

Impacts of water level fluctuation on mesotrophic rich fens: acidification vs. eutrophication

Casper Cusell^{1,2*}, Leon P. M. Lamers², Geert van Wirdum³ and Annemieke Kooijman¹

¹Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, NL-1090, GE Amsterdam, The Netherlands; ²Aquatic Ecology and Environmental Biology, Institute for Water and Wetland Research, Radboud University Nijmegen, NL-6525, AJ Nijmegen, The Netherlands; and ³Deltares Subsurface and Groundwater Systems, P.O. Box 85467, NL-3584, CB Utrecht, The Netherlands

Summary

1. Water levels in areas with intensive agriculture have often been strictly controlled for decades. Recently, more natural fluctuating water levels have been propagated to improve the ecological quality of wetlands in these areas. This study investigated the effects of water levels on protected base-rich mesotrophic fens during winter and summer.
2. We used a mesocosm approach to simulate water level fluctuations under winter and summer conditions (light and temperature) to test the effects of water level on the biogeochemistry and vegetation of two highly endangered fen types with the brownmosses *Scorpidium scorpioides* and *Hamatocaulis vernicosus* as dominant mosses.
3. Both species showed decreased photosynthetic capacity at lower water levels. These levels also resulted in decreased alkalinity due to oxidation processes in the moss layer. High water levels led to increased pH and alkalinity, due to reduction processes and infiltration of base-rich water.
4. For the P-limited *S. scorpioides* mesocosms, high water levels did not lead to P mobilization or to significant changes in biomass production, P uptake and N:P ratios. However, for the mesocosms with *H. vernicosus*, where P limitation was not obvious, high water levels did result in P mobilization and increased P availability due to iron reduction. The lower P mobilization for *S. scorpioides* appeared to be related to lower total soil P content and higher Fe:P and Ca:P ratios. Although high water levels resulted in the accumulation of ammonium under winter conditions, this did not happen under summer conditions, making ammonium toxicity unlikely.
5. *Synthesis and applications.* Our results suggest that low water levels could hamper protected brownmosses in rich fens, especially during the summer. In contrast, inundations may have a positive effect on rich fens by increasing the alkalinity. However, inundations may lead to eutrophication due to internal P mobilization in soils with a high total P content and low Fe:P ratio. Therefore, we recommend that soils with high total P content and low Fe:P ratios should not be flooded.

Key-words: alkalization, buffer capacity, desiccation, *Hamatocaulis vernicosus*, inundation, mesocosm, phosphorus mobilization, *Scorpidium scorpioides*, water management, water-table fluctuation

Introduction

Water levels in pristine wetlands vary with meteoric and groundwater balances in and around these wetlands (Baker, Thompson & Simpson 2009). However, water levels in most non-pristine land systems are rather strictly controlled in view of a variety of human interests, including

agriculture and flood protection. The surface waters in most northwest European wetlands are managed for this control, which usually implies more constant water levels. This is accomplished by a water supply from rivers and storage basins during dry periods, which frequently has a questionable water quality. In addition, large-scale drainage and groundwater abstractions have resulted in lowered potential groundwater levels and reduced exfiltration of groundwater into the remaining wetlands (Grootjans & ten

*Correspondence author. E-mail: c.cusell@uva.nl

Klooster 1980; van Wirdum 1991; van Diggelen *et al.* 2006; van Loon 2010).

In the situation described, artificial supply of water in dry seasons reduces the risks of desiccation and acidification by oxidation processes (e.g. Roelofs 1991) and decreases the influence of base-poor rainwater (van Wirdum 1991). At the same time, however, the surface water inflow may lead to eutrophication as a result of high phosphorus and nitrogen inputs (Koerselman, Bakker & Blom 1990; Wassen *et al.* 1996; Bollens 2000; Olde Venterink *et al.* 2002b) or nutrient mobilization through high sulphate concentrations (Patrick & Khalid 1974; Caraco, Cole & Likens 1989; Roelofs 1991; Lamers, Tomassen & Roelofs 1998). Solving problems regarding acidification and desiccation may thus lead to unwanted eutrophication. Hydrological isolation of fens (e.g. by building dams) can provide a solution to exclude eutrophic water. However, this often accelerates acidification because of the enlarged relative influence of rain water, which does not have buffering capacity (e.g. van Wirdum 1991).

This friction between preventing acidification and eutrophication in wetlands under severe human pressure is especially problematic for mesotrophic base-rich fens, which are protected under the European Habitats Directive (transition mires and quaking bogs, H7140). Both acidification and eutrophication can result in fast transformation of these species-rich communities, with many endangered brownmosses such as *Scorpidium scorpioides* (Hedw.) Limpr. and *Hamatocaulis vernicosus* (Mitt.) Hedenäs, being replaced by more acid and species-poor *Sphagnum* dominated vegetation (Sjörs 1950; van Wirdum 1991, 1993; Kooijman & Bakker 1995; Wheeler & Proctor 2000; Kooijman & Paulissen 2006).

As neither the isolation of specific conservation areas nor the input of polluted surface water seems satisfying, alternative management options are needed. The allowance of more varying water levels has recently been advocated to improve the ecological water quality of wetlands and to reduce management costs (Loeb, Lamers & Roelofs 2008). This may cause water levels to decrease in fens during dry periods, while inundation of fens with base-rich water may occur during wetter periods. However, these more natural water levels may also have unwanted side effects, such as increased net mineralization and acidification by oxidation processes during periods of decreased water levels (Oomes, Kuikman & Jacobs 1997; Lamers, Roozendaal & Roelofs 1998; Lucassen, Smolders & Roelofs 2002; Olde Venterink *et al.* 2002a; Geurts *et al.* 2010). In periods with high water levels and inundations, the increased nutrient load of supplied surface water may lead to eutrophication and mobilization of phosphates, as well as to sulphide and ammonium toxicity as a result of reduction processes (Smolders & Roelofs 1996; Lamers, Tomassen & Roelofs 1998). The allowance of more varying water levels in wetland systems must therefore be considered in view of the potentially most sensitive, but also most valuable, rich fens.

We used a mesocosm approach to test the impact of low and high water levels in rich fens and unravel different processes like acidification and eutrophication. The aim was to analyse the effects of water level on biogeochemistry and vegetation development under summer and winter conditions in two characteristic types of rich fen with moss layers dominated by either *H. vernicosus* or *S. scorpioides*. The experiment was set up using intact soil cores, including the bryophyte and vascular vegetation. The questions addressed, were as follows: (i) what are the responses of pore water chemistry and vegetation to high and low water levels, (ii) what are the responses to increased concentrations of ammonium and sulphate in the supply water, and (iii) how are these responses influenced by season. Our hypothesis for (i) was that low water levels would reduce the growth of *H. vernicosus* and *S. scorpioides* and increase the acidity and nutrient concentrations in the pore water. In contrast, periods of inundation with base-rich water would increase the concentration of base cations and alkalinity in the pore water and may lead to eutrophication by internal P mobilization. For (ii), we hypothesized that high ammonium or sulphate concentrations in the inundation water lead to ammonium or sulphide toxicity. Our investigation did not allow for separate analysis of the partly antagonistic, temperature and light controlled processes for hypothesis (iii).

Materials and methods

EXPERIMENTAL DESIGN

Intact soil cores (upper 30 cm) including living mosses and higher plants were collected in 64 PVC columns (radius = 9.5 cm, length = 50 cm) in April 2009. They were collected from two summer-mown rich fens in National Park Weerribben-Wieden (the Netherlands), which is a Ramsar site that receives special protection in view of the European Habitats Directive. We collected 24 cores in 'Het Kiersche Wiede' (52°42'N, 6°8'E) where *H. vernicosus* dominated the moss layer (H-cores; *Caricion nigrae* – *Carex nigra-Agrostis canina* type), and 40 cores in 'De Stobbenribben' (52°47'N, 5°59'E) where *S. scorpioides* dominated the moss layer (S-cores; *Caricion davallianae* – *Scorpidium-Carex diandra* type). Fewer cores with *H. vernicosus* were collected, because it concerns one of the few locations in the Netherlands with this EU-habitat directive species.

