



Aquatic macrophytes as bioindicators of carbon dioxide in groundwater fed rivers

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ABSTRACT

Aquatic plants have been used as hydrological tracers in groundwater fed river systems. In nature, patterns in plant distribution have been attributed to ammonium (NH₄) toxicity and phosphate (PO₄) limitation, while some laboratory studies have focused on the role of the partial pressure of CO₂ (pCO₂). The aims of this study were (i) to test whether plant distribution was more related to pCO₂ than NH₄ and PO₄ in nature, (ii) to develop and test the predictive power of new plant indices for pCO₂, NH₄ and PO₄, and (iii) to test the potential causality of the relationships using species eco-physiological traits. These tests were carried out with field data from the Rhine, Rhône and Danube river basins. Species composition was best related to the effect of pCO₂. The pCO₂ plant index was well calibrated ($r^2 = 0.73$) and had the best predictive power ($r^2 = 0.47$) of the three indices tested on independent datasets. The plant-pCO₂ relationship was supported by a biological mechanism: the ability of strictly submerged species of aquatic vascular plants to use HCO₃ under low pCO₂. This was not the whole story: the effects of pCO₂, NH₄ and PO₄ on plant distribution were partially confounded and interacted all together with temperature. However, neither NH₄ toxicity nor P limitation could be asserted using species eco-physiological traits. Moreover, the predictive power of the NH₄ and PO₄ plant indices was not as strong as pCO₂, at $r^2 = 0.24$ and $r^2 = 0.27$, respectively. Other potentially confounding variables such as spatial structure, biotic and physical factors were unlikely to confound the findings of this study.

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1. Introduction

Many essentially terrestrial and amphibious plants can grow completely submerged in spring fed chalk streams (Kohler et al., 1973). This has been ascribed, in large part, to the high pCO₂ typically found in these environments (Sand-Jensen et al., 1992; Carbiener et al., 1995; Sand-Jensen and Frost-Christensen, 1998). Yet the effect of CO₂ on species distribution in groundwater fed rivers remains to be tested. Indeed, previous studies on plant species distribution using physico-chemical data, in groundwater fed systems, have generally focused on the role of PO₄ and NH₄, considering that CO₂ was not a limiting factor (Kohler et al., 1973; Carbiener et al., 1990; Bornette and Amoros, 1991). However, more recent studies have shown that the role of PO₄ and NH₄ on species composition in rivers is either not significant or subordinate (Demars and Harper, 2005a; Demars and Thiébaud, 2008; cf Paal et al., 2007).

Most strictly submerged (obligate aquatic) plant species living in alkaline rivers (HCO₃ > 2 meq L⁻¹) have evolved the capacity to use HCO₃ to cope with potentially low and fluctuating supplies of CO₂ for photosynthesis (Spence and Maberly, 1985). Most amphibious species

growing in alkaline rivers, however, lack this adaptation (Sand-Jensen et al., 1992).

The concentration of CO₂ needed to half-saturate the photosynthesis of aquatic plants typically lies between 6–13 times air-equilibrium concentrations (Sand-Jensen and Borum, 1991). The averaged excess partial pressure of CO₂ (EpCO₂) in small streams is around 8 times air-equilibrium concentrations (Rebsdorf et al., 1991). Where large groundwater upwellings occur, however, the stream excess partial pressure of CO₂ (EpCO₂) can be more than 30 times air-equilibrium concentrations (Massabuau and Fritz, 1984), which is enough to stimulate plant growth in water to a rate approaching that in air where the rate of CO₂ diffusion is 10,000 times faster (Sand-Jensen and Frost-Christensen, 1999). Although, the growth rate of plants with aerial leaves is typically 3–9 times higher than the rate of fully submerged plants at the air equilibrium concentration, this difference can be eliminated by increasing the CO₂ to 15–22 times air-equilibrium concentrations (Madsen and Sand-Jensen, 1991; Madsen and Breinholt, 1995).

As the concentration of CO₂ tends to decrease rapidly along a spring fed water course towards the air-equilibrium (Massabuau and Fritz, 1984), plants with the ability to uptake CO₂ from the atmosphere with aerial leaves, or to uptake HCO₃ from the water have a competitive advantage (Madsen and Sand-Jensen, 1987, 1991; Vadstrup and Madsen, 1995). Hence, species composition is predicted to change along a gradient of CO₂ concentrations, under high HCO₃ concentrations (>2 meq L⁻¹).

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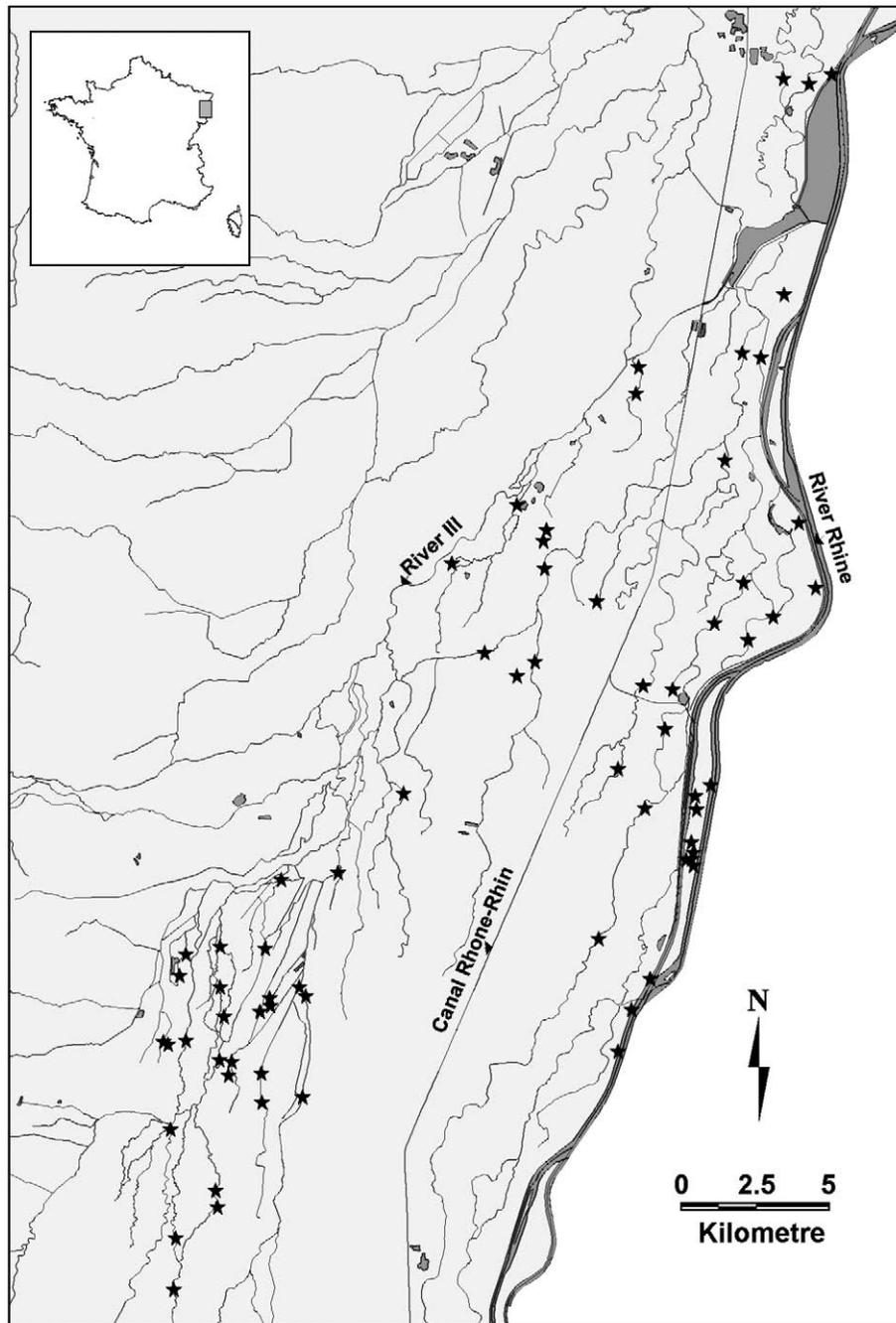


Fig. 1. Alsace floodplain, north east France, with stream sampling sites (stars). Water flows from south to north. Note that some small streams with a sampling site do not appear on this map.

