



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

E.C.H.E.T. LUCASSEN*, A.J.P. SMOLDERS, A.L. VAN DER SALM
and J.G.M. ROELOFS

*Environmental Biology, Department of Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands; *Author for correspondence (e-mail: estheluc@sci.kun.nl; phone: +31-243652912; fax: +31-243652134)*

Received 1 July 2002; accepted in revised form 21 August 2003

Key words: Eutrophication, Nitrate, Phosphate, Redox buffer, Sulphate

Abstract. During the last 60 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 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 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 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 reduced release of Fe and S^{2-} and increased release of SO_4^{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 PO_4^{3-} from Fe – PO_4^{3-} complexes. At a higher redox potential, reduced Fe, including FeS_x , was oxidised, increasing the content of Fe(III) capable of binding PO_4^{3-} . This prevented increased 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 ($<32 \mu\text{mol l}^{-1}$) (EEA 1998). During the last 60 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. 2001).

Once accumulating in groundwater, NO_3^- causes oxidation of pyrite in the subsoil (Arevana and Robertson 1998; Pauwels et al. 1998; Tesoriero et al. 2000). In the province of Limburg (The Netherlands) 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 $\text{Fe} - \text{PO}_4^{3-}$ complexes in the sediment to produce FeS_x (FeS_2 and FeS), resulting in mobilisation of phosphate (PO_4^{3-}) (Boström et al. 1982; Caraco et al. 1989; Roelofs 1991; Smolders and 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 3 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 and Stortelder 2000; Lucassen et al. 2000). Enclosure experiments have shown that high SO_4^{2-} concentrations can cause 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 for publication). 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 $\text{Fe}(\text{OOH})$ 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 (including black alder carrs) which were differently affected by NO_3^- . The findings were supplemented by laboratory work.

Materials and methods

Field work

Monitoring the black alder carrs

From May 1998 to June 2001, pore water samples were taken in the alder carrs Beeselsbroek ($51^\circ 15' \text{N}$; $6^\circ 03' \text{E}$), Kaldenbroek ($51^\circ 26' \text{N}$; $6^\circ 08' \text{E}$) and Dubbroek in the Netherlands. Five to 10 locations were sampled five times a year. Pore water

was collected anaerobically with nitrogen-preflushed vacuum bottles connected to ceramic cups (Eijkkamp 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'; 6°05'E) were monitored from April 2000 to January 2003. In the past, both fens had been highly eutrophied and dominated by *Salix* sp. (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 2 m. 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 (five 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 and 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 min using a Milestone microwave type mls 1200 Mega. Digestates were stored in iodated polyethylene bottles at 4 °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 sulfur. Five hundred grams of this fresh sediment was gradually dried in a funnel ($r = 7.5$ cm, $h = 10$ cm) placed in a glass flask at 20 °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 h 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 2 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^- addition 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 $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, 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 2 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 2 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 5 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 $\mu\text{mol L}$ fresh sediment) of Fe, SO_4^{2-} , S^{2-} and 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:

$$\text{ER} = \frac{(x_{2-20} \cdot F \cdot t)}{V}$$

with: 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).

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 sulphide anti oxidant buffer (SAOB) 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) equipped 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 ‘Oceanography International’ model 0525 HR infrared carbon analyser. After citric acid had been added, the remaining samples were

Table 1. Chemical composition of the medium ($\mu\text{mol L}^{-1}$) resembling alkaline groundwater from Beeselsbroek with and without added NO_3^- .

	+ NO_3^-	- NO_3^-
NaHCO_3^-	2000	2000
KCl	250	250
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	200	200
CaCl_2	—	2500
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	2500	—

stored in iodated polyethylene bottles at -23°C until further analysis. In these samples, the following compounds were measured using Technicon II auto-analysers: NO_3^- and PO_4^{3-} (Kamphake et al. 1967) and NH_4^+ (Grasshoff and Johansen 1977). K^+ was determined by flame photometry (Jarell Ash IL Plasma-200). 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 \pm 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 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 PO_4^{3-} , NH_4^+ and Fe in the pore water decrease with increasing NO_3^- concentrations. Above NO_3^- concentrations of $100 \mu\text{mol L}^{-1}$, the PO_4^{3-} , Fe and NH_4^+ concentrations are very low. The 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

