Biogeochemical Constraints
for Restoration of
Sulphate-Rich Fens

een wetenschappelijke proeve op het gebied
van de Natuurwetenschappen, Wiskunde en Informatica

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Stichting
het Limburgs
Landschap

"Twenty years from now you will be more disappointed
by the things you didn't do than by the ones you did do.
So explore, dream, discover"

Mark Twain

Aan mijn ouders en grootouders
Voor Wesley

Contents

CHAPTER 1

General introduction: hydrological changes and biogeochemical problems in fens along the river Meuse

Esther C.H.E.T. Lucassen

Fens

Peaty wetlands can be divided into bogs and fens. The term bog is used for ombrotrophic (sometimes weakly minerotrophic) mires, roughly corresponding the phytosociological units Oxycocco-Sphagnetea, Scheuchzeretalia and Caricetalia nigrae. Fens are defined as minerotrophic base-rich mires, with pH values exceeding 5.5. They develop by vegetational succession from open water, which changes into a reedswamp of *Phragmites*, *Cladium* and large *Carex* species, and then to semi-terrestrial fen communities including fen carrs (woodlands) dominated by *Alnus*. When ongoing peat accumulation rises above the water level, *Alnus* woodland is transformed into *Pinus-Betula* woodland (Wheeler & Proctor, 2000). The diversity of fen types is large and mainly depends upon the nutrient status of the substratum (Table 1).

Figure 1. Photograph of a black alder carr with *Alnus glutinosa, Caltha palustris*, *Iris pseudacorus*, *Carex elongata* and *Equisetum fluviatile* at sites where iron-rich groundwater discharges to the surface. Photo: E. Lucassen.

Black alder carrs are dominated by *Alnus glutinosa* (L.) Gaertner, which is an indigenous tree species in a large part of Europe (Weeda et al., 1985). At sites where regional base-rich

groundwater permanently discharges to the surface, wetland plants like *Carex elongata* L., *Caltha palustris* L., *Equisetum fluviatile* L., *Cardamine amara* L. and *Carex acutiformis* Ehrh. occur (Figure 1) (Falinska, 1979; Jalink & Jansen, 1995; Klijn & Witte, 1999; Rosenberry et al., 2000). Groundwater inputs can be relatively high during the winter, while temporary droughts may occur in large parts of the forests during summer.

Table 1. Fen vegetation types in relation to trophic status and higher phytosociological units (according to Wheeler & Proctor, 2002).

Vegetation/Habitat type	Trophic Status	Phytosociological class, order or alliance
Small sedge fen	Oligotrophic/Mesotrophic	Scheuchzerio-Caricetea: Caricion davallianae
Slender sedge fen	Oligotrophic/Mesotrophic	Scheuchzerio-Caricetea: Caricion lasiocarpa
Fen meadow	Mesotrophic/Eutrophic	Molinietalia: Calthion, Molinion
Tall herb fen, reed fen	Mesotrophic/Eutrophic	Molinietalia: Filipendulion; Phragmitetea: Phragmition, Magnocaricion; Galio-Urticetea: Convolvulion
Tall sedge fen	Mesotrophic/Eutrophic	Phragmitetea: Magnocaricion
Fenn carr (fen woodland)	Mesotrophic/Eutrophic	Alnetea glutinosae: Alnion glutinosae; Salicion cinereae.
Wet woodland	Eutrophic	Salicetea purpureae: Salicion albae; Querco-Fagetea: Alno Ulmion.

Origin of fens along the river Meuse

Terrestrialised parts of former meanders of the river Meuse feature many fens. The Meuse, which is fed by rainwater and groundwater, originates in France on the Plateau de Langres, and flows through France, Belgium and the Netherlands, where it enters a lowland area before it flows into the North Sea. Over the last 700,000 years, the course of the river Meuse has gradually shifted to the north-east, due to movements in the earth's crust. Some 700,000 years ago, the Meuse meandered through the Centrale Slenk/Roerdalslenk area. As the Roerdalslenk, and the Peel Horst in a latter stage, subsided and turned, the river Meuse finally shifted towards the Venlo Slenk, a shift which occured some 400,000 years ago.

During the last ice ages of the Pleistocene, sea levels were constantly dropping, resulting in very high flow rates and increased erosion, resulting in the creation of river terraces (Figure 2). In the Holocene (the last 10,000 interglacial years) the river's reduced flow rates led to the deposition of thick clay layers. The course of the meanders changed constantly, and isolated wetlands developed on the clayey grounds of former meanders. The wetlands were fed by local groundwater infiltrating at higher terraces and/or by regional base-rich groundwater. Faults in the Peel Horst formed barriers to groundwater flow, which led to the development of seepage zones and the formation of peatlands (including black alder carrs)

during the Pleistocene and Holocene periods (Bense & de Vries, 2000). Despite the fact that the wetlands are no longer in permanent open contact with the Meuse, the river still has influences on water tables in the wetlands, depending on the water table in the river and the geomorphological conditions (Figure 2). During extremely wet winters, groundwater discharge is greatly reduced, and fens situated on the lower terraces are flooded by the river (Figure 3).

The total surface area covered by fens in Europe is relatively small. The fact that these ecosystems are restricted to extreme environments plays an important role. During the 19th and 20th century, large-scale drainage activities for agricultural purposus, as well as land re-allotments and cultivation, have led to a decrease in the area covered by fens and/or a reduction of their quality due to increased desiccation and eutrophication.

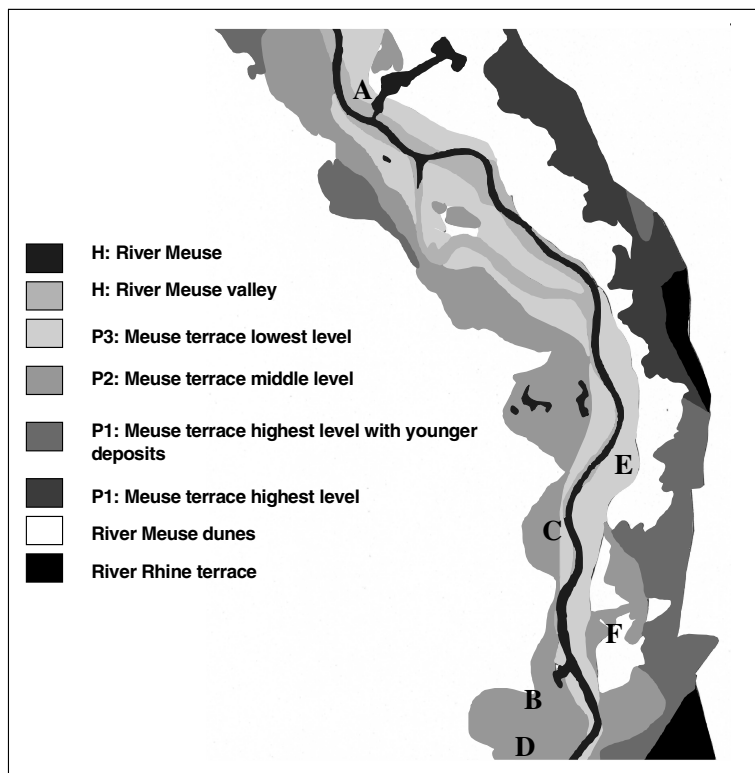**Figure 2.** Geomorphology of the river Meuse (in the province of Limburg, the Netherlands) with river terraces formed in the ice ages of the Pleistocene (P1-P3) and the current river valley formed during the Holocene (H) (modified from Wolfert & de Lange, 1990). A-E are black alder carrs which have developed in former meanders of the river Meuse: A, Heuloërbroek; B, Koelbroek; C, Kaldenbroek; D, Dubbroek; E, Lommerbroek; F, Venkoelen.

Figure 3. Heuloërbroek (near Bergen), situated at the lowest river terrace in December 2002 (left) and flooded by the river Meuse in January 2003. Photos: E. Lucassen & R. Peters.

Hydrological changes and impacts

Around 1970, groundwater tables in areas along the river Meuse were greatly lowered by building activities and/or deepening of drainage ditches, in order to create more land for cultivation and to enable re-allotments (Figure 4). The effect of drainage on fens was clearly documented for the "Castenrayse vennen" (near Venray) and "Spurkt" (near Kessel) where groundwater tables fell by 1 m, even when the drainage brooks were still dammed (Van den Munckhof, in prep). Groundwater levels in undrained fens clearly showed that these drops in groundwater table could not have been the result of climate changes. Lowered groundwater tables have led to severer, more persistent and more frequent summer droughts. These drier conditions in wetlands were accompanied by the disappearance of characteristic black alder carr species and a dominant growth of more drought-resistant species like *Rubus idaeus* L., *Rubus fructicosus* L., *Urtica dioica* L. and *Calamagrostis canescens* (Weber) Roth (Stortelder, 1998).

During the last 5 years, attempts have been made to dam drainage brooks, in order to raise water levels again and re-expand the area covered by characteristic black alder carr vegetation. It was found that complete damming of ditches resulted in high water tables but stagnating water in several fens. Contrary to what had been expected, however, the quality of the characteristic vegetation at seepage zones declined within one year, while massive development of *Lemna minor* L. and wetland grasses occurred (Boxman & Stortelder, 2000; Lucassen et al., 2000) (Figure 5).

Figure 4. Deepening of the Lobeek brook in the Spurkt area in August 1978. Photos: P. van den Munckhof.

Changes in groundwater quality

The quality of the groundwater in the Netherlands has changed during the 19th and 20th centuries. Increased agricultural activities have not only resulted in falling groundwater tables but also in increased NO_3^- and SO_4^{2-} concentrations in the groundwater. Increased desiccation and NO_3^- leaching from farmland have both favoured the oxidation of geological iron(di)sulphides (FeS_x) in the subsoil. The SO_4^{2-} mobilised by these processes contributes, together with increased SO_4^{2-} leaching from farmlands and increased atmospheric S deposition, to higher SO_4^{2-} concentrations in the groundwater (Kölle & Schreeck, 1982; Kölle et al., 1985).

Figure 5. Kaldenbroek in a dry condition in November 1977 (left) and after the damming of the drainage brook in 1998 (centre) and 1999 (right), showing massive development of *Lemna minor* and *Glyceria fluitans*. Photos: P. van den Munckhof (left) and E. Lucassen (centre & right).

Increased availability of SO_4^{2-} in wetlands has received much attention (Gorham, 1976; Johnson, 1979; Roelofs, 1983; Schindler et al., 1988; Smolders & Roelofs, 1993; Lamers et al., 1998). Increased availability of SO_4^{2-} as an electron acceptor in the breakdown processes of organic matter leads to increased SO_4^{2-} reduction rates in freshwater wetlands, which increases nutrient availability due to mobilisation of nutrients inside the system. This phenomenon is called internal eutrophication (Roelofs, 1991). Sulphide (S^{2-}) reduces the availability of free iron (Fe) by forming FeS_x and interferes with $o\text{-}PO_4^{3-}$ binding in iron phosphate complexes, leading to the release of $o\text{-}PO_4^{3-}$ to the water layer (Sperber, 1958; Boström et al., 1982; Caraco et al., 1990; Roelofs, 1991; Smolders & Roelofs, 1993). Oxidation of FeS_x leads to the production of acid and SO_4^{2-}, resulting in acidification of poorly buffered wetlands (Vangenechten et al., 1981; Schuurkes et al., 1988; Van Haesebroeck et al., 1997).
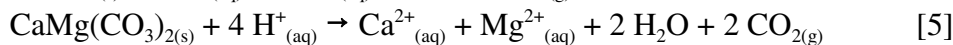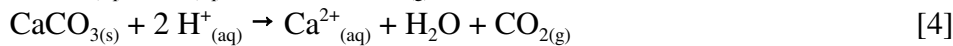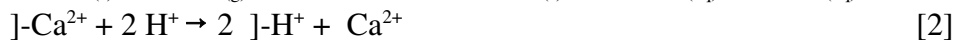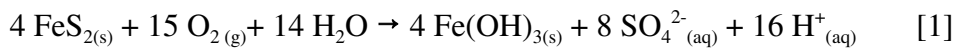
If NO_3^- is incompletely reduced in the subsoil, it is discharged to groundwater-fed wetlands. NO_3^- reduction is known to lead to oxidation of reduced Fe and inhibition of Fe and SO_4^{2-}

reduction, as it is an energetically more favourable electron acceptor than Fe and SO_4^{2-} (Uhlmann & Paul, 1994; Smolders et al., 1997). This indicates that NO_3^- might restrict the mobilisation of o-PO_4^{3-} in S-rich fens.

Desiccation and decreased groundwater input

Falling groundwater lables lead to more intense, persistent and frequent summer droughts. Aerobic conditions in the sediment promote the oxidation of FeS_x and production of SO_4^{2-} and protons (Eq. [1]). Protons react with the cation exchange buffer of the sediment (Eq. [2]), and bicarbonate (HCO_3^-) (Eq. [3]) and carbonates in the sediment, including calcite ($CaCO_3$) (Eq. [4]) and dolomite ($CaMg(CO_3)_2$) (Eq. [5]). Acidification and mobilisation of heavy metals might occur when the buffering capacity of the sediment is insufficient to compensate for the acids produced due to FeS_x oxidation (Drever, 1997).

In the surface water of the Spurkt black alder carr, SO_4^{2-} concentrations were 465 ± 93 µmol/l during the 1967-1973 period (21 measurements), while concentrations were 1443 ± 338 µmol/l during the 1993-1996 period (15 measurements) (Van den Munckhof, in prep). It is very likely that the change in SO_4^{2-} concentration is the result of the falling groundwater tables caused by the construction of drainage ditches in the 1970s. Although the black alder carrs along the river Meuse are fed by base-rich groundwater, it is possible that these processes cause acidification and mobilisation of heavy metals during dry summers or in dredged sediments. Differences between wetland species in the sensitivity to acidification and heavy metals, might stimulate the growth of more acid-tolerant wetland plants in parts that dry out during very dry summers. Input and reduction of Fe^{3+} and SO_4^{2-} from the groundwater can lead to Fe^{2+} and sulphide (S^{2-}) concentrations that are toxic to plants (Ponnamperuma, 1984; Koch & Mendelssohn, 1989; Koch et al., 1990; Armstrong et al., 1996; Smolders & Roelofs, 1996; Lamers et al., 1998). Therefore, decreased groundwater input might also stimulate the growth of wetland plants that are less tolerant to these toxins.

$$4\ FeS_{2(s)} + 15\ O_{2\,(g)} + 14\ H_2O \rightarrow 4\ Fe(OH)_{3(s)} + 8\ SO_4^{2-}{}_{(aq)} + 16\ H^+{}_{(aq)} \qquad [1]$$
$$]\text{-}Ca^{2+} + 2\ H^+ \rightarrow 2\ ]\text{-}H^+ +\ Ca^{2+} \qquad [2]$$
$$HCO_3^-{}_{(aq)} + H^+{}_{(aq)} \rightarrow H_2O + CO_{2(g)} \qquad [3]$$
$$CaCO_{3(s)} + 2\ H^+{}_{(aq)} \rightarrow Ca^{2+}{}_{(aq)} + H_2O + CO_{2(g)} \qquad [4]$$
$$CaMg(CO_3)_{2(s)} + 4\ H^+{}_{(aq)} \rightarrow Ca^{2+}{}_{(aq)} + Mg^{2+}{}_{(aq)} + 2\ H_2O + 2\ CO_{2(g)} \qquad [5]$$

On the other hand, decreased input of base-rich groundwater might also affect plant distribution more directly. Decreased input of groundwater rich in calcium (Ca) and iron (Fe) might affect nutrient-richness, as these ions effectively bind o-PO_4^{3-} (Stumm & Morgan, 1996; Golterman, 1998; Smolders et al., 2001) and as they can interact with other nutrients at

the cation exchange complex, like ammonium (NH_4^+) or potassium (K^+) (Koenig & Pan, 1996; Holmboe & Kristensen, 2002). Nitrogen, phosphorus and potassium are important factors that control plant productivity and species-richness in fens and meadows (Olde Venterink, 2000). Therefore desiccation might play an important role in stimulating the growth of rapidly growing wetland plants.

Damming of drainage brooks and decreased groundwater input

As the black alder carrs are fed by SO_4^{2-}-rich groundwater (Lucassen et al., 2002), the observed eutrophication could be caused by increased SO_4^{2-} reduction rates. Although SO_4^{2-} concentrations in the groundwater were already high before drainage brooks were dammed most systems had never shown signs of serious eutrophication under these conditions. This supports the view that a stagnating water layer favours accumulation of o-PO_4^{3-} in the water layer. Damming of groundwater prevents periodic droughts during the summer, which probably prevents regeneration of Fe^{3+} from reduced Fe compounds. Stagnating groundwater also leads to a restricted input of Fe (hydr)oxides and NO_3^- in wetlands. High NO_3^- loads might inhibit Fe and SO_4^{2-} reduction and might lead to oxidation of reduced Fe (Uhlmann and Paul, 1994; Smolders, 1997). Fe (hydr)oxides have a strong binding capacity for o-PO_4^{3-} (Smolders et al., 1995, 2001; Lamers et al., 2002). Stimulation of SO_4^{2-} reduction, in combination with decreased free Fe contents in the sediment, favours o-PO_4^{3-} mobilisation and may have caused eutrophication of black alder carrs after drainage ditches were dammed.

Outline of the thesis

This thesis investigates biogeochemical processes that occur in fens as a consequence of desiccation and the damming of groundwater. The main questions and hypotheses are presented in Table 2, with references to the chapters in which they are discussed. The answers to these questions as well as the consequences for restoration management are discussed in Chapter 9.

Table 2 Questions and hypotheses.

1. How sensitive are fens in the river Meuse area to drought-induced acidification and mobilisation of heavy metals ?

Desiccation will only result in acidification if the buffering capacity of the sediment is too low to neutralise the acid produced by FeS_x oxidation. The buffering capacity and the reduced S content will depend upon groundwater influence and seasonal variations in groundwater table. *Chapters 5 & 6.*

2. Which processes at the seepage sites in fens influence plant distribution under semi-natural conditions ?

Input of base cations might affect the sensitivity of seepage sites to desiccation, acidification and mobilisation of heavy metals. Subsequently, high inputs of Ca and Fe might affect nutrient availability, and high inputs of Fe and SO_4^{2-} may give rise to high concentrations of reduced toxins. *Chapters 2, 3 & 4.*

3. Which factors cause or prevent eutrophication in SO_4^{2-}-rich fens ?

Short water retention times might prevent the accumulation in the water layer of o-PO_4^{3-} transferred from the sediment. A constant input of groundwater and natural water table fluctuations will ensure input of Fe and regeneration of Fe^{3+} from reduced Fe compounds, respectively. High concentrations of Fe^{2+} might prevent the interaction of S^{2-} with Fe~PO_4^{3-} complexes, while high concentrations of Fe^{3+} favour reduction of Fe instead of SO_4^{2-}. Stagnation probably restricts groundwater input and prevents water table fluctuations, favouring Fe depletion and eutrophication. *Chapters 6 ,7 & 8.*

4. What are the effects of high NO_3^- concentrations in groundwater on the biogeochemistry of fens ?

NO_3^- might act as a more favourable electron acceptor than Fe and SO_4^{2-}. In addition, NO_3^- might oxidise reduced Fe compounds. Both might favour decreased availability of Fe^{2+} and o-PO_4^{3-}. *Chapters 7 & 8.*

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CHAPTER 2

Effects of base-rich groundwater discharge on ammonium availability and the distribution of fen plants

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To investigate which factors play an important role in the distribution of plants in groundwater-fed fens, a vegetation survey was carried out in 17 black alder carrs (fens dominated by *Alnus glutinosa*) along the river Meuse in the Netherlands. We sampled four different vegetation types of black alder carrs characterised by species groups: species-rich vegetation characteristic of groundwater discharge (Caltha palustris group), species-poor vegetations characteristic of eutrophic environments with stagnating groundwater (Glyceria fluitans group and Calla palustris group) and vegetations without these plant species, with fluctuating groundwater tables (Carex remota group). The results of a Canonical Correspondence Analysis (CCA) indicated that characteristics indicating groundwater discharge (such as total Ca, Mg and Fe in the sediment pore water and/or sediment), together with NH_4^+ in the pore water, best explained the distribution of the major fen vegetation types. The total Ca concentration in the sediment and the concentration of Ca, Mg, S^{2-} and o-PO_4^{3-} in the pore water differed significantly between the groups. The Caltha palustris group had the highest Ca concentration in the sediment and Ca and Mg concentrations in the pore water, whereas NH_4^+, S^{2-} and o-PO_4^{3-} concentrations in the pore water were lowest. NH_4^+ concentration in the pore water was negatively correlated with the total Ca, Mg and Fe concentrations in the sediment. We propose that high inputs of bivalent cations via the groundwater lead to saturation of the sediment adsorption complex in seepage zones, preventing the binding of NH_4^+, which is then continuously removed via the flowing groundwater. Sites with more fluctuating groundwater levels (Carex remota group) and sites with stagnating groundwater (Glyceria fluitans group and Calla palustris group) had a smaller influence of base-rich groundwater. This was accompanied by a lower saturation of the sediment adsorption complex with Ca, enabling a higher binding capacity for NH_4^+ which was accompanied by higher NH_4^+ concentrations in the pore water. The results are discussed in relation to rewetting strategies for desiccated wetlands.

Introduction

Black alder carrs are fens fed by base-rich groundwater, dominated by *Alnus glutinosa* (L.) (black alder) (Wheeler & Proctor, 2000). Groundwater influence is relatively large during the winter, whereas temporary desiccation may occur in large parts of the fens during the summer (Stortelder et al., 1998). Sites that are constantly fed by groundwater are species-rich and dominated by *Caltha palustris* L. (marsh marigold)*, Equisetum fluviatile* L. (swamp horsetail), *Carex pseudocyperus* (cyperus sedge)*, Ranunculus lingua* (greater spearwort) and *Carex acuta* L. (slender tufted sedge). These plant species are indicative of groundwater discharge (Falinska, 1979; Jalink & Jansen, 1995; Klijn & Witte, 1999; Rosenberry et al., 2000). Groundwater tables in the Netherlands have been greatly lowered in recent decades, largely in order to favour agricultural acitivities, resulting in the partial desiccation of many wetlands in agricultural areas. As a result, the above characteristic plant species have declined in abundance, while their accompanying species and plant species characteristic of eutrophic environments, like *Glyceria fluitans* (L.) (flote-grass)*, G. maxima* (Hartman) Holmberg (reed sweetgrass) and *Lemna minor* L. (common duckweed) have increased. Plants indicative of eutrophic environments mainly dominate in depressions with stagnating groundwater (Schaminée et al., 1995; Jalink & Jansen, 1995; Stortelder et al., 1998).

The distribution of wetland plants can be influenced by several physico-chemical factors. High concentrations of reduced toxins, such as reduced iron (Fe^{2+}), may influence the distribution of various wetland plant taxa depending upon their tolerance mechanisms (Talbot & Etherington, 1987; Snowden & Wheeler, 1993; Wheeler et al., 1985; Snowden & Wheeler, 1995). Sulphide (S^{2-}) is also known to be toxic to several wetland species, thus affecting plant distribution (Smolders et al., 1995,1996; Armstrong et al., 1996; Lamers et al., 1998; Fogli et al., 2002; Lucassen et al., submitted[a]). In addition, nutrient availability can play an important role in the distribution of fen plants (Olde Venterink, 2000). Increased phosphate (o-PO_4^{3-}) concentrations cause serious eutrophication and a dominance of floating species, such as *Lemna* spp. and algae. High nutrient concentrations are likely to promote the growth of fast-growing wetland grasses (Lucassen et al., 2000[b]; Lucassen et al., submitted[a]).

The above factors might all play a role in determining changes in the vegetation composition due to fen desiccation. Falling water tables and infiltration of nitrate (NO_3^-) from agricultural land both increase groundwater sulphate (SO_4^{2-}) concentrations, by favouring the oxidation of pyrite in the subsoil (Stumm and Morgan, 1981; Nordstrom, 1982; Kölle et al., 1985). The feeding groundwater of many fens in the Netherlands therefore contains high SO_4^{2-} concentrations, up to 1.5 mmol l^{-1} (Lucassen et al., 2002[a]). Toxic S^{2-} concentrations may build up in organic sediments as a result of SO_4^{2-} reduction (Lamers et al., 1998). In addition, many wetlands near the river Meuse are partly fed by regional groundwater containing high concentrations of iron (Fe) (Lucassen et al., 2002[a]). Fe^{2+} strongly binds to S^{2-}, under

formation of iron (di)sulphides ($FeS_{(x)}$) (Smolders et al., 1995) and it therefore remains unclear whether both toxins play a major role in the distribution of plant species in fens. In addition, the regional groundwater is rich in Ca and Fe, and zones that are strongly influenced by groundwater are likely to have relatively low o-PO_4^{3-} concentrations, as these ions immobilise o-PO_4^{3-} under formation of $CaPO_4$ and $FePO_4$ precipitates (Stumm & Morgan, 1981; Golterman, 1998; Lucassen et al., submitted[b]). The base-richness of the groundwater may also affect NH_4^+ availability, due to its effect on composition of exchangeable cations (Nommik & Vatras, 1982; Scheffer & Schachtschabel, 2002). It is also possible that increased drought periods during the summer lead to increased NH_4^+ concentrations in the pore water by stimulating mineralisation (Schuurkes et al., 1988).

To investigate which factors (e.g. nutrients, toxins) play an important role in causing changes in the composition of the vegetation due to changed groundwater inputs, we sampled 17 fens near the river Meuse. Most fens are situated in terrestialized meanders of the river Meuse and are only very locally fed by groundwater at sites were the clay layer is disrupted. These seepage zones can be visibly distinguished from other parts of the fens as groundwater is continuously discharged and, as a consequence, the surface water at these locations does not freeze during winter time. Vegetation types sampled included, species-rich vegetation stands indicative of groundwater discharge, species-poor vegetations indicative of eutrophic environments (with stagnating groundwater) and vegetation types without these species. Sediment and pore water were collected and vegetation and abiotic data were analysed by Canonical Correspondence Analysis (CCA).

Materials and Methods

Vegetation survey
Seventeen black alder carrs in the river Meuse region of the Netherlands were sampled during the summer of 1999 (Figure 1). The fens have a high groundwater discharge rate during the winter, while large parts dry out temporarily during the summer. We sampled species-rich vegetations dominated by plant species characteristic of groundwater discharge (including *Caltha palustris*, *Equisetum fluviatile* and *Carex pseudocyperus*) and sites with stagnating groundwater dominated by plant species characteristic of eutrophic environments (like *Glyceria fluitans*, *G. maxima* and *Lemna minor*). In addition, we sampled sites where these vegetation types were absent.

At each location, the coverage of each species was estimated in 1 m^2 plots. In the field, pore water was collected anaerobically at a depth of 5-15 cm with the help of Rhizon SMS soil moisture samplers (Rhizon SMS-5 cm; Eijkelkamp Agrisearch Equipment, the Netherlands) that were connected to a 100 ml vacuum serum bottle that was flushed with N_2 before drawing out the gas. A 1 kg sediment sample was collected at the same location and depth. A

50 g portion of each sediment sample was dried (24 h at 70 ^{0}C) in duplicate to determine its moisture content. Half of the dried samples were homogenised and a 200 mg portion was digested for 17 minutes with 4 ml concentrated nitric acid and 1 ml 30% hydrogen peroxide, using a Milestone type mls 1200 Mega microwave. Digestates were stored in iodated polyethylene bottles at 4 ^{0}C, until further chemical analysis. The other half of the dried sediment samples were incinerated (6 h at 550 ^{0}C) to determine the organic matter content. In order to estimate the sensitivity of the sediment to desiccation and acidification, 250 g portions of fresh sediment were gradually dried in a funnel (r = 7.5 cm; h = 10 cm) and placed in an Erlenmeyer flask (500 ml) at room temperature. The outflow of the funnel was covered with plastic gauze to prevent loss of sediment. After a standardised period of 2 months, the by then completely dry sediments were rewetted with demineralised water up to 250 g to obtain the original moisture content, and rehomogenised by shaking for 24 hours at 100 rpm in a 1-l polyethylene bottle. Subsequently, pore water samples were collected with the help of Rhizon SMS soil moisture samplers connected to 100 ml vacuum serum bottles (Lucassen et al., 2002[b]).

Chemical analysis
pH of the sediment pore water samples was measured using a standard Ag/AgCl$_2$ electrode connected to a Radiometer Copenhagen type PHM 82 standard pH meter. HCO$_3^-$ analyses were carried out using an 'Oceanography International' model 0525 HR infrared carbon analyser. The concentration of free S^{2-} was determined in a 10.5 ml subsample, fixed with S^{2-} antioxidant buffer (containing sodium hydroxide, sodium EDTA and ascorbic acid) immediately after collection (Van Gemerden, 1984), using a S^{2-} ion-specific Ag elecrode and a double junction calomel reference electrode. Half of the remaining samples were stored at 4 ^{0}C in glass test tubes with 2% concentrated HNO$_3$ to avoid metal precipitation. Total Ca, Mg, S, Mn, Fe, Al, P, Zn was analysed using an inductively coupled plasma emission spectrophotometer (ICP, Spectroflame). The remaining samples were stored at -23 ^{0}C in iodated polyethylene bottles and analysed for NO$_3^-$, NH$_4^+$ and o-PO$_4^{3-}$ using Technicon II autoanalysers according to Technicon (1969), Grasshoff and Johansen (1977) and Henriksen (1965) respectively.

Data analysis
Vegetation and abiotic data were analysed by Canonical Correspondence Analysis (CCA) (CANOCO; Ter Braak & Šmilauer, 1998). In a CCA, species and environmental data are used to arrange the sites along the ordination axes. Prior to statistical analysis, the vegetation data (% cover) were subjected to a ln(y+1) transformation so that the data more closely approximated a normal distribution. Species occurring in less than 5% of the relevés were removed from the data set (using the option 'downweighting of rare species' in the

CANOCO program). To rank the environmental variables, they were subjected to forward selection (Ter Braak & Šmilauer, 1998). The significance of the relationship between the species ordination and the environmental variables, plus the significance of the axes, was tested using a Monte Carlo permutation test (Ter Braak & Šmilauer, 1998). Only those variables that were significant ($p < 0.05$) and had a correlation coefficient > 0.15 were retained in the models. In addition, a one-way Analysis of Variance (ANOVA) with Tukey post hoc-test (GLM procedure, SPSS 11.0) was performed to assess differences in concentrations of elements between the vegetation types.
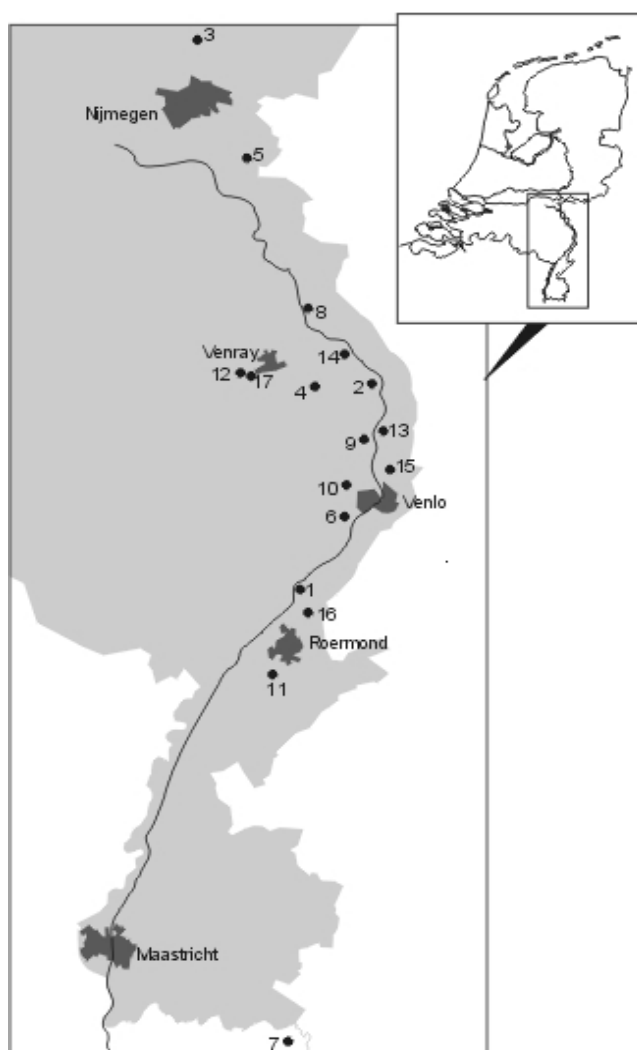

Figure 1. Geographical positions of the sampled fens. 1, Beeselsbroek; 2, Broekhuizerschuitewater; 3, Bronbeek; 4, Castenrayse Vennen; 5, De Bruuk; 6, Dubbroek; 7, Geuldal; 8, Heuloërbroek; 9, Kaldenbroek; 10, Koelbroek; 11, Landgoed Hoosden; 12, Spurkt; 13, Lommerbroek; 14, Roekenbosch; 15, Schaapsbroek; 16, Swalmdal; 17, Weversloosbroek.

Figure 2. Photographs of the 4 investigated fen vegetation types. A, **Caltha palustris group** with dominant growth of *Caltha palustris* (front) and *Equisetum fluviatile* (back) accompanied by *Carex elongata* and *Iris pseudacorus*; B, **Glyceria fluitans group** with monotonous growth of *Glyceria fluitans*; C, **Calla palustris group** with dominant growth of *Calla palustris* accompanied by *Lemna minor* and *Juncus effusus*; D, **Carex remota group** with dominant growth of other plant species than above, in this case *Carex paniculata*.

Results

Species-rich vegetation types were dominated by *Caltha palustris*, *Equisetum fluviatile*, *Carex pseudocyperus*, *Ranunculus lingua* and *Carex acuta* (**Caltha palustris group**). The vegetation type with plant species characteristic of eutrophic environments was dominated by *Glyceria fluitans, Lemna minor* and *Glyceria maxima* (**Glyceria fluitans group**). *Calla palustris* (water arum) is known to be a plant species of oligotrophic conditions (Vanden Berghen, 1952). However in the former meanders of the river Meuse area it used to grow predominantly in depressions with stagnating groundwater or at drier sites with clayey subsoils, accompanied by plant species characteristic of eutrophic environments (Schaminée et al., 1995) (**Calla palustris group**). The sites without the above plant species were dominated by *Carex remota* L. (remote sedge)*, Solanum dulcamara* L. (woody nightshade)*, Calamagrostis canescens* (Weber) Roth (purple small reed)*, Mentha aquatica* L. (water

mint)*, Lycopus europaeus* L. (gipsywort) and *Carex paniculata* L. (greater tussock sedge) (**Carex remota group**) (Table 1). Photographs of the four fen vegetation groups are given in Figure 2.

Table 1. Vegetation composition at locations continuously fed by groundwater (**Caltha palustris group**); Vegetation composition of locations dominated by plant species characteristic of eutrophic environments (**Glyceria fluitans group**); Vegetation composition of locations dominated by Calla palustris accompanied by plant species characteristic of eutrophic environments (**Calla palustris group**); Vegetation compostion of locations without the dominant species of the Caltha palustris, Glyceria fluitans or Calla palustris groups (**Carex remota group**). The dominant and accompanying species are given as well as the % cover (mean ± standard deviation). n= total number of locations sampled; freq = frequency / number of locations where the species was present.

n	dominant species	freq	cover	accompanying species	freq	cover
	Caltha palustris group					
44	*Caltha palustris*	18	27 ± 24	*Filipendula ulmaria*	14	11 ± 12
	Equisetum fluviatile	11	12 ± 21	*Solanum dulcamara*	14	3 ± 4
	Carex pseudocyperus	13	20 ± 25	*Cardamine pratensis*	12	7 ± 13
	Ranunculus lingua	5	36 ± 29	*Iris pseudacorus*	9	14 ± 22
	Carex acuta	4	29 ± 20	*Carex elongata*	8	13 ± 28
				Lycopus europaeus	8	8 ± 11
				Mentha aquatica	7	<5
				Lysimachia vulgaris	7	1
				Cardamine amara	6	14 ± 20
				Phragmites australis	5	18 ± 17
				Sparganium erectum	5	<5
				Ranunculus repens	4	<5
				Lemna minor	4	1
				Galium palustre	3	19 ± 27
				Rorippa nasturtium-aquat.	3	4 ± 2
				Carex acuta	2	<5
				Calla palustris	1	1
				Carex paniculata	1	10
				Glyceria maxima	1	1
	Glyceria fluitans group					
21	*Glyceria fluitans*	9	66 ± 12	*Solanum dulcamara*	4	<5
	Lemna minor	9	21 ± 36	*Iris pseudacorus*	3	30 ± 10
	Glyceria maxima	8	55 ± 30	*Calamagrostis canescens*	2	1
				Galium palustre	2	<5
				Sparganium erectum	2	15
				Lysimachia vulgaris	2	8
				Calla palustris	1	1

Table 1. Continued

n	dominant species	freq	cover	accompanying species	freq	cover
				Caltha palustris	1	25
				Carex paniculata	1	20
				Carex pseudocyperus	1	20
				Equisetum fluviatile	1	1
				Mentha aquatica	1	<5
				Potentilla palustris	1	10
	Calla palustris group					
12	*Calla palustris*	12	49 ± 37	*Lemna minor*	5	17 ± 15
				Galium palustre	4	21 ± 28
				Juncus effusus	3	<5
				Lycopus europaeus	3	1
				Solanum dulcamara	3	1
				Sparganium erectum	2	8
				Sphagnum squarrosum	2	<5
				Carex acuta	1	50
				Glyceria fluitans	1	1
				Iris pseudacorus	1	30
				Lysimachia vulgaris	1	1
				Potentilla palustris	1	10
				Ranunculus lingua	1	<5
				Ranunculus repens	1	1
	Carex remota group					
31	*Carex remota*	6	49 ± 37	*Carex pseudocyperus*	8	8 ± 11
	Solanum dulcamara	6	30 ± 29	*Sparganium erecetum*	5	3 ± 4
	Calamagrostis canescens	5	39 ± 37	*Lysimachia vulgaris*	4	5 ± 5
	Mentha aquatica	5	27 ± 38	*Galium palustre*	3	5 ± 5
	Lycopus europaeus	5	11 ± 16	*Iris pseudacorus*	3	1
	Carex paniculata	4	75 ± 0	*Sphagnum squarrosum*	2	7,5
				Lemna minor	2	1
				Glyceria maxima	2	<5
				Glyceria fluitans	2	15
				Phragmites australis	2	1
				Caltha palustris	1	1
				Cardamine pratensis	1	1
				Equisetum fluviatile	1	1
				Carex elongata	1	1
				Carex acuta	1	40
				Ranunculus lingua	1	1
				Ranunculus repens	1	30

Figure 3 shows the results of the CCA of the relevés, together with the characteristics of sediment and sediment pore water that best explained the variation in plant distribution. Characteristics indicating groundwater discharge, such as Ca, Mg, Fe and HCO_3^- in the pore water, explained an important part of the variation in species composition. Figure 3 shows a difference in range between sites with groundwater discharge, characterised by high Ca, Mg and Fe in the sediment (pore water), and sites with less groundwater discharge, characterised by increased NH_4^+ concentrations. The Caltha palustris group mainly appears in the right part of the diagram, with high concentrations of Ca, Mg and HCO_3^- in the pore water and high total Fe, Mg and Ca concentrations in the sediment. The Glyceria fluitans group and the Calla palustris group are mainly located in the opposite, lower left part of the diagram, indicating high NH_4^+ concentrations. The Carex remota group is not restricted to any particular part of the diagram but mainly occurs in the lower right and upper left parts.

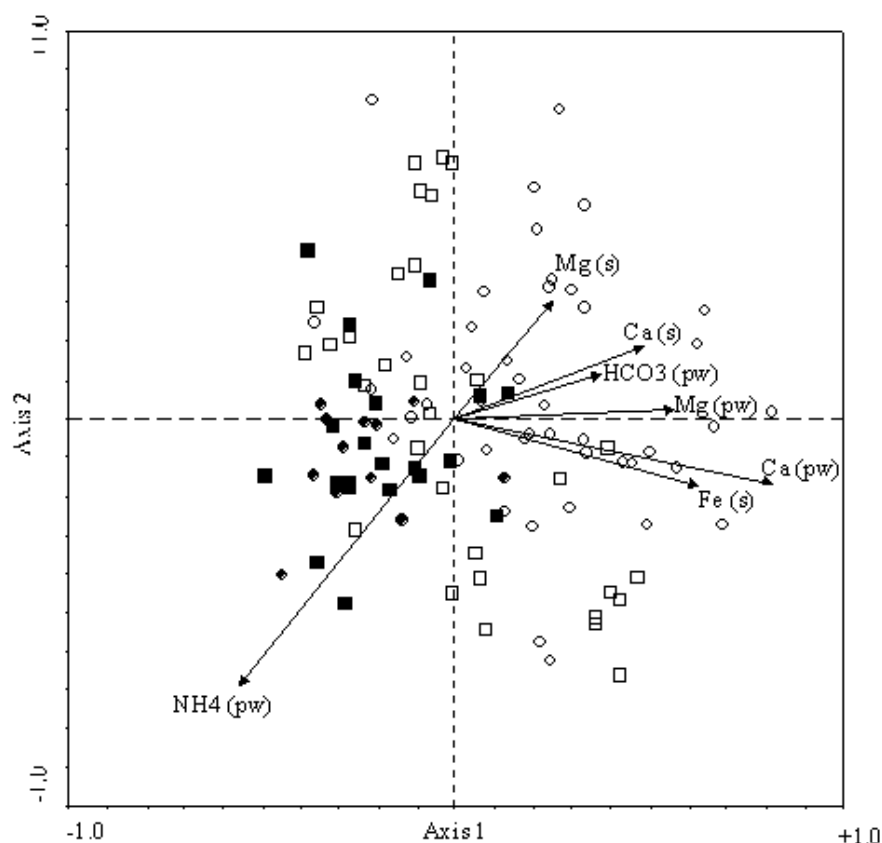**Figure 3.** CCA ordination diagram for the first two axes with sites and main environmental variables (arrows). Eigenvalues for axes 1 and 2 are 0.47 and 0.27, respectively; the sum of all canonical values is 1.55; Monte Carlo test: significant (F-ratio = 1.964, *P* = 0.005). ○, Caltha palustris group; ■, Glyceria fluitans group; ●, Calla palustris group; □, Carex remota group. pw = pore water; s = sediment.