Aquatic plants are widely used for biomonitoring purposes, yet the underlying environmental basis for their distribution is poorly understood, hampering data interpretation (Paal et al., 2007; Demars and Edwards, 2009). The aims of this study were therefore (i) to test whether plant distribution responded more to CO_2 than inorganic N and P, (ii) to test whether it is possible to disentangle the previously ascribed effects of NH_4 and PO_4 from CO_2 (and HCO_3^-) on species distribution and abundance (Sand-Jensen, 1989), (iii) to develop new plant indices, based on species indicator values, and test whether these were able to predict CO_2 , PO_4 and NH_4 concentrations, and finally (iv) to test the potential causality of the relationships by using eco-physiological traits relevant to the ecosystem studied. These tests were carried out with field data of running waters from continental Europe: Rhine floodplain, Rhône floodplain, and Danube river basin.

2. Material and methods

2.1. Study areas

The factors determining in stream CO_2 super-saturation include the groundwater origin and its relative proportion to stream water discharge, stream metabolism and stream reaeration rate. The Rhine floodplain offers an exceptional set of hydrological conditions where the variability of CO_2 concentrations (Massabuau and Fritz, 1984) and nutrient concentrations (Carbiener et al., 1990; Carbiener and Trémolières, 1990) is extremely large. There was also a large amount of information on stream water chemistry and aquatic plants (Carbiener et al., 1990; Eglin and Robach, 1992; Trémolières et al., 1993, 1994; Eglin et al., 1997). Hence, the dataset available in the Rhine

Table 1

Range and percentiles of the averaged physico-chemical variables of the 74 sites surveyed in the Rhine floodplain.

	pH	EC ^a μS cm ⁻¹	T ^b °C	HCO ₃ ^c meq L ⁻¹	Cl mg L ⁻¹	PO ₄ μg P L ⁻¹	NH ₄ μg N L ⁻¹	NO ₃ μg N L ⁻¹	NO ₂ μg N L ⁻¹	COD ^d mg O ₂ L ⁻¹	O ₂ % saturation	EpCO ₂ ^e x times atm
Minimum	7.2	381	10.2	2.5	30	5	3	190	3	0.3	13	3
10th	7.3	517	11.4	2.9	40	9	12	974	7	0.9	56	4
50th	7.7	669	13.4	3.5	83	25	39	2950	24	1.8	85	12
90th	8.1	747	15.3	4.5	132	215	189	6900	53	3.7	101	28
Maximum	8.3	979	17.4	5.1	156	358	973	9000	114	7.9	111	43

^a Electrical conductivity.^b Temperature.^c Calculated from calcium hardness measurements (see text).^d Chemical oxygen demand.^e Excess partial pressure of CO₂, atm = atmospheric pressure.

floodplain may allow the differentiation of free CO₂ from the effects of inorganic N and P on aquatic macrophyte distribution. Two other similar areas for which sufficient published data of the same quality existed were selected to test the findings of the Rhine floodplain: the Rhône floodplain and the River Moosach within the Danube river basin. All the sites investigated were running waters and excluded the main stems (Rhine, Ill, Rhône rivers). Sites with still water reported in other studies (e.g. Robach et al., 1997) were not included in the present study.

2.1.1. The stream network of the Rhine floodplain (Alsacian Ried)

The main study area is situated alongside the left bank of the Rhine river (7°40' E, 48°20' N). It lies on a thick layer of sand and gravel alluvium, rich in carbonate, coming from the alpine Rhine river basin. The Rhine River has been straightened, confined to a single channel, and embanked 0.5–2 km from the channel (period 1830–1860) and finally completely canalised (period 1930–1970) which now prevents the flooding of its floodplain – Carbiener and Trémolières (1990); Eglin and Robach (1992). Despite this, the calcareous alluvium supports one of the largest networks of groundwater fed rivers in Europe, characterised by stable flows and temporally stable physico-chemistry (Carbiener et al., 1990). The groundwater fed streams (central part of the Ried) provide optimal biotopes for the *Potamogeton coloratus* Hornem. plant community (Carbiener et al., 1990). The groundwater stream network is partly affected however by the Ill, a tributary of the Rhine, draining water from the Vosges mountains (Trémolières et al., 1994) and Rhine water seepage (Trémolières et al., 1993). Other streams, such as those running on Rhinau and Gerstheim islands, are directly fed by the Rhine water (Eglin and Robach, 1992). The averaged depth range of these streams is 0.3–2 m with clear water.

2.1.2. Rhône floodplain

The history, physico-chemistry and aquatic vegetation of the Rhône River floodplain (5°40' E, 45°40' N) are similar to the Rhine River floodplain (Bravard et al., 1986; Bornette and Amoros, 1991). The field sites are cut-off channels of the Rhône river and tend to be much wider than the streams draining the Alsacian floodplain, and partially subject to the Rhône flood pulses (Bornette et al., 1994).

2.1.3. Danube river basin (River Moosach)

The Bavarian calcareous streams (11°44' E, 48°24' N) have similar physico-chemistry and aquatic vegetation to the Rhine floodplain, and drain fenlands comparable to the groundwater fed rivers of the central Alsacian Ried (Kohler et al., 1971, 1973).

2.2. Macrophyte data

2.2.1. Macrophyte survey

Seventy-four sites in 39 streams were surveyed in the hydrographic network of the Alsacian Ried (Fig. 1). These data were essentially the same as those published by Robach et al. (1996a),

although sixteen 'sites' (out of the original 90 sites) were surveyed twice (both for vegetation and physico-chemistry) and so the oldest surveys were removed from the original dataset. Plants were surveyed during the summer months, within a five year period (1988–1993), along a 50–100 m long stream reach. The cover-abundance of the species was assessed visually using the scale of Braun Blanquet (1932): +, present; 1, <5%; 2, 5–25%; 3, 25–50%; 4, 50–75%; 5, >75% cover (coded from 1 to 6 for the statistical analyses). Forty-nine aquatic species were recorded in total, of which 36 were selected for the analysis because they occurred in at least three sites (Appendix A). There were on average about seven species per site (range 1–26).

The groundwater fed running waters investigated were characterised by amphibious plants growing completely submerged (especially close to groundwater upwelling). Examples include *Juncus subnodulosus* Schrank, *Cardamine amara* L., *Agrostis stolonifera* L., *Phalaris arundinacea* L., *Schoenoplectus lacustris* (L.) Palla, *Sparganium emersum*, *Berula erecta*, *Mentha aquatica*, *Veronica anagallis-aquatica*, *Myosotis scorpioides*, *Rorippa nasturtium-aquaticum* (see Appendix A). All the species considered in this study (Appendix A) were growing completely or mostly submerged (Carbiener et al., 1995) apart from the free floating taxa *Azolla filiculoides*, *Lemna minor/minuta* and *Spirodela polyrhiza*.

2.2.2. Species traits of aquatic vascular plants

Several species traits (biological attributes) were taken from the literature relevant to spring-fed chalk streams: strictly submerged (Willby et al., 2000); bicarbonate user (Gessner, 1959; Kohler and Schoen, 1984; Madsen and Sand-Jensen, 1991; Rybová et al., 1991; Sand-Jensen et al., 1992; Maberly and Madsen, 2002), nitrate reductase activity (Melzer, 1980; Melzer and Exler, 1982), and phosphorus tissue concentration (Robach et al., 1996b). Most of these traits can effectively vary with external environmental conditions (phenotypic variability) but this was found to be small compared to the differences between species (genetic variability), e.g. Melzer (1980); Demars and Edwards (2007, 2008). The most important was to select results on traits from the same area studied (e.g. Melzer, 1980; Melzer and Exler, 1982; Robach et al., 1996b) or from similar environmental conditions (e.g. bicarbonate utilisation by plant was from studies with low EpCO₂ and high HCO₃) in order to have comparable relative values of species traits. The analyses were restricted to the vascular plants (dominant taxa) because other macrophyte types (bryophytes, algae) have different C:N:P stoichiometry (Demars and Edwards, 2007).