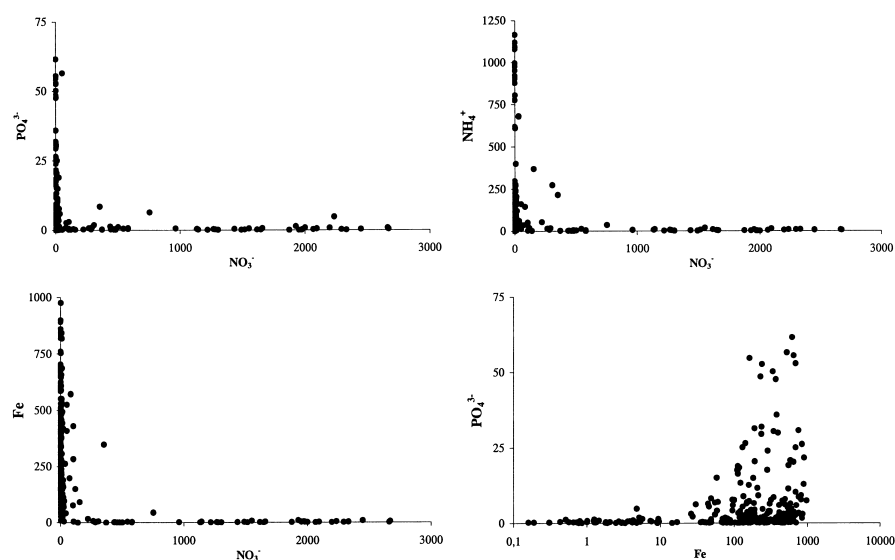


Figure 1. Pore water scatterplots with PO_4^{3-} , NH_4^+ and Fe concentrations as a function of the NO_3^- concentration, and PO_4^{3-} as a function of the Fe concentration. Concentrations are given in $\mu\text{mol L}^{-1}$.

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-} ($1000 \mu\text{mol L}^{-1}$) but poor in NO_3^- ($< 35 \mu\text{mol L}^{-1}$), 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 ($1000 \mu\text{mol L}^{-1}$) but also very high NO_3^- concentrations ($600\text{--}4900 \mu\text{mol L}^{-1}$). Concentrations of 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).

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

The redox potential (+247 mV) and the NO_3^- concentration ($2030 \mu\text{mol L}^{-1}$) 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 $348 \mu\text{mol L}^{-1}$, respectively). The Fe concentration was extremely low ($0.5 \mu\text{mol L}^{-1}$) at the NO_3^- -rich site, while HCO_3^- , NH_4^+ , Mn and Al concentrations

Table 2. Vegetation composition in 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	
<i>Alisma plantago-aquatica</i>	o	<i>Callitriche</i> sp	o
<i>Ceratophyllum demersum</i>	ld	<i>Chara globularis</i> var. <i>globularis</i>	o
<i>Lemna trisulca</i>	d	<i>Elodea canadensis</i>	la
<i>Lemna minor</i>	cod	<i>Hydrocharis morsus-ranae</i>	r
<i>Nymphaea alba</i>	o	<i>Nymphaea alba</i>	r
<i>Potamogeton natans</i>	o	<i>Potamogeton crispus</i>	o
<i>Ranunculus flammula</i>	r	<i>Potamogeton natans</i>	o
<i>Riccia fluitans</i>	o	<i>Rorippa nasturtium-aquaticum</i>	o

in the pore water were also significantly lower and 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 ($8 \mu\text{mol g}^{-1}$ DW) than the non-chlorotic plants of the NO_3^- -poor site ($1198 \mu\text{mol g}^{-1}$ 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 chlorophyll A, chlorophyll B and carotenoids were lower in chlorotic plants. The Fe concentrations in roots of chlorotic plants ($2 \mu\text{mol g DW}^{-1}$) were much lower than those in non-chlorotic plants ($1527 \mu\text{mol g DW}^{-1}$), while Ca, N and P concentrations were also significantly lower, and Zn, K^+ and Mg concentrations were significantly higher. Fe, Ca, N and carbon concentrations in the shoots were also significantly lower in chlorotic plants. Fe concentrations in the shoots were up to four times lower ($3 \mu\text{mol g DW}^{-1}$), 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 to $15000 \mu\text{mol l}^{-1}$. 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).