Table 2. Means (± and standard error of the mean) of parameters, including those that best explain variation in plant species distribution according to CANOCO. **N** = number of sublocations; **pw** = pore water (μmol l^{-1}); **s** = sediment (μmol g dw^{-1}); org = organic content; moist = moisture content. Means with the same letter do not differ significantly.

Group	N	NH$_4^+$ (pw)	HCO$_3^-$ (pw)	Ca (pw)	Mg (pw)	Fe (s)	Ca (s)
Caltha palustris	44	33 (5)a	2602 (31)a	2064 (113)b	430 (33)b	1508 (215)a	619 (145)b
Glyceria fluitans	21	213 (46)bc	1777 (342)a	1001 (141)a	263 (26)a	587 (89)a	190 (35)a
Calla palustris	12	198 (25)c	1427 (279)a	886 (186)a	262 (33)a	755 (169)a	241 (40)ab
Carex remota	31	139 (22)b	2230 (374)a	1745 (208)b	309 (24)ab	1038 (217)a	346 (30)ab
		Mg (s)	org (%)	moist (%)	S^{2-} (pw)	o-PO$_4^{3-}$ (pw)	pH (pw)
Caltha palustris		44 (8)a	56 (3)a	83 (1)a	0.6 (0.3)a	1.6 (0.3)ab	6.9 (0.1)a
Glyceria fluitans		45 (6)a	52 (7)a	78 (4)a	8 (3)bc	7 (3)b	6.4 (0.5)a
Calla palustris		66 (13)a	68 (8)a	84 (5)a	23 (8)c	0.9 (0.2)a	6.3 (0.1)a
Carex remota		44 (5)a	64 (3)a	84 (5)a	6 (2)ab	4 (2)ab	6.5 (0.1)a

The mean NH$_4^+$ concentration in the pore water of the Caltha palustris group was significantly lower (33 μmol l^{-1}) than the corresponding values for the Carex remota group (139 μmol l^{-1}), the Glyceria fluitans group (213 μmol l^{-1}) and the Calla palustris group (198 μmol l^{-1}). The Caltha palustris group showed a significantly higher mean Ca and Mg concentration and a significantly lower S^{2-} concentration in the pore water compared to the other three groups. Also the mean total Ca concentration in the sediment of the Caltha palustris group was significantly higher than those of the other groups. There were no significant differences in other parameters with the exception of the mean o-PO$_4^{3-}$ concentration in the pore water, which was significantly higher for the Glyceria fluitans group compared to the other groups (Table 2).

Figure 4 shows that there was a negative correlation between the NH$_4^+$ concentration in the pore water and the total content of the cations Ca, Mg and Fe in the sediment. There were no such correlations between NH$_4^+$ in the pore water and the organic content, the moisture content or the pH of the pore water.
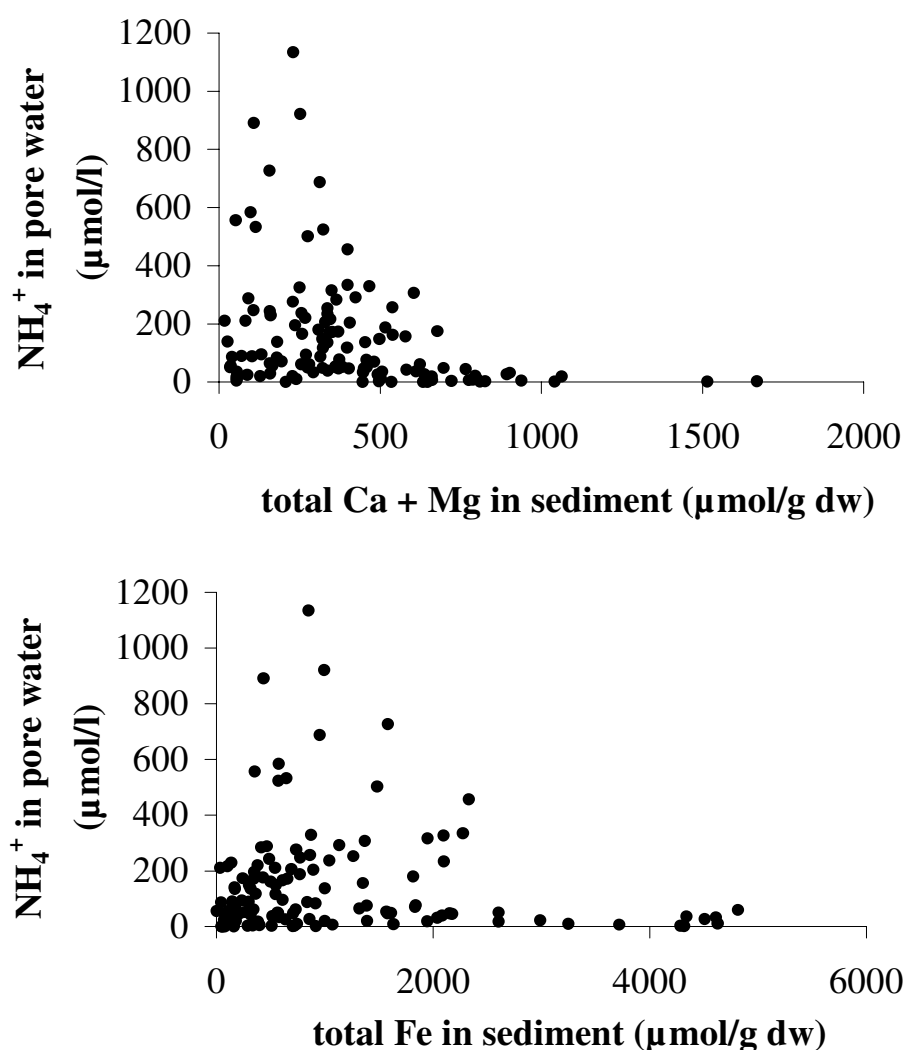
Figure 4. Scatterplots showing NH_4^+ concentration in the pore water as a function of the total Ca + Mg and the total Fe content of the sediment.

Discussion

The results of the CCA show that parameters indicating base-rich groundwater in combination with the NH_4^+ concentration in the pore water best explained the variation in distribution of the major fen vegetation types (Figure 3). The total Ca concentration in the sediment and the Ca, Mg, S^{2-} and $o\text{-}PO_4^{3-}$ concentrations in the pore water differed significantly between groups. In contrast to the other vegetation types, the Caltha palustris group was characterised by a relatively high Ca concentration in the sediment and relatively high concentrations of Ca and Mg in the pore water, whereas NH_4^+, S^{2-} and $o\text{-}PO_4^{3-}$

concentrations in the pore water were relatively low (Table 2). The NH_4^+ concentration in the pore water was found to be negatively correlated with the total Ca, Mg and Fe content of the sediment (Figure 4).

NH_4^+ availability seemed to be an important factor in explaining the distribution of plant species in these groundwater-fed wetlands. NH_4^+ adsorption in riparian sediment can be extensive and is coupled to supplies from the groundwater, ammonification and transformation of dissolved inorganic nitrogen by nitrification, denitrification and nitrate reduction (Triska et al., 1994). The NH_4^+ adsorption capacity is further influenced by the presence of competitive cations with bivalent and trivalent cations in particular suppressing the binding of NH_4^+, more than monovalent cations (Nommik & Vahtras, 1982; Scheffer & Schachtschabel, 2002). Suppressed binding of NH_4^+ by bivalent cations occurs in mangrove sediments where high Fe concentrations reduce the NH_4^+ adsorption capacity of the sediment (Holmboe & Kristensen, 2002). In addition, Koenig & Pan (1996) showed that Ca addition increases NH_4^+ availability and adsorption to plants in NH_4^+ fertilised dry soils. The NH_4^+ concentration in pore water is linearly related to the concentration of NH_4^+ adsorbed onto sediment solids in a dynamic equilibrium, whereby the adsorptive capacity for NH_4^+ depends on the ion exchange capacity associated with the organic matter and the clay content of the sediment (Rosenfeld, 1979; Boatman & Murray, 1982; Raaphorst & Malschaert, 1996). It might therefore be expected that a decreased adsorption of NH_4^+ would lead to decreased NH_4^+ concentrations in the sediment pore water.

The differences in NH_4^+ concentrations between the vegetation types cannot be explained by differences in the availability of organic matter. Nor can differences in NH_4^+ accumulation be explained by desiccation, resulting in acidification during dry summers (Schuurkes et al., 1988). The vegetations were all sampled at wet locations and there were no differences in moisture content and pH between the locations in the summer of 1999 (Table 2). Thus, there was no correlation between NH_4^+ and moisture content, nor between NH_4^+ and the organic matter content, although NH_4^+ in the pore water did correlate with the total Fe, Ca and Mg content of the sediment (Figure 4). The black alder carrs we examined in the river Meuse region are continuously fed with groundwater rich in Ca, Mg and Fe (Stortelder, 1998). Locations dominated by the Caltha palustris group, indicative of base-rich groundwater influence (Falinska, 1979; Jalink & Jansen, 1995; Klijn & Witte, 1999; Rosenberry et al., 2000), had the highest total Ca, Mg and Fe contents in the sediment and the lowest NH_4^+ concentration in the pore water. Apparently, the continuous flow of base-rich groundwater has led to the organic-rich sediment adsorption complex being saturated with Ca, Mg and Fe, thereby preventing NH_4^+ from being adsorbed by and accumulating in the system. NH_4^+ in the interstitial pore water is probably continuously removed by the flowing groundwater. These processes probably lead to very low NH_4^+ concentrations in the pore water at seepage sites. The Carex remota group dominates the vegetation in black alder carrs with lower

groundwater discharge rates. This is in agreement with the lower Ca concentration in the sediment compared to the Caltha palustris group. The Glyceria fluitans group and the Calla palustris group often occur massively at sites fed by stagnating groundwater. This observation is supported by the very low total Ca concentration in the sediment. The adsorption sites of the soils at the locations dominated by species from the Carex remota, Calla palustris and Glyceria fluitans groups are therefore less saturated with Ca, enabling more NH_4^+, originating from mineralisation processes, to be adsorbed. This results in higher NH_4^+ pore water concentrations in these groups as compared to the Caltha palustris group. Higher NH_4^+ concentrations enable the development of fast-growing wetland grasses like *G. fluitans, G. maxima* and *C. canescens* and plants that invest relatively much energy in shoot biomass, like *C. paniculata. Calla palustris*, which is known to grow under oligotrophic conditions (Vanden Bergh, 1952) is also able to sustain eutrophic conditions but is then accompanied by other plant species which are always characteristic of eutrophic environments (Schaminée et al., 1995).

Locations fed by groundwater also had relatively low o-PO_4^{3-} and S^{2-} concentrations in the pore water. High concentrations of Fe and Ca in the feeding groundwater may immobilise o-PO_4^{3-} by forming $CaPO_4$ and $FePO_4$ precipitations (Stumm & Morgan, 1986; Golterman, 1998; Lucassen et al., submitted[b]). In addition, Fe^{2+} strongly binds S^{2-} by forming FeS_x (Smolders et al., 1995, 2001). It is likely that species of the Glyceria fluitans and Calla palustris groups have adapted to exclude, tolerate or avoid high S^{2-} concentrations in the sediment. In general, highly regulable radial oxygen losses at the roots of wetland plants oxidise and detoxify S^{2-} and Fe^{2+} in the sediment (Justin & Armstrong, 1987; Laan et al., 1989; Armstrong et al., 1994; Blom & Voesenek, 1996; Smolders et al., 1996). This mechanism occurs in *Glyceria maxima*, which is highly aerenchymatous (Smirnoff & Crawford, 1983; Bodelier, 1997). In addition, wetland plants can detoxify S^{2-} by forming thiols in the root system, making rhizomes less sensitive to high S^{2-} concentrations than roots (Furtig et al., 1996). This might explain the tolerance shown by *Calla palustris,* which grows from thick sprawling rhizomes that are buried in mud or grow under the water surface. Fe can also affect the distribution of wetland plants by acting as a phytotoxin (Ponnamperuma et al., 1955; Albano et al., 1996). Fe toxicity often becomes visible in the development of brown necrotic leaf spots (Tanaka et al, 1966; Laan et al., 1991), which have been found to develop in *G. fluitans* after groundwater discharge in a desiccated mesotrophic wetland meadow was reincreased (Lucassen et al., 2000[a]). This is in agreement with our present results, which show that *G. fluitans* occurred at sites with the lowest Fe concentrations. High Fe concentrations might restrict the growth of Fe-intolerant species at seepage zones.

Our results indicate that base-rich groundwater has an oligotrophicating effect on fens. High base saturation in minerotrophic soils might easily lead to NH_4^+ limitation, even in highly productive late successional wooded fens like alder carrs. Black alder is adapted to nitrogen

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limitation, as it can take up nitrogen for assimilation via nitrogen-fixing actinomycetes that can induce NH_4^+ assimiliation in root nodules (Blom et al., 1981; Guan et al., 1996). By assimilating atmospheric nitrogen, alder trees can supply the entire system with nitrogen. If the groundwater influence decreases, NH_4^+ accumulation may easily occur in black alder dominated systems because the nitrogen input will remain high. This effect is enhanced by the high atmospheric background deposition in many European countries. Increased NH_4^+ concentrations probably stimulate the growth of the Carex remota group, the Glyceria fluitans group and the Calla palustris group. During the last decades, hydraulic measures for agricultural purposes have led to falling groundwater tables in fens, causing severer, more persistent and more frequent summer droughts. Therefore, attempts have been made during the last years, to raise water levels by damming drainage ditches to retain groundwater. Damming of groundwater leads to rising water tables, while the retention time of the surface water increases, resulting in decreased groundwater input. Our results suggest that expansion of the Caltha palustris group in fens does not occur as long as the groundwater pressure is not restored. Rewetting of fens by damming ditches leads to decreased groundwater input and probably stimulates the growth of species from the Carex remota group and/or the Glyceria fluitans group.

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CHAPTER 3

Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill.

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Abstract

The decline of *Cirsium dissectum* in dessicating wetlands is attributed to acidification and eutrophication. Experimental evidence was obtained for the first time on ammonium (NH_4^+) toxicity under low pH. In a hydroculture experiment, interactive effects of nitrogen forms (250 µmol l^{-1} NH_4^+ or 250 µmol l^{-1} nitrate (NO_3^-)) and pH (4, 5 or 6) were studied with regard to the vitality of *C. dissectum* seedlings. The results show that 250 µmol l^{-1} NH_4^+ as sole nitrogen source only had negative effects on *C. dissectum* in combination with a low pH. NH_4^+ uptake at a rhizosphere pH of 4, resulted in lower nitrogen contents of both roots and shoots, lower internal pH of roots and shoots and increased contents of basic amino-acids, resulting in decreased survival rate and biomass development. At higher pH, or when NO_3^- was the nitrogen source, these processes did not take place. This phenomenon stresses the importance of periodic influence of base-rich groundwater during the winter in wet species-rich heathlands and grasslands, necessary to restore the acid neutralising capacity of the soil. Anthropogenic lowering of the groundwater table will lead to acidification enabling ammonium to become toxic to herbaceous plant species such as *C. dissectum*.

Introduction

During the past decades, the biodiversity of former species-rich grasslands has declined, as many of the characteristic herbaceous species disappeared. One of these species is *C. dissectum*, characteristic of relatively nutrient-poor wet heathlands and adjacent grasslands that are periodically influenced by base-rich groundwater (Westhoff & Den Held, 1969; Rodwell, 1991). Grasses like *Calamagrostis canescens* (Weber) Roth. and *Molinia caerulea* (L.) Moench. have become dominant. This process has been attributed to lower (local and regional) groundwater tables and strongly increased atmospheric nitrogen (N) and sulfur (S) deposition (Grootjans et al., 1986; Bakker et al., 1987; Soukupová, 1992; Jansen et al., 1996). Decreased groundwater tables can cause acidification by stimulating hydrogen producing processes in the soil including nitrification and the oxidation of iron-sulphides (Roelofs, 1993; Berendse et al., 1994; Lamers et al., 1998). It is known from dwarf-shrub dominated heathland soils that nitrification is inhibited below pH 4.5, resulting in ammonium (NH_4^+) accumulation and increased NH_4^+ to nitrate (NO_3^-) ratios in the soil (Roelofs et al., 1985; Van Breemen & Van Dijk, 1988; Erisman & Bobbink, 1997). Berendse & Aerts (1984) showed in micro-ecosystem experiments that grasses in particular can easily increase biomass with increasing NH_4^+ availability, thereby overshadowing and outcompeting herbaceous plant species. The fact, however, that many herbaceous plant species already disappeared before the grasses became dominant (Houdijk et al., 1993), indicates that mechanisms other than increased competitive vigour of grasses must be involved in the decline of original plant species.

Van Dobben (1991), showed that pH values as low as 4.0 did not affect growth of several heathland species, indicating that plants were not susceptible to proton toxicity itself. Therefore De Graaf et al. (1998) hypothesized that the decline of heathland species had to be attributed to enhanced NH_4^+ concentrations in the soil, to a shift in NH_4^+ to NO_3^- ratio or to increased aluminium (Al) concentrations, all resulting from soil acidification. In a hydroculture experiment they showed that NH_4^+ concentrations above 100 µmol l^{-1} caused deficiencies of several cations above pH 4. However, from a vegetation survey in 19 intact heathlands in the Netherlands (Bobbink et al., 1996), *C. dissectum* appeared to grow without problems at NH_4^+ concentrations of 100 µmol l^{-1} and a soil pH between 4.8 - 6.0. This difference in tolerance to NH_4^+ may be related to the difference in pH of the rhizosphere. NH_4^+ uptake is coupled to the active excretion of protons by the roots (Raven & Smith, 1976). The hydrogen concentration in the rhizosphere of acidified soils is already high, which is expected to complicate hydrogen transport outside the root cells as it is driven against an electrochemical gradient. We therefore hypothesize that NH_4^+ only becomes toxic to *C. dissectum* at a low rhizosphere pH. To test this hypothesis, a hydroculture experiment was carried out in which seedlings were grown on culture media containing either 250 µmol

l^{-1} NH_4^+ or NO_3^- at pH 4, 5 and 6. As far as we know this is the first time a plant species, disappearing from acidified and eutrophicated wet heathlands, is experimentally exposed to high NH_4^+ concentrations combined with different pH's of the rooting medium.

Materials and methods

Experimental design

In December 1997 seedlings of *C. dissectum* were obtained by germination of seeds (collected in July 1996) originating from a natural population in the nature reserve "De Bruuk" near Nijmegen (51°46'N; 5°53'E), the Netherlands. The soil pore water at this location is nutrient poor (NO_3^- and NH_4^+ concentrations are 7 and 11 µmol l^{-1} respectively) and base-rich (the pH is 5.6, the alkalinity 197 µeq l^{-1} and the calcium (Ca) concentration 1317 µmol l^{-1}). Germination took place at room temperature on filter paper wetted with demineralised water. Two weeks after germination, six seedlings (root length 2-4 cm) were transferred to 2 l opaque containers with a nutrient solution containing 50 µmol l^{-1} NO_3^- and 50 µmol l^{-1} NH_4^+ at pH 4. Following one week of acclimatisation, 6 treatments were applied in fourfold (n=4) differing in type of nitrogen source (250 µmol l^{-1} NO_3^- or NH_4^+) and pH (pH 4, 5 or 6). For amino acid and pH analyses, 30 plants were grown up in 25 l containers in NH_4^+ or NO_3^- containing culture media at pH 4 and pH 6. Each week the culture medium was replaced and after 2 months plants were harvested and pooled in 5 groups.

Concentrations of nutrients were low as to represent natural conditions (De Graaf et al., 1994) and had the following concentrations: 250 µmol l^{-1} K^+, 100 µmol l^{-1} Mg^{2+}, 100 µmol l^{-1} Ca^{2+}; 5 µmol l^{-1} Fe, 125 µmol l^{-1} SO_4^{2-}, 0.7 µmol l^{-1} Zn^{2+}, 0.8 µmol l^{-1} Mn^{2+}, 0.2 µmol l^{-1} Cu^{2+}, 0.8 µmol l^{-1} H_3BO_3 and $8.1*10^{-3}$ µmol l^{-1} Mo. Fe was added as Fe-EDTA. Phosphate (o-PO_4^{3-}) concentrations were rather high (250 µmol l^{-1}) to prevent growth inhibition due to o-PO_4^{3-} limitation. Exposure media of the containers were continuously refreshed by pumping 12.5 l medium per week per container from 25 l reservoir tanks. Containers were continuously aerated to prevent development of anoxic conditions. To all reservoir tanks 0.5 µmol l^{-1} 1-cyanguanidine was added to prevent nitrification. During the experiment no changes in NH_4^+ and NO_3^- concentrations could be detected. Acidity in each reservoir was maintained by the addition of 100 µmol l^{-1} $NaHCO_3^-$ buffer and by daily checking of pH. When necessary, pH was adjusted. Furthermore, pH of the effluent was measured several times. Plants were grown in a climate chamber at 10/14 hours day/night, 27/21 °C day/night, a light intensity of 200 µmol $m^{-2}s^{-1}$ and a relative air humidity of 45-65 %.

Plant growth

During the experimental period, the number of dead individuals, the number of leaves (N), and the maximum leaf length (L_{max}) and width (W_{max}) were recorded for all plants at two

week intervals. Estimated shoot size was calculated by multiplication of N, L_{max} and W_{max} (Van der Sman et al., 1988). Plants were harvested after six weeks. During this period there was no light competition. Roots and shoots were separated and total dry weights per container were determined after 24 hours drying at 70 °C.

Nutrient analyses

Dried root and shoot material was ground and mixed in liquid nitrogen, and redried for 24 hours at 70 °C. For the analyses of the chemical composition, 100 mg of material was digested in 4 ml HNO_3^- and 1 ml H_2O_2 using a Milestone destruction microwave (MLS 1200 Mega). Total fosfor (P), iron (Fe), Ca, magnesium (Mg), manganese (Mn) and zinc (Zn) concentrations were measured by inductively coupled plasma emission spectrometry (ICP, Spectroflame), Potassium (K^+) using a Technicon Flame photometer IV, and o-PO_4^{3-} using a Technicon AAII system (Henriksen, 1965). Carbon (C) and nitrogen (N) content of the plant material were determined using a Carlo Erba NA 1500 CNS analyser.

Internal pH and amino acid analyses of roots and shoots

For pH analyses, 0.2-2 ml demineralised water was added to 0.01-0.1 g of dried material and subsequently homogenised using a glass-to-glass Potter homogenising device. Hereafter pH was measured using a Sentron pH sensor coupled to a Sentron 1001 pH meter.

Free amino acids from fresh root and shoot material were extracted using 70 % ethanol containing 10 ml thiodiglycol and 700 mg citric acid l^{-1}. The combined fractions were washed with 40 ml chloroform, freeze-dried, dissolved in 2 ml lithium citrate (0.2 M Li, pH 2.6) and stored at -20 °C until analyses. Norleucine (150 nmol) was added as an internal standard at the beginning of the extraction procedure. Amino acid concentrations were determined by High Performance Liquid Chromatograhy (HPLC) (Varian Liquid Chromatograph 5000) using a cation exchange column (LKB UP8) and mixtures of lithium citrate (pH 2.6) and lithium borate (pH 10.6) of increasing molarity and pH. For quantification, fluorescence was measured (excitation wavelength 390 nm, emission wavelength 460 nm) after pre-column derivatization with 9-Fluorenylmethyl-chloroformate (Van Dijk & Roelofs, 1988). A subsample was dried (24 h at 60 °C) in order to express the amino acid concentrations on dry weight base.

Statistical analyses

Plants from one container were pooled. Data are expressed as mean ± S.E.M. (n=4 or n=5). After log transformation of plant growth parameters (e.g. estimated shoot size and dry weights (DW) of roots and shoots), paramaters were statistically analysed by a two-way analysis of variance (GLM procedure, SPSS 7.5) with nitrogen type and pH as class

variables. Differences between treatments (P < 0.05) were subsequently tested with Tukey post tests.

Results

Plant characteristics

Plants growing on 250 µmol l^{-1} NH$_4^+$ were negatively affected by a lower pH. At pH 4, NH$_4^+$ significantly lowered survival and the estimated shoot size finally resulting in a three times lower total dry weight (DW) compared to growth on NH$_4^+$ at pH 6. Total DW of plants growing on NH$_4^+$ at pH 6 did not significantly differ from plants growing on NO$_3^-$. Although the growth on NO$_3^-$ did not differ significantly at different pH values, there seemed to be a positive effect by a lower pH (Figure 1). Table 1 shows that the interaction between N-source type with pH is responsible for the observed effects on shoot development of *C. dissectum*, and not N-source type or pH alone.

Plant nutrient analyses

Plant nutrient analyses are shown in Table 2a-b. For all pH treatments, the C and N concentration of roots and shoots were significantly higher when grown on NH$_4^+$. Furthermore, Ca and especially Mg concentrations were lower in the roots and shoots of plants growing on NH$_4^+$. K$^+$ and Zn concentrations were significantly lower in the NH$_4^+$ treatments, but only in the roots.

The higher N concentrations of roots and shoots of plants growing on NH$_4^+$ were also positively related to pH. At pH 4, the NH$_4^+$ concentration of the roots and shoots was lower compared to pH 5 and pH 6. Furthermore, the C concentrations of roots were significantly higher in plants growing on NH$_4^+$ at lower pH. These pH-induced differences did not take place in plants grown on NO$_3^-$. In Table 1 it is shown that the C concentrations in the roots and the N concentrations in the roots and shoots are indeed significantly affected by the interaction between N-type and pH, in contrast to the Ca, Mg and K$^+$ concentrations, which are only affected by N-source type and not by pH.

Internal pH and amino acid analyses of roots and shoots

Internal pH analyses and amino acid analyses are shown in Table 3. Internal pH of roots and shoots of plants growing on NH$_4^+$ was significantly lower than pH of roots and shoots of plants growing on NO$_3^-$. Furthermore, the internal pH of roots and shoots of plants growing on NH$_4^+$ at pH 4 was significantly lower compared to plants growing on NH$_4^+$ at pH 6. This was also, in a lesser extent, the case for the roots of plants growing on NO$_3^-$. The internal pH of roots and shoots is significantly affected by N-type and pH as well as by their interactive effect (Table 1).

The total amino acid concentrations of roots and shoots of *C. dissectum* growing on NH_4^+ was significantly higher at pH 4 compared to pH 6. This was the result of higher arginine, aspargine, glutamine and proline concentrations of the roots, accounting for 70 % and 84 % of the total amino acid content of the roots and shoots respectively. In the NO_3^--fed plants no such differences in total amino acid concentrations of the shoots and roots were found. The NO_3^--fed plants had amino acid concentrations that did not significantly differ from plants grown on NH_4^+ at pH 6. The concentration of amino acids is significantly affected by N-type and pH as well as by their interactive effect (Table 1).
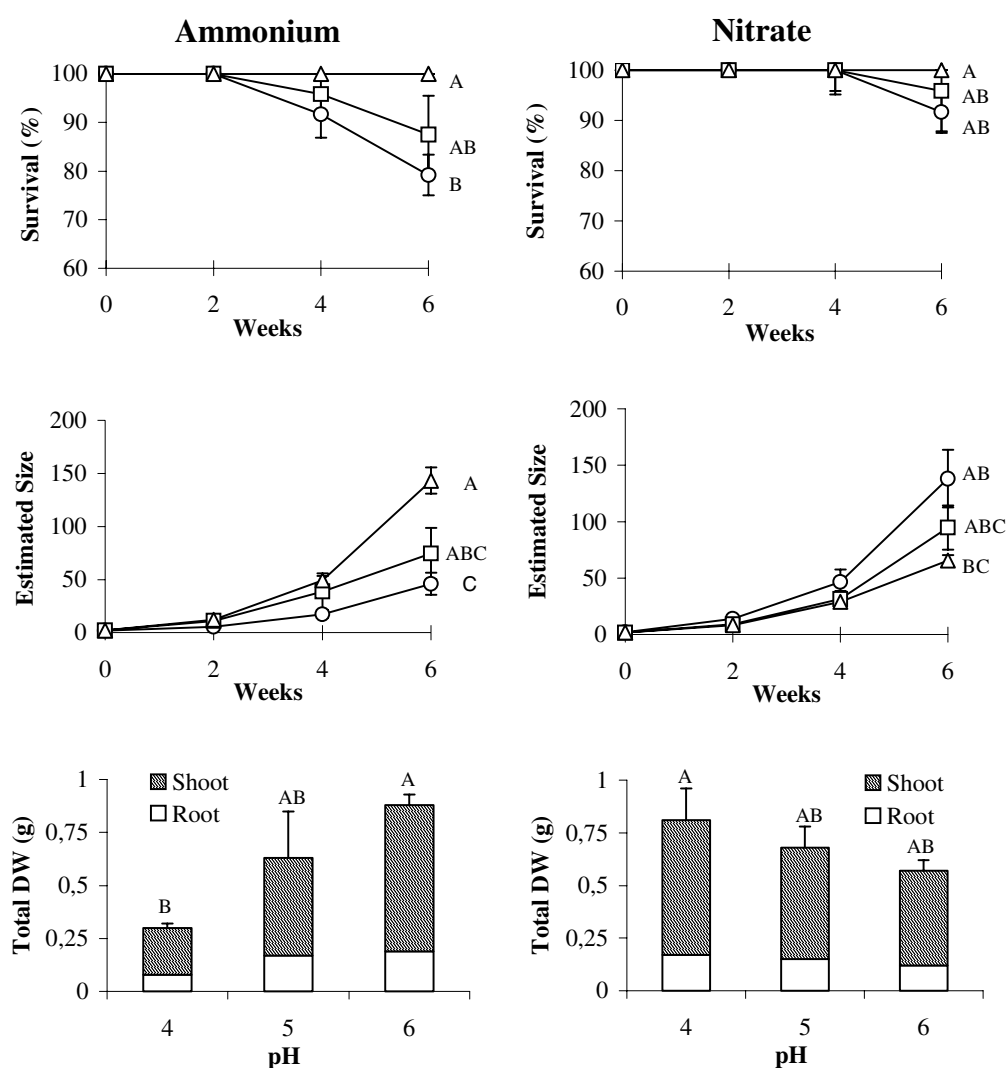

Figure 1. Survival (%), estimated shoot size (g) and total dry weight (g) of *C. dissectum* during six weeks of growth on medium containing 250 µmol l⁻¹ ammonium or nitrate. Means ± (SEM) are given (n=4). Means with the same letter are not significantly different; comparisons between pH and nitrogen sources were made. ○, pH 4; □, pH 5; Δ, pH 6.

Table 1. Anova table with the (interactive) effects of nitrogen source and pH on growth parameters, internal pH and concentration of C, N and amino acids of roots and shoots. ***, $p < 0.001$; **, $p < 0.01$, *, $p < 0.05$; ns, not significant.

	N_{source}	pH	$N_{source}*pH$
Root			
Dry weight	ns	ns	ns
C	***	ns	*
N	***	*	*
Ca	**	ns	ns
Mg	***	ns	ns
K^+	***	ns	ns
Internal pH	***	***	**
Total amino acid	***	***	***
Shoot			
Dry weight	ns	ns	**
Estimated biomass	ns	ns	**
C	***	ns	ns
N	***	*	*
Ca	**	ns	ns
Mg	*	ns	ns
K^+	ns	ns	ns
Internal pH	***	***	***
Total amino acid	***	***	**

Discussion

The results show that NH_4^+ as sole nitrogen source only can negatively affect survival and growth of *C. dissectum* when combined with a low rhizosphere pH. NH_4^+ concentrations of 250 µmol l^{-1}, combined with a rhizosphere pH of 4, significantly reduced biomass development compared to NH_4^+ concentrations of 250 µmol l^{-1} at pH 6. In the latter case, biomass development did not significantly differ from growth on 250 µmol l^{-1} NO_3^- at different pH' s.

It is well known that assimilation of NH_4^+ produces protons in almost an equimolar ratio (3 $NH_4^+ \rightarrow 3$ R·$NH_2 + 4$ H^+ (Raven, 1986)). As the capacity of the shoots to dispose of protons is rather limited (Raven, 1986), NH_4^+ assimilation is thought to take place mainly in the roots

(Engels & Marschner, 1993). The protons will be excreted into the rhizosphere, which can result in acidification of the rhizosphere (Raven & Smith, 1976). The proton excretion process takes place by H^+-ATPase pumps in the root hairs which may consume up to 25-50% of the cellular ATP (Felle, 1982). At lower external pH, this transport is driven against a stronger electrochemical gradient which will cost more energy, decreasing carbohydrate levels in the roots. Lower carbohydrate levels directly decrease the net extrusion of protons in the apoplast, resulting in a lower cytosolic pH (Schubert & Mengel, 1986).

Table 2a. Root nutrient concentrations of *C. dissectum* after six weeks of growth on medium containing 250 µmol l^{-1} NH_4^+ or NO_3^- at pH 4, 5 or 6. Means ± Standard error of the Mean (SEM) are given (n=4). Means with the same letter are not significantly different, comparisons between pH values and between nitrogen species were made. Nutrient concentrations are given in µmol g^{-1} DW with the exception of C and N concentrations which are given in mmol g^{-1} DW.

Root	C	N	K^+	P	Fe	Ca	Mg	Mn	Zn
ammonium									
pH 4	35.2^A	4.3^B	618^B	470^A	17^B	47^{AB}	50^A	1.1^E	3.4^B
pH 5	34.5^{AB}	5.3^A	560^B	488^A	15^B	49^{AB}	57^A	2.8^D	3.4^B
pH 6	33.6^B	5.7^A	537^B	441^A	18^B	43^B	47^A	5.3^C	3.2^B
nitrate									
pH 4	32.2^C	3.0^C	1071^A	533^A	26^{AB}	67^A	188^B	9.3^{BC}	7.7^A
pH 5	32.4^C	3.1^C	1072^A	538^A	50^A	79^{AB}	229^B	12.7^B	7.5^A
pH 6	32.3^C	2.9^C	1056^A	461^A	21^B	83^{AB}	203^B	23.8^A	5.8^A

Table 2b. Shoot nutrient concentrations of *C. dissectum* after six weeks of growth on medium containing 250 µmol l^{-1} NH_4^+ or NO_3^- at pH 4, 5 or 6. Means ± SEM are given (n=4). Means with the same letter are not significantly different, comparisons between pH values and nitrogen form were made. Nutrient concentrations are given in µmol g^{-1} DW with the exception of C and N concentrations which are given in mmol g^{-1} DW.

Shoot	C	N	K^+	P	Fe	Ca	Mg	Mn	Zn
ammonium									
pH 4	32.8^A	4.1^B	731^A	375^A	5^A	309^A	247^A	3.7^B	5^A
pH 5	31.9^A	4.6^A	750^A	411^A	6^A	414^A	291^A	3.0^B	4^A
pH 6	32.1^A	4.7^A	788^A	446^A	4^A	448^A	296^A	3.3^{AB}	3.9^A
nitrate									
pH 4	29^B	3.5^C	790^A	498^A	3.5^A	742^A	511^A	4.9^{AB}	5.3^A
pH 5	28.9^B	3.5^C	754^A	424^A	3.3^A	736^A	456^A	6.1^{AB}	5.0^A
pH 6	28.8^B	3.5^C	882^A	408^A	3.8^A	714^A	441^A	7.5^A	4.5^A

Table 3. Internal pH and (total) free amino acid concentration (μmol g^{-1} DW) of roots and shoots from *C. dissectum* after six weeks of exposure to 250 μmol l^{-1} NH$_4^+$ or NO$_3^-$ at pH 4 and 6. Means $\pm$ SEM are given (n=5). Comparisons between all groups (pH, nitrogen source type and roots and shoots) were made; means with the same letter are not significantly different.

	Root		Shoot	
	pH 4	pH 6	pH 4	pH 6
ammonium				
Internal pH	3.98 (0.04)[D]	4.99 (0.06)[B]	4.25 (0.07)[C]	4.79 (0.02)[B]
Total amino acids	366.7 (28.8)[A]	136.9 (19)[B]	283.5 (38.3)[A]	71.5 (15.2)[B]
Arginine	78.1 (5.1)[A]	19.5 (2)[B]	31.2 (6)[A]	2.3 (0.4)[B]
Glutamine	16.1 (2.9)[A]	4.8 (0.7)[B]	2.2 (0.5)[A]	1.7 (0.2)[A]
Aspargine	121 (9.4)[A]	29.8 (3.7)[BC]	109.3 (8.9)[A]	5.3 (0.7)[B]
Proline	91.9 (9.9)[A]	16.9 (4.7)[B]	59.8 (17.4)[A]	6.5 (1.4)[B]
nitrate				
Internal pH	4.73 (0.07)[C]	5.32 (0.04)[A]	4.99 (0.04)[A]	5.02 (0.03)[A]
Total amino acids	159.9 (8.8)[B]	106.8 (8.5)[B]	69.5 (7)[B]	106.7 (20.2)[B]
Arginine	19.9 (1.3)[C]	13.8 (2)[C]	26 (0.7)[B]	1.4 (0.3)[B]
Glutamine	5.7 (0.7)[B]	3 (0.6)[B]	1.9 (0.9)[A]	1.8 (0.2)[A]
Aspargine	21.6 (2.9)[BC]	15 (4.3)[C]	3.4 (0.6)[B]	3.9 (0.9)[B]
Proline	15.7 (0.7)[B]	9.3 (1.1)[B]	7.4 (1)[B]	6.8 (1)[B]

As a result, the uptake of NH$_4^+$ is decreased (Weissman, 1950) while the di- and polyamine content of the root is usually increased. These processes serve as a homeostatic mechanism for maintaining intra cellular pH (Smith & Wilshire, 1975). Marschner (1995) hypothesized that these processes could explain the relationship between growth retardation and lower internal pH in NH$_4^+$- fed *Sorghum bicolor* plants as found by Findenegg et al. (1982).

The same processes occur in *C. dissectum* as a consequence of NH$_4^+$ uptake at a low rhizosphere pH. NH$_4^+$ uptake at pH 4 resulted in a strong acidification of the roots and a strong increase in the content of basic amino acids with a high N/C ratio in both roots and shoots. As a result, survival and growth strongly decreased. The same NH$_4^+$ concentrations did not affect *C dissectum* at a rhizophere pH of 6. In this case proton excretion was not impaired and no severe acidification of roots and shoots occured. De Graaf et al. (1998) investigated the toxic effects of NH$_4^+$ on *C. dissectum* only at pH 4. It was concluded that NH$_4^+$ toxicity was caused by the interference of NH$_4^+$ with cation uptake by the roots, resulting in deficiencies of Ca, Mg and K$^+$. Our results show that NH$_4^+$ uptake at higher pH also decreases the Ca and Mg content of roots and shoots compared to NO$_3^-$ uptake, but does

not negatively affect growth and development. Decreased uptake of cations is, like decreased growth and development, not caused by interactive effects of N-source with pH, but by the type of N-source solely. This indicates that NH_4^+ has negative effects on the development of *C. dissectum* at low external pH values via impairment of proton excretion by the roots resulting in root and shoot acidification.

It is generally assumed that during the past decades *C. dissectum* and other herbaceous species have disappeared from wet heathlands and adjacent grasslands due to increased atmospheric NH_4^+ deposition and decreased groundwater levels (Aerts & Berendse, 1988, Houdijk et al., 1993). NH_4^+ deposition not only leads to higher NH_4^+ levels but may also lead to soil acidification due to increased nitrification rates. Under acidic conditions, NH_4^+ accumulation in the soil is enhanced due to impaired nitrification and increased mobilisation of NH_4^+ from cation exchange sites. In anoxic soils, dissimilatory NO_3^- reduction to NH_4^+, may contribute to the accumulation of NH_4^+. Our results show that *C. dissectum* can sustain very high NH_4^+ concentrations of at least 250 µmol l^{-1} when pH is relatively high. Only after a drop in pH, as a result of acidification, does NH_4^+ become toxic. This phenomenon shows the importance of the periodic influence of base-rich groundwater during the winter in these ecosystems. As groundwater tables are lowered by human intervention, the influence of base-rich groundwater in the winter becomes too low to counteract the acid producing soil processes that take place during dry periods in the summer. As a result, the soil acidifies and accumulated NH_4^+ becomes toxic to once-characteristic plant species including *C. dissectum*.

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CHAPTER 4

Increased groundwater levels cause iron toxicity in *Glyceria fluitans* (L.) R.Br.

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Abstract

After restoration of the hydrology in a eutrophic wetland meadow seepage strongly increased and the helophyte *Glyceria fluitans* intensively developed brown necrotic spots on 67 % of the leaves at a site where groundwater table was highest. Sites where plants intensively developed brown necrotic spots and sites where plants did not develop these spots were compared using soil pore water chemistry, root plaque content and plant nutrient concentration. At affected sites, Fe concentrations in the soil pore water were 3.2 times higher (2616 µmol l^{-1}) resulting in 9.4 times higher Fe plaque concentrations on the roots (473 µmol g^{-1} DW), 3.4 times higher Fe concentrations in the youngest unaffected leaves (6.2 µmol g^{-1} DW) and 18.2 times higher Fe concentrations in the affected leaves (32.8 µmol g^{-1} DW). No such differences were found for other elements. The location is known to be an Fe-rich site with Fe-rich groundwater in the subsoil. Furthermore it is known that the reduction of insoluble Fe(III) oxides to Fe(II) in waterlogged soils is stimulated. The site with the highest groundwater table possessed the highest Fe concentration in the soil pore water and it is therefore likely that this relatively high Fe concentration is responsible for the development of brown necrotic leaf spots after restoration of the hydrology. This is the first observation of brown necrotic leaf spot development, caused by Fe toxicity, for wild plants growing under field conditions.