At the day of collection, all cores were placed for 2 weeks in a climate room at 4 °C with a relative air humidity of 50–60% and a light level of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Philips MGR 102, 400 W; 12 h day^{-1}). After this, a second acclimatization period of 3 weeks started, in which half of the cores (12 H-cores and 20 S-cores) were randomly selected and transported to a climate room at 18 °C and 16 h of light per day (summer conditions). The other half stayed at 4 °C and 12 h of light per day (winter conditions). Water levels were maintained at surface level during both acclimatization periods, corresponding to the field situation.

The experiment started after the second acclimatization period (week 0). In both climate rooms, 12 H and S-cores randomly received three different treatments: a control treatment with water level at the surface (0), a treatment with a 15 cm lower water

level (−15) and a treatment with a 15 cm higher water level (+15) ($n = 4$). Table 1 shows the chemical composition of the supplied inundation water. The remaining 16 cores with *S. scorpioides* were used to test for possible ammonium and sulphide toxicity during high water levels under winter and summer conditions, by raising the water levels with NH_4 -enriched or SO_4 -enriched water (+15N and +15S) ($n = 4$). Ammonium and sulphate inputs represented the quality of surface water in agricultural areas adjacent to National Park Weerribben-Wieden. Standing water was refreshed completely every 3 weeks, to compensate for changing concentrations in the surface water caused by infiltration, plant uptake and/or exchange between water and soil.

During the experiment, water levels were checked twice weekly and replenished with demineralized water when necessary to compensate for evapotranspiration. Unfortunately, due to leakages, we had to exclude one control core (0) under summer conditions and one core with low water level (−15) under winter conditions, both collected from the H-fen, from analyses ($n = 3$ for both).

SAMPLING

Permanent soil moisture samplers (Rhizons SMS 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were inserted to collect pore water at two depths: one in the moss layer (−1 cm) and the other in the soil (−10 cm). Samples were taken anaerobically by connecting vacuumed plastic syringes of 50 mL. The first 10 mL of each sample was discarded to exclude stagnant sampler water. Moss pore water samples were taken in weeks 0, 5, 12, 21 and 31. Soil pore water samples were taken more often in weeks 0, 3, 5, 8, 12, 15, 21, 27 and 31, because most changes were expected in this layer. Samples from week 0 were taken after the incubation periods, just before starting the treatments.

At the end of the experiment, the photosynthetic yields at the apex of three randomly selected individuals of *H. vernicosus* or *S. scorpioides* were measured for each core (Junior-PAM fluorometer; Heinz Walz GmbH, Effeltrich, Germany) to get an indication of their vitality. Fluorescence yields were determined after 30 min of dark adaptation as $(F_m - F_0)/F_m$, where F_0 is the minimum fluorescence of chlorophyll at a low intensity of light needed to keep the reaction centres of photosynthetic system II open, while F_m is the maximum fluorescence at an intense light pulse. Subsequently, all above-ground plant biomass was harvested and separated into four groups: *Carex* species [predominantly *Carex elata* (All.), *Carex lasiocarpa* (Ehrh.), *Carex rostrata* (Stokes) and *Carex oederi oedocarpa* (Lange)], *Juncus* species [in particular *Juncus subnodulosus* (Schränk)], grass species [principally *Phragmites australis* (Steud.), *Agrostis canina* (L.) and *Calamagrostis stricta* (Koeler)] and a rest group [e.g. *Equisetum fluviatile* (L.), *Galium palustre* (L.), *Menyanthes trifoliata* (L.) and *Utricularia minor*

Table 1. Chemical composition of the inundation water

Chemical composition	Added to	Concentration (μM)
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	All inundation water	1500
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	All inundation water	300
KCl	All inundation water	100
NaHCO_3	All inundation water	2000
NH_4Cl	Inundation with NH_4 -addition	100
Na_2SO_4	Inundation with SO_4 -addition	1000

(L.)). Dry weights were determined after drying at 70 °C until constant weight. Finally, soil samples of the uppermost 10 cm were taken.

CHEMICAL ANALYSES

Immediately after sampling, sulphide concentrations in the pore water were measured with a sulphide ion-specific Ag-electrode (Orion Research, Beverly, MA, USA) and a double junction calomel reference electrode in 10.5 mL pore water with 10.5 mL sulphide antioxidant buffer (van Gemerden 1984). The pH-values were measured, and alkalinities were determined by titration to pH 4.2 using 0.01 M HCl. After dividing all samples into two subsamples, 1% of concentrated HNO_3 was added to one of them to prevent metal precipitation. Both subsamples were stored in iodated polyethylene bottles at −24 °C until further analysis. Total concentrations of Ca, Fe, Mn and S were measured in the acidified subsamples by ICP-OES (Optima 3000 XL; PerkinElmer, Waltham, MA, USA). NH_4 , NO_3 , o- PO_4 , Cl and dissolved organic matter (DOC) concentrations were analysed colorimetrically with continuous flow auto-analysers (Skalar Analytical BV, Breda, The Netherlands).

Dry weight and gravimetric moisture content of the soil samples, collected from the uppermost 10 cm at the end of the experiment, were determined by drying at 70 °C until constant weight. To determine total element concentrations in soils and plants (all plant groups together), dried and ground samples were digested for 17 min with 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) (Milestone srl, Sorisole, Italy). Digestates were analysed by ICP, as described previously. Nitrogen contents in plant material were determined with a Carlo Erba NA1500 elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA). Vascular plant N:P ratios were calculated on dry weight basis.

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS for Windows (version 20.0; SPSS, Chicago, IL, USA). QQ-plots were used to assess the normality of the distribution of all repeatedly measured chemical variables. All variables except pH and alkalinity were $\log(x + 1)$ -transformed to compensate for skewness and kurtosis. A three-way ANOVA with Tukey HSD post hoc test has been performed to determine significant differences due to season, water level or source area at the start of the experiment. Some variables differed between both source areas, but there were no significant differences for season and water level at the start (see Table S1 in Supporting information). In the subsequent analyses, the results of H and S-cores were tested as two separate data sets, to determine the effect of season and water level for both types of fen. As subsamples were taken several times from the same cores, a linear mixed model with time as random effect was used to determine the response to fixed factors (season and water level) for both fen types (West, Welch & Galecki 2007). This model was used instead of GLM repeated measures, because the required assumption of independent and identically distributed residual errors was violated. Differences between water levels, whenever significant in the linear mixed model, were further examined by comparing their estimated marginal means in a Bonferroni post hoc test (correction for multiple comparisons). To further examine interaction effects of season and water level, pairwise comparisons, with a 95% confidence interval, of the final measurements

were used. *P*-values in the text are indicated by asterisks: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ^{NS}not significant.

A three-way ANOVA with Tukey HSD post hoc test was performed to determine the effect of treatments on variables that were only measured at the end of the experiment: total soil concentrations, nutrient contents of plant tissue, biomass of plant groups and fluorescence yields of mosses. These variables were not log-transformed, because QQ-plots indicated normality of distribution for all these variables.

Results

PH AND ACID BUFFERING

Initial values

At the start of the experiment, the pH differed significantly between cores from fens with *H. vernicosus* (H-cores) and

S. scorpioides (S-cores), having mean values of 5.8 and 6.3, respectively (moss: $F_{1,34} = 58.1^{***}$, soil: $F_{1,34} = 60.8^{***}$; Fig. 1, see Table S1, Supporting information). Alkalinity and calcium concentrations also differed between both core types, with initial alkalinities of about 0.4 and 0.9 meq L⁻¹ (moss: $F_{1,34} = 57.6^{***}$, soil: $F_{1,34} = 34.7^{***}$) and calcium concentrations of around 400 and 1000 μM (moss: $F_{1,34} = 94.9^{***}$, soil: $F_{1,34} = 51.4^{***}$), for H and S-cores, respectively.

