



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

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**Abstract.** During the last 60 years, pollution of the groundwater with  $\text{NO}_3^-$  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  $\text{NO}_3^-$  concentrations had the lowest Fe and  $\text{PO}_4^{3-}$  concentrations in the pore water. A comparison of two restored open water fens, differing in  $\text{NO}_3^-$  supply via the groundwater, indicated that the redox potential and the sulphate ( $\text{SO}_4^{2-}$ ) reduction rate were lower when the groundwater contained not only  $\text{SO}_4^{2-}$  but also high  $\text{NO}_3^-$  concentrations. The lower  $\text{SO}_4^{2-}$  reduction rates in the  $\text{NO}_3^-$ -rich open water fen were associated with lower  $\text{PO}_4^{3-}$  concentrations and the presence of plant species characteristic of clear water. In contrast, the higher  $\text{SO}_4^{2-}$  reduction rates in the  $\text{NO}_3^-$ -poor open water fen were associated with very high  $\text{PO}_4^{3-}$  concentrations and massive development of plant species characteristic of eutrophic environments. Investigations at  $\text{NO}_3^-$ -rich seepage sites in black alder carrs, showed that high  $\text{NO}_3^-$  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  $\text{NO}_3^-$ -rich seepage sites contained no oxidisable  $\text{FeS}_x$ , contrary to  $\text{NO}_3^-$ -poor locations, which became acidified and mobilised extremely high amounts of  $\text{SO}_4^{2-}$  due to  $\text{FeS}_x$  oxidation. A laboratory experiment showed that  $\text{NO}_3^-$  addition to sediments led to reduced release of Fe and  $\text{S}^{2-}$  and increased release of  $\text{SO}_4^{2-}$ , very likely due to the oxidation of reduced Fe and S compounds. Overall, the results confirmed that  $\text{NO}_3^-$  is an energetically more favourable electron acceptor in anaerobic sediments than Fe and  $\text{SO}_4^{2-}$ , and that high  $\text{NO}_3^-$  loads function as a redox buffer, preventing reduction of Fe and  $\text{SO}_4^{2-}$ . Limited  $\text{SO}_4^{2-}$  reduction prevents  $\text{S}^{2-}$ -mediated mobilisation of  $\text{PO}_4^{3-}$  from Fe –  $\text{PO}_4^{3-}$  complexes. At a higher redox potential, reduced Fe, including  $\text{FeS}_x$ , was oxidised, increasing the content of Fe(III) capable of binding  $\text{PO}_4^{3-}$ . This prevented increased  $\text{PO}_4^{3-}$  availability and the concomitant massive development of plant species characteristic of eutrophic environments.

### Introduction

Nitrate ( $\text{NO}_3^-$ ) concentrations in groundwater are low under natural conditions ( $<32 \mu\text{mol l}^{-1}$ ) (EEA 1998). During the last 60 years, however, groundwater  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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 ( $\text{NH}_4^+$ ) deposition rates in especially pine forests, and increased  $\text{NO}_3^-$  concentrations in the groundwater, as  $\text{NH}_4^+$  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,  $\text{NO}_3^-$  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  $\text{NO}_3^-$  and the appearance of increased  $\text{SO}_4^{2-}$  concentrations in the groundwater at this depth. Increased availability of  $\text{SO}_4^{2-}$  in wetlands is known to cause serious eutrophication problems, as  $\text{S}^{2-}$  produced by  $\text{SO}_4^{2-}$  reduction, interacts with Fe –  $\text{PO}_4^{3-}$  complexes in the sediment to produce  $\text{FeS}_x$  ( $\text{FeS}_2$  and  $\text{FeS}$ ), resulting in mobilisation of phosphate ( $\text{PO}_4^{3-}$ ) (Boström et al. 1982; Caraco et al. 1989; Roelofs 1991; Smolders and Roelofs 1993; Lamers et al. 1998; Smolders et al. 2001). Thus, increased  $\text{SO}_4^{2-}$  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  $\text{SO}_4^{2-}$  concentrations can cause  $\text{PO}_4^{3-}$  mobilisation and eutrophication. However, no eutrophication occurs, if there is a constant input of groundwater that contains not only  $\text{SO}_4^{2-}$  but also contains high  $\text{NO}_3^-$  concentrations (Lucassen et al. submitted for publication). This indicates that  $\text{NO}_3^-$  in some way may prevent eutrophication of wetlands. Research in drinking water reservoirs has shown that  $\text{NO}_3^-$  can oxidise Fe in the top layer of the soil (creating bond sites for phosphates) and that it can inhibit  $\text{SO}_4^{2-}$  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  $\text{NO}_3^-$  'pollution' via the groundwater might prevent eutrophication of  $\text{SO}_4^{2-}$ -enriched wetlands, field work was carried out in fens (including black alder carrs) which were differently affected by  $\text{NO}_3^-$ . 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 (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'; 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 $\text{NO}_3^-$ -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  $\text{NO}_3^-$ -rich seepage at Beeselsbroek. The sites directly fed by  $\text{NO}_3^-$ -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  $\text{FeS}_x$  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<sub>3</sub><sup>-</sup> 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<sup>-2</sup> s<sup>-1</sup>, 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<sub>3</sub><sup>-</sup> (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<sub>4</sub> production. The Estimated Release (ER in μmol L fresh sediment) of Fe, SO<sub>4</sub><sup>2-</sup>, S<sup>2-</sup> and PO<sub>4</sub><sup>3-</sup> from the sediments during the experimental flow-through period (from  $t = 2$  to  $t = -20$  weeks) was calculated for each container as follows:

