Preslia 85: 405–420, 2013

Do high iron concentrations in rewetted rich fens hamper restoration?

Zabraňuje velká koncentrace železa úspěšné obnově minerálně bohatých slatinišť?

Camiel J. S. Aggenbach[1,2], Hans Backx[2], Willem Jan Emsens[2],
Ab P. Grootjans[3,4], Leon P. M. Lamers[3], Alfons J. P. Smolders[3,5],
Pieter J. Stuyfzand[1,6], Lesław Wołejko[7] & Rudy Van Diggelen[2]

Dedicated to Kamil Rybníček and Eliška Rybníčková on the occasion of their 80th birthdays

[1]KWR Watercycle Research Institute, P. O. Box 1072, 3430 BB Nieuwegein, The Netherlands, email: camiel.aggenbach@kwrwater.nl; [2]Ecosystem Management Research Group, Department of Biology, University of Antwerp, Universiteitsplein 1C, 2610 Wilrijk, Belgium, email: hans.backx@ua.ac.be, ruurd.vandiggelen@ua.ac.be; [3]Department of Aquatic Ecology and Environmental Biology, Institute for Wetland and Water Research, Radboud University Nijmegen, Heyendaalseweg 135, NL-6525 ED Nijmegen, The Netherlands, email: a.smolders@science.ru.nl, l.lamers@science.ru.nl; [4]Centre for Energy and Environmental Studies, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands, e-mail: A.P.Grootjans@rug.nl; [5]B-WARE Research Centre, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands; [6]Amsterdam Critical Zone Hydrology Group, FALW, VU University Amsterdam, Boelelaan 1085, 1081 HV Amsterdam, The Netherlands, email: pieter.stuyfzand@falw.vu.nl; [7]West Pomeranian University of Technology in Szczecin, Slowackiego 17, 71-434 Szczecin, Poland, e-mail: ales@asternet.pl

Aggenbach C. J. S., Backx H., Emsens W. J., Grootjans A. P., Lamers L. P. M., Smolders A. J. P., Stuyfzand P. J., Wołejko L. & Van Diggelen R. (2013): Do high iron concentrations in rewetted rich fens hamper restoration? – Preslia 85: 405–420.

In this study we address the question of the extent to which iron may be a limiting factor in restoring rich fens in the temperate climate zone of Europe. Rewetted fens that were heavily degraded in the past by draining over a long period, were compared with pristine fens or fens with slightly altered hydrological systems. The chemical composition of peat and of pore water was analysed and related to the composition of the vegetation of the fens. The species composition and chemistry of the topsoil of restored fens differed markedly from that of the other fens, while the chemistry of the pore water from deeper layers showed only minor differences. Multivariate analysis revealed that differences in species composition between both categories were strongly related to the concentration of Fe in the pore water in the topsoil. Restored sites with high iron concentrations in the pore water ($> 100\ \mu mol \cdot L^{-1}$) lacked many vascular plants and mosses typical of peat forming fens. Iron and inorganic phosphorus pools in the topsoil of most restored fens were much greater than in the reference fens. A higher soil phosphorus pool originated mainly from the iron-bound fraction. We conclude that these differences are strongly governed by local processes and not by regional differences in climate, which were associated with geographical distribution of the different fens studied. The strong accumulation of iron and phosphorus in restored fens is attributed to a long history of drainage, which enhanced the accumulation of oxidized iron in the topsoil and also lowered the concentrations of calcium, magnesium and sulphur through drainage-caused reoccurring oxidation-reduction and leaching processes. A high iron and associated high phosphorous content appears to be an important and possibly irreversible bottleneck to restoring biodiversity and accumulation of peat with a low degree of humification in degraded fens. If a degraded fen has a low iron content then it is more likely to be restorable.

Keywords: brown mosses, *Carex,* helophytes, iron toxicity, peat formation, redox, rewetting, rich fen

Introduction

Rich fens are peat-forming ecosystems with vegetation dominated by *Amblystegiaceae* (brown mosses) and low productive sedges. They are mainly fed by base-rich ground- or surface water and are characterized by a very high biodiversity (Middleton et al. 2006). For instance, many species of orchids, such as *Dactylorhiza incarnata, Epipactis palustris* and *Liparis loeselii,* occur in such fens. Some centuries ago they were common in Europe, in particular in lowland river valleys and lakes in the process of terrestrialization, but now they are rare, especially in western Europe. Many were drained for agriculture and forestry, others were severely damaged by peat mining, groundwater abstraction, pollution of surface water and groundwater, and atmospheric deposition (Van Diggelen et al. 2006). They are now categorized as EU habitats in the Habitat Directive, which implies that remaining sites not only have to be conserved, but degraded sites also have to be restored.

The most important measure used to restore damaged mires is rewetting (Klimkowska et al. 2010) but unfortunately the outcomes of many fen rewetting projects are often not very successful (Schrautzer et al. 2013). In many cases they result in the dominance of large helophytes (Timmermann et al. 2006), which results in species-poor vegetation (Olde Venterink et al. 2001, Wołejko 2002) due to shading of brown mosses and small vascular plants (Kotowski et al. 2010, Štechová et al. 2012). An important reason for this is that drained fens often have a large pool of iron-bound inorganic phosphorus in the topsoil (Olde Venterink et al. 2009) and rewetting may cause mobilization of this phosphorus (Lucassen et al. 2005, Zak et al. 2008). Despite lowered N- and P mineralization under wet conditions rewetting-induced P mobilization may prevent the establishment of conditions of a low nutrient status, which is necessary for fen plant community restoration.

Rewetting may also induce or enhance toxicity effects and hamper the recovery of fen vegetation (Lamers et al. 2006). Well-known examples are free sulphide (S^{2-}, HS^-, H_2S) (Adema et al. 2003, Geurts et al. 2009) and ammonium (Paulissen et al. 2005, Verhoeven et al. 2011). Iron toxicity is less well-known but possibly more common in fens. Ferrous iron is toxic for many wetland species even at low concentrations of 20 $\mu mol \cdot L^{-1}$ (Snowden & Wheeler 1993, Van der Welle et al. 2007). Drained fens with groundwater discharge can potentially accumulate large amounts of oxidized iron in the aerobic topsoil and in the case of rewetting this can lead to high iron concentrations in the pore water of 600–3000 $\mu mol \cdot L^{-1}$ (Lucassen et al. 2000). Only plants that can tolerate high Fe levels can survive in such an environment. A well-known mechanism to counteract Fe-toxicity effects is internal gas transport to the roots and radial oxygen release (ROL) at the root tips (Jackson & Armstrong 1999). This adaptation causes Fe^{2+} oxidation in the rhizosphere (Begg et al. 1994).

Iron may also directly affect the accumulation of peat. Under the anoxic conditions that develop after rewetting Fe^{3+} is reduced to Fe^{2+}, thereby oxidizing organic matter to inorganic carbon (Li et al. 2012). It is therefore very likely that after rewetting of iron-rich fens decomposition rates of organic matter will remain high. It might even be questioned whether peat formation is possible at all under extreme iron-rich conditions.