High water levels

High water levels led to a continuous increase in chloride concentrations in both moss and soil layers of H (moss: $F_{2,24} = 169.6^{***}$, soil: $F_{2,14} = 100.0^{***}$; Table 2) and S-cores (moss: $F_{2,10} = 159.0^{***}$, soil: $F_{2,24} = 76.0^{***}$; Table 3, see Figs S1 and S2, Supporting information). A similar pattern was found for calcium concentrations in H (moss:

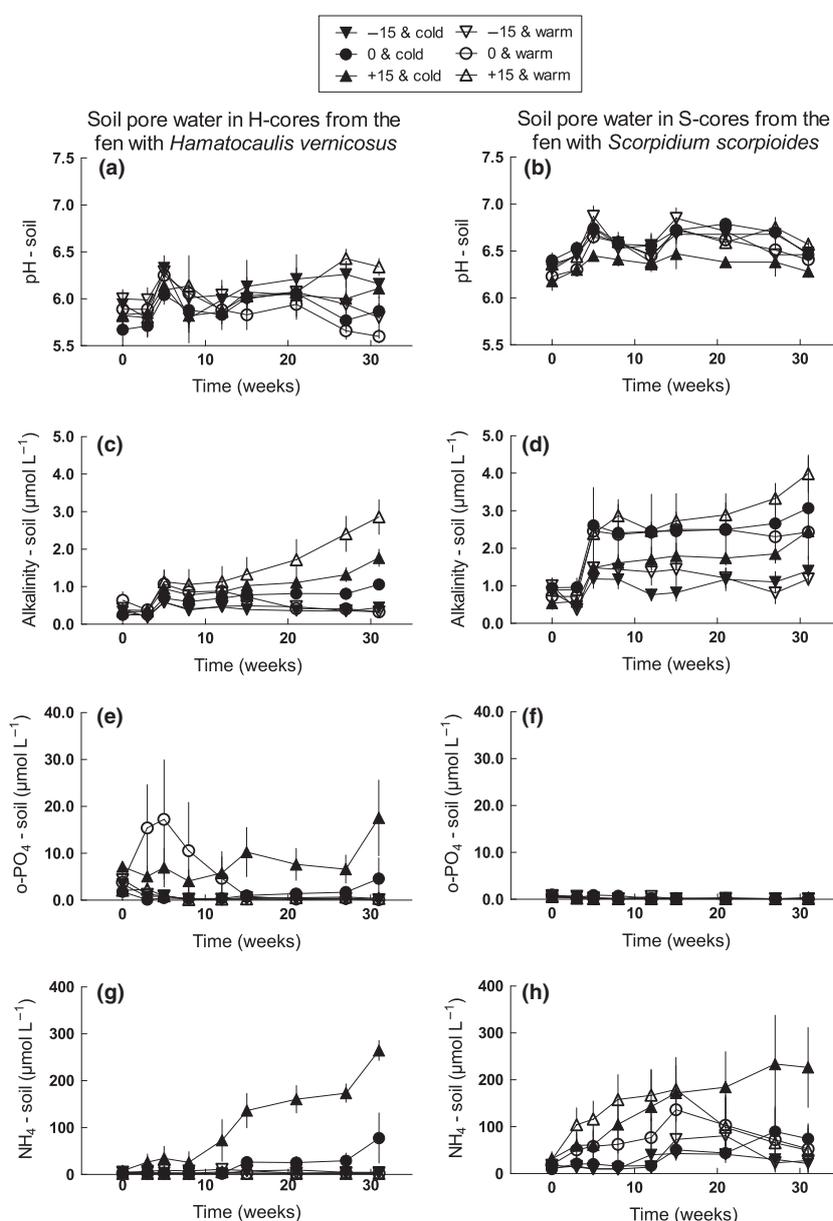


Fig. 1. pH, alkalinity, ortho-phosphate and ammonium concentrations in the soil pore water during 31 weeks of raised (+15), control (0) and lowered (-15) water levels in H and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*. Means are given with their standard errors ($n = 3-4$).

Table 2. Effects of season, water level and interaction effects on chemical variables in the pore water of the moss layer (–1 cm) and soil (–10 cm) of H-cores with *Hamatocaulis vernicosus*

Dependent variable	d.f. denominator	Season (d.f. = 1)	Level (d.f. = 2)	Season × level (d.f. = 2)	–15	0	15
Moss pore water							
pH	28.0	13.11**	24.28**	4.02*	a	a	b
Alkalinity	21.7	9.10**	81.65**	4.59*	a	b	c
Ca	20.4	0.04	130.21**	1.50	a	a	b
Cl	24.0	0.01	169.60**	0.97	a	a	b
Fe	16.2	2.03	11.05**	4.25*	a	a	b
S	14.4	55.28**	217.39**	61.37**	c	b	a
Sulphide	33.9	0.11	2.34	2.58	a	a	a
OrthoP	17.3	1.76	3.95*	5.21*	a	a	b
NO ₃	35.1	0.20	0.22	4.24*	a	a	a
NH ₄	18.2	5.61*	3.54*	7.36**	a	ab	b
DOC	12.6	7.22**	7.44**	5.01*	b	ab	a
Soil pore water							
pH	43.3	0.69	1.29	0.15	a	a	a
Alkalinity	22.2	4.14	3.45*	1.26	a	a	b
Ca	16.3	9.08**	71.40**	3.89*	a	a	b
Cl	14.1	1.90	100.03**	3.70*	a	a	b
Fe	23.0	0.60	0.83	3.24	a	a	a
S	14.8	16.77**	13.42**	3.19	b	a	a
Sulphide	39.3	2.38	2.11	1.76	a	a	a
OrthoP	23.8	9.80**	6.22**	7.13**	a	a	b
NO ₃	21.5	1.85	1.81	2.33	a	a	a
NH ₄	14.9	0.48	4.49*	4.61*	a	ab	b
DOC	25.7	0.68	0.15	5.96**	a	a	a

Significant differences between water levels are indicated by different letters. See Table S2 (Supporting information) for further information about interaction effects.

F-ratios with their level of significance: * $P \leq 0.05$ and ** $P \leq 0.01$.

Table 3. Effects of season, water level and interaction effects on chemical variables in the pore water of the moss layer (–1 cm) and soil (–10 cm) of S-cores with *Scorpidium scorpioides*

Dependent variable	d.f. denominator	Season (d.f. = 1)	Level (d.f. = 2)	Season × level (d.f. = 2)	–15	0	15
Moss pore water							
pH	22.7	1.02	1.61	2.82	a	a	a
Alkalinity	50.2	0.72	100.09**	6.97**	a	b	c
Ca	18.9	0.28	126.92**	3.07	a	a	b
Cl	10.4	1.59	159.01**	0.79	a	a	b
Fe	28.9	4.69*	7.30**	3.88*	a	ab	b
S	22.8	6.23*	112.76**	6.04**	c	b	a
Sulphide	17.0	1.63	0.59	2.53	a	a	a
OrthoP	40.1	0.79	2.24	2.73	a	a	a
NO ₃	20.1	0.07	67.32**	0.14	b	a	a
NH ₄	21.0	21.31**	11.20**	9.41**	a	ab	b
DOC	24.8	10.90**	12.78**	7.84**	b	a	a
Soil pore water							
pH	22.2	0.21	0.36	1.54	a	a	a
Alkalinity	33.9	3.80	19.63**	2.44	a	b	b
Ca	28.5	3.88	32.74**	0.71	a	b	c
Cl	24.0	0.37	75.98**	1.42	a	a	b
Fe	23.2	0.91	4.55*	0.84	a	b	b
S	19.7	0.52	14.15**	1.80	b	a	a
Sulphide	38.6	0.51	2.17	3.16	a	a	a
OrthoP	22.4	0.89	1.01	0.50	a	a	a
NO ₃	32.4	0.73	6.86**	0.38	b	a	a
NH ₄	19.5	0.15	6.78**	5.55*	a	ab	b
DOC	45.0	19.14**	3.03	2.72	a	a	a

Significant differences between water levels are indicated by different letters. See Table S3 (Supporting information) for further information about interaction effects.

F-ratios with their level of significance: * $P \leq 0.05$ and ** $P \leq 0.01$.