Introduction

Glyceria fluitans (L.) R.Br. is a helophyte that often dominates the vegetation in highly degenerated sites in formerly species-rich wet meadows. In one of these degenerated wet meadows, "De Ravenvennen", near Lomm, The Netherlands ($51^0$28'N; $6^0$11'E) attempts have been made to restore the natural hydrology. Hereafter, groundwater tables increased greatly resulting in strongly increased seepage in the lower parts. At locations where seepage was highest, *G. fluitans* intensively developed brown necrotic leaf spots. These kind of spots have often been observed in rice (*Oryza sativa* L.) cultivars in tropical and sub-tropical countries and in the floriculture industries where they have been attributed to iron toxicity (Ponnamperuma et al., 1955; Tanaka et al., 1966; Howeler, 1973; Yoshida and Tadano, 1978; Biernbaum et al., 1988; Halbrooks and Albano, 1990; Albano and Miller, 1993; Albano et al., 1996). From many solution culture experiments and from greenhouse experiments it is known that high iron (Fe) concentrations can negatively affect the root system and growth of various wetland and river ecosystem species depending upon their tolerance mechanisms (Wheeler et al., 1985; Laan et al., 1989; Snowden and Wheeler 1993; 1995). However, the observation of brown necrotic leaf spots has never been reported for wild plants growing under field conditions. The location we are investigating is known as an Fe-rich site with Fe-rich groundwater in the subsoil. Therefore the changed increased groundwater table and increased seepage may have resulted in increased Fe levels in the root zone and subsequently caused iron toxicity in *G. fluitans*. To investigate whether Fe toxicity was indeed responsible for the observed development of brown necrotic leaf spots, sites where plants developed brown necrotic spots and sites where plants did not develop these spots (both located in "De Ravenvennen") were compared using parameters of soil pore water chemistry, root plaques and plant nutrient concentrations.

Materials and methods

Field material

In July 1998, both affected and unaffected stands of *G. fluitans* were sampled from five locations by collecting five plants from each stand. At each location, soil pore water was collected anaerobically using ceramic cups which were connected to 100 % vacuum serum bottles. Ceramic cups were installed in the upper 10 cm of the soil. The pH and alkalinity were determined immediately. pH was measured using a radiometer Copenhagen type PHM 82 standard pH meter (Danmark). Alkalinity was measured by titrating 5 ml of sample with 0.01 mmol l^{-1} HCl down to pH 4.2. Hereafter citric acid was added (0.25 g l^{-1}) to avoid precipitation of metals in the oxidizing samples and samples were stored in iodated polyethylene bottles at -28 ^{0}C until further analysis.

After washing the roots in demineralised water, roots and shoots were separated and root plaques were extracted according to Christensen and Wigand (1998) to determine their chemical composition. Shoot length was measured and the affected and unaffected shoots of the affected plants were separated. Shoots were then divided into stems, sheaths and leaves. After drying (24 h at 70 ^{0}C) plant material was ground in liquid nitrogen and 100 mg was digested with 4 ml concentrated nitric acid and 1 ml 30 % hydrogen peroxide using a Milestone microwave type mls 1200 Mega (Germany). Digests were used to determine chemical composition.

Chemical analysis

The following compounds in the sediment pore water and root plaques were measured using Technicon II autoanalysers (America): nitrate (NO_3^-) and phosphate (o-PO_4^{3-}) according to Kamphake et al. (1967) and ammonium (NH_4^+) according to Grasshof and Johansen (1977). Potassium (K^+) was determined flame photometrically by use of a Technicon autoanalyser. Analyses for total iron (Fe), calcium (Ca), magnesium (Mg), silicium (Si), zinc (Zn), fosfor (P), sulfur (S) and aluminum (Al) in soil pore water, root plaques and plant digests were determined by inductively coupled plasma emission spectrometry (ICP, Spectroflame). Carbon (C) and nitrogen (N) content of ovendried plant tissue material were determined using a Carlo Erba NA 1500 CNS analyser (Italy).

Statistical analysis

Results are presented as means ± standard error of the mean (SEM) (n=5). The Mann-Whitney U-test was used to assess differences in soil pore water chemistry, root plaques and plant nutrient content between sites with affected and unaffected *G. fluitans*; P < 0.05 was accepted for statistical significance.

Results

At the location where the groundwater table was highest (18 cm above surface level), 67 ± 7 % of the leaves of *G. fluitans* developed brown necrotic spots (Figure 1). At the site where plants did not develop necrotic spots, the groundwater table was 10 cm above surface level. The length and the number of leaves of affected plants (length 60 ± 4 cm, total number of leaves 6 ± 0.3) was higher than those of unaffected plants (length 51 ± 2 cm, total number of leaves 5 ± 0.3).

Table 1 shows that mean S, NO_3^- and Fe concentrations in the pore water of affected plants were significantly higher than in the soil pore water of unaffected plants, while o-PO_4^{3-} levels in the soil pore water of the affected stands were significantly lower. Fe concentrations in the soil pore water were three times higher at the affected site (2,5 mmol l^{-1}). In the root plaque

of affected plants, concentrations of Fe, Mn and Ca were significantly higher. Fe concentrations in the root plaque were 9.4 times higher in affected plants (473 µmol g^{-1} DW). In affected leaves, Fe concentrations were 18.2 times higher (32.8 µmol g^{-1} DW) compared to leaves of unaffected plants (Table 2). Affected plants also contained higher N, S and Si concentrations in all plant parts, while Mn and Ca concentrations were lower than in unaffected plants. These differences however were less pronounced than the difference in Fe content. Furthermore, older leaves of affected plants contained 5.3 times more Fe compared to the unaffected younger leaves of the same plants. The difference in S content between these leaves was less pronounced and no differences were found regarding N, Ca, Si and Mn content.

Figure 1. Unaffected leaf of *G. fluitans* (right) and leaf with development of brown necrotic spots (left).

Table 1. Soil pore water chemistry (µmol l^{-1}) and concentrations of root plaque extractable components (µmol g^{-1} DW) of *G. fluitans*, unaffected and affected by iron toxicity. Means ± (standard errors) are given (n = 5). *, P ≤ 0.05; **, P ≤ 0.005.

	Pore Water		Root plaque	
	unaffected	**affected**	**unaffected**	**affected**
pH	6.43 (0.02)	6.29 (0.06)*		
alkalinity	1886 (209)	2912 (514)		
NH$_4^+$	112 (42)	783 (438)		
NO$_3^-$	12 (0.2)	17 (2.4)*		
K$^+$	340 (36)	195 (112)	110 (21)	110 (14)
o-PO$_4^{3-}$	90 (27)	28 (20)*	4.2 (0.9)	3.3 (0.5)
Ca	879 (107)	1113 (208)	19.8 (2.3)	32 (5)*
Mg	350 (32)	354 (30)	6.3 (1)	9.2 (1.1)
Mn	127 (31)	96 (32)	2.8 (0.9)	9.3 (2.5)*
Fe	806 (184)	2612 (451)**	50.5 (7.4)	473 (13.5)**
Si	109 (9)	349 (69)**	7.3 (3.7)	10.2 (2.2)
Zn	3.9 (0.6)	4.1 (0.7)	0.3 (0.2)	0.6 (0.1)
Al	38 (4)	44 (5)	6.7 (1.5)	9.5 (1.7)
S	246 (32)	357 (47)*		

Table 2. Nutrient concentrations in roots and shoot parts sampled from *G. fluitans* stands, unaffected (U) and affected (A) by iron toxicity. Means ± (standard errors) are given (n=5). Concentrations are given in µmol g^{-1} DW with the exception of C which is given in mmol g^{-1} DW. Sheaths and leaves of affected plants are divided in A- (healthy looking shoots) and A+ (affected shoots). Affected plants (A- and A+) are compared with unaffected plants. **a**, $p < 0.05$; **b**, $p < 0.005$.

		Root	Stem	Sheath	Leaf
C	U	31.4 (1.5)	35.7 (0.2)	36.2 (0.2)	37.9 (0.3)
	A+	29 (1)	35.2 (0.2)**a**	35.4 (0.2)**a**	36.6 (1.2)
	A-			34.9 (0.2)**a**	37.2 (0.4)
N	U	941 (129)	598 (45)	1009 (72)	2504 (120)
	A+	1300 (17)**a**	1048 (54)**b**	1529 (82)**a**	3006 (120)**a**
	A-			1971 (109)**a**	3087 (104)**a**
K$^+$	U	116 (18)	142 (14)	265 (23)	252 (9)
	A+	133 (15)	240 (23)**a**	355 (16)**a**	268 (8)
	A-			355 (23)**a**	277 (7)
Ca	U	27.5 (4.9)	21.3 (1.5)	31.7 (2.8)	65.1 (3.6)
	A+	22.9 (2.8)	15.1 (1.3)**b**	21 (2.0)**a**	32.4 (2.4)**b**
	A-			18.5 (1.6)**b**	30.9 (0.9)**b**
Mg	U	16.8 (2.9)	22.1 (1.7)	27.8 (2.5)	55.5 (4.3)
	A+	37 (2.5)**b**	25.1 (1.4)	26.3 (2.1)	48.6 (4.2)
	A-			37.5 (2.8)**a**	56.3 (3.1)
Mn	U	3.8 (0.4)	3.9 (0.6)	6.6 (0.8)	4.2 (0.6)
	A+	2.7 (0.2)**a**	2.2 (0.2)**a**	3.5 (0.3)**b**	1.6 (0.2)**b**
	A-			2.0 (0.2)**b**	1.2 (0.1)**b**
Fe	U	8.2 (0.7)	1.2 (0.5)	3.1 (0.6)	1.8 (0.2)
	A+	87.7 (7.8)**b**	6.6 (1)**b**	26.7 (4.9)**b**	32.8 (7.7)**b**
	A-			6.4 (1)**a**	6.2 (0.4)**b**
Si	U	5.3 (0.5)	6.7 (0.6)	8.5 (0.6)	9.9 (0.4)
	A+	6.2 (1)	15.3 (1.1)**b**	18 (1.4)**b**	17.6 (1.2)**b**
	A-			13.1 (1.2)**b**	19.3 (4.8)**b**
Zn	U	1.1 (0.1)	1.6 (0.5)	1.0 (0.3)	1.8 (0.8)
	A+	0.97 (0.16)	0.99 (0.07)	0.83 (0.15)	2.58 (1.14)
	A-			0.99 (0.28)	2.44 (1.38)
P	U	100.8 (8.1)	86.8 (16.4)	125.8 (9.5)	134.5 (10)
	A+	61.6 (5.3)**b**	110.7 (8.5)	132.3 (13.1)	107.5 (6.2)**a**
	A-			139.5 (11.1)	130.3 (7.6)
S	U	968 (173)	25.4 (2.8)	33.7 (2)	70.6 (3.2)
	A+	1458 (204)	88.4 (4)**b**	111 (6.2)**b**	226.6 (8.3)**b**
	A-			95.1 (5.4)**b**	164.9 (5.1)**b**
Al	U	1.87 (0.31)	0.40 (0.14)	0.47 (0.12)	0.8 (0.34)
	A+	4.51 (0.58)**b**	0.12 (0.04)**a**	1.03 (0.15)**b**	1.08 (0.3)
	A-			0.23 (0.08)	0.68 (0.4)

Discussion

The results clearly show that high Fe levels in soil pore water resulted in increased Fe levels in the plants. The degree of Fe accumulation in plant leaves is related to the Fe concentration in the soil, the oxidising activity of the roots and the transpiration rate of the shoot (Jones 1971; Bienfait 1989; Laan et al., 1989). The 3.2 times higher Fe concentrations (2612 µmol l^{-1}) in the soil pore water of the affected plants caused 9.4 times higher Fe plaque concentrations (473 µmol g^{-1} DW) on the roots, 3.4 times higher Fe concentrations (6 µmol g^{-1} DW) in the young unaffected leaves and 18.2 times higher Fe concentrations (33 µmol g^{-1} DW) in the old affected leaves. No such differences were found for other elements. The oxidising activity of the roots resulted in lowering of Mn concentrations in the affected plants. At the affected sites, Mn concentrations were 3.3 times higher in the root plaque (9.3 µmol g^{-1} DW), and 1.4 times lower in the roots (2.7 µmol g^{-1} DW) compared to the affected sites, while concentrations in the soil pore water did not differ significantly. Decreased uptake of Fe, Mn and other toxic compounds formed in waterlogged soils, due to the formation of root plaque, has been reported before (Tanaka et al., 1966; Iremonger & Kelly, 1988). Apparently, in the present work, the Fe concentrations in the soil pore water were so high that the oxidising activity of the roots could not prevent excessive uptake of Fe.

The leaf Fe concentrations of the affected plants agree well with values that are known to cause the development of brown necrotic spots in rice plants and several *Rumex* species growing on hydroculture. From rice plants it is known that Fe toxicity symptoms do not develop at Fe concentrations lower than 8 µmol g^{-1} DW (Yoshida and Tadano, 1978; Ottow et al., 1982). In hydroculture experiments with *Rumex thyrsiflorus*, *R. crispus* and *R. maritimus,* brown necrotic leaf spots developed at mean Fe concentrations of 26, 32 and 24 µmol g^{-1} DW, respectively (Laan et al., 1991). It is therefore likely that high Fe concentrations in soil pore water are the main cause in the development of the brown necrotic spots on the leaves of *G. fluitans*. This then is the first observation of brown necrotic leaf spot development, caused by Fe toxicity, for wild plants growing under field conditions. In "De Ravenvennen", Fe concentrations in the soil pore water have increased due to the restoration of the local hydrology. The high Fe levels in the soil pore water (2.6 mmol l^{-1}) are the result of the increased supply of Fe from the deeper groundwater and the stimulated reduction of insoluble Fe(III) oxides to Fe(II) in waterlogged soils (Ponnamperuma et al., 1955; Howeler, 1973; Yoshida and Tadano, 1978; Laan et al., 1991). From culture experiments, it is known that wetland species show differential tolerance to high Fe concentrations (Cook, 1990; Snowden and Wheeler, 1993; 1995). In Fe-rich alder carrs in The Netherlands, it has been observed that *G. fluitans* and *G. maxima*, which both are helophytes, increased in abundance after the groundwater level dropped due to drainage of surrounding agricultural areas. Under natural conditons, nutrient levels in these systems are

already high (van Duren et al., 1997) so it is unlikely that differences in nutrient level between natural and drained conditions can explain the increased abundance of these species under drained conditions. However, under drained conditions the input of Fe-rich groundwater decreases and Fe(II) is oxidised into insoluble Fe(III) oxides. Therefore, drained conditions lead to lower Fe concentrations in the root zones. We hypothesize that the abundance of *G. fluitans* and other plant species is limited due to Fe toxicity in systems fed by Fe-rich groundwater (under natural hydrological conditions).

Acknowledgements

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CHAPTER 5

Potential sensitivity of mires to drought, acidification and mobilisation of heavy metals: the sediment S/(Ca+Mg) ratio as diagnostic tool

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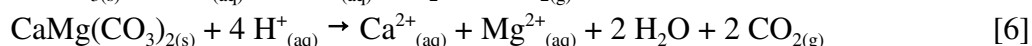*Environmental Pollution (2002) 120: 635-646*

Abstract

In recent decades sulphate concentrations in the groundwater in many parts of the Netherlands have increased dramatically resulting in increased production of Fe-(di)sulphides in sediments of ecosystems fed by this water. A sediment survey was carried out to study the potential sensitivity of wetlands to drought and subsequent acidification as a consequence of Fe-(di)sulphide oxidation. Desiccation led to severe acidification and mobilisation of heavy metals when the sediment S/(Ca+Mg) ratio exceeded 2/3. A total of 47 % of the investigated locations contained S/(Ca+Mg) ratios higher than 2/3 and in 100 %, 75 % and 50 % of the locations, mobilisation of Zn, Cd and Ni exceeded the Dutch signal value for groundwater quality. Consistent with the sediment survey, lime addition experiments confirmed that increasing the buffer capacity, down to a S/(Ca+Mg) ratio 2/3, led to a drastic inhibition of the acidification and heavy metal percolation from dredged sediments. The performance of the same processes under drained field conditions demonstrates the relevance of these processes during dry summers.

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Introduction

In recent decades sulphate (SO_4^{2-}) concentrations have increased considerably in groundwater in many parts of the Netherlands. Between 1966 and 1991, SO_4^{2-} concentrations in the eastern and southern part of the Netherlands increased from $\pm$ 250 to $\pm$ 1600 μmol l^{-1} (Jansen & Roelofs, 1996). This increase has been attributed to increased atmospheric sulphur (S) deposition and leakage from agricultural lands. Increased drought and infiltration of nitrate (NO_3^-) from agricultural lands into the groundwater also cause elevated SO_4^{2-} concentrations in the groundwater, by favoring the oxidation of pyrite in the subsoil (Stumm & Morgan, 1981; Nordstrom, 1982; Kölle et al., 1985) (Eq. [1] & [2]).

Increased SO_4^{2-} concentrations can have important consequences for wetlands fed by groundwater. SO_4^{2-} reduction leads to the production of sulphides in reductive organic sediments (Berner, 1970). If the seepage is also rich in iron (Fe) then Fe-sulphides, including pyrite, will strongly accumulate. Heavy metals also will accumulate as metal-sulphides, or will be adsorbed to Fe-hydroxides. This might have consequences during dry periods. Acidification and mobilisation of heavy metals as a consequence of pyrite and metal-sulphide oxidation is well known from drained acid mines but also from ecosystems on coastal plains (Van Breemen, 1973; Barton, 1978; Harries & Ritchie, 1983; De Jong et al., 1994; Anisfeld & Benoit, 1997; Banks et al., 1997; Portnoy, 1999). The phenomenon also has been documented for oligotrophic pools contaminated with S via the atmosphere. These pools all had in common that they were too poorly buffered to counteract the acid production during pyrite oxidation in dry periods (Vangenechten et al., 1981; Van Dam, 1987; Schuurkes et al., 1988; Van Haesebroeck et al., 1997). From earlier research it was already known that the presence of (bi)carbonate buffers in the sediment can retard acidification resulting from desiccation (Van der Sluijs, 1970; Ritsema & Groenenberg, 1993). The produced hydrogen ions react with the cation exchange buffer of the sediment (Eq. [3]), the alkalinity of the sediment pore water including bicarbonate (HCO_3^-) (Eq. [4]) and with carbonates in the sediment including calcite (Eq. [5]) and dolomite (Eq. [6]). Acidification will only occur when the buffering capacity of the sediment is insufficient to compensate for the acids produced due to pyrite oxidation (Drever, 1997).

$$2\ FeS_{2(s)} + 6\ NO_{3\ (aq)}^- + 2\ H_2O \rightarrow 2\ FeOOH_{(s)} + 3\ N_2 + 4\ SO_{4\ (aq)}^{2-} + 2\ H_{(aq)}^+ \qquad [1]$$

$$4\ FeS_{2\ (s)} + 15\ O_{2(g)} + 14\ H_2O \rightarrow 4\ Fe(OH)_{3(s)} + 16\ H_{(aq)}^+ + 8\ SO_{4\ (aq)}^{2-} \qquad [2]$$

$$]\text{-}Ca^{2+} + 2\ H^+ \rightarrow 2\ ]\text{-}H^+ +\ Ca^{2+} \qquad [3]$$

$$HCO_{3\ (aq)}^- + H_{(aq)}^+ \rightarrow H_2O + CO_{2(g)} \qquad [4]$$

$$CaCO_{3(s)} + 2\ H_{(aq)}^+ \rightarrow Ca_{(aq)}^{2+} + H_2O + CO_{2(g)} \qquad [5]$$

$$CaMg(CO_3)_{2(s)} + 4\ H_{(aq)}^+ \rightarrow Ca_{(aq)}^{2+} + Mg_{(aq)}^{2+} + 2\ H_2O + 2\ CO_{2(g)} \qquad [6]$$

Chapter 5 The S/(Ca+Mg) ratio in the soil

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In the province of Limburg, the Netherlands, many black alder carrs (forested wetlands dominated by black alder (*Alnus glutinosa*)) are situated in old terrestrialised meanders of the river Meuse. This region is very rich in Fe and therefore, most of these ecosystems have been fed by groundwater rich in Fe for decades (Van den Munckhof, 2000). Hydraulic measures for agricultural purposes have led to lower groundwater tables, resulting in long, severe and frequent drought periods during the summer. This might increase the susceptibility of these ecosystems to S input via seepage that takes place during wet winters or for S that has accumulated in preceeding years or decades. Next to this, drainage due to ecosystem restoration activities, and the concomitant dredging of degraded sediments, could cause acidification and heavy metal contamination of surrounding soils. However, black alder carrs are fed by deep groundwater which also contains relatively high amounts of (bi)carbonate, calcium (Ca) and magnesium (Mg) (Stortelder et al., 1998) and might therefore be less susceptible to desiccation than the above mentioned slightly buffered pools.

To investigate the potential susceptibility to drought, sediment of 17 alder carrs (124 locations) in the south-eastern part of the Netherlands were sampled for determination of the potential sensitivity to desiccation and acidification. As far as we know this is the first study concerning the sensitivity of non-coastal minerotrophic wetlands to drought. A laboratory experiment was done to test whether addition of $CaCO_3$ can prevent acidification and percolation of heavy metals from dredged sediments. Finally, the effect of temporary drainage by restoration activities in the Venkoelen was measured (the sediment of the lime addition experiment originated from this location and the loaction also took part in the sediment survey). We hypothesise that the susceptibility of wetlands to desiccation, following drought, depends upon the fixed balance between the Ca and Mg buffering capacity and the reduced S content of the sediment.

Materials and Methods

Potential sensitivity of wetlands to desiccation: a sediment survey
Sediments from 17 black alder carrs in the south-eastern part of the Netherlands were sampled during the summer of 1999. Most of the black alder carrs are situated in old terrestrialised meanders of the river Meuse (Figure 1). In each black alder carr, 2-17 locations were sampled resulting in a total number of 124 locations. At each location, pore water was collected anaerobically at 5-15 cm depth with Rhizon SMS soil moisture samplers (Rhizon SMS- 5 cm; Eijkelkamp Agrisearch Equipment, the Netherlands) connected to 100 ml N-flushed vacuum serum bottles. A sediment sample of 1 kg was collected at the same location and depth.

From each sediment sample 50 g was dried in duplo (24 h at 70 ^{0}C) for determination of the moisture content. Hereafter half of the the dried samples were homogenised and 200 mg was

digested for 17 minutes with 4 ml concentrated nitric acid and 1 ml 30 % hydrogen peroxide using a Milestone microwave type mls 1200 Mega. Digestates were stored in iodated polyethylene bottles at 4 ^{0}C until further chemical analyses. The other half of the dried sediment samples were incinerated (6 h at 550 ^{0}C) for determination of the organic matter content. To determine the effect of desiccation of the sediments, 500 g of the fresh sediments were gradually dried in a funnel (r=7.5 cm, h=10 cm) placed in a glass flask at 20 ^{0}C. The outflow of the funnel was covered with plastic gauze to prevent the loss of sediment. After 2 months, the dried sediments were homogenised, rewetted with distilled water up to 500 g to obtain the original moisture content, and rehomogenised by shaking for 24 hours at 100 rpm in a 1 l polyethylene bottle. Hereafter pore water samples were collected with Rhizon SMS soil moisture samplers connected to 100 ml vacuum serum bottles.
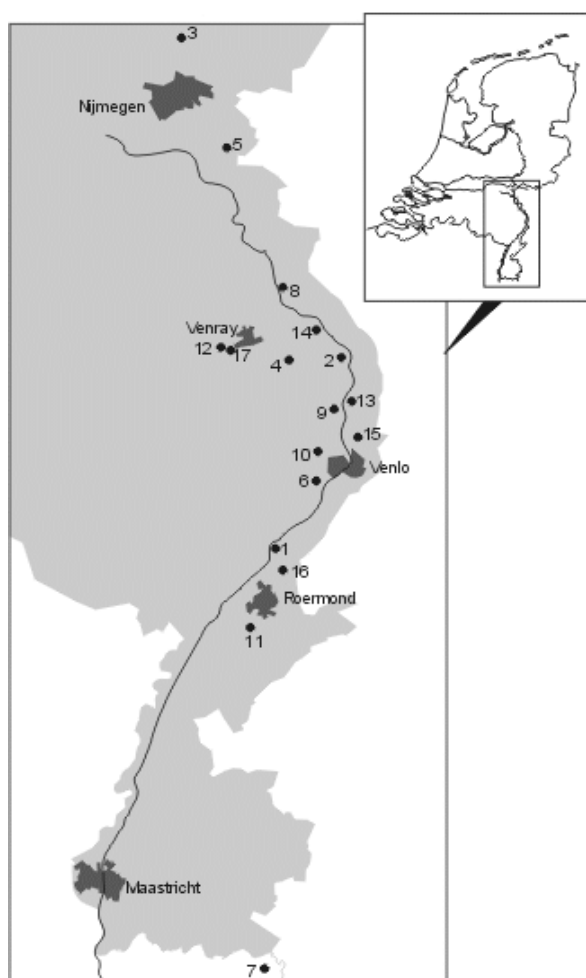

Figure 1. Geographical positions of the sampled fens. 1, Beeselsbroek; 2, Broekhuizerschuitewater; 3, Bronbeek; 4, Castenrayse Vennen; 5, De Bruuk; 6, Dubbroek; 7, Geuldal; 8, Heuloërbroek; 9, Kaldenbroek; 10, Koelbroek; 11, Landgoed Hoosden; 12, Spurkt; 13, Lommerbroek; 14, Roekenbosch; 15, Schaapsbroek; 16, Swalmdal; 17, Weversloosbroek.

Restoration of the Venkoelen: CaCO₃-addition to removed peaty and silty sediment and the effect of temporary drainage.

The Venkoelen is a partly terrestrialised lake situated in an old meander of the river Meuse in the nature reserve "Het Zwartwater" near Venlo, the Netherlands (51° 24'N; 6° 11'E). 1/3 of the water volume of the lake originates from local seepage rich in SO_4^{2-} and Fe (in 1998 maximum SO_4^{2-} and Fe concentrations of 1792 µmol l⁻¹ and 1532 µmol l⁻¹ were measured in the groundwater for example). In the sixties the lake was limed on behalf of restoration of the fish populations. As a consequence, SO_4^{2-} reduction and mineralisation in the originally slightly buffered sediment were strongly stimulated resulting in sulphide (S^{2-}) accumulation and eutrophication (Smolders and Roelofs, 1999). In order to restore the ecosystem it was temporary drained in November 2000 and degraded sediment was removed to a depot (Figure 2).

Figure 2. Drainage of the Venkoelen during restoration activities (removal of degraded sediment to a depot).

To study the effect of dredging and liming on the quality of the percolating groundwater, 100 l of peaty and silty surface sediment was collected at different locations situated in the middle of the mire. These subsamples were mixed and the following procedures were carried

out: from both sediment types 10 samples of 50 g were dried (24 h at 70 ^{0}C) and digested for chemical composition. 0.5, 2.5, 5 and 7.5 g CaCO$_3$ was mixed with 500 g fresh sediment (n=4) and sediments, including untreated sediment, were gradually dried as mentioned before. Percolating water of the first week was collected. After three and five weeks percolating water was collected after the addition of 125 ml distilled water to the surface of the funnel. To study the effect of drainage, pore water was collected with ceramic cups (as mentioned before) in December 2000. The results were compared with measurements taken at the same site in December 1997.

Chemical analyses

pH of the sediment pore water samples was measured using a standard Ag/AgCl$_2$ electrode connected to a radiometer Copenhagen type PHM 82 standard pH meter. Samples were stored at 4 ^{0}C in glass test tubes with 2 % concentrated HNO$_3$ to avoid precipitation of metals. Pore water samples and destruates of the sediment samples were measured for total Ca, Mg, S, manganese (Mn), Fe, aluminum (Al), fosfor (P), zinc (Zn), nickel (Ni), cobalt (Co), copper (Cu) and cadmium (Cd) using an inductively coupled plasma emission spectrophotometer (ICP, Spectroflame).

Data processing and statistical analyses

The sensitivity to acidification as a consequence of desiccation is expressed as the net production of free hydrogen ions per g of dry sediment. The sensitivity to mobilisation of heavy metals is expressed as the net mobilised fraction per g of dry sediment. The net mobilised fraction of elements per g dry sediment was calculated as follows:

$((D_e-F_e)* M /(1-M))/ S_e$

D_e = pore water concentration after desiccation and rewetting (μmol l^{-1})
F_e = pore water concentration under field conditions (μmol l^{-1})
S_e = total sediment content (μmol kg^{-1} dw).
M = moisture factor

Data of the lime addition experiment are presented as means ± standard error of the mean and are plotted against the sediment S/(Ca+Mg) ratio which differs per sediment type and per treatment as a consequence of liming. Differences between the chemistry of the percolating water due to liming were tested with a one-way analysis of variance (GLM procedure for repeated measures, SPSS 7.5). Significance of differences was subsequently tested with a Tukey post test at the 0.05 confidence limit. In Figures 6 and 7 the significance levels after five weeks are given.

Results

Potential sensitivity of wetlands to desiccation: a sediment survey

In Figure 3 the net mobilised fractions of SO_4^{2-}, the consumption of HCO_3^-, and the net mobilised fractions of Ca+Mg and heavy metals from the desiccated sediments are plotted against the net H^+ production. The net H^+ production increased with increasing mobilised SO_4^{2-} fraction. HCO_3^- was consumed when the net H^+ production increased. At net H^+ production higher than $\pm 10^{-5}$ µmol g^{-1} DW, all HCO_3^- was consumed and the net mobilised fractions of Ca+Mg and heavy metals, including Zn, Ni, Cd, Co, Al and Mn, strongly increased. Contrary to these metals, mobilisation of Fe, Cu and Pb only occured at high net H^+ production ($> 10^{-1}$ µmol g^{-1} DW).

The degree of acidification due to desiccation was correlated with the total S/(Ca+Mg) ratio in the sediment and with the total S/(Ca+Mg) ratio in the pore water after rewetting. The strongly acidified sediments contained total sediment S/(Ca+Mg) ratios higher than 2/3. In the pore water of the strongly acidified (pH < 4) rewetted sediments, the S concentration was higher than the Ca+Mg concentration (Figure 4a-b).
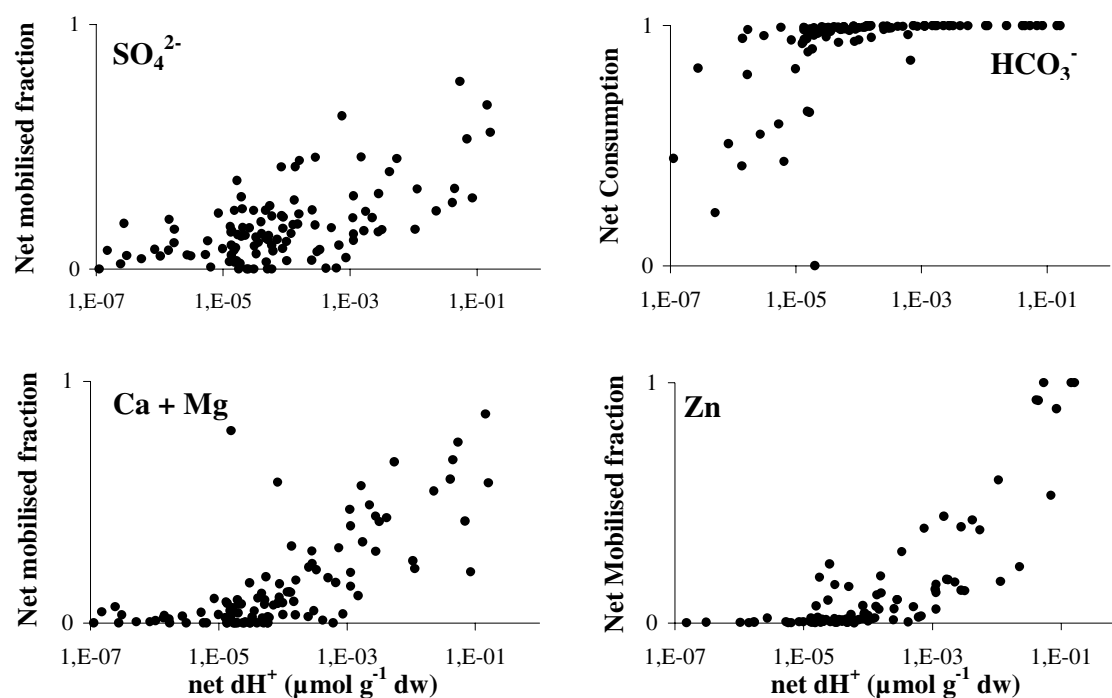

Figure 3. The net mobilised SO_4^{2-} fraction, the consumption of HCO_3^- and the net mobilised fractions of Ca+Mg and several (heavy) metals, plotted against the net H^+ production in the dredged sediments of the investigated alder carrs (figure continues on next page).
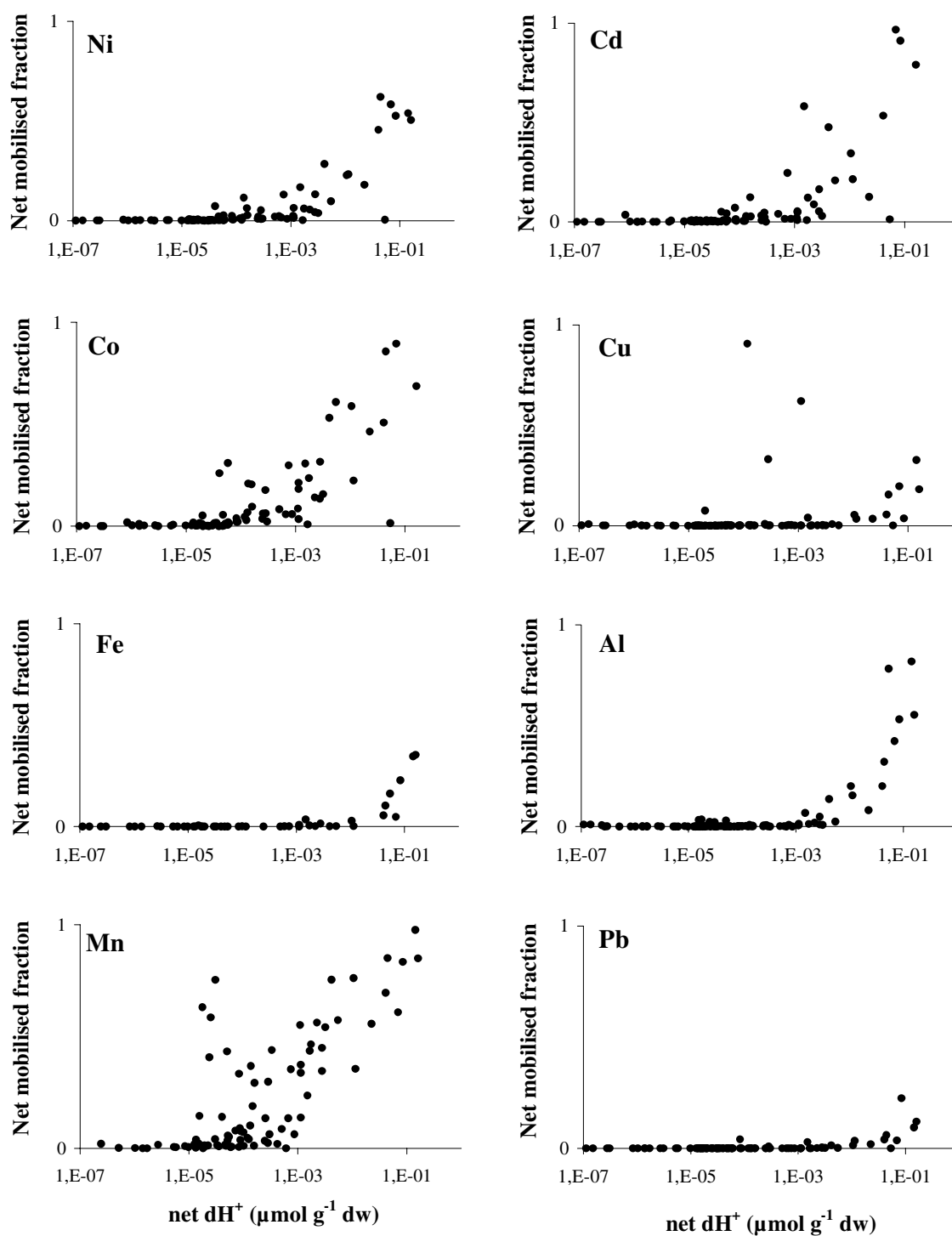
Figure 3 Continued. The net mobilised SO_4^{2-} fraction, the consumption of HCO_3^- and the net mobilised fractions of Ca+Mg and several (heavy) metals, plotted against the net H^+ production in the dredged sediments of the investigated alder carrs.
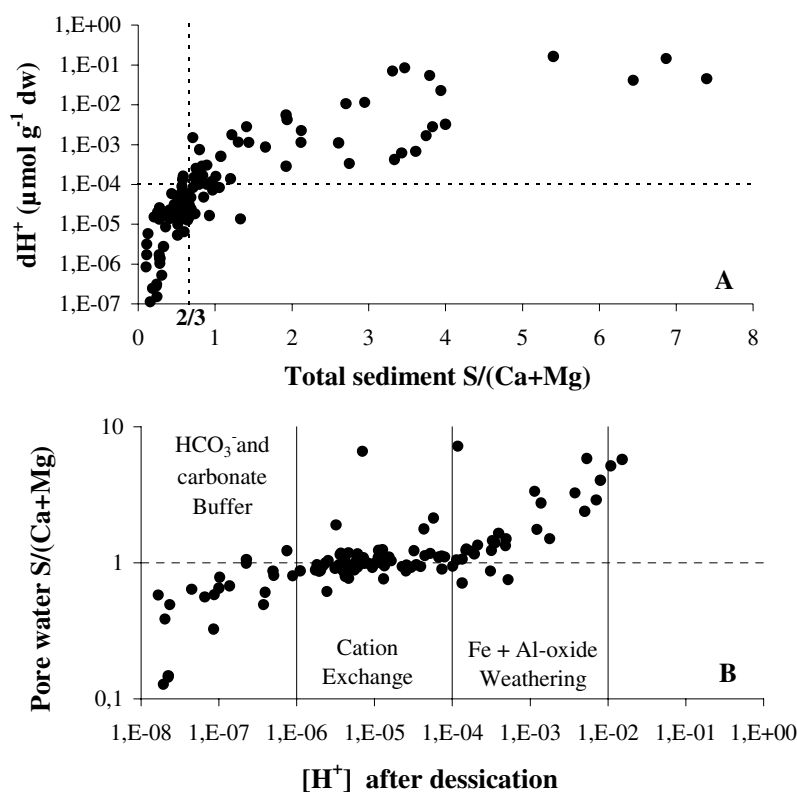
Figure 4. The sediment S/(Ca+Mg) ratio under field conditions plotted against the net H^+ production (**A**) and the SO_4^{2-}/(Ca+Mg) ratio in the pore water after rewetting of dredged sediments, plotted against the net logaritmic H^+ concentration (**B**).

The mean pH in the pore water of the 124 investigated sediments before desiccation was 6.65 ± 0.54 while the mean pH of the dried sediments dropped to 4.97 ± 1.33 (Figure 5a). Due to desiccation, concentrations of Zn, Cd, Ni, Co, Pb and Cu increased and exceeded the Dutch B-reference values for groundwater quality (Ministry of VROM, 1983) in respectively 100%, 75%, 50%, 45 %, 15% and 13% of the investigated locations (Figure 5b). The Dutch B-reference values for groundwater quality are given on top of the bars in Figure 5b.

CaCO₃-addition to dredged peaty and silty sediment of the Venkoelen
In Figure 6 & 7 the pH and the concentration of various elements in the percolating water of dredged peaty and silty sediment is plotted against the total S/(Ca+Mg) ratios in the sediments. The differences in S/(Ca+Mg) ratios are the result of differences in sediment type and liming treatments. Five weeks after dredging, the SO_4^{2-} concentration in the percolating water of both sediment types was significantly higher than at the start of the experiment. The SO_4^{2-} concentration in the percolating water of the peaty and silty soils increased, independently of the amount of lime added, from 400 to 7500 µmol l⁻¹ and from 100 to 3000

μmol l^{-1}, respectively. The HCO$_3^-$ concentration in the percolating water of both untreated sediment types decreased to zero (p < 0.05). In contrast, the concentrations of Ca and Mg strongly increased from 200 to 4000 μmol l^{-1} and 100 to 800 μmol l^{-1} (p < 0.05) in the peaty sediment and from 500 to 1500 μmol l^{-1} and from 200 to 400 μmol l^{-1} (p < 0.05) in the silty sediment. In the percolating water of the untreated peaty and silty sediments, the pH decreased from pH 4.5 to 3 and from pH 7.5 to 3.5 (p < 0.05), respectively. This decrease in pH was also accompanied by an increase in the concentration of heavy metals, including Zn, Ni, Cd, Co and Al (p < 0.05). Concentrations were especially very high in the percolating water of the peaty sediments. The concentration of Fe and Cu, however, significantly decreased in both sediment types following desiccation.

The drop of pH in the percolating water of both sediment types decreased with increasing CaCO$_3$ addition. Due to CaCO$_3$ additions higher than 2.5 g and 7.5 g, to the peaty and silty sediment respectively, pH remained unaffected compared to original conditions. As well as pH, the concentrations of heavy metals in the percolating water of both sediment types also decreased with increasing addition of CaCO$_3$. In the percolating water of the peaty and silty sediment, addition of at least 2.5 g CaCO$_3$, strongly reduced mobilisation of Zn, Ni, Cd, Co and Al after five weeks of desiccation. Addition of CaCO$_3$ instantly decreased Fe and Cu concentrations of the percolation water, especially in the organic sediment.