In the present study we address the question to what extent iron affects the restoration of degraded rich fens. We studied 16 sites in the temperate climate zone of north-western Europe, ranging from pristine fens to rewetted heavily disturbed fens. We measured the composition of the vegetation, chemistry of soil pore water and physical and chemical properties of the soil. We discuss our findings on iron toxicity, P availability, vegetation structure and vegetation composition in the context of fen development.

Methods

Study sites

We selected 16 sites in lowland regions of The Netherlands, Belgium and Poland, which had thick sandy aquifers (Appendix 1). As selection criteria we used: (i) the site is very wet with the water table less than 10 cm below the surface, (ii) is covered by a layer of peat more than 20 cm deep, (iii) which developed in a terrestrial situation, (iv) the location is fed by base-rich groundwater, (v) flooding by surface water does not occur or is very rare, and (vi) the variation in soil degradation ranges from almost pristine to heavily degraded. Fens that were selected as reference areas consist of sites that have never been drained, or only very superficially, and rewetted after abandonment, and with most of the typical fen species remaining. The restored fens were rewetted at least a decade ago after having being drained over a long period of time. The management of the vegetation at the reference sites is variable ranging from no management to regular haymaking, whereas most restored sites were subjected to regular haymaking. The restored sites are in Belgium and The Netherlands. Undrained groundwater-fed rich fens no longer exist there and therefore the reference sites were situated in Poland.

Sampling

Each study site consisted of a 5 × 5 m plot, where plant species and vegetation structure were recorded. One metre from the plot centre we recorded the profile of the peat using a Russian peat gauge. We recorded the level of humification of the peat using the Von Post scale and classified the peat type on the basis of its colour and the macroremnants it consisted of. In the centre of the plot we sampled groundwater using 60 mL vacuum syringes connected to ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Normally we placed the ceramic sampler in the soil layer 10–20 cm below the peat in order to sample the groundwater before it entered the peat but at sites with a thick peat layer we placed it at a depth of 150 cm in the peat. Two samples of pore water were collected from the upper 10 cm of the soil along two opposite borders of the plot with macrorhizons (pore size 0.22 μm). Fieldwork and sampling were carried out between 18 May and 24 June 2009.

Vegetation

The cover and abundance of vascular plants, mosses and litter were recorded in the 5 × 5 m plots. We used the Doing scale (Londo 1976) and converted the classes therein to percentage cover. The cover of the category 'large helophytes' was calculated as the sum of the cover of *Carex acuta*, *C. acutiformis*, *C. paniculata*, *C. rostrata*, *C. ×elytroides, Equisetum palustre*, *E. fluviatile*, *Juncus acutiflorus*, *J. subnodulosus*, *Menyanthes trifoliata*, *Phragmites australis*, *Scirpus lacustris*, *S. sylvaticus*, *Sparganium erectum*, *Typha angustifolia* and *T. latifolia*. The cover of characteristic fen species of vascular plants and mosses was calculated as the sum of the covers of the individual species.

Taxonomy follows Van der Meijden (1996) for vascular plants and Siebel & During (2006) for mosses.

Pore water sampling and chemical analyses

Before sampling the ceramic samplers and rhizons were flushed with pore water. Subsamples for ICP analysis were acidified by adding 0.7 ml 65% ultrapure HNO_3 per 100 mL of sample. Subsamples for sulphide were only taken from the topsoil and stored in air tight glass tubes and mixed with sulphide antioxidant buffer SAOB, while pH and alkalinity were measured directly in the field: pH with portable equipment and alkalinity by titration to pH 4.2 with 0.01 M HCl. Samples were stored at 4 °C before transportation to the laboratory. Ca^{2+}, Mg^{2+}, K^+, Na^+, Cl^- , Fe_t, Mn_t, Al_t and Si_t were measured using ICP (IRIS Intrepid II), soluble reactive P, NH_4^+, NO_3^- using an Auto Analyzer 3 system (Bran+ Luebbe) and ammonium molybdate, hydrazine sulphate and salicylate. SO_4^{2-} was measured using Technicon Autoanalyzer Methodology (1981) and TIC measurements on an Infrared Gas Analyzer (ABB Advance Optima). Sulphide was measured using a sulphide-specific Ag electrode (Orion Research) in a double conjunction calomel reference electrode.

Soil sampling and chemical analyses

Close to where each sample of pore water was collected a 10 × 10 × 10 cm sample of soil was collected to measure bulk density. These were dried for 72 h at 105 °C and then weighed. We took five other subsamples, each of 5 × 5 × 10 cm, in the 5 × 5 m plot and pooled these, sealed them and stored them at 4 °C. In the laboratory they were frozen at –18 °C and stored. Dried subsamples (48 h at 70 °C) were ground in liquid N and homogenized for further analyses. We measured organic matter content by loss of ignition for 4 h at 550 °C and that of C and N using a Carlo Erba NA1500 elemental analyzer (Thermo Fisher Scientific). We then digested 200 mg in 4 mL HNO_3 (65%) and 1 mL H_2O_2 (30%) using a microwave labstation (Milestone srl) and diluted this to measure Ca, Mg, Na, K, S, Fe, Al, Mn and Si using ICP. We used sequential P fractioning after (Golterman 1996) to measure Fe+Al-bound P, Ca-bound P, organic P and labile P (NH_4Cl-extractable) in 10 g of fresh sediment. The total P content was calculated as the sum of all fractions. The concentration of Olsen-P was analysed and used as a measure of the P fraction, which is easily available to plants (Olsen et al. 1954). Extractions of fresh sediments were corrected for their water content. All values were calculated in terms of dry weight (dw) of soil.

Numerical analyses

We started our analysis by comparing the vegetation parameters of the reference and restored sites and checked the significance using a Mann-Whitney U-test. We then carried out a canonical correspondence analysis (CCA) in order to detect which abiotic characteristics of the topsoil are correlated most strongly with the composition of the vegetation (ter Braak & Šmilauer 2002). Most of the abiotic data had to be log(x+1) transformed in order to achieve normality. Species cover values were also log(x+1) transformed. Abiotic variables were selected by forward stepwise selection and significance tests based on permutations ($P < 0.05$ at 499 permutations) to evaluate the importance of each variable. A term was selected if its correlation with all previously added terms was lower than 0.6. The remaining variables were added as passive variables. We used pore-water variables (pH, alkalinity and concentrations of TIC, Al, Ca^{2+}, Fe, K^+, Mg^{2+}, Mn, P, SO_4^{2-} , Si and sum (NH_4^++NO_3^-) in the CCA because these are more relevant to the root environment. We added Ellenberg moisture indicator values (F-ind; Ellenberg et al. 1992) as proxy values for wetness and cover of herbaceous plants as a proxy for light conditions at soil level. F-ind was calculated per relevé as the average of the indicator values for individual species.

