Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands

Abstract—Increased sulfur loads originating from polluted surface water and groundwater, and from enhanced atmospheric input, are a major threat to the biogeochemical functioning and biodiversity of freshwater wetlands. Sulfate reduction, normally playing a modest role in these systems, becomes the most important biogeochemical process, inducing severe eutrophication and sulfide toxicity. In field enclosure experiments we observed striking differences between the responses of two freshwater marshes to sulfate. On one location sulfate addition resulted in strong phosphorus mobilization without sulfide accumulation, whereas high sediment sulfide concentrations, known to be toxic to wetland macrophytes, were reached in the other marsh without eutrophication occurring. The results could be explained by differences in groundwater iron discharge and nutrient contents of the peat sediments. Sulfate reduction rates appeared to be limited by either electron donor availability (first marsh) or electron acceptor availability (second marsh). The implications of these findings are explained in relation to freshwater wetland management.

Sulfate reduction rates (SRR) in anaerobic sediments of freshwater wetlands are generally rate limited by the availability of sulfate (SO_4^{2-}). However, SO_4^{2-} pollution of surface water and groundwater, due to runoff from mining areas and agricultural areas and the use of SO_4^{2-} enriched river water to combat desiccation, has caused a significant increase in the availability of the microbial electron acceptor in many freshwater wetlands (Roelofs 1991; Koerselman et al. 1993; Smolders and Roelofs 1993; Lamers et al. 1998*a*). Concentrations have increased from less than 200 μmol L^{-1} to values of 500 μmol L^{-1} and higher (over 3,000 μmol L^{-1}). In addition, sulfate is mobilized from recent or geological pyrite deposits by oxygen intrusion during desiccation (Vangenechten et al. 1981; Schuurkes et al. 1988; Heathwaite 1990; Lamers et al. 1998*b*) and chemolithotrophic denitrification in nitrate-polluted areas (Kölle et al. 1985; Lamers et al. 1999). In addition to these SO_4^{2-} sources, the increased atmospheric deposition of sulfur (S) may provide another important anthropogenic contribution to the total S input of these wetlands, the extent depending on the S emission rates of the area (Gorham 1976; Johnson 1979; Schindler et al. 1980; Cook et al. 1986).

The resulting enhancement of SO_4^{2-} availability stimulates microbial SRR in anaerobic freshwater sediments. For marine sediments it has been shown that SO_4^{2-} reducers play an important role in the mineralization of organic matter (Jørgensen 1982). In addition, SO_4^{2-} reduction indirectly influences nutrient kinetics by interacting with phosphate (PO_4^{3-}) binding to iron (Fe). Disturbance of Fe $\sim PO_4^{3-}$ binding in both freshwater and marine sediments leads to the release of PO_4^{3-} to the water layer and undesirable eutrophication effects (Boström et al. 1982; Caraco et al. 1989; Roelofs 1991; Smolders and Roelofs 1993; Lamers et al. 1998*a*).

The high availability of SO_4^{2-} in marine systems is even thought to be the reason why biomass production is mostly not PO_4^{3-} limited here, provided that the availability of electron donors from microbial decomposition is not limiting SRR (Caraco et al. 1989).

In addition to eutrophication, increased SRRs may lead to the accumulation of dissolved sulfide (HS^-) in the sediment, generating a (phyto)toxic effect even at low concentrations (Smolders and Roelofs 1996; Lamers et al. 1998*a*). The actual concentration is, however, mainly governed by the availability of reactive Fe in the sediment, sequestering and detoxifying HS^- as FeS_x (Smolders et al. 1995; Lamers et al. 2001). The same may hold for PO_4^{3-}, whereby Fe counteracts sulfide-induced P mobilization.

Both eutrophication and HS^- toxicity form serious threats to freshwater wetland communities, and especially to freshwater peatlands, because the potential supply of electron donors provided by organic matter breakdown is generally high. In addition, these wetland types are generally characterized by the dominance of rooting aquatic macrophytes sensitive to HS^-. Most aquatic peatland habitats are shallow and so sediment biogeochemistry strongly controls nutrient cycling in the water layer. Species-rich peatlands are known to develop into monotonous systems due to SO_4^{2-} pollution, becoming dominated by fast-growing and HS^--resistant species (Lamers et al. 1998*a*). It is therefore of major importance to understand the biogeochemical changes in sulfate-polluted peatlands and the factors regulating the extent of disturbance.

