Understanding the realised niche of an amphibious softwater plant, *Eleocharis multicaulis*

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With 4 figures and 3 tables

Abstract: The West European amphibious plant *Eleocharis multicaulis*, characteristic of softwater lakes, is rare and endangered in many regions. The present study aimed to evaluate several presumed niche features suggested in syntaxonomic studies, to reveal other important niche variables affecting the cover of this species and to quantify these responses. A dataset of 724 environmental and vegetation variables was built from a survey of 59 plots in The Netherlands and Belgium. Discriminant analysis, Pearson goodness-of-fit calculations, weighted averages and presence profiles were used. We found that *Eleocharis multicaulis* performs best in base-poor environments with an organic top layer and an established vegetation. The species is apparently N limited and profits from N input as long as NO_3^- is dominant over NH_4^+. A high atmospheric N deposition is detrimental, because the resulting strong acidification leads to dominance of NH_4^+ over NO_3^-. Intense acidification in agricultural areas has probably been an important extinction factor in the recent past. We hypothesise that current variability of soil Si, a very good predictor for the species' performance, might reflect these acidification processes. This hypothesis is supported by the fact that Si is correlated with trophic status, which is likely higher in agricultural regions. Nowadays, *Eleocharis multicaulis* suffers from agricultural P, K and alkalinity inputs as well as reduced oxygen supply (e. g. by reduced water-level dynamics), all leading to a lower redox state. These factors could bring about competitive suppression by other species, a high NH_4^+/NO_3^- ratio and P mobilisation.

Key words: Acidification, ammonium, *Eleocharis multicaulis*, eutrophication, niche, nitrification, nitrogen limitation, silicon, soft water.

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Table 1. Literature-derived averages of environmental variables, with respect to the presence of *Eleocharis multicaulis*. Cover-weighted averages (WA) from the current study are presented for summer and winter. For the formula of WA, see Material and methods. Soil data of the present study apply to the mineral soil layer. The WA of de Lyon & Roelofs (1986) is weighted by presence of *Eleocharis multicaulis*. + = positive effect on *Eleocharis multicaulis*, – = negative effect, ^ = optimum at intermediate values; / = not tested. The number of signs is a measure for the indication value, calculated with a modified chi-square statistic.

A. Chemical conditions	Pietsch 1978	de Lyon & Roelofs 1986		This study	
Study area	Central Europe	The Netherlands		The Netherlands & Belgium	
Sites with *Eleocharis multicaulis* total number of sites	109/109	18/ca. 600		41/59	
Variable	Mean	WA	Indication value	Summer WA	Winter WA
Water					
Alkalinity (μeq L^{-1})	510	200	–––	58.6	78.7
NH_4^+ (μmol L^{-1})	52	33.4		12.5	13.0
HCO_3^- (μmol L^{-1})	510			53.9	71.2
HCO_3^- proportion	0.18		–––	0.04	0.05
Ca^{2+} (μmol L^{-1})	963	130	–––	76.5	93.1
CO_2 (μmol L^{-1})	166	high	/	52.2	79.6
Cl^- (μmol L^{-1})	513	420	–––	251.6	336.1
Cl^- proportion	0.26		/	0.43	0.54
Fe (μmol L^{-1})	11.5	11.3	^	10.7	4.3
Mg^{2+} (μmol L^{-1})	274	80	–––	28.0	36.2
Mn (μmol L^{-1})	3.5	1.3	––	0.73	0.51
NO_3^- (μmol L^{-1})	66.9	2.9	–	56.8	27.5
PO_4^{3-} (μmol L^{-1})	7.8	2.6	+	0.2	0.1
pH	6.3	4.5	––	5.0	5.0
K^+ (μmol L^{-1})		65	–	52.2	35.6
Salinity (μeq L^{-1})		1600	–––	1024	1106
Si (μmol L^{-1})	108			6.2	29.2
Na^+ (μmol L^{-1})		260	–––	184.7	236.9
SO_4^{2-} (μmol L^{-1})	967	200	–––	176.0	125.8
SO_4^{2-} proportion	0.56		/	0.52	0.39
Soil					
Ca digestion (μmol kg DW^{-1})		16000	––	3923	3497
Fe digestion (μmol kg DW^{-1})		38000	––	18626	15820
Loss on ignition (LOI) (%)		25	+	2.8	3.0
Mg digestion (μmol kg DW^{-1})		9000	–––	6494	6133
Mn digestion (μmol kg DW^{-1})		1000	–––	95.6	85.7
Total N content (mmol kg DW^{-1})		127	^	65	125
P digestion (μmol kg DW^{-1})		4200	––	1455	1589
K digestion (μmol kg DW^{-1})		12000	––	6028	6671

Introduction

Because of their international decline since the 20th century, several plant species of the syntaxonomic class *Littorelletea* have been studied for their relation with environmental processes. Especially the macrophytes *Littorella uniflora, Lobelia dortmanna, Isoetes* spp., *Juncus bulbosus* and *Sphagnum* spp. have been considered, resulting in an extensive literature record, e.g. Schuurkes et al. (1986), Farmer & Spence (1987), Rørslett & Brettum (1989), Paffen & Roelofs (1991), van Dam & Buskens (1993), Szmeja (1994), Lucassen et al. (1999), Smolders et al. (2002) and Arts (2002).

An important plant community of the *Littorelletea*, the *Eleocharition multicaulis* (Vanden Berghen 1969) alliance, has not been subject to focused research programs as yet. It can be found higher up sandy lake shores and is characterised by *Eleocharis multicaulis, Hypericum elodes* and *Scirpus fluitans*. In the present study, we consider *Eleocharis multicaulis* (Many-stalked Spike-rush), an uncommon perennial with an Atlantic distribution area from North-West Africa to North-West and Central Europe. Several authors have commented on its rarity and potentially or actually endangered state (Schoof-van Pelt 1973, Dierssen 1975, Pietsch 1978, Dierssen 1981, Kaplan 1993, Weeda et al. 2000).