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

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<sup>2-</sup> by means of an Orion type 94-16A S<sup>2-</sup> ion-specific silver electrode (Van Gemerden 1984). CH<sub>4</sub> 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<sub>3</sub><sup>-</sup> and CO<sub>2</sub> 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  $\text{NO}_3^-$ .

	+ $\text{NO}_3^-$	- $\text{NO}_3^-$
$\text{NaHCO}_3^-$	2000	2000
KCl	250	250
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	200	200
$\text{CaCl}_2$	–	2500
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	2500	–

stored in iodated polyethylene bottles at  $-23^\circ\text{C}$  until further analysis. In these samples, the following compounds were measured using Technicon II auto-analysers:  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  (Kamphake et al. 1967) and  $\text{NH}_4^+$  (Grasshoff and Johansen 1977).  $\text{K}^+$  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  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$  and Fe in the pore water as a function of  $\text{NO}_3^-$  concentration in the pore water show that the concentrations of  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$  and Fe in the pore water decrease with increasing  $\text{NO}_3^-$  concentrations. Above  $\text{NO}_3^-$  concentrations of  $100 \mu\text{mol L}^{-1}$ , the  $\text{PO}_4^{3-}$ , Fe and  $\text{NH}_4^+$  concentrations are very low. The  $\text{PO}_4^{3-}$  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

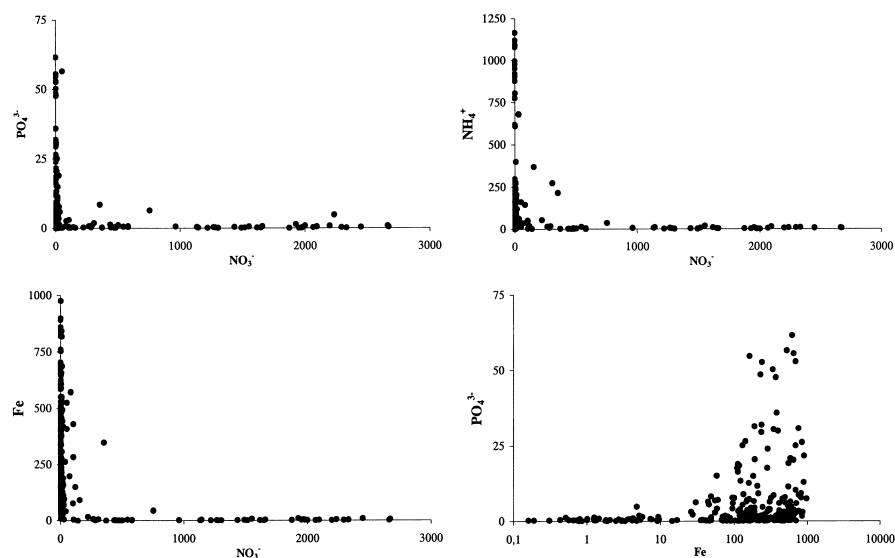


Figure 1. Pore water scatterplots with  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$  and Fe concentrations as a function of the  $\text{NO}_3^-$  concentration, and  $\text{PO}_4^{3-}$  as a function of the Fe concentration. Concentrations are given in  $\mu\text{mol L}^{-1}$ .