The high variability of factors interacting with the effects of SO_4^{2-} enrichment in freshwater wetlands makes it very difficult to investigate these effects using correlations between data collected from different wetlands. Moreover, SO_4^{2-} concentrations of the surface water are regulated by influx, efflux, and consumption rates and are thereby useless as a descriptive variable for SRR and induced adverse effects. For this reason, field enclosure experiments were set up to investigate the effects of increased SO_4^{2-} concentrations in the water layer of freshwater peatlands. Because Fe is known to be a major determinant for the degree to which adverse SO_4^{2-} effects (eutrophication and HS^- accumulation) occur, two locations differing significantly in pore water Fe concentration were chosen. The difference is caused by the high discharge of Fe-rich groundwater in one of the locations. To enable a correct comparison, the surface water alkalinity of one location had to be adjusted to the level characteristic of river water used to raise the water tables in these wetlands. It was hypothesized that increasing SO_4^{2-} availability would lead to increasing HS^- concentrations in the sediment and higher PO_4^{3-} mobilization to the water layer in the low-Fe site. The high-Fe location was expected to show

Table 1. Surface water, sediment and pore water characteristics for both research locations (control values). Concentrations in μmol L^{-1}, alkalinity in μeq L^{-1}, unless indicated otherwise.

| | Tienhoven (TH) | | Weerribben (WR) | | |
	mean	(SEM)	mean*	(SEM)	ANOVA†
Surface water					
SO_4^{2-} (winter)	177	(6)	42	(3)	‡
SO_4^{2-} (summer)	107	(10)	54	(7)	†
HPO_4^{2-} (winter)	0.2	(0.0)	0.2	(0.0)	§
HPO_4^{2-} (summer)	0.8	(0.2)	0.5	(0.0)	§
alkalinity (winter)	4062	(129)	139/2057	(9/161)	‡/‡
alkalinity (summer)	1576	(113)	148/2384	(17/301)	‡/§
pH (winter)	7.97	(0.03)	6.07/7.91	(0.10/0.09)	‡/§
pH (summer)	7.90	(0.29)	6.24/8.00	(0.04/0.16)	†/§
Sediment					
Loss on ignition (%)	30.1	(1.4)	77.6	(2.6)	‡
Total Fe	41.3	(7.9)	7.4	(0.6)	‡
Total Al	92.3	(14.3)	6.4	(0.4)	‡
Total S	38.8	(6.4)	21.4	(2.0)	†
Fe/S (mol mol^{-1})	1.1	(0.1)	0.3	(0.1)	‡
Sediment pore water					
Alkalinity (winter)	6982	(540)	465/893	(95/32)	‡/‡
Alkalinity (summer)	6325	(355)	708/1067	(63/12)	‡/‡
pH (winter)	6.50	(0.05)	5.16/6.06	(0.03/0.18)	‡/§
pH (summer)	6.44	(0.07)	6.19/6.27	(0.07/0.06)	§/§
HPO_4^{2-} (winter)	0.6	(0.3)	1.3	(0.6)	§
HPO_4^{2-} (summer)	1.8	(0.5)	3.4	(1.2)	§
Fe (winter)	267	(85)	13	(1)	†
Fe (summer)	65	(16)	15	(3)	†
Al (winter)	2	(1)	4	(1)	§
Al (summer)	2	(0)	4	(1)	§
HS$^-$ (winter)	2	(0)	7	(1)	†
HS$^-$ (summer)	0	(0)	16	(2)	†
NH_4^+ (winter)	321	(22)	68	(18)	†
NH_4^+ (summer)	289	(58)	181	(41)	§
K$^+$ (winter)	50	(14)	44	(3)	§
K$^+$ (summer)	45	(15)	48	(4)	§

* Two values are given for alkalinity and pH: control treatment/2 mmol L^{-1} HCO$_3^-$ treatment (no SO_4^{2-} added).
† $p \leq 0.01$.
‡ $p \leq 0.001$.
§ Not significant.

only moderate or even no effects of SO_4^{2-} pollution because of the 'shielding' effect of Fe.

Methods—Site description: Two freshwater marshes were selected for the enclosure experiment. The first location was a small turf pond near Tienhoven (Utrecht, the Netherlands; 52°11′N, 5°06′E). Enclosures were installed in open water next to a floating helophyte vegetation (dominated by *Carex elata, Typha latifolia,* and *Lythrum salicaria*) in March 1997, and the experiment continued until January 1998. The arable grassland adjacent to the pond was fertilized with manure every year. The water layer was 0.4 m deep, above a fen peat layer of 0.7–0.8 m. The aquatic vegetation on this location comprised *Potamogeton trichoides, P. obtusifolius, Stratiotes aloides, Hydrocharis morsus-ranae, Elodea nutallii, Chara vulgaris,* and *Nitella flexilis.* The area received groundwater rich in Fe, originating from the higher Hilversum plateau. Seepage was indicated by the hydraulic head values recorded in piezometers with filters between 0.3 and

0.4 m below sediment surface. The second location was one of the many turf ponds (former turbaries) in the Weerribben National Park (Overijssel, the Netherlands; 52°48′N, 5°58′E), where the experiment was conducted from March 1998 until January 1999. At this location there was neither discharge nor recharge of groundwater (as indicated by the hydraulic head values of piezometers installed in the sediment), and dissolved iron concentrations in the sediment were low. The water layer was 0.9–1.1 m and the fen peat sediment (unharvested layer) between 2 and 2.5 m. The pond showed low abundance of *Nuphar lutea, Stratiotes aloides,* and *Nymphaea alba,* and the water was surrounded by a fringe of *Phragmites australis, Carex paniculata,* and *C. elata.* The chemical characteristics of both locations are presented in Table 1.