Table 1 gives an overview of environmental characteristics of *Eleocharis multicaulis*, found previously by Pietsch (1978) and de Lyon & Roelofs (1986). The work by de Lyon & Roelofs (1986) in The Netherlands is the only autecological niche study we have encountered. Dierssen (1975) and Pietsch (1977, 1978) stressed the broad tolerance of the plant towards pH (4.3 to 8.1), total salt concentration, Ca^{2+} and SO_4^{2-} and various other variables. More recent accounts (mostly phytosociological) make specific statements: preference for an acid situation rich in NH_4^+ and CO_2 (Pietsch 1985, de Lyon & Roelofs 1986, Arts et al. 2002), nutrient-poor water and soil (most

Table 1. Continued.

B. Granulometry (ln %)

de Lyon & Roelofs 1986			This study	
Class (μm)	WA	Indication value	Class (μm)	WA
<2	2	––	<2	2.4
2–50	13	–	2–16	10.1
50–200	51		16–63	10.3
200–2000	31	/	63–125	11.1
			125–250	33.8
			250–500	28.5
			500–1000	2.6
			>1000	0.0

authors) and a mineral substrate devoid of organic material (sapropelium) (Schaminée et al. 1992, 1995).

In Western Europe, it has been suggested that the plant can sustain higher intensities of acidification than other characteristic species (Kaplan 1992, 1993, 1998, Weeda et al. 2000). Where the *Eleocharition multicaulis* alliance disappeared, *Eleocharis multicaulis* often persisted the longest (Schoof-van Pelt 1973, Weeda et al. 2000), which leads to the hypothesis that it might be a competitive dominant under these conditions.

It can be assumed that still other environmental variables are influential to the performance of *Eleocharis multicaulis*. In particular, ratios of ion concentrations (ionic ratios) could be meaningful. Some authors used ionic ratios for chemical classification of ecosystems, without reference to plants. Several others used them in respect to the requirements of plants. In the latter case, ratios are often chosen to reflect the intensity of ecologically relevant biogeochemical processes, such as acidification. Examples include Al/Ca, NH_4^+/NO_3^-, S/(Ca + Mg), (Ca + Mg)/(Na + K), Ca/(Ca + Cl) and N/P (Bloemendaal & Roelofs 1988, van Wirdum 1991, de Graaf et al. 1998, Lamers et al. 2001, Lucassen et al. 2002). Macro-ionic concentrations, available from standard chemical analyses, might be important as well.

The hypotheses we wanted to test in this study are: 1. that the species is confined to an acid situation rich in NH_4^+ and CO_2, nutrient-poor water and soil and a mineral substrate devoid of organic material, 2. that the species is a competitive dominant under certain conditions, and 3. that other factors, such as ionic ratios, influence the performance of this species.

To achieve our aims, we conducted a field survey in The Netherlands and Belgium (Ruysschaert 2002). To reveal potentially important variables for the performance of the species, we used discriminant analysis and univariate screening methods. The response to these variables was quantified using presence profiles, percentiles and cover-weighted averages.

Material and methods

Study design

Fifty-nine plots of 2 m^2 each were sampled in 33 softwater lakes of Pleistocene, sandy areas in Belgium and The Netherlands (Fig. 1), in which *Eleocharis multicaulis* was either present or absent, but potentially present based on the presence of other characteristic species of the *Eleocharition* alliance. Some lake properties are listed in Table 2. One to three plots were selected per lake. In this way, the plots were not completely independent. We assumed we could ignore this effect, because plots within one lake were chosen on the basis of obvious ecological differences. The response variable of interest was the cover of *Eleocharis multicaulis*, which was split up into three ecologi-

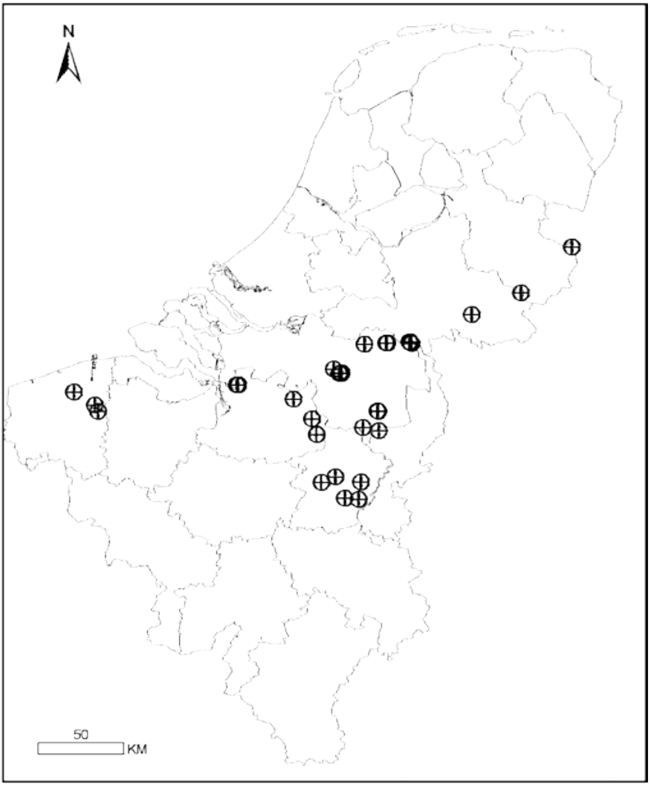


Fig. 1. Map of sampling locations (Belgium and The Netherlands).

Table 2. Some characteristics of the sampled lakes. IQR = interquartile range (75 th minus 25 th percentile).