$F_{2,20} = 130.2^{***}$, soil: $F_{2,16} = 71.4^{***}$) and S-cores (moss: $F_{2,19} = 126.9^{***}$, soil: $F_{2,29} = 32.7^{***}$). In inundated H-cores, chloride and calcium concentrations in the soil pore water increased especially under summer conditions, as indicated by interaction effects of season and water level (Cl: $F_{2,14} = 3.7^*$, Ca: $F_{2,16} = 3.9^*$; see Table S2, Supporting information).

Alkalinity increased in most H and S-cores with high water levels compared with control cores (H moss: $F_{2,21} = 81.7^{***}$, H soil: $F_{2,22} = 3.5^*$, S moss: $F_{2,50} = 100.1^{***}$, S soil: $F_{2,34} = 19.6^{***}$; Figs 1 and 2, Tables 2 and 3), although alkalinity also increased in many control cores. Unlike chloride and calcium concentrations, which increased immediately in cores with high water levels, alkalinity stayed constant during the first 3 weeks of inundation. After 3 weeks, however, alkalinity increased sharply in the subsequent 2 weeks from 0.4 to 1.1 meq L⁻¹ in H-cores and from 0.9 to 2.5 meq L⁻¹ in S-cores. The pH also increased between weeks three and five from 5.8 to 6.2 in H-cores and from 6.3 to 6.7 in S-cores.

After 5 weeks, alkalinity kept rising in all inundated cores, though less fast, while pH did not change anymore in H (soil: $F_{2,43} = 1.3^{NS}$) and S-cores (soil: $F_{2,22} = 0.4^{NS}$).

High water levels also resulted in significantly lower sulphur concentrations in the moss layer (H: $F_{2,14} = 217.4^{***}$, S: $F_{2,23} = 112.8^{***}$), especially after 3–5 weeks (Tables 2 and 3, see Figs S1 and S2, Supporting information). In contrast, iron concentrations in the moss layer increased, but only under winter conditions (H: $F_{2,16} = 4.3^*$, S: $F_{2,29} = 3.9^*$; see Tables S2 and S3, Supporting information).

Low water levels

In the moss layer, alkalinity showed a significant decrease (H: $F_{2,21} = 81.7^{***}$, S: $F_{2,50} = 100.1^{***}$) from 0.5 to 0.2 μM in H-cores and from 1.0 to 0.5 μM in S-cores (Figs 1 and 2, Tables 2 and 3). This was accompanied by

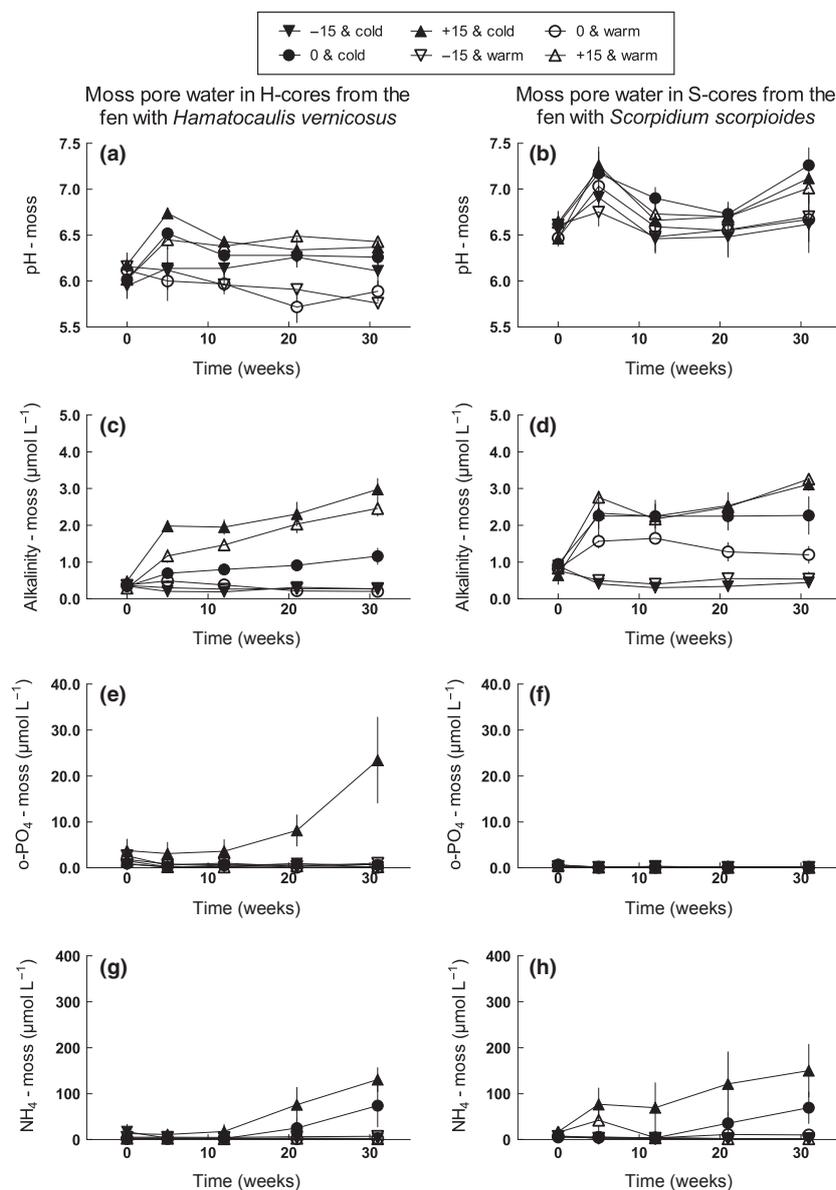


Fig. 2. pH, alkalinity, ortho-phosphate and ammonium concentrations in the moss pore water during 31 weeks of raised (+15), control (0) and lowered (–15) water levels in H and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*. Means are given with their standard errors ($n = 3-4$).

decreased iron concentrations in S-cores (moss: $F_{2,29} = 7.3^{**}$, soil: $F_{2,23} = 4.6^*$) and increased sulphur concentrations in both H (moss: $F_{2,14} = 217.4^{***}$, soil: $F_{2,15} = 13.4^{***}$) and S-cores (moss: $F_{2,23} = 112.8^{***}$, soil: $F_{2,20} = 14.1^{***}$; see Figs S1 and S2, Supporting information). In the moss layer, sulphur concentrations particularly increased under summer conditions, as shown by interaction effects of season and water level for both H ($F_{2,14} = 61.4^{***}$) and S-cores ($F_{2,20} = 6.0^{**}$; see Tables S2 and S3, Supporting information). It was only under these summer conditions in H-cores that low water levels led to decreased pH-values in the moss layer ($F_{2,28} = 4.0^*$; see Table S2, Supporting information). For S-cores, low water levels did not affect the pH in the moss layer ($F_{2,23} = 2.8^{NS}$). In the soil pore water, pH was not affected for either H ($F_{2,43} = 1.3^{NS}$) or S-cores ($F_{2,22} = 0.4^{NS}$).

NUTRIENTS

Initial concentrations

At the start of the experiment, ortho-phosphate concentrations significantly differed between H and S-cores in the moss layer ($F_{1,34} = 7.6^{**}$) and soil ($F_{1,34} = 11.1^{**}$; Figs 1 and 2, see Table S1, Supporting information). H-cores contained about 1.0 μM ortho-phosphate in the moss layer and about 6.0 μM in the soil pore water, while S-cores showed much lower concentrations of about 0.2 and 0.6 μM in the moss layer and soil pore water, respectively. Also, the total P content of soils was significantly higher in H-cores ($F_{1,33} = 232.6^{***}$), leading to significantly lower total Fe:P ($F_{1,33} = 97.9^{***}$) and Ca:P ratios ($F_{1,33} = 424.8^{***}$; Table 4).

High water levels

High water levels led to an increase in ortho-phosphate concentrations in the pore water (moss and soil) of

H-cores, but only under winter conditions as shown by season–water level interactions (moss: $F_{2,17} = 5.2^*$, soil: $F_{2,24} = 7.1^{**}$; Figs 1 and 2, see Table S2, Supporting information). In contrast, S-cores were not affected by high water levels (moss: $F_{2,40} = 2.2^{NS}$, soil: $F_{2,22} = 1.0^{NS}$), not even in cores with extra SO_4 -input (Fig. 3).