In order to test the potential causality of species-environment relationships, vascular plant species traits were regressed against species indicator values (see Section 2.6.). *Potamogeton coloratus* was reported as a bicarbonate user by Kohler and Schoen (1984), but not its 'sterile' submersed life form associated with the spring area (Buchwald et al., 1995). Due to these conflicting reports, *P. coloratus* was excluded from the regression analyses.

Species with high tissue phosphorus concentrations may be species with fast growth rate capacity (leaf P tissue of terrestrial herbaceous

species has been related positively to maximum potential relative plant growth rate, Thompson et al., 1997). Hence, species phosphorus tissue content was expected to be positively related to species associated with increasing PO₄ concentrations. Whether this expected positive relationship is ecologically relevant depends on whether P limitation occurs, i.e. whether observed tissue P concentrations exceed plant tissue critical P threshold for 95% maximum yield or growth rate (Gerloff, 1975; Demars and Edwards, 2007).

The nitrate reductase activity (NRA) may not respond positively to increasing nitrate species optimum concentrations (as expected), but may respond negatively to increasing ammonium species optimum concentrations, because the presence of ammonium has been reported to block or slow the NRA (Melzer, 1980; Rolland and Trémolières, 1995; Cedergreen and Madsen, 2003).

2.3. Physico-chemical data

Eleven physico-chemical variables of the stream water column were measured monthly at each site for at least one year during the year of plant survey (Table 1). Portable instruments were used to measure *in situ* pH (Hanna, HI 8424), temperature and dissolved oxygen concentration (WTW, OXI 96). In the laboratory, water samples were filtered. Nitrate and nitrite analyses were determined by spectrometry following AFNOR (1986, method NF T 90-012 and NF T 90-013). Soluble reactive phosphorus (hereafter referred to as phosphate) and ammonium were also determined by spectrometry (NF T 90-023 and NF T 90-015 respectively; AFNOR, 1986) but using an automated microflux method (Alliance Instruments). Chemical oxygen demand (COD) was determined by chemical oxidation (NF T 90-101; AFNOR, 1986) and chloride with an Ag-selective electrode. Calcium concentration (calcium hardness) was analysed by EDTA titrimetry (Standing Committee of Analysts, 1982).

Oxygen solubility in water at equilibrium with air (at 1013.25 hPa), as a function of temperature, was calculated with a third order polynomial regression equation, using data published by the Standing Committee of Analysts (1989, p. 32), to estimate the percentage of dissolved oxygen saturation.

The EpCO₂ can be calculated from measurements of pH, temperature and titration alkalinity (e.g. Neal et al., 1998). The latter was not measured but calcium concentrations correspond generally very well to titration alkalinity measurements (Sigg et al., 1994, p. 85). In the Alsacian Ried, titration alkalinity (HCO₃⁻, meq L⁻¹) can be predicted from calcium concentration (meq L⁻¹) determined by atomic absorption spectrometry (AAS, Bertrand Fritz, personal communication) as follows: [HCO₃⁻] = 0.6879 [Ca] + 0.7358 (r² = 0.99, P < 0.001; from data with alkalinity range 2.6–4.5 meq L⁻¹ in Massabuau and Fritz, 1984). It was assumed that the calcium concentration determined by AAS on water filtered through Millipore 0.45 µm is comparable to the EDTA titrimetry method since it was also recommended by the Standing Committee of Analysts (1982). Hence, the EpCO₂ (units are x times atmospheric pressure units, with atmospheric pCO₂ = 340 ppm) was calculated using the calculated titration alkalinity, pH and water temperature as follows (see Eq. (2) in Neal et al., 1998, Table 1):

$$\text{EpCO}_2 = \frac{(\text{HCO}_3^- + 10^{6-\text{pH}}) 10^{6-\text{pH}} \gamma}{(6.46 - 0.0636T)}$$

with alkalinity (HCO₃⁻) in µeq L⁻¹, ion activity (γ = 0.95) and observed stream water temperature (T, in °C). The calculated EpCO₂ was very similar to the results of Massabuau and Fritz (1984) and fitted within the observed range of the Rhône floodplain (Bornette and Amoros, 1991; Bornette et al., 1996) and Moosach River within the Danube river basin (Kohler et al., 1973), all based on measured titration alkalinity, pH and water temperature measurements.

Dissolved nitrate, nitrite, ammonium and phosphate concentrations of the stream water were log transformed prior to statistical analysis to reflect previously established relationships between nutrient (NH₄, PO₄) concentrations and aquatic macrophyte communities (e.g. Carbiener et al., 1990) and increase the normalisation of the data. Pearson coefficient correlations were run between pairs of variables to check out the strength of their inter-relationships.

2.4. Spatial analyses

The sampling was designed to minimize spatial effects (74 sites across 39 streams). The length of the survey reach was most appropriate for our study on pCO₂, because the maximum rate of degassing observed did not exceed 4–8% over 50–100 m (Massabuau and Fritz, 1984). More detailed mapping of the vegetation and monitoring of the physico-chemistry in the Alsace floodplain from older studies showed that sharp changes can happen at finer resolution than the present study (see e.g. Trémolières et al., 1993, 1994). Hence, the spatial structure was not expected to be important. The relative independence of the sites was confirmed with spatial autocorrelation tests (Section 2.4.1.) and the effect of the spatial structure on species composition was tested with a trend surface analysis (Section 2.4.2.).

2.4.1. Multivariate Mantel correlograms

Multivariate Mantel correlograms were constructed to study the spatial structure of species composition and water physico-chemistry. The geographical distances between sites were computed with ArcGIS using Hawthstools. The geographical distance matrix, the first four axes of the DCA (species composition data) and first four axes of the PCA (water chemistry data), were used to build similarity matrices of the sites based on Euclidean distance using GENSTAT 10. Excel was then used to produce 11 similarity matrices for the various distance classes (0–2, 2–4, ..., 20–22 km) as in Legendre and Legendre (1998, p. 737). All similarity matrices were then standardized to zero mean and unit variance. The cross product of the unfolded matrices was calculated with GENSTAT and multiplied by 1/(1/d), where d = [n(n–1)/2] is the number of distances in the upper triangular part of each matrix (Legendre and Legendre, 1998, p. 554), in order to calculate the standardized Mantel statistic (range –1 to 1). The Mantel test was computed in GENSTAT. This test has the advantage of working at finer resolution than the trend-surface analysis, but it is non-directional.

2.4.2. Trend surface analysis

The geographical coordinates X (easting) and Y (northing) and their combinations (XY, X², Y², X²Y, XY², X³, Y³) were used to build a third degree spatial polynomial model. X and Y were centered on their respective means prior to model fitting to alleviate the problem of multicollinearity (Legendre and Legendre, 1998, p. 526, 739). This trend-surface analysis was relevant to detect smooth transition across the Alsace floodplain.

2.5. Multivariate data analyses

All the data were centred and standardised. All ordinations were run on correlation matrices with Canoco 4.5 (ter Braak and Šmilauer, 2002). A principal component analysis (PCA) was run to investigate the water chemistry data. A detrended correspondence analysis (DCA) was run using the species × site table to investigate how the species relate to each other. As there was a complete turnover of species composition along the first axis of the DCA (length > 5 SD), a unimodal model was used for the constrained ordination analyses. Canonical correspondence analysis (CCA) was performed to find out which chemical and spatial factors would significantly explain the species composition (Monte Carlo permutation test, 1000 unrestricted random permutations). Partial CCAs were run to partition the variation of selected variables.

Table 2
Pearson correlation coefficient between pairs of physico-chemical variables.