*Lemma 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).

$\text{SO}_4^{2-}$  concentrations in the pore water and surface water of the eutrophic fen, which was fed by groundwater rich in  $\text{SO}_4^{2-}$  ( $1000 \mu\text{mol L}^{-1}$ ) but poor in  $\text{NO}_3^-$  ( $< 35 \mu\text{mol L}^{-1}$ ), decreased immediately after the influence of groundwater was reinforced. However,  $\text{SO}_4^{2-}$  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  $\text{SO}_4^{2-}$  concentrations ( $1000 \mu\text{mol L}^{-1}$ ) but also very high  $\text{NO}_3^-$  concentrations ( $600\text{--}4900 \mu\text{mol L}^{-1}$ ). Concentrations of  $\text{PO}_4^{3-}$ , Fe,  $\text{NH}_4^+$  and  $\text{CH}_4$  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 $\text{NO}_3^-$ -rich seepage.

The redox potential ( $+247 \text{ mV}$ ) and the  $\text{NO}_3^-$  concentration ( $2030 \mu\text{mol L}^{-1}$ ) in the pore water of the soil at sites directly fed by  $\text{NO}_3^-$ -rich groundwater were much higher than those at sites indirectly fed by groundwater ( $-160 \text{ mV}$  and  $348 \mu\text{mol L}^{-1}$ , respectively). The Fe concentration was extremely low ( $0.5 \mu\text{mol L}^{-1}$ ) at the  $\text{NO}_3^-$ -rich site, while  $\text{HCO}_3^-$ ,  $\text{NH}_4^+$ , 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