Low water levels

In neither H nor S-cores did low water levels influence the ortho-phosphate concentrations in the pore water (Figs 1 and 2, Tables 2 and 3). Low water levels did, however, led to increased DOC concentrations in the moss layer of both core types, but only under summer conditions (H: $F_{2,13} = 5.0^{**}$, S: $F_{2,25} = 7.8^{**}$; see Tables S2 and S3, Supporting information). Low water levels also led to increased nitrate concentrations in S-cores (moss: $F_{2,20} = 67.3^{***}$, soil: $F_{2,32} = 6.8^{**}$), but not in H-cores (moss: $F_{2,35} = 0.2^{NS}$, soil: $F_{2,22} = 1.8^{NS}$).

POTENTIAL TOXIC EFFECTS OF AMMONIUM AND SULPHIDE

Ammonium accumulation only occurred in cores with high water levels and only under winter conditions, as shown by the interaction effects of season and water level, for both H (moss: $F_{2,21} = 9.4^{***}$, soil: $F_{2,18} = 7.3^{**}$) and S-cores (moss: $F_{2,20} = 5.6^*$, soil: $F_{2,15} = 4.6^*$; Figs 1 and 2, see Tables S2 and S3, Supporting information). For both core types, concentrations of 100–150 μM in the moss pore water and 200–300 μM in the soil pore water were reached under winter conditions. Extra NH_4 -input into S-cores with high water levels did, however, not lead to a further increase ($F_{1,20} = 0.1^{NS}$). Here, ammonium concentrations in the pore water were already higher than in the supplied water.

Table 4. Chemical soil composition at the end of different treatments in H and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*

Treatment	Fe_{tot}^* (mmol kg^{-1} dw)	Ca_{tot}^* (mmol kg^{-1} dw)	P_{tot}^* (mmol kg^{-1} dw)	$\text{Fe}_{\text{tot}}/\text{P}_{\text{tot}}^*$ (mol mol^{-1})	$\text{Ca}_{\text{tot}}/\text{P}_{\text{tot}}^{\dagger\dagger}$ (mol mol^{-1})
<i>H. vernicosus</i>					
–15 & cold	21.2 (4.4)	273 (37)	35.0 (1.1)	0.61 (0.12)	7.8 (1.0)
0 & cold	28.4 (10.7)	275 (54)	34.0 (3.3)	0.84 (0.23)	8.2 (2.0)
+15 & cold	27.0 (5.1)	340 (63)	34.5 (5.2)	0.79 (0.06)	9.9 (1.6)
–15 & warm	24.2 (5.9)	301 (24)	36.2 (4.4)	0.66 (0.09)	8.4 (0.6)
0 & warm	21.5 (4.0)	275 (77)	36.2 (4.3)	0.69 (0.11)	6.8 (2.2)
+15 & warm	20.2 (5.5)	372 (70)	28.1 (6.7)	0.72 (0.09)	12.0 (3.4)
<i>S. scorpioides</i>					
–15 & cold	48.1 (21.4)	466 (60)	21.9 (3.7)	2.19 (1.00)	21.5 (3.1)
0 & cold	59.3 (15.8)	443 (15)	20.9 (1.7)	2.88 (0.92)	21.3 (1.9)
+15 & cold	47.0 (16.9)	422 (22)	19.3 (2.6)	2.41 (0.72)	22.1 (2.0)
–15 & warm	55.0 (23.7)	461 (39)	22.5 (3.7)	2.36 (0.71)	20.7 (2.0)
0 & warm	72.3 (7.0)	445 (56)	21.1 (2.2)	3.44 (0.35)	21.1 (1.9)
+15 & warm	34.6 (21.9)	435 (36)	18.2 (2.0)	1.84 (1.00)	22.2 (1.6)

Mean values and standard deviations ($n = 3-4$).

Significant differences ($P \leq 0.01$) between source areas, seasons and water levels are indicated by *, † and ‡, respectively.

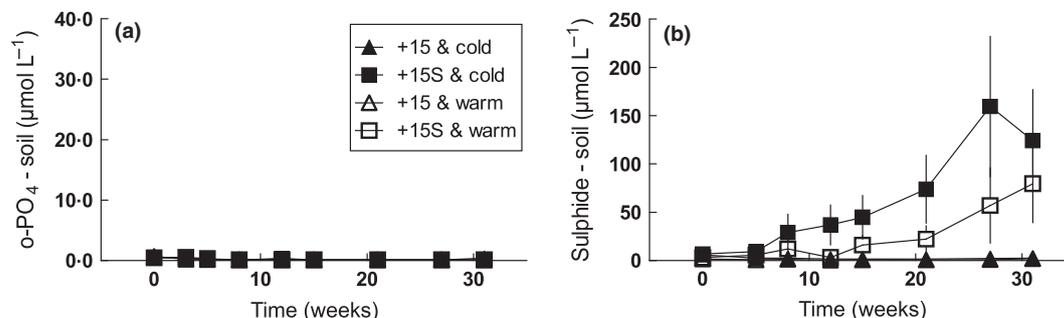


Fig. 3. Ortho-phosphate and sulphide concentrations in the soil pore water during 31 weeks of raised water levels with either 0 or 1000 μM sulphate in S-cores with *S. scorpioides*. Means with their standard errors ($n = 4$).

Sulphide concentrations were, in contrast, not affected by water level, neither for H (moss: $F_{2,34} = 2.3^{\text{NS}}$, soil: $F_{2,39} = 2.1^{\text{NS}}$), nor for S-cores (moss: $F_{2,17} = 0.6^{\text{NS}}$, soil: $F_{2,39} = 2.2^{\text{NS}}$). They stayed low in almost all cores, with values below 2 μM . Strong sulphide accumulation, to levels of 200 μM , only occurred in SO_4 -enriched S-cores, and especially under winter conditions (soil: $F_{1,20} = 9.0^{**}$; Fig. 3).

VEGETATION RESPONSE

As expected, the above-ground biomass at the end of the experiment was significantly higher under summer than winter conditions ($F_{1,34} = 60.4^{***}$; Fig. 4). Furthermore, the more eutrophic H-cores had a significantly higher total biomass than S-cores ($F_{1,34} = 8.9^{**}$). Water level treatments did not influence the total biomass ($F_{2,34} = 1.8^{\text{NS}}$), except for H-cores with low water levels under summer conditions, which showed a decrease. This was mainly caused by lower biomass of *Juncus* species. Addition of ammonium at high water levels, which only occurred in S-cores, did also not affect above-ground biomass, but

addition of sulphate led to a strong decline in *Carex* species and an increase in *Juncus* and grass species.

Different water levels did not lead to differences in plant nitrogen ($F_{2,31} = 4.9^{\text{NS}}$) and phosphorus ($F_{2,31} = 3.6^{\text{NS}}$) contents, nor in plant N:P ratios ($F_{2,31} = 1.8^{\text{NS}}$; Table 5). However, the source area of the core and season seemed to be important. In accord with the higher nutrient status of H-cores, the vegetation contained significantly higher phosphorus contents in control H-cores compared with S-cores ($F_{1,31} = 102.4^{***}$), leading to N:P ratios of about 15 and 37 ($F_{1,31} = 11.4^{**}$), respectively. The higher phosphorus contents and above-ground biomass of H-cores resulted in a much higher P consumption compared with S-cores, especially under summer conditions ($F_{1,30} = 13.4^{***}$; Table 6). Plant N:P ratios were also affected by season ($F_{1,31} = 32.1^{***}$). Values were significantly higher under summer than winter conditions for both core types, due to lower phosphorus contents ($F_{1,31} = 147.1^{***}$), but equal nitrogen contents. The ratios increased from 4 to 15 in control H-cores and from 15 to 37 in control S-cores.