Cl	-0.15										
DO	0.46	-0.41									
EpCO ₂	-0.47	0.10	-0.61								
HCO ₃	-0.43	0.20	-0.09	0.55							
NH ₄ *	0.71	-0.04	0.34	-0.44	-0.35						
NO ₂ *	0.49	-0.02	0.25	-0.46	-0.25	0.54					
NO ₃ *	-0.17	-0.40	0.17	0.43	0.48	-0.25	-0.21				
PO ₄ *	0.71	-0.11	0.28	-0.55	-0.56	0.79	0.56	-0.29			
Temperature	0.62	-0.05	0.30	-0.52	-0.42	0.63	0.63	-0.49	0.66		
EC	-0.49	0.49	-0.34	0.48	0.57	-0.30	-0.46	0.18	-0.54	-0.45	
pH	0.44	-0.03	0.69	-0.93	-0.37	0.46	0.48	-0.39	0.48	0.54	-0.36
	COD	Cl	DO	EpCO ₂	HCO ₃	NH ₄ *	NO ₂ *	NO ₃ *	PO ₄ *	Temp	EC

The 5% level significance (after Bonferroni correction) is indicated by italics. Strongest relationships ($r > 0.5$) are highlighted.

*log ($x + 1$) transformed.

2.6. Species indicator values

Gaussian logistic regression can be used to estimate species indicator values from species presence-absence data (binomial distribution) and measured environmental variables from a range of sites (ter Braak and Looman, 1986). A Poisson distribution should be assumed for the discrete species abundance data of the present study. While this is often not the case, the variance in the data was proportional to the mean ($r^2 = 0.90$), and so the assumption of the log-linear regression could be relaxed (Jongman et al., 1995, p.50).

The response of a species describes the probability $p(x)$ that the species occurs as a function of an environmental variable x . The Gaussian log-linear curve models the expected abundance (Ey) response of a species:

$$\ln(Ey) = \ln[p(x)] = b_0 + b_1x + b_2x^2 = a - 0.5(x-u)^2 / t^2$$

where u is the species optimum or indicator value (the value of x with highest probability of occurrence), t is its tolerance (a measure of ecological amplitude), and a is the species maximum expected abundance. The parameters b_0 , b_1 , and b_2 can be estimated by log-linear regression to obtain the following:

$$u = -b_1 / 2b_2$$

$$t = 1 / \sqrt{-2b_2}$$

$$Ey = p(u) = \exp(b_0 + b_1u + b_2u^2)$$

Gaussian log-linear regressions were performed by the statistical packages GENSTAT 8.2 (Payne, 2005) and Canodraw 4.0 (ter Braak and Šmilauer, 2002). The Gaussian log-linear response curve is bell-shaped and symmetrical, and therefore its optimum is identical to its mean.

The results were interpreted similarly to Roy et al. (2000). The significance of a species optimum can be judged by whether the quadratic coefficient b_2 is significantly less than zero. The majority of optima (>80% of species) were significant at the 5% level (strong optima) or non-significant but with negative b_2 (weak optima). For a few species, the fitted optimum was beyond the maximum or minimum of the observed range of the environmental variable (truncated optima). The truncation consisted of reassigning their optimum to the minimum or maximum of the observed range. If b_2 was positive (no optima), the log-linear regression was re-run without the quadratic term; and if b_1 was significant, the optimum was assigned to the minimum or maximum of the observed range. Finally, if b_2 was positive and b_1 not significant, the species was simply rejected.

2.7. Plant index

A simple plant index (I) was calculated for selected chemical factors at every site (j) based on the observed abundance (A) of

species ($1, \dots, i, \dots, n$) and the species optimum (u) and tolerance (t) derived from the generalized linear models (GLM, ter Braak and Barendregt, 1986):

$$I_j = \left(\sum_{i=1}^n \frac{A_i u_i}{t_i^2} \right) / \left(\sum_{i=1}^n \frac{A_i}{t_i^2} \right)$$

The index was calculated for the 74 sites of the Rhine floodplain that had been used to derive the species optimum and tolerance (calibration step), and applied to three independent datasets: 16 sites of the Rhine floodplain surveyed earlier, 9 sites surveyed within the Rhône floodplain (published data from Bornette and Amoros, 1991; Bornette et al., 1996), and 12 sites from the Danube river basin (published data from River Moosach, Kohler et al., 1973).

3. Results

3.1. Environmental variables

The environmental gradients (physico-chemical variables) on which the analyses were based had a very wide range, generally spanning about one order of magnitude (Table 1). While there were

Table 3

Percentage of explained variation in species composition by individual physico-chemical and spatial variables, based on 74 sites \times 36 species, analysed by Canonical Correspondence Analysis, tested against 1000 unrestricted Monte Carlo random permutations (significance at the 5% level after Bonferroni correction $P \leq 0.002$).

	% variation explained	P
EpCO ₂	9.3	<0.001
pH	8.0	<0.001
PO ₄ -P*	7.9	<0.001
HCO ₃	7.7	<0.001
Temperature	7.0	<0.001
NH ₄ -N*	6.7	<0.001
COD	6.7	<0.001
NO ₃ -N*	5.4	<0.001
Conductivity	4.6	<0.001
NO ₂ -N*	4.3	<0.001
X (Easting)	3.8	0.001
Dissolved O ₂	3.8	0.002
X ³	2.9	n.s.
Chloride	2.3	n.s.
X ² Y	2.1	n.s.
XY ²	2.0	n.s.
X ²	1.9	n.s.
Y ³	1.7	n.s.
XY	1.6	n.s.
Y (northing)	1.3	n.s.
Y ²	1.3	n.s.
All significant variables together	33.5	<0.001

*log ($x + 1$) transformed.

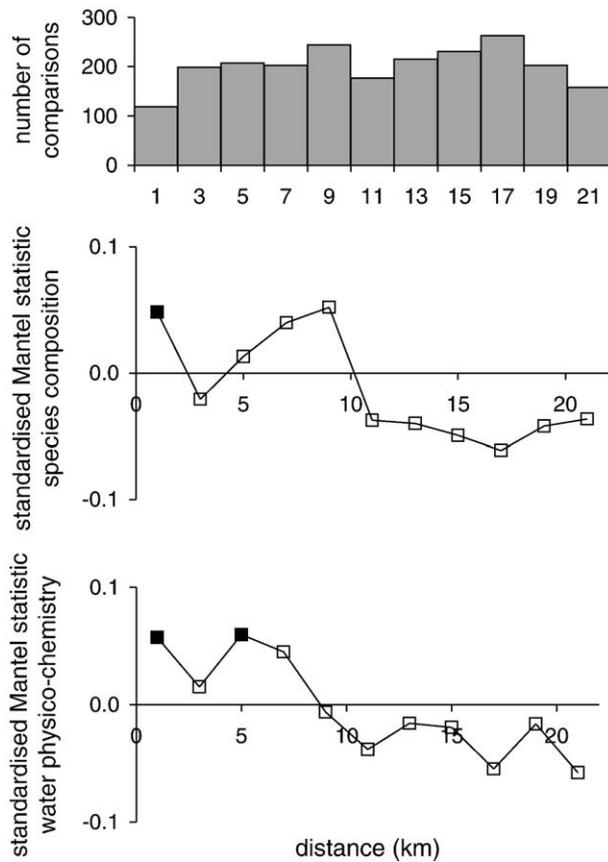


Fig. 2. Spatial auto-correlograms (non-directional) of plant species composition and stream water physico-chemistry across the Alsace floodplain. Significant tests are indicated by a filled square (after progressive Bonferroni correction of $\alpha=0.05/k$ for the k th distance class). Positive Mantel statistics represent positive autocorrelation.

many significant correlations between variables, very few were strong (Table 2). Some were trivial such as pH versus $EpCO_2$ ($r=-0.93$). Others were more inconvenient for the purpose of this study, such as PO_4 versus NH_4 ($r=0.79$) or COD ($r=0.71$). $EpCO_2$ was also significantly but relatively weakly related to PO_4 ($r=-0.55$) and NH_4 ($r=-0.44$).

3.2. Linking species composition to environmental and spatial variables

Together, the predictive variables explained 33% of the variation in the species data (Table 3). The main predictor was $EpCO_2$ (9.3% of explained variation in species composition). All the other physico-chemical variables were also significant, except chloride. Dissolved oxygen saturation was the least significant factor. While PO_4 and NH_4 were subordinate, they individually explained a similar amount of variation in species composition to $EpCO_2$.

The partial effect of $EpCO_2$, after taking into account the effect of PO_4 (or NH_4), explained 5.6% of the variation in species composition ($P<0.001$). The partial effect of PO_4 (or NH_4) after taking into account the effect of $EpCO_2$, explained 4.2% of the variation in the species data ($P<0.001$).