Effect of drainage in the Venkoelen

The effect of drainage on the pore water chemistry of the Venkoelen is shown in Table 1. As a consequence of drainage the mean pH decreased from 6.37 to 4.27 with a minimum value of 3.57. The mean SO$_4^{2-}$ concentrations increased from 0.12 mmol l^{-1} to 16.77 mmol l^{-1} with a maximum value of 52.38 mmol l^{-1}. Next to this also Ca, Mg and the concentration of various metals increased. For example the mean Al concentration increased ten times up to 1025 μmol l^{-1}.

Table 1. Some chemical properties of the sediment pore water of the Venkoelen under undrained conditions in December 1997 and under drained conditions during restoration activities in December 2000 (one month after the start of drainage). Mean (minimum and maximum) values are given in μmol l^{-1}.

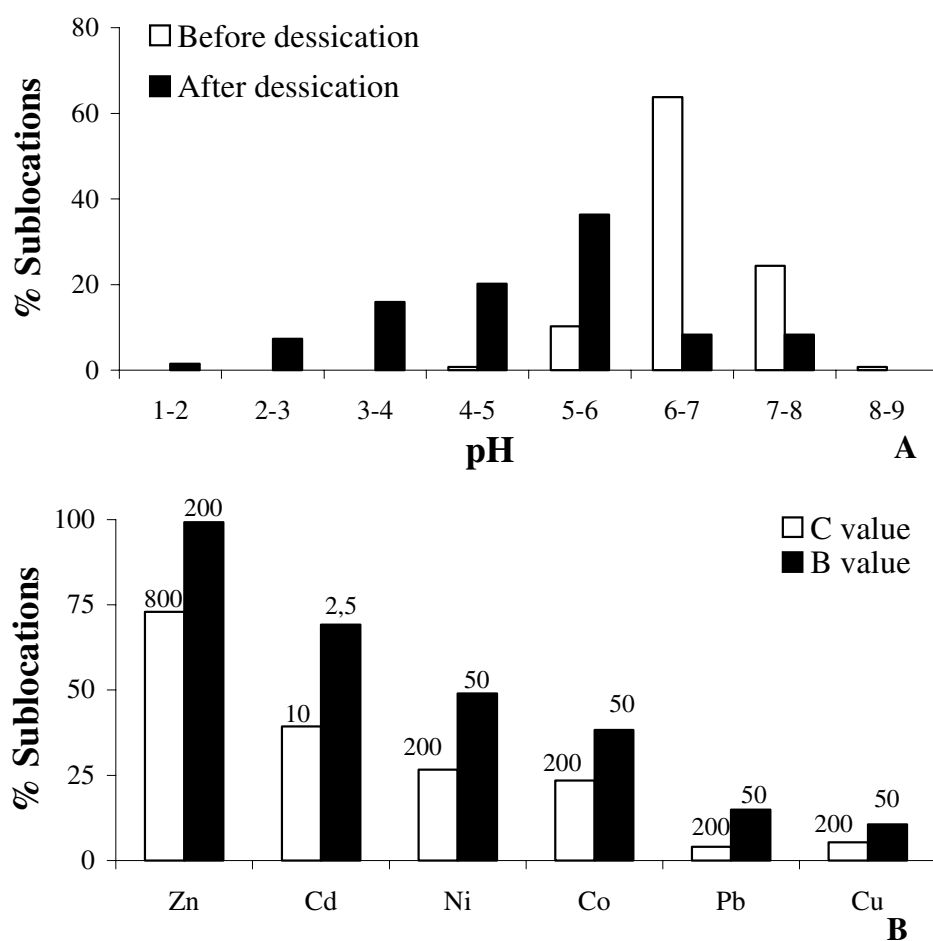|	undrained (n=10)	drained (n=5)
pH	6.37 (6.24-6.45)	4.27 (3.57-5.48)
SO$_4^{2-}$	121 (51-472)	16774 (260-52380)
Ca	631 (517-871)	15138 (256-49982)
Mg	359 (228-446)	909 (193-2005)
Al	10 (2-45)	1025 (11-2828)
Zn	1.6 (1.2-2.3)	191 (3-890)

Figure 5. pH of the sediment pore water samples of the investigated alder carrs under field conditions and after rewetting following desiccation **(A)** and the percentage of the locations that exceeded the Dutch B and C reference values for groundwater quality after rewetting of dredged sediments concerning Zn, Ni, Cd, Co, Pb and Cu **(B)**. The Dutch B and C reference values for the various elements are given on top of the bars (ppb).
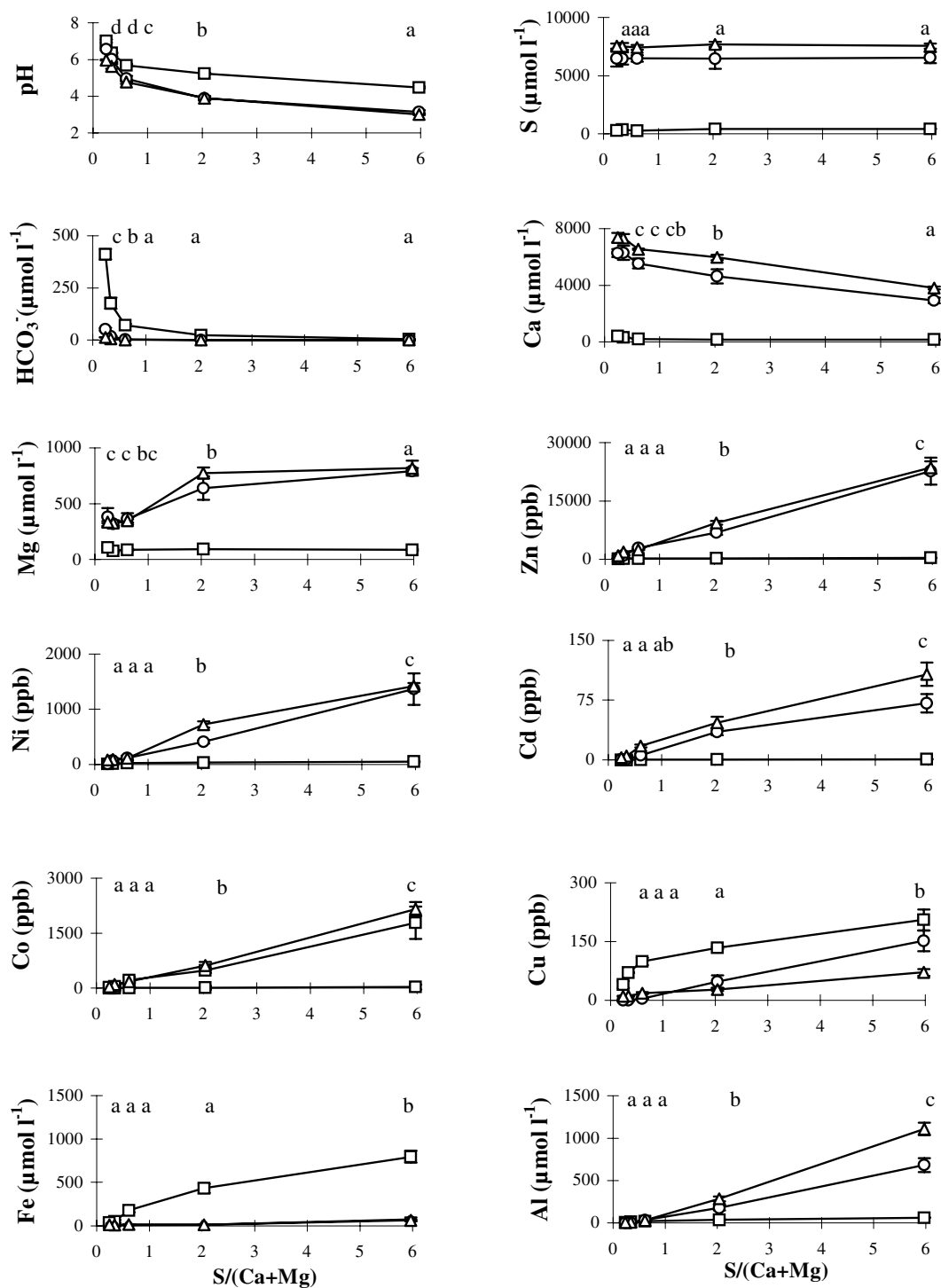
Figure 6. pH and concentrations of HCO$_3$-, total S, Ca, Mg, Fe, Al (μmol l^{-1}) and Zn, Ni, Cd, Co, Cu (ppb) in the percolating water of the unlimed and limed peaty sediment of the Venkoelen, one (□), three (○) and five (Δ) weeks after desiccation. Means with a same number do not significantly differ after five weeks. The differences in S/(Ca+Mg) ratios are a result of liming.

Figure 7. pH and concentrations of HCO_3^-, total S, Ca, Mg, Fe, Al (μmol l^{-1}) and total Zn, Ni, Cd, Co, Cu (ppb) in the percolating water of the unlimed and limed silty sediment of the Venkoelen, one ($\square$), three ($\bigcirc$) and five ($\triangle$) weeks after desiccation. Means with a same number do not significantly differ after five weeks. The differences in S/(Ca+Mg) ratios are a result of liming.
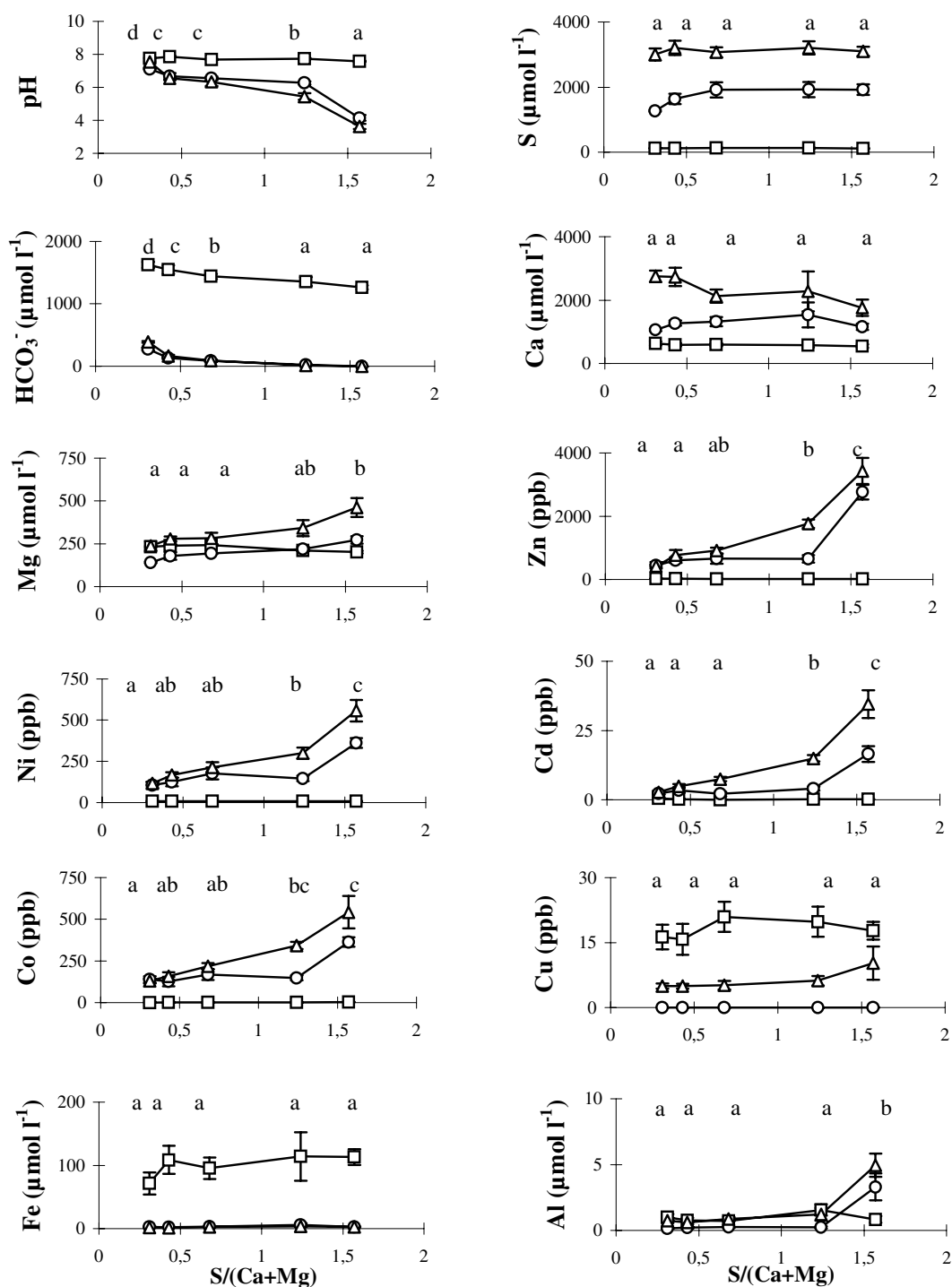
Discussion

The results of the sediment survey indicated that complete desiccation of black alder carr sediments is associated with greater acidity and mobilisation of heavy metals when the total S/(Ca+Mg) ratio of the sediment is higher than ± 2/3. A total of 47 % of the 124 investigated locations had S/(Ca+Mg) ratios higher than 2/3. Depending on the metal content of the sediment, mobilisation of Zn, Cd and Ni exceeded the Dutch reference signal for groundwater in 100, 75 and 50 % of the locations respectively. Results of the lime addition experiment confirmed that increasing the buffering capacity of dredged sediment of the Venkoelen, by adding $CaCO_3$ down to S/(Ca+Mg) ratios lower than 2/3 (5 g $CaCO_3$/kg FW), strongly inhibited percolation of heavy metals due to desiccation following dredging. Field measurements in the Venkoelen, under drained conditions, indicated that freshwater wetland sediments may indeed be very sensitive to acidification after aeration under field conditions when sediment S/(Ca+Mg) ratios are high. In the experimentally dredged sediments, the SO_4^{2-} concentrations in pore water increased as a consequence of Fe-(di)sulphide oxidation. Depending on the amount of Fe-(di)sulphides, a concomitant acid production initially led to consumption of HCO_3^-, and at high net H^+ production, to consumption of carbonates in the sediment. This was indicated by the increased Ca and Mg concentrations in the pore water of the acidified sediments. Our results indicated that when the S/(Ca+Mg) ratio in the sediments exceeds approximately 2/3, the cation exchange and carbonate buffer content are insufficient to neutralise acid production as a result of Fe-(di)sulphide oxidation during desiccation. Consequently, the sediments acidified strongly and potentially toxic metals were mobilised. The occurence of these processes is also confirmed by the higher concentrations of SO_4^{2-} in pore water, produced as a result of Fe-(di)sulphide oxidation, compared to the concentrations of Ca and Mg that are generated by carbonate buffering of produced acid. In general the pore water of the rewetted acidified sediments had a SO_4^{2-}/(Ca+Mg) ratio > 1. This ratio clearly indicates the different buffer ranges operating during drought. From pH 8 to pH 6 bicarbonate in the pore water and Ca and Mg carbonates in the sediment will predominantly buffer the produced acid. When the H^+ concentration increases, Ca and Mg cations from the cation complex of the sediment will be exchanged. When pH drops below 4, Fe and Al oxide weatering takes place.

The results indicated that only a fraction of the total sediment S content was mobilised. Gypsum ($CaSO_4$) formation reduces the SO_4^{2-} and Ca concentrations in the desiccating sediments (Wagner, 1982; Ritsema et al., 1992) and thus the net mobilised S fraction. However, pyrite and FeS may be present in various forms and sizes of particles and the formation of Fe-oxide coatings during oxidation may strongly affect pyrite oxidation rates (Morse, 1991). Ca and Mg carbonates also may differ strongly in solubility (Scheffer & Schachtschabel, 1992). Some of the S will also be present in organic form and thus not be

mobilised following oxidation. Despite these uncertainties the sediment S/(Ca+Mg) ratio appeared to be a reliable indicator for the potential sensitivity of the studied systems to acidification and concomitant heavy metal mobilisation.

Metal-sulphides that are formed under reduced conditions are oxidised as a result of desiccation and the metals are bound to insoluble carbonates or (hydr)oxides (Drever, 1997). When pH drops below 4.5, almost all carbonates are dissolved and adsorption to Fe-oxide is the dominant mechanism. The fact that net mobilisation of various metals below this pH differed, is due to differences in adsorption capacities to Fe-hydroxides that are formed during pyrite oxidation. It is known that the adsorption capacity of Pb and Cu to Fe-hydroxides is much higher than the adsorption capacity of Zn and Cd. The adsorption capacity of Pb and Cu only decreases when pH becomes very low and Fe-hydroxides start dissolving (Dzombak & Morel, 1990). This explains why net low fractions of Fe, Pb and Cu were mobilised only in the sediments that became extremely acid (pH < 2) following desiccation).

The results of the laboratory experiment indicated that mobilisation and percolation of heavy metals, as a consequence of oxidation, also took place in dredged peaty and silty sediments of the Venkoelen. By adding $CaCO_3$ to the fresh sediments, the buffer capacitiy increased and pH remained higher, hereby preventing the dissolution of oxidised heavy metals. Due to the addition of at least 2.5 g $CaCO_3$ to 500 g fresh peaty and silty sediment (71.5 g and 16.7 g $CaCO_3$ per kg dry sediment respectively), the total S/(Ca+Mg) ratio in sediment decreased from 6 and 1.6 to 0.61 and 0.68, respectively and net mobilisation of heavy metals decreased drastically. This is in agreement with results of the sediment survey which indicated that strong acidification (pH < 4) and mobilisation of heavy metals, as a consequence of desiccation, only took place in sediments with S/(Ca+Mg) ratios > 2/3. Fe and Cu concentrations decreased upon desiccation due to formation of Fe-(hydr)oxides and adsorption of Cu to the Fe-(hydr)oxide surface. New formation of Fe and Cu carbonates led to an immediate decrease in concentration of these elements, after addition of $CaCO_3$ (Drever, 1997).

Under laboratory conditions, relative rates of oxygen supply and oxidation rates will be higher than under field conditions. Therefore one might question the relevance to processes in the field. The results of the field measurements in the Venkoelen indicated that severe acidification, as a result of drainage, takes place even when there is still contact with the buffering groundwater. The fact that desiccation, subsequent oxidation of Fe-(di)sulphides, and acidification really do take place in the Venkoelen also appears from historical data. After extreme dry summers of 1990 and 1995, pH of the Venkoelen dropped from 7 to 3.5-3.2 and SO_4^{2-} concentrations in surface water increased from 500 to 2600 µmol l^{-1}. In these periods it was reported that the fish and amphibian populations displayed drastic declines. *Equisetum fluviatile* also disappeared locally during the following years while other species

like *Juncus effusus* and *J. acutiflorus* seemed to benefit from the temporary acid conditions (Oranjewoud, 2000).

The natural presence or production of Fe-(di)sulphides in the top layer of non-coastal wetlands is uncommon. Therefore the question arises why the concentrations in surface sediments of the investigated ecosystems are so high. In the region we studied, the presence of a pyrite-containing soil layer at a depth of 20 m was detected years ago. It was shown that the SO_4^{2-} concentrations in groundwater at this depth increased strongly while infiltrating NO_3^- disappeared (Van Steenwijk, 1986). Monitoring of groundwater quality in 3 of the investigated alder carrs from 1998 to 2000, indicated that the mean SO_4^{2-} concentrations in the groundwater were up to 1750 µmol l^{-1} (Lucassen et al., 2000). SO_4^{2-} reduction results in the production of Fe-(di)sulphides in the organic Fe-rich sediments. Therefore it is possible that the formation of Fe-(di)sulphides in the investigated alder carrs has increased as an indirect result of the increased NO_3^- concentrations in the groundwater, caused by excess fertilisation of pastures during recent decades. In fact, pyrite is broken down in the deeper subsoil and rebuild in the shallow sediments of groundwater fed marshes. The actual oxidation of pyrite in the subsoil by NO_3^- has been demonstrated previously in field isotope tracer experiments (Aravena & Robertson, 1998; Pauwels et al., 1998) and in field mass balance calculations (Tesoriero et al., 2000). The degree of pyrite accumulation in ecosystems fed by groundwater will depend on the SO_4^{2-} and Fe concentrations of the seepage and the period of inundation. The highest total S and Fe concentrations are encountered in the zones that constantly receive seepage and never dry out. These zones are dominated by plants characteristic of alder carr forests like *Caltha palustris*, *Equisetum fluviatile* and *Ranunculus lingua*. Especially in these regions, temporal regional drainage by human intervention and dry summers might have severe consequences for these endangered vegetation types. This idea is supported by the strong decline of *Equisetum fluviatile*, a plant species typically growing in Ca rich seepage zones, after the extreme dry summers of 1990 and 1996 in the Venkoelen. Locations that dry out periodically do not build up that much pyrite and are less sensitive to desiccation (S/(Ca+Mg) < 2/3).

It can be concluded that the S/(Ca+Mg) ratio in the sediment can be used as a simple diagnostic tool in determining the sensitivity of mires to drought and acidification. It remains to be determined whether this also is the case on a large geographic scale.

Acknowledgements

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CHAPTER 6

Effects of temporary desiccation on the mobility of phosphorus and metals in sulphur-rich fens: differential responses of sediments and consequences for water table management

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Wetland Ecology and Management: acceptable after revision

Abstract

In the Netherlands, permanently damming of sulphate (SO_4^{2-})-rich surface water, in order to rewet desiccated wetlands, has resulted in stagnation and eutrophication of surface water. Permanently damming of surface water prevents periodic drought during summer and leads to suppressed iron (Fe) input via the groundwater and to stimulated SO_4^{2-} reduction, all resulting in depletion of reducible Fe in the sediment. A laboratory experiment was conducted to assess the importance of temporary desiccation, its differential effects on various sediment types and the consequences for water table management. Permanently high SO_4^{2-}-rich surface water levels above sediments that are indirectly affected by shallow groundwater flows, resulted in severe eutrophication. The effect of temporary desiccation on P mobilization and immobilization strongly depended on the sediment Fe and P pools in combination with the buffering capacity of the sediment. Desiccation of sediment that is indirectly affected by shallow groundwater flows, led to a long-lasting reduction in phosphate (o-PO_4^{3-}) release from the sediment because a reduced Fe pool is present, resulting in the release of Fe upon oxidation. Formerly dry sediments that have not been influenced by groundwater for a long time do not possess such a reduced Fe pool and desiccation did not lead to a reduced P-release from these sediments resulting in considerable eutrophication of the water layer. In sediment of seepage zones that are directly and permanently influenced by deeper groundwater, reduced Fe and calcium (Ca) levels are so high that o-PO_4^{3-} was effectively immobilized under oxidized as well as reduced conditions. The results indicate that restoration of desiccated wetlands can not be achieved by simply retaining water by means of constructed dams. If water retention is artificially increased, temporary drops in water level during the summer are necessary to recharge the reducible P-binding Fe pool in large zones of the wetlands in order to prevent eutrophication.

Introduction

Sulphate (SO_4^{2-}) concentrations in groundwater and surface water have increased greatly during the last decades as a consequence of increased atmospheric sulphur (S) deposition and leakage from agricultural lands. In addition, falling water tables and infiltration of nitrate (NO_3^-) from agricultural lands into the groundwater also increases groundwater SO_4^{2-} concentrations by favouring the oxidation of FeS_x in the subsoil (Stumm & Morgan, 1981; Nordstrom, 1982; Kölle et al., 1985). SO_4^{2-} pollution of surface and groundwater is known to cause severe problems in freshwater wetlands (Roelofs, 1991; Smolders & Roelofs, 1993; Lamers et al., 1998). High SO_4^{2-} loads often lead to eutrophication (Caraco et al., 1989; Boström et al., 1982) and accumulation of phytotoxic sulphide (S^{2-}) (Smolders & Roelofs, 1996; Lamers et al., 1998). SO_4^{2-} reduction and concomitant S^{2-} production leads to reduction of Fe(III), including $FePO_4$ complexes, and concomitant phosphate ($o\text{-}PO_4^{3-}$) mobilization (Boström et al., 1982; Caraco et al., 1989). Furthermore, SO_4^{2-}-rich systems tend to be more sensitive to acidification and heavy metal mobilization following desiccation (Lucassen et al., 2002[b]). In general, this can lead to an increased dominance of a small number of fast growing S^{2-} and metal resistant plant species and thus to a loss of biodiversity. The response of wetlands to SO_4^{2-} pollution, however, depends on several factors. SO_4^{2-} reduction rates are lower in acidic and/or oxidative sediments than in more alkaline and/or reductive environments (Roelofs, 1991; Lamers et al., 1999), while C/P ratio and Fe concentration also play an important role in the extent of $o\text{-}PO_4^{3-}$ following SO_4^{2-} reduction (Lamers et al., 2001; Smolders et al., 2001).

In recent years, attempts have been made to restore desiccated alder carrs (fens dominated by black alder (*Alnus glutinosa*)) in the south eastern part of the Netherlands. This area is very rich in Fe (Van den Munckhof, 2000) and wetlands in this region are therefore enriched by groundwater rich in SO_4^{2-} and Fe (Lucassen et al., 2000). Since the 1970' s, regional water tables have been greatly lowered, largely in order to favour agricultural practices in the areas surrounding the wetlands. During the summer, groundwater influence decreases and the water table drops below the sediment surface in large parts of the alder carrs. This leads to undesirable desiccation during very dry periods, especially in the more elevated parts. Attempts to retain water by permanently damming SO_4^{2-}-rich groundwater, prevented periodic drought during summer and suppressed the input of Fe-rich groundwater. This was accompanied by increased availability of $o\text{-}PO_4^{3-}$ and S^{2-} in the surface water, massive development of algae, lemnids and fast-growing wetland grasses (e.g. *Glyceria maxima* L. and *G. fluitans* L.), and a strong decline of the original vegetation (e.g. *Caltha palustris* L., *Equisetum fluviatile* L., *Carex elongata* L.) (Lucassen et al., in press, Lucassen et al., submitted). Thus, permanent high water levels also appeared to have a negative effect on the development of the species-rich characteristic fen vegetation (Boxman & Stortelder, 2000;

Lucassen et al., 2000; Lucassen et al., 2002[a]).

Temporary desiccation of the top layer of the sediment might be important in preventing an undesirable vegetation development. Oxidation of dissolved Fe leads to precipitation of Fe as Fe(III) hydroxides, which effectively adsorb o-PO_4^{3-}. However after reflooding, this oxidized Fe is reduced again, possibly resulting in the re-release of the immobilized o-PO_4^{3-}. Hence, one would expect only a minor effect of desiccation. However, if significant amounts of insoluble reduced Fe, for instance iron-sulphides (FeS_x) or siderite ($FeCO_3$), are present, oxidation of the sediment results in the net release of Fe(III), which would greatly increase the amount of reducible oxidized Fe in the sediment capable of binding o-PO_4^{3-} and thus probably produce a more lasting beneficial effect of desiccation.

We hypothesized that the water regime, as well as the presence of various sediment types varying in reduced Fe content, play an important role in the nutrient (o-PO_4^{3-}) availability and plant composition of wetlands. To test this hypothesis, we studied the effect of two water regimes, with and without temporary desiccation, on three sediment types: sediment originating from seepage zones with a high reduced Fe content (S-sediment), sediment from zones that temporarily dry out during the summer, with a moderately high reduced Fe content (T-sediment) and sediment of formerly dry zones without reduced Fe (D- sediment).

Methods

*Origin of sediment*s

In the spring of 1999, 100 l of wet sediment (S-sediment) and 100 l of semi wet sediment (T-sediment) were collected from the Dubbroek alder carr (51° 22' N; 6° 05' E) while 100 l of dry sediment (D-sediment) were collected from the Koelbroek alder carr (51° 23' N; 6° 07' E). Both fens have vegetations belonging to the *Carici elongatae alnetum* and are situated in old, terrestrialized meanders of the river Meuse. S-sediment represents locations constantly receiving Fe and SO_4^{2-}-rich seepage and thus having a high reduced Fe content. T-sediment represents locations with fluctuating water tables having lower FeS_x contents. D-sediment represents locations from strongly desiccated alder carrs that are very poor in FeS_x or other reduced Fe forms. The S-sediment from Dubbroek was from a location dominated by *Equisetum fluviatile* L., *Caltha palustris* L. and *Rorippa nasturtium-aquaticum* (L.) Hayek, while the T-sediment was from a location dominated by *Carex acuta* L., *Ranunculus repens* L. and *Filipendula ulmaria* (L.). The D-sediment from Koelbroek was from a location dominated by *Rubus fructicosus* L.

Sediment characteristics

To determine the concentrations of several elements, 50 g of each sediment type was dried in sixfold (n=6) (24 h at 70 ^{0}C). Half of the dried samples were homogenised and a 500 mg

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portion of each was digested for 17 minutes with 4 ml concentrated nitric acid (Merck, Darmstadt, Germany) and 1 ml 30 % hydrogen peroxide (Merck, Darmstadt, Germany) using a Milestone mls 1200 Mega microwave (Sorisole, Italy). Digestates were stored in iodated polyethylene bottles at 4 ^{0}C until further chemical analysis.

To estimate the oxidizable reduced S content of the sediments at the start of the experiment, 500 g portions of the fresh sediments were gradually dried in a funnel (r=7,5 cm, h=10 cm) and placed in an Erlenmeyer flask (500 ml) at room temperature. The outflow of the funnel was covered with plastic gauze to prevent loss of sediment. After 2 months of drying, sediments were rewetted with distilled water to a total weight of 500 g (to regain the original moisture content) and rehomogenised by shaking for 24 hours at 100 rpm in a 1 l polyethylene bottle to dissolve all mobilized SO_4^{2-}. Subsequently, pore water samples were collected with teflon Rhizon SMS soil moisture samplers (Eijkelkamp Agrisearch Equipment, the Netherlands) connected to 100 ml vacuum serum bottles (Lucassen et al., 2002[b]).

Laboratory experiment: experimental design and treatments

The experiment was carried out between March 1999 and April 2000 in a climate controlled room (MC 785-Klima, Koel-Combi, the Netherlands) with an air temperature of 20 °C, an air humidity of 60 % saturation, a light level of 100 μmol m^{-2} s^{-1} and a daily photoperiod of 12 hrs. For each sediment type, 8 aquaria (25 x 25 x 30 cm) were filled with sediment up to 1/3 of their height, and then topped off with a solution that reflected the composition of the local groundwater (used chemicals: Merck, Darmstadt, Germany) (Table 1). Flow was created by means of peristaltic pumps that pumped solution from 10 l reservoirs through black silicone tubes at a flow speed of 10 l a month. In August 1999, the water layers in half (4) of the aquaria were removed and the water table was kept at a constant low level (-5 cm beneath ground surface) for 2,5 months. Demineralized water was added to the aquaria to compensate for evaporation. The surface water in the untreated aquaria were also removed after 2,5 months and all aquaria were carefully refilled with solution to be able to compare water chemistry development. Then, 5 g of fresh *L. minor* was added to each aquarium and after 3 months the dry weight of algae and lemnids was determined (24 hrs at 70 °C). Dried algae and lemnids were subsequently ground in liquid nitrogen, dried (24 hrs at 70 °C), and a 100 mg portion was digested as mentioned earlier. P is the limiting nutrient in development of algae and lemnids in black alder carr fens (Lucassen et al., in press, Lucassen et al., submitted) and therefore the digestates were only used to determine the total amount of o-PO_4^{3-} taken up from the water layer. Each four weeks, pore water samples were collected anaerobically in 100 ml vacuum serum bottles by means of teflon Rhizon SMS soil moisture samplers (Eijkelkamp Agrisearch Equipment, the Netherlands). Samples of the surface water were taken with 50 ml iodated polyethylene bottles.

Chemical analyses

CH_4 was measured using ethane as an internal standard on a 'Pye Unicam' gas chromatograph (Unicam Cambridge, UK) equipped with a flame photometric detector and a 'Porapak Q' (80/100 mesh) column ('Waters Chromatography', Etten-Leur, the Netherlands). The pH of the water samples was measured using a standard $Ag/AgCl_2$ electrode connected to a Radiometer Copenhagen type PHM 82 standard pH meter. HCO_3^- analyses were carried out using an 'Oceanograpy International' model 0525 HR infrared carbon analyser. The concentration of free S^{2-} was determined in a 10 ml subsample, fixed immediately after collection with S^{2-} antioxidant buffer containing sodium hydroxide, sodium EDTA and ascorbic acid (Van Gemerden, 1984). A S^{2-} ion-specific Ag elecrode and a double junction calomel reference electrode were used (Thermo Orion, Beverly). Half of the remaining samples were stored at 4 ^0C in glass test tubes with 2 % concentrated HNO_3 to avoid precipitation of metals. Total Ca, Mg, S, Mn, Fe, Al, P, Zn was analysed using an inductive coupled plasma emission spectrophotometer (ICP, Spectroflame). The remaining samples were stored at -24 ^0C in iodated polyethylene bottles and analysed for NH_4^+ according to Grasshoff and Johansen (1977), NO_3^- according to Kamphake et al. (1967) and o-PO_4^{3-} according to Henriksen (1965), using Technicon II autoanalysers.

Table 1. Composition of the basic medium (pH 7,8).
Concentrations are given in μmol l^{-1} .

KCl	MgCl$_2$.6H$_2$O	CaCl$_2$	NaHCO$_3^-$	Na$_2$SO$_4$
100	350	1500	3000	1000

Statistical analyses

Results are presented as means ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used to assess differences between sediment characteristics at the start of the experiment (n=3). A two-way ANOVA with repeated measures (GLM procedure, SPSS 7.5) was used to test effects of the sediment type and/or periodic desiccation on pore water chemistry and total biomass development of algae and lemnids during the complete experimental period. Differences between treatments (p $\leq$ 0,05) were subsequently tested with Tukey post tests. Significance was accepted at a level of p $\leq$ 0,05.

Results

Sediment characteristics

Sediment characteristics at the start of the experiment are shown in Table 2. T-sediment contained a significantly lower moisture and organic matter content compared to S and D-

sediment. All three sediment types did significantly differ in composition of elements with the exception of the Fe concentration in T and D-sediment. S-sediment had a significantly higher oxidizable S pool as well as higher concentrations of S, Ca, Fe and P than T and D-sediment, while Al and Mg concentrations were significantly lower. The Fe concentration and particularly the oxidizable S pool were lowest in D-sediment.

Table 2. Sediment characteristics at the start of the experiment. Total Ca, Mg, Fe, Al, P and S content in the sediment are given in µmol g^{-1} dry sediment (n=3). S concentrations in the pore water under field conditions (S field) and after temporary desiccation (S desicc.) are given in µmol l^{-1} (n=3). ΔS=Sdesicc. - S field. Means with the same symbol do not differ significantly.

	org. matter content	moist. content	tot Ca	tot Mg	tot Fe	tot Al	tot P	tot S	S field	S desicc.	Δ S
S	66.0 (1.4)[b]	84.8 (0.3)[b]	511 (16)[c]	31 (1)[a]	2043 (171)[b]	62 (3)[a]	116 (8)[c]	297 (4)[c]	1233 (52)[b]	11272 (1251)[c]	10039 (774)[c]
T	14.8 (0.4)[a]	54.4 (1.0)[a]	131 (1)[a]	121 (5)[c]	450 (5)[a]	418 (12)[c]	17 (1)[a]	41 (1)[a]	358 (77)[a]	4448 (583)[b]	4090 (438)[b]
D	78.6 (1.1)[b]	85.0 (0.1)[b]	183 (5)[b]	60 (1)[b]	206 (10)[a]	269 (18)[b]	38 (2)[b]	150 (9)[b]	987 (61)[b]	1021 (52)[a]	34 (70)[a]

Laboratory experiment: effects of temporary desiccation on the quality of pore water and surface water and the development of algae and lemnids

The effect of desiccation on sediment chemistry is shown in Table 3 and Figs 1a-c. Values of pH, alkalinity, HCO_3^-, S, Ca, Mg, NH_4^+ and NO_3^- concentration in the pore water were influenced by sediment type, water table and their interactive effect. As a consequence of desiccation, S concentrations generally increased while pH, alkalinity and HCO_3^- decreased. The increase in S was very high in S-sediment (22000 µmol l^{-1}) compared to T-sediment (5000 µmol l^{-1}) and D-sediment (1250 µmol l^{-1}). The drop in pH was largest in S-sediment (from 7.3 to 3.7) followed by D-sediment (6.2 to 4.7) and T-sediment (from pH 7.1 to 5.8).

Table 3. ANOVA table with (interactive) effects of sediment type and water table on sediment pore water chemistry and total biomass of algae and lemnid species. P-values are given as well as symbols of the Tukey-post test for each sediment type. [#], due to the contradictionary responses between S-sediment and T-sediment, no overall (interactive) effect of the water table was tested.

|	sediment	water table	sediment*water table	Tukey-test sediment		
				S	T	D
Biomass	0,000	0,25	0,000	a	b	c
pH	0,000	0,000	0,001	b	b	a
Alkalinity	0,000	0,000	0,011	b	c	a
HCO$_3^-$	0,000	0,000	0,000	b	c	a
Ca	0,000	0,000	0,000	b	c	a
Mg	0,000	0,010	0,000	b	c	a
S	0,000	0,000	0,000	b	a	a
S^{2-}	0,000	0,899	0,011	a	a	b
P	0,000	0,525[#]	0,432[#]	a	a	b
NH$_4^+$	0,000	0,000	0,000	a	b	a
NO$_3^-$	0,000	0,003	0,000	a	b	b
CH$_4$	0,000	0,000	0,007	a	s	b
Fe	0,000	0,078	0,000	b	c	a
Mn	0,000	0,091	0,014	b	c	a
Zn	0,001	0,001	0,000	b	b	a
Al	0,000	0,149	0,209	a	a	b
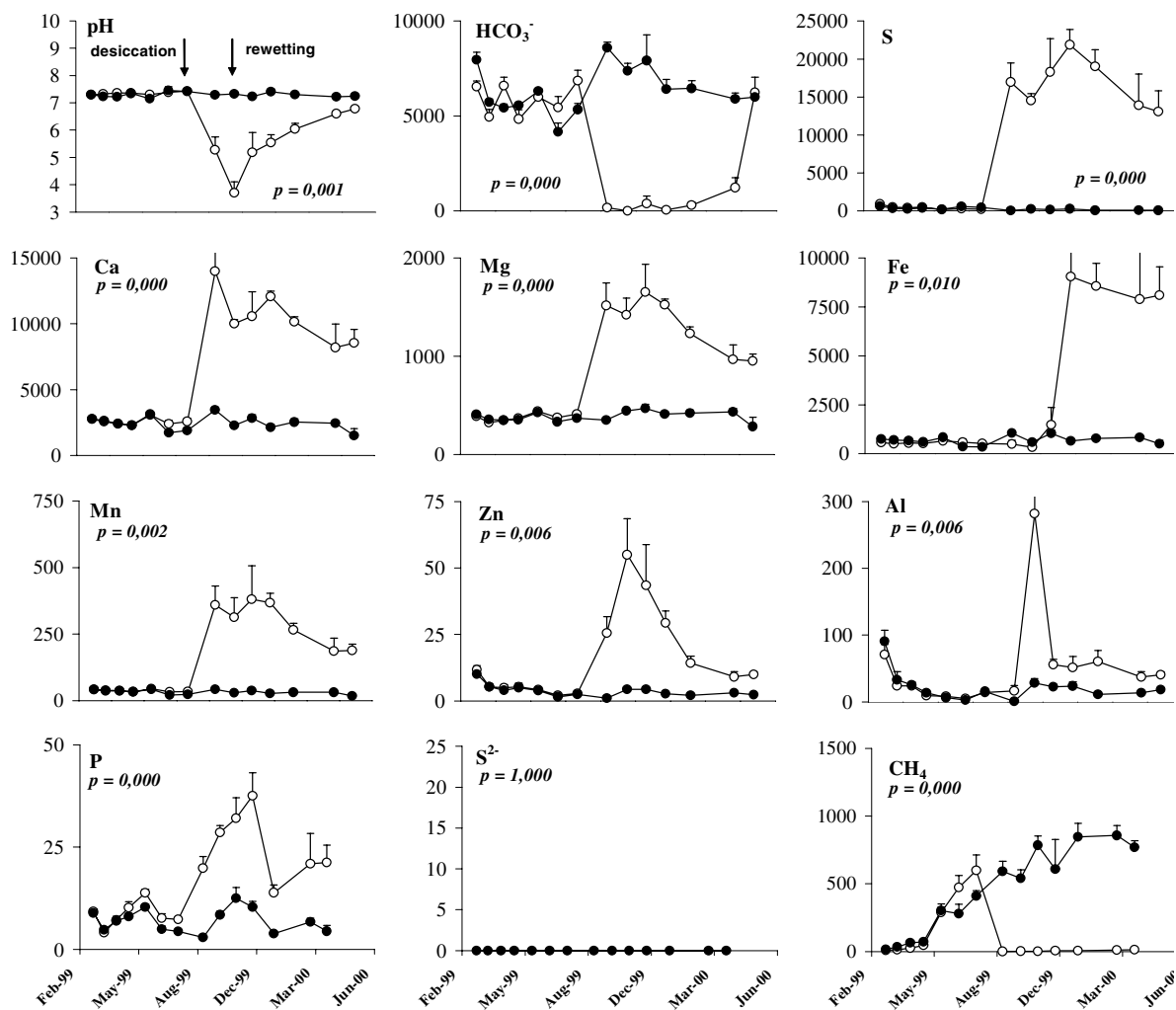
Figure 1a. Sediment pore water chemistry of **S-sediment** in a state of permanent inundation (●) and in a situation including a 2-month drought period (-5 cm) from August to October (○). Concentrations are shown in μmol l^{-1}. P-values are given for the overall experimental period.