Water level treatments clearly influenced the vitality of mosses ($F_{2,32} = 25.5^{***}$). Low water levels led to

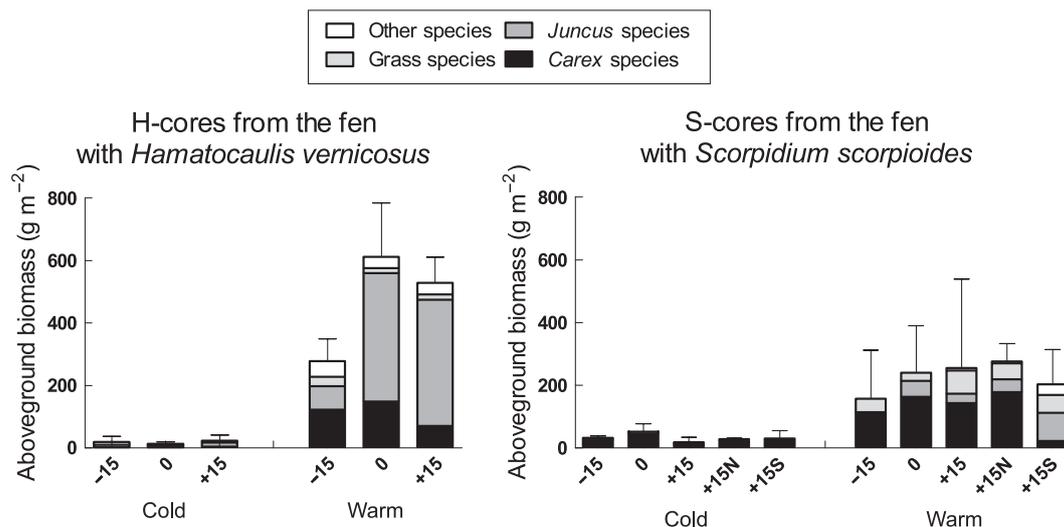


Fig. 4. Above-ground biomass of different vegetation groups after 31 weeks of high (+15), control (0) and low (−15) water levels in H and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*, including inundated S-cores to which 100 μM ammonium (+15N) or 1000 μM sulphate (+15S) was added. Means with their standard deviations ($n = 3\text{--}4$).

Table 5. Nitrogen and phosphorus contents and N:P ratios of the above-ground vascular plant tissue at the end of the experiment for H and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*

Treatment	Plant N (g kg ⁻¹ dp)	Plant P*† (g kg ⁻¹ dp)	Plant N/P*† (g g ⁻¹)
<i>H. vernicosus</i>			
-15 & cold	6.4 (1.3)	2.35 (0.08)	2.7 (0.5)
0 & cold	10.8 (2.3)	2.89 (0.29)	3.7 (0.5)
+15 & cold	13.7 (9.4)	3.57 (1.12)	3.6 (2.0)
-15 & warm	8.3 (0.5)	0.69 (0.10)	12.2 (1.9)
0 & warm	10.4 (3.4)	0.72 (0.11)	15.2 (7.0)
+15 & warm	24.4 (3.6)	0.70 (0.24)	38.2 (15.6)
<i>S. scorpioides</i>			
-15 & cold	12.1 (5.3)	0.83 (0.19)	14.4 (4.6)
0 & cold	11.7 (3.1)	0.84 (0.29)	14.6 (4.3)
+15 & cold	10.4 (3.5)	1.11 (0.32)	9.5 (2.8)
-15 & warm	16.8 (5.0)	0.55 (0.26)	34.0 (14.8)
0 & warm	13.7 (7.6)	0.42 (0.08)	36.6 (29.5)
+15 & warm	19.2 (1.3)	0.46 (0.18)	46.1 (14.5)

Mean values and standard deviations ($n = 3-4$). Significant differences between source areas and seasons are indicated by * and †, respectively ($P \leq 0.01$).

Table 6. Vitality of both mosses, as indicated by their fluorescence yields ($F_m - F_0$)/ F_m , and phosphorus amounts in above-ground vascular plant tissue (P concentration * biomass ha⁻¹) at the end of the experiment for H and S-cores with *Hamatocaulis vernicosus* and *Scorpidium scorpioides*

Treatment	($F_m - F_0$)/ F_m of mosses †‡	P in above-ground biomass*† (mol ha ⁻¹)
<i>H. vernicosus</i>		
-15 & cold	0.42 (0.11)	22 (12)
0 & cold	0.54 (0.15)	16 (6)
+15 & cold	0.58 (0.08)	39 (23)
-15 & warm	0.26 (0.11)	62 (18)
0 & warm	0.21 (0.03)	143 (45)
+15 & warm	0.48 (0.05)	104 (41)
<i>S. scorpioides</i>		
-15 & cold	0.29 (0.06)	9 (2)
0 & cold	0.62 (0.12)	10 (7)
+15 & cold	0.55 (0.07)	7 (6)
+15N	0.66 (0.06)	
+15S	0.40 (0.03)	
-15 & warm	0.27 (0.11)	26 (26)
0 & warm	0.43 (0.12)	31 (16)
+15 & warm	0.60 (0.05)	32 (29)
+15N	0.61 (0.04)	
+15S	0.36 (0.18)	

Mean values and standard deviations ($n = 3-4$). Significant differences between source areas, seasons and water levels are indicated by *, † and ‡, respectively ($P \leq 0.01$).

significantly lower fluorescence yields of between 0.25 and 0.35 for both mosses, compared with yields of about 0.50 in control cores (Table 6). These values indicate that *H. vernicosus* and *S. scorpioides* were vital in control cores, but significantly less vital at low water levels. Inundation, in contrast, did not lead to vitality changes. Both

species showed fluorescence yields of between 0.50 and 0.65 under both seasonal conditions. Under summer conditions, growth rates were higher than in control cores, with many individuals growing more than 15 cm in 31 weeks to position their apices above the water level (results not shown). The addition of ammonium in S columns with high water levels did not influence fluorescence yields of *S. scorpioides*, but SO₄-enrichment led to a decrease.

Discussion

DIFFERENCES BETWEEN BOTH FEN TYPES

Cores with *H. vernicosus* (H-cores) not only showed lower pH, alkalinity and calcium concentrations than cores with *S. scorpioides* (S-cores), which is consistent with previous studies (e.g. Hedenäs 2003; Štechová *et al.* 2008), but also higher P availability. Low P availability in S-cores corresponded with strong P limitation, as indicated by high plant N:P ratios of around 35 in control S-cores under summer conditions (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003; Güsewell 2004). This is supported by significantly lower plant N:P ratios under winter than summer conditions, suggesting that phosphorus became less readily available than nitrogen during the growing season.

RISKS OF LOW WATER LEVELS

At low water levels, oxidation of iron and sulphides led to decreased alkalinity in the moss layer of both fens. This process was also described by Loeb, Lamers & Roelofs (2008) for soils of riverine meadows. Under summer conditions, this decrease in buffer capacity led to a significant lowering of the pH in the moss layer of H-cores from 6.0 to 5.5, while S-cores still showed sufficient buffer capacity to prevent such a decrease in pH. Furthermore, increased DOC concentrations under summer conditions suggested an increase in decomposition rates in both core types with low water level (Oomes, Kuikman & Jacobs 1997; Olde Venterink *et al.* 2002a). Ortho-phosphate concentrations remained, however, low. In H-cores, this was probably due to increased P uptake by plants. In the strongly P-limited S-cores, this is presumably caused by P sorption to oxidized iron or calcium (Patrick & Khalid 1974; Koerselman, van Kerkhoven & Verhoeven 1993).

Low fluorescence yields for *H. vernicosus* and *S. scorpioides* in treatments with low water levels suggest that both mosses do not like long periods of drought, which corresponds to other studies (Boryslawski 1978; Mälson, Backéus & Rydin 2008). Although temporary desiccation and acidification seem to be a rather common phenomenon in rich fens (e.g. Kooijman & Whilde 1993; Štechová & Kučera 2007), long periods of drought may lead to the disappearance of brownmosses (den Held, Schmitz & Wirdum 1992; van Diggelen *et al.* 2006).