The interaction $EpCO_2 \times PO_4$ explained 3.8% of the variation after taking into account the individual effects of $EpCO_2$ and PO_4 ($P=0.002$). Using stepwise selection, the effect of temperature, temperature $\times PO_4$ and temperature $\times EpCO_2$ explained an additional 2.1, 2.8 and 2.3% of the remaining variation in species composition ($P=0.021$, $P=0.003$, $P=0.009$) respectively. $EpCO_2$, PO_4 , temperature and their interaction factors explained, all together, 24.5% of the variation in species composition.

The spatial structure was significant for the 0–2 km distance class with respect to species composition and in the 0–2 and 4–6 km classes for physico-chemistry (Fig. 2). However, the Mantel statistic was very small, not exceeding 0.06.

Out of all the spatial variables entered in the CCA, only easting (X) was significant and explained 3.8% of the variation in plant composition (Table 3). After taking into account all the significant chemical variables, easting explained only 1.7% of variation in plant composition. So only 2.1% of the variation explained by the supplied chemical variables was spatially structured.

3.3. Plant indices

The species optima are reported in Appendix A and Appendix B with the tolerance and expected abundance. The plant indices were calculated for three selected variables: $EpCO_2$, PO_4 and NH_4 (Fig. 3).

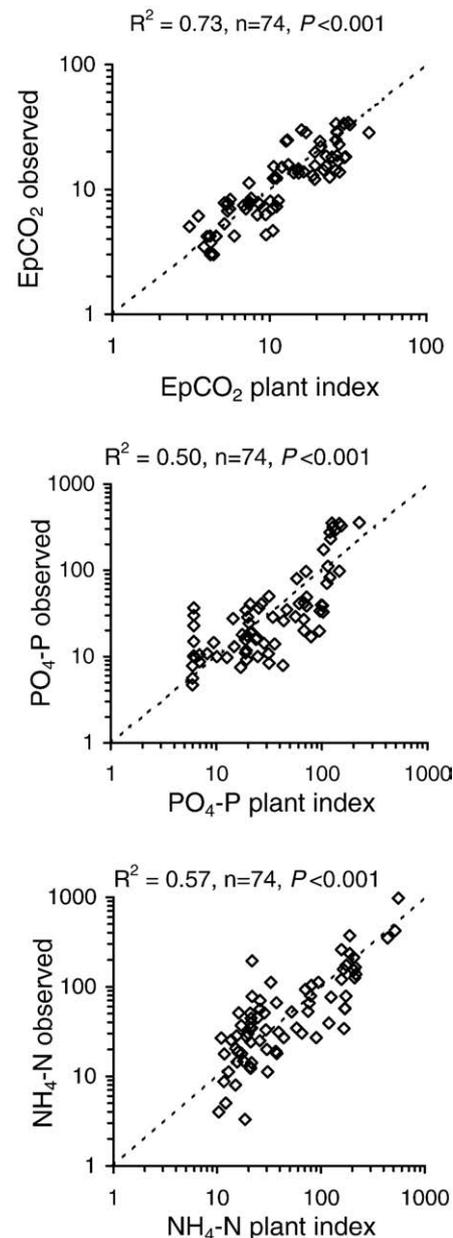


Fig. 3. Calibration of selected plant indices to observed nutrient concentrations (in $\mu g L^{-1}$) based on 74 sites. The dotted lines represent the expected 1:1 slope.

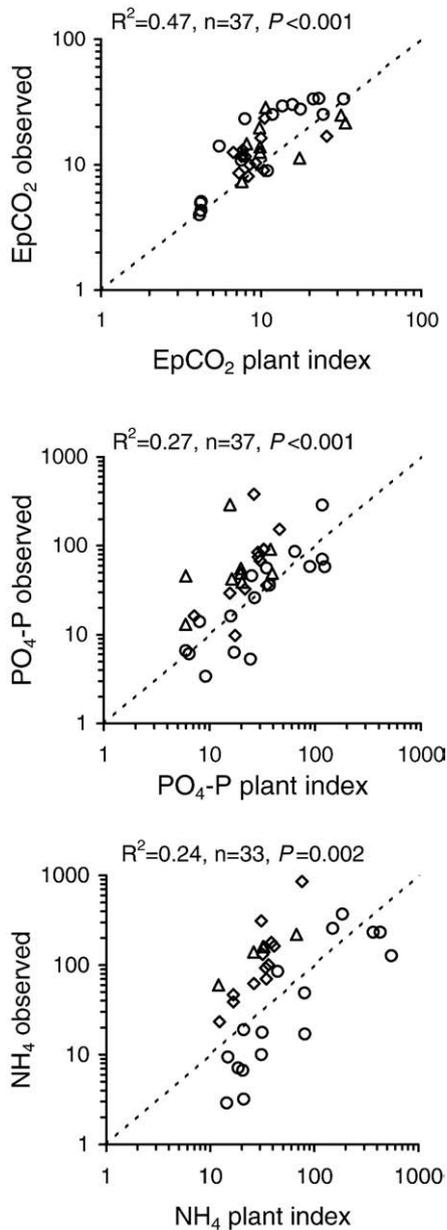


Fig. 4. Test of selected plant indices to observed nutrient concentrations based on 16 sites from the Rhine floodplain (circles), nine sites from the Rhône floodplain (triangles), and 12 sites from the Danube river basin (diamonds). The dotted lines represent the expected 1:1 slope.

There was a good fit between the plant indices and the selected variables based on the 74 sites surveyed (calibration): adjusted $r^2 = 0.50\text{--}0.73$ (based on a linear regression of log transformed values with intercept set to zero). The test showed that the plant indices predicted the observed environmental gradients as follows (adjusted r^2 ; based on a linear regression of log transformed values with intercept set to zero): EpCO_2 ($r^2 = 0.47$), NH_4 ($r^2 = 0.24$), PO_4 ($r^2 = 0.27$) — Fig. 4. The EpCO_2 plant index underestimated the expected EpCO_2 at intermediate concentrations from the sites of the Rhine floodplain (Fig. 4). The NH_4 and PO_4 plant indices underestimated the observed nutrient concentrations from the Rhône floodplain and the Danube river basin (Fig. 4). The NH_4 plant index also overestimated NH_4 concentrations when expected values were below $10 \mu\text{g N L}^{-1}$. While the EpCO_2 plant index fitted better the observed values than the NH_4 and PO_4 plant indices, the EpCO_2 index was highly correlated with the NH_4 and PO_4 indices ($r = -0.92$ and $r = -0.85$, $P < 0.001$, respectively). There was generally

enough bioindicator taxa per site to calculate the indices, in the calibration dataset (average 6, range 1–21) and the test dataset (average 7, range 2–14), with $>80\%$ of sites having ≥ 3 bioindicator species.

3.4. Species traits of aquatic vascular plants

The data used for the analyses are given in Appendix A. There were more bicarbonate users at low EpCO_2 (logit regression, $P = 0.047$, Fig. 5). Strictly submerged species were more likely to be associated with low EpCO_2 , approaching air equilibrium (logit regression, $P = 0.036$, Fig. 5). There were no significant relationships between the NRA and species optima for NO_3 and NH_4 (Fig. 5). Note that if the outlier (*Potamogeton coloratus*) was removed from the NRA- NO_3 regression, the relationship would be contrary to what is biologically expected (see Section 2.2.2). Although phosphorus concentrations of the plant tissue were significantly related to the species optima for PO_4 ($P < 0.007$), all tissue concentrations were above plant tissue critical threshold for 95% maximum growth rate (Fig. 5).

4. Discussion

4.1. Potential causality of the relationships

4.1.1. $p\text{CO}_2$

The results (especially from the plant indices) showed that there is a stronger relationship between plant distribution and EpCO_2 than with PO_4 and NH_4 . This is supported by known biological mechanisms: aquatic vascular plant species associated with low EpCO_2 , approaching air equilibrium, were more likely to be strictly submerged and bicarbonate users, and amphibious species were generally associated with higher EpCO_2 (Fig. 5). The capacity of some populations of *Potamogeton coloratus* (excluded from the logit regression, see Section 2.2.2.) to uptake bicarbonate may result from adaptation to other environments where EpCO_2 is either moderate (up to 8 times air-equilibrium concentrations) or variable, such as in standing waters (Talling, 1976).