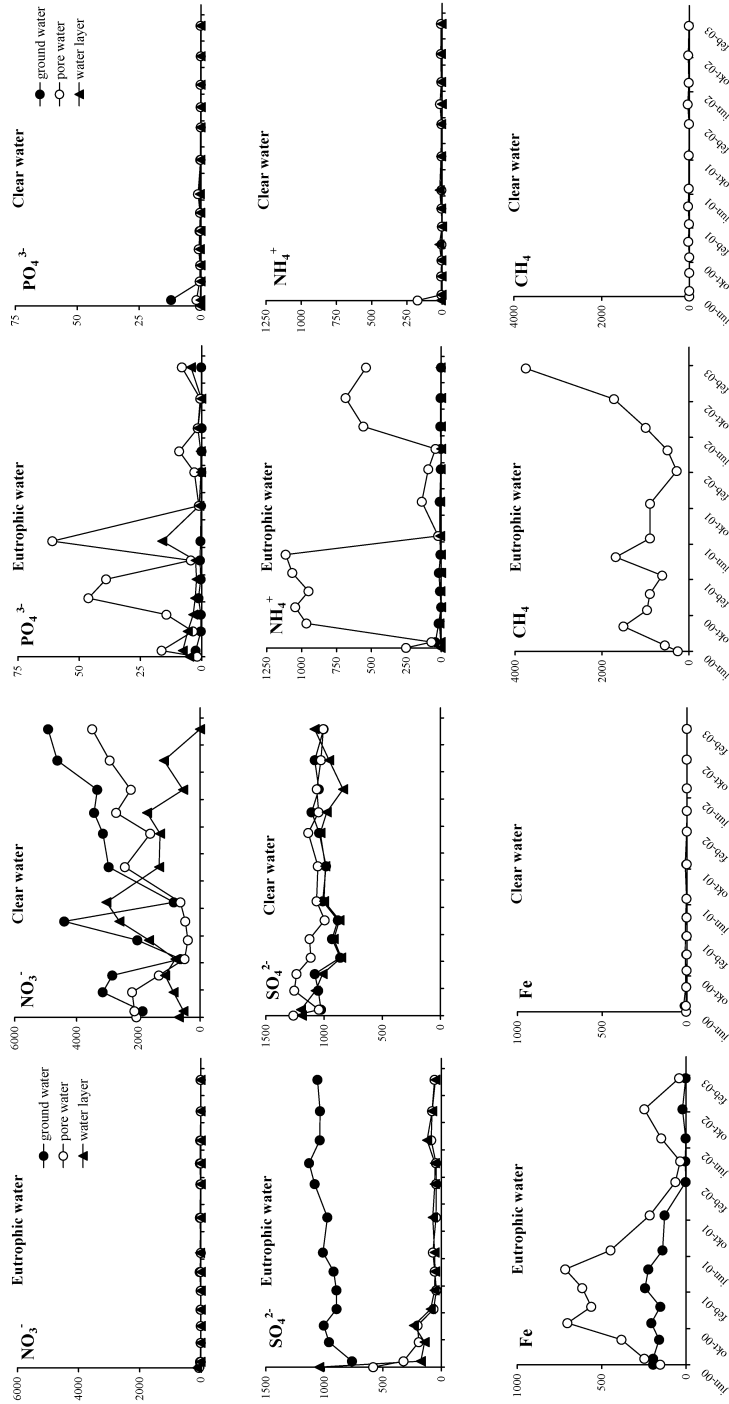


Figure 2. Chemical composition of the groundwater, pore water and water layer in the eutrophying fen (left) and in the fen developing clear water (right) between April 2000 and January 2003. Concentrations are given in  $\mu\text{mol L}^{-1}$ .



Table 3. Pore water (PW) chemistry ( $\mu\text{M}$ ), E (mV) and concentrations of root plaque extractable components (RP) ( $\mu\text{mol/g DW}$ ) of *C. pseudoocyperus* at locations fed by  $\text{NO}_3^-$ -poor and  $\text{NO}_3^-$ -rich groundwater. Means  $\pm$  (standard errors) are given ( $n = 6$ ). \* $P \leq 0.05$ ; \*\* $P \leq 0.005$ .

	$\text{NO}_3^-$	E	pH	$\text{HCO}_3^-$	$\text{SO}_4^{2-}$	$\text{NH}_4^+$	$\text{PO}_4^{3-}$	Fe	Mn	Ca	Mg	$\text{K}^+$	Zn	Al
<i>PW</i>														
- $\text{NO}_3^-$	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)
+ $\text{NO}_3^-$	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>														
- $\text{NO}_3^-$								1198 (154)	13 (2)	112 (9)	11 (0.4)	82 (11)	0.5 (0.1)	2.1 (0.5)
+ $\text{NO}_3^-$								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  $\text{NO}_3^-$ -poor and  $\text{NO}_3^-$ -rich seepage. Means  $\pm$  (standard errors) are given ( $n=6$ ). \* $P \leq 0.05$ , \*\* $P \leq 0.005$ , \*\*\* $P \leq 0.0005$ . Concentration of C is given in  $\text{mmol/g DW}$ .

	Chl A	Chl B	Carot	C	N	Fe	P	Mn	Ca	Mg	K <sup>+</sup>	Zn	Al	S
<i>Root</i>														
- $\text{NO}_3^-$				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)
+ $\text{NO}_3^-$				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>														
- $\text{NO}_3^-$	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)
+ $\text{NO}_3^-$	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)

