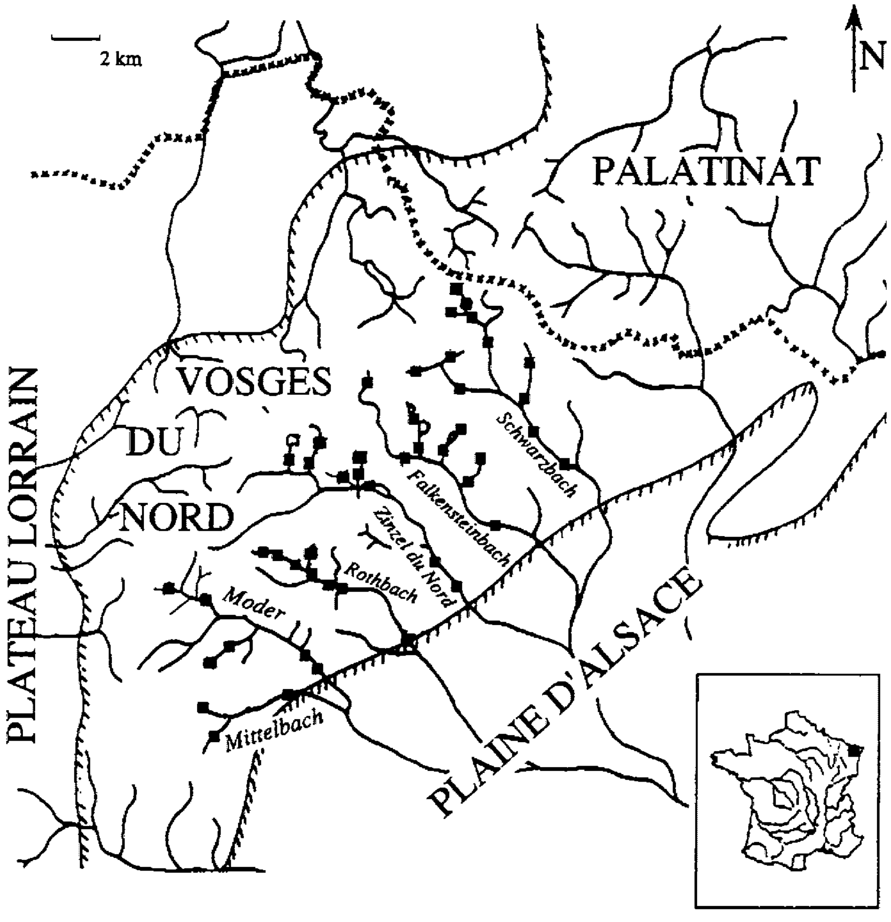

Figure 1. Location of sampling sites within the Northern Vosges river network.

aluminium, at eight sites were replaced by calculated values ($Al = 12050e^{-0.78*pH}$, $r^2 = 0.50$). Alkalinity, NO_3, NH_4, PO_4 and Al were $\log_{10}(x+1)$ transformed prior to statistical analysis. The excess partial pressure of CO_2 ($EpCO_2$, units are x times atmospheric pressure) was calculated (see Neal *et al.*, 1998) using the averaged alkalinity, pH and an estimated water temperature of 10°C. Errors in calculation of $EpCO_2$ are negligible ($\pm 5\%$) within the expected ± 5°C variability between sites. The spatial structure of the river network was coded simply using a catchment approach. The six main catchments were binary coded as a dummy variable and the distance from river's source to site (hereafter referred to as 'distance') was measured from 1:50 000-scale maps. The habitat table was therefore composed of 14 surface water chemistry variables and two spatial variables (distance and catchment, the latter including six modalities).

Forty-seven aquatic species were recorded, of which 34 were selected because they occurred in at least three sites. The number of species per site varied between two and 18. The six classes of the Braun-Blanquet index of cover-abundance were coded from 1 to 6 and these scores were used to weight the species in the multivariate analyses.

All the data in the present study were centred and standardized. All ordinations were run on correlation matrices. A principal component analysis (PCA) was run using the site attribute data to investigate the strength of the variables and their inter-relationships. Detrended correspondence analysis (DCA) was run using the 'detrended by segment' option to obtain estimates of gradient length in standard deviation units of species turnover (ter Braak and Šmilauer, 2002: 90). DCA was run to investigate how the species were related to each other. The axis eigenvalues were divided by the total inertia, so that the strength of the axes could be expressed in percentage of explained variance.