The inclusion of free floating taxa in the multivariate analyses and in the EpCO_2 index was questionable. They were not excluded because *Lemna minor/minuta* is a bicarbonate user (this could be an important feature at other submerged life stages in the development of that taxa such as wintering, although, alternatively it may just reflect the evolutionary history of the species). The multivariate analyses also requested a common list of taxa for the multivariate analyses (especially in partial CCAs and testing for interaction effects). Without the free floating taxa EpCO_2 explained 9.6% (instead of 9.3%) of the variation in species composition. The plant indices were also virtually unchanged in both the calibration and test datasets (adjusted r^2 reported in Section 3.3. were unchanged). This is mostly because the abundance of the free floating taxa was small and so did not affect much the index. None of the results of this study were therefore biased by the free floating taxa.

4.1.2. Nutrients (N, P)

Previously, ammonium toxicity and phosphorus limitation were identified as the key factors regulating plant distribution (Glänzer et al., 1977; Carbiener et al., 1995). Original ammonium toxicity tests in aquaria on the supposedly most sensitive species, *Potamogeton coloratus*, showed, however, no significant effects of $\text{NH}_4\text{-N}$ concentrations up to 12 mg L^{-1} (Glänzer, 1974). In general, ammonium toxicity (in alkaline freshwater) has not been detected below $\text{NH}_4\text{-N}$ concentrations of $1\text{--}2 \text{ mg L}^{-1}$ (Melzer and Exler, 1982; Dendène et al., 1993; Rolland and Trémolières, 1995). These concentrations are 100–1000 times higher than the $10\text{--}20 \mu\text{g L}^{-1}$ $\text{NH}_4\text{-N}$ nutrient concentrations above which the *Potamogeton coloratus* community is not found in the groundwater fed streams of the Rhine floodplain (Carbiener et al., 1990). As there was also no negative relationship between NRA and species optimum ammonium concentrations (Fig. 5), there was no direct evidence of ammonium toxicity from field data.

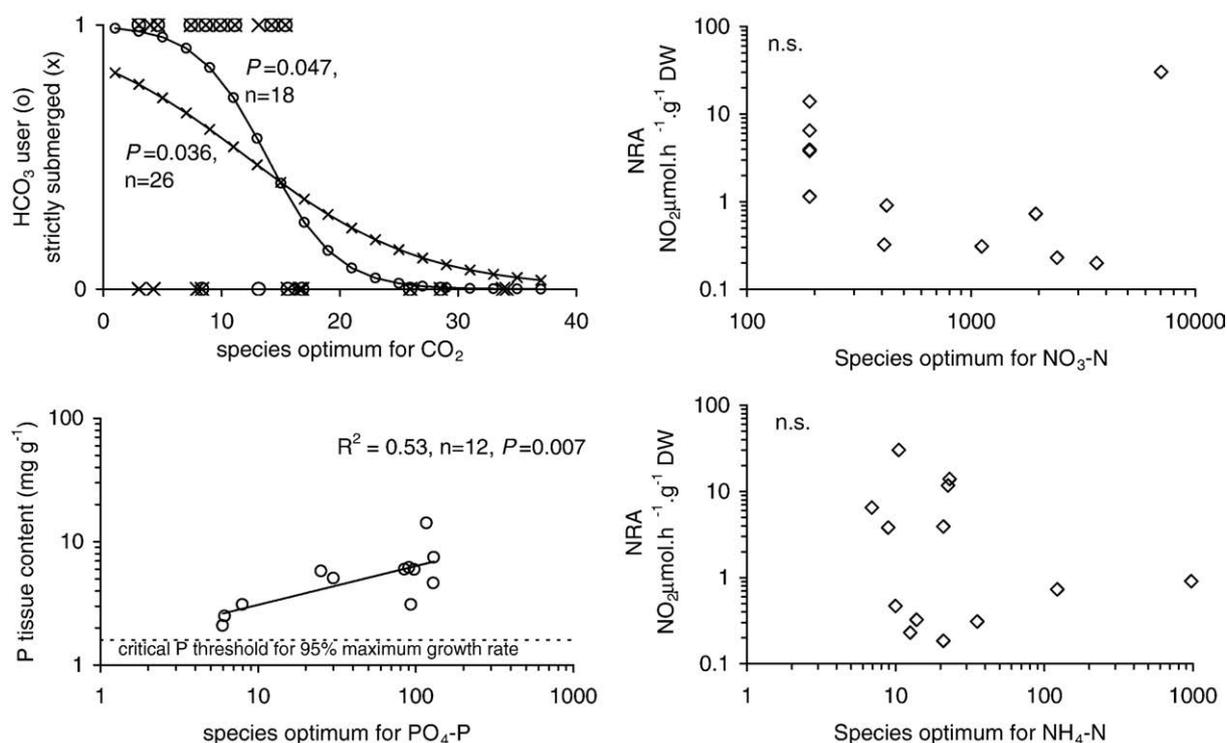


Fig. 5. Species eco-physiological traits regressed against species indicator values (optimum determined by Gaussian log-linear regression). NRA, nitrate reductase activity.

Nutrient (N, P) plant tissue concentrations are generally only weakly related to external phosphorus concentrations and this is likely to be due mostly to 'luxury consumption' (Demars and Edwards, 2007). In the Alsace Ried, the correlation between stream water PO_4 and tissue P concentrations (*Berula erecta*, *Callitriche obtusangula*, *Elodea canadensis*, *E. nuttallii*) was relatively strong ($r^2 = 0.64$, $n = 113$, $P < 0.001$; from data published in Robach et al., 1996b). This may partly be the product of the correlation between stream EpCO_2 and phosphate due to their inverse relationship ($r = -0.55$; Table 2), because increasing pCO_2 (while controlling for pH) has been shown to decrease P plant tissue concentration (Titus and Andorfer, 1996).

However, the P leaf tissue concentration only increased from 2 to 11 mg g^{-1} along a gradient of PO_4 of 3–440 $\mu\text{g L}^{-1}$. Virtually all the samples (>98%) were also above the critical nutrient threshold for 95% maximum growth rate, indicating that there is probably no strong P limitation as previously thought, even at high pCO_2 , since the bioassays were conducted under 0.5–1% CO_2 air enriched conditions (Colman et al., 1987). Further field experiments are needed to find out whether this is really just 'luxury consumption' or whether species with higher P concentration may have a competitive advantage (faster maximum potential growth rate, Thompson et al., 1997) as nutrient enrichment increases in this physically stable environment.

Nitrate was also a significant, although somewhat subordinate, predictor of species composition. Although this goes against previous findings (Carbiener et al., 1995), high nitrate concentrations have been shown to have negative effects on the physiology of some freshwater aquatic species (Robe and Griffiths, 1994; Boedeltje et al., 2005). Hence the potential negative effects of nitrate should not be completely dismissed.

Finally macrophytes generally play a negligible role in regulating N and P fluxes in running waters compared to upwelling groundwater nutrient fluxes (e.g. Canfield and Hoyer 1988).

4.1.3. Multiple factors

The overall plant distribution, however, was best predicted by a combination of factors such as EpCO_2 , PO_4 (or NH_4) and temperature.

Since dissolved inorganic carbon acquisition depends on ATP supply, it seems possible that P limitation would affect carbon concentration mechanisms (Giordano et al., 2005; Beardall et al., 2005). It is also known that the plant tissue N concentration needed to saturate the growth rate of *Elodea canadensis* is higher at low than at high CO_2 concentrations (Madsen et al., 1998). The temperature range observed in this study (annual average 10–17 °C) is strong and so a significant effect of temperature on species composition was expected, also in interaction with CO_2 , because some species responded differently in laboratory experiments (Olesen and Madsen, 2000).

4.1.4. Oxygen

The correlation between PO_4 and COD was not a problem in this study because virtually all COD values were falling within 'high' or 'good' water quality classes 1A (<3 $\text{mg O}_2 \text{L}^{-1}$) or 1B (<5 $\text{mg O}_2 \text{L}^{-1}$), i.e. not polluted by organic matter.