We continued by comparing the water chemistry of the topsoil of reference and restored sites. We did the same for the deeper groundwater in order to check whether differences in the chemistry of water from the top layer are related to differences in the chemical composition of inflowing groundwater. Then we compared the alkalinity of pore water of the topsoil with that calculated based on the assumption that the dissolution of carbonates are the only sources of HCO_3^- in order to check whether other alkalinity generating processes have occurred. Next we compared pool sizes and phosphate fractions in the topsoil and checked for significant differences between reference and restored sites using a Mann-Whitney U-test. Spearman correlation coefficients and P-values were calculated for soil and pore-water variables. Finally we assessed Fe tolerances of the species that were present at five or more sites, on the basis of their distribution in this dataset.

Results

Vegetation structure and species diversity

Reference and restored sites differed greatly in the structure of their vegetation (Table 1). At restored sites the cover of the herbaceous layer was greater and that of the moss layer lower than at reference sites. Also the cover of large helophytes was two times greater at the restored sites. Values for litter cover at the two sites did not differ. Cover of vascular plants characteristic of rich fen did not differ while that of mosses was much greater at the reference sites. Differences in species diversity reflected the greater number of species of moss and vascular plants characteristic of rich fens at the reference sites.

Relation between plant species composition and abiotic conditions

In the CCA only few variables were selected for the model, because of strong covariation between the environmental variables (Fig. 1). Pore-water Fe, F-ind, Mg^{2+} and SO_4^{2-} were selected as explanatory variables. Fe and F-ind correlated strongly with, respectively, axes 1 and 2. The reference and restored sites were separated from each other only along axis 1. Along axis 2 the groups overlapped completely but the restored sites were more variable than the reference sites, suggesting more variation in wetness in the first category. Cover of herbaceous plants was significantly correlated ($r_S = 0.52$, $P < 0.05$) with the first axis.

Pore water chemistry

Pore water of both restored and reference sites was weakly acid to neutral and had a relatively high TIC, alkalinity and Ca^{2+} concentration, whereas the values for SO_4^{2-} , NH_4^+, NO_3^- and reactive P were mostly rather low. Most of the differences in the chemistry recorded at reference and restored sites were for the topsoil (Table 2). Concentrations of

Table 1. – Average values ± standard deviations for features of the vegetation structure at reference (n = 7) and restored sites (n = 9). P-values of the Mann-Whitney U-test for difference are listed, with significant values in bold.

Parameter	Unit	Reference sites	Restored sites	P
Structure				
Cover of herbaceous plant layer	% surface	27.9±10.7	51.7±19.0	**0.004**
Cover of characteristic vascular plant species	% surface	32.7±20.3	33.9±16.7	0.681
Cover of large helophytes	% surface	17.6±14.4	41.3±20.2	**0.008**
Cover of moss layer	% surface	90.0±13.8	48.8±40.2	**0.006**
Cover of characteristic moss species	% surface	39.3±27.2	4.1±6.9	**0.001**
Cover of litter	% surface	11.3±22.1	11.4±16.4	0.478
Height herbaceous plant layer	cm	33.3±14.7	70±31.1	**0.003**
Number of species				
Total number of plant species		29.6±12	26.7±5.7	1.000
Vascular species of plants		22.1±8.4	22.7±4.9	0.388
Characteristic species of vascular plants		10.7±2.8	5.8±3.1	**0.009**
Mosses		7.4±4.7	4.0±1.9	**0.046**
Characteristic species of moss		4.6±2.1	1.4±0.5	**0.001**

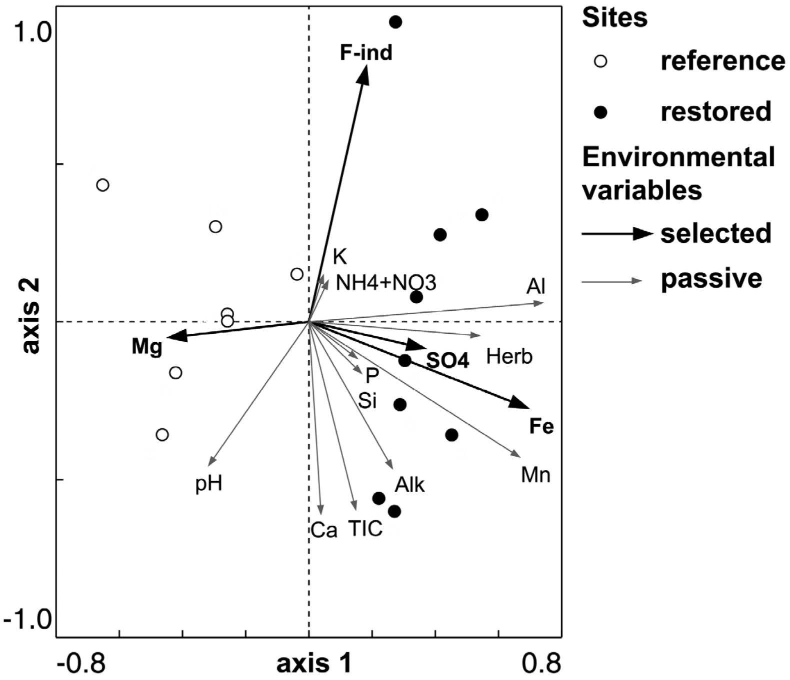

Fig. 1. – CCA biplot with scores for sites and environmental variables consisting of pore-water variables, indicator value for moisture (F-ind) and cover of herbaceous plant layer (Herb). Fe, SO_4^{2-}, Mg^{2+} and F-ind were selected for the model and the other environmental variables were incorporated as passive variables.

Table 2. – Mean values ± standard deviation of chemical features of the pore water collected shallowly (0–10 cm) and deep (40–150 cm) at reference (n = 7) and restored sites (n = 9). P-values of the Mann-Whitney U-test for difference are listed (significant in bold). TIC is total inorganic carbon, sulphide is water dissolved sulphide (H_2S, HS^-, S^{2-}). TIC is in µmol C·L^{-1}, all other variables except for pH are in µmol·L^{-1}.