Canonical correspondence analyses (CCA) were performed (i) to investigate which factors would significantly explain the species composition (Monte Carlo permutation test); (ii) to include in the final CCA model a minimum number of factors (through a stepwise selection procedure); and (iii) to quantify the variance partitioning of the two groups of factors: water chemistry and spatial isolation. A Bonferroni correction was applied as in Demars and Harper (2005). The significance of the axes of the final CCA model was also tested with the Monte Carlo permutation test (1000 unrestricted random permutations). The species-habitat table (table of weighted averages) produced by the CCA shows even more detail than the CCA ordination diagram. Association between species and habitat variables were arbitrarily retained when the weighted average exceeded 0.5. The species occurrence and the cumulative (first and second CCA axis) fit per species, as fraction of variance of species, are also reported (ter Braak and Šmilauer, 2002: 176). The species tolerance (niche breadth) was statistically adjusted (see ter Braak and Šmilauer, 2002: 178) and a weighted average was calculated for the first two axes of the CCAs. The range in species niche breadth was then divided into four equal classes and represented by a disc of increasing area as a simple way of improving the visualization of the result. All the multivariate analyses were performed with Canoco 4.5 and the ordination diagrams with Canodraw 4.0 (ter Braak and Šmilauer, 2002). As in previous studies, the percentage of explained variance for the different factors was expected to be relatively low overall (see, for example, Demars and Harper (2005)).

RESULTS

Environmental variables

Surface water chemistry was weakly mineralized, mildly acid to circumneutral, and poorly to well buffered (Table 1). There was also a wide range of inorganic N and P, and $EpCO_2$ (Table 1). The ion concentrations and buffering capacity of the water increased with distance to source. Some variables are highly correlated with each other (pH-alkalinity, NH_4-PO_4, NO_3-conductivity), others partially unrelated (pH-PO_4) or

Table 1. Surface water chemistry of the Northern Vosges rivers, based on 47 sites. Units: conductivity (µS cm^{-1}); alkalinity (µeq L^{-1}); Ca, Na, K, Mg, SO_4, Cl, NO_3-N (mg L^{-1}); NH_4-N, PO_4-P, Al (µg L^{-1}); pCO_2 (x times atmospheric pressure)

	pH	Conductivity	Alkalinity	Ca	Na	K	Mg	SO_4	Cl	NO_3	NH_4	PO_4	Al	pCO_2
Min.	5.1	39	2	2.8	1.2	2.1	0.9	5.6	2.5	0.06	16	8	22	2
10th	5.7	46	67	3.7	1.5	2.5	1.1	7.5	2.8	0.20	40	12	43	5
50th	6.5	59	209	5.2	2.0	2.9	1.5	9.7	4.5	0.44	67	38	61	9
90th	7.1	93	429	9.4	3.7	3.6	3.0	15.0	6.4	0.95	195	124	162	18
Max.	7.3	130	634	15.0	6.5	4.8	4.5	20.2	10.1	1.95	443	552	217	35