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, PO_4^{3-} , P, S^{2-} , Mn, K^+ and Si significantly decreased, as did the production of CH_4 . The changes in PO_4^{3-} and SO_4^{2-} concentrations were most obvious during the first 3 weeks, when the medium was stagnating (Figure 4, Table 6). The period with a flow rate of 25 L medium per week led to significantly lower net releases of Fe, S^{2-} and PO_4^{3-} and a

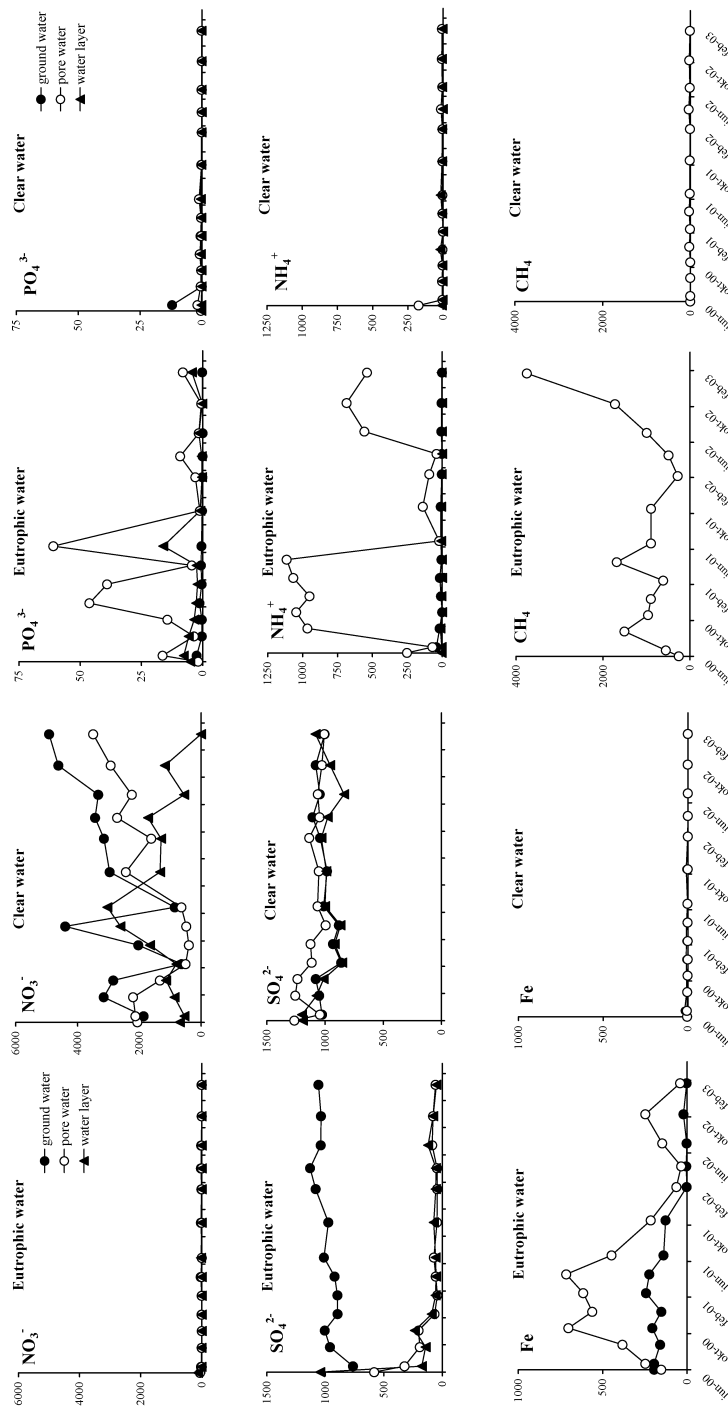


Figure 2. Chemical composition of the ground water, pore water and water layer in the eutrophying fen (left) and in the fen developing clear water (right) between April 2000 and January 2003. Concentrations are given in $\mu\text{mol L}^{-1}$.

Table 3. Pore water (PW) chemistry (μM), E (mV) and concentrations of root plaque extractable components (RP) ($\mu\text{mol/g DW}$) of *C. pseudocyperus* at locations fed by NO_3^- -poor and NO_3^- -rich groundwater. Means \pm (standard errors) are given ($n = 6$). * $P \leq 0.05$; ** $P \leq 0.005$.