Variable	Shallow layer			Deep layer		
	Reference sites	Restored sites	P	Reference sites	Restored sites	P
TIC	5687±1517	6490±3864	0.482	6657±2242	5831±4233	0.470
Alkalinity	4179±1277	4917±3550	0.747	4950±1528	4407±3586	0.252
pH	6.8±0.4	6.3±0.5	**0.003**	6.8±0.3	6.4±0.3	0.071
Ca^{2+}	1569±438	1548±1230	0.414	1861±686	1692±1604	0.408
Mg^{2+}	373±196	137±60	**0.000**	385±154	201±97	**0.016**
Na^+	235±115	266±281	0.608	184±97	314±248	0.351
K^+	16.1±18.9	11.4±9.2	0.690	15.6±17.3	17.6±17.3	0.758
Fe	44±46	1568±1478	**0.004**	75±92	412±722	0.055
Mn	3.0±2.3	20.0±22.9	**0.010**	3.1±1.9	12.8±23.5	0.607
Al	1.3±1.8	9.7±9.0	**0.009**	1.5±1.6	6.6±9.6	0.351
Si	197±70	212±130	0.635	384±234	274±123	0.470
Cl–	246±208	404±573	0.894	213±197	469±568	0.351
SO_4^{2-}	19±13	337±881	**0.042**	53±64	48±40	1.000
NH_4^+	9.3±8.9	25.7±37.1	0.115	103±141	21±21	0.071
NO_3^-	13.0±42.3	2.9±2.8	0.247	31.5±79.1	8.5±21.6	0.408
Soluble reactive P	1.4±1.8	1.5±2.0	0.835	1.7±1.8	0.3±0.2	**0.012**
Sulphide	0.3±0.3	1.2±1.7	0.311			

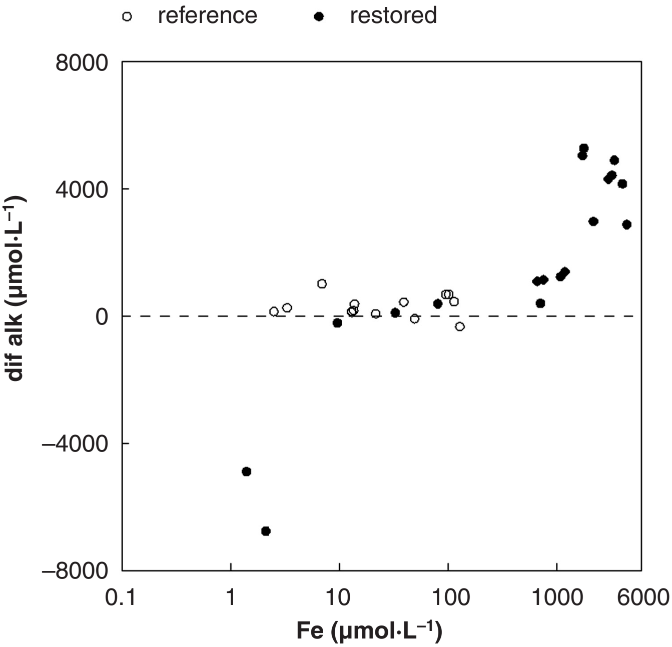

Fig. 2. – Fe concentration in topsoil pore water in relation to the difference between measured alkalinity and calculated carbonate derived alkalinity (dif alk = 2·(Ca^{2+} + Mg^{2+}) – alkalinity; concentrations in µmol·L^{-1}) of topsoil pore water. Points represent subsamples from reference and restored sites.

Fe, Mn, Al and SO_4^{2-} were higher at the restored sites, while pH and Mg^{2+} concentrations were higher at the reference sites. Most striking were differences in Fe concentrations. At some restored sites Fe was the dominant cation, while at reference sites this was always the case for Ca^{2+}. Sulphide concentrations were very low (< 10 $\mu mol \cdot L^{-1}$) at all sites and there were no differences between sites.

At restored sites the pore-water Fe concentration in the topsoil was 5 to 38 times greater than in the deeper layer, while it was not different between both layers at the reference sites. The Mn concentration showed a similar pattern.

A comparison of topsoil pore-water alkalinity with the values expected when alkalinity originates only from carbonate dissolution (Fig. 2) shows that subsamples from the reference sites were close to this value. Most subsamples from restored sites, however, had a much higher alkalinity than the calculated carbonate-derived alkalinity. This excess was closely associated with Fe pore-water concentrations.

Composition of topsoil

The topsoil at nearly all reference sites consisted of weakly-humified small sedge and brown moss peat. At all restored sites the organic matter was strongly decomposed and plant species were no longer recognizable in the upper peat layer. At these sites the topsoil was very black and contained small organic particles, and was of low consistency. Total C, Ca, Mg and S were greater in the topsoil at the reference sites, N content did not differ, and P, Fe, Mn, Al and Si were greater at the restored sites (Fig. 3). Differences were most striking for Fe and Mn. At restored sites Fe made up a large fraction of on average 4.8% dw, which is very different from that recorded at the reference sites (average 0.6% dw). Total Fe was positively correlated with total P ($r = 0.90$, $P < 0.001$) and Mn ($r = 0.51$, $P < 0.05$), and negatively correlated with Mg ($r = -0.73$, $P < 0.01$) and S ($r = -0.87$, $P < 0.001$).

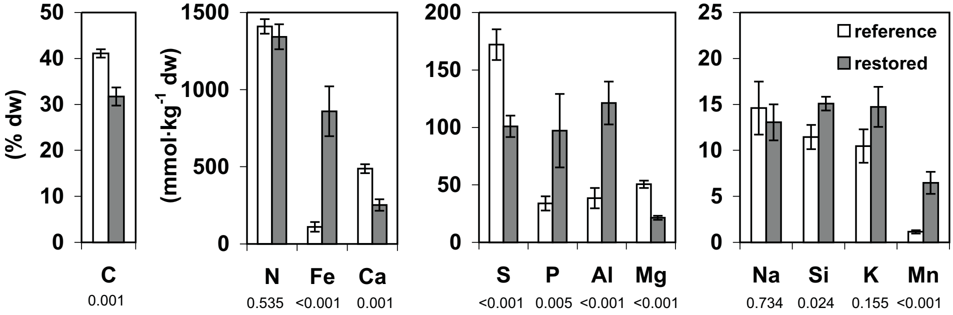

Fig. 3. – Pool sizes of different elements for reference and restored sites. Bars indicate mean values and stacks the standard error. P-values of the Mann-Whitney U-test for difference are indicated.

Phosphorus fractions in topsoil

The total P content of the topsoil was much greater at the restored sites than at the reference sites but organic P contents were similar (Fig. 4). The fraction of Fe+Al-bound P formed a major part of the inorganic P pool at the restored sites and was five times greater

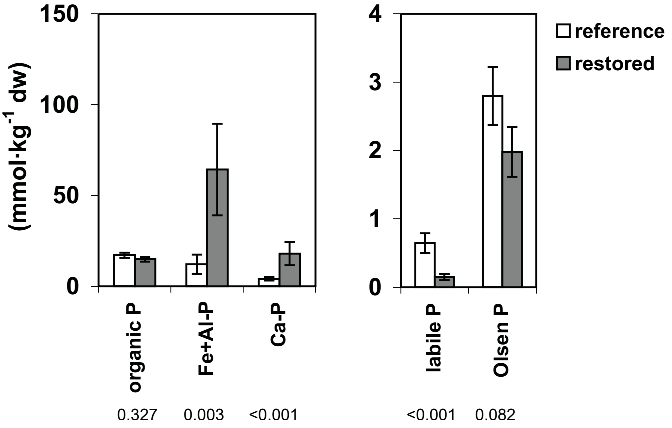

Fig. 4. – P-fractions for reference and restored sites. Bars indicate mean values and stacks the standard error. P-values of the Mann-Whitney U-test for difference are indicated.