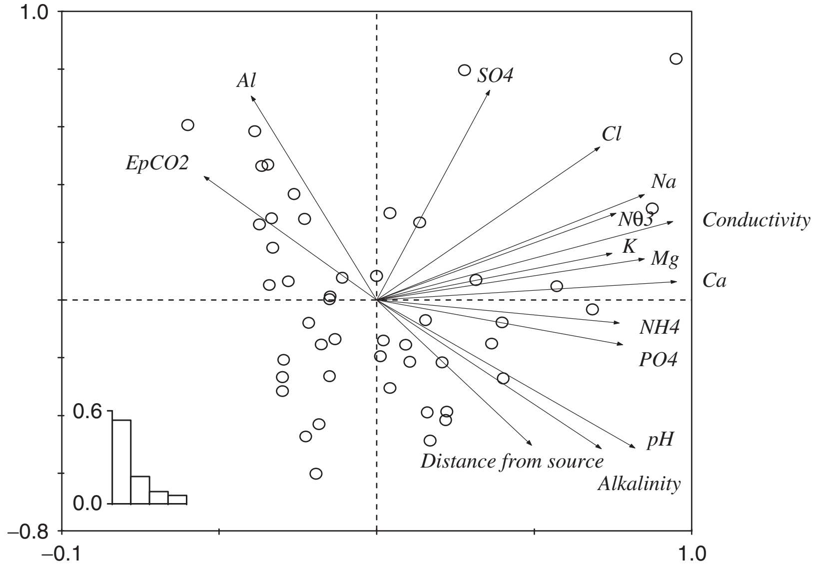

Figure 2. PCA ordination diagram showing correlations between environmental variables used in the 47 sites. The angle between variables indicates the extent of correlation: acute = correlated, 90° = not correlated, 180° = inversely correlated. Inset depicts the pattern of change in eigenvalues of the first four axes. Axis 1 is horizontal and axis 2 vertical.

independent (pH-SO_4) — see Figure 2. Hence it may be possible to disentangle the partial effect of some variables on species distribution.

Species

There was a large species turnover of six standard deviation units along the first two axes of the DCA (Figure 3). The species at opposite ends of axes were never found together in a site. There were more species associated with the positive end of axis 1 than the negative end. The DCA showed strong differentiation between taxonomic groups. Algae and mosses were distributed along axis 1 but were associated with the positive end of axis 2. Vascular plants were mostly associated with the positive end of axis 1 (except *P. polygonifolius*) and negative end of axis 2.

Linking species and environmental variables

The main predictors (see Table 2) were pH, alkalinity and distance. The other significant variables seemed to play a subordinate role (e.g. $EpCO_2$, PO_4, Ca and NH_4). Neither NO_3 nor catchment identity were significant. Together, the supplied predictive variables explained 38% of the variance in the species data.

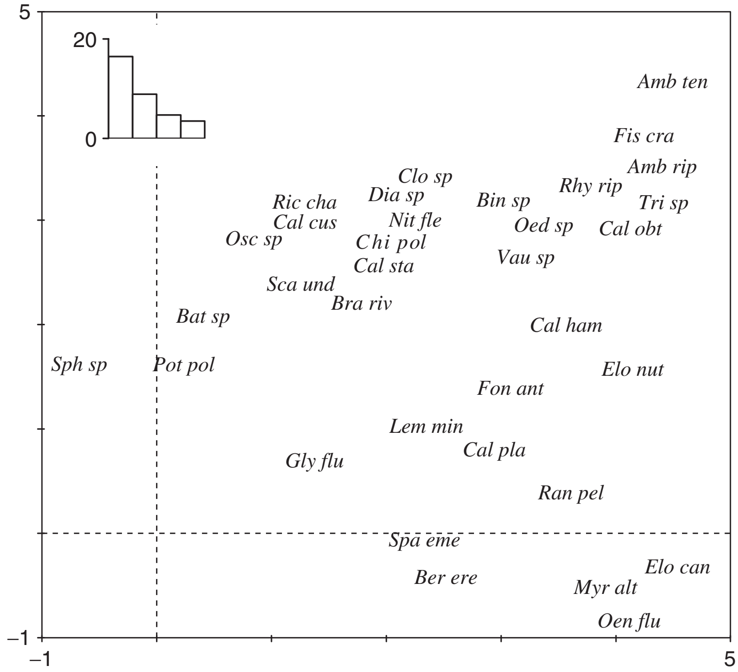

Figure 3. DCA ordination diagram showing the inter-relationships of species. The labels are centred on the scores, with minor adjustments in some cases to avoid overlap. Inset depicts the pattern of change in eigenvalues (percentage explained variance) of the first four axes. Axis 1 is horizontal and axis 2 vertical. Full species names are listed in Table 3.

Table 2. Percentage of variance explained by each significant variable in the species data (47 sites × 34 species) using canonical correspondence analysis: singly and after stepwise selection

Significant variables	Percentage of explained variance				
	Singly	*p*	After stepwise selection	*p*	Order
pH	12	<0.001	12	<0.001	1
Alkalinity[a]	11	<0.001	5	0.002	2
Distance	10	<0.001	4	0.002	3
$EpCO_2$	9	<0.001	3	0.048	5
PO_4[a]	8	<0.001	3	0.006	4
Ca	8	<0.001		n.s.	
NH_4[a]	7	<0.001		n.s.	
Conductivity	6	<0.001		n.s.	
Mg	6	0.002		n.s.	
Al[a]	5	0.002		n.s.	
All together	38	<0.001	27	<0.001	

[a] $\log_{10}(x+1)$ transformed prior to statistical analysis.