The concentration of P in the pore water was also influenced by sediment type, water table and their interactive effect. The effect of desiccation on the P concentration in the pore water depended strongly on sediment type. The concentration of P decreased strongly in D-sediment (from 100 μmol l^{-1} to 3 μmol l^{-1}) during desiccation but immediately increased following rewetting. P concentration in T-sediment also decreased strongly as a consequence of desiccation (from 33 μmol l^{-1} to 3 μmol l^{-1}) and, unlike D-sediment, the P concentration increased slowly after rewetting and lower P concentrations persisted for a long period following rewetting. Desiccation of S-sediment led to an increase in P concentration, which remained higher than in the control treatment, for the entire experimental rewetting period. Apart from nutrients, the concentrations of metals in the pore water were also influenced by sediment type and/or water table and their interactive effect. Desiccation led to a

considerable increase in the concentrations of Zn (from 3 to 55 µmol l^{-1}), Mn (from 35 to 380 µmol l^{-1}) and Al (from 17 to 282 µmol l^{-1}) in S-sediment. The concentrations decreased slightly after rewetting, but remained higher than those in the control treatment during the entire experiment. These effects were less obvious in the other sediments. Desiccation led to a slight drop in Fe and Mn concentrations in T-sediment and Fe and Al concentrations in D-sediment. After rewetting, these concentrations returned to the original values.
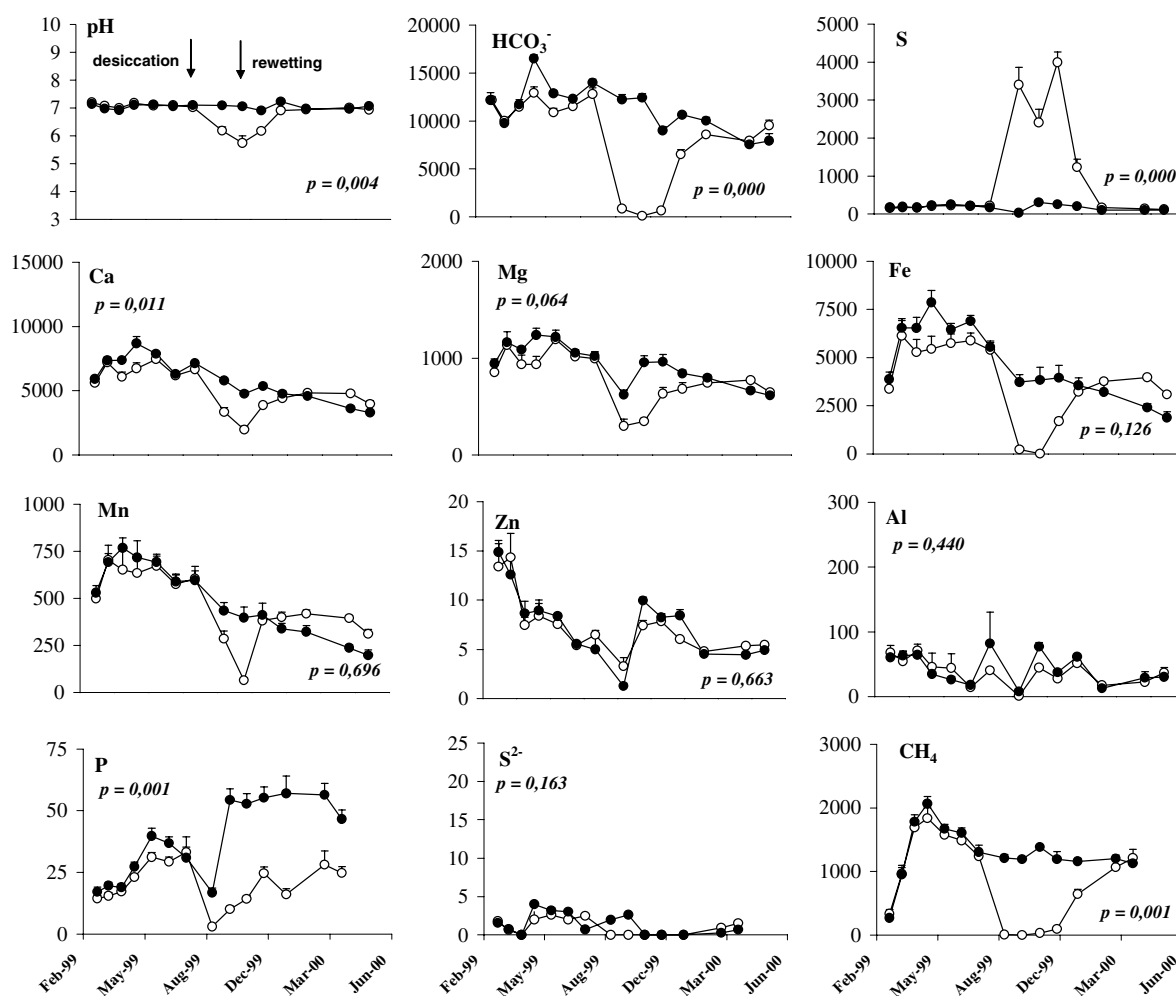
Figure 1b. Sediment pore water chemistry of **T-sediment** in a state of permanent inundation (●) and in a situation including a 2-month drought period (-5 cm) from August to October (○). Concentrations are shown in µmol l^{-1}. P-values are given for the overall experimental period.

The effect of desiccation on water quality, P uptake and algal and lemnid biomass development after rewetting of desiccated sediment is shown in Fig. 2, Fig. 3 and Table 3. In general, desiccation led to a significant drop in pH and concentration of HCO$_3^-$ (with the

exception of pH in T-sediment) and a significant increase in Ca, Mg and S concentrations in the surface water. o-PO_4^{3-} concentration of the surface water above S-sediment and D-sediment were significantly increased and decreased respectively, while desiccation had no significant effect on o-PO_4^{3-} concentration of the surface water above T-sediment. P-uptake and biomass development of algae and lemnids was highest in the surface water above D-sediment, independently of the water regime. A comparable effect took place in the surface water above T-sediment without desiccation of the sediment. Desiccation of T-sediment, however, strongly restricted P-uptake and algal and lemnid biomass development.
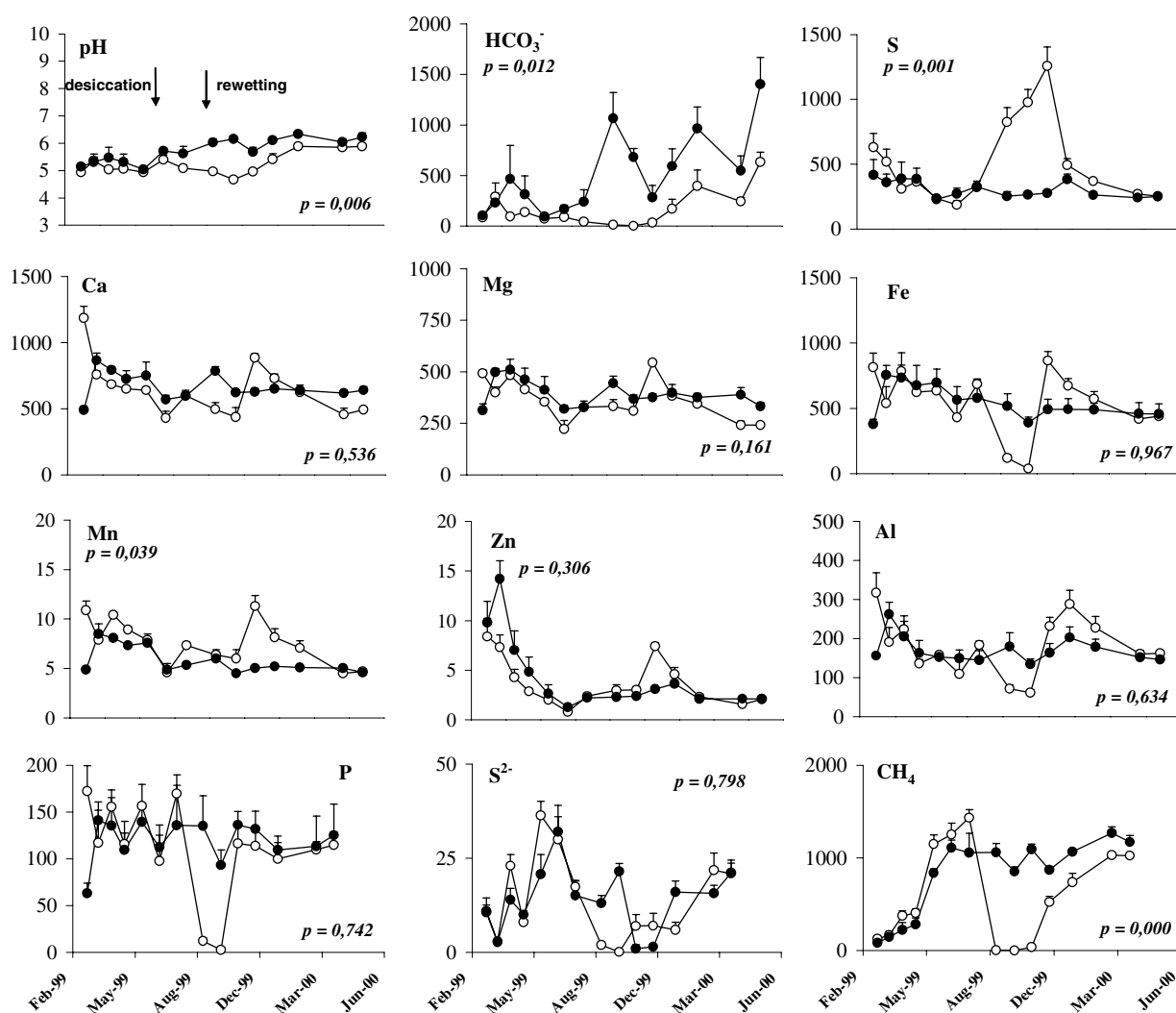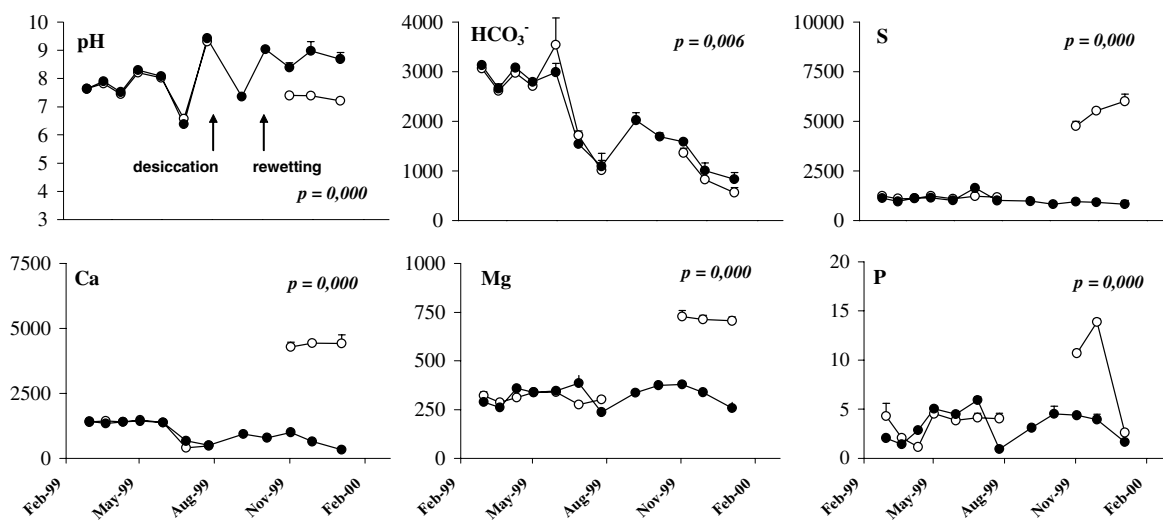

Figure 1c. Sediment pore water chemistry of **D-sediment** in a state of permanent inundation (●) and in a situation including a 2-month drought period (-5 cm) from August to October (○). Concentrations are shown in µmol l^{-1}. P-values are given for the overall experimental period.

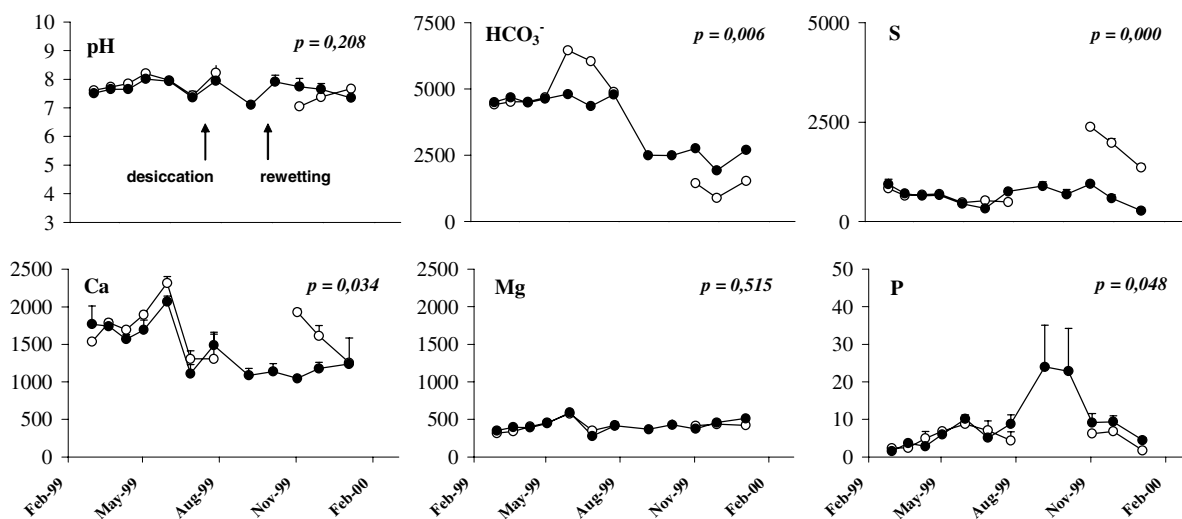**S-sediment**

T-sediment

Figure 2. Surface water chemistry above the sediments, in a state of permanent inundation (●) and in a situation including a 2-month drought period (-5 cm) from August to October (○). Concentrations are shown in µmol l^{-1}. The first surface water sample after the desiccation period, was taken three weeks after refilling of the aquaria. P-values are given for the 2 month period after rewetting.
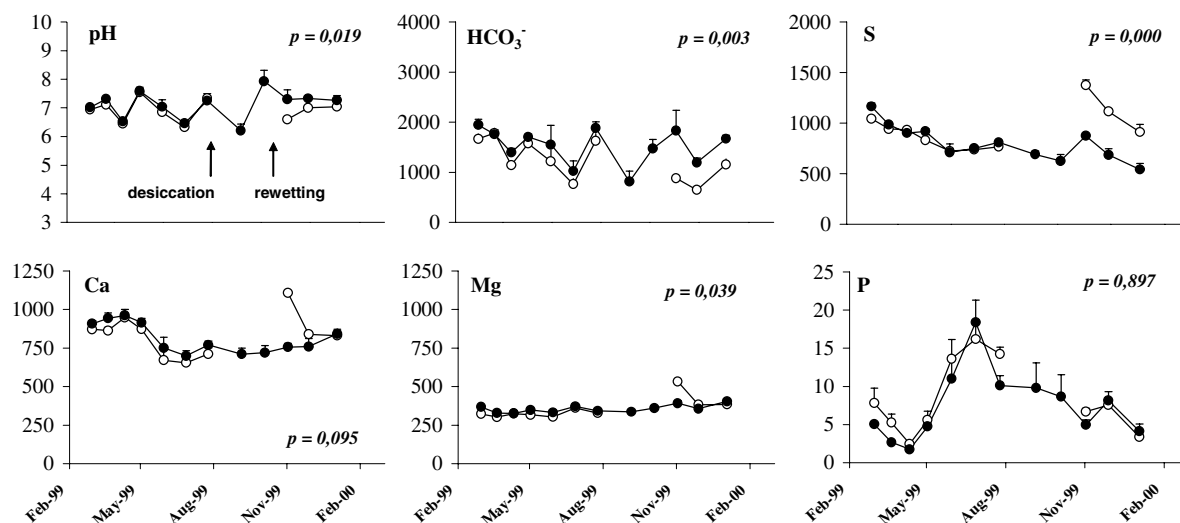
81

D-sediment

Figure 2. **(Continued).**

The P-uptake and development of algae and lemnids in the surface water above S-sediment was limited but unlike that in T-sediment, desiccation led to a slight increase in biomass development. In general, desiccation had little to no effect on metal concentration in the water layer following rewetting, with the exception of Al and Mn, which increased in the water layer above D-sediment and S-sediment respectively (results not shown).
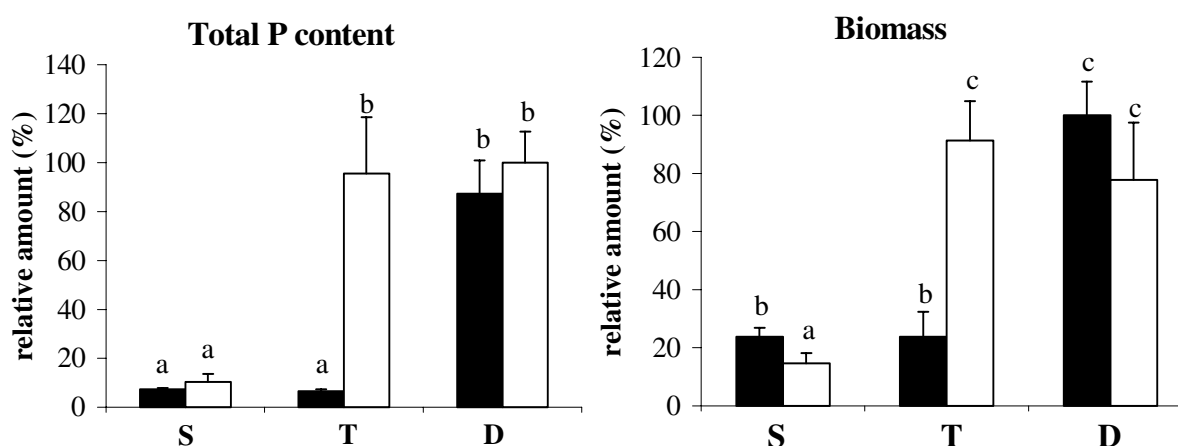

Figure 3. Relative amounts (%) of P uptake and biomass development by algae and lemnids in the water layer. ■ = desiccation period involved; □ = without desiccation period involved. 100 % dry weight = 88.64 gram m^{-2}. 100 % P content = 6.67 mmol P m^{-2}. Means with the same letter do not significantly differ.

Discussion

Effect of temporary desiccation on P-availability in sediment

The results show that the P concentrations in pore water depended upon the sediment type and the interaction between sediment type and water table fluctuations.

Inundation of strongly desiccated parts of alder carrs (D-sediment) caused severe eutrophication of the water layer. This can be attributed to the high o-PO_4^{3-} concentrations in the sediment pore water and the low levels of Fe in the sediment. In desiccated sediments, Fe is present in the oxidized form and is highly immobile. As a consequence, o-PO_4^{3-} is largely immobile and mostly present as $FePO_4$ or adsorbed to Fe(hydr)oxides. Anoxic conditions in the sediment after inundation lead to the reduction of oxidized Fe compounds and result in the mobilization of the previously immobile o-PO_4^{3-} (Roelofs, 1991; Wetzel, 2001). This process is accelerated by SO_4^{2-} reduction, as S^{2-} can also reduce Fe(III) from Fe-PO_4^{3-}-complexes, resulting in the formation of FeS_x and mobilization of o-PO_4^{3-} (Sperber, 1958; Patrick and Khalid, 1974; Boström et al., 1982; Smolders and Roelofs, 1993; Golterman, 1995; Lamers et al., 1998). The degree of o-PO_4^{3-} mobilization resulting from waterlogging will therefore depend upon the free Fe content of the sediment, the SO_4^{2-} concentration of the groundwater and the period of inundation. Thus, inundation of previously dry soils generally results in increased availability of o-PO_4^{3-}. Desiccation, and hence re-oxidation, of the sediment strongly decreased o-PO_4^{3-} concentrations, but levels rapidly re-established upon rewetting when the process is reversed. On the whole, there was no net beneficial effect of temporal desiccation, and a strong eutrophication of the water layer was observed in both treatments (Figure 1c & 3).

Well-developed alder carr vegetation can be found at locations that always used to be waterlogged or shallowly inundated by groundwater in winter and that become desiccated only during the drier summer months (T-sediment) (Stortelder, 1998). Permanent inundation of these sediment resulted in very high P concentrations in the pore water even when the organic matter content is relatively low (15 %). The temporal desiccation at these locations resulted in a more persistent decrease in the P levels in sediment pore water after rewetting. In our experiment, the temporarily desiccated sediments had significantly lower sediment pore water P concentrations than the permanently inundated sediment, even a half year after rewetting (Fig. 1b). These sediments have been influenced by SO_4^{2-}- and Fe-rich groundwater for many years, resulting in higher total Fe concentrations than in the formerly dry alder carr sediment (D-sediment). In the T-sediment, part of the Fe supplied via the groundwater is present in reduced form (as FeS_x and siderite) as these sediments are anaerobic during most of the year. Desiccation leads to the oxidation of at least part of this insoluble reduced Fe pool, as is clearly indicated by the increased pore water SO_4^{2-} concentrations resulting from FeS_x oxidation following desiccation. As a result, the reducible (oxidized) Fe pool increases strongly upon temporal desiccation. As the same amount of o-PO_4^{3-} is now immobilized by a

much greater reducible Fe pool, it takes much longer before this P pool is mobilized again upon rewetting (Fig. 1b). In a laboratory experiment, Golez and Kyuma (1997) showed that oxidation of pyritic sediments can indeed lead to decreased $o\text{-}PO_4^{3-}$ availability because of the increased oxidized Fe concentration in the sediment. In T-sediments, temporal desiccation even seems to be a prerequisite for maintaining good pore water quality, as is clearly shown by the high P concentrations in the pore water when these sediments are permanently flooded (Fig. 1b & 3). A good pore water quality (low P concentrations) can only be maintained when these sediments temporarily desiccate, as is the case under field conditions.

In the Fe- and SO_4^{2-}-rich seepage sediment (S-sediment), by contrast, desiccation increased the availability of P in the sediment, leading to concentrations in the water layer that only slightly stimulate the growth of lemnids. These sediments have been permanently influenced by SO_4^{2-}-, Ca- and Fe-rich seepage. Since they never dry out, FeS_x and siderite have accumulated constantly and have never been broken down by temporal desiccation. As a result, FeS_x and total Fe concentrations are extremely high in these sediments. Upon desiccation, the amount of insoluble reduced Fe that is oxidized will be very high and the newly formed oxidized Fe-pool is correspondingly large. However, in contrast to the T-sediment, total P concentrations were not decreased but showed a significant increase (Fig. 1a). At seepage locations, $o\text{-}PO_4^{3-}$ is efficiently immobilized in the form of $CaPO_4$ and $FePO_4$, as the groundwater contains very high calcium (Ca) and Fe concentrations (Stumm and Morgan, 1981; Golterman, 1998). This is reflected by the high total Ca, Fe and P concentrations in the S-sediment and the low $o\text{-}PO_4^{3-}$ concentrations in the pore water. However, sediments with high FeS_x concentrations acidify upon desiccation when more acid is produced than can be buffered by cation exchange or carbonate dissolution (Lucassen et al., 2002[b]). The solubility of $o\text{-}PO_4^{3-}$ complexes, including apatite $Ca_5(PO_4)_3(OH,F,Cl)$, strengite $(FePO_4)$ and variscite $(AlPO_4)$, is strongly influenced by pH. With decreasing pH, apatite will be the first to dissolve, followed by variscite and strengite. Binding capacities are strongest at pH 6, but below pH 4 dissolution of metal-phosphates occurs, resulting in P mobilization (Stumm & Morgan, 1981; Golterman, 1998). Beltman et al., (1996) showed that P availability did indeed increase in fens at pH values below 4.1 under field conditions. Temporary desiccation of S-sediment led to a very strong increase in SO_4^{2-}, and severe acidification down to pH 3.7, resulting in dissolution of apatite and variscite as indicated by the increased Ca and Al concentrations in the pore water (Fig. 1a). After rewettting, pH increased and re-immobilization of $o\text{-}PO_4^{3-}$ was to be expected. This did indeed occur (Fig. 1a), but the overall $o\text{-}PO_4^{3-}$ concentrations remained higher than those in the permanently inundated treatment for at least six months after rewetting.

The effect of desiccation on P mobilization or immobilization strongly depended on the FeS_x and P contents, in combination with the buffering capacity of the sediment. Seepage zones contain the highest amounts of FeS_x and might easily acidify upon desiccation depending

upon the buffering capacity of the sediment. Depending upon the amount of P present as apatite or variscite, this may lead to a net mobilization of P in the sediment. Whether there is a net mobilization of P depends on the amount of P that is immobilized at the same time in the newly formed reducible (oxidized) Fe pool. In sediments that are indirectly influenced by seepage and tend to dry out during dry periods (T-sediments), FeS_x levels tend to be moderately high, and FeS_x oxidation results in an increase in the reducible Fe pool, though it does not lead to severe acidification. As no extra P is mobilized from other P complexes, this results in an increase in the ratio of reducible Fe to P and hence in a lower P availability after rewetting. The formerly dry sediment hardly contained any insoluble reduced Fe. The mobile reduced Fe is oxidized but desiccation does not lead to an additional increase in the reducible Fe pool. The ratio of the reducible Fe pool to P is not increased, so no extra immobilization occurs after rewetting.

Effects of temporary desiccation on P availability in the water layer

The results show that P concentrations in water layer and the concomitant development of algae and lemnids in the water layer depended upon the sediment type and the interaction between sediment type and water table fluctuations. P concentrations in the water layer are determined by the P concentrations in the sediment pore water and various processes that influence the exchange of P from the sediment to the water layer (Wetzel, 2001). In D-sediment, temporal desiccation did not lead to significant differences in sediment P or in P concentrations in the water layer after rewetting. In both treatments, the water layer was extremely eutrophied, with a very high production of algae and lemnids. In the T-sediment, much lower P concentrations were observed in both pore water and water layer after temporal desiccation, and the desiccation treatment strongly diminished the uptake of P and the concomitant biomass production of lemnids and algae in the water layer. In the S-sediment, the P concentrations in the sediment pore water were increased upon desiccation, but the P concentration in the water layer remained relatively low. Apparently, the exchange of P from the sediment to the water layer was much less in the temporarily desiccated S-sediment than in the permanently flooded treatment. Oxidation processes in the top layer of the sediment may play an important role in counteracting the release of P to the water layer. Diffusion of Fe(II) to the aerobic top layer of the sediment increases the Fe(III) content of the sediment/water interface, resulting in an increased P binding capacity of the sediment and restricting P mobilization to the water layer (Mortimer, 1941; Baccini, 1985; Smolders et al. 1995). The high Fe concentrations in the pore water in the S-sediment after temporal desiccation may therefore have reduced the release of P to the water layer.

In addition, gas ebullition can increase turbulence in soft organic sediments stimulating P release from the sediment to the water layer. Since methane (CH_4) concentrations are generally high in anaerobic organic alder carr sediments (Figs. 1a-c), ebullition of CH_4 gas may increase the release of P to the water layer. Unlike D-sediments and T-sediments, CH_4

concentrations remained very low in desiccated S-sediments after rewetting. This can be explained by the high SO_4^{2-} concentrations in this sediment as a consequence of FeS_x oxidation. SO_4^{2-} reducing bacteria compete with methanogens for the organic substrates (such as acetate) but are much better competitors. Therefore, high SO_4^{2-} reduction rates strongly decrease CH_4 production rates in reductive sediments (Lovley and Klug, 1983; Bhattacharya et al., 1995; Lamers et al., 1999; Smolders et al., 2002). The lack of CH_4 ebullition may also have contributed to the lower release of P in the temporarily desiccated S-sediment.

Effects of temporary desiccation on heavy metal concentrations
Apart from effects on the nutrient balance, periodic desiccation also affected the mobilization of (heavy) metals, depending upon sediment type. Metal-sulphides formed under reduced conditions oxidize during desiccation after which the metals are bound to insoluble carbonates or (hydr)oxides. Depending upon the drop in pH during desiccation, carbonates and (hydr)oxides dissolve and metals are released (Drever, 1997). Therefore, desiccation of S-sediment led to strong acidification and subsequent mobilization of Mn, Zn and Al in the pore water. Fe levels were not increased by acidification, as Fe-(hydr)oxides are highly insoluble and become mobile only when pH drops below 3.0 (Drever, 1997). After rewetting, the pH of the sediment gradually increased as a consequence of reduction processes, resulting in decreased Al and Zn concentrations. Fe concentrations, however, increased greatly as Fe(III) (hydr)oxides, formed during desiccation, are reduced again to highly mobile Fe(II). In T-sediment and D-sediment, the lower FeS_x concentration did not lead to severe acidification of the sediment, preventing strong dissolution of metal hydroxides and resulting in decreased concentrations of Fe, Mn and Al during desiccation and increased concentrations following rewetting due to (hydr)oxide formation and reduction of (hydr)oxides respectively. Due to the relatively high pH of the basic medium, pH of the surface water remained relatively high following rewetting, preventing mobilization of metals to the surface water (results not shown).

Implications for wetland management
Permanently damming of ditches in order to retain groundwater results in permanently wet conditions in wetlands. This usually results in a more or less stagnant water layer, in contrast to the previous natural situation, in which a flow of ground water from the seepage sites through the upper soil layers of the surrounding area permanently supplied Fe and Ca to a large part of the wetland (Lucassen et al., 2000). These components are known to effectively bind P in wetlands (Golterman, 1998). In areas formerly indirectly affected by shallow groundwater flows (T-sediments), permanent high water levels result in eutrophication. In these areas, desiccation leads to a long lasting reduction in P release from the sediment because a reduced Fe pool is present (mainly FeS_x and siderite). This reduced Fe is oxidized

upon desiccation, resulting in an increased oxidized Fe pool, which is the most effective factor in immobilizing P in the sediments. In the seepage zones, which are directly influenced by deeper groundwater, Ca and Fe levels are so high that $o-PO_4^{3-}$ is effectively immobilized under oxidized as well as reduced conditions. Ca probably plays the dominant role in immobilizing $o-PO_4^{3-}$ in these seepage zones.

Thus, permanently high water levels are bound to have negative effects in the short term, because permanently reduced conditions exhaust the pool of oxidized Fe. In the longer term sediments will undergo a much smaller influence of the groundwater in terms of Fe supplied to the sediments, as seepage is suppressed by the higher water levels in the wetlands. Temporal desiccation of the parts surrounding the seepage zones probably plays an important role in maintaining a low $o-PO_4^{3-}$ exchange during wet seasons, because part of the reduced Fe pool (supplied and accumulated in the wet season) is oxidized and becomes available for $o-PO_4^{3-}$ adsorbtion/immobilization. Parts that were formerly dry, however, and have not been influenced by groundwater for a long time (D-sediments), do not possess a reduced Fe pool. In these parts, a stagnating water layer will result in a highly eutrophic water layer if $o-PO_4^{3-}$ levels in the soil are relatively high, as was the case in our study. Oxidation in summer does not reduce $o-PO_4^{3-}$ release from these sediments after rewetting, as no additional Fe is released upon oxidation.

The supply of groundwater is of paramount importance and will determine the ability of the sediment to retain P under reduced conditions. In a well-functioning system, the seepage zones of the forests will never dry out. However, the surrounding zones tend to dry out each summer for a certain period, when seepage pressure is relatively low. Our results show that this temporal desiccation is probably essential, as it renews the oxidized Fe pool in the sediment and hence the $o-PO_4^{3-}$ retention capacity of the sediment. Damming of groundwater decreases the supply of this groundwater and thus that of $o-PO_4^{3-}$ immobilizing compounds such as Fe, resulting in permanent reduced conditions in the sediment layer. This generally results in strong eutrophication. The size of the part of the alder carr forest in which appropriate water quality can be maintained, depends very much on the seepage pressure, which is largely determined by the regional hydrology. Water extraction and agricultural activities have led to considerable drops in regional groundwater levels, resulting in less seepage and therefore desiccation of alder carr forests. Our results demonstrate that it is not possible to restore these forests by simply retaining water in the systems by means of constructed dams. This will especially be the case if the forests are relatively rich in P. If water retention is artificially increased, one should try to mimic the natural hydrology by allowing parts of the wetlands to dry out in summer.

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CHAPTER 7

Effects of stagnating sulphate-rich groundwater on the mobility of phosphate in freshwater wetlands: a field experiment

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Abstract

Water tables in the Netherlands have been greatly lowered in recent decades, largely in order to favour agricultural activities. Drought and increased nitrate (NO_3^-) leaching from agricultural land lead to oxidation of iron-sulphide in the subsoil, resulting in increased sulphate (SO_4^{2-}) concentrations in the groundwater. In addition, increased atmospheric sulphur (S) deposition and leaching of SO_4^{2-} from agricultural land have increased SO_4^{2-} concentrations in the groundwater. Attempts to retain groundwater in desiccated wetlands by simply damming drainage ditches have resulted in greatly increased retention times of the water, die back of the original vegetation and massive development of algae and lemnids in summer. In order to determine whether stagnation of SO_4^{2-}-rich groundwater plays an important role in eutrophication, nine bottomless enclosures were placed in the sediment of an open water body to block the supply of groundwater. Na_2SO_4 was added to three enclosures twice a year to simulate a minimised input of SO_4^{2-}-rich groundwater. In addition NaCl was added to three enclosures, while three enclosures were left untreated. (Pore) water quality and vegetation development were compared with those at seepage-fed sites outside the enclosures. The results show that stagnation of SO_4^{2-}-rich groundwater led to increased SO_4^{2-} reduction in the sediment, resulting in P mobilisation from the sediment and eutrophication of the water layer, due to the interference of S^{2-} with the iron-phosphorous cycle. This was accompanied by massive growth of common duckweed (*Lemna minor*) and filamentous algae and a decreased growth of broadleaved pondweed (*Potamogeton natans*). The consistently high input of SO_4^{2-} via seepage did not lead to eutrophication. The results indicated that SO_4^{2-} reduction rates at sites fed by seepage were very low. This was probably caused by the consistently high input of Fe and NO_3^- via the groundwater, which prevents reduction of SO_4^{2-} by providing energetically more favourable electron acceptors. In fens with stagnating groundwater, Fe and NO_3^- will be rapidly depleted, stimulating SO_4^{2-} reduction resulting in development of toxic S^{2-} concentrations and eutrophication of the water layer.

Introduction

Sulphate (SO_4^{2-}) pollution of surface water and groundwater can caues eutrophication in freshwater wetlands (Boström et al., 1982; Caraco et al., 1989; Roelofs, 1991; Smolders & Roelofs, 1993; Murray, 1995; Lamers et al., 1998; Beltman et al., 2000). Free sulphide (S^{2-}), originating from SO_4^{2-} reduction, can disturb the iron-phosphorus cycle by favouring the reduction of oxidised iron (Fe^{3+}) to reduced iron (Fe^{2+}) in iron phosphate, iron (hydr)oxide-phosphate, and humic iron phosphate complexes. As a result of increased S^{2-} production, Fe from these complexes gradually becomes fixed as FeS_x, while formerly immobile P is released (Sperber, 1958; Patrick & Khalid, 1974; Boström et al., 1982; Smolders & Roelofs, 1993; Moore & Reddy, 1994; Smolders et al., 1995). In the Netherlands, SO_4^{2-} concentrations in groundwater and surface water have greatly increased over the last decades, as a consequence of increased atmospheric sulphur (S) deposition and leaching of SO_4^{2-} from agricultural land. In addition, falling water tables and infiltration of nitrate (NO_3^-) from agricultural land and forest soils into the groundwater also increases groundwater SO_4^{2-} concentrations by favouring the oxidation of FeS_x in the subsoil (Stumm & Morgan, 1981; Nordstrom, 1982; Kölle et al., 1985).

Water tables in the Netherlands have been greatly lowered in recent decades, largely in order to favour agricultural activities, resulting in the partial desiccation of many wetlands in agricultural areas. Many wetlands near the river Meuse are often rich in iron (Fe), as the feeding deeper groundwater also contains large amounts of Fe (Van den Munckhof, 2000). Despite the consistently high SO_4^{2-} input via the groundwater, many systems have never shown signs of serious eutrophication. The retention time of the water might play an important role in preventing eutrophication, because P released from the sediment will be rapidly removed from the system if the retention time of the water is short. Attempts to retain groundwater by simply damming drainage ditches has resulted in greatly increased water retention times and a concomitant development of filamentous algae and *L. minor* in summer (Boxman & Stortelder, 2000; Lucassen et al., 2000). By contrast, situations in which the supply of seepage was maintained were not affected by eutrophication. This supports the idea that groundwater stagnation favours the mobilisation and accumulation of P from the sediment into the water layer. Stagnation leads to restricted input of Fe-hydroxides, which have a strong binding capacity for P (Smolders et al., 1995, 2001; Golterman, 1998; Lamers et al., 2002). Ongoing reduction of Fe will then lead to depletion of oxidised Fe, which might stimulate the use of available SO_4^{2-} as electron acceptor.

In order to study whether SO_4^{2-} promotes P accumulation in Fe-rich wetlands with a stagnating groundwater input, nine bottomless enclosures were placed in an open water body that is constantly fed by Fe- and SO_4^{2-}- rich seepage. The enclosures blocked the local groundwater flow, thus isolating small parts of the system. Na_2SO_4 was added to three of the enclosures twice a year (resulting in surface water SO_4^{2-} concentrations comparable to

groundwater concentrations) over a period of two years, to simulate a restricted input of surface water with SO_4^{2-}. Three other enclosures were enriched with a comparable amount of NaCl. Water quality and vegetation development were compared with control enclosures (CTR) and with sites fed by seepage outside the enclosures.

Materials and methods

Experimental design

In October 2000, 9 bottomless polycarbonate transparant enclosures (1 m height, 1 m diameter) were placed in the peaty sediment of the Dubbroek fen (51^0 22' N; 6^0 05' E) at sediment and water depths of respectively 30 and 50 cm. As Dubbroek is only very locally fed by groundwater, placement of the enclosures led to stagnation of groundwater in the enclosures.

One week after placement, 250 g Na_2SO_4 was added to three of the enclosures to increase the original SO_4^{2-} concentration in the water layer by 2 mmol l^{-1}. To three other enclosures, an equivalent molar amount of sodium (Na^+) was added as NaCl. The remaining enclosures were left untreated (CTR). Treatments were repeated in April 2001, December 2001 and May 2002. Monthly, surface and pore water samples of the enclosures were taken over a period of two years. Samples were also taken from sites outside the enclosures, which were fed by seepage (n=3). Pore water was collected anaerobically with 100 ml glass vacuum serum bottles connected to ceramic cups that had been permanently installed in duplicate in the sediment (5-15 cm). Water samples were collected in iodated 500 ml polyethylene bottles. The vegetation of the enclosures was harvested in October 2001 and October 2002. Macrophytes and filamentous algae were washed with demineralised water and dried (24 hrs, 100 ^{0}C) to determine their dry weight. In addition, sediment samples were collected at nine locations outside the enclosures. To determine the concentrations of several elements, 50 g of sediment was dried in duplicate (24 h at 70 ^{0}C). Half of the dried samples were homogenised and a 500 mg portion of each was digested for 17 minutes with 4 ml concentrated nitric acid and 1 ml 30% hydrogen peroxide, using a Milestone mls 1200 Mega microwave. The other half of the dried sediment samples were incinerated (6 h at 550 ^{0}C) for determination of the organic matter content. Digestates were stored in iodated polyethylene bottles at 4 ^{0}C untill further chemical analyses.

Chemical analyses

The pH of the water samples was measured using a standard $Ag/AgCl_2$ electrode connected to a Radiometer Copenhagen type PHM 82 standard pH meter. Methane (CH_4) was measured with ethane as an internal standard on a 'Pye Unicam' gas chromatograph Porapak Q' (80/100 mesh) column ('Water Chromatograpy', Etten-Leur, the Netherlands). Bicarbonate (HCO_3^-) analyses were carried out using an 'Oceanograpy International' model

0525 HR infrared carbon analyser. The concentration of free S^{2-} was determined in a 10 ml subsample, fixed immediately after collection with S^{2-} antioxidant buffer containing sodium hydroxide, sodium EDTA and ascorbic acid (Van Gemerden, 1984). A S^{2-} ion-specific Ag elecrode and a double junction calomel reference electrode were used. A part of all water and pore water samples was stored at 4 ^{0}C in glass test tubes with 2 % concentrated HNO_3 to avoid precipitation of metals. Total concentrations of Ca, Mg, S, Mn, Fe, Al, P and Zn in the (pore) water and sediment samples, as well as in the digestates, were measured using an inductively coupled plasma emission spectrophotometer (ICP, Spectroflame). The other part of the water and pore water samples were stored in iodated polyethylene bottles at -23 ^{0}C, untill analyses for nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (o-PO_4^{3-}) using Technicon II autoanalysers according to Technicon (1969), Grasshoff and Johansen (1977) and Henriksen (1965) respectively.

Statistical analyses

Results are presented as means with standard error of the mean (SEM). A one-way analysis of variance (ANOVA) (GLM procedure, SPSS 11.0) with Tukey post-test was used to assess differences in plant biomass. A one-way ANOVA with repeated measures was used to assess differences in the chemistry of the surface and pore water. All values were log transformed. Significance was accepted at $p < 0.05$.

Results

The organic-rich sediment at the Dubbroek fen was very rich in Fe, Ca, P and S (Table 1).