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

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

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

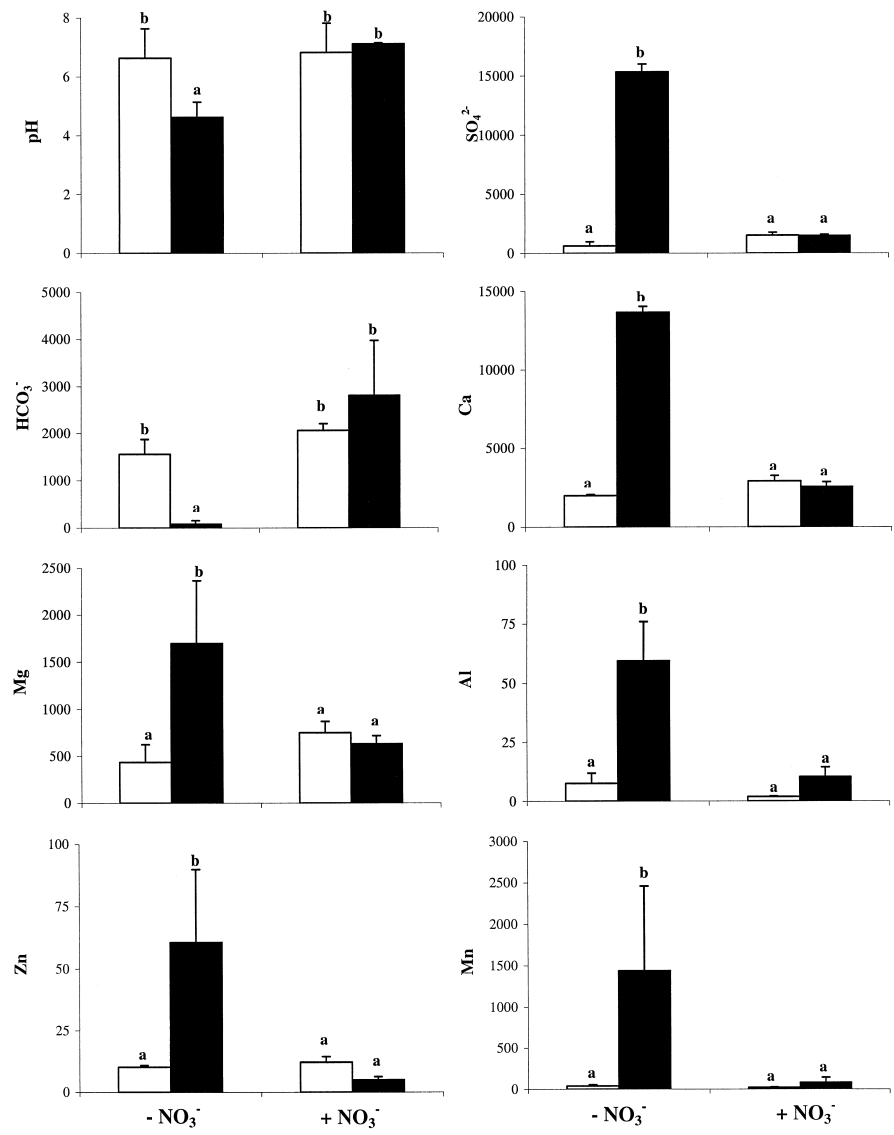


Figure 3. Chemical composition of the pore water of sediments fed directly (NO_3^- -rich) and indirectly (NO_3^- -poor) by groundwater under field conditions (white bars), and after rewetting following experimental desiccation (black bars). Concentrations are given in $\mu\text{mol L}^{-1}$.

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 (Table 5).