	Minimum	Median	Maximum	IQR/range
Surface class	<0.1 a	0.5–1 ha	>10 ha	
pH	4.1	5.0	7.6	0.53
Alkalinity (μeq L^{-1})	−81	29	1508	0.23
Electric conductivity EC_{25} (μS cm^{-1})	24	79	455	0.18
Median granule size of mineral layer (μm)	22	167	254	0.48

cally relevant classes: absent (cover = 0; 18 plots), low cover (0 < cover ≤ 10 %; 26 plots) and high cover (cover > 10 %; 15 plots). For each plot, 35 vegetation variables were measured (including cover of the plant species occurring in at least five plots), as well as 24 physical variables regarding lake, plot, water layer and soil, 27 chemical variables of the surface water, and 31 chemical variables of both mineral (sandy) soil and organic top layer, the latter only if present. 79 derived chemical variables were constructed from equivalent concentrations, mostly ionic ratios. A list of all measured and derived variables can be obtained from the corresponding author. Most chemical variables were sampled once in summer (2001) and once in winter (2002). The difference between winter and summer was a measure of seasonality. Winter nutrient values were an estimate of availability for the next growing season. The full dataset comprised 724 compound predictor variables (variable × season).

Chemical sampling and analyses

In each plot, a surface water sample was taken, part of which was used for the determination of pH (WTW Sentix electrode), electric conductivity (WTW Tetracon 325 electrode), redox potential (WTW Sentix ORP electrode) and alkalinity (titration with sulphuric acid down to pH 4.2, using bromcresol green-methyl red indicator, by means of a Hach Digital Titrator, model 16900). The rest of the sample was filtered through a Whatman GF/C filter (pore diameter 0.45 μm). A subsample was prepared for inorganic carbon analysis through injection in a Venoject Glass Vacuum Tube, stored directly at 2–6 °C. The remaining part of the filtered water was divided over two iodated 50 mL polyethylene tubes, one with 1 % of 65 % HNO_3 solution, the other with 0.8 % of 31.24 g L^{-1} citric acid solution, and immediately frozen below –10 °C using a transportable freezer.

Sediment samples up to 0.5 L were taken from the upper 20 cm of soil, separating mineral layer and organic top layer (threshold at 10 % organic matter content). The samples were immediately stored air-tight at 2–6 °C. One part (approximately 50 g) was oven-dried at 65 °C for 24 h to determine moisture content. About 10 mg of dried sediment was burnt with a Flash EA 1112 NC Soil analyser (ThermoQuest, Interscience), to determine total C and N content. Granulometric composition of fresh sediment, between 0.4 μm and 2000 μm, was measured volumetrically with a Malvern Mastersizer (S Long Bench) by means of laser diffraction.

Digestates of dry sediment were prepared according to Smolders et al. (2002), water- and NaCl-extracts of fresh sediments according to Brouwer & Roelofs (2002) and ammonium lactate extracts according to Egnér et al. (1960). They were stored in a iodated polyethylene tube at –20 °C. Water- and NaCl-extracts were acidified with citric acid (like the water samples). Lucassen et al. (2004) was followed for determination of organic matter content (loss on ignition) of the sediment and inorganic carbon analysis of water samples. Macro-ionic concentrations of all samples and derivates were measured according to Lamers et al. (1998).

Statistical analyses

Different types of analysis can be expected to provide different results, which complement each other. Therefore, we carried out three sets of analyses. To reveal previously unknown major predictor variables, these analyses were done with the maximum available number of variables, though each time a different subset of the data was used because of missing values. None of the analyses had specific distributional requirements.

First, discriminant analysis (DA) (Manly 1994) was performed on a data-subset of 30 plots and 392 predictor variables. For reliable application of DA (Williams & Titus 1988), variables were (further) discarded in two steps. First, all predictor variables were categorised into three adjacent classes of equal frequency. The association between the predictor variables and the response variable was then evaluated with Pearson chi-square calculations by means of 3×3 contingency tables. A *p*-level of 0.05 was used as a threshold to select a preliminary subset of response-related variables. Secondly, stepwise DA selected those variables (continuous scale) from this subset that, acting together, most successfully separated the three response classes of *Eleocharis multicaulis*. With three response classes, DA yields two discriminant functions. As 46 plots had no missing values for the selected variables, DA scores were obtained for 46 plots. Homogeneity of variances among groups was subjectively evaluated on the basis of the ordination diagram, as proposed by Quinn & Keough (2002).

Next, Spearman correlations were calculated between the variables and the first discriminant function. This univariate screening was done for the 46 plots and all variables. The third analysis consisted of Pearson chi-square calculations like the ones above for the total dataset [see also de Lyon & Roelofs (1986) and Bloemendaal & Roelofs (1988)]. Variables that yielded *p*-values < 0.1 in either of these univariate screening analyses were considered as relevant. *P*-levels in these procedures are merely criteria to delimit a subset of variables. They have no value for multiple statistical inference, since global type-I error is not under control. Care must be taken in presuming causal effects of these variables on the response because of multicollinearity (Mac Nally 2000, Graham 2003). Therefore, intercorrelations were investigated using non-metric multidimensional scaling (MDS), complemented by cluster analysis with Ward's method. For both multivariate analyses, $1-r^2$ was used as a distance measure between two variables. Analyses were done with the statistical package SPSS 11.0 for Windows (SPSS Inc. 2001).