After stepwise selection Ca, NH_4, conductivity, Mg and Al were not significant and, therefore, these variables were only introduced in the final CCA as passive predictive variables. The CCA diagram (Figure 4) shows *P. polygonifolius*, *Sphagnum* sp. and *Scapania undulata* to be associated with the upper parts of the rivers. These were characterized by low pH, low alkalinity, low PO_4 and high pCO_2. Other species, such as

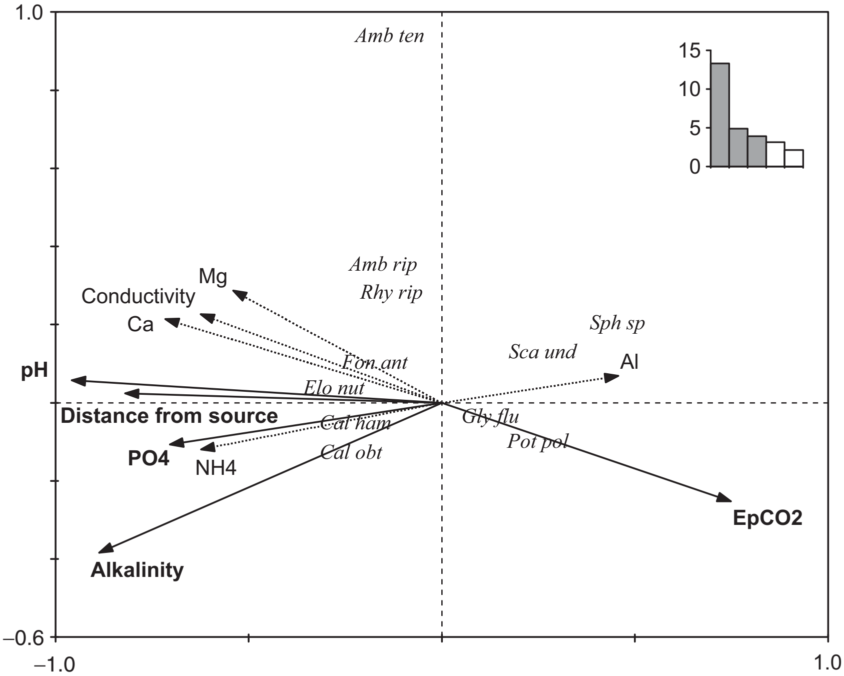

Figure 4. Projection of the species (with fit greater than 20%) and significant habitat variables (see Table 1, single effects) into the CCA ordination diagram. The habitat variables represented by dotted arrows were introduced as passive variables because the information was redundant (see Table 1, partial effects after stepwise selection). Axis 1 is horizontal and axis 2 vertical. Inset depicts the pattern of change of the five constrained axes in percentage of explained variance. Shaded bars represent the significant axes. Alkalinity, NH_4, PO_4, Al were $\log_{10}(x+1)$ transformed prior to analysis. Full species names are listed in Table 3.

Elodea nuttallii, *Callitriche hamulata*, *Callitriche obtusangula* and *Fontinalis antipyretica* were associated with the lower part of the rivers. Since the first three axes of the CCA were significant, the species associations with environmental variables have been further detailed in Table 3. It is obvious, from this table, that many species are eurytopic (not particularly associated with any variable, having large niche breadth). Table 3 shows that virtually no species can be assigned to any specific environmental variable, not even stenotypic species such as *P. polygonifolius*. The best indicator species for groups of variables are those with high occurrence and good fit. Only 12 taxa had their occurrence exceeding 20% and only 11 taxa, out of 34, had a species fit greater than 20%. Only one was an alga (*Vaucheria* with an occurrence of 21%). Mosses and vascular plants were equally represented and separated along axis 2. Mosses tended to be associated with the upper/middle part of the watercourses, while vascular plants spread all along the watercourses, *P. polygonifolius* and *Callitriche stagnalis* being associated with the upper part and *Elodea*, *Myriophyllum alterniflorum* with the lower parts (Table 3). Two species may be reliably related to a single factor: *C. stagnalis* and *Callitriche platycarpa*, associated with short distance from a river's source and high NH_4 concentrations, respectively (Table 3). The position of *Amblystegium tenax* in Figure 4 should not be over-interpreted because it occurred in only 6% of the sites (see Table 3).