BENEFITS AND RISKS OF HIGH WATER LEVELS

High vitality of both mosses during inundation suggest that long periods of high water levels can be beneficial for *H. vernicosus* and *S. scorpioides*. Inundation with base-rich water led to an increase in soil buffer capacity. The sharp increase in alkalinity and pH after 3 weeks of inundation was at least partly caused by internal reduction processes, as sulphur concentrations decreased simultaneously (Smolders *et al.* 2006; Loeb, Lamers & Roelofs 2008). Apart from internal alkalization, increasing chloride and calcium concentrations also suggested continuous infiltration of bicarbonate-rich water, which seemed to be highest under summer conditions, when infiltration was easier due to higher evapotranspiration. The lack of increase in alkalinity and pH during the first 3 weeks of inundation, despite the immediate infiltration of base-rich inundation water at the start of the experiment, was probably caused by the consumption of bicarbonate through oxidation processes (Loeb, Lamers & Roelofs 2008).

Reducing conditions may not only lead to desired alkalization, but also to undesired P mobilization (e.g. Patrick & Khalid 1974). The effect of high water levels on net P availability differed between both core types. P availability remained low in strongly P-limited cores with *S. scorpioides*, as can be concluded from low ortho-phosphate concentrations and high plant N:P ratios, while ortho-phosphate concentrations increased in inundated cores with *H. vernicosus*. Relatively low total soil Fe:P and Ca:P ratios in H-cores compared with S-cores made them more sensitive to P mobilization (Smolders & Roelofs 1993; Zak, Gelbrecht & Steinberg 2004; Geurts *et al.* 2008). Ortho-phosphate concentrations, however, only increased in H-cores under winter conditions, because mobilized ortho-phosphate was rapidly taken up by plants under summer conditions, as can be concluded from the increased P consumption of plants.

In addition to internal alkalization and P mobilization, reduced conditions may also lead to high sulphide and ammonium concentrations (e.g. Lamers, Tomassen & Roelofs 1998). Despite sulphate reduction in all inundated cores, iron concentrations seemed to be high enough to bind most produced sulphide and prevent toxic effects (Smolders & Roelofs 1993, 1996; van der Welle *et al.* 2006). This was, however, not the case in SO₄-enriched inundated S-cores. Sulphide concentrations up to levels of 200 µM were not only toxic for *S. scorpioides*, but also resulted in a vegetation change from dominance of *Carex* species to *Juncus* and grass species. This is in accordance with results found by Lamers, Tomassen & Roelofs (1998), who found a similar change already at sulphide levels of about 20 µM.

Ammonium concentrations, in contrast, did increase in all inundated cores due to reduction processes, but only under winter conditions. Despite high ammonium concentrations of well above 100 µM, a level above which toxic effects can be expected for brownmosses under summer

conditions (Paulissen *et al.* 2004), none of the mosses showed decreased fluorescence yields, probably due to lack of growth. Although anaerobic microbial activity must have been higher under summer conditions (e.g. Dunfield *et al.* 1993), increased plant activity of graminoid species, such as *Carex elata* and *Carex rostrata* (Visser *et al.* 2000), *Phragmites australis* (e.g. Gries, Kappen & Löscher 1990) and *Juncus* species (Visser *et al.* 2000) probably led to increased radial oxygen loss (ROL) from roots, which stimulates nitrification and prevents ammonium accumulation. Increased influence of ROL under summer conditions is supported by relatively low concentrations of methane and manganese (II) (results not shown), iron (II) and sulphide.

IMPLICATIONS FOR FEN CONSERVATION

Rich fens can only persist under mineral-rich and nutrient-poor conditions (Sjörs 1950; van Wirdum 1991; Wheeler & Proctor 2000; Kooijman & Paulissen 2006). Managers of rich fens should therefore focus on the preservation and restoration of these conditions (e.g. Grootjans *et al.* 2006). More varying water levels have recently been advocated as a successful management tool (Loeb, Lamers & Roelofs 2008). However, our mesocosm experiment showed that more varying water levels may be risky for certain sensitive and endangered types of rich fen.

Periods of low water levels should be prevented as much as possible to prevent desiccation, acidification and eutrophication of brownmoss dominated rich fens (e.g. Mälson, Backéus & Rydin 2008). This study shows that such periods may indeed lead to increased net mineralization and acidification by oxidation processes, especially during the summer.

By contrast, periods of inundation with base-rich water led to mineral supply and soil alkalization in rich fens. This study shows that infiltration of base-rich water will especially occur during the summer, when infiltration is easier due to high evapotranspiration rates. However, phosphorus concentrations in the inundation water should be low to prevent eutrophication and concomitant species loss, especially in fen types in which phosphorus strongly limits biomass production. This may require additional measures, such as P stripping of the surface water. In addition, inundation water should also contain low sulphate concentrations to prevent sulphide toxicity to characteristic moss and *Carex* species. Finally, soils with high total P content and low Fe:P ratios should not be flooded, because this will increase the risk of internal P mobilization and increased P availability, even without external loading.

Acknowledgements

We wish to thank Robin Mook and Martin Versteeg for their help in the field, Roy Peters and Deef van Houdt for their assistance during the experiment, Leon van den Berg and Philippine Vergeer for statistical advice and Ton van Wijk, Piet Wartenbergh and Bert de Leeuw for

analytical assistance. We also want to thank Natuurmonumenten and State Forestry (SBB) for their approval to collect sods in their nature reserves. This study was funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation as a part of the Research Programme 'Ontwikkeling + Beheer Natuurkwaliteit'.