Presence profiles were constructed for the most influential predictor variables, in order to directly visualise the presence of *Eleocharis multicaulis* (between 0 and 1). For each predictor variable, presence was calculated for each of five adjacent classes of equal frequency, e.g. for the five classes of water pH. The presence was calculated as the ratio of the number of plots in which *Eleocharis multicaulis* was present to the total number of plots belonging to the predictor variable class. Weighted averages of predictor variables (*WA*) were calculated in a similar way as by de Lyon & Roelofs (1986), in order to make comparisons. The medians of five adjacent classes of equal frequency were calculated for each predictor variable, in order to eliminate outliers (M_1 to M_5). Sometimes only two or three classes were distinguished because of insufficient different values. For each class, the mean cover was calculated arithmetically, including absence data (C_1 to C_5). We then used the following formula to obtain the weighted average:

$$WA = \Sigma\, (M_i \cdot C_i)/\Sigma\, C_i$$

Results

Discriminant analysis

Discriminant analysis selected five variables which, acting together, separated the three response groups (absent/low cover/high cover) of *Eleocharis multi-*

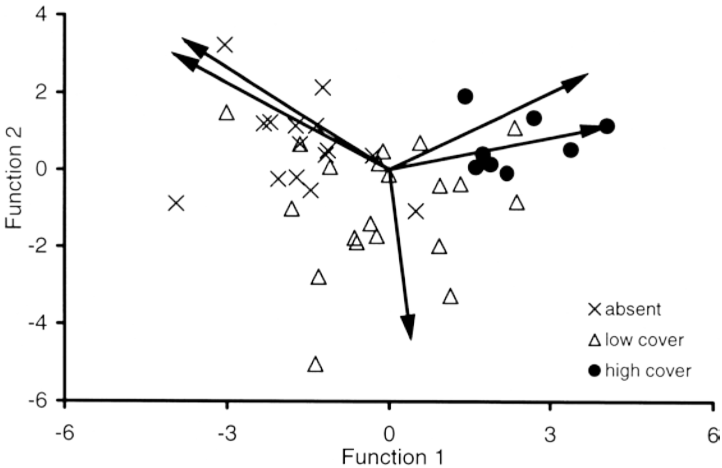


Fig. 2. The two discriminant functions separate the response classes of *Eleocharis multicaulis*. Vectors represent the standardised discriminant function coefficients of the transformed original variables. They have been scaled with a fixed factor to fit properly in the graph.

caulis (Fig. 2). There was no significant intercorrelation among these variables ($p > 0.1$) and group variances were of comparable magnitude (visual evaluation of the ordination diagram). The first discriminant function represented a gradient from absent to high cover, while the second separated the low cover group. The first discriminant function explained 76% of between-group variance (eigenvalue 2.705); the second 24% (eigenvalue 0.852). Three abiotic variables were important to the first discriminant function: NH_4^+/NO_3^- ratio of the water in summer (–), seasonality of the N/K ratio of the mineral layer (NaCl-extraction) (+) and seasonality of divalent/monovalent cation ratio of the water (+) (DMR = (Ca + Mg)/(Na + K)), with the +/– signs indicating the response direction of *Eleocharis multicaulis*. Seasonality of N/K had a strong positive correlation with its winter values ($r = 0.93$; $p < 0.001$), while the seasonality of DMR was negatively related with its summer values ($r = -0.37$, $p = 0.006$). The remaining two variables were the cover of the neighbouring species *Hydrocotyle vulgaris* and *Mentha aquatica* (–). The cover of *Hydrocotyle vulgaris* was related to the low cover response of *Eleocharis multicaulis*.

Univariate screening

Table 3 shows a selection of significant variables ($p < 0.1$ in at least one test). A full list of results is available from the corresponding author. A first cluster of interrelated variables contained redox potential of the surface water (+), both Si measurements of the mineral soil in winter [digestion (+) and NaCl-extraction (–)] and the NH_4^+/NO_3^- ratio of the water in summer (–) (Table 3).

Table 3. The response of *Eleocharis multicaulis* to some significant variables of univariate screening tests ($p < 0.1$), involving Pearson chi-square tests as well as Spearman correlations with the first discriminant function. Variables are grouped according to intercorrelation structure. Weighted averages are presented, as well as the 10 th and 90 th percentiles for the plots where *Eleocharis multicaulis* was present (range). MIN = mineral soil layer; ORG = organic top soil layer; S = summer; W = winter; D = winter minus summer (seasonality); S/W = significant effect in summer and winter, with summer values shown in table; + = positive effect on *Eleocharis multicaulis*, – = negative effect; WA = weighted average (weighted by mean cover, see Materials and Methods).

Compartment	Season	Variable	Response	WA	Range
Vegetation					
	S	Cover Drosera intermedia (%)	+		0–3.6
	S	Cover Molinia caerulea (%)	+		0–12
	S	Cover Juncus bulbosus (%)	+		0–20
	S	Cover Agrostis canina (%)	–		0–8.8
	S	Cover Juncus effusus (%)	–		0–4
	S	Cover Hypericum elodes (%)	–		0–18.4
	S	Cover Galium palustre (%)	–		0–0.8
	S	Cover Lysimachia vulgaris (%)	–		0–1.8
	S	Cover Mentha aquatica (%)	–		0–0
	S	Total plant cover (%)	+	75.9	32–100
Variables correlated with redox potential of surface water (winter)					
Water	W	Redox potential (mV)	+	265.3	178.2–315.4
ORG	S	Presence of organic top layer	+		0–1
MIN	S/W	Si digestion (μmol kg DW^{-1})	+	27241	2887–50216
Water	S	NH_4^+/NO_3^-	–	0.66	0.07–2.81
MIN	W	Si NaCl-extraction (μmol kg DW^{-1})	–	523.2	18.1–4278.7
Acidity & base richness					
Water	S/W	Alkalinity (μeq L^{-1})	–	58.6	–66.8–410
Water	S/W	pH	–	5.0	4.2–7.5
Water	S/W	Ion Ratio (IR) = Ca/(Ca+Cl)	–	26.4	12.2–64.9
Water	S/W	Divalent/monovalent cation ratio (DMR)	–	0.75	0.21–2.27
Winter replenishment of base cations in the soil: moderate correlation with acidity complex					
MIN	D	Mg digestion (μmol kg DW^{-1})	+	–1130.43	–6550.65–1620.55
MIN	D	K digestion (μmol kg DW^{-1})	+	1082.07	–2706.67–4402.52
ORG	D	Ca digestion (μmol kg DW^{-1})	+	–9133.73	–53162.41–15950.38
Variables correlated with NH_4^+/NO_3^- ratio of surface water (summer)					
ORG	S	Cover of organic layer (%)	+	85.4	99.6–100
Water	S	NO_3^- (μmol L^{-1})	+	56.8	4.6–220.1
Water	S	NO_3^-/PO_4^{3-}	+	536.5	25.2–2320.1
Water	S	NO_3^-/K^+	+	1.33	0.11–3.70
Nitrogen availability in mineral layer (winter)					
MIN	W	N/K (NaCl-extraction)	+	714	0–2155
MIN	W	Total N content (mmol kg DW^{-1})	+	125	0–186
MIN	W	N/P (NaCl-extraction)	+	17844	0–28930
Phosphorus availability in mineral layer: correlated with silicon in mineral layer (winter) – digestion					
MIN	W	P digestion (μmol kg DW^{-1})	+	1589	119–2881
MIN	W	P NaCl-extraction/digestion ratio	–	0.006	0.002–0.021
Organic layer nutrient richness: correlated with silicon in mineral layer (winter) – digestion					
ORG	S	S digestion (μmol kg DW^{-1})	–	69088	12113–174221
ORG	W	Total N content (mmol kg DW^{-1})	–	482	131–1146
ORG	W	Loss on ignition (LOI) (%)	–	22.8	8.0–56.9
ORG	W	NO_3^- water-extraction (μmol kg DW^{-1})	–	46.4	8.2–212.8