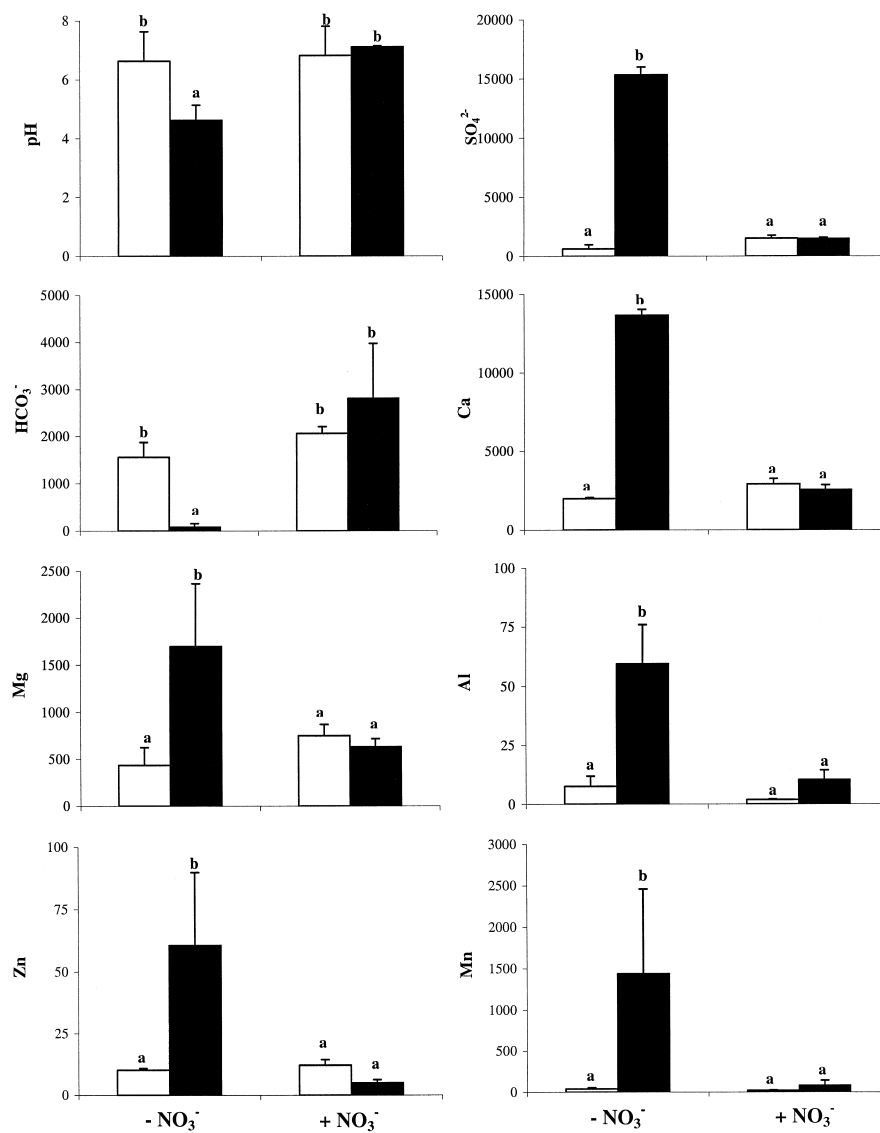


Figure 3. Chemical composition of the pore water of sediments fed directly ( $NO_3^-$ -rich) and indirectly ( $NO_3^-$ -poor) by groundwater under field conditions (white bars), and after rewetting following experimental desiccation (black bars). Concentrations are given in  $\mu\text{mol L}^{-1}$ .

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  $\text{NO}_3^-$  concentrations positively affect groundwater fed  $\text{SO}_4^{2-}$  enriched wetlands by restricting the mobilisation of  $\text{PO}_4^{3-}$  from the sediment to the water layer. Under anaerobic conditions, reduction of Fe from Fe –  $\text{PO}_4^{3-}$  complexes leads to the mobilisation of  $\text{PO}_4^{3-}$  from sediments (Hosomi 1980; Furumai and Ogaki 1982; Roelofs 1991; Wetzel 2001). Mobilisation of  $\text{PO}_4^{3-}$  from these complexes probably occurred in the wetlands examined in the present study, as was indicated by the positive correlation between  $\text{PO}_4^{3-}$  and Fe in the pore water in the monitoring study (Figure 1). It is well known that the release of Fe-bound  $\text{PO}_4^{3-}$  is accelerated by increased  $\text{SO}_4^{2-}$  reduction rates. The  $\text{S}^{2-}$  produced, reduces Fe(III) in iron phosphate, iron (hydr)oxide phosphate and humic iron phosphate complexes, mobilising  $\text{PO}_4^{3-}$  (Sperber 1958; Patrick and Khalid 1974; Boström et al. 1982; Smolders and Roelofs 1993; Moore and Reddy 1994; Lamers et al. 1998).  $\text{S}^{2-}$  also forms iron (di)sulphides ( $\text{FeS}_x$ ) with Fe(II), reducing the amounts of Fe available for  $\text{PO}_4^{3-}$  binding and decreasing the Fe/ $\text{PO}_4^{3-}$  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  $\text{PO}_4^{3-}$  from the sediment to the overlying water layer increases as more  $\text{PO}_4^{3-}$  escapes the Fe trap in the oxidised surface layer of the sediment (Roden and Edmonds 1997; Smolders et al. 2001; Wetzel 2001).