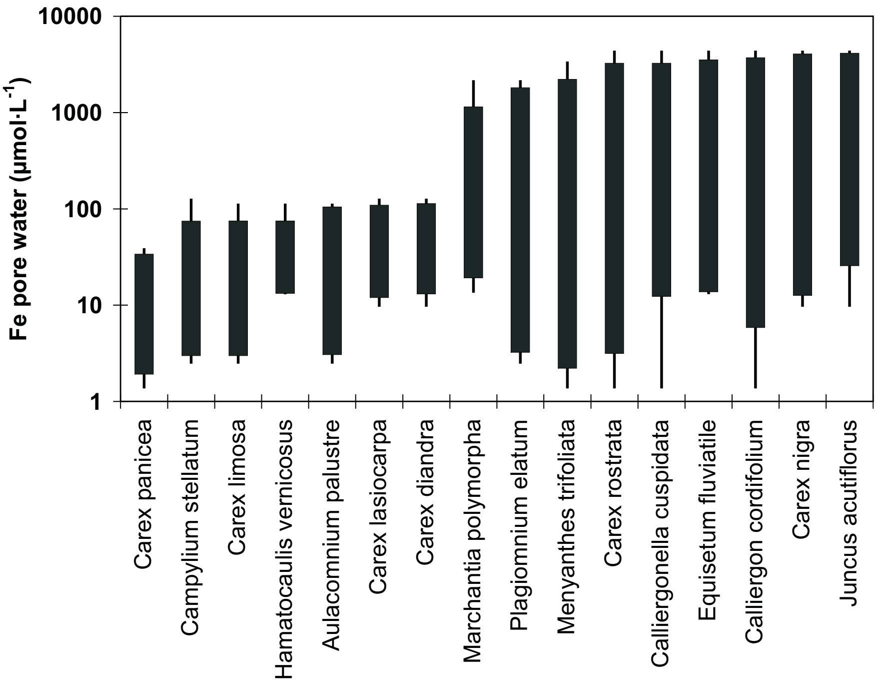

Fig. 5. – Occurrences of plant species in relation to Fe in the topsoil pore-water. Bars indicate 10 and 90 percentile and stacks minimum and maximum values. Species are sorted based on the value of the 90 percentile.

there than at the reference sites. The fraction of Ca-bound P was the second largest inorganic P fraction and was significantly greater at the restored sites. The labile P fraction was small at both but somewhat greater at the reference sites. The fractions of Ca-bound P, Fe+Al-bound P were positively correlated with total Fe content and not significantly

Table 3. – Correlation matrix for the organic P, Fe+Al-bound P, Ca-bound P, labile P, Olsen P and total Fe, Ca, Al content for the sites (n = 16). Spearman correlation coefficients are listed with indicators of P-values (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

	P(org)	P(FeAl)	P(Ca)	P(lab)	P(Olsen)	Total Fe	Total Ca
P_org							
P_FeAl	0.24						
P_Ca	0.27	0.98***					
P_lab	0.27	–0.30	–0.29				
P_olsen	0.22	0.50*	0.51*	0.14			
total Fe	–0.02	0.82***	0.75***	–0.52*	0.07		
total Ca	0.38	–0.48	–0.47	0.19	0.07	–0.64**	
total Al	–0.08	–0.16	–0.09	–0.31	–0.38	0.06	–0.30

correlated with total Ca and Al (Table 3). There were no significant differences in Olsen-P concentrations between the two groups, but this parameter did show a weak correlation with Ca-bound and Fe+Al-bound P fractions. There was a much stronger correlation with the ratio total Fe:Fe+Al-bound P ($P_{Olsen} = 21577 \cdot (\text{total Fe/Fe+Al-P})^{-0.8053}$; $r_{adj}^2 = 0.60$). At ratios of total Fe:Fe+Al-bound P < 22 $\text{mol} \cdot \text{mol}^{-1}$ Olsen-P increased strongly. We did not find any significant correlations between Olsen-P levels and the ratio of total Al:Fe+Al-bound P or of total Ca:Ca-bound P.

Plant species occurrence in relation to iron content

Most small-stature *Carex* species (*Carex diandra*, *C. lasiocarpa*, *C. limosa* and *C. panicea*) and several moss species (*Aulocomnium palustre*, *Campylium stellatum*, *Hamatocaulis vernicosus* and *Plagiomnium elatum*) were found only at sites with a low Fe pore-water concentration (Fe < 150 $\mu\text{mol} \cdot \text{L}^{-1}$). They were practically limited to reference sites (Fig. 5). Also the cover of all other species of moss except *Calliergonella cuspidata* was greater at these sites. There was a high cover of *C. cuspidata* and to a less extent also of *Menyanthes trifoliata* at sites with a high Fe concentration, whereas *Carex rostrata* and *Equisetum fluviatile* were almost completely restricted to sites with a high Fe concentration. However, even the cover of these species was low at sites where the Fe concentration was very high (> 2000–3000 $\mu\text{mol} \cdot \text{L}^{-1}$).

Discussion

Factors affecting the composition of the vegetation in rich fens

Although the restored sites in the Netherlands and Belgium were rewetted at least one decade ago their vegetation still differs considerably from that at the reference sites in Poland. The first axis of the CCA shows a clear separation between the two groups, correlated with differences in topsoil Fe, Mn and the concentration of Al in pore water. Fe concentration has the highest loadings on this axis, suggesting a dominant effect of variation in Fe on the composition of the vegetation. In addition, cover of herbaceous plants is also significantly correlated with the first axis, indicating that light availability at soil level is another important factor affecting the cover of mosses and low-growing vascular plants in fens (Kotowski et al. 2010). The second axis is related to Ellenberg moisture indicator values and the complex Ca-TIC alkalinity. Interestingly, the average loadings for restored and reference sites are similar on this axis but there is more scatter within the restored sites, suggesting more variation in wetness and pH between restored sites.

Unfortunately, the differences between restored fens and reference sites cannot be separated entirely from geographical effects. The reference sites, especially the most eastern ones, are subject to colder winters, longer snow cover and a smaller precipitation excess. These differences may affect rates of nutrient supply, length of the growing season and water regime (Richardson & Marshall 1986, Nicholson et al. 1996, Verhoeven et al. 1996). Despite such climatic effects we believe our comparison is sound. Palaeoecological research has shown that the composition of peat layers in river valleys in the Netherlands and nearby Germany is not very different from that of Polish systems (Grosse-Brauckmann 1979, Van Diggelen et al. 1991). Both consist of remnants of rich fen vegetation with a high frequency of *Carex limosa*, *Aulocomnium palustre*, *Calliergon giganteum*, *Scorpidium cossonni* and *S. scorpioides*. Moreover, the large differences between restored and reference sites we recorded in many chemical features of the top layer are not reflected in the characteristics of the groundwater in deeper layers, suggesting they are not caused by regional differences but by local processes. Despite climatic differences there is no difference in the Ellenberg moisture indicator values in the two regions. All these phenomena suggest that the effects of local processes are dominant and overrule any differences in climate.