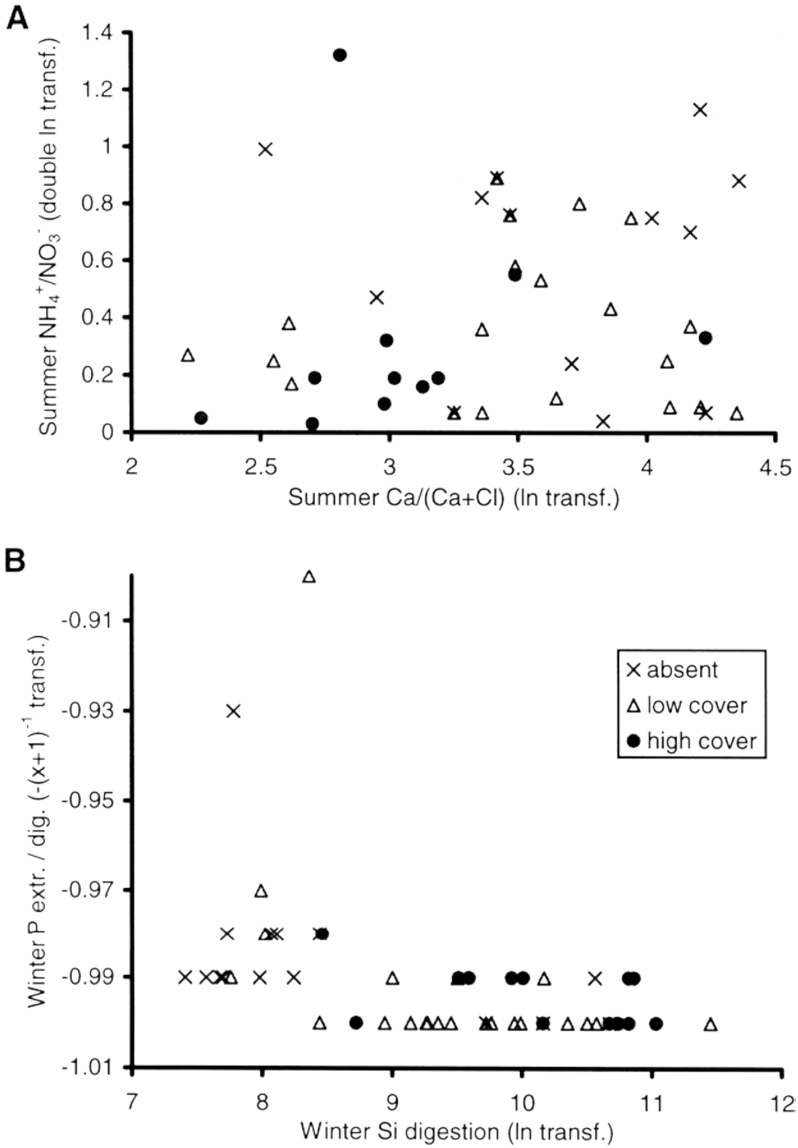


Fig. 3. Some influential variables are interrelated, others are not. A. The NH_4^+/NO_3^- concentration has no significant relationship with the ion ratio Ca/(Ca + Cl) (surface water, summer; $p = 0.124$). B. Digestion-Si and the extraction/digestion ratio of P have an inverse relationship (mineral soil, winter; $r = -0.45$, $p = 0.001$).