Partial CCAs showed that surface water chemistry and longitudinal connectivity were both playing a significant and partially independent role, surface water chemistry being the dominant component (17% alone and 6% confounded with distance). The partial effect of distance, after taking into account all the significant chemical variables (after stepwise selection), still explained 4% of the variance in the species data ($p < 0.001$). The partial effect of PO_4, after taking into account the effect of pH, explained 3.4% of the

Table 3. Species association (positive +, negative −, none o) with significant environmental variables derived from a species × environment table, produced by canonical correspondence analysis, based on 47 sites. Species were ranked by the strength of association with pH. Fit (%) = cumulative fit per species, as fraction of variance of species; niche breadth = species tolerance

	pH	Conductivity	Alkalinity[a]	Ca	Mg	NH_4[a]	PO_4[a]	Al[a]	$EpCO_2$	Distance	Occurrence (%)	Fit (%)	Niche breadth
Sphagnum sp.	−	−	−	−	−	−	−	+	+	−	17	34	⬤
Scapania undulata	−	−	−	−	−	−	−	+	○	−	30	25	⬤
Potamogeton polygonifolius	−	−	−	−	−	−	−	○	+	−	47	44	●
Batrachospermum sp.	−	○	−	○	○	−	−	+	+	○	9	9	⬤
Riccardia chamaedryfolia	−	−	−	−	○	−	−	+	○	−	6	7	•
Glyceria fluitans	−	○	−	−	○	○	○	○	+	○	72	28	⬤
Nitella flexilis	○	○	○	○	−	○	○	○	○	○	6	2	●
Oscillatoria sp.	○	−	○	○	−	○	○	○	○	−	6	1	⬤
Brachythecium rivulare	○	○	−	−	○	−	○	○	○	○	9	1	⬤
Callitriche stagnalis	○	○	○	○	○	○	○	○	○	−	30	10	⬤
Callergonella cuspidata	○	○	○	○	○	−	−	○	○	○	6	0	⬤
Sparganium emersum	○	○	○	○	○	○	○	○	○	○	34	1	⬤
Berula erecta	○	○	○	○	○	○	○	−	○	○	9	12	⬤
Diatomea sp.	○	○	○	○	○	○	○	○	○	○	13	2	⬤
Lemna minor	○	○	○	○	○	○	○	○	○	○	30	1	⬤
Closterium sp.	○	○	○	○	○	○	+	+	○	○	11	2	⬤
Binuclearia sp.	○	○	○	○	○	+	+	○	○	○	15	15	●
Chiloscyphus polyanthus	○	○	○	○	○	○	○	○	−	○	17	10	⬤
Oedogonium sp.	○	○	○	○	○	○	○	○	○	○	15	16	•
Vaucheria sp.	○	○	○	○	○	○	○	○	○	○	21	15	•
Rhynchostegium riparioides	○	○	○	○	○	○	○	○	○	○	19	19	⬤
Callitriche hamulata	○	○	○	○	○	○	○	○	○	○	45	28	⬤
Fissidens crassipes	○	○	○	○	○	+	+	○	○	○	11	22	•
Callitriche platycarpa	○	○	○	○	○	+	○	○	○	○	53	15	⬤
Oenanthe fluviatilis	+	○	+	○	○	○	○	−		+	11	6	•
Fontinalis antipyretica	+	○	○	○	○	○	○	○	−	+	32	16	⬤
Tribonema sp.	+	+	+	+	○	○	+	○	○	+	6	15	•
Ranunculus peltatus	+	+	+	+	○	○	○	○	○	+	21	20	⬤
Callitriche oblusangula	+	+	+	+	○	+	+	○	○	+	17	34	•
Amblystegium riparium	+	+	○	+	+	○	+	○	−	○	15	26	•
Myriophyllum alterniflorum	+	○	+	○	○	○	○	○	−	+	6	12	•
Elodea canadensis	+	+	+	+	○	○	○	−	−	+	17	45	●
Elodea nuttallii	+	+	+	+	+	○	○	○	−	+	17	23	•
Amblystegium tenax	+	+	○	+	+	+	+	−	−	−	6	17	•

[a] $\log_{10}(x+1)$ transformed prior to statistical analysis.

variance in the species data ($p = 0.012$). The partial effect of NH_4, after taking into account the effect of pH, explained 2.3% of the variance in the species data ($p = 0.23$). The partial effect of pH, after taking into account the effect of PO_4 or NH_4, explained respectively 6.1% and 8.1% of the variance in the species data ($p < 0.001$). The same results were obtained when pH was substituted by alkalinity.