Discussion

The results show that high groundwater NO_3^- concentrations positively affect groundwater fed SO_4^{2-} enriched wetlands by restricting the mobilisation of PO_4^{3-} from the sediment to the water layer. Under anaerobic conditions, reduction of Fe from $\text{Fe} - \text{PO}_4^{3-}$ complexes leads to the mobilisation of PO_4^{3-} from sediments (Hosomi 1980; Furumai and Ogaki 1982; Roelofs 1991; Wetzel 2001). Mobilisation of PO_4^{3-} from these complexes probably occurred in the wetlands examined in the present study, as was indicated by the positive correlation between 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 PO_4^{3-} is accelerated by increased SO_4^{2-} reduction rates. The S^{2-} produced, reduces Fe(III) in iron phosphate, iron (hydr)oxide phosphate and humic iron phosphate complexes, mobilising PO_4^{3-} (Sperber 1958; Patrick and Khalid 1974; Boström et al. 1982; Smolders and Roelofs 1993; Moore and 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 PO_4^{3-} binding and decreasing the Fe/ PO_4^{3-} ratio in the pore water (Boström et al. 1982; Caraco et al. 1989; Roelofs 1991; Smolders and Roelofs 1993; Murray 1995). As a result, the release of PO_4^{3-} from the sediment to the overlying water layer increases as more PO_4^{3-} escapes the Fe trap in the oxidised surface layer of the sediment (Roden and 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 and 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 and Nielsen 1998; Weber et al. 2001). It is indeed known that NO_3^- reduction can lead to oxidation of Fe(II) (Uhlmann and Paul 1994; Smolders et al. 1997; Aravena and Robertson 1998; Pauwels et al. 1998; Tesoriero et al. 2000) and metal sulphides (Klapper 1989; Ripl 1976; Golterman 1991, 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 the oxidation of FeS_x , was much higher in sediments receiving NO_3^- -rich medium than in those receiving medium without NO_3^- (Tables 5 and 6 and Figure 4). At high pH, Fe^{3+} precipitates as insoluble iron (hydr)oxides (Drever 1997). Schippers and 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 $\mu\text{mol/L}$) and poor in Mn (<10 $\mu\text{mol/L}$). 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 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