Toxicity

The high pore-water Fe concentration recorded in most restored fens is well above the 180–450 $\mu mol \cdot L^{-1}$ limit and likely to be toxic for many species of fen plants (Snowden & Wheeler 1993). Because we only measured total Fe we cannot relate the sensitivity of species to ferrous-iron, but given the high pH and low redox value most of the pore-water iron will consist of ferrous-iron (Appelo & Postma 2005). A high Fe content in the pore water also seems to inhibit rich fen bryophytes, e.g. *Hamatocaulis vernicosus* occurs in our dataset only at sites where the Fe content is in the range of 13–114 $\mu mol \cdot Fe\ L^{-1}$. This covers most of the range of 3 to 48 $\mu mol \cdot L^{-1}$ reported in other studies (Štechová & Kučera 2007, Štechová et al. 2012). Only *Calliergonella cuspidata* can establish high cover under iron-rich conditions but at very high levels (> 3000 $\mu mol \cdot L^{-1}$) the cover of this species is low. At most iron-rich sites vascular plants with high stem porosity, like *Equisetum fluviatile* and *Carex rostrata* (Mainiero & Kazda 2004), are dominant, suggesting these species use ROL to counteract iron toxicity.

Under field conditions Mn toxicity is strongly associated with toxicity of other metals (El-Jaoual & Cox 1998) and cannot be separated from the effects of the latter. We did not find any indication of sulphide toxicity. The free sulphide concentrations were far below the threshold values of 50 to 700 $\mu mol \cdot L^{-1}$ reported by Geurts et al. (2009) and were not significantly different at the reference and restored sites. The high iron concentration obviously acts as a detoxicant by inducing the formation of iron sulphides (Van der Welle et al. 2008). There were also no differences in NH_4^+ between reference and restored sites and pore-water NH_4^+ concentrations were very low. We therefore consider ammonium toxicity to be unlikely.

Phosphorus availability

The inorganic P pool at the restored sites consists mainly of Fe-bound P and is much greater there than at reference sites. Interestingly, this is not reflected in greater Olsen-P levels, presumably because of a huge surplus of iron. Olsen-P levels at all sites are close to the threshold of 300 $\mu mol \cdot L^{-1}$ for which P limitation is assumed (Smolders et al. 2008). However, the question is whether plant-available P is really low at these restored sites. The high cover of large helophytes indicates a productive environment. All these species are capable of ROL and can acidify their rhizosphere by oxidizing ferrous iron and export of protons from their roots (Conlin & Crowder 1989, Begg et al. 1994), which enhances P availability (Hinsinger 2001). For such plants P accumulation under Fe-rich conditions may not necessarily imply a low P availability. This hypothesis contradicts the assumption that a high iron:phosphorus ratio is essential for low phosphorus availability (Koopmans et al. 2004) and is supported by high levels of acid-extractible P in rewetted fens (Zak et al. 2008).

Changes in soil chemistry caused by draining and subsequent rewetting

The greater pool of iron at restored than reference sites is partly the result of a concentration effect due to organic matter loss during the drainage period. However, this cannot explain the parallel loss of Ca, Mg and S. We hypothesize this is caused by recurring oxidation and reduction of iron (and sulphides) during the draining of the fen, together with a continuous supply via the groundwater of ferrous iron. When the water table is low during summer the topsoil is aerated and ferrous iron and sulphides are oxidized. The protons yielded by these reactions cause desorption of base cations from the exchange complex, and the released cations and sulphate partly leave the system together with the drainage water (McLaughlin & Webster 2010). Iron, however, has a much lower solubility under oxidizing conditions and remains in the topsoil. When the water table is high in winter and spring iron is reduced again. This process yields alkalinity and causes desorption of protons and partial readsorption of metals. This hypothesis implies that both the amount of iron that accumulates and the rate at which Ca and Mg are leached increase with the length of the drainage period.

Such alternating oxidation/reduction of iron strongly accelerates the anaerobic mineralization of organic material because ferric iron is available as an electron acceptor (Li et al. 2012). Decomposition will be further enhanced by a high availability of P (Tomassen et al. 2004). Accumulation of organic matter, and especially peat with a low degree of humification, is bound to be slow in fens where peat has accumulated with a large pool of iron in the topsoil. These may change irreversibly into systems with a strong cycling of carbon and nutrients.

Restoration prospects

Large iron and phosphorus pools in the topsoil can seriously affect the restoration potential of degraded fens. High ferrous iron concentrations in rewetted fens can reach high levels and act as a toxicant and exclude typical (rich) fen species, thereby lowering the prospects for restoring the biodiversity. In addition, peat-forming brown mosses and small *Carex* species may be affected and peat formation may be strongly reduced. In most cases the vegetation becomes dominated by iron-tolerant helophytes with high ROL capabilities, which can acidify the rhizosphere and enhance P mobilization. Removal of the top layer is then an option for restoring fen vegetation (Klimkowska et al. 2010). However, the efficiency of this measure depends at least on the vertical distribution of iron and phosphorus in the peat layer. Degraded fens with a low iron content seem to have much better prospects for restoration.

Acknowledgements

This study was funded by the Dutch O+BN Programme ‘Knowledge Network for Restoration and Management of Nature’. The contribution of Willem Jan Emsens was funded by the Bijzonder Onderzoek Fonds BOF of the University of Antwerp. We thank Mark Schoenmaeckers for his contribution to the field work, Martin Konert for his help with laboratory analyses, Dominik Zak for his suggestions and Tony Dixon for his improvements of the English.