DISCUSSION

Aquatic plant distribution in the Northern Vosges

One of the strengths of this dataset is that it comes from a geologically homogeneous sandstone region where many studies have been published. The effect of pollution on plant distribution in the Northern Vosges was suggested as long ago as the 1960s (Engel and Kapp, 1964). Muller (1990) established four plant communities organized along the longitudinal gradient of the rivers, mostly based on inorganic N and P

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concentrations, as inspired by Carbiener (e.g. Carbiener and Ortscheit, 1987; Carbiener *et al.*, 1990). Further studies did not disentangle the potential effects of pH-alkalinity (Thiébaut *et al.*, 1995) from inorganic N and P (Thiébaut and Muller, 1995), and therefore the two effects were eventually amalgamated (Thiébaut and Muller, 1999).

This study shows either that inorganic N played a very subordinate role or that its effect could not be separated from that of pH or alkalinity. It also shows that the effect of pH (or alkalinity) is more important than PO_4, yet both parameters are still significant after removing the effect of the other. However, the causality of both relationships is not straightforward.

In the case of inorganic P, mass balance studies of entire river reaches in calcareous lowland rivers bearing high standing biomass (see Westlake *et al.*, 1972), have shown that plant phosphorus retention was small compared with the phosphorus fluxes: -1.1% in Bere Stream with PO_4-P concentrations $\sim 10\,\mu g\,L^{-1}$ (Ladle and Casey, 1971); 2.5% in River Frome with PO_4-P $\sim 100\,\mu g\,L^{-1}$ (Westlake, 1968); and $<1\%$ in River Thame with PO_4-P ~ 400–$2000\,\mu g\,L^{-1}$ (House *et al.*, 2001).

In the case of pH, signs of necrosis in parenchyma of *P. polygonifolius* only appeared at $pH = 4.4$, an order of magnitude lower than the observed range of the present study (Thiébaut *et al.*, 2002a). This site, however, was characterized by high concentrations of total aluminium and low dissolved organic carbon (DOC) concentrations. At $pH = 4.4$ the concentration of labile toxic Al^{3+} would therefore be high (Stumm and Morgan, 1970). This is not the case for the other headwaters included in this study where aluminium would be less soluble ($pH > 5.5$) and Al^{3+} could be complexed by the DOC. The lack of floating leaves observed during the transplant experiments at the site with $pH = 4.4$ (Thiébaut *et al.*, 2002a) may be due to high partial pressure of CO_2 in the water.

Spatial isolation (longitudinal connectivity) was also significant, even after removing the effects due to surface-water chemistry. However its role seemed much more limited than in lowland rivers (Demars and Harper, 2005), with none of the catchments having a significantly distinct set of species. This may be caused by the presence of many small ponds situated across the small streams and peat bogs towards the head of the watercourses. Birds visiting the wetlands and ponds may disseminate seeds and vegetative propagules between the catchments (Sculthorpe, 1967: 328; Hutchinson, 1975: 244).

This study showed that as much as 38% of the variance in the species data could be explained by surface-water chemistry and distance of the site from the river's source. It did not take into account, however, the physical aspect of the streams. It is therefore very likely that some of the variance explained by distance and water chemistry would be confounded by other physical variables. The physical properties of the sites may explain better the segregation of mosses and vascular plants than the variables considered in this study (e.g. Haslam, 1978; Haury and Muller, 1991; Haury *et al.*, 1995; Holmes *et al.*, 1999b; Paal and Trei, 2004). Generally the overall percentage cover of aquatic plants was very low because the river bed consists predominantly of mobile sand, particularly in the upper part of the stream catchments. Peak flows easily uproot established plants in sand as found out during transplant experiments with *P. polygonifolius* (Thiébaut, 1997; Thiébaut *et al.*, 2002a). It is also unlikely that competition was so strong as to displace or exclude species in the headwaters because the overall percentage cover is generally extremely low. It is more likely that the fluctuation of spatial and temporal availability of resources creates an open window for new colonization events (Thiébaut, 2005). Hence species with high dispersal and resilience capacity may be more frequent, although the stochasticity of the system should prevent large abundances, even by invasive plants such as *Elodea* (Thiébaut, 2005). Species with high resistance capacity may not be very frequent owing to a lack of appropriate habitats but some can be locally abundant, such as *Ranunculus peltatus* (Thiébaut, 2005). Competition may be more important in the lower part of the rivers (e.g. Thiébaut *et al.*, 1997) where there is a higher species diversity (Thiébaut *et al.*, 2002b), although other physical disturbance (siltation, river bank management) may be responsible for a degradation in aquatic macrophyte populations (Thiébaut *et al.*, 2004).