Table 1. Some sediment characteristics of the Dubbroek fen (n=9). Total concentrations of elements in the sediment are given in µmol g^{-1} dw. The moisture and organic matter content are given in %. Mean, standard error of the mean (SEM) and the mediane are given.

	Ca	Mg	Mn	Fe	Si	Zn	P	S	Al	moisture content	organic content
MEAN	719	57	27	2059	77	8	170	414	128	88	68
SEM	51	6	6	439	22	1	50	68	22	1	4
MEDIANE	669	54	26	1993	56	7	113	378	119	89	72

Results of the water layer chemistry are shown in Figure 1 and Table 2. Addition of Na_2SO_4

to stagnating water in the enclosures resulted in temporarily increased SO_4^{2-} concentrations in the water layer of up to 2-4 mmol l^{-1}. After applications in summer, concentrations rapidly decreased to the original concentration, while concentrations remained relatively high after applications in winter. In enclosures to which no Na_2SO_4 was added, SO_4^{2-} concentrations
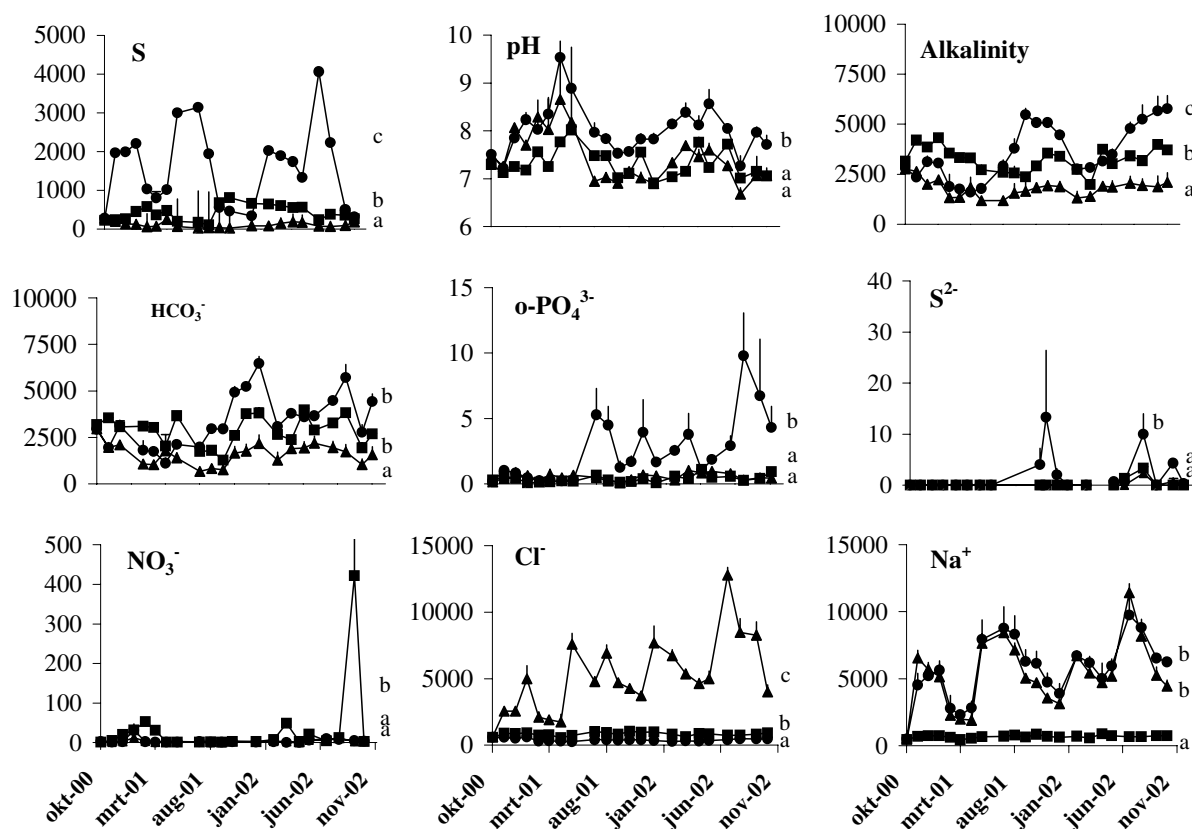

Figure 1. Surface water chemistry of sites fed by seepage and of stagnating water in enclosures enriched with Na_2SO_4 or NaCl. Treatments with the same letter do not differ significantly over the experimental period. Treatments: Stagnating water of enclosures enriched with NaCl (▲); Stagnating water of enclosures enriched with Na_2SO_4 (●), Sites fed by seepage water outside the enclosures (■). Concentrations are given in µmol l^{-1}, alkalinity is given in µeq l^{-1}.

remained very low during the entire experimental period (mean concentration 0.1 mmol l^{-1}). SO_4^{2-} concentrations in the water layer of seepage-fed sites outside the enclosures were significantly higher than those in the stagnating water of untreated enclosures and enclosures to which NaCl had been added. SO_4^{2-} concentrations in the water layer of sites fed by seepage fluctuated from 0.5 to 0.8 mmol l^{-1} in winter to 0.2 mmol l^{-1} in summer. Surface water in the enclosures contained low NO_3^- concentrations (<10 µmol l^{-1}) while the water layer of seepage-fed sites sometimes contained significantly increased NO_3^- concentrations.

After the first summer, pH, alkalinity and concentrations of HCO_3^-, $o\text{-}PO_4^{3-}$, total P and S^{2-} were significantly higher in the water layer of enclosures to which Na_2SO_4 had been added than in all other treatments. HCO_3^- in the water layer of sites fed by seepage was, however, not significantly lower than in enclosures treated with Na_2SO_4. The mean concentrations of Ca, Mg, Al and Si were significantly higher in the water layer of sites fed by seepage while
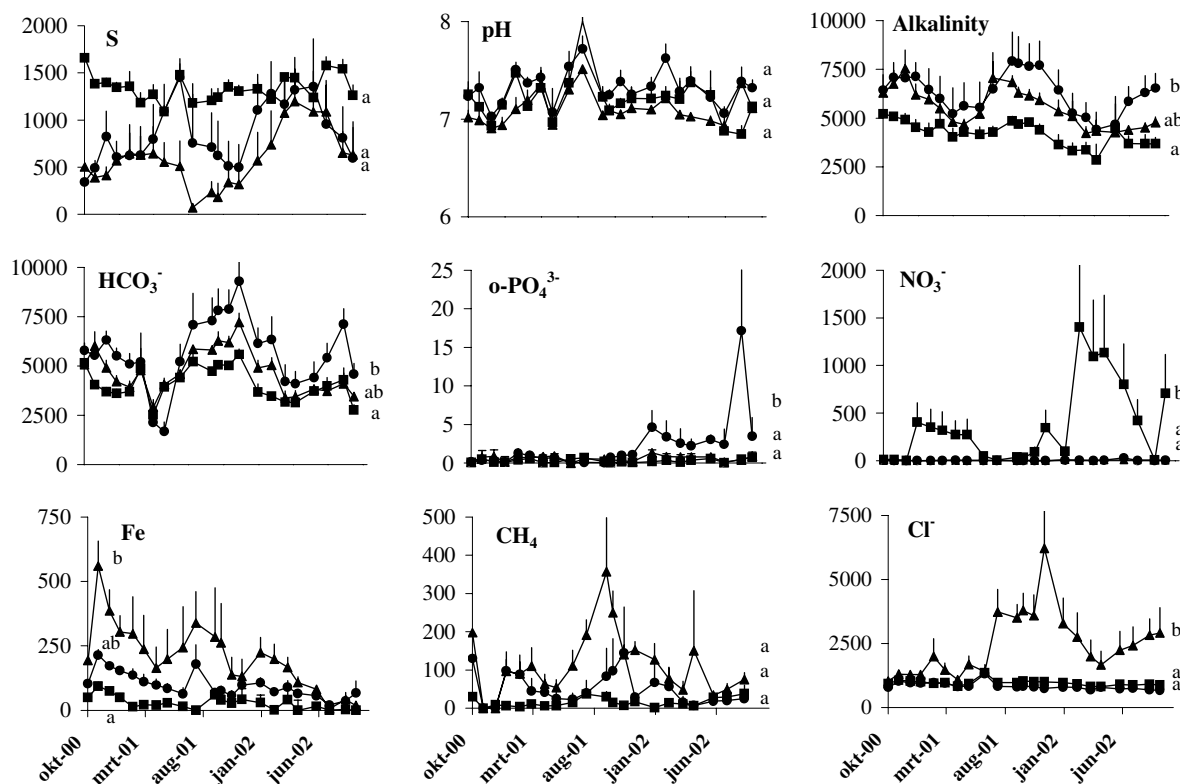

Figure 2. Pore water chemistry of sites fed by seepage and of stagnating sediments in enclosures enriched with Na_2SO_4 or NaCl. Treatments with the same letter do not differ significantly over the experimental period. Treatments: Stagnating pore water of enclosures enriched with NaCl (▲); Stagnating pore water of enclosures enriched with Na_2SO_4 (●); Sites fed by seepage water outside the enclosures (■). Concentrations are given in $\mu mol \, l^{-1}$, alkalinity is given in $\mu eq \, l^{-1}$.

NH_4^+ was significantly lower. The mean concentrations of these elements did not differ between the water layer of enclosures to which NaCl and Na_2SO_4 had been added, with the exception of Ca which was significantly lower in the Na_2SO_4 enclosure (Na_2SO_4: 488 µmo/l; NaCl: 716 µmo/l; seepage: 1737 µmo/l). There were no differences in surface water chemistry between enclosures enriched with NaCl and untreated enclosures (CTR), except for Na^+, Cl^- (results of CTR enclosures not shown).

Results of the pore water chemistry are shown in Figure 2 and Table 2. The SO_4^{2-}

concentration in the pore water of sites fed by seepage was consistently high (1.5 mmol l^{-1}). In the spring of 2001 and 2002, SO_4^{2-} concentration in stagnating pore water in enclosures enriched with Na_2SO_4 gradually increased from 0.5 to 1.5 mmol l^{-1}, which is comparable to that at sites fed by seepage. After July 2001 and 2002, however, concentrations rapidly decreased again to the original concentrations. In stagnating pore water of enclosures enriched with NaCl, SO_4^{2-} concentrations remained low during the first year but increased to 1.2 mmol l^{-1} in 2002.

Table 2. ANOVA table with effects of treatments on surface water and sediment pore water chemistry inside the enclosures and at sites outside the enclosures fed by seepage. ***, P < 0.001; **, p < 0.005; *, p < 0.05; ns, not significant.

	PORE WATER	**SURFACE WATER**
SO_4^{2-}	ns	***
Na^+	**	***
Cl^-	**	***
pH	ns	**
Alkalinity	*	**
HCO_3^-	*	**
$o\text{-}PO_4^{3-}$	*	***
P	*	**
NO_3^-	**	***
Fe	**	ns
CH_4	ns	–
S^{2-}	ns	*
Ca	ns	***
Mg	ns	**
Mn	*	ns
Al	ns	***
Si	**	**
Zn	*	**
K^+	**	ns
NH_4^+	***	***

In the same period, chloride (Cl^-) concentrations in the pore water decreased by an equivalent amount. NO_3^- concentrations in pore water of sites fed by seepage were

significantly higher (up to 1.5 mmol l^{-1}) compared to those in stagnating pore water in the enclosures (< 10 µmol l^{-1}). NO_3^- concentrations in the pore water of sites fed by seepage strongly fluctuated and decreased to zero in summer, while Fe concentrations were relatively low, especially when compared to those in stagnating pore water of enclosures that had not been enriched with Na_2SO_4. After the first summer, alkalinity and concentrations of HCO_3^- and o-PO_4^{3-} were higher in stagnating pore water of enclosures enriched with Na_2SO_4 than in stagnating water of enclosures poor in SO_4^{2-} and sites fed by SO_4^{2-}-rich seepage. S^{2-} concentrations in the pore water were very low under all conditions and did not differ significantly (mean concentration 1.3 µmol l^{-1}, results not shown). The mean concentrations of Ca, Mg and Al did not differ between treatments whereas the concentrations of Si, K^+ and NH_4^+ was significantly lower in pore water of sites fed by seepage compared to stagnating pore water in the enclosures. There were no differences in the chemistry of stagnating pore water between enclosures enriched with NaCl and untreated enclosures (CTR), with the exception of Na^+, Cl^- and SO_4^{2-}, which remained low during the entire experimental period in the CTR enclosures (results of CTR enclosures not shown) (Fig. 2, Table 2).

In stagnating SO_4^{2-}-rich water there was a strong development of filamentous algae or common duckweed (*L. minor*) during both summers. This did not occur in stagnating water poor in SO_4^{2-} or at sites fed by SO_4^{2-}-rich seepage. The biomass of broad-leaved pondweed *(Potamogeton natans)* was significantly lower in stagnating water rich in SO_4^{2-} than in all other situations. The growth of stoneworth (*Chara globularis*) remained unaffected (Fig. 3 & 4).

Discussion

Effect of high SO_4^{2-} concentrations in stagnating water

The results show that addition of Na_2SO_4 to stagnating water in enclosures leads to increased o-PO_4^{3-} concentrations in the pore water and increased o-PO_4^{3-} and S^{2-} concentrations in the water layer, resulting in mass development of filamentous algae or *L. minor* and die-back of aquatic macrophytes.

After each addition of Na_2SO_4 to the enclosures, diffusion of SO_4^{2-} took place from the water layer to the sediment, resulting in SO_4^{2-} concentrations in the pore water rising from 0.5 to 1.5 mmol l^{-1}, which was comparable to concentrations in the seepage-fed sites. SO_4^{2-} reduction rates in anaerobic sediments of freshwater wetlands are generally rate limited by the availability of SO_4^{2-} (Lamers et al., 1998; Lamers et al., 2002). In summer, when microbial activity was high, the increased availability of SO_4^{2-} in the stagnating pore water led to increased SO_4^{2-} reduction rates in the sediment (as indicated by the loss of SO_4^{2-} from the sediment pore water), accumulation of S^{2-} in the water layer and increased alkalinity and HCO_3^- concentrations in the water layer and pore water since SO_4^{2-} reduction is an alkalinity generating process (Baker et al., 1986; Lamers et al., 1998). In addition, our results indicated

that high Cl^- concentrations in the enclosures exchange with SO_4^{2-} at the soil adsorption complex. A part of the Cl^- might have disappeared from the pore water due to downward diffusion. However, pore water Cl^- concentrations in the NaCl enclosures increased to 6 mmol l^{-1}, following the application of Cl^- to the water layer in December 2001. Subsequently pore water Cl^- concentrations showed a gradual decrease, whereas pore water SO_4^{2-} concentrations increased to values comparable to those at seepage-fed sites without any external additions.

In summer, SO_4^{2-} was reduced, as can be observed from the gradual decrease in SO_4^{2-} concentrations. Alternatively, this pattern could be explained by re-adsorption of SO_4^{2-}. In the CTR enclosures this effect did not occur. As natural Cl^- concentrations at Dubbroek (and comparable groundwater-fed freshwater wetlands) are much lower (< 1 mmol l^{-1}), it is to be expected that exchanging of SO_4^{2-} by Cl^- at the soil adsorption complex, makes no significant contribution to eutrophication in this type of wetland.
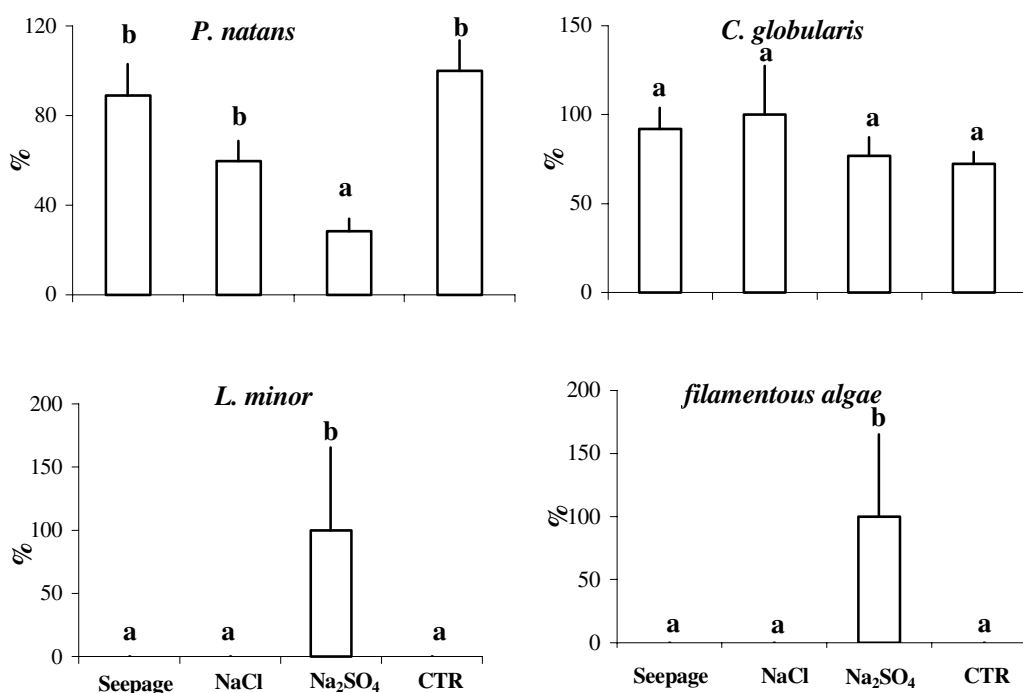

Figure 3. Relative cumulative dry biomass in % (October 2001 + 2002) of *P. natans*, *C. globularis*, *L. minor* and filamentous algae in stagnating waters (Na$_2$SO$_4$, NaCl or CTR) and seepage-fed sites outside the enclosures. 100 % *P. natans* = 112.0 g m^{-2}; 100 % *C. globularis* = 249.6 g m^{-2} ; 100 % *L. minor* = 33.1 mg m^{-2}; 100 % algae = 1.5 g m^{-2}. Means with the same letter do not differ significantly.

The site where the experiment was carried out is very rich in Fe (Table 1) as it is constantly fed with Fe-rich seepage (Lucassen et al., 2002). S^{2-} formed by SO_4^{2-} reduction was therefore bound to Fe^{2+} in the sediment, as was clear from the lower Fe^{2+} concentrations in the

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stagnating pore water of enclosures enriched with Na_2SO_4 compared to that of enclosures enriched with NaCl and untreated enclosures (CTR). o-PO_4^{3-} concentrations in the deeper sediment (5-15 cm) of enclosures enriched with Na_2SO_4 remained very low during the first
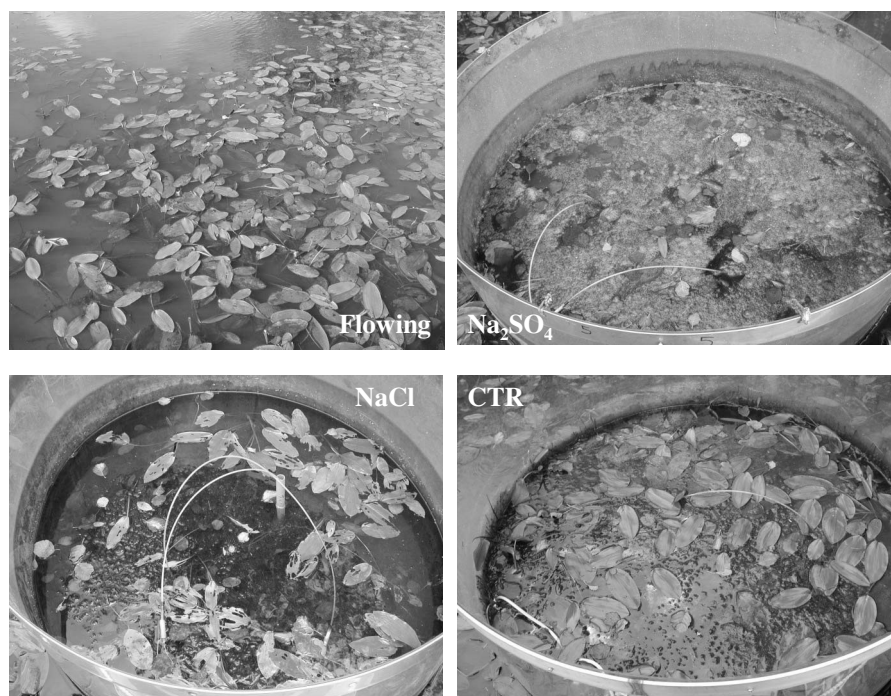

Figure 4. Plant development in stagnating waters of the enclosures (Na_2SO_4, NaCl, CTR) and at sites fed by seepage outside the enclosures (flowing) (October 2001).

year (1 µmol l^{-1}), probably because it could still be sufficiently immobilised by Fe complexation. During the second year, however, free Fe^{2+} concentrations had greatly decreased, and the availability of Fe for P complexation was no longer sufficient to completely immobilise o-PO_4^{3-} in the sediment, resulting in increased o-PO_4^{3-} concentrations in the pore water of up to 17 µmol l^{-1}. In addition, the increase in pH of the water layer that was caused by SO_4^{2-} reduction, could also have stimulated P mobilisation from the sediment top layer as the binding capacity of o-PO_4^{3-} to Fe(OOH) decreases at higher pH. On the other hand, o-PO_4^{3-} coprecipitates with $CaCO_3$ at higher pH (Golterman, 1995; 1998).

In the stagnating water of enclosures to which no Na_2SO_4 had been added, o-PO_4^{3-} concentrations remained low during the entire experimental period. In the summer of 2001, o-PO_4^{3-} concentrations in the stagnating water layer of enclosures enriched with Na_2SO_4 greatly increased (from 0.5 to 5 µmol l^{-1}) while pore water concentrations remained low (1 µmol l^{-1}). The reason may be that the pore water samples were collected from a depth of approximately 5-15 cm, while the release of P from the sediment is strongly determined by processes in the uppermost sediment layer. In this uppermost layer, SO_4^{2-} derived from the

water layer is primarily reduced and produced S^{2-} interacts with the $Fe-PO_4$ complexes mobilising o-PO_4^{3-}. Oxidation processes in the top layer of the sediment play an important role in counteracting the release of P to the water layer (Wetzel, 2001). The diffusion of Fe^{2+} to the aerobic top layer of the sediment increases the Fe^{3+} content of the sediment/water interface, resulting in an increased P binding capacity of the sediment and restricted P mobilisation to the water layer (Mortimer, 1941; Baccini, 1985; Smolders et al. 1995). SO_4^{2-} reduction processes in the upper sediment layer can reduce the thickness of the aerobic layer and the mobility of Fe in the sediment. During the second year, the o-PO_4^{3-} concentrations in the water layer increased further, up to 10 µmol l^{-1}, probably due to ongoing SO_4^{2-} reduction in the sediment top layer and higher o-PO_4^{3-} concentrations in the pore water deeper in the sediment profile.

The increased o-PO_4^{3-} availability in the stagnant water layer of enclosures enriched with Na_2SO_4 resulted in massive growth of filamentous algae and *L. minor* in summer, while the growth of *P. natans* was greatly decreased. Very low S^{2-} concentrations of 10 µmol l^{-1} can already strongly reduce the vitality of aquatic plants (Smolders et al., 1995,1996; Armstrong et al., 1996; Lamers et al., 1998). Although S^{2-} concentrations remained low in the pore water of the Fe-rich sediments, relatively high S^{2-} concentrations (up to 40 µmol l^{-1}) were present in the stagnating water of enclosures enriched with Na_2SO_4 and thus even higher concentrations may have been present in the anaerobic top layer of the sediment. Therefore, apart from competition by filamentous algae and *L. minor*, S^{2-} toxicity may have affected the vitality of *P. natans* in the SO_4^{2-} treated enclosures. Remarkably the biomass of *C. globularis* remained unaffected, although the lower parts of the shoots of these plants were clearly blackened, probably due to FeS_x formation. Comparable hydrological changes in marshes in the Camargue (Southern France) also caused permanent anoxic conditions leading to development of phyto-toxic S^{2-} concentrations (Van Wijck et al., 1992; Van Wijck & De Groot, 1993).

Comparison of SO_4^{2-}-rich stagnating water and SO_4^{2-}-rich seepage.
The results show that the consistently high supply of SO_4^{2-} via the seepage does not lead to excessive o-PO_4^{3-} mobilisation or the development of filamentous algae and *L. minor*, as is the case under more or less stagnating conditions.
The seepage is not only rich in SO_4^{2-} but also in NO_3^-. High NO_3^- concentrations can act as a redox buffer preventing reduction of Fe and SO_4^{2-} by being an energetically more favourable electron acceptor (Uhlmann & Paul, 1994; Izzo et al., 1997; Lucassen et al., in press). In addition NO_3^- is known to oxidise Fe^{2+} (Uhlmann and Paul, 1994; Smolders et al., 1997) and metal-sulphides (Ripl, 1976; Golterman, 1991) increasing the content of oxidised Fe capable to bind P in the sediment (Golterman, 1995). The fact that NO_3^- is a more favourable electron acceptor is also indicated by our results. To begin with, the reduced Fe concentration in the groundwater was much lower, despite the constant input of Fe via the

seepage. In addition, the concentration of CH_4 was consistently low at the sites fed by seepage water, in contrast to the stagnant water in the enclosures, indicating a higher redox potential. The inhibition of SO_4^{2-} reduction by high NO_3^- concentrations at sites fed by seepage is also supported by the consistently high SO_4^{2-} concentrations in the pore water during the experimental period and the consistently high SO_4^{2-} concentrations in the water layer in winter. Only during the first summer, when NO_3^- concentrations were relatively low, some lower SO_4^{2-} concentrations were observed in the water layer. In the following year, when the groundwater NO_3^- concentrations were much higher (1.5 vs 0.5 mmol l^{-1}), there were no such lower SO_4^{2-} concentrations.

Fe from reduced groundwater springs is oxidised in the top layer of seepage zones. However, we observed that Fe-colloids were also distributed throughout the wetland during certain periods of the year. Constant inputs of Fe and NO_3^- inhibit reduction of SO_4^{2-} and stimulate oxidation of reduced Fe-compounds, increasing the P-binding capacity of the soil. The results indicate that high concentrations of SO_4^{2-} in the seepage do not lead to eutrophication of the water layer as long as the input of Fe and NO_3^- via the groundwater is high. If the inputs of Fe and NO_3^- are low, however, SO_4^{2-} reduction leads to eutrophication. Raising water levels by simply damming drainage ditches, instead of restoring the original local groundwater pressure, leads to stagnation of surface water, which blocks groundwater input and thus the supply of Fe and NO_3^-. These compounds will be rapidly depleted stimulating SO_4^{2-} reduction and resulting in eutrophication if the groundwater is rich in SO_4^{2-}. This was also observed in the stagnating water of the enclosures. Immediately after their placement, the SO_4^{2-} concentration decreased and CH_4 rose compared to sites fed by seepage. This can be attributed to the impaired water flow, which blocked the NO_3^- and Fe inputs and thus led to more reduced conditions. In addition, blocked input of Ca might have attributed to development of higher o-PO_4^{3-} concentrations due to lower co-precipitation rates of o-PO_4^{3-} with $CaCO_3$ (Golterman, 1998). In order to prevent eutrophication, the increase in water level must not exceed groundwater pressure, so that a continuous supply of groundwater is maintained.

Acknowledgements

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CHAPTER 8

High groundwater nitrate concentrations inhibit eutrophication of sulphate-rich freshwater wetlands.

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Abstract

During the last sixty years, pollution of the groundwater with NO_3^- has greatly increased in many parts of Europe, as a consequence of excessive use of manure and synthetic fertilisers. Monitoring of groundwater-fed wetlands indicated that sediments with high NO_3^- concentrations had the lowest Fe and o-PO_4^{3-} concentrations in the pore water. A comparison of two restored open water fens, differing in NO_3^- supply via the groundwater, indicated that the redox potential and the sulphate (SO_4^{2-}) reduction rate were lower when the groundwater contained not only SO_4^{2-} but also high NO_3^- concentrations. The lower SO_4^{2-} reduction rates in the NO_3^--rich open water fen were associated with lower o-PO_4^{3-} concentrations and the presence of plant species characteristic of clear water. In contrast, the higher SO_4^{2-} reduction rates in the NO_3^--poor open water fen were associated with very high o-PO_4^{3-} concentrations and massive development of plant species characteristic of eutrophic environments. Investigations at NO_3^--rich seepage sites in black alder carrs, showed that high NO_3^- concentrations in the pore water caused chlorosis in the alder carr vegetation, due to lower availability of Fe in the pore water and less Fe uptake by the plants. Experimental desiccation of sediments proved that the NO_3^--rich seepage sites contained no oxidisable FeS_x, contrary to NO_3^--poor locations, which became acidified and mobilised extremely high amounts of SO_4^{2-} due to FeS_x oxidation. A laboratory experiment showed that NO_3^- addition to sediments led to an increased release of SO_4^{2-} and reduced releases of Fe and S^{2-}, very likely due to the oxidation of reduced Fe and S compounds. Overall, the results confirmed that NO_3^- is an energetically more favourable electron acceptor in anaerobic sediments than Fe and SO_4^{2-}, and that high NO_3^- loads function as a redox buffer, preventing reduction of Fe and SO_4^{2-}. Limited SO_4^{2-} reduction prevents S^{2-}-mediated mobilisation of o-PO_4^{3-} from Fe-PO_4^{3-} complexes. At a higher redox potential, reduced Fe, including FeS_x, is oxidised, increasing the content of Fe(III) capable of binding o-PO_4^{3-}. This prevented increased o-PO_4^{3-} availability and the concomitant massive development of plant species characteristic of eutrophic environments.

Introduction

Nitrate (NO_3^-) concentrations in groundwater are low under natural conditions ($< 32 \, \mu$mol l^{-1}) (EEA, 1998). During the last sixty years, however, groundwater NO_3^- concentrations have greatly increased in many parts of Europe, due to increased pollution. Excessive use of manure and synthetic fertilisers has resulted in leaching of NO_3^- from agricultural lands. In Europe, the largest net applications of nitrogen (N) on agricultural land occur in the Netherlands and Belgium, with surpluses of 200 and 125 kg N/ha/year, respectively (Iversen et al., 1998). In addition, leakage of NO_3^- from forests soils to the groundwater has increased as gaseous ammonia and ammonium sulphate aerosols are effectively filtered by tree crowns. This causes high ammonium (NH_4^+) deposition rates in especially pine forests, and increased NO_3^- concentrations in the groundwater, as NH_4^+ is rapidly nitrified in forest soils, even under acid conditions (Van Breemen, 1982; Roelofs et al., 1985; Hagedorn et al., 2000).

Once accumulating in groundwater, NO_3^- causes oxidation of pyrite in the subsoil (Arevana & Robertson, 1998; Pauwels et al., 1998; Tesoriero et al., 2000). In the province of Limburg, Van Steenwijk (1986) indicated the presence of a pyrite containing layer at a depth of 20 m, which coincided with the disappearance of infiltrating NO_3^- and the appearance of increased SO_4^{2-} concentrations in the groundwater at this depth. Increased availability of SO_4^{2-} in wetlands is known to cause serious eutrophication problems, as S^{2-} produced by SO_4^{2-} reduction, interacts with Fe-PO_4^{3-} complexes in the sediment to produce FeS_x (FeS_2 and FeS), resulting in mobilisation of phosphate (o-PO_4^{3-}) (Boström et al., 1982; Caraco et al., 1989; Roelofs, 1991; Smolders & Roelofs, 1993; Lamers et al., 1998; Smolders et al., 2001). Thus, increased SO_4^{2-} concentrations in the groundwater may negatively affect groundwater fed wetlands.

During the last three years attempts have been made in the Netherlands, to restore desiccated fens by damming drainage ditches. In addition to increased water levels, this has also resulted in stagnation of groundwater and prevention of periodic droughts in summer. As a consequence, the wetlands have become highly eutrophied (Boxman & Stortelder 2000, Lucassen et al., 2000). Enclosure experiments have shown that high SO_4^{2-} concentrations can cause o-PO_4^{3-} mobilisation and eutrophication. However, no eutrophication occurs, if there is a constant input of groundwater that contains not only SO_4^{2-} but also contains high NO_3^- concentrations (Lucassen et al., submitted). This indicates that NO_3^- in some way may prevent eutrophication of wetlands. Research in drinking water reservoirs has shown that NO_3^- can oxidise Fe in the top layer of the soil (creating bond sites for phosphates) and that it can inhibit SO_4^{2-} reduction by being an energetically more favourable electron acceptor (Uhlmann and Paul, 1994). Golterman (1995) also found a link between S, N and P cycles indicating that denitrification by FeS produces Fe(OOH) enhancing P binding onto sediments. To study whether NO_3^- "pollution" via the groundwater might prevent

eutrophication of SO_4^{2-}-enriched wetlands, field work was carried out in fens which were differently affected by NO_3^-. The findings were supplemented by laboratory work.

Materials and methods

<u>Field work</u>

Monitoring the black alder carrs

From May 1998 to June 2001, pore water samples were taken in the alder carrs Beeselsbroek (51° 15' N; 6° 03' E), Kaldenbroek (51° 26' N; 6° 08' E) and Dubbroek in the Netherlands. Five to 10 locations were sampled 5 times a year. Pore water was collected anaerobically with nitrogen-preflushed vacuum bottles connected to ceramic cups (Eijkelkamp Agrisearch Equipment, the Netherlands), which were permanently installed in the upper 15 cm of the sediment.

Restoration of fens

Two open water fens at Dubbroek (51° 22' N; 6° 05' E) were monitored from April 2000 to January 2003. In the past, both fens had been highly eutrophied and dominated by *Salix spec.* (willow) and *Phragmites australis* (common reed)*,* as a consequence of drainage. Degraded sediment layers were removed in April 2000 and groundwater influence was reinforced by filling in drainage ditches in May 2000. The water layer, pore water and groundwater were sampled monthly. Groundwater was collected from a piezometer installed at a depth of two metres. Pore water samples (n=4) were collected as described above. In the summer of 2001, the vegetation was recorded according to Tansley (1946).

Examination of locations directly and indirectly fed by NO_3^- -rich seepage

In September 1998, plant material (5 plants), sediment pore water and sediment were sampled at sites (n=6) directly and indirectly fed by NO_3^--rich seepage at Beeselsbroek. The sites directly fed by NO_3^--rich seepage were covered with chlorotic *Carex pseudocyperus* (cyperus sedge) plants, whereas the plants at sites indirectly fed by groundwater were non-chlorotic. At the laboratory, roots and shoots were separated and washed in demineralised water. To determine chemical composition, root plaques were extracted according to Christensen & Wigand (1998). The two youngest leaves were ground up and mixed in liquid nitrogen, after which 20 ml of 96 % ethanol was added to 200 mg of plant material. Samples were shaken for 24 h in the dark at 4 °C. Chlorophyll A, chlorophyll B and carotenoid content were determined in the supernatant according to Wellburn and Lichtenthaler (1984). To determine chemical composition, dried (24 h at 70 °C) root and shoot material was ground up and mixed in liquid nitrogen and redried (24 h at 70 °C). Subsequently, 100 mg of dry material was digested with 4 ml concentrated nitric acid and 1 ml 30% hydrogen

peroxide for 17 minutes using a Milestone microwave type mls 1200 Mega. Digestates were stored in iodated polyethylene bottles at 4 ^{0}C until further analysis.

In addition, the redox potential was measured at a depth of 10 cm beneath the sediment surface, using a platinum electrode and a Metrohm AgCl/KCl reference electrode connected to a mV/pH meter. Sediment pore water was collected as described above.

A 1 kg sediment sample was collected to estimate the content of oxidisable sulphur. Five hundred g of this fresh sediment was gradually dried in a funnel (r = 7.5 cm, h = 10 cm) placed in a glass flask at 20 ^{0}C. The outflow of the funnel was covered with plastic gauze to prevent the loss of sediment. After a period of 2 months, the dry sediments were homogenised, rewetted with demineralised water up to 500 g to obtain the original moisture content, and rehomogenised by shaking for 24 hours at 100 rpm in a 1 l polyethylene bottle (Lucassen et al., 2002). (From experiments it was known that the largest amount of oxidisable FeS_x is oxidised after two months). Subsequently, water samples were sucked from the rewetted sediments by means of teflon Rhizon SMS soil moisture samplers (Eijkelkamp Agrisearch Equipment, the Netherlands). In addition 50 g of fresh sediment was dried (24 h at 70 °C) and 200 mg was digested as described above, to determine chemical composition.

Laboratory experiment

Effect of NO$_3^-$ additon to S-rich sediment.

Ten monoliths originating from Beeselsbroek were placed in 10 l plastic containers in a climate room that had a light level of 100 µmol m^{-2} s^{-1} , an air temperature of 20 °C and a humidity of 60 %. Perforations in the bottoms of the containers were covered with plastic gauze to prevent loss of sediment. The containers were placed in larger 12 l containers with overflow levels. During the first two weeks, the monoliths were inundated with a medium whose composition resembled the local alkaline groundwater at Beeselsbroek, with or without NO$_3^-$ (Table 1).

After these two weeks, an experimental flow-through unit was created by means of peristaltic pumps that pumped the medium from ten 25 l tanks through black silicone tubes at a flow rate of 25 l per week. Over a period of five months, pore water was collected twice a month with teflon Rhizon SMS soil moisture samplers with a length of 10 cm (Eijkelkamp Agrisearch Equipment, the Netherlands), which were permanently installed (two samplers per container). At the end of the experiment the redox potential was measured at a depth of - 10 cm (as described above). Sediment was also incubated at 20 °C to determine CH$_4$ production. The Estimated Release (ER in µmol l^{-1} fresh sediment) of Fe, SO$_4^{2-}$, S^{2-} and o-PO$_4^{3-}$ from the sediments during the experimental flow-through period (from t =2 to t=-20 weeks) was calculated for each container as follows:

ER = ($\bar{x}_{2-20}$ * F * t)/V

with:

$\bar{x}_{2-20}$= mean elemental concentration from t=2 to t=20 weeks
F= flow rate in l/week (=25)
t= number of weeks (=18)
V= volume of the container in l (=10)

Table 1. Chemical composition of the medium (μmol l^{-1}) resembling alkaline groundwater from Beeselsbroek with and without added NO_3^-.

	$+NO_3^-$	$-NO_3^-$
$NaHCO_3^-$	2000	2000
KCl	250	250
$MgCl_2.6H_2O$	200	200
$CaCl_2$	-	2500
$Ca(NO_3)_2. 4H_2O$	2500	-

Chemical analysis of the water samples

pH of the water samples was measured immediately after collection, using a Radiometer Copenhagen type PHM 82 standard pH meter. A 10.5 ml quantity of (pore)water was fixed with 10.5 ml SAOB (sulphide anti oxidant buffer) and used for the detection of S^{2-} by means of an Orion type 94-16A S^{2-} ion-specific silver electrode (Van Gemerden, 1984). CH_4 was measured with ethane as an internal standard on a 'Pye Unicam' gas chromatograph (Unicam Cambridge, UK) equiped with a flame photometric detector and a 'Porapak Q' (80/100 mesh) column ('Waters Chromatography', Etten-Leur, the Netherlands). HCO_3^- and CO_2 analyses were carried out using an 'Oceanograpy International' model 0525 HR infrared carbon analyser. After citric acid had been added, the remaining samples were stored in iodated polyethylene bottles at -23 °C until further analysis. In these samples, the following compounds were measured using Technicon II autoanalysers: NO_3^- and o-PO_4^{3-} (Kamphake et al., 1967) and NH_4^+ (Grasshoff & Johansen, 1977). K^+ was determined by flame photometry using a Technicon autoanalyser. Total Fe, Ca, Mg, Si, Zn, P, S and Al concentration in the (pore) water samples and destruates were determined by means of an inductively coupled plasma emission spectrometer (ICP, Spectroflame). As the pore water was sampled anaerobically, the total Fe measured by the ICP very likely represents reduced

Fe (the oxidising Fe was dissolved by the addition of citric acid). Total C and N content of the oven-dried plant tissue were determined using a Carlo Erba CNS analyser.

Statistical analysis

Results are presented as means ± standard error of the mean (SEM). A one-way analysis of variance (with or without repeated measures) (GLM procedure, SPSS 11.0) was used to assess differences in elemental composition at the various locations and in the various experimental treatments. Significance was accepted if $p \leq 0.05$.

Results

Field studies

Monitoring of alder carrs

Scatterplots of concentrations of $o\text{-}PO_4^{3-}$, NH_4^+ and Fe in the pore water as a function of NO_3^- concentration in the pore water show that the concentrations of $o\text{-}PO_4^{3-}$, NH_4^+ and Fe in the pore water decrease with increasing NO_3^- concentrations. Above NO_3^- concentrations of 100 μmol l^{-1}, the $o\text{-}PO_4^{3-}$, Fe and NH_4^+ concentrations are very low. The $o\text{-}PO_4^{3-}$ concentrations also increase with increasing Fe concentrations in the pore water (Figure 1).

Restoration of fens

One year after restoration, one fen was entirely dominated by plant species characteristic of eutrophic environments like *Lemna trisulca* (leaf duckweed) and *Lemna minor* (common duckweed), while other non-rooting plant species like *Riccia fluitans* (crystalwort) and *Ceratophyllum demersum* (coontail) were also present. These species, indicating very nutrient-rich conditions of the water layer, did not grow in the other fen, which instead showed development of plant species characteristic of moderately nutrient-rich clear water, like *Chara globularis* (stonewort*), Elodea canadensis* (canadian waterweed) and *Rorippa nasturtium-aquaticum* (white watercress) (Table 2).