Souhrn

Tato studie řeší otázku, do jaké míry může koncentrace železa v prostředí ovlivňovat úspěšnost obnovy minerálně bohatých slatinišť mírného pásma Evropy. Srovnávala obnovená slatiniště, která byla v minulosti dlouhodobě odvodněna a v současnosti jsou znovu zavodněna, s nenarušenými nebo jen slabě narušenými referenčními slatiništi a vztahovala chemické složení půdy (rašeliny) a půdní vody k druhovému složení vegetace. Druhové složení vegetace i chemismus svrchní vrstvy půdy na obnovených a referenčních slatiništích se výrazně lišily, zatímco chemismus vody v hlubších vrstvách (40–150 cm) vykazoval jen malé rozdíly. Rozdíly v druhovém složení mezi obnovenými a referenčními slatiništi nejlépe vysvětlovala koncentrace železa v půdní vodě a ve svrchní vrstvě půdy. Na obnovených slatiništích s koncentrací železa ve vodě vyšší než 100 μmol·L^{-1} chyběla řada druhů cévnatých rostlin a mechorostů, které jsou typické pro minerálně bohatá slatiniště hromadící rašelinu. Na většině obnovených slatinišť byla celková zásoba železa a anorganického fosforu (většinou vázaného na železo) výrazně větší než na referenčních slatiništích. Pozorované rozdíly jsou zjevně způsobeny lokálními procesy a nikoliv regionálními rozdíly v klimatu, které vyplývají z geografického rozmístění lokalit (referenční lokality se nacházely v Polsku, zatímco obnovená slatiniště v Nizozemsku a Belgii). Velká akumulace železa a fosforu na obnovených slatiništích je v této studii přisouzena dlouhé historii odvodnění, během níž vzrostla koncentrace oxidované formy železa ve svrchní vrstvě půdy a naopak poklesly koncentrace vápníku, hořčíku a síry. Důvodem jsou opakované oxidačně-redukční procesy, které nastávaly na odvodněných slatiništích během letních poklesů hladiny vody. Vysoká koncentrace železa, spojená rovněž s velkým obsahem fosforu, je tudíž důležitým a patrně i nevratným omezením při obnově jak biodiverzity, tak tvorby nehumifikované rašeliny. Pokud má ale obnovené slatiniště nízkou koncentraci železa, pravděpodobnost úspěšné obnovy je vyšší.

References

Adema E. B., van Gemerden H. & Grootjans A. P. (2003): Is succession in wet calcareous dune slacks affected by free sulfide? – J. Veg. Sci. 14: 153–162.

Appelo C. A. J. & Postma D. (2005): Geochemistry, groundwater and pollution. – CRC Press, Amsterdam.

Begg C. B. M., Kirk G. J. D., Mackenzie A. F. & Neue H. U. (1994): Root-induced iron oxidation and pH changes in the lowland rice rhizosphere. – New Phytol. 128: 469–477.

Conlin T. S. S. & Crowder A. A. (1989): Location of radial oxygen loss and zones of potential iron uptake in a grass and two non-grass emergent species. – Can. J. Bot. 67: 717–722.

El-Jaoual T. & Cox D. A. (1998): Manganese toxicity in plants. – J. Plant Nutr. 21: 353–386.

Ellenberg H., Weber H. E., Düll R., Wirth V., Werner W. & Paulißen D. (1992): Zeigerwerte von Pflanzen in Mitteleuropa. 2. Aufl. – Scr. Geobot. 18: 1–248.

Geurts J. J. M., Sarneel J. M., Willers B. J. C., Roelofs J. G. M., Verhoeven J. T. A. & Lamers L. P. M. (2009): Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: a mesocosm experiment. – Environ. Poll. 157: 2072–2081.

Golterman H. L. (1996): Fractionation of sediment phosphate with chelating compounds: a simplification, and comparison with other methods. – Hydrobiologia 335: 87–95.

Grosse-Brauckmann G. (1979): Zur Bedeutung einiger Macrofossil-Vergesellschaftungen unter dem Gesichtspunkt der Torfbilding. – In: Wilmanns O. & Tüxen R. (eds), Werden und Vergehen von Pflanzengesellschaften, p. 111–132, J. Cramer, Vaduz.

Hinsinger P. (2001): Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. – Plant and Soil 237: 173–195.

Jackson M. B. & Armstrong W. (1999): Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. – Plant Biol. 1: 274–287.

Klimkowska A., Grootjans A. P., Kotowski W. & Van Diggelen R. (2010): Prospects for fen meadow restoration on severely degraded fens. – Persp. Plant Ecol. Evol. Syst. 12: 245–255.

Koopmans G. F., Chardon W. J., Ehlert P. A. I., Dolfing J., Suurs R. A. A., Oenema O. & van Riemsdijk W. H. (2004): Phosphorus availability for plant uptake in a phosphorus-enriched noncalcareous sandy soil. – J. Environ. Qual. 33: 965–975.

Kotowski W., Beauchard O., Opdekamp W., Meire P. & van Diggelen R. (2010): Waterlogging and canopy interact to control species recruitment in floodplains. – Funct. Ecol. 24: 918–926.

Lamers L. P. M., Loeb R., Antheunisse A. M., Miletto M., Lucassen E. C. H. E. T., Boxman A. W., Smolders A. J. P. & Roelofs J. G. M. (2006): Biogeochemical constraints on the ecological rehabilitation of wetland vegetation in river floodplains. – Hydrobiologia 565: 165–186.

Li Y. C., Yu S., Strong J. & Wang H. L. (2012): Are the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus driven by the "Fe-III-Fe-II redox wheel" in dynamic redox environments? – J. Soils Sedim. 12: 683–693.

Londo G. (1976): The decimal scale for releves of permanent quadrats. – Vegetatio 33: 61–64.

Lucassen E. C. H. E. T., Smolders A. J. P., Lamers L. P. M. & Roelofs J. G. M. (2005): Water table fluctuations and groundwater supply are important in preventing phosphate-eutrophication in sulphate-rich fens: consequences for wetland restoration. – Plant and Soil 269: 109–115.

Lucassen E. C. H. E. T., Smolders A. J. P. & Roelofs J. G. M. (2000): Increased groundwater levels cause iron toxicity in *Glyceria fluitans* (L.). – Aquat. Bot. 66: 321–327.

Mainiero R. & Kazda M. (2004): Effects of *Carex rostrata* on soil oxygen in relation to soil moisture. – Plant and Soil 270: 311–320.

McLaughlin J. W. & Webster K. L. (2010): Alkalinity and acidity cycling and fluxes in an intermediate fen peatland in northern Ontario. – Biogeochemistry 99: 143–155.

Middleton B., Grootjans A. P., Jensen K., Olde Venterink H. & Margóci K. (2006): Fen management and research perspectives: an overview. – In: Verhoeven J. T. A., Beltman B. B. & Bobbink R. (eds), Wetlands: functioning, biodiversity, conservation, and restoration, p. 247–268, Springer Verlag, Berlin.

Nicholson B. J., Gignac L. D. & Bayley S. E. (1996): Peatland distribution along a north-south transect in the Mackenzie River Basin in relation to climatic and environmental gradients. – Vegetatio 126: 119–133.

Olde Venterink H., Kardel I., Kotowski W., Peeters W. & Wassen M. J. (2009): Long-term effects of drainage and hay-removal on nutrient dynamics and limitation in the Biebrza mires, Poland. – Biogeochemistry 93: 235–252.

Olde Venterink H., Wassen M. J., Belgers J. D. M. & Verhoeven J. T. A. (2001): Control of environmental variables on species density in fens and meadows: importance of direct effects and effects through community biomass. – J. Ecol. 89: 1033–1040.