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Implications for bioindication and conservation

It is clear from this study that there is no species able to separate the single effect of inorganic P enrichment from that of pH or alkalinity. The role of inorganic N seemed to be very subordinate or indistinguishable from stronger gradients such as pH or alkalinity. This extends previous findings based on single species response analyses within the Schwarzbach and Rothbach catchments (Thiébaut and Muller, 1998). The low occurrence of algae may be due to their ephemeral development (blooms outside the sampling dates of the survey). The algal abundance is also difficult to ascertain. Better correlations between algae and environmental variables may arise when algae are identified taxonomically down to species (rather than genus). The lack of strong correlations between inorganic N and P and aquatic plant composition and abundance seriously question the ability of the current macrophyte indices or vegetation-based methods for ecological diagnosis (Holmes *et al.*, 1999a; Amoros *et al.*, 2000; AFNOR, 2003; Kohler and Schneider, 2003; Haury *et al.*, 2006). It also questions the relative importance of inorganic N and P in riverine ecosystems, particularly compared with alkalinity in streams with $5.5 < \text{pH} < 7.5$.

There is an obvious need for clarification of the term 'trophic status'. The first indices clearly included alkalinity as part of the definition (Newbold and Palmer, 1979; Holmes and Newbold, 1984), but the new indices (*opere citato*) are striving to respond reliably to inorganic N and P enrichment. This would not hold true for the Northern Vosges because alkalinity was a stronger predictor. More detailed studies could try to disentangle the role of inorganic nutrients from alkalinity in the headwaters versus lower parts of the watercourses, and in sluggish versus fast-flowing reaches.

The next step will be to calculate the optimum, tolerance and expected abundance of all the species for which there is sufficient information either using the output from the CCA or through a species approach using a generalized linear model regression analysis (Gaussian Poisson log-linear). The weighted (by species abundance) average of the species optimum will define an objective macrophyte index that will allow back-calculation of the water-quality parameters (calibration exercise, see Jongman *et al.* (1995)). Then classes could be produced and the uncertainty quantified using multiple discriminant analysis. Macrophyte indices on alkalinity (or pH) and phosphorus will be partly correlated and so it will be important to calculate the indices for both water-quality parameters: not just phosphorus as this could lead to misinterpretation through over-simplification by those using these indices (e.g. nature conservation bodies).

The indices, and to a large extent the implementation of the European Water Framework Directive 2000/60/EC, also rely on the assumption that ecological responses are due simply to local environmental conditions. More work is needed to consider the regional perspective (e.g. Ricklefs, 1987), particularly the importance of the species pool for aquatic plants, and the connectivity between wetlands, ponds and the river network. What would happen if the peat bogs were drained, and the ponds devoid of submerged macrophytes? The protection of these regional habitats may be crucial in order to protect rare plant populations.

Unfortunately, in the Northern Vosges, the populations of protected species are declining owing to habitat degradation (Thiébaut *et al.*, 2004), and the present expansion of water-crowfoot (*Ranunculus peltatus*) leads to a decrease in plant diversity in the lower part of the watercourses generally enriched with nutrients (Garbey *et al.*, 2004; Thiébaut, unpublished data). Further action is therefore required to implement the aims of the Biosphere Reserve. However, without a broader understanding of the socio-economic context, management and conservation efforts may be inefficient. One important step would be to find out how different focus groups (particularly fish farmers, water industry, local communities) value their water resources and quality, before and after being briefed on the ecological issues (such as acidification, eutrophication, species diversity). Then processes such as river basin management planning may be used better to solve conflicts of interests through environmental cost–benefit analysis and implementation of existing European, national and regional legislation.

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