As  $\text{NO}_3^-$  is an energetically more favourable electron acceptor in anaerobic sediments than Fe and  $\text{SO}_4^{2-}$ , high  $\text{NO}_3^-$  loads function as a redox buffer limiting the reduction of Fe and  $\text{SO}_4^{2-}$  (Scheffer and Schachtschabel 1992; Drever 1997). In addition,  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  as a redox buffer also became obvious from our laboratory experiment. A continuous flow of  $\text{NO}_3^-$ -rich medium led to a higher redox potential in the sediment than the same medium without  $\text{NO}_3^-$ . In addition, the release of  $\text{SO}_4^{2-}$ , due to the oxidation of  $\text{FeS}_x$ , was much higher in sediments receiving  $\text{NO}_3^-$ -rich medium than in those receiving medium without  $\text{NO}_3^-$  (Tables 5 and 6 and Figure 4). At high pH,  $\text{Fe}^{3+}$  precipitates as insoluble iron (hydr)oxides (Drever 1997). Schippers and Jørgensen (2001, 2002) showed that Mn reduction plays an important role in oxidation of  $\text{FeS}_x$  in marine sediments. This will only play a minor role in our investigated systems, as they are fed by groundwater rich in  $\text{NO}_3^-$  (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  $\text{NO}_3^-$  concentrations. It is also possible that some of the mobilised  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  in the laboratory experiment originated from anion exchange due to  $\text{NO}_3^-$  addition.  $\text{SO}_4^{2-}$  can bind to positively charged clay minerals and iron-oxides, but binding of  $\text{NO}_3^-$  or  $\text{Cl}^-$  to these adsorption sites is known to be very weak. Therefore addition of these