Experimental setup and sampling: For the Tienhoven (TH) experiment 15 transparent polycarbonate cylinders (diameter 1.0 m, height 1.75 m, open on both sides) were used as

enclosures for five different treatments in triplicate. The cylinders were gently pressed into the peat sediment to a depth of about 0.4 m below sediment surface. To prevent movement during storm events, all cylinders were anchored using lines and pegs. Inside each enclosure, two ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment) connected each to a 1.5-m polyethylene tubing (Dayco Imperial Poly-Flo 3 mm) with needles at the other end were placed at 10 cm below the sediment surface. Pore water samples were collected by inserting the needles of both tubes through the septa into the nitrogen-flushed vacuum infusion flasks (500 ml; 50 ml for sulfide sampling). The first sample of about 25 ml was discarded in order to correct for the dead volume of the sampling system. Overall sediment redox potentials were measured in triplicate in each enclosure at 5–10-cm depth (after stabilization of the value), using a platinum wire electrode, a Ag/AgCl (3 mol L^{-1} KCl) reference electrode at the water surface, and a portable pH/mV meter (Radiometer Copenhagen PHM201). Redox values were expressed as standard potentials (E_h) relative to a standard hydrogen reference electrode. The electrode readings were calibrated using a Metrohm standard redox buffer. Surface water samples were collected from 10 cm below water surface in a dark polyethylene bottle. In order to collect the samples without disturbing the sediment, a small inflatable boat (Seahawk, Intex) was used.

The following treatments were randomly assigned to the TH cylinders: no addition (control, C), 0.5 mmol SO_4^{2-} L^{-1} (0.5 S), 1 mmol SO_4^{2-} L^{-1} (1 S), 2 mmol SO_4^{2-} L^{-1} (2 S), and 4 mmol Cl^- L^{-1} (4 Cl). The chloride treatment was performed to test for possible anion replacement effects, the equivalents of charge per liter equaling that of the 2 S treatment. For all treatments sodium salts (pro analysi grade) were used, dissolved in stock solutions of which appropriate volumes were added, and gently mixed with the surface water without disturbing the sediment. In addition, pore water samplers were installed on three outside-control sites to check for possible enclosure artifacts.

The same setup was used for the Weerribben (WR) experiment, but here the cylinders were placed around a wooden raft, from which sampling was conducted. To avoid the enclosures from sinking too deep into the sediment, they were attached to a wooden frame (Fig. 1). All SO_4^{2-} treated cylinders additionally received 2 mmol HCO_3^- L^{-1} (sodium salt) in order to mimic the influence of allochthonous SO_4^{2-} enriched water, which generally shows this level of alkalinity (Roelofs 1991). Instead of a chloride treatment, a 2 mmol HCO_3^- L^{-1} only (HCO_3) treatment was used as an extra control. Pore water and surface water sampling were carried out as on the TH location. On both locations, concentrations of the added chemicals were restored to original levels after sampling.

Careful time analysis of the concentrations of Na and Cl in the cylinders showed that there was no loss of ions from inside the enclosures through the sediment on both locations.

For phosphorus fractionation, five sediment cores were collected outside the cylinders from the upper 10 cm using a metal underwater sediment corer (diameter 7 cm). Samples were transported to the laboratory in airtight bags and kept in the dark at 4°C until further processing within the same week.

Sample processing and chemical analyses: The pH was determined using a combined glass electrode (with standard Ag/AgCl electrode), and alkalinity was estimated by titrating part of the sample down to pH 4.2 using 0.01 mol L^{-1} HCl. After the addition of citric acid to a final concentration of 0.6 mmol L^{-1} in order to prevent metal precipitation, the extinction at 450 nm was measured for colorimetric background correction. The samples were stored (for a maximum of 3 weeks) in iodated 100-ml bottles at -28°C until further analysis. The concentration of free sulfide was determined in 10.5-ml sample collected in a 50-ml anaerobic bottle and fixed immediately (1 : 1 v : v) after collection with sulfide antioxidant buffer containing NaOH, Na-EDTA, and ascorbic acid. The concentration was measured on the same day according to Lamers et al. (1998*a*).