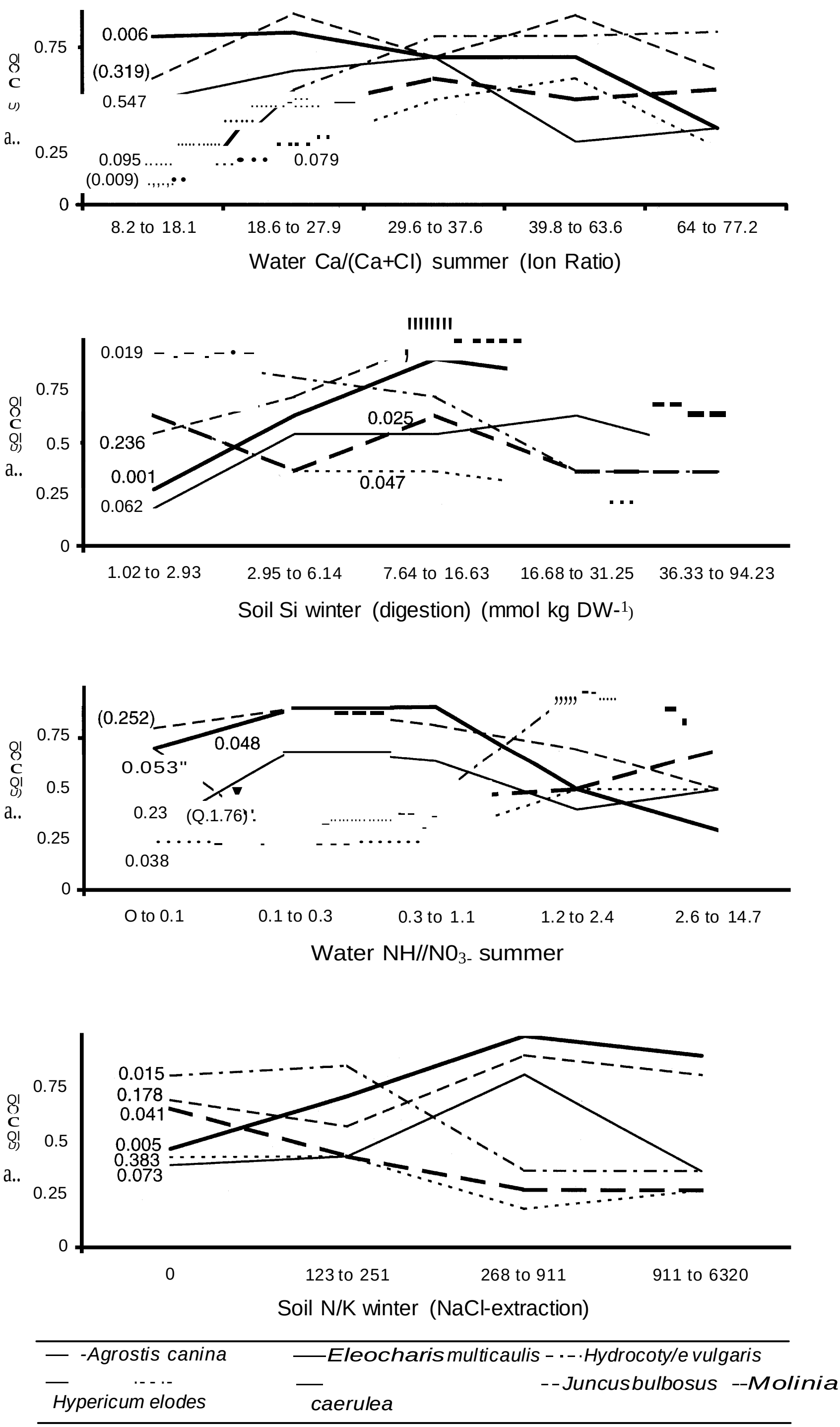
Eleocharis multicaulis performed best under the most acidic circumstances. These acidity variables were correlated with variables of the redox cluster and primarily consisted of surface water variables. Among these, the strongest univariate predictor of the species' performance was the so called 'ion ratio' (–) (IR = Ca/(Ca + Cl); Fig. 3 A). The acidity cluster had moderate correlations with base cation concentrations of the mineral or organic soil layer (–). Winter replenishment of Mg and K (mineral soil) and Ca (organic top layer) was positively related to the species' performance. Also the presence of an organic top layer was positively related with the cover of the species. Such a layer mostly occurred at lower pH values. Digestion-K content of the mineral soil was negatively correlated with latitude and longitude ($p < 0.001$), which both had a positive relation with *Eleocharis multicaulis* (not shown).

CO_2 had a slightly negative correlation with the first discriminant function in winter (not shown). It was not associated with acidity variables. NO_3^- (or N) dominance in the water, in absolute terms or relative to NH_4^+, PO_4^{3-} or K^+, had a significant positive relation with the performance of *Eleocharis multicaulis*. These relations only existed in summer. N in the mineral soil (winter), absolute and also relative to P or K, had a positive relation as well. The digestion-Si of the mineral soil in winter (+) was positively correlated with mineral soil ratios that have digestion-P in the numerator (relative to base cations), as well as with P itself (+). Especially the digestion/extraction ratio of P had a positive effect on *Eleocharis multicaulis* (Fig. 3 B). Si was negatively correlated with organic matter content (loss on ignition, LOI) (–), S concentration (–), NO_3^- and N content (–) of the organic top layer. Organic layers with LOI > 20 % generally occurred when they were thinner than 5 cm.

Eleocharis multicaulis responded positively to total plant cover (weighted average: 76 %). Positively associated species were *Drosera intermedia, Molinia caerulea* and *Juncus bulbosus*. Negatively associated species were *Juncus effusus, Agrostis canina, Hypericum elodes, Galium palustre, Lysimachia vulgaris* and *Mentha aquatica*. For *Hydrocotyle vulgaris*, no clear association was found.