SO_4^{2-} concentrations in the pore water and surface water of the eutrophic fen, which was fed by groundwater rich in SO_4^{2-} (1000 μmol l^{-1}) but poor in NO_3^- (< 35 μmol l^{-1}), decreased immediately after the influence of groundwater was reinforced. However, SO_4^{2-} concentrations in the pore water and water layer of the clear water fen remained high. Contrary to the eutrophic fen, the clear water fen was fed by groundwater that had not only high SO_4^{2-} concentrations (1000 μmol l^{-1}) but also very high NO_3^- concentrations (600-4900 μmol l^{-1}). Concentrations of $o\text{-}PO_4^{3-}$, Fe, NH_4^+ and CH_4 were much higher in the pore water and water layer of the eutrophic fen compared to the clear water fen (Figure 2).
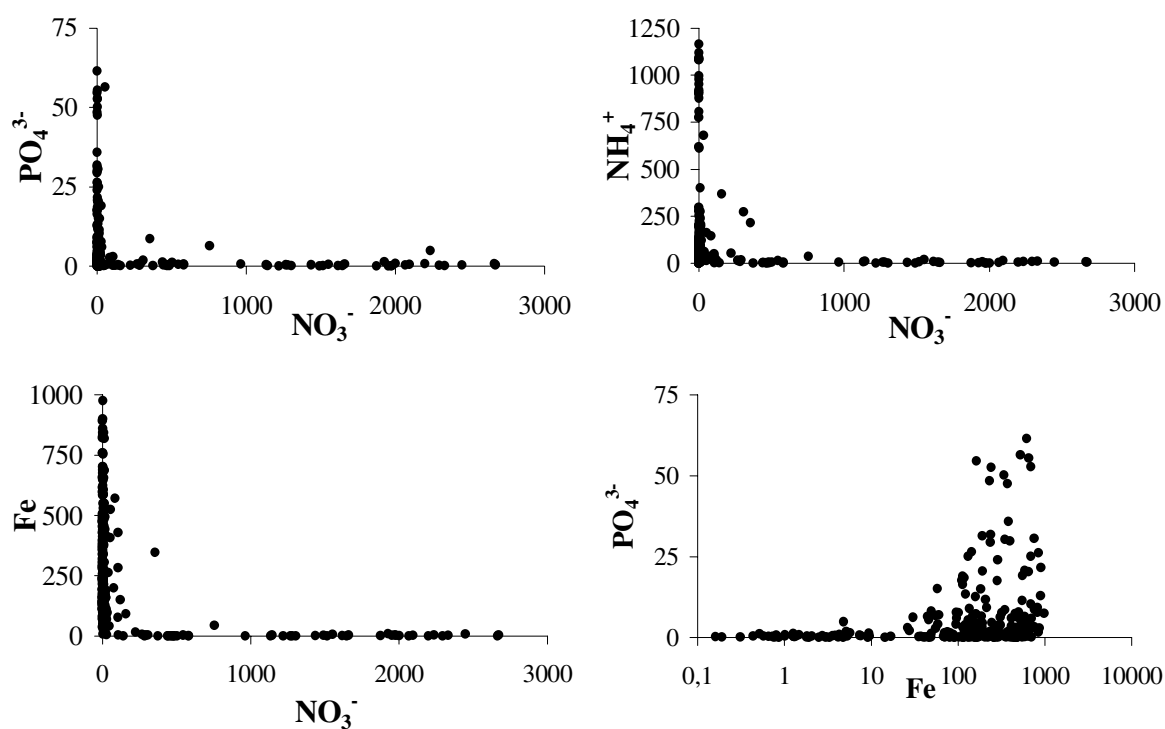
Figure 1. Pore water scatterplots with o-PO_4^{3-}, NH_4^+ and Fe concentrations as a function of the NO_3^- concentration, and o-PO_4^{3-} as a function of the Fe concentration. Concentrations are given in μmol l^{-1}.

Table 2. Vegetation composition of the eutrophic and clear water fens in the summer of 2001. d, dominant; cod, codominant; a, abundant; f, frequent; l, local; o, occasional; r, rare; s, seldom.

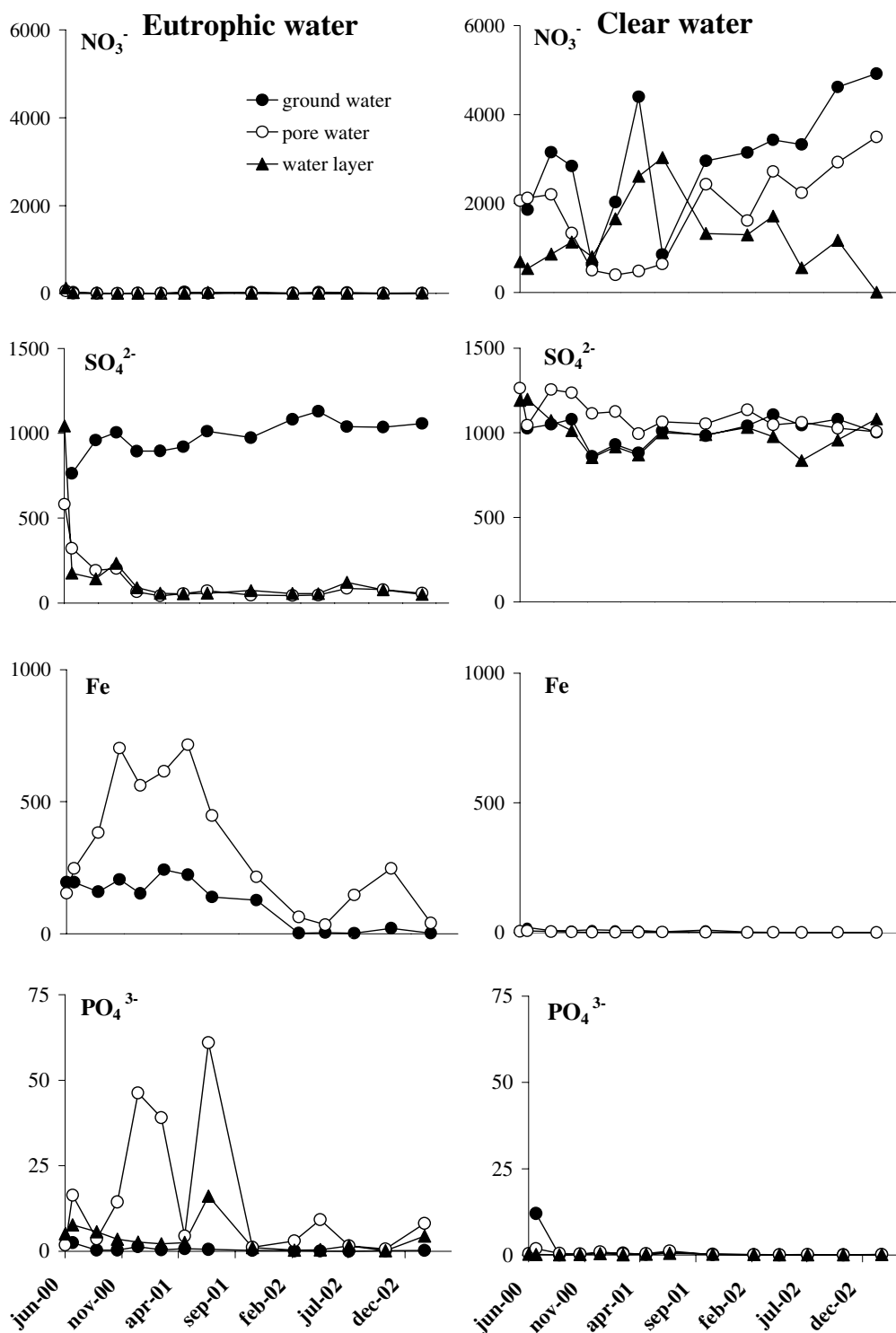| Eutrophic water		Clear water	
Alisma plantago-aquatica	o	*Callitriche spec.*	o
Ceratophyllum demersum	ld	*Chara globularis var. globularis*	o
Lemna trisulca	d	*Elodea canadensis*	la
Lemna minor	cod	*Hydrocharis morsus-ranae*	r
Nymphea alba	o	*Nymphea alba*	r
Potamogeton natans	o	*Potamogeton crispus*	o
Ranunculus flammula	r	*Potamogeton natans*	o
Riccia fluitans	o	*Rorippa nasturtium-aquaticum*	o

Figure 2. Chemical composition of the groundwater, pore water and water layer in the eutrophying fen (left) and in the fen developing clear water (right) between April 2000 and January 2003. Concentrations are given in µmol l⁻¹.
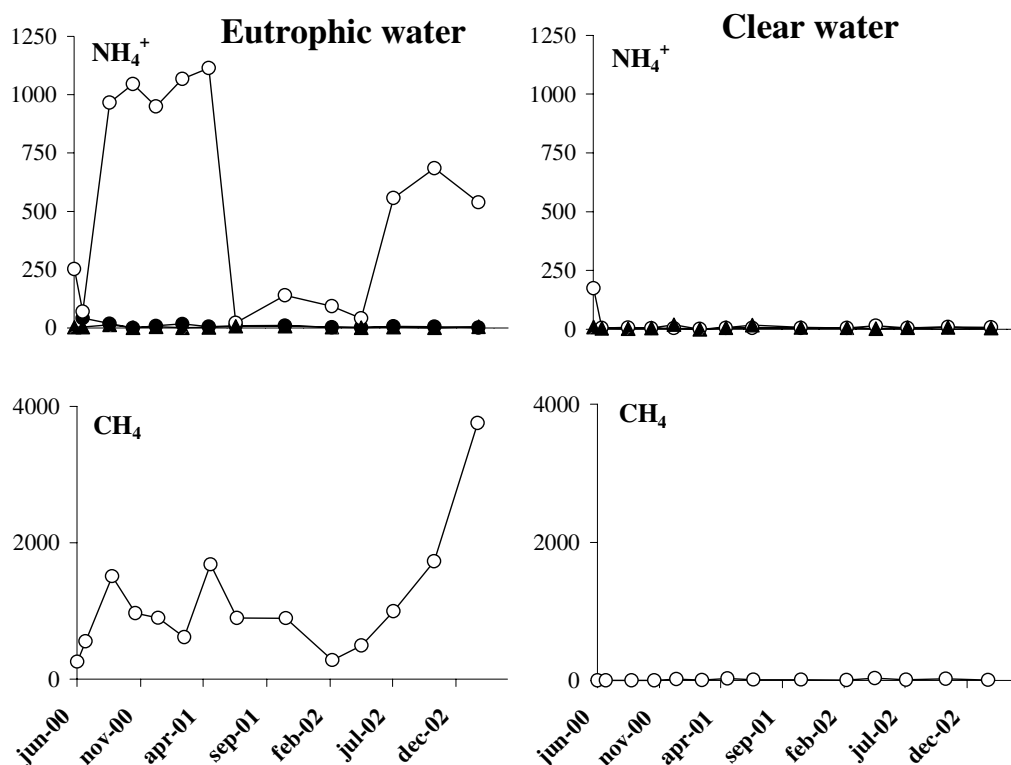
Figure 2 Continued.

Examination of locations directly and indirectly fed by NO_3^- -rich seepage.

The redox potential (+247 mV) and the NO_3^- concentration (2030 µmol l^{-1}) in the pore water of the soil at sites directly fed by NO_3^- rich groundwater were much higher than those at sites indirectly fed by groundwater (-160 mV and 348 µmol l^{-1} respectively). The Fe concentration was extremely low at the NO_3^--rich site (0.5 µmol l^{-1}). HCO_3^-, NH_4^+, Mn and Al concentrations in the pore water were also significantly lower while S, Ca, Mg and K^+ concentrations were significantly higher. The root plaques of the chlorotic *C. pseudocyperus* plants at the NO_3^--rich site also had significantly lower Fe concentrations (8 µmol g^{-1} DW) than the non-chlorotic plants of the NO_3^--poor site (1198 µmol g^{-1} DW). The Ca concentration in the root plaques was also significantly lower, whereas Zn, K^+, Mg and Mn concentrations were significantly higher (Table 3).

As expected, the concentrations of chlorophyl A, chlorophyl B and carotenoids were lower in chlorotic plants. The Fe concentrations in roots of chlorotic plants were much lower (2 µmol g dw $^{-1}$) than those in non-chlorotic plants (1527 µmol g dw $^{-1}$). Ca, N and P concentrations were also significantly lower while Zn, K^+ and Mg concentrations were significantly higher. Fe, Ca, N and carbon concentrations in the shoots were also significantly lower in clorotic plants. Fe concentrations in the shoots were up to 4 times lower (3 µmol g dw $^{-1}$), while the

differences in Ca, N and C concentrations were smaller. Zn, K^+ and Al concentrations were higher in the shoots of chlorotic plants (Table 4).

At NO_3^--poor sites, desiccation led to a marked increase in SO_4^{2-} concentrations, from 1000 µmol l^{-1} to 15000 µmol l^{-1}. In addition, pH, alkalinity and HCO_3^- concentrations strongly decreased, whereas Ca, Mg and concentrations of several metals, including Al, Zn and Mn, strongly increased. These changes did not occur as a consequence of desiccation at NO_3^--rich sites (Figure 3).

Table 3. Pore water chemistry (µM), E (mV) and concentrations of root plaque extractable components (µmol/gr DW) of *C. pseudocyperus* at locations fed by NO_3^--poor and NO_3^--rich groundwater. Means ± (standard errors) are given (n=6). *, P ≤ 0.05; **, P ≤ 0.005.

	Pore water		Root plaque	
	- NO_3^-	**+ NO_3^-**	**- NO_3^-**	**+ NO_3^-**
NO_3^-	348 (92)	2030 (186)**		
E	-140 (19)	247 (36)**		
pH	6.59 (0.05)	6.69 (0.02)		
HCO_3^-	2738 (391)	1576 (17)*		
SO_4^{2-}	1243 (69)	1572 (17)**		
NH_4^+	31.2 (5.5)	7.6 (0.3)**		
o-PO_4^{3-}	0.8 (0.3)	0.6 (0.1)		
Fe	373 (96)	0.5 (0.1)**	1198 (154)	8 (2)**
Mn	29.4 (3.2)	7.6 (1.6)**	13 (2)	35 (6)*
Ca	2084 (145)	2732 (110)*	112 (9)	35 (2)**
Mg	301 (35)	765 (93)**	11 (0.4)	16 (1.3)**
K^+	184 (52)	633 (103)**	82 (11)	174 (21)**
Zn	3.3 (0.2)	3.9 (0.2)	0.5 (0.1)	2.6 (0.3)**
Al	7 (1.3)	3.3 (0.2)**	2.1 (0.5)	3.1 (1.2)

Table 4. Pigment concentrations in the shoots (mg/g FW) and nutrient concentrations in roots and shoots (μmol/g DW) of *C. pseudocyperus* at locations fed by NO_3^--poor and NO_3^--rich seepage. Means ± (standard errors) are given (n=6). *, $P \leq 0.05$; **, $P \leq 0.005$. Concentration of C is given in mmol/g DW.

	Roots		Shoots	
	$- NO_3^-$	$+ NO_3^-$	$- NO_3^-$	$+ NO_3^-$
Chlorophyl A			2.6 (0.4)	0.54 (0.1)**
Chlorophyl B			1.5 (0.2)	0.48 (0.1)**
Carotenoids			0.35 (0.05)	0.11 (0.02)**
C	26.6 (1.9)	31 (0.9)*	36.8 (0.3)	36 (0.2)*
N	830 (80)	665 (70)*	1960 (30)	1440 (50)*
Fe	1527 (472)	2 (1)*	12 (2)	3 (0.1)**
P	170 (43)	43 (9)**	113 (17)	122 (6)
Mn	12 (3)	15 (3)	12 (3)	19 (2)
Ca	118 (35)	39 (4)*	132 (9)	109 (4)
Mg	44 (6)	61 (4)*	77 (14)	99 (3)
K^+	524 (81)	880 (60)*	934 (71)	1410 (24)**
Zn	2.3 (0.5)	6.6 (0.8)**	2 (0.4)	4.5 (0.9)**
Al	4.5 (0.5)	13.7 (6.7)	1.7 (0.4)	4.6 (0.8)*
S	880 (56)	1053 (177)	133 (29)	161 (6)

Laboratory study

Effect of NO_3^- addition to S-rich sediments.

Addition of NO_3^- led to a significant increase in the redox potential and the concentrations of NO_3^- and SO_4^{2-} in the pore water. Concentrations of Fe, o-PO_4^{3-}, P, S^{2-}, Mn, K^+ and Si significantly decreased, as did the production of CH_4. The changes in o-PO_4^{3-} and SO_4^{2-} concentrations were most obvious during the first three weeks, when the medium was stagnating. The period with a flow rate of 25 l medium per week led to significantly lower net releases of Fe, S^{2-} and o-PO_4^{3-} and a significantly higher net release of SO_4^{2-} from the monoliths treated with NO_3^-. There was no difference in the total content of these elements at the start of the experiment (Figure 4, Table 5 & 6).
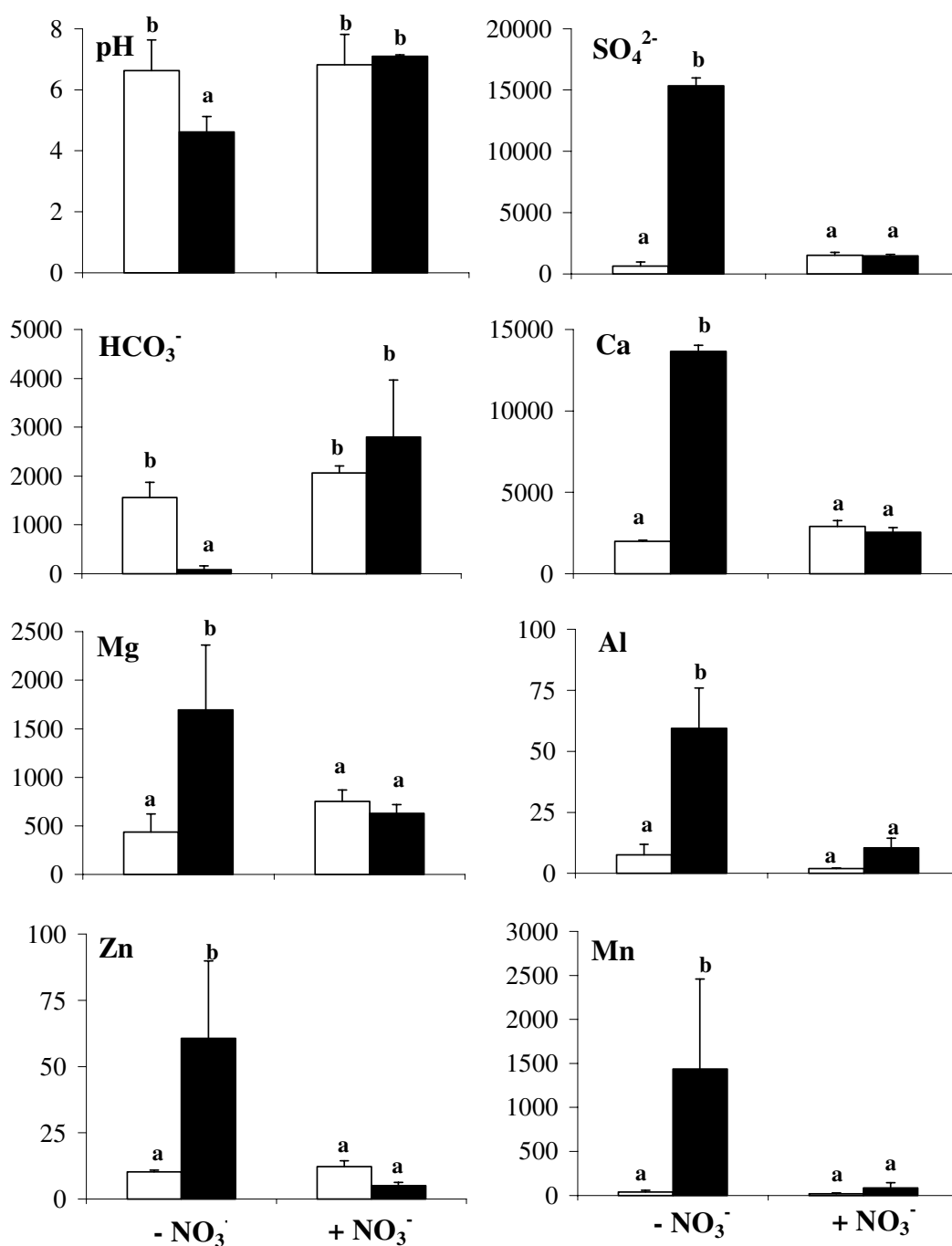
Figure 3. Chemical composition of the pore water of sediments fed directly (NO_3^- -rich) and indirectly (NO_3^--poor) by groundwater under field conditions (□), and after rewetting following experimental desiccation (■). Concentrations are given in $\mu mol\, l^{-1}$.
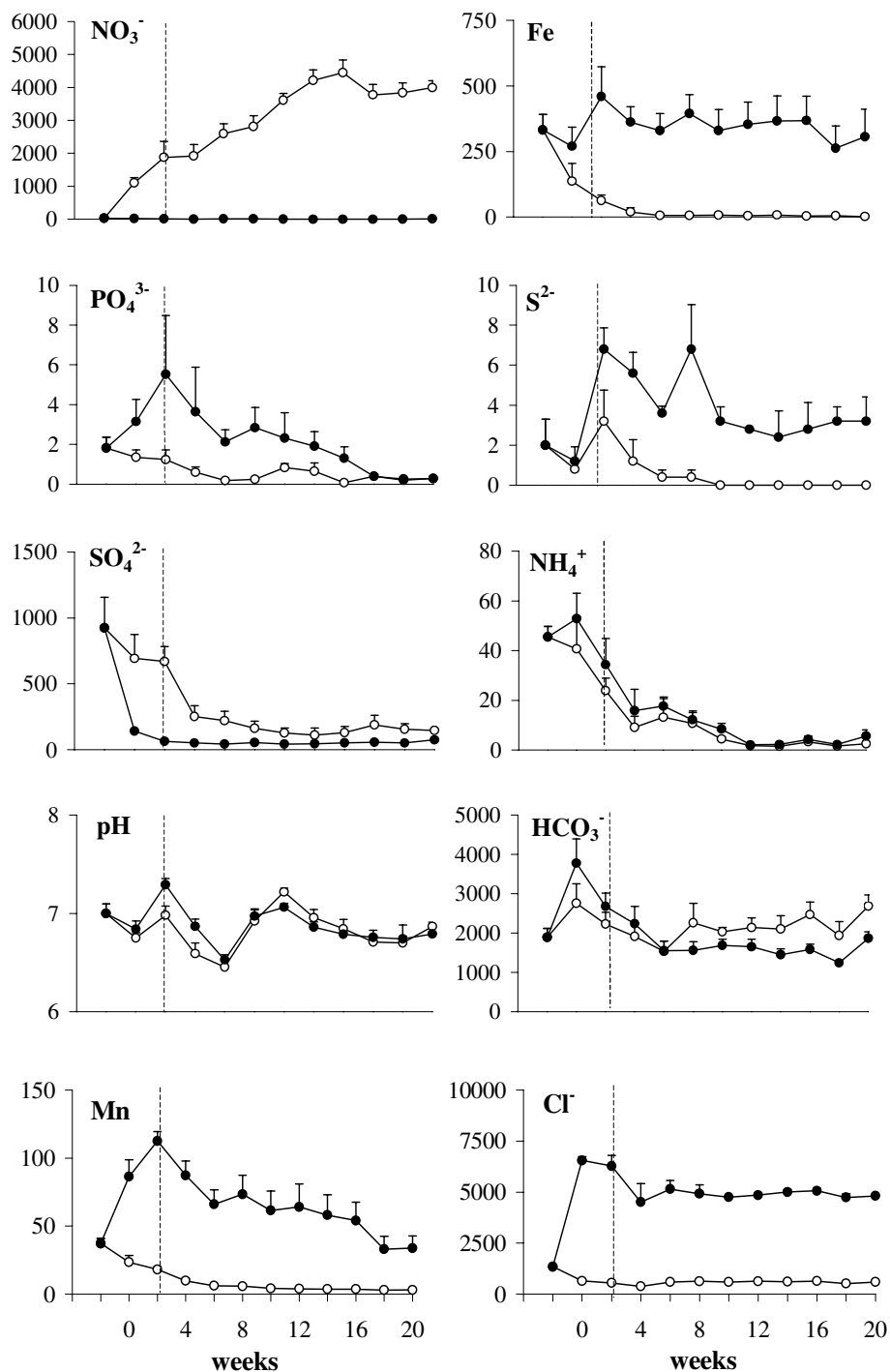
Figure 4. Chemical composition of the pore water in S-rich monoliths from Beeselsbroek after stagnation (first 3 weeks) and during flow-through (last 18 weeks) with NO_3^- -rich ($\circ$) and NO_3^- -poor ($\bullet$) medium at the laboratory. Concentrations are given in μmol l^{-1}.

Table 5. Total concentrations of Fe, S and P at the start of the experiment (μmol/g dw), redox potential (mV) at -10 cm, CH_4 production (μmol/kg fresh sediment/day) at the end of the experiment, and estimated release (ER) of Fe, SO_4^{2-}, S^{2-} and o-PO_4^{3-} during the experimental period (μmol l^{-1} fresh sediment). Means ± (SEM) are given (n=5). a, $P \leq 0.05$; b, $P \leq 0.005$; c, $P \leq 0.001$.

	total Fe	total S	total P	Redox	CH_4	ER Fe	ER SO_4^{2-}	ER S^{2-}	ER o-PO_4^{3-}
- NO$_3$-	974 (242)	321 (35)	53 (3)	57 (36)	397 (19)	15748 (3486)	2310 (123)	163 (33)	88 (36)
+ NO$_3^-$	875 (161)	364 (38)	53 (4)	233 (17)a	3 (0.2)b	470 (279)c	8760 (3122)a	18 (13)a	20 (5)a

Table 6. ANOVA table with effects of NO_3^- treatment, time and their interaction (treatement*time) on pore water chemistry of S-rich monoliths from Beeselsbroek. A one-way ANOVA with repeated measures was used (after natural-log transformation). *, $p < 0.05$; **, $P < 0.005$; ***, $p < 0.001$; ns, not significant.

	treatment	time	time*treatment
pH	ns	ns	ns
HCO$_3^-$	ns	ns	*
NO$_3^-$	***	**	*
Fe	***	**	*
o-PO$_4^{3-}$	*	***	ns
P	**	ns	ns
S^{2-}	***	*	ns
SO$_4^{2-}$	*	**	*
NH$_4^+$	ns	**	ns
Mn	***	***	**
Na$^+$	ns	ns	ns
CO$_2$	ns	**	ns
K$^+$	*	***	ns
Ca	*	ns	**
Mg	ns	***	*
Zn	ns	ns	ns
Al	ns	**	ns
Si	**	**	ns

Discussion

The results showed that high groundwater NO_3^- concentrations positively affected groundwater fed SO_4^{2-} enriched wetlands by restricting the mobilisation of o-PO_4^{3-} from the sediment to the water layer. Under anaerobic conditions, reduction of Fe from Fe-PO_4^{3-} complexes leads to the mobilisation of o-PO_4^{3-} from sediments (Hosomi, 1980; Furumai & Ogaki, 1982; Roelofs, 1991; Wetzel 2001). Mobilisation of o-PO_4^{3-} from these complexes probably occurred in the wetlands examined in the present study, as was indicated by the positive correlation between o-PO_4^{3-} and Fe in the pore water in the monitoring study (Figure 1). It is well known that the release of Fe-bound o-PO_4^{3-} is accelerated by increased SO_4^{2-}-reduction rates. The S^{2-} produced, reduces Fe(III) in Fe-phosphate, Fe-(hydr)oxide-phosphate and humic Fe-phosphate complexes, mobilising o-PO_4^{3-} (Sperber, 1958; Patrick & Khalid, 1974; Boström et al., 1982; Smolders & Roelofs, 1993; Moore & Reddy, 1994; Lamers et al., 1998). S^{2-} also forms iron (di)sulphides (FeS_x) with Fe(II), reducing the amounts of Fe available for o-PO_4^{3-} binding and decreasing the Fe/o-PO_4^{3-} ratio in the pore water (Boström et al., 1982; Caraco et al., 1989; Roelofs, 1991; Smolders & Roelofs, 1993; Murray, 1995). As a result, the release of o-PO_4^{3-} from the sediment to the overlying water layer increases as more o-PO_4^{3-} escapes the Fe trap in the oxidised surface layer of the sediment (Roden & Edmonds, 1997; Smolders et al., 2001; Wetzel, 2001).

As NO_3^- is an energetically more favourable electron acceptor in anaerobic sediments than Fe and SO_4^{2-}, high NO_3^- loads function as a redox buffer limiting the reduction of Fe and SO_4^{2-} (Scheffer & Schachtschabel, 1992; Drever, 1997). In addition, NO_3^- reducing bacteria have the capacity to grow anaerobically with Fe(II) as the electron donor, resulting in the production of Fe(III) (Straub et al., 1996; Nielsen & Nielsen, 1998; Weber et al., 2001). It is indeed known that NO_3^- reduction can lead to oxidation of Fe(II) (Uhlmann & Paul, 1994; Smolders et al., 1997; Aravena & Robertson, 1998; Pauwels et al., 1998; Tesoriero et al., 2000) and metal sulphides (Klapper, 1989; Ripl, 1976; Golterman, 1991 and 1995) under field conditions. The role of NO_3^- as a redox buffer also became obvious from our laboratory experiment. A continuous flow of NO_3^--rich medium led to a higher redox potential in the sediment than the same medium without NO_3^-. In addition, the release of SO_4^{2-}, due to oxidation of FeS_x, was much higher in sediments receiving medium with NO_3^- (Table 5-6 & Figure 4). At high pH, Fe^{3+} precipitates as insoluble iron (hydr)oxides (Drever, 1997). Schippers & Jørgensen (2001, 2002) showed that Mn reduction plays an important role in oxidation of FeS_x in marine sediments. This will only play a minor role in our investigated systems, as they are fed by groundwater rich in NO_3^- (up to 5000 µmol/l) and poor in Mn (< 10 µmol/l). In addition, our laboratory experiment showed that Mn reduction does not take place at high NO_3^- concentrations. It is also possible that some of the mobilised SO_4^{2-} and o-PO_4^{3-} in the laboratory experiment originated from anion exchange due to NO_3^- addition.

SO_4^{2-} can bind to positively charged clay minerals and iron-oxides, but binding of NO_3^- or Cl^- to these adsorption sites is known to be very weak. Therefore addition of these components can not be expected to mobilise large amounts of SO_4^{2-} and o-PO_4^{3-} (Drever, 1997). This is confirmed by the fact that the addition of $CaCl_2$ amounts in the control treatment did not increase SO_4^{2-} concentrations to the same levels as happened in the NO_3^- treatment. Field locations that were directly fed by groundwater had a much lower reduced S content than locations indirectly fed by the same groundwater. The locations that were directly fed by groundwater, had high NO_3^- concentrations, preventing the reduction of SO_4^{2-} and thus the formation of FeS_x. Ongoing NO_3^- reduction reduces the concentration of NO_3^- in the groundwater, and therefore locations indirectly fed by groundwater accumulated large amounts of FeS_x due to SO_4^{2-} reduction. SO_4^{2-} reduction was not inhibited at these locations, as the groundwater was poor in NO_3^- (Figure 3).

By maintaining a high redox potential, NO_3^- inhibits the release of o-PO_4^{3-} from iron-phosphate complexes in anaerobic sediments (Ripl, 1976; Ripl, 1978; Andersen, 1982; Jensen & Andersen, 1992). The monitoring study revealed that wetlands fed by groundwater with high NO_3^- concentrations were characterised by the lowest o-PO_4^{3-} concentrations (Figure 1 & 2) and the development of aquatic plants characteristic of clear waters (Table 2). The laboratory study showed that addition of NO_3^- was accompanied by a significantly lower release of o-PO_4^{3-} from the monoliths as it oxidised reduced Fe and thus increased the amount of oxidised Fe capable of binding o-PO_4^{3-} (Figure 4, Table 5 & 6). The comparison between the NO_3^--poor and NO_3^--rich restored fens indicated that NO_3^- prevented SO_4^{2-} reduction (Figure 2). The occurrence of SO_4^{2-} reduction in the NO_3^--poor fen was indicated by the higher SO_4^{2-} concentration in the groundwater as compared to concentrations in the pore water and in the water layer, which decreased immediately after the groundwater discharge was reinforced. As a result, o-PO_4^{3-} concentrations in pore water and water layer increased, resulting in a rapid development of species characteristic of eutrophic environments (Table 2). These changes did not take place in the NO_3^--rich fen. Oxidised conditions enhance nitrification, which explains the relatively low NH_4^+ concentration in the pore water of the NO_3^--rich fen, as opposed the the very high NH_4^+ concentrations in the NO_3^--poor fen.

The high NO_3^- concentrations in the groundwater of the wetlands investigated did not result in eutrophication. Black alder carrs are not limited by nitrogen, as alder trees fix nitrogen in root nodules (Blom et al., 1981; Guan et al., 1996). Thus, increased availability of nitrogen is in itself not likely to change the trophic status in these systems. All investigated alder carrs that were fed with NO_3^--rich seepage, were dominated by species of the *Calthion palustris* association, which is characteristic of well-developed alder carrs. Smolders et al. (1997) showed that high NO_3^- concentrations in the groundwater caused Fe deficiency chlorosis in *Juncus acutiflorus*. The same effect was clearly visisble in some of the species in the black

alder carrs that were fed with NO_3^--rich seepage (Table 4). High NO_3^- concentrations acted as a redox buffer under wet conditions and decreased the free Fe concentrations in the sediment (Table 3). Increased NO_3^--assimilation may also increase the apoplastic pH, leading to immobilisation of Fe and/or less Fe reduction. This in turn results in decreased free internal Fe concentrations, causing Fe deficiency chlorosis (Smolders et al., 1997).

In addition to influencing o-PO_4^{3-} availability, NO_3^- might also affect the sensitivity of wetland sediments to acidification during dessication. Oxidation of FeS_x produces sulphuric acid, causing acidification and mobilisation of heavy metal sulphides, depending on the buffer capacity of the sediment (Lucassen et al., 2002). This was also evident in the sediments originating from locations indirectly fed by seepage (and thus poor in NO_3^-). These sediment types mobilised large amounts of SO_4^{2-} as a consequence of desiccation, and the acid produced led to exhaustion of the buffer capacity, as indicated by decreased alkalinity and HCO_3^- concentration and increased Ca and Mg concentrations (as a consequence of cation exchange buffering). This caused a drop in pH and mobilised (heavy) metals including Zn, Al and Mn. These processes did not take place at locations directly fed by NO_3^--rich groundwater, where SO_4^{2-} reduction was impaired and only minor amounts of FeS_x had accumulated (Figure 3).

We propose the following scenario for the current situation in the Netherlands. NO_3^--leaching from agricultural lands and forest soils leads to increased NO_3^- concentrations in the groundwater. When NO_3^- reaches FeS_x-containing subsoil layers it may oxidise FeS_x which leads to the mobilisation of SO_4^{2-} and a decrease of the NO_3^- concentration. The resulting NO_3^-/SO_4^{2-} ratio will strongly affect the quality of groundwater fed fens. If SO_4^{2-} concentrations are high and NO_3^- concentrations are low, eutrophication may occur as a result of SO_4^{2-} reduction-related processes such as o-PO_4^{3-} mobilisation and Fe immobilisation (FeS_x accumulation). However, if NO_3^- concentrations are also high, SO_4^{2-} and Fe reduction are impaired and mobilisation of o-PO_4^{3-} from iron-phosphate complexes is prevented. In addition, NO_3^- may oxidise reduced Fe compounds increasing the amount of Fe^{3+} capable of binding o-PO_4^{3-}. So NO_3^- leaching into the groundwater increases the risk of eutrophication of the discharge areas by mobilising SO_4^{2-} in FeS_x containing aquifers. However, if it reaches a discharge area in sufficiently high amount, NO_3^- may prevent actual eutrophication (at least of the directly fed parts) by functioning as a redox buffer.

Acknowledgements

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CHAPTER 9

Synthesis: Biogeochemical processes in S-rich wetlands subjected to changes in groundwater quantity and quality; implications for wetland management

Esther C.H.E.T. Lucassen

Changes in groundwater quantity and quality of fens

Throughout the last decades, fens in the Netherlands have suffered from anthropogenic threats like desiccation, cultivation, acidification and eutrophication, all resulting in loss of biodiversity (Roelofs, 1991; Koerselman & Verhoeven, 1995; Lamers et al, 2002). Many fens along the river Meuse have been severely drained between the 1930s and the 1970s, in order to lower local groundwater tables and allow agricultural activities. Apart from changes in groundwater levels, the increased agricultural activities have also led to changes in groundwater quality. NO_3^- and SO_4^{2-} concentrations in groundwater have increased. In the river Meuse region, restoration of desiccated fens by simply damming drainage ditches has often failed to produce the desired results. Stagnation of surface water led to decreased groundwater discharge, decline of the original vegetation and severe eutrophication (Boxman & Stortelder, 2000; Lucassen et al., 2000).

This thesis is devoted to the understanding of biogeochemical processes in fens fed by SO_4^{2-}-rich groundwater, as well as the implications for wetland management. The present synthesis tries to give an overview of the processes in fens that prevent eutrophication under semi-natural conditions and the adverse processes that occur when groundwater inputs are impaired due to extreme desiccation or stagnation of surface water. Special emphasis is put on the remarkable role of NO_3^- in these processes. The chapter ends with recommendations for the restoration of desiccated fens and a table with answers to the questions posed in the general introduction.

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Processes preventing eutrophication in SO_4^{2-}-rich fens under semi-natural conditions

Processes preventing eutrophication in SO_4^{2-}–rich fens under semi-natural conditions are summarised in Figure 1. A characteristic of fens is their base-richness (Gore, 1983; Wheeler & Proctor, 2000). Base-rich groundwater plays an important role in determining the concentrations of nutrients in fens (Chapter 2). Ca and Fe suppress binding of NH_4^+ to the soil adsorption complex (Koenig & Pan, 1996; Holmboe & Kristensen, 2002). The NH_4^+ concentration in pore water is linearly related to the concentration of NH_4^+ adsorbed onto sediment solids in a dynamic equilibrium, whereby the NH_4^+ adsorption capacity depends on the ion exchange capacity associated with the organic matter and clay content of the sediment (Rosenfeld, 1979; Boatman & Murray, 1982; Raaphorst & Malschaert, 1996). The continuous supply of base cations prevents adsorption of NH_4^+ to the soil, and the NH_4^+ is constantly removed from the system via the flowing groundwater. In addition, high concentrations of Fe and Ca in the feeding groundwater immobilise o-PO_4^{3-} by forming $CaPO_4$ and $FePO_4$ precipitations (Stumm & Morgan, 1986; Golterman, 1998; Smolders et al., 2001; Chapter 6). Base-rich groundwater clearly has an oligotrophying effect on fens (Chapter 2). The seepage zones of black alder carrs, for instance, have low NH_4^+ and o-PO_4^{3-} concentrations in the pore water and have a very species-rich vegetation, dominated by plant species characteristic of groundwater discharge, like *Caltha palustris* and *Equisetum fluviatile*. Desiccated parts or parts that are fed by stagnating groundwater receive less groundwater, and NH_4^+ can more easily bind to the adsorption complex, while o-PO_4^{3-} is less immobilised by complexation with Ca or Fe. The characteristic fen species are absent from these locations. Lemnids and fast growing wetland grasses, like *Glyceria fluitans* and *Glyceria maxima*, become dominant if the groundwater stagnates (Chapter 7).

Seepage zones are fed by groundwater, which can be rich in NO_3^-, Fe and SO_4^{2-}. These components are subjected to reduction in the microbial breakdown of organic matter under wet conditions. In seepage zones, reduction of Fe(III) mainly takes place beneath the aerobic layer of the sediment. Beneath this layer, Fe is present in the reduced form and SO_4^{2-} reduction leads to the formation of S^{2-}, which reacts with free Fe^{2+} under formation of FeS_x (Chapter 5). At seepage sites fed by groundwater rich in NO_3^-, reduction of Fe and SO_4^{2-} is inhibited, as NO_3^- is a more favourable electron acceptor (Chapters 7 & 8). In addition, reduction of NO_3^- may even lead to oxidation of reduced Fe compounds, further increasing the content of Fe(III) capable of binding o-PO_4^{3-} (Uhlmann & Paul, 1994; Chapters 7 & 8). Outside the seepage zones, concentrations of NO_3^- and Fe in the groundwater decrease rapidly and SO_4^{2-} also acts as an electron acceptor in the microbial breakdown of organic matter (Chapter 8).

In summer, groundwater input is restricted and large parts outside the seepage zones dry out. Periodic drought is important in oxidising reduced Fe compounds in the sediment and thus in

re-increasing the content of Fe(III) capable of binding o-PO_4^{3-}. Chapter 6 shows that beneficial effects of temporary desiccation of fens depend on the oxidisable Fe pool of the sediment in combination with the buffering capacity. Seepage zones never dry out under natural circumstances, but temporary desiccation of sediments outside the seepage zones leads to a long-lasting reduction in P release from the sediment because of the presence of a reduced Fe pool (FeS$_x$), resulting in the release of Fe(III) upon oxidation. In addition, NH_4^+ is oxidised to NO_3^- under aerobic conditions, which is then denitrified in anaerobic sediment layers, resulting in a loss of N.