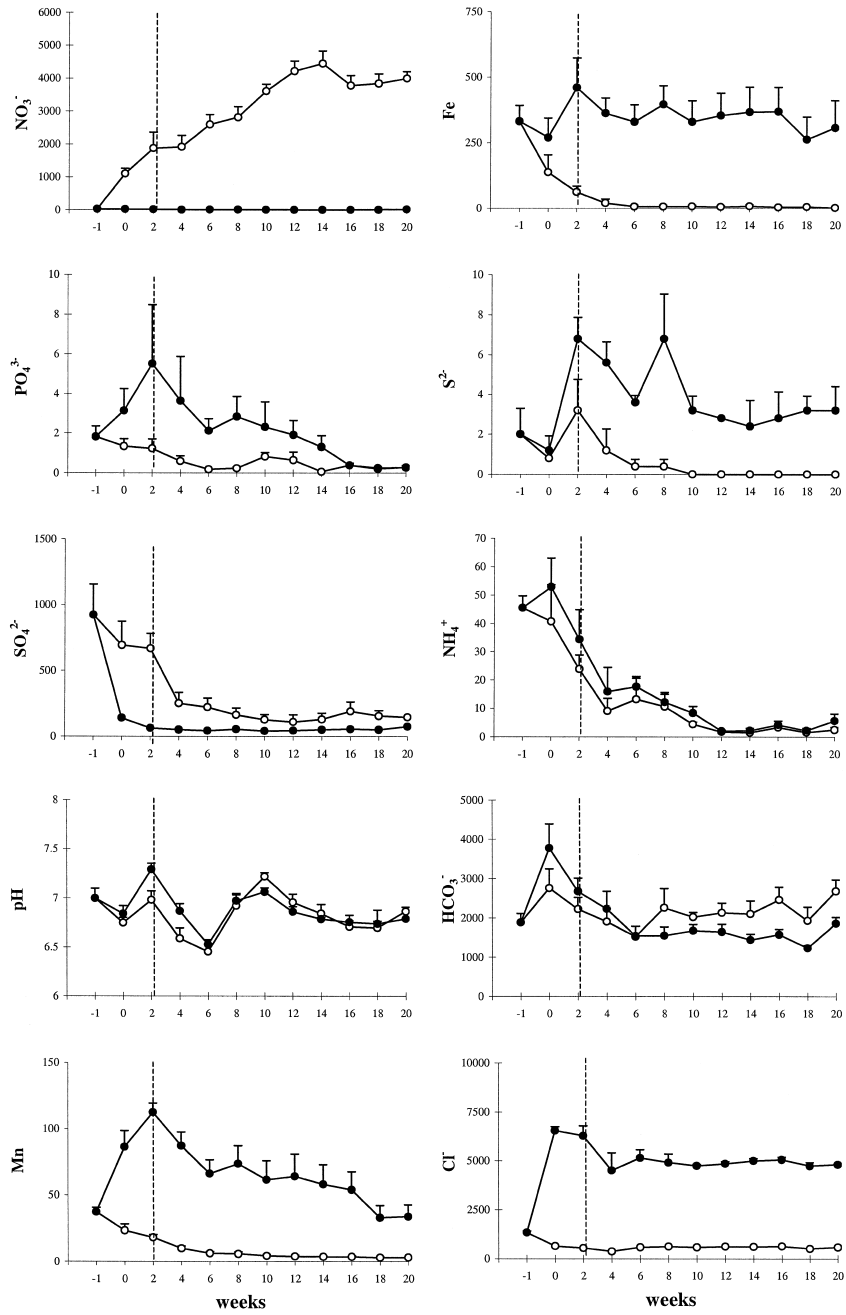


Figure 4. Chemical composition of the pore water in S-rich monoliths from Beeselsbroek after stagnation (first 3 weeks) and during flow-through (last 18 weeks) with  $\text{NO}_3^-$ -rich ( $\circ$ ) and  $\text{NO}_3^-$ -poor ( $\bullet$ ) medium at the laboratory. Concentrations are given in  $\mu\text{mol L}^{-1}$ .

Table 5. Total concentrations of Fe, S and P at the start of the experiment ( $\mu\text{mol/g DW}$ ), redox potential (mV) at  $-10\text{ cm}$ ,  $\text{CH}_4$  production ( $\mu\text{mol/kg fresh sediment/day}$ ) at the end of the experiment, and estimated release (ER) of  $\text{Fe}$ ,  $\text{NO}_3^-$ ,  $\text{S}^{2-}$  and  $\text{PO}_4^{3-}$  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	$\text{CH}_4$	ER Fe	ER $\text{SO}_4^{2-}$	ER $\text{S}^{2-}$	ER $\text{PO}_4^{3-}$
$-\text{NO}_3^-$	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  $\text{NO}_3^-$  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
$\text{HCO}_3^-$	ns	ns	*
$\text{NO}_3^-$	***	**	*
Fe	***	**	*
$\text{PO}_4^{3-}$	*	***	ns
P	**	ns	ns
$\text{S}^{2-}$	***	*	ns
$\text{SO}_4^{2-}$	*	**	*
$\text{NH}_4^+$	ns	**	ns
Mn	***	***	**
$\text{Na}^+$	ns	ns	ns
$\text{CO}_2$	ns	**	ns
$\text{K}^+$	*	***	ns
Ca	*	ns	**
Mg	ns	***	*
Zn	ns	ns	ns
Si	**	**	ns
Al	ns	**	ns

components can not be expected to mobilise large amounts of  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  (Drever 1997). This is confirmed by the fact that the addition of  $\text{CaCl}_2$  amounts in the control treatment did not increase  $\text{SO}_4^{2-}$  concentrations to the same levels as happened in the  $\text{NO}_3^-$  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  $\text{NO}_3^-$  concentrations, preventing the reduction of  $\text{SO}_4^{2-}$  and thus the formation of  $\text{FeS}_x$ . Ongoing  $\text{NO}_3^-$  reduction reduces the concentration of  $\text{NO}_3^-$  in the groundwater, and therefore locations indirectly fed by groundwater accumulated large amounts of  $\text{FeS}_x$  due to  $\text{SO}_4^{2-}$  reduction.  $\text{SO}_4^{2-}$  reduction was not inhibited at these locations, as the groundwater was poor in  $\text{NO}_3^-$  (Figure 3).