A sequential phosphorus (P) fractionation procedure of the sediment samples was performed according to Psenner et al. (1988). The buffered dithionite and hydroxide fraction were pooled and presented as Fe/Al fraction. Total P, Fe, Al, and S were determined after microwave destruction (Milestone MLS 1200 Mega) of 500 mg dry sample (dried at 105°C until constant weight) in 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%). Loss on ignition was determined after heating dry sediment samples at 550°C for 4 h in order to estimate the percentage organic matter. Concentrations of orthophosphate (SRP, soluble reactive P), NO_3^- (and NO_2^-), NH_4^+, and Cl^- were measured colorimetrically with Technicon AA II systems and corrected for background color signals (Lamers et al. 1998*a*). K was determined by flame photometry, and total concentrations of Ca, Mg, Fe, P, Al, and S in the moisture samples were determined by inductively coupled plasma emission spectrometry (Jarrell Ash IL Plasma-200). At the (relatively high) concentrations used in the experiment, the total S concentrations in the water layer provided a good estimate of sulfate because only a small percentage of the element is present in organic form. This was verified by parallel analysis of various samples for different treatments using capillary ion analysis (Waters Technologies), in which sulfate concentrations were shown to match the total S concentrations within the uncertainty of both methods. Net sulfate consumption rates were estimated from the differences in the amounts of SO_4^{2-} between the sampling dates (including additions). For each treatment and location, the average net sulfate consumption rate for the period between the beginning of April and the second half of October (in which both the PO_4^{3-} and the sulfide response occurred) was calculated from SO_4^{2-} disappearance rates between sampling times. The data presented are the means and standard error of the means (SEM) of these three (replicate) values. For both locations, no loss of anions by diffusion through the sediment could be detected. Therefore only SO_4^{2-} reduction, SO_4^{2-} consumption, and SO_4^{2-} binding could be responsible for SO_4^{2-} loss.

Data analyses: A repeated measures analysis was used to examine the response to the treatments, using the SAS procedure GLM (General Linear Models), model one-way AN-

Fig. 1.   Part of the experimental setup at the Weerribben location. Arrow indicates tubing connected to ceramic moisture samplers in the sediment.

OVA, for repeated measures (SAS 1989; Potvin et al. 1990). Dunnett's test was used as a posttest to examine whether PO_4^{3-} and sulfide (HS^-) concentrations had increased at a given time as a result of SO_4^{2-} treatment (one-sided). Data were log-transformed to make the variances less dependent on the sample means and to fit a normal distribution. Differences between P fractions, sulfate consumption rates, and pore water and surface water characteristics were tested using a two-tailed ANOVA at the 0.05 confidence limit. For clarity of presentation, the figures present the arithmetic means and their SEMs.

Results—Site control comparison: The most important biogeochemical characteristics for both locations are presented in Table 1. Control SO_4^{2-} concentrations in surface water of both marshes were below 200 μmol L^{-1} and lowest for Weerribben (WR). Alkalinity and pH in Tienhoven (TH) were higher for both surface and pore water. After bicarbonate addition to the surface water, surface water alkalinities and pH were equal. There were no significant differences between PO_4^{3-} concentrations in surface water and sediment pore water, but Fe concentrations in pore water and total values in the sediment were much higher in the TH marsh. Pore water NH_4^+ concentrations were higher on this location in winter, but not in summer. There were no differences between the K concentrations of both marshes.

P fractionation showed that total P concentrations were four times as high in the TH sediment as compared to WR (Fig. 2). By far the largest fraction was organic P, accounting for 85% and 91% of total P for TH and WR, respectively.
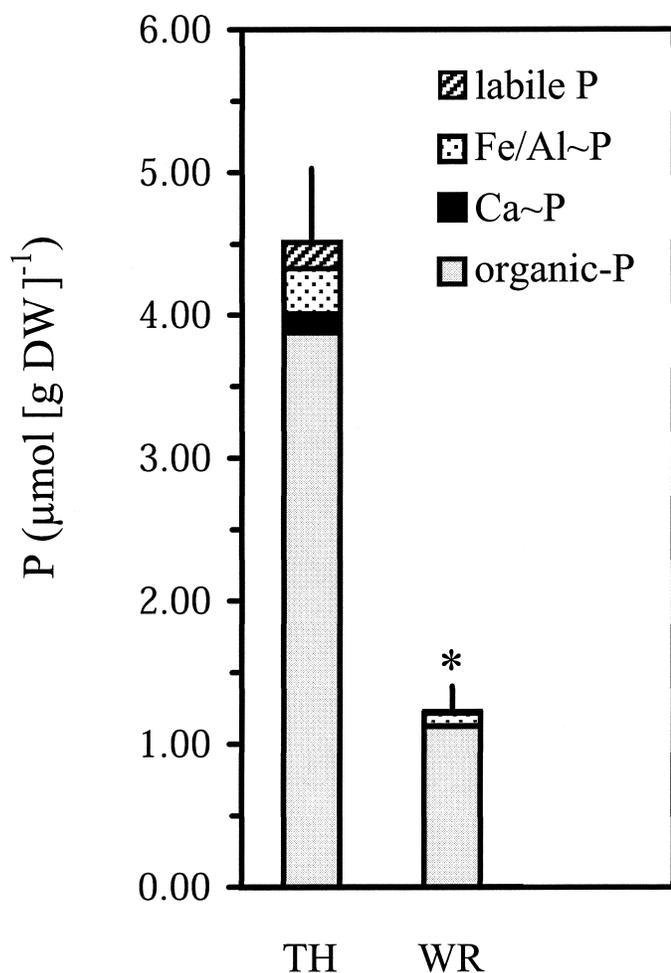
Fig. 2. Total phosphorus concentrations, and fractionation (Psenner et al. 1988), for the sediments of Tienhoven (TH) and Weerribben (WR) marsh. *$p = 0.0006$ (ANOVA).