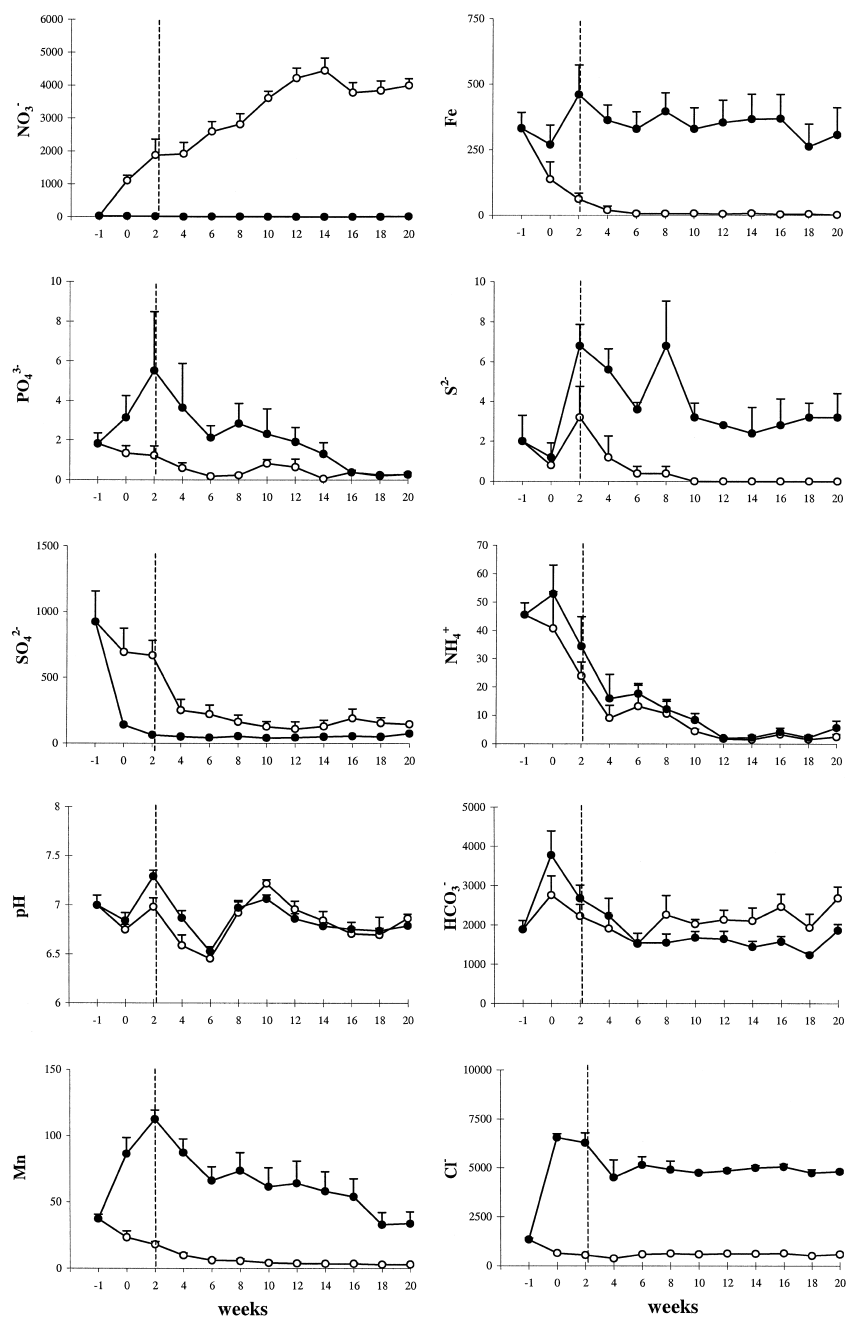


Figure 4. Chemical composition of the pore water in S-rich monoliths from Beeselsbroek after stagnation (first 3 weeks) and during flow-through (last 18 weeks) with NO_3^- -rich (\circ) and NO_3^- -poor (\bullet) medium at the laboratory. Concentrations are given in $\mu\text{mol L}^{-1}$.

Table 5. Total concentrations of Fe, S and P at the start of the experiment ($\mu\text{mol/g DW}$), redox potential (mV) at -10 cm , CH_4 production ($\mu\text{mol/kg fresh sediment/day}$) at the end of the experiment, and estimated release (ER) of Fe , NO_3^- , S^{2-} and PO_4^{3-} during the experimental period ($\mu\text{mol L}^{-1}$ fresh sediment). Means \pm (SEM) are given ($n=5$). * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.001$.

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

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

	Treatment	Time	Time \times treatment
pH	ns	ns	ns
HCO_3^-	ns	ns	*
NO_3^-	***	**	*
Fe	***	**	*
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
Si	**	**	ns
Al	ns	**	ns

components can not be expected to mobilise large amounts of SO_4^{2-} and 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 PO_4^{3-} from iron phosphate complexes in anaerobic sediments (Ripl 1976, 1978; Andersen 1982; Jensen and Andersen 1992). The monitoring study revealed that wetlands fed by groundwater with high NO_3^- concentrations were characterised by the lowest PO_4^{3-} concentrations (Figures 1 and 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 PO_4^{3-} from the monoliths as it oxidised reduced Fe and thus increased the amount of oxidised Fe capable of binding PO_4^{3-} (Figure 4, Tables 5 and 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, 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 to 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 visible 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 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 $\text{NO}_3^-/\text{SO}_4^{2-}$ 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 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 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 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

The authors would like to thank Roy Peters and Martin Versteeg for their assistance in the field, and Jelle Eygensteyn, Liesbeth Pierson and Rien van der Gaag for their assistance with the chemical analyses. Furthermore, we would like to thank Jan Klerkx for improving the English. This research was sponsored by 'Stichting het Limburgs Landschap', the Netherlands.