The lack of strong relationship between dissolved oxygen and species composition stemmed mostly from the inability of dissolved oxygen saturation to discriminate the origin of the groundwater. Low dissolved oxygen concentrations were either indicating seepage from the canalised Rhine River (e.g. low EpCO_2 , low NO_3 , high PO_4 and NH_4 concentrations) or upwelling of the deep aquifer of the central Alsacian floodplain (high EpCO_2 , high NO_3 , low PO_4 and NH_4 concentrations). The influence of in-stream metabolic activity (respiration and photosynthesis) on the partial pressure of O_2 and CO_2 was small compared to the groundwater influence, despite an expected high rate of photosynthesis in a site choked with vegetation (Massabuau and Fritz 1984). In this study pCO_2 and O_2 were annual averages (based on monthly data) and this has also alleviated the effect of the plant on the water chemistry.

4.2. Potential confounding factors

4.2.1. Physical variables

The photosynthetic rate is also known to be dependent on the interactions between CO_2 , temperature and light availability

(Blackmann and Smith 1911; Steemann Nielsen, 1947; Maberly, 1985). Light was identified as the main predictor of macrophyte standing crops and daily maximum primary production rates in Florida spring fed streams (Canfield and Hoyer, 1988; Duarte and Canfield, 1990).

The effect of $p\text{CO}_2$, sediment fertility and $p\text{CO}_2 \times$ sediment fertility has been shown to affect yield, N and P accumulation in plant tissue of *Vallisneria americana* Michx. (Titus and Andorfer, 1996). The relative growth rate of several species growing at low pH responded differently to inorganic carbon enrichment and sediment type and this has been attributed to the carbon source (Pagano and Titus, 2004, 2007). So sediment fertility may be an important factor.

However, species growing in rivers have generally not shown strong preferences for sediment inorganic N and P (Clarke and Wharton, 2001) except for *Chara hispida* L. and *P. coloratus* growing in more organic sediments (Schneider and Melzer 2004). Sediment P in the main area investigated was related to stream water PO_4 (Trémolières, unpublished) as generally found along such a strong PO_4 gradient (e.g. Demars and Harper, 2002, 2005b); and nutrient uptake from the sediment by rooted macrophytes depends on water column nutrient concentrations and the species eco-physiological abilities (cf. Robach et al., 1995; Pelton et al., 1998; versus Chambers et al., 1989; Xie et al., 2005). Free floating macrophytes may also access sediment P indirectly either via rooted plant P translocation (e.g. DeMarte and Hartman, 1974) or via the dynamic equilibrium of P concentration between the sediment and the water column (e.g. Jarvie et al., 2005).

A recent study of the streams of the right bank floodplain of the Rhine showed that physical factors (shade, depth, water velocity, sediment, turbidity) were significantly explaining about 10% of the variance in species composition (Trempe, 2007), a similar finding to the calcareous rivers of eastern England (Demars and Harper, 2005a). Stream hydrology of lowland Danish rivers, however, did not explain plant community composition (Riis et al., 2008). Water current velocity also had a very limited effect on tissue P concentration of vascular plants (Baldy et al., 2007). Hence, the role of physical factors at our scale of study should not compromise our results.

4.2.2. Spatial structure

This study was designed to avoid as much as possible the effect of spatial autocorrelation due to plant dispersal or chemical dispersion, hence the lack of spatial effects at the scale of observation. By reducing the sampling interval and focusing on individual streams, or more simple river networks, the spatial structure becomes an important driver of plant composition (Demars and Harper, 2005a; Trempe, 2007). The size of the sampling area varied slightly between sites but this is unlikely to have introduced a strong bias (Trempe, 2007). The sampling area was also sufficiently large to avoid strong effects of asexual reproduction at the plant patch scale ($\sim 10^2$ m). It is likely that some of the unexplained variability in plant distribution may come from that scale effect.

The significance of easting (X) in the CCA analyses may be related to the four broad hydrological zones from east to west: the active floodplain of the Ill (Trémolières et al., 1994), the central Ried fed only by deep upwelling groundwater (Carbiener et al., 1990), the zone affected by polluted seepage from the Rhine water (Eglin et al., 1997) and the stream (Schaftheu) on Rhinau Island fed directly by the Rhine water (Eglin and Robach, 1992). The weakness of that effect may be due to the high spatial heterogeneity at finer scale due to point source pollution (e.g. treated effluent from towns and fish farms) or deep groundwater upwelling.

4.2.3. Biotic interactions

Species plant palatability (i.e. grazing) was found to be unrelated to nutrient enrichment in undisturbed aquatic ecosys-

tems such as groundwater fed rivers (Elger et al., 2002, 2004). Competition between plant species may not be very strong, even in groundwater fed streams due to alternative species life history characteristics (Greulich and Bornette, 2003). Competition with epiphytes is unlikely to explain plant distribution in relatively stable systems, as indicated by laboratory experiments that showed no effect of N, P enrichment on epiphyte biomass per unit of surface area in three elodeid species (James et al., 2006). Invertebrate grazing during the plant regenerative phase may also influence the ultimate structure of macrophyte communities (Elger et al., 2009). Direct nutrient effects may not be observed due to complex interactions between nutrient fluxes, epiphytes, grazers (macrophytes and/or epiphytic biofilm) and top predators (Jones and Sayer 2003).

4.3. Plant indices

The species approach adopted in this study has allowed the calculation of plant indices in units directly comparable to the observed variables. This is an improvement on previous indices where the resulting scores were not directly comparable to observed units (e.g. Kohler and Schneider, 2003; Dodkins et al., 2005). Furthermore, the EpCO_2 plant index was directly supported by biological mechanisms. While all the indices were well calibrated ($r^2 = 0.50\text{--}0.73$), the PO_4 and NH_4 plant indices performed much less well against independent data ($R^2 = 0.27$, $r^2 = 0.24$, respectively) than the CO_2 plant index ($r^2 = 0.47$). The drop of performance of the indices may be partly due to the lack of common 'indicator' species between the different areas, i.e. some species recorded in the independent datasets were not part of the training set from the River Rhine floodplain. Better predictions may also be achieved if the submerged and floating/emergent growth-forms of the same species were recorded separately.

The univariate approach adopted with the GLM took no account of the correlations or interactions between different variables. Therefore, it is important to present the results of several indices together, rather than in isolation to avoid potential misinterpretation (Demars and Edwards, 2009).

5. Conclusions

While plant species distribution was more strongly related to CO_2 than inorganic N and P, the three factors were partially confounded and interacted all together with temperature. Hence, CO_2 is one important factor, but not the whole story. The plant- CO_2 relationship was supported by a biological mechanism (the use of HCO_3^- under low EpCO_2) and a better predictive power of the CO_2 plant index than NH_4 or PO_4 plant indices.

The present field study should stimulate further work such as screening eco-physiological traits of aquatic vascular plants under standard conditions, and testing the role of $p\text{CO}_2$, N and P on plant growth rate and regeneration rate under controlled conditions (light, velocity). Further work on the role of biotic interactions should also increase our predictive abilities.