The percentage of P bound to Fe and aluminum (Al) was equal (± 7–7.5%) for both locations. However, dithionite-extracted P, representing reductant soluble P (mainly from iron hydroxide surfaces; Psenner et al. 1988), accounted for almost 50% of this fraction in TH; while in contrast almost no P could be detected in this fraction for WR (results not shown). In TH, 3% of P was bound to Ca against 0% in WR. Finally, not only the absolute, but also the relative pool of labile P was higher in TH as compared to WR (4% and 2%, respectively).

Induced sulfate consumption rate: The addition of SO_4^{2-} resulted in remarkable differences between both marsh locations. Independent of the added concentration, the net consumption rates were raised from undetectable in the control to 2.5–3.5 mmol m^{-2} d^{-1} in TH (Fig. 3). There were no significant differences between the levels of addition. The WR site showed a completely different reaction to SO_4^{2-} addition, the consumption rates increasing rapidly (and significantly) with increasing SO_4^{2-} concentration to a rate of 11 mmol m^{-2} d^{-1}. At 0.5 mmol SO_4^{2-} L^{-1}, however, the net
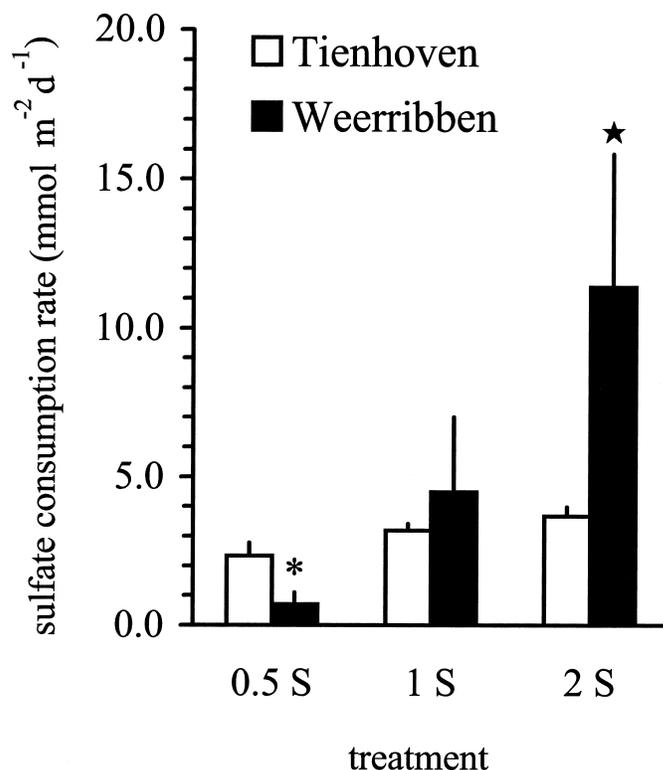
Fig. 3. Net sulfate consumption rates for both locations as a result of the addition of 0.5, 1.0, or 2.0 mmol SO_4^{2-} L^{-1} (indicated as 0.5 S, 1 S, and 2 S, respectively. *$p \leq 0.10$ (between sites); ★ $p \leq 0.05$ (between 0.5 S and 2 S).

SO_4^{2-} consumption rate was significantly lower than TH, but still much higher than its control (undetectable).

Eutrophication: In TH, PO_4^{3-} concentrations in the water layer were raised by the addition of SO_4^{2-} (Fig. 4). There was a characteristic seasonal pattern that correlated with temperature for all groups, but the level of PO_4^{3-} mobilization was much higher for the SO_4^{2-} treatments. Although the overall treatment effect was not significant, there was a significant interaction between time and treatment (Table 2), and 1 S and 2 S treatments were significantly higher during several weeks. Higher concentrations of SO_4^{2-} tended to induce higher PO_4^{3-} concentrations in the water layer. In the sediment pore water, collected at -10 cm, no differences could be detected between control and treatments (results not shown). In late autumn and winter, PO_4^{3-} concentrations in the surface water decreased again for all treatments. Cl treatment proved to be unable to boost the PO_4^{3-} concentration. The concentrations of NH_4^+, NO_3^-, and K in surface water and pore water remained equal to the control values (results not shown).