References

- Baker, C., Thompson, J.R. & Simpson, M. (2009) Hydrological dynamics I: surface waters, flood and sediment dynamics. *The Wetlands Handbook* (eds E. Maltby & T. Barker), pp. 120–168. Wiley-Blackwell, Oxford.
- Bollens, U. (2000) *Effects of nutrient inputs and water regime on wetland vegetation and the performance of wetland species*. PhD thesis, Swiss Federal Institute of Technology, Zürich.
- Boryslawski, Z.R. (1978) Notes on the ecology and biology of *Scorpidium scorpioides* (Hedw.) Limpr. *Acta Societatis Botanicorum Poloniae*, **47**, 15–23.
- Caraco, N.F., Cole, J.J. & Likens, G.E. (1989) Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. *Nature*, **341**, 316–318.
- van Diggelen, R., Middleton, B., Bakker, J., Grootjans, A. & Wassen, M.J. (2006) Fens and floodplains of the temperate zone: present status, threats, conservation and restoration. *Applied Vegetation Science*, **9**, 157–162.
- Dunfield, P., Knowles, R., Dumont, R. & Moore, T.R. (1993) Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH. *Soil Biology & Biochemistry*, **25**, 321–326.
- van Gernerden, H. (1984) The sulfide affinity of phototrophic bacteria in relation to the location of elemental sulfur. *Archives of Microbiology*, **139**, 289–294.
- Geurts, J.J.M., Smolders, J.P., Verhoeven, J.T.A., Roelofs, J.G.M. & Lamers, L.P.M. (2008) Sediment Fe:PO₄ ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. *Freshwater Biology*, **53**, 2101–2116.
- Geurts, J.J.M., Smolders, A.J.P., Banach, A.M., van de Graaf, J.P.M., Roelofs, J.G.M. & Lamers, L.P.M. (2010) The interaction between decomposition, net N and P mineralization and their mobilization to the surface water in fens. *Water Research*, **44**, 3487–3495.
- Gries, C., Kappen, L. & Lösch, R. (1990) Mechanism of flood tolerance in reed, *Phragmites australis* (Cav.) Trin. Ex Steudel. *New Phytologist*, **114**, 589–593.
- Grootjans, A.P. & ten Klooster, W.P. (1980) Changes of groundwater regime in wet meadows. *Acta Botanica Neerlandica*, **29**, 541–554.
- Grootjans, A.P., Adema, E.B., Bleuten, W., Joosten, H., Madaras, M. & Janakova, M. (2006) Hydrological landscape settings of base-rich fen mires and fen meadows: an overview. *Applied Vegetation Science*, **9**, 175–184.
- Güsewell, S. (2004) N:P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, **164**, 243–266.
- Hedenäs, L. (2003) The European species of the Calliergon–Scorpidium–Drepanocladus complex, including some related or similar species. *Meylania*, **28**, 1–117.
- den Held, A.J., Schmitz, M.B. & van Wirdum, G. (1992) Types of terrestrializing fen vegetation in the Netherlands. *Fens and Bogs in the Netherlands: Vegetation, History, Nutrient Dynamics and Conservation* (ed. J.T.A. Verhoeven), pp. 237–321. Kluwer Academic Publishers, Dordrecht.
- Koerselman, W., Bakker, S.A. & Blom, M. (1990) Nitrogen, phosphorus and potassium budgets for two small fens surrounded by heavily fertilized pastures. *Journal of Ecology*, **78**, 428–442.
- Koerselman, W. & Meuleman, A.F.M. (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, **33**, 1441–1450.
- Koerselman, W., van Kerkhoven, M. & Verhoeven, J.T.A. (1993) Release of inorganic N, P and K in peat soils; effect of temperature, water chemistry and water level. *Biogeochemistry*, **20**, 63–81.
- Kooijman, A.M. & Bakker, C. (1995) Species replacement in the bryophyte layer in mires: the role of water type, nutrient supply and interspecific interactions. *Journal of Ecology*, **83**, 1–8.
- Kooijman, A.M. & Paulissen, M.P.C.P. (2006) Higher acidification rates in fens with phosphorus enrichment. *Applied Vegetation Science*, **9**, 205–212.
- Kooijman, A.M. & Whilde, J. (1993) Variation in growth rates between populations of *Scorpidium scorpioides* with different habitats. *Journal of Bryology*, **17**, 567–577.
- Lamers, L.P.M., Roozendaal, S.M.E. & Roelofs, J.G.M. (1998) Acidification of freshwater wetlands: combined effects of non-airborne sulphur pollution and desiccation. *Water, Air, and Soil Pollution*, **105**, 95–106.
- Lamers, L.P.M., Tomassen, H.B.M. & Roelofs, J.G.M. (1998) Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. *Environmental Science & Technology*, **32**, 199–205.
- Loeb, R., Lamers, L.P.M. & Roelofs, J.G.M. (2008) Effects of winter versus summer flooding and subsequent desiccation on soil chemistry in a riverine hay meadow. *Geoderma*, **145**, 84–90.
- van Loon, A.H. (2010) *Unravelling hydrological mechanisms behind fen deterioration in order to design restoration strategies*. PhD thesis, University Utrecht, Utrecht.
- Lucassen, E.C.H.E.T., Smolders, A.J.P. & Roelofs, J.G.M. (2002) Potential sensitivity of mires to drought, acidification and mobilization of heavy metals: the sediment S/(Ca+Mg) ratio as diagnostic tool. *Environmental Pollution*, **120**, 635–646.
- Mälson, K., Backeus, I. & Rydin, H. (2008) Long-term effects of drainage and initial effects of hydrological restoration on rich fen vegetation. *Applied Vegetation Science*, **11**, 99–106.
- Olde Venterink, H., Davidsson, T.E., Kiehl, K. & Leonardson, L. (2002a) Impact of drying and re-wetting on N, P and K dynamics in a wetland soil. *Plant and Soil*, **243**, 119–130.
- Olde Venterink, H., Pieterse, N.M., Belgers, J.D.M., Wassen, M.J. & de Ruiter, P.C. (2002b) N, P, and K budgets along nutrient availability and productivity gradients in wetlands. *Ecological Applications*, **12**, 1010–1026.
- Olde Venterink, H., Wassen, M.J., Verkroost, A.W.M. & de Ruiter, P.C. (2003) Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology*, **84**, 2191–2199.
- Oomes, M.J.M., Kuikman, P.J. & Jacobs, F.H.H. (1997) Nitrogen availability and uptake by grassland in mesocosms at two water levels and two water qualities. *Plant and Soil*, **192**, 249–259.
- Patrick, W.H. & Khalid, R.A. (1974) Phosphate release and sorption by soils and sediments – effect of aerobic and anaerobic conditions. *Science*, **186**, 53–55.
- Paulissen, M.P.C.P., van der Ven, P.J.M., Dees, A.J. & Bobbink, R. (2004) Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. *New Phytologist*, **164**, 451–458.
- Roelofs, J.G.M. (1991) Inlet of alkaline river water into peaty lowlands: effects on water quality and *Stratiotes aloides* L. stands. *Aquatic Botany*, **39**, 267–293.
- Sjörs, H. (1950) On the relation between vegetation and electrolytes in North Swedish mire waters. *Oikos*, **2**, 241–258.
- Smolders, A. & Roelofs, J.G.M. (1993) Sulphate-mediated iron limitation and eutrophication in aquatic ecosystems. *Aquatic Botany*, **46**, 247–253.
- Smolders, A.J.P. & Roelofs, J.G.M. (1996) The roles of internal iron hydroxide precipitation, sulphide toxicity and oxidizing ability in the survival of *Stratiotes aloides* roots at different iron concentrations in sediment pore water. *New Phytologist*, **133**, 253–260.
- Smolders, A.J.P., Lamers, L.P.M., Lucassen, E.C.H.E.T., van der Velde, G. & Roelofs, J.G.M. (2006) Internal eutrophication: how it works and what to do about it - a review. *Chemistry and Ecology*, **22**, 93–111.
- Štechova, T. & Kučera, J. (2007) The requirements of the rare moss, *Hamatocaulis vernicosus* (Calliergonaceae, Musci), in the Czech Republic in relation to vegetation, water chemistry and management. *Biological Conservation*, **135**, 443–449.
- Štechova, T., Hájek, M., Hájková, P. & Navrátilová, J. (2008) Comparison of habitat requirements of the mosses *Hamatocaulis vernicosus*, *Scorpidium cossonii* and *Warnstorfia exannulata* in different parts of temperate Europe. *Preslia*, **4**, 399–410.
- Visser, E.J.W., Bögemann, G.M., van de Steeg, H.M., Pierik, R. & Blom, C.W.P.M. (2000) Flooding tolerance of *Carex* species in relation to field distribution and aerenchyma formation. *New Phytologist*, **148**, 93–103.
- Wassen, M.J., van Diggelen, R., Wolejko, L. & Verhoeven, J.T.A. (1996) A comparison of fens in natural and artificial landscapes. *Vegetatio*, **126**, 5–26.
- van der Welle, M.E.W., Cuppens, M.L.C., Lamers, L.P.M. & Roelofs, J.G.M. (2006) Detoxifying toxicants: interactions between sulphide and iron toxicity. *Environmental Toxicology & Chemistry*, **25**, 1592–1597.
- West, B.T., Welch, K.B. & Galecki, A.T. (2007) *Linear Mixed Models: A Practical Guide Using Statistical Software*. Chapman & Hall/CRC, Boca Raton, FL.
- Wheeler, B.D. & Proctor, M.C.F. (2000) Ecological gradients, subdivisions and terminology of north-west European mires. *Journal of Ecology*, **88**, 187–203.
- van Wirdum, G. (1991) *Vegetation and hydrology of floating rich-fens*. PhD thesis, University of Amsterdam, Amsterdam.

van Wirdum, G. (1993) An ecosystems approach to base-rich freshwater wetlands, with special reference to fenlands. *Hydrobiologia*, **265**, 129–153.

Zak, D., Gelbrecht, J. & Steinberg, C.E.W. (2004) Phosphorus retention at the redox interface of peatlands adjacent to surface waters in Northeast Germany. *Biogeochemistry*, **70**, 357–368.

Received 21 August 2012; accepted 22 March 2013

Handling Editor: Angela Strecker

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Calcium, chloride, iron and sulphur concentrations in the soil pore water during 31 weeks of raised (+15), control (0) and lowered (–15) water levels in H and S-cores from fens with *H. vermicosus* and *S. scorpioides*, respectively.

Fig. S2. Calcium, chloride, iron and sulphur concentrations in the moss pore water during 31 weeks of raised (+15), control (0) and lowered (–15) water levels in H and S-cores from fens with *H. vermicosus* and *S. scorpioides*, respectively.

Table S1. Effects of source area, season, water level and their interactions on chemical variables in the pore water of the moss layer (–1 cm) and soil (–10 cm) at the start of the experiment ($t = 0$).

Table S2. Pairwise comparisons (95% confidence interval) of the final measurements in H-cores with *H. vermicosus*.

Table S3. Pairwise comparisons (95% confidence interval) of the final measurements in S-cores with *S. scorpioides*.