Acknowledgments

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Appendix A. Species occurrence, indicator values, and traits used for the statistical analyses

Species	Occurrence %	EpCO2 x atm	PO ₄ ^a µg L ⁻¹	NH ₄ ^a µg L ⁻¹	NO ₃ ^a mg L ⁻¹	HCO ₃ ^b user	Strictly submerged	NRA ^c	Species P tissue mg g ⁻¹ DW
<i>Azolla filiculoides</i> Lam.	12		86	973	0.190		0		
<i>Berula erecta</i> (Huds) Coville	69	29	6	21	0.190	0	0	3.9	2.5
<i>Butomus umbellatus</i> L.	4	4	133	616	1.547		0		
<i>Callitriche obtusangula</i> Le Gall	70	17	30	22			0	11.7	5.1
<i>Ceratophyllum demersum</i> L.	35	3	90	213	1.371	1	1		6.2
<i>Chara vulgaris</i> L.	4	39	13	35	0.190	1	1		
<i>Elodea canadensis</i> Michx.	26	14	25	10		1	1	0.5	5.8
<i>Elodea callitrichoides</i> (Rich.) Casp.	19	3	135	973	1.094		1		
<i>Elodea nuttallii</i> (Planch.) H. St. John.	38	5	130	147	0.190	1	1		7.5
<i>Enteromorpha intestinalis</i> (L.) Link	7	3	126	973	1.004		1		
<i>Fontinalis antipyretica</i> Hedw.	18	3	23	21		0	0	0.2	
<i>Groenlandia densa</i> (L.) Fourr.	9	7	32	35	1.113	1	1	0.3	
<i>Hydrodictyon reticulatum</i> (L.) Bory	8	3	343		1.193	1	1		
<i>Lamprocystis roseopersicina</i> (Kützing) Schroeter	4	30	8				1		
<i>Lemna minor</i> L./ <i>minuta</i> Kunth	53	3	133	973	0.253	1	0		
<i>Lemna trisulca</i> L.	27	15	21	14	0.409	1	1	0.3	
<i>Mentha aquatica</i> L.	15		13	9	0.190	0	0	3.8	
<i>Myosotis scorpioides</i> L.	18					0	0		
<i>Myriophyllum spicatum</i> L.	36	3	129	338	1.541	1	1		4.6
<i>Myriophyllum verticillatum</i> L.	5	13	8	12	2.412	0	1	0.2	3.1
<i>Nuphar lutea</i> (L.) Sm.	9	16	18			0	0		
<i>Potamogeton coloratus</i> Hornem.	9	34	6	10	7.035	0/1	0	30.3	2.1
<i>Potamogeton crispus</i> L.	18	9	85	7	0.190	1	1	6.5	6.0
<i>Potamogeton friesii</i> Rupr.	18	11	102	80	0.190	1	1		
<i>Potamogeton pectinatus</i> L.	32	3	93	116	1.270	1	1		3.1
<i>Potamogeton perfoliatus</i> L.	12	3	98		1.208	1	1		6.0
<i>Potamogeton pusillus</i> L.	4		22	57	0.190	1	1		
<i>Potamogeton</i> × <i>fluitans</i> Roth.	12	8	397	208	3.102		0		
<i>Ranunculus fluitans</i> Lam.	11	4	117	122	1.938		1	0.7	14.2
<i>Rorippa nasturtium-aquaticum</i> (L.) Hayek	35	26	27	3	9.000	0	0		
<i>Sparganium emersum</i> Rehmman	23	8	397		3.624	0	0	0.2	
<i>Spirodela polyrhiza</i> (L.) Schleid.	18	3	397	973	0.493		0		
<i>Spirogyra</i> Link	4	30	8			1	1		
<i>Veronica anagallis-aquatica</i> L.	20	17	20		0.190	0	0	1.2	
<i>Veronica beccabunga</i> L.	8	34	66	973	0.419		0	0.9	
<i>Zannichellia palustris</i> L.	11	10	27	23	0.190	1	1	14.0	

Values in italics represent optimums reassigned to the minimum or maximum of the observed range.

^a backtransformed optimum values.

^b under high alkalinity (2–4 meq L⁻¹) and low pCO₂ (near air equilibrium concentration).

^c NO₂ µmol.h⁻¹.g⁻¹ DW.

Appendix B. Parameters to calculate the plant indices

Species	EpCO ₂		a	u ^a	SRP			NH ₄	
	u	t			t ^a	a	u ^a	t ^a	a
<i>Azolla filiculoides</i> Lam.				1.94	0.27	1.0	2.99	1.00	1.2
<i>Berula erecta</i> (Huds) Coville	29	17	2.7	0.85	0.63	2.9	1.34	0.47	2.8
<i>Butomus umbellatus</i> L.	4	0	0.8	2.13	0.15	0.8	2.79	0.15	2.4
<i>Callitriche obtusangula</i> Le Gall	17	11	2.4	1.49	0.64	2.1	1.37	0.93	2.0
<i>Ceratophyllum demersum</i> L.	3	10	2.7	1.96	0.39	2.0	2.33	0.56	1.5
<i>Chara vulgaris</i> L.	39	8	0.4	1.15	0.24	0.2	1.56	0.35	0.1
<i>Elodea canadensis</i> Michx.	14	6	1.1	1.41	0.43	0.7	1.04	1.10	0.6
<i>Elodea callitrichoides</i> (Rich.) Casp.	3	11	1.5	2.13	0.44	1.3	2.99	1.00	2.6
<i>Elodea nuttallii</i> (Planch.) H. St. John.	5	11	1.4	2.12	0.81	1.4	2.17	0.92	1.2
<i>Enteromorpha intestinalis</i> (L.) Link	3	11	0.5	2.10	0.40	0.4	2.99	1.00	2.2
<i>Fontinalis antipyretica</i> Hedw.	3	33	0.3	1.39	0.35	0.4	1.34	0.42	0.4
<i>Groenlandia densa</i> (L.) Fourr.	7	1	1.5	1.52	0.34	0.4	1.56	0.22	0.5
<i>Hydrodictyon reticulatum</i> (L.) Bory	3	15	0.5	2.54	0.78	0.4			
<i>Lamprocystis roseopersicina</i> (Kützing) Schroeter	30	7	0.2	0.94	0.32	0.1			
<i>Lemna minor</i> L./ <i>minuta</i> Kunth	3	21	1.9	2.13	0.67	2.2	2.99	1.16	2.6
<i>Lemna trisulca</i> L.	15	6	1.3	1.34	0.27	1.0	1.17	0.57	0.9
<i>Mentha aquatica</i> L.				1.14	0.22	0.6	1.00	0.33	0.8
<i>Myosotis scorpioides</i> L.									
<i>Myriophyllum spicatum</i> L.	3	7	1.8	2.11	0.50	1.7	2.53	0.63	1.6
<i>Myriophyllum verticillatum</i> L.	13	4	0.3	0.95	0.38	0.2	1.13	0.23	0.4
<i>Nuphar lutea</i> (L.) Sm.	16	4	0.6	1.29	0.22	0.4			
<i>Potamogeton coloratus</i> Hornem.	34	5	2.0	0.84	0.10	3.0	1.06	0.29	1.0
<i>Potamogeton crispus</i> L.	9	5	0.6	1.93	0.55	0.5	0.90	1.49	0.4

(continued on next page)

Appendix B (continued)

Species	EpCO ₂				SRP			NH ₄	
	<i>u</i>	<i>t</i>	<i>a</i>	<i>u</i> ^a	<i>t</i> ^a	<i>a</i>	<i>u</i> ^a	<i>t</i> ^a	<i>a</i>
<i>Potamogeton pectinatus</i> L.	3	5	2.8	1.97	0.44	2.0	2.07	0.49	1.5
<i>Potamogeton perfoliatus</i> L.	3	6	0.7	1.99	0.32	1.0			
<i>Potamogeton pusillus</i> L.				1.35	0.18	0.2	1.76	0.29	0.1
<i>Potamogeton</i> × <i>fluitans</i> Roth.	8	3	0.8	2.60	0.56	2.4	2.32	0.16	2.0
<i>Ranunculus fluitans</i> Lam.	4	4	0.9	2.07	0.50	0.8	2.09	0.38	0.7
<i>Rorippa nasturtium-aquaticum</i> (L.) Hayek	26	18	0.8	1.44	0.53	0.8	0.63	1.00	1.6
<i>Sparganium emersum</i> Rehmman	8	6	0.8	2.6	1.00	1.8			
<i>Spirodela polyrhiza</i> (L.) Schleid.	3	11	1.0	2.60	0.83	0.9	2.99	1.48	1.9
<i>Spirogyra</i> Link	30	7	0.4	0.94	0.32	0.3			
<i>Veronica anagallis-aquatica</i> L.	17	26	0.3	1.33	0.59	0.4			
<i>Veronica beccabunga</i> L.	34	48	0.1	1.82	0.43	0.2	2.99	1.00	0.1
<i>Zannichellia palustris</i> L.	10	7	0.3	1.45	0.22	0.4	1.38	0.55	0.3

Blank cells indicate that the species was simply not a bioindicator.

^a $\log(x + 1)$ transformed; with *u* species optimum or indicator value, *t* species tolerance, *a* species maximum expected abundance.

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