In WR, by contrast, there was neither a seasonal pattern in PO_4^{3-} concentrations nor a response to SO_4^{2-} treatment. PO_4^{3-} concentrations remained low throughout the year, although the temperature profiles mimicked those of the TH location. The bicarbonate treatment also showed the same response as the control. As in TH, concentrations of NH_4^+,
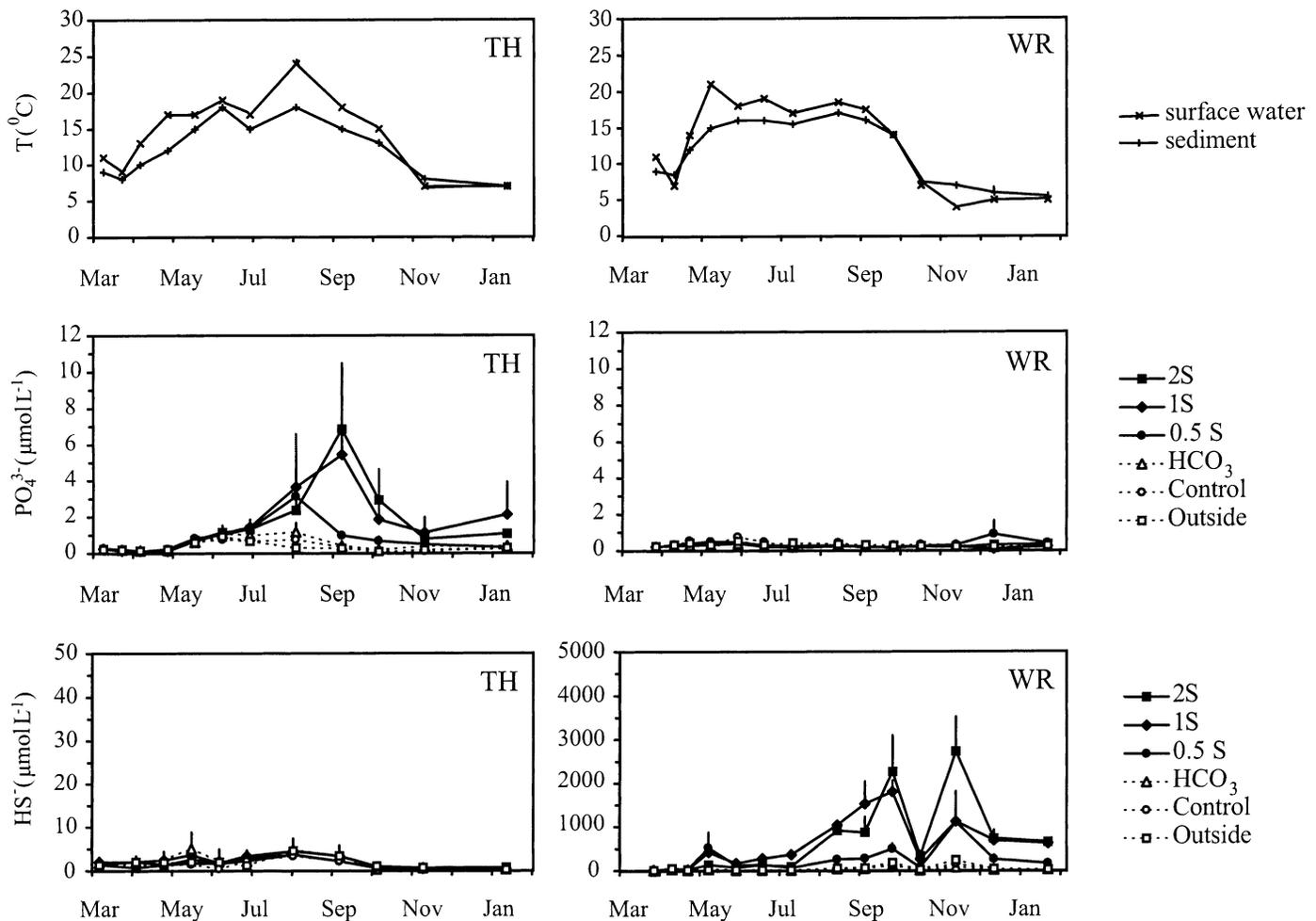
Fig. 4.   Temperatures of surface water and sediment and concentrations of phosphate and sulfide (HS^-) in the surface water at Tienhoven (TH) and Weerribben (WR) as a result of the addition of 0.5, 1.0, or 2.0 mmol SO_4^{2-} L^{-1} (indicated as 0.5 S, 1 S, and 2 S, respectively). Outside refers to samples taken outside the enclosures. In TH, an additional series was treated with 4 mmol chloride L^{-1} (4 Cl). In WR, all SO_4^{2-} treatments received 2 mmol L^{-1} of bicarbonate. One extra series received only bicarbonate treatment (HCO_3). PO_4^{3-} was significantly higher than the control in August and October for 2 S ($p \leq 0.05$) and in July for 1 S. As from June, all SO_4^{2-} treated groups had significantly higher sulfide concentrations than the control ($p \leq 0.05$).