By maintaining a high redox potential,  $\text{NO}_3^-$  inhibits the release of  $\text{PO}_4^{3-}$  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  $\text{NO}_3^-$  concentrations were characterised by the lowest  $\text{PO}_4^{3-}$  concentrations (Figures 1 and 2) and the development of aquatic plants characteristic of clear waters (Table 2). The laboratory study showed that addition of  $\text{NO}_3^-$  was accompanied by a significantly lower release of  $\text{PO}_4^{3-}$  from the monoliths as it oxidised reduced Fe and thus increased the amount of oxidised Fe capable of binding  $\text{PO}_4^{3-}$  (Figure 4, Tables 5 and 6). The comparison between the  $\text{NO}_3^-$ -poor and  $\text{NO}_3^-$ -rich restored fens indicated that  $\text{NO}_3^-$  prevented  $\text{SO}_4^{2-}$  reduction (Figure 2). The occurrence of  $\text{SO}_4^{2-}$  reduction in the  $\text{NO}_3^-$ -poor fen was indicated by the higher  $\text{SO}_4^{2-}$  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,  $\text{PO}_4^{3-}$  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  $\text{NO}_3^-$ -rich fen. Oxidised conditions enhance nitrification, which explains the relatively low  $\text{NH}_4^+$  concentration in the pore water of the  $\text{NO}_3^-$ -rich fen, as opposed to the very high  $\text{NH}_4^+$  concentrations in the  $\text{NO}_3^-$ -poor fen.

The high  $\text{NO}_3^-$  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  $\text{NO}_3^-$ -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  $\text{NO}_3^-$  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  $\text{NO}_3^-$ -rich seepage (Table 4). High  $\text{NO}_3^-$  concentrations acted as a redox buffer under wet conditions and decreased the free Fe concentrations in the sediment (Table 3). Increased  $\text{NO}_3^-$  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  $\text{PO}_4^{3-}$  availability,  $\text{NO}_3^-$  might also affect the sensitivity of wetland sediments to acidification during desiccation. Oxidation of  $\text{FeS}_x$  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  $\text{NO}_3^-$ ). These sediment types mobilised large amounts of  $\text{SO}_4^{2-}$  as a consequence of desiccation, and the acid produced led to exhaustion of the buffer capacity, as indicated by decreased alkalinity and  $\text{HCO}_3^-$  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  $\text{NO}_3^-$ -rich groundwater, where  $\text{SO}_4^{2-}$  reduction was impaired and only minor amounts of  $\text{FeS}_x$  had accumulated (Figure 3).

We propose the following scenario for the current situation in the Netherlands.  $\text{NO}_3^-$  leaching from agricultural lands and forest soils leads to increased  $\text{NO}_3^-$  concentrations in the groundwater. When  $\text{NO}_3^-$  reaches  $\text{FeS}_x$ -containing subsoil layers it may oxidise  $\text{FeS}_x$  which leads to the mobilisation of  $\text{SO}_4^{2-}$  and a decrease of the  $\text{NO}_3^-$  concentration. The resulting  $\text{NO}_3^-/\text{SO}_4^{2-}$  ratio will strongly affect the quality of groundwater fed fens. If  $\text{SO}_4^{2-}$  concentrations are high and  $\text{NO}_3^-$  concentrations are low, eutrophication may occur as a result of  $\text{SO}_4^{2-}$ -reduction related processes such as  $\text{PO}_4^{3-}$  mobilisation and Fe immobilisation ( $\text{FeS}_x$  accumulation). However, if  $\text{NO}_3^-$  concentrations are also high,  $\text{SO}_4^{2-}$  and Fe reduction are impaired



and mobilisation of  $\text{PO}_4^{3-}$  from iron–phosphate complexes is prevented. In addition,  $\text{NO}_3^-$  may oxidise reduced Fe compounds increasing the amount of  $\text{Fe}^{3+}$  capable of binding  $\text{PO}_4^{3-}$ . So  $\text{NO}_3^-$  leaching into the groundwater increases the risk of eutrophication of the discharge areas by mobilising  $\text{SO}_4^{2-}$  in  $\text{FeS}_x$  containing aquifers. However, if it reaches a discharge area in sufficiently high amount,  $\text{NO}_3^-$  may prevent actual eutrophication (at least of the directly fed parts) by functioning as a redox buffer.

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