Processes in SO_4^{2-}-rich fens when groundwater influence is impaired: extreme drought

If regional groundwater tables are greatly lowered, more severe, longer lasting and more frequent drought periods occur in fens, resulting in decreased growth of the type of species-rich vegetation characteristic of habitats with groundwater discharge (Chapter 2).
Desiccated sediments only receive groundwater during extremely wet periods. Immediately after desiccation, oxidation of FeS$_x$ leads to the production of acid, which exchanges with Ca at the adsorption complex. Due to the smaller input of Fe and the base cations Mg and Ca, the adsorption complex becomes less saturated with cations, and NH_4^+ originating from the breakdown of organic matter, atmospheric deposition or fixation by alder trees is easily bound to the adsorption complex, resulting in high NH_4^+ concentrations in the sediment. In addition, the decreased Fe input increases o-PO_4^{3-} availability in wet periods, as less o-PO_4^{3-} can be complexed. Higher NH_4^+ concentrations are accompanied by the absence of plant species characteristic of groundwater discharge and by a monotonous growth of wetland grasses (like *Glyceria fluitans* and *G. maxima*) and *Lemna minor* in depressions with stagnating surface water (Chapter 2). Desiccation leads to acidification, depending upon the S/(Ca+Mg) ratio of the sediment (Chapter 5). High NH_4^+ concentrations combined with a low pH can be toxic to plant species that are characteristic of well-developed fen meadows such as *Cisium dissectum* (Chapter 3).
Although seepage zones never dry out under natural conditions, extreme drops in regional groundwater tables may lead to the desiccation of such seepage zones. Depending upon the buffering capacity of the sediment and the amount of accumulated FeS$_x$, oxidation results in acidification of the sediment. Extreme acidification occurs when the total S/(Ca+Mg) ratio in the soil exceeds 2/3 (Chapter 5). As seepage zones contain very large amounts of strengite (FePO$_4$) and apatite (Ca$_5$(PO$_4$)$_3$(OH,F,Cl)), due to complexation of o-PO_4^{3-} with Ca and Fe from the groundwater, acidification may lead to mobilisation of extremely high amounts of o-PO_4^{3-} in the sediment (Chapter 6). The reason is that Fe~PO_4^{3-} complexes dissolve under extremely acid conditions. The solubility of apatite, strengite and variscite (AlPO$_4$) is
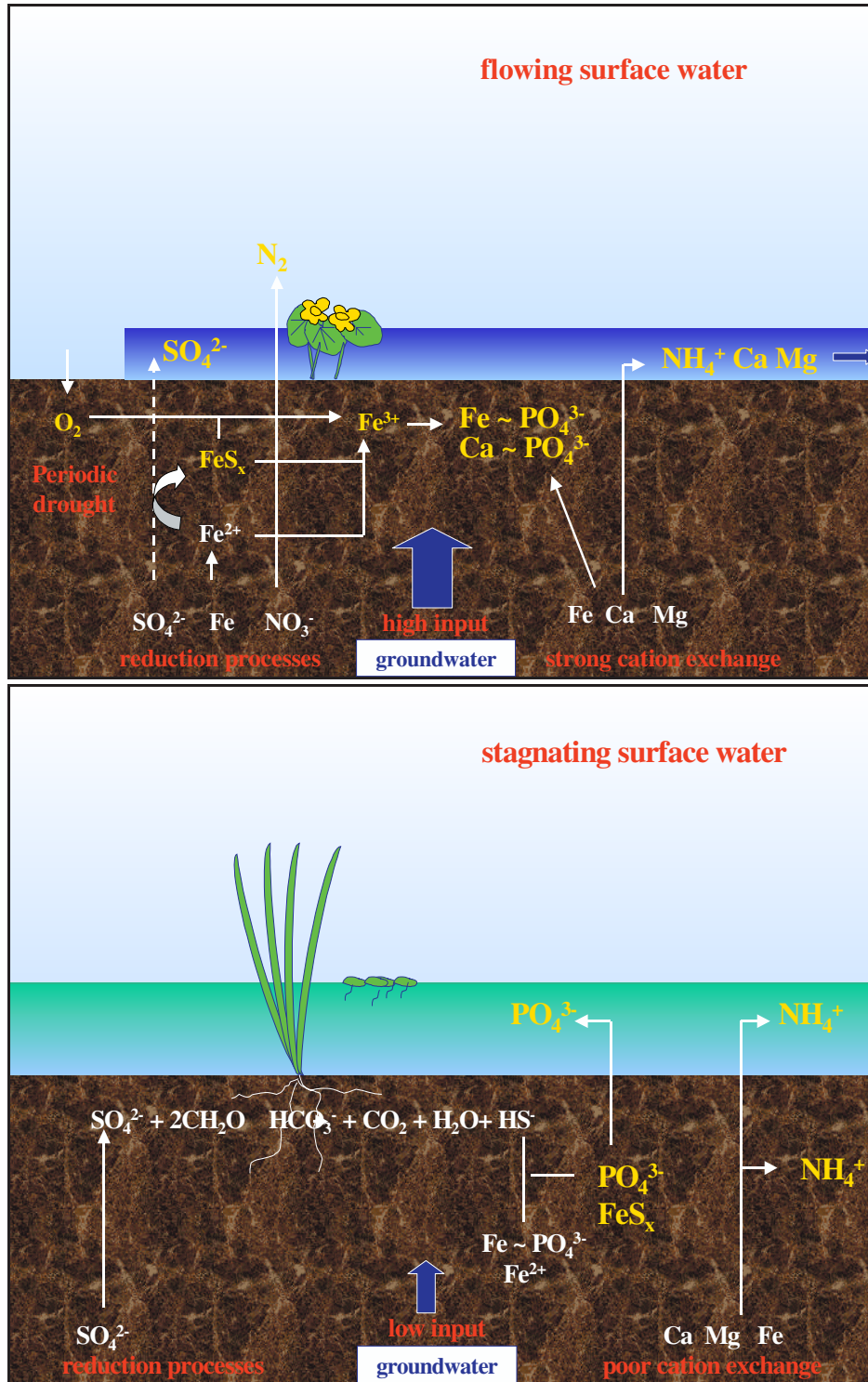
Figure 1. Oligotrophying processes in fens under semi-natural conditions, including continuous input of base-rich groundwater and periodic droughts in summer (top panel) and the absence of these processes in fens with stagnating surface water, favouring SO_4^{2-} reduction and Fe depletion, resulting in eutrophication (bottom panel).
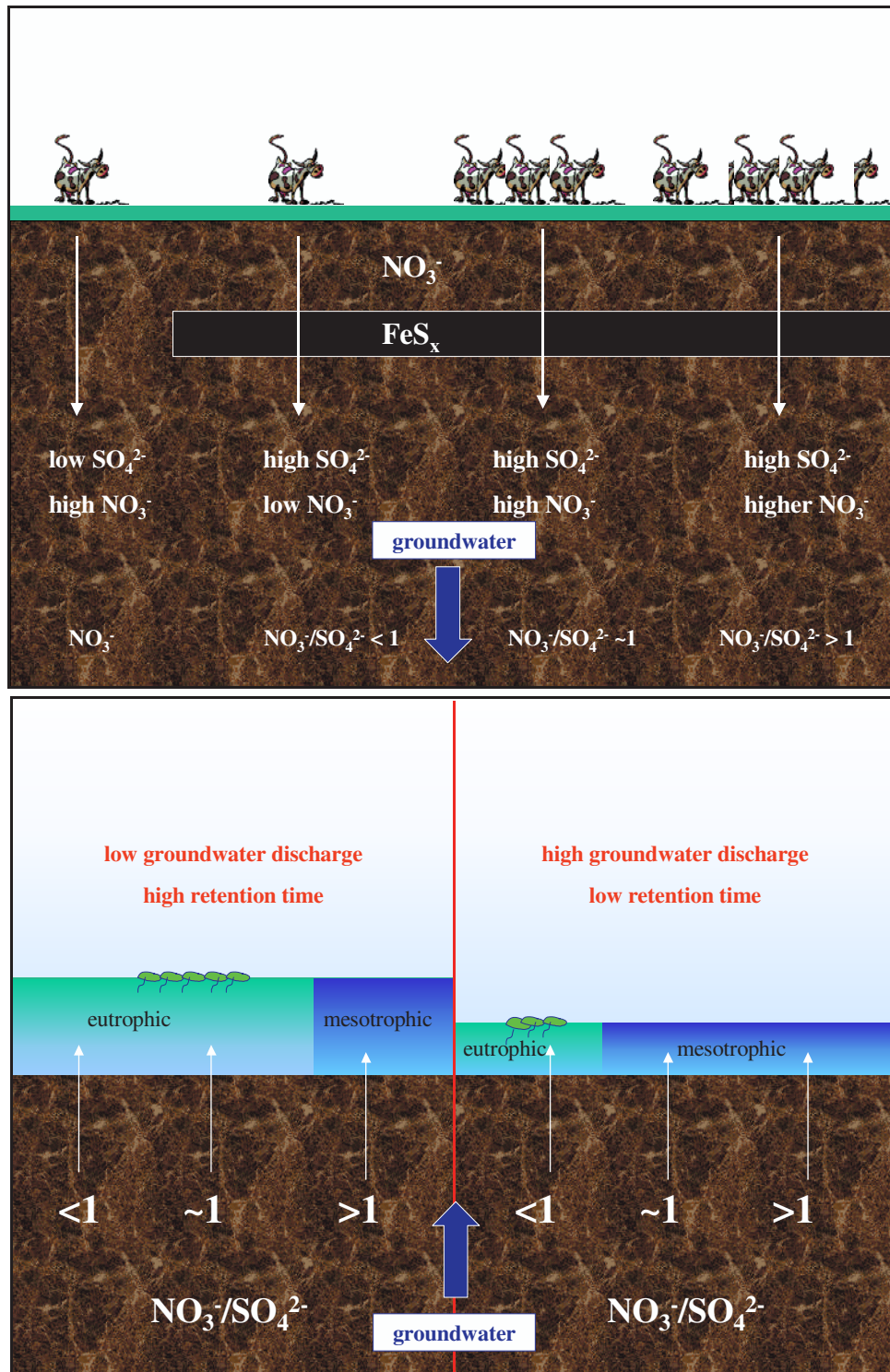
Figure 2. Nitrate leaching from agricultural lands leads to denitrification and oxidation of FeS_x in the subsoil, resulting in mobilisation of SO_4^{2-} in the groundwater (top panel). The NO_3^-/SO_4^{2-} ratio in the groundwater resulting from this process, in combination with the retention time of the surface water, determines the o-PO_4^{3-} concentration in fens (bottom panel).

strongly influenced by pH. With decreasing pH, apatite is the first to dissolve, followed by variscite and strengite. Binding capacities are greatest at pH 6, but below pH 4, dissolution of metal phosphates occurs, resulting in P mobilisation (Stumm & Morgan, 1981). These processes do indeed play a role under field conditions (Beltman et al., 1996; Golterman, 1998). As the total Fe content is extremely high in seepage zones, mobilised $o\text{-}PO_4^{3-}$ in the sediment is bound to Fe in the aerobic top layer of the sediment, preventing mobilisation of $o\text{-}PO_4^{3-}$ to the water layer after rewetting (Chapter 6).

Severe desiccation and subsequent acidification not only results in mobilisation of $o\text{-}PO_4^{3-}$ at seepage zones but can also mobilise high concentrations of heavy metals. Fens in the river Meuse region are generally very sensitive to desiccation. Dredging of sediments for the purpose of restoration activities may lead to the massive percolation of heavy metals from dredged sediments to the groundwater (Chapter 5). Long-term monitoring of the Venkoelen fen has shown that acidification and mobilisation of heavy metals do indeed occur in fens during dry summers (like those of 1990 and 1995) or when fens are artificially drained (Oranjewoud, 2000; Smolders & Roelofs, 2002; Chapter 5).

Processes in SO_4^{2-}-rich fens when groundwater influence is impaired: permanently stagnating surface water

Damming of drainage ditches in order to raise water levels in desiccated fens results in permanently high stagnating water tables, accompanied by eutrophication and accumulation of phytotoxic S^{2-} (Chapters 6 & 7).

As the groundwater input decreases, the input of the base cations Ca, Mg and Fe is decreased, which might result in a greater NH_4^+ binding capacitiy of the soil adsorption complex. In addition, as temporary desiccation ceases to occur during the summer, no denitrification takes place, resulting in high NH_4^+ concentrations in the stagnating pore water. Decreased input of groundwater also blocks the input of the electron acceptors NO_3^-, Fe and SO_4^{2-} in seepage zones. This leads to the depletion of NO_3^- and Fe as electron acceptors, resulting in increased reduction of SO_4^{2-} (Chapter 7). In addition, since periodic desiccation is prevented in most of the fen, no Fe is regenerated from reduced Fe complexes. Sulphide (S^{2-}) produced by SO_4^{2-} reduction reduces the availabaility of free Fe(II) under formation of iron (di)sulphides (FeS_x). As the concentration of free Fe(II) decreases, S^{2-} interacts with Fe from $Fe\text{~}PO_4^{3-}$ complexes, leading to the release of $o\text{-}PO_4^{3-}$ to the water layer (Figure 1, Boström et al., 1982; Caraco et al., 1990). SO_4^{2-} reduction thus leads to eutrophication in fens, depending upon the free Fe content of the sediment. Desiccated parts of fens have a low Fe pool, and $o\text{-}PO_4^{3-}$ concentrations are extremely high, regardless of SO_4^{2-} reduction processes. Parts of fens that become desiccated during the summer have a moderately high Fe content, and SO_4^{2-} reduction leads to a gradual increase in $o\text{-}PO_4^{3-}$ concentration. Seepage

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zones have a very high Fe content, and o-PO$_4^{3-}$ concentrations can stay low for a very long time even though SO$_4^{2-}$ reduction takes place (Chapter 6).

The role of nitrate on the biogeochemistry of fens

NO$_3^-$ leaching from agricultural lands and forest soils leads to increased NO$_3^-$ concentrations in the groundwater (Kölle & Schreeck, 1982; Hagedorn et al., 2000). When NO$_3^-$ reaches FeS$_x$-containing subsoil layers, it may oxidise FeS$_x$, which leads to the mobilisation of SO$_4^{2-}$ and a decrease in the NO$_3^-$ concentration (Van Steenwijk, 1986). The resulting NO$_3^-$/SO$_4^{2-}$ ratio strongly affects the quality of groundwater-fed fens. On the one hand, NO$_3^-$ leaching into the groundwater increases the risk of eutrophication in discharge areas by increasing SO$_4^{2-}$ concentrations in the groundwater and stimulating processes such as Fe immobilisation and o-PO$_4^{3-}$ mobilisation due to S^{2-} and FeS$_x$ formation. However, if NO$_3^-$ concentrations in the groundwater are sufficiently high, NO$_3^-$ may prevent SO$_4^{2-}$-induced eutrophication by functioning as a redox buffer and by oxidising reduced Fe compounds, depending upon the retention time of the surface water (Figure 2, Chapters 7 & 8).

Implications for wetland management in fens

Groundwater discharge has to be restored in desiccated fens in order to re-increase the saturation of the soil adsorption complex with base cations during summer, which is necessary to prevent accumulation of NH$_4^+$ in the pore water and the accompanying growth of fast-growing wetland grasses and lemnids (Chapter 2). In addition, the increased influence of such base-rich groundwater increases the buffering capacity of the soil and prevents acidification in summer due to oxidation of FeS$_x$. This is important as NH$_4^+$ may be toxic to plants that are characteristic of groundwater discharge in combination with a low pH (Chapter 3).

A natural water regime, characterised by a continuous flow of groundwater in combination with periodic droughts during the summer, must be maintained to prevent eutrophication of SO$_4^{2-}$-rich fens. Rewetting by means of maintaining permanently high water levels blocks groundwater input and prevents periodic droughts resulting in Fe depletion, stimulation of SO$_4^{2-}$ reduction and massive eutrophication. In addition, phytotoxic S^{2-} may accumulate in the water layer. At the Kaldenbroek fen, these processes resulted in the decline of characteristic plant species and massive development of lemnids within one year after rewetting.

First of all, desiccated fens have to be gradually rewetted, with regular desiccation periods included in the process, in order to allow an Fe pool to build up in the desiccated sediments. The adverse effects of SO$_4^{2-}$ accelerate with rising water tables (Figure 4), and building up an

Fe pool makes the previously desiccated parts of fens less sensitive to SO_4^{2-} reduction in periods when groundwater inputs are relatively high. Water tables can be raised without causing stagnation of surface water by raising them to below the potential groundwater table. This maintains a positive groundwater pressure, which enables continuous flow-through of groundwater, supplying base cations and electron acceptors to the system. The success of these restoration measures is apparent from the persistently good water quality at the Dubbroek fen as compared to the Kaldenbroek fen (Figure 3).

The potential groundwater pressure determines the potential rewettable fen area. If the potential groundwater pressure is relatively low, the retention time of the surface water will be relatively high in order to rewet a certain area. In addition, the composition of the groundwater may also play an important role in determining the potential rewettable area. It is likely that the SO_4^{2-}/NO_3^- ratio in the groundwater influences the maximum retention time of the surface water. If the SO_4^{2-}/NO_3^- ratio in the groundwater is low, NO_3^- depletion takes longer, allowing a longer retention time of the surface water and thus the maintenance of a relatively high water table. The same will be true for the concentration of base cations in the groundwater. However, decreases in base cation and Fe supplies will lead to a decrease in the biodiversity of the fens. Thus, restoration will be optimal if the regional groundwater pressure can be restored.
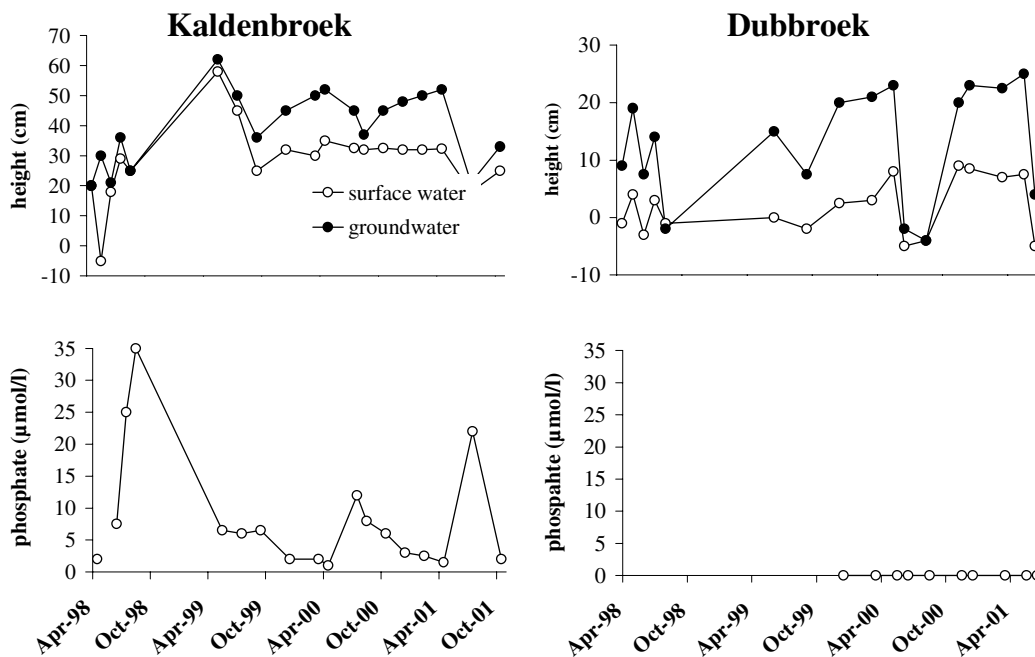
Figure 3. The influence of periodic droughts during the summer on o-PO_4^{3-} concentrations in the water layer of previously desiccated fen parts. At the Kaldenbroek fen, surface water was dammed resulting in stagnating groundwater input resulting in high o-PO_4^{3-} concentrations in the water layer and severe eutrophication. At the Dubbroek fen, a continuous input of groundwater was maintained in combination with periodic droughts in summer, resulting in low o-PO_4^{3-} concentrations in the water layer and expansion of the characteristic vegetation (Lucassen, unpublished results).
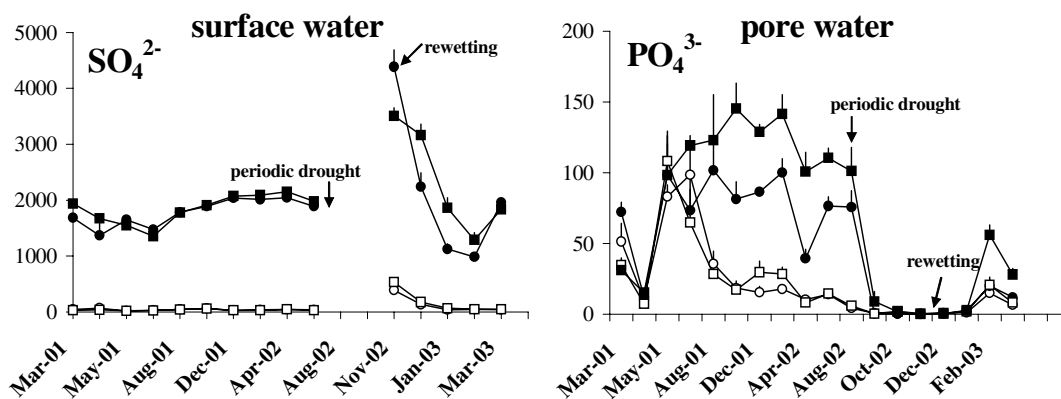

Figure 4. Interactive effects of SO_4^{2-} concentration in the surface water and water table height on o-PO_4^{3-} concentrations in the pore water of sediment originating from an Fe-rich zone in the Kaldenbroek fen (as studied in a laboratory experiment). ■; 2 mmol/l SO_4^{2-} and 25 cm water table;●; 2 mmol/l SO_4^{2-} and 5 cm water table; □; 0 mmol/l SO_4^{2-} and 25 cm water table; ○; 0 mmol/l SO_4^{2-} and 5 cm water table. Concentrations in µmol/l. (Lucassen, unpublished results).

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Chapter 9 Synthesis

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Table 1 Answers to questions in Chapter 1.

1. How sensitive are fens in the river Meuse regions to drought-induced acidification and mobilisation of heavy metals ?

Desiccation only results in acidification when the S/(Ca+Mg) ratio of the sediment exceeds 2/3. Fens in the river Meuse region are very sensitive to desiccation, and dredging of sediments for the purpose of restoration activities will always lead to percolation of heavy metals to the groundwater. To prevent contamination, dredged sediments can be limed to a S/(Ca+Mg) ratio < 2/3.

2. Which processes at seepage sites in fens influence plant distribution under semi-natural conditions?

A distribution study has shown that the input of Fe and the base cations Ca and Mg, in relation to NH_4^+ concentration in the pore water, plays a major role. Groundwater in fens is rich in Fe and the base cations Ca and Mg, which prevents adsorption of NH_4^+ to the soil adsorption complex, resulting in very low NH_4^+ concentrations in the pore water. With decreasing groundwater influence, NH_4^+ concentration increases, resulting in the disappearance of plant species characteristic of groundwater discharge and the growth of plant species characteristic of eutrophic environments, like fast-growing wetland grasses *Glyceria maxima* and *G. fluitans*. Differences in Fe tolerance between plant species might also play a role.

3. Which factors prevent or cause eutrophication in SO_4^{2-}-rich fens ?

A constant input of groundwater ensures input of Fe and NO_3^- into the system, which are more favourable electron acceptors than SO_4^{2-}. In addition, the large Fe input increases the capacity of the aerobic top layer of the sediment to bind o-PO_4^{3-}, and free Fe(II) restricts interference of S^{2-} with Fe from Fe~PO_4^{3-}. If the surface water is dammed to raise water levels, groundwater input is blocked and no desiccation may take place during dry summers. SO_4^{2-} reduction is then stimulated and free Fe is depleted, resulting in eutrophication.

4. What are the effects of high NO_3^- concentrations in groundwater on the biogeochemistry of fens ?

NO_3^- is a more favourable electron acceptor than Fe and SO_4^{2-}. NO_3^- prevents SO_4^{2-} reduction and SO_4^{2-}-induced eutrophication of fens. In addition, it lowers the sensitivity of fen sediment to acidification and mobilisation of heavy metals following desiccation. NO_3^- does not cause changes in vegetational composition of the seepage zones, but it causes Fe-deficiency chlorosis of some fen plant species at seepage sites, as it oxidises reduced Fe compounds, lowering the availability of Fe^{2+}.

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Field Work Picture Gallery

Samenvatting

In het begin van de vorige eeuw is door toenemende drainageactiviteiten ten behoeve van landbouwactiviteiten de stand van het grondwater in het stroomgebied van de rivier de Maas sterk verlaagd. Deze verlaging van de grondwaterstand heeft tot sterke verdroging in door grondwater gevoede laagvenen geleid, met name in de drogere zomerperioden. Hierdoor is in deze laagvenen, waaronder elzenbroekbossen, de soortenrijke karakteristieke vegetatie grotendeels verdwenen en heeft zich een soortenarmer droogte-resistente vegetatie ontwikkeld. De toenemende landbouwactiviteiten hebben naast hun invloed op de kwantiteit ook geleid tot verandering van de kwaliteit van het grondwater. Door de toegenomen bemesting en ammoniakemissie vanuit de landbouw is de nitraatuitspoeling naar het diepere grondwater in deze gebieden sterk toegenomen. Het nitraat reageert vervolgens met mariene geologische pyrietafzetting in de ondergrond waarbij sulfaat wordt gevormd. Afhankelijk van de mate van nitraatuitspoeling en de hoeveelheid pyriet in de ondergrond zijn de concentraties aan nitraat en/of sulfaat in het grondwater dus sterk toegenomen.

Sinds de jaren 90 wordt getracht de verdroogde laagvenen te herstellen. Omdat herstel van het regionale grondwaterpeil vaak onmogelijk is, is getracht de laagvenen te restaureren door de stand van het oppervlaktewater te verhogen middels het plaatsen van stuwen. Door permanent hoge oppervlaktewaterpeilen te handhaven traden geen periodieke waterstandschommelingen meer op waardoor de laagvenen niet meer, zoals van nature wel het geval was, droogvielen. Het te hoog opstuwen van oppervlaktewater leidde echter in de zomer niet tot een uitbreiding van de karakteristieke laagveenvegetatie maar tot een snelle achteruitgang hiervan gepaard gaande met de ontwikkeling van toxisch sulfide en massale ontwikkeling van soorten kenmerkend voor zeer voedselrijke condities waaronder klein kroos (*Lemna minor*), liesgras (*Glyceria maxima*) en mannagras (*Glyceria fluitans*).

In dit proefschrift zijn de biogeochemische processen in zwavelrijke grondwatergevoede laagvenen onderzocht die optreden onder verschillende hydrologische condities: semi-natuurlijke condities waarin continue doorstroming met basen- en ijzerrijk grondwater plaatsvindt en condities waarbij door te hoog opstuwen van het oppervlaktewater de invloed van grondwater sterk is afgenomen. Het doel van dit onderzoek is te onderzoeken welke processen bepalend zijn voor de geobserveerde veranderingen in de vegetatie van een mesotroof naar uiterst eutroof karakter, om uiteindelijk randvoorwaarden te kunnen stellen aan herstelmaatregelen voor verdroogde wetlands.

Onder semi-natuurlijke condities voorziet de continue aanvoer van grondwater een laagveen van basische kationen (calcium en magnesium) en electronacceptoren voor de microbiële afbraak van organisch materiaal (ijzer, nitraat, sulfaat). Gebleken is dat basische kationen en ijzer een verlagende invloed op de nutriëntenbeschikbaarheid hebben. Calcium, magnesium en ijzer binden namelijk sterker aan het bodemabsorptiecomplex dan ammonium waardoor het ammonium continu afgevoerd wordt uit het systeem, resulterend in een lage ammoniumbeschikbaarheid. Continue aanvoer van ijzer verhoogt tevens de concentratie van

ijzer(hydr)oxiden in de aerobe toplaag van de bodem en hiermee de capaciteit om fosfaat in de bodem vast te leggen waardoor de mobilisatie van fosfaat naar de waterlaag beperkt blijft. Daarnaast is gereduceerd ijzer in staat om sulfide te binden waardoor interactie van sulfide met ijzer uit ijzer-fosfaatcomplexen, resulterend in fosfaatmobilisatie, beperkt blijft. Onder semi-natuurlijke condities wordt de vorming van ijzer-sulfiden beperkt omdat sulfaatreductie in aanwezigheid van hoge ijzer- en nitraatconcentraties geremd wordt doordat ijzer en nitraat thermodynamisch gezien betere electronenacceptoren zijn. Ten gevolge van tijdelijke droogval in de zomer worden eventueel gevormde immobiele ijzer-sulfiden weer geoxideerd waardoor fosfaatbindende ijzer(hydr)oxiden geregenereerd worden terwijl het gevormde mobiele sulfaat uit het systeem afgevoerd wordt in nattere tijden. Bij oxidatie van ijzersulfiden wordt tevens zuur gevormd. De gevoeligheid van een bodem voor verzuring ten tijde van verdroging hangt af van de hoeveelheid ijzersulfiden in verhouding tot het bufferend vermogen van de bodem. Verdroging zal buiten de kwellocaties (die nooit droogvallen) meestal echter niet tot verzuring leiden omdat door jaarlijkse droogval geen accumulatie van ijzersulfiden heeft kunnen optreden. Door droogval treedt tevens oxidatie van ammonium op waarbij nitraat gevormd wordt. Het nitraat kan na diffusie in het dieper gelegen anaërobe gedeelte van de bodem worden gedenitrificeerd waarbij stikstofgas gevormd wordt dat naar de lucht verdwijnt (gekoppelde nitrificatie-denitrificatie). Onder deze mesotrofe omstandigheden vindt groei plaats van een soortenrijke *Calthion*-vegetatie die gekenmerkt wordt door o.a. dotterbloem (*Caltha palustris*), holpijp (*Equisetum fluviatile*), grote boterbloem (*Ranunculus flammula*) en diverse Carex-soorten waaronder de elzenzegge (*Carex elongata*) en hoge cyperzegge (*Carex pseudocyperus*).

Opstuwen van oppervlaktewater om het waterpeil in verdroogde laagvenen te verhogen leidt tot een afname van de grondwaterinvloed. Hierdoor wordt de aanvoer van basische kationen en van ijzer en nitraat geremd. Door de afname van basische kationen neemt de binding van ammonium aan het bodemadsorptiecomplex toe waardoor ook de ammoniumconcentratie in het stagnerende bodemvocht stijgt. Door de verminderde aanvoer van ijzer en nitraat wordt de sulfaatreductie niet meer geremd. Sulfide dat gevormd wordt zal nu ijzer uit ijzerfosfaatcomplexen reduceren waarbij fosfaat gemobiliseerd wordt. Door het permanent hoog opstuwen van het oppervlaktewater vindt in de zomer geen droogval meer plaats. Hierdoor treedt er ook geen oxidatie van ijzersulfiden op waardoor de beschikbaarheid van fosfaatbindend ijzer laag blijft. Doordat tevens geen doorstroming van grondwater plaatsvindt treedt er een accumulatie op van nutriënten in de waterlaag. De verhoogde sulfideconcentraties in de bodem zijn toxisch voor de karakteristieke *Calthion*-vegetatie. Zwarte elzen (*Alnus glutinosa*) sterven af en vallen om waardoor de lichtinval toeneemt. De verhoogde lichtinval in combinatie met de sterk verhoogde nutriëntenbeschikbaarheid leidt tot een massale groei van met name kroos, liesgras en mannagras. Liesgras en mannagras zijn in staat sulfide te detoxificeren buiten de plant doordat de wortels zuurstof uitscheiden.

Het kroos kan hierbij de waterlaag afdichten waardoor de waterlaag anaëroob wordt hetgeen de nalevering van fosfaat uit de bodem verder versterkt.

Om verdroogde laagvenen succesvol te restaureren is het ten eerste van belang een gebied geleidelijk te hervernatten. Door ijzeraanvoer en door droogval van de bodem in de zomer kan een ijzervoorraad opgebouwd worden in de voormalig verdroogde bodem. Tevens wordt door geleidelijk vernatten de sulfaatreductie beperkt doordat deze afneemt bij een verlaging van het waterpeil. Stagnatie van oppervlaktewater moet te allen tijde voorkomen worden. Door het stuwpeil beneden het stijgniveau van het grondwater op te zetten wordt een positieve kweldruk gehandhaafd waardoor de input van basische kationen en electronenacceptoren via het grondwater gegarandeerd blijft. Periodieke droogval van een onderwaterbodem in de zomer is een vereiste voor het handhaven van mesotrofe condities, hetgeen bereikt kan worden via het plaatsen van een regelbare stuw.

Summary

In the beginning of the 19th century, increased agricultural activities were accompanied by building and deepening of drainage ditches, resulting in reduced groundwater tables in many fens along the river Meuse. Especially during dry summers, the reduced groundwater tables led to strong desiccation of groundwater-fed fens, including black alder carrs. As a consequence, the characteristic species-rich vegetation was replaced by a more species-poor and drought-resistant vegetation. Next to influencing groundwater quantity, the increased agricultural activities also led to changes in groundwater quality. Increased manuring of pastures and ammonia emission have led to increased leaching of nitrate to the groundwater. Nitrate reacts with marine geological pyrite-containing deposits in the subsoil resulting in mobilisation of sulphate. Thus, the concentrations of nitrate and sulphate in the groundwater have increased during the last decades depending upon the concentration of nitrate leaching to the groundwater and the amount of pyrite in the subsoil.

Some years ago, restoration measures were taken in some desiccated fens. As recovery of the regional groundwater table is often impossible, water levels in many fens were increased by simply damming surface water. Under semi-natural conditions, groundwater input is restricted in summer and periodic drought takes place. However, by damming surface water, permanent high water tables were maintained and no periodic droughts took place anymore. Damming of surface water was accompanied by the disappearance of the characteristic vegetation at the seepage zones and by massive growth of common duckweed (*Lemna minor*) and fast growing wetland grasses including reed sweetgrass (*Glyceria maxima*) and floating sweetgrass (*Glyceria fluitans*).

In this thesis, biogeochemical processes occuring in sulphate-rich groundwater fed fens are studied under two hydrological conditions: semi-natural conditions with a continuous input of base-rich and iron-rich groundwater, and conditions in which groundwater input is strongly decreased as a consequence of damming surface water. The aim of this study was to find out which factors and processes are responsible for the observed change in vegetation (from plants characteristic of mesotrophic to highly eutrophic conditions) and to determine biogeochemical constraints for the restoration of desiccated sulphate-rich fens.

Under semi-natural conditions, the continuous input of groundwater supplies fens with bivalent cations (iron, calcium and magnesium) and electron acceptors for the microbial breakdown of organic matter (iron, nitrate, sulphate). High inputs of bivalent cations via the groundwater lead to saturation of the sediment adsorption complex in seepage zones, preventing the binding of NH_4^+, which is then continuously removed via the flowing groundwater. So a high input of bivalent cations via the groundwater results in restricted ammonium availability in fens. The continuous input of iron(hydr)oxides also increases the capacity of the aerobic sediment top layer to bind phosphate resulting in a decreased mobilisation of phosphate from the sediment to the water layer. Next to this, reduced iron is capable of binding sulphide in the sediment restricting mobilisation of phosphate in the

Summary

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sediment due to the interaction of sulphide with iron-phosphate complexes. Under semi-natural conditions the formation of iron-sulphides is restricted as sulphate reduction is inhibited in the presence of high iron and nitrate concentrations which are energetically more favourable electron acceptors. Immobile iron-sulphides are oxidised as a consequence of periodic drought during summer, regenerating immobile iron(hydr)oxides capable of binding phosphate, while mobile sulphate is removed via the flowing water layer when groundwater input reincreases. Oxidation of iron-sulphides also leads to the production of acid. The sensitivity of sediments to drought, acidification and mobilisation of heavy metals depends upon the amount of iron-sulphides and the buffering capacity of the sediment. In contrast to seepage zones that never dry out, periodic drought of parts outside seepage zones mostly will not lead to strong acidification as no accumulation of iron-sulphides takes place. Periodic drought also leads to oxidation of ammonium and formation of nitrate which diffuses to deeper anaërobic sediment layers where it is denitrified resulting in the production of nitrogen gas that disappears into the atmosphere (a process called coupled nitrification-denitrification). Under these mesotrophic conditions, fens are dominated by a species-rich *Calthion*-vegetation characterised by species like marsh marigold (*Caltha palustris*), swamp horsetail (*Equisetum fluviatile*), greater spearwort (*Ranunculus flammula*) and many Carex-species including elongated sedge (*Carex elongata*) and cyperus sedge (*Carex pseudocyperus*).

Increasing water levels in desiccated fens by damming surface water leads to a decreased groundwater input and thus to a restricted supply of base cations and the electron acceptors (iron and nitrate). The lower input of bivalent cations leads to increased binding of ammonium to the sediment adsorption complex resulting in a higher ammonium availability in the pore water. Reduction of sulphate is no longer inhibited as iron and nitrate are depleted. Produced sulphide will reduce iron from iron-phosphate complexes resulting in phosphate mobilisation. By maintaining permanent high water tables, no periodic drought occurs during summer. As a consequence, iron-sulphides are not oxidised and the concentration of iron capable of binding phosphate remains low. Thus in stagnating surface water, nutrient availability in the sediment is high and accumulation of nutrients to the stagnating water layer takes place. The increased sulphide concentrations in the sediment are toxic to the characteristic *Calthion*-vegetation. Black alder (*Alnus glutinosa*) dies, falls down and light intensity increases. The increased light intensity, in conjunction with the increased nutrient availability, leads to massive growth of *Lemna minor* and fast growing wetland grasses. *Glyceria fluitans* and *Glyceria maxima* are capable of detoxifying sulphide outside the plant as they have high radial oxygen losses at the roots. *Lemna minor* can completely cover the water surface when phosphate concentrations in the water layer are high. As a consequence, less oxygen diffuses into the water layer and the water layer can become anaërobic which further stimulates the mobilisation of phosphate from the sediment to the

water layer.

For a successful restoration of desiccated fens, they have to be gradually rewetted, with regular desiccation periods included, in order to allow an Fe pool to build up in the desiccated sediments. The adverse effects of SO_4^{2-} accelerate with rising water tables. Building up an Fe pool makes the previously desiccated parts of fens less sensitive to SO_4^{2-} reduction in periods when groundwater inputs are relatively high. In addition, stagnation of surface water should be avoided. Water tables can be raised without causing stagnation of surface water by raising them to below the potential groundwater table. This maintains a positive groundwater pressure, which enables continuous flow-through of groundwater, supplying base cations and electron acceptors to the system. Finally, periodic drought during summer is important in oxidising reduced Fe compounds in the sediment and thus in re-increasing the content of Fe(III) capable of binding o-PO_4^{3-}. Periodic drought can be achieved by making use of a controllable dam.

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Curriculum vitae

Op 10 april 1974 werd ik geboren in Gennep. Toen ik twee jaar was besloten mijn ouders naar Bergen te verhuizen waar ik naar de openbare school "De Klimop" ging. Van 1986 tot 1992 fietste ik dagelijks en via het het pontje over de Maas in Afferden, naar het Elzendaal College in Boxmeer waar ik het VWO volgde. Vervolgens ging ik in 1992 Biologie studeren aan de Katholieke Universiteit Nijmegen (KUN). Deze studie heb ik in 1997 afgerond met een hoofdvak en twee bijvakken. Het hoofdvak Aquatische Ecologie & Milieubiologie (Prof. Roelofs) werd uitgevoerd in Noorwegen in samenwerking met het Noors Instituut voor Water Onderzoek (NIVA) in Oslo (Dr. Brandrud). De effecten van verzuring, bekalking en herverzuring op de waterkwaliteit, sedimentkenmerken en vegetatieontwikkeling van zachte wateren werden onderzocht. Tijdens het eerste bijvak Organismale Dierfysiologie (Dr. Lock en Dr. Stouthart) werden de mogelijkheden tot een stress-respons in vroege levensstadia van de gewone karper onderzocht met behulp van chemische (PCB's, insecticiden) en mechanische (druk) stressoren. Het tweede bijvak Organismale Dierfysiologie (Prof. Wendelaar Bonga) werd uitgevoerd in samenwerking met het NIVA in Grimstad (Drs. Kroglund). In veldexperimenten is de aluminiumtoxiciteit in zalm in mixing-zones van neutraal water (rivier- en zeewater) met zuur rivierwater onderzocht, met als doel het vaststellen van bekalkingsstrategieën voor verzuurde Noorse rivieren.

Na mijn studie heb ik bij de werkgroep Milieubiologie (KUN) enkele korte onderzoeksopdrachten verricht. De effecten van slibophoping op de biogeochemie van open en gesloten kanaalbakken werd in tal van kanalen en rivieren in Nederland onderzocht. Daarnaast werd meegewerkt aan projecten ter bepaling van kritische stikstofdepositieniveaus voor droge heideterreinen (met Paul van der Ven) en aan projecten ter bepaling van de effecten van bekalking van inzijggebieden op de biogeochemie van verzuurde vennen. Tenslotte heb ik meegewerkt aan onderzoek naar herintroductiemogelijkheden van zeegrassen in Nederland. In 1998 werd ik aangesteld als 3^e geldstroom junior onderzoeker bij de werkgroep Milieubiologie van de KUN. Het werk werd verricht in opdracht van de Stichting het Limburgs Landschap. Direct na mijn aanstelling besloot ik al mijn vrije dagen op te nemen om samen met Dimphy Kuijpers Nieuw-Zeeland en de Fiji-eilanden te kunnen bezoeken. Na terugkomst werd het onderzoek hervat. Herstelmogelijkheden voor verdroogde, verzuurde en geëutrofieerde voormalig natte Limburgse natuurgebieden, waaronder moerasbossen, vennen en landbouwgronden werden onderzocht en gerapporteerd. Om dergelijke opdrachten goed te kunnen uitvoeren komt altijd een deel fundamenteel onderzoek om de hoek kijken. Dit onderzoek is beschreven in dit proefschrift. In 2001 ontving ik de dr I.B.M. Frye-Stipendium-Prijs van de KUN, een prijs die uitgereikt wordt aan jonge onderzoeksters. Dankzij deze prijs kon ik in september 2003 mijn promotieonderzoek presenteren op het "2nd International Symposium on Phosphorus Dynamics in the Soil-Plant Continuum" in Perth, West Australië. Vanuit Australië ben ik naar Indonesië gereisd om deel te nemen aan het samenwerkingsproject van de KUN met Indonesië "Seagrass systems of

East Kalimantan: preserving by understanding".