References

- Andersen J.M. 1982. Effect of NO_3^- concentration in lake water on phosphate release from sediment. *Water Res.* 16: 1119–1126.
- Aravena R. and Robertson W.D. 1998. Use of multiple tracers to evaluate denitrification in ground water: Study of nitrate from a large-flux septic system plume. *Ground Water* 36(6): 975–982.
- Blom J., Roelofs W. and Akkermans A.D.L. 1981. Assimilation of nitrogen in root nodules of alder (*Alnus glutinosa*). *New Phytol.* 89: 321–326.
- Boström B., Jansson M. and Forsberg C. 1982. Phosphorus release from lake sediments. *Arch. Hydrobiol.* 18: 5–59.
- Boxman A.W. and Stortelder A.H.F. 2000. Hoe natter, hoe beter? De invloed van het waterpeil bij maatregelen tegen verdroging in elzenbroekbossen. *Vakblad Natuurbeheer* 5: 75–77.
- Caraco N.F., Cole J.J. and Likens G.E. 1989. Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. *Nature* 341: 316–318.
- Christensen K.K. and Wigand C. 1998. Formation of root plaques and their influence on tissue phosphorus content in *Lobelia dortmanna*. *Aquat. Bot.* 61: 111–122.
- Drever J.I. 1997. *The Geochemistry of Natural Waters, Surface and Ground Water Environments*. 3th edn. Prentice Hall, Upper Saddle River, NJ 07458.
- EEA, Europe's Environment, the Second Assessment. 1998. European Environment Agency, Copenhagen.
- Furumai H. and Ogaki S. 1982. Fractional composition of phosphorus in sediments related to release. *Water Sci. Tech.* 14: 215–226.
- Golterman H.L. 1991. The influence of FeS on the denitrification rate. *Verh. Int. Ver. Limnol.* 24: 3025–3028.
- Golterman H.L. 1995. The labyrinth of nutrient cycles and buffers in wetlands: results based on research in the Camargue (southern France). *Hydrobiol.* 315: 39–58.
- Grasshoff K. and Johannsen H. 1977. A new sensitive method for the determination of ammonia in seawater. *Wat. Res.* 2: 516.
- Guan C., Ribeiro A., Akkermans A., Jing Y., van Kammen A., Bisseling T. and Pawlowski K. 1996. Nitrogen metabolism in actinorhizal nodules of *Alnus glutinosa*: expression of glutamine synthetase and acetylornithine transaminase. *Plant Mol. Biol.* 32: 1177–1184.

- Hagedorn F., Schleppi P., Bucher J. and Flühler H. 2001. Retention and leaching of elevated N deposition in a forest ecosystem with gleysols. *Water, Air, Soil Pol.* 129: 119–142.
- Hosomi M., Okada M. and Sudo R. 1980. Release of phosphorus from sediments. *Verh. Int. Ver. Limnol.* 21: 628–633.
- Iversen T.M., Grant K. and Nielsen K. 1998. Nitrogen enrichment of European inland and marine waters with special attention to Danish policy measures. *Env. Poll.* 102: 771–780.
- Jensen H.S. and Andersen F.Ø. 1992. Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. *Limnol. Oceanogr.* 37: 577–589.
- Kamphake L.J., Hannah S.A. and Cohen J.M. 1967. Automated analysis for nitrate by hydrazine reduction. *Wat. Res.* 1: 205–206.
- Klapper H. 1989. Restaurierung von Seen. In: Busch K.F., Uhlmann D. and Weise G. (ed) *Ingenieurökologie 2. Aufl.*, Fischer Verlag, Jena, pp. 278–284.
- Lamers L.P.M., Tomassen H.B.M. and Roelofs J.G.M. 1998. Sulphate-induced eutrophication and phytotoxicity in freshwater wetlands. *Environ. Sci. Technol.* 32: 199–205.
- Lucassen E., Smolders A. and Roelofs J. 2000. De effecten van verhoogde sulfaatgehalten op grondwater gevoede ecosystemen. *H₂O* 25/26: 28–31.
- Lucassen E.C.H.E.T., Smolders A.J.P. and Roelofs J.G.M. 2002. Potential sensitivity of mires to drought, acidification and mobilisation of heavy metals: the sediment S/(Ca + Mg) ratio as diagnostic tool. *Environ. Pollut.* 120: 635–646.
- Lucassen E.C.H.E.T., Smolders A.J.P., Van de Crommenacker J. and Roelofs J.G.M. Effects of stagnating sulphate-rich groundwater on the mobility of phosphate in freshwater wetlands: a field experiment (submitted for publication).
- Moore Jr. P.A. and Reddy K.R. 1994. Role of Eh and pH on phosphorus geochemistry in sediments of lake Okeechobee, Florida. *J. Environ. Qual.* 23: 955–964.
- Murray T.E. 1995. The correlation between iron sulphide precipitation and hypolimnetic phosphorus accumulation during one summer in a soft water lake. *Can. J. Fish. Aquat. Sci.* 52: 1190–1194.
- Nielsen J.L. and Nielsen P.H. 1998. Microbial nitrate-dependent oxidation of ferrous iron in activated sludge. *Environ. Sci. Technol.* 32: 3556–3561.
- Patrick W.H. and Khalid R.A. 1974. Phosphate release and sorption by soils and sediments; effect of aerobic and anaerobic conditions. *Science* 186: 53–55.
- Pauwels H., Kloppmann W., Foucher J.C., Martelat A. and Fritsche V. 1998. Field tracer test for denitrification in a pyrite-bearing aquifer. *Appl. Geochem.* 13: 767–778.
- Ripl W. 1976. Biochemical oxidation of polluted lake sediment with nitrate. *Ambio* 5: 132–135.
- Ripl W. 1978. Oxidation of Lake Sediment with Nitrate: a Restoration Method for Former Recipients. *Inst. Limn. Lund. Sweden* 151 p.
- Roden E.E. and Edmonds J.W. 1997. Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron-sulfide formation. *Arch. Hydrobiol.* 139: 347–378.
- Roelofs J.G.M. 1991. Inlet of alkaline river water into peaty lowlands: effects of water quality and *Stratiotes aloides* L. stands. *Aquat. Bot.* 39: 267–293.
- Roelofs J.G.M., Kempers A.J., Houdijk A.L.F.M. and Jansen J. 1985. The effect of air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands. *Plant Soil* 84: 45–56.
- Scheffer F. and Schachtschabel P. 1992. *Lehrbuch der Bodenkunde*. Enke, Stuttgart.
- Schippers A. and Jørgensen B.B. 2001. Oxidation of pyrite and iron sulphide by manganese dioxide in marine sediment. *Geochim. Cosmochim. Acta* 65: 915–922.
- Schippers A. and Jørgensen B.B. 2002. Biogeochemistry of pyrite and iron sulphide oxidation in marine sediments. *Geochim. Cosmochim. Acta* 66: 85–92.
- Sperber J.I. 1958. Release of phosphate from soil minerals by hydrogen sulphide. *Nature* 181: 934.
- Smolders A.J.P. and Roelofs J.G.M. 1993. Sulphate mediated iron limitation and eutrophication in aquatic ecosystems. *Aquat. Bot.* 46: 247–253.
- Smolders A.J.P., Hendriks R.J.J., Campscreur H.M. and Roelofs J.G.M. 1997. Nitrate induced iron deficiency chlorosis in *Juncus acutiflorus*. *Plant Soil* 196: 37–45.