Table 2.   Repeated measures analysis of variance (general linear models) for the data shown in Fig. 4.

Dependent variable	Independent variable			
	Treatment	Time	Interaction	
Tienhoven (TH)				
PO_4^{3-} (water layer)	2.10*	14.47†	1.87‡	($df = 5, 11, 55$)
HS^- (pore water)	0.22*	18.92†	0.85*	
Weerribben (WR)				
PO_4^{3-} (water layer)	2.18*	6.45†	1.18*	($df = 5, 13, 65$)
HS^- (pore water)	37.07†	90.16†	4.76†	

* Not significant.
† $p \leq 0.0001$.
‡ $p \leq 0.05$.

NO_3^-, and K in surface water and pore water were not influenced by the treatments (results not shown).

For both locations, the controls outside the cylinder did not differ from the inside controls for PO_4^{3-} and temperature, so it could be concluded that there was no artifact generated as a result of the experimental setup.

Dissolved sulfide accumulation: Although net SO_4^{2-} consumption rates were strongly raised by the addition of SO_4^{2-}, there was no extra accumulation of free HS^- in the TH sediments (Fig. 4). All treatments showed the same seasonal profile, concentrations staying below 5 μmol L^{-1}. The WR sediments, on the other hand, accumulated free HS^- to high levels. HS^- concentrations were significantly higher in all SO_4^{2-} treatments as compared to the controls from June until the end of the experiment. The higher the concentration of SO_4^{2-} added, the more HS^- accumulated. Although HS^- concentrations decreased in winter, they remained high in all SO_4^{2-} treatments.

Discussion—The addition of 0.5 mmol SO_4^{2-} L^{-1} to the water layer (background concentrations 180 and 40 μmol L^{-1}, respectively) raised net SO_4^{2-} consumption rates from undetectable levels to 2 mmol m^{-2} d^{-1} in Tienhoven (TH) and 1 mmol m^{-2} d^{-1} in Weerribben (WR; Fig. 3). The net SO_4^{2-} consumption rate is the resultant of SO_4^{2-} reduction, heterotrophic SO_4^{2-} consumption (microbes, vegetation, fauna), SO_4^{2-} binding in the sediment, diffusion through the sediment, and sulfide (re)oxidation. The comparison of water layer and sediment pore water did not reveal any major anion fluxes, and sulfate binding at anion exchange sites is low in fen peat at pH 6–6.5 (Scheffer and Schachtschabel 1992). Precipitation is unlikely, as the solubility products for calcium and magnesium sulfate were not reached. Although the reoxidation of sulfide from iron sulfides like pyrite is very unlikely because these compounds are very stable in reduced sediments, sulfide (re)oxidation in the sediment top layer may strongly influence the calculated net sulfate consumption rates (Holmer and Storkholm 2001).

In order to compare both locations, 2 mmol HCO_3^- L^{-1} was added in WR because it is known that sulfate reduction rates (SRRs) are strongly reduced at low (bicarbonate) alkalinity (Roelofs 1991). HCO_3^- treatment had no effect on any variables other than alkalinity and pH (Table 1). There was a clear difference in response to a further increase in the SO_4^{2-} concentration. Whereas net SO_4^{2-} consumption rates in TH remained equal, the values for WR strongly increased with increasing SO_4^{2-} concentration. This seems to indicate that SRRs were rate limited by the availability of the electron acceptor SO_4^{2-} in WR, but not in TH. Another explanation, however, may be that sulfide reoxidation is more efficient in TH, e.g., by oxidized Fe that is highly available. Pore water analysis, however, showed very low free sulfide concentrations at this location due to FeS_x formation. The high Fe concentrations in TH, but not in WR, may imply that Fe (III) reduction is an important microbial redox process at the first location. SO_4^{2-} addition, however, accelerated SO_4^{2-} consumption, indicating that SO_4^{2-} reducers were able to compete with Fe reducers. In sediments with high doses of SO_4^{2-}, overall redox potential (E_h) was lowered from values around +150 mV to values between 0 and +50 mV in the WR sediments, while this potential remained around +150 mV in the TH sediments (results not shown). Although the values must be regarded with caution, as potentials measured with electrodes in natural sediments do not always match actual E_hs (Stumm and Morgan 1996; Drever 1997), they may indicate redox buffering by Fe reducing activity in TH possibly impeding SRRs.