Presence profiles

The species presence profiles for ion ratio (IR) and digestion-Si were concordant with the species associations (Fig. 4). The profiles for the NH_4^+/NO_3^- ratio of the surface water (summer), similar to those for extraction-Si (not shown), showed that *Eleocharis multicaulis* and positively associated species reached their optimum within the ratio interval 0.1 to 1 (where NO_3^- is dominant). *Hydrocotyle vulgaris* had a low presence in this region, while it attained a high presence at both very low (0 to 0.1) and higher (> 1) values. For the N/K ratio of the mineral layer in winter (NaCl-extraction), *Eleocharis multicaulis* and companions attained a maximum response at higher values, while the inverse pattern was true for the other species. These presence profiles were similar to those for the N/P ratio of the mineral layer in winter (NaCl-extrac-


0.006
(0.319)
0.547
0.095
(0.009)
0.079
0.75
0.25
0
8.2 to 18.1
18.6 to 27.9
29.6 to 37.6
39.8 to 63.6
64 to 77.2
Water Ca/(Ca+Cl) summer (Ion Ratio)
0.019
0.236
0.001
0.062
0.025
0.047
0.75
0.5
0.25
0
1.02 to 2.93
2.95 to 6.14
7.64 to 16.63
16.68 to 31.25
36.33 to 94.23
Soil Si winter (digestion) (mmol kg DW-1)
(0.252)
0.048
0.053
0.23
0.038
0.75
0.5
0.25
0
0 to 0.1
0.1 to 0.3
0.3 to 1.1
1.2 to 2.4
2.6 to 14.7
Water NH/NO3- summer
0.015
0.178
0.041
0.005
0.383
0.073
0.75
0.5
0.25
0
0
123 to 251
268 to 911
911 to 6320
Soil N/K winter (NaCl-extraction)
Agrostis canina
Eleocharis multicaulis
Hydrocotyle vulgaris
Hypericum elodes
Juncus bulbosus
Molinia caerulea

tion) and the NO_3^-/K^+ and NO_3^-/PO_4^{3-} ratio of the surface water during summer (not shown).

Discussion

The most important predictor variables for the cover of *Eleocharis multicaulis* were the digestion-Si content of the mineral layer (+), the NH_4^+/NO_3^- ratio of the surface water (–), N measures of the water and mineral soil layer (+) and base cation richness variables such as ion ratio (IR) and DMR (–), with the signs (+/–) indicating the response direction. Our study thus confirms the species' presumed optimum in acidic and oligotrophic conditions, taking notice that oligotrophy concerns low P or K levels of the soil. In contradiction to earlier studies, we did not find significant results for NH_4^+ or CO_2; neither were they correlated with acidity variables. Also the preference for a mineral soil could not be supported, as we primarily found the species on organic substrate.

Nutrient limitation

Weighted averages of N, K and P (in water and soil) were lower than those measured by Pietsch (1978) and de Lyon & Roelofs (1986). The positive response to soil N and water NO_3^- indicates that *Eleocharis multicaulis* is favoured in times of atmospheric N deposition or other ways of N input. This is true on condition that NH_4^+ does not become the dominant N form in the water and P or K do not increase more than N. As atmospheric N deposition eventually leads to NH_4^+ dominance (Schuurkes et al. 1988), this form of N input should remain moderate, however. Probably, excess of soil N is depleted by the vegetation during the growing season, as relationships could not be established for summer data. Soils in the southern and western part of the investigated area were richer in K. This could be an explanation for lower performance of *Eleocharis multicaulis* in these areas, reflected by its response to latitude and longitude.

Fig. 4. Presence profiles of *Eleocharis multicaulis* and some positively or negatively associated species in relation to selected predictor variables. For each profile, the *p*-value of the corresponding Pearson chi-square test is shown. Low *p*-values indicate a significantly changing presence profile. *P*-values in brackets refer to tests in which more than 50 % of the expected frequencies in the chi-square test were lower than 5, which makes test results less reliable.

The extraction/digestion ratio of soil P indicates its availability to plants. The more tightly P is bound to the sediment (digestion-P) and thus unavailable to the extract and the vegetation, the better the performance of *Eleocharis multicaulis*, which is in agreement with observations on the N/P ratio.

It seems that CO_2 was not a limiting nutrient for the plant. Potentially it is only limiting in inundated conditions (Ruysschaert 2002). Neither did we find a relation with absolute NH_4^+, in contradiction to de Lyon & Roelofs (1986). At the time of their study (1970 s and 1980 s), intense acidification occurred in Dutch and Belgian softwater lakes, driven by strong atmospheric deposition of $(NH_4)_2SO_4$ and H_2SO_4 (Schuurkes et al. 1987 a, 1988). NH_4^+ deposition was strongest in agricultural areas (Schuurkes et al. 1987 b) and led to acidification through nitrification. A temporary increase of CO_2 often occurred due to protonation of soil CO_3^{2-} and water-dissolved HCO_3^- (Roelofs 1983, Roelofs et al. 1995, Smolders et al. 2002). Yet before this period (mid 20 th century), we can assume that a moderate N-enrichment and subsequent acidification (e. g. to pH 4.5–6) in agricultural areas made these lakes optimal for *Eleocharis multicaulis*. This is a plausible explanation why Pietsch (1985) and de Lyon & Roelofs (1986) found a significant effect of CO_2. The NH_4^+/ NO_3^- ratio became high as acidification got more intense (1970 s and 1980 s). Our survey revealed that the high NH_4^+/NO_3^- ratio is detrimental to the species, from which we infer that de Lyon & Roelofs (1986) made their measurements during a transitional stage. We propose that *Eleocharis multicaulis* disappeared soon afterwards from these sites, as was observed by van Beers (1994) for the South of The Netherlands.

Acidification may have caused fixed Si to dissolve in the interstitial water, and leach from the system. Extraction-Si mainly includes interstitial Si, while digestion-Si is the amount yielded by extreme weathering, as can be obtained by intense acidification. We presume that we measured the lowest digestion-levels and the highest extraction-levels in the historically most acidified places. As Si is an accurate predictor of the performance of *Eleocharis multicaulis*, the intensity of historical acidification would be of utmost importance to understand the species' current distribution.

In more recent times, N deposition levels have become lower as a consequence of better fertilization practice and reduced emissions from the energy and transport sector (Erisman & Bobbink 1997, Van Laer & Van Steertegem 2003). Agricultural eutrophication is nowadays often linked with an increase of alkalinity, due to the inlet of buffered water (e. g. Vanderhaeghe 2000). The fact that acidification was most intense in agricultural areas, could explain why Si is now a general measure of agricultural eutrophication and water alkalinity.