- Smolders A.J.P., Lamers L.P.M., Moonen M., Zwaga K. and Roelofs J.G.M. 2001. Controlling the phosphate release from phosphate-enriched sediments by adding various iron compounds. *Bio-geochemistry* 54: 219–228.
- Straub K.S., Benz M., Schink B. and Widdel F. 1996. Anaerobic, nitrate dependent microbial oxidation of ferrous iron. *Appl. Environ. Microbiol.* 62: 1458–1460.
- Tansley A.G. 1946. *Introduction to Plant Ecology*. Allen and Unwin, London, 260 p.
- Tesoriero A.J., Liebscher H. and Cox S.E. 2000. Mechanisms and rates of denitrification in an agricultural watershed: electron and mass balance along ground water flow paths. *Water Resour. Res.* 36: 1545–1559.
- Uhlmann D. and Paul L. 1994. Causes and effects of 'nitrate saturation' in phosphate-deficient water bodies. *Water Sci. Technol.* 30: 281–288.
- Van Breemen N., Burrough P.A., Velthorst E.J., Van Dobben H.F., De Wit T., Ridder T.B. and Reijnders H.F.R. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature* 299: 548–550.
- Van Gernerden H. 1984. The sulphide affinity of phototrophic bacteria in relation to the location of elemental sulphur. *Arch. Microbiol.* 139: 289–294.
- Van Steenwijk J.M. 1986. *De chemie van het grondwater in het eerste watervoerend pakket rond het pompstation Vierlingsbeek*. KIWA, SWE 86.018., The Netherlands.
- Weber K.A., Picardal F.W. and Roden E.E. 2001. Microbially catalyzed nitrate-dependent oxidation of biogenic solid-phase Fe(II) compounds. *Environ. Sci. Technol.* 35: 1644–1650.
- Wellburn A.R. and Lichtenthaler H. 1984. Formulae and program to determine total carotenoids and chlorophylls A and B of leaf extracts in different solvents. In: Sybesma C. *Photosynthesis Research*. Vol 2. (ed) Martinus Nijhoff/Dr. W. Junk Publ., The Hague.
- Wetzel R.G. 2001. *Limnology*. W.B. Saunders Company, Philadelphia.