Olsen S. R., Cole C. W., Watanabe R. & Dean L. A. (1954): Estimation of available phosphorus in soils by extraction with sodium bicarbonate. – US Department of Agriculture Circular No. 939, Washington, DC.

Paulissen M., Besalu L. E., De Bruijn H., Van der Ven P. J. M. & Bobbink R. (2005): Contrasting effects of ammonium enrichment on fen bryophytes. – J. Bryol. 27: 109–117.

Richardson C. J. & Marshall P. E. (1986): Processes controlling movement, storage, and export of phosphorus in a fen peatland. – Ecol. Monogr. 56: 279–302.

Schrautzer J., Sival F., Breuer M., Runhaar H. & Fichtner A. (2013): Characterizing and evaluating successional pathways of fen degradation and restoration. – Ecol. Indic. 25: 108–120.

Siebel H. & During H. (2006): Beknopte mosflora van Nederland en België [Moss flora of the Netherlands and Belgium]. – KNNV Publishing, Utrecht.

Smolders A. J. P., Lucassen E. C. H. E. T., van der Aalst M., Lamers L. P. M. & Roelofs J. G. M. (2008): Decreasing the abundance of *Juncus effusus* on former agricultural lands with noncalcareous sandy soils: possible effects of liming and soil removal. – Restor. Ecol. 16: 240–248.

Snowden R. E. D. & Wheeler B. D. (1993): Iron toxicity to fen plant species. – J. Ecol. 81: 35–46.

Štechová 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. – Biol. Conserv. 135: 443–449.

Štechová T., Kučera J. & Šmilauer P. (2012): Factors affecting population size and vitality of *Hamatocaulis vernicosus* (Mitt.) Hedenas (*Calliergonaceae*, Musci). – Wetl. Ecol. Manage. 20: 329–339.

ter Braak C. J. F. & Šmilauer P. (2002): CANOCO reference manual and Canodraw for windows user's guide: software for canonical community ordination (version 4.5). – Microcomputer Power, Ithaca.

Technicon Autoanalyzer Methodology (1981): Industrial method 635-81W. – Technicon, New York.

Timmermann T., Margóczi K., Takács G. & Vegelin K. (2006): Restoration of peat-forming vegetation by rewetting species-poor fen grasslands. – Appl. Veget. Sci. 9: 241–250.

Tomassen H. B. M., Smolders A. J. P., Lamers L. P. M. & Roelofs J. G. M. (2004): Development of floating rafts after the rewetting of cut-over bogs: the importance of peat quality. – Biogeochemistry 71: 69–87.

Van der Meijden R. (1996): Heukels' flora van Nederland. – Wolters-Noordhoff, Groningen.

Van der Welle M. E. W., Niggebrugge K., Lamers L. P. M. & Roelofs J. G. M. (2007): Differential responses of the freshwater wetland species *Juncus effusus* L. and *Caltha palustris* L. to iron supply in sulfidic environments. – Environ. Poll. 147: 222–230.

Van der Welle M. E. W., Roelofs J. G. M. & Lamers L. P. M. (2008): Multi-level effects of sulphur-iron interactions in freshwater wetlands in the Netherlands. – Sci. Total Environ. 406: 426–429.

Van Diggelen R., Middleton B., Bakker J., Grootjans A. & Wassen M. (2006): Fens and floodplains of the temperate zone: present status, threats, conservation and restoration. – Appl. Veg. Sci. 9: 157–162.

Van Diggelen R., Molenaar W., Casparie W. A. & Grootjans A. P. (1991): Paläoökologische Untersuchungen als Hilfe in der Landschaftsanalyse im Gorecht-Gebiet (Niederlande). – Telma 21: 57–73.

Verhoeven J. T. A., Beltman B., Dorland E., Robat S. A. & Bobbink R. (2011): Differential effects of ammonium and nitrate deposition on fen phanerogams and bryophytes. – Appl. Veg. Sci. 14: 149–157.

Verhoeven J. T. A., Keuter A., Van Logtestijn R., Van Kerkhoven M. B. & Wassen M. (1996): Control of local nutrient dynamics in mires by regional and climatic factors: a comparison of Dutch and Polish sites. – J. Ecol. 84: 647–656.

Wolejko L. (2002): Soligenous wetlands of north-western Poland as an environment for endangered mire species. – Acta Soc. Bot. Pol. 71: 49–61.

Zak D., Gelbrecht J., Wagner C. & Steinberg C. E. W. (2008): Evaluation of phosphorus mobilization potential in rewetted fens by an improved sequential chemical extraction procedure. – Eur. J. Soil Sci. 59: 1191–1201.

Received 31 January 2013
Revision received 29 March 2013
Accepted 1 April 2013

Appendix 1. – The fen sites investigated in Poland (PL), the Netherlands (NL) and Belgium (B).

Site code	Site name	Latitude	Longitude	Hydrology	Vegetation management
Reference sites >					
BZ1	southern basin Biebrza (PL)	53°17'11.9"	22°36'32.3"	not drained	in the past irregular haymaking and recently haymaking every winter
BZ2	Biebrza near Lipsk (PL)	53°42'5.8"	22°57'7.1"	not drained	in the past irregular haymaking and over the last decades periodical removal of shrubs and trees
DZ	Debrzynka (PL)	53°31'45.3"	16°59'36.5"	superficially drained in the past for several decades, rewetted during last decade	for several decades no management
MA	Luboń (PL)	54°01'33.0"	17°29'80.3"	not drained	last decades no management
RS1	Rospuda (PL)	53°54'24.9"	22°57'0.5"	not drained	idem
RS2	Rospuda (PL)	53°54'19.1"	23°21'36.8"	not drained	idem
SP	Gogolewko in Słupia Landscape Park (PL)	54°21'31.8"	17°25'65.6"	superficially drained in the past for several decades, rewetted during last decade	in the past haymaking, recently no management
Restored sites >					
BM	Bennekomse Meent (NL)	52°00'29.6"	5°35'47.5"	drained for centuries, rewetted during last decade	haymaking for many years
DA1	Drentse Aa (NL)	53°00'51.1"	6°37'46.2"	idem	idem
DA2	Drentse Aa (NL)	53°01'04.4"	6°37'43.7"	idem	idem
DA3	Drentse Aa (NL)	53°01'28.3"	6°40'00.8"	idem	idem
ES	Elperstroom (NL)	52°53'03.2"	6°40'00.1"	idem	idem
LS	Langstraat (NL)	51°41'17.1"	5°01'18.8"	idem	idem
ZB1	Zwarte Beek (B)	51°04'48.7"	5°17'10.1"	drained for centuries, rewetted several decades ago	idem
ZB2	Zwarte Beek (B)	51°05'28.2"	5°19'20.7"	drained for centuries, rewetted 2 decades ago	idem
ZB3	Zwarte Beek (B)	51°05'33.3"	5°19'29.2"	drained for centuries, rewetted several decades ago	in the past haymaking but over the, last 3 decades no management