Redox state

In the lake we studied, we found a positive correlation between the NH_4^+/ NO_3^- ratio and pH ($r = 0.36$; $p = 0.01$), indicating that a high ratio is not an indication of an acid state anymore. We believe that high values are nowadays caused by a low redox potential and subsequent reduced nitrification. The system can turn into a low redox state through several processes. The first is oxygen consumption, caused by a high decomposition rate of organic substrates under supply of mostly agricultural P, K and buffer capacity (Lamers et al. 1998, Smolders et al. 2002). Secondly, reduced oxygen-rich shallow groundwater flows can lower the redox state. Flow reduction is caused by the drainage of catchment areas. Finally, damped water level dynamics also induce a lower redox potential. For *Eleocharis multicaulis*, we did indeed find an optimal seasonal water level change of ca. 25 cm ($p < 0.1$) and an optimal alkalinity of only 60 to 80 μeq L^{-1}. Also Dierssen (1975), Schaminée et al. (1992) and Runge (1996) stress that water level dynamics are important. Furthermore, oxic summer conditions will immobilise PO_4^{3-} as insoluble $FePO_4$ due to the oxidation of Fe^{2+} (Smolders et al. 2002, Lucassen et al. 2004). They will also prevent reduction of sulphate and consequential accumulation of phytotoxic sulphide in organic top layers (Smolders et al. 2003).

Base richness

Despite the species' negative reaction towards base cation richness, an addition of base cations during winter seems to be favourable. An increase of base cations during winter probably prevents extreme acidification of the system. In sandy lake shores these base cations can be provided by discharge of shallow, moderately base-rich groundwater. This is in agreement with Schaminée et al. (1992, 1995), who mention mixed influence of base-poor rainwater and base-rich groundwater as an optimal situation.

On average, we found lower macro-ionic values than de Lyon & Roelofs (1986) and especially Pietsch (1978) (Table 1). We presume that this is the consequence of leaching of base cations from the system in times of strong acidification. Probably, acidification first caused an increase of most ions in the surface water, due to the protonation of soil particles. Being dissolved, these ions were subject to gradual removal from the system. Also, data from a softwater lake district at Turnhout (northern Belgium) show a continuous decline of ion concentrations from 1973 to 2000 (Vanderhaeghe 2000). Beside this, it is unclear which analytical methods Pietsch (1978) used, so that comparison of some concentrations may be unreliable.

Organic top layer

The species' predominant occurrence on organic sediment was also mentioned by Dierssen (1975), Pietsch (1978), Szmeja & Clément (1990), Vahle (1990), Kaplan (1992), Drengemann et al. (1995) and Urban (1999). The opposite view of Schaminée et al. (1992, 1995) may have arisen because they considered this species together with other species of the *Eleocharitetum multicaulis* association like *Deschampsia setacea*, which may be more intolerant of an organic top layer. However, growing on an organic top layer has the risk of living in an NH_4^+ and PO_4^{3-} rich environment if this layer is decomposed under anoxic conditions. Oxygenation of the organic substrate, limited P and K supplies or restriction of buffer capacity might therefore be prerequisites for *Eleocharis multicaulis*.

Competition

In contrast to statements in vegetation science (Pietsch 1978, Schaminée et al. 1995), *Eleocharis multicaulis* is not a real pioneer species; it has its optimum in a succession stage with an established vegetation under which an organic layer has already developed. Our study indicates that *Eleocharis multicaulis* can be a competitive dominant. The optimum of *Eleocharis multicaulis* at NH_4^+/NO_3^- ratios between 0.1 and 1, and the coinciding retreat of *Hydrocotyle vulgaris*, indicates that the former is a competitive dominant over the latter under these circumstances. Such an environment will exist as long as acidification is not too strong. The negatively associated species *Hydrocotyle vulgaris, Agrostis canina* and *Hypericum elodes* are apparently limited by P or K, as their presence profiles for N/P and N/K (NaCl-extraction) have the inverse form of that of *Eleocharis multicaulis*. For all these species, the regions of low presence are probably due to competitive suppression.

Conclusions

The main results and ideas in this paper are:

- *Eleocharis multicaulis* performs best in an established vegetation where an organic top layer has formed;
- *Eleocharis multicaulis* profits from N input as long as NO_3^- dominates over NH_4^+ and P and K remain poorly available;
- the dominance of NH_4^+ over NO_3^- in the water has a negative influence on the species' performance, and can be caused by insufficient water level dynamics, rapid oxygen consumption in organic top layers due to P–K

eutrophication in buffered conditions, or by intense acidification through strong atmospheric N deposition;

- *Eleocharis multicaulis* profits from replenishment with base cations by shallow groundwater flows during winter, although the resulting level should remain low in order to maintain an acidic environment;
- intense historical acidification has been detrimental to the plant and strongly influenced its current distribution;
- both Si-determinations of the mineral soil (digestion and NaCl-extraction) reflect this historical acidification;
- *Eleocharis multicaulis* has a higher competitive ability when NO_3^- is dominant over NH_4^+ in the water;
- *Eleocharis multicaulis* suffers under competition with *Hydrocotyle vulgaris, Agrostis canina* and *Hypericum elodes* when (agricultural) supply of P or K is too high or when redox potential is not high enough to immobilise soil P;
- both Si-determinations of the mineral layer (digestion and NaCl-extraction) are measures for this agricultural influence, which is an inheritance of former intense acidification in the same areas.

Currently, experiments are being carried out to confirm hypotheses on nutrient limitation and competition. In order to establish the link with agriculture, a field investigation should be done, relating site parameters like Si, N, P and K levels to landscape variables such as distance to agricultural land and inlet of eutrophied water.

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