At all concentrations of SO_4^{2-} added, there was strong PO_4^{3-} eutrophication of the surface water in TH (Fig. 4). This response, showing the resultant of PO_4^{3-} mobilization and consumption, was much more distinct than the natural PO_4^{3-} pattern during the growing season in which microbial activity is stimulated by increasing temperature. High consumption rates earlier in the growing season (mainly by macrophytes and algae) will most probably have slowed down the SO_4^{2-} induced increase in PO_4^{3-} concentrations. It is very unlikely that PO_4^{3-} mobilization was caused by anion replacement at sediment binding sites. Aspecific ion binding capacity is generally very low at circumneutral pH, and

PO_4^{3-} binds much more strongly to these binding sites (Scheffer and Schachtschabel 1992). Moreover, Cl treatment at equal normality as the highest SO_4^{2-} treatment showed no PO_4^{3-} mobilization at all. It is known that the production of sulfide disturbs iron-phosphorus cycling. Fe (III) is reduced to Fe (II) in iron phosphate (strengite), iron(hydr)oxide-phosphate, and humic-iron-phosphate complexes, by which PO_4^{3-} is released (Sperber 1958; Patrick and Khalid 1974; Boström et al. 1982; Smolders and Roelofs 1993; Lamers et al. 1998*a*). In addition, sulfide binds iron, forming iron sulfides like FeS and FeS_2 and strongly reducing the pool of free iron available for PO_4^{3-} binding (Boström et al. 1982; Caraco et al. 1989; Roelofs, 1991; Smolders and Roelofs 1993; Lamers et al. 1998*a*). There was no increase in PO_4^{3-} concentrations at 10 cm below the sediment surface, strongly suggesting that the (re)mobilization processes only took place in the upper layer. Since organic matter concentrations in the surface water were low, and nitrogen and potassium concentrations (both not expected to limit primary production) did not increase during the season (results not shown), mineralization processes in the water layer seem to be of minor importance. Although Fe concentrations in sediments may be high enough to immobilize PO_4^{3-} to a large extent (like in TH), increased SRRs due to high SO_4^{2-} concentrations are known to counteract this effect by FeS_x formation, leading to net PO_4^{3-} mobilization (Roden and Edmonds 1997). The high total Al concentrations in the TH sediment indicate that a significant part of the metal-bound PO_4^{3-} may be bound to Al. Sulfide-induced PO_4^{3-} mobilization from this source is, however, unlikely since Al does not precipitate with sulfide (Stumm and Morgan 1996).

The results of the experiment imply that the use of riverine water to compensate for water shortage, containing SO_4^{2-} concentrations of 0.5 mmol L^{-1} and higher, can indeed induce P eutrophication in fens and marshes (Roelofs 1991; Smolders and Roelofs 1993; Lamers et al. 1998*a*). In addition, increased atmospheric SO_4^{2-} fluxes, SO_4^{2-} mobilization by desiccation, and chemolithotrophic denitrification contribute to (strongly) increased SO_4^{2-} concentrations in groundwater and surface water. Since P is the main nutrient limiting primary production in many freshwater environments, internal P mobilization due to SO_4^{2-} pollution is of major concern. Especially in shallow aquatic fen systems, such as fen lakes and marshes, the interaction of SO_4^{2-} from the water layer with sediment processes is extremely important. There is only a moderate dilution effect because the water column is relatively small. In addition, stratification as seen in deeper lakes is absent and all nutrients released from the sediment will cycle through the entire water column.

The comparison of both marshes indicated, however, that equal or even higher SRRs do not necessarily lead to P mobilization. In the WR experiment, no increase in the PO_4^{3-} concentration in the water layer could be detected. The fact that the water layer was twice as deep as in TH cannot account for the lack of detection, as shown by Fig. 4. The temperature values in time were equal for both sites. The lack of response must therefore lay in the sediment characteristics. Indeed, P concentration of the WR sediment was less than 25% of the value of TH (Fig. 2). Moreover, the amount of P that could be mobilized from Fe ~ P and Al

~ P complexes was much lower. In TH, 50% of this fraction was dithionite extractable (mainly composing of iron (hydr)oxide ~ P), while this fraction contained hardly any P for WR (results not shown). This means that sulfide-driven phosphate mobilization could be expected to be much more important in TH as compared to WR, which is in accordance with the results. P concentrations of the sediments on both mesotrophic locations were, however, much lower than those in more eutrophic lakes, ranging between 18 and 222 μmol g^{-1} (Pettersson et al. 1988). The upward flow of groundwater in TH is likely to have enhanced mass flow through the sediment, thereby facilitating PO_4^{3-} transfer from the peat to the water layer.

An additional factor influencing PO_4^{3-} mobilization is the pH. In TH, pH of the water layer increased from 7.5 to 9–9.5 during the growing season, which was strongly correlated to temperature (results not shown) as a result of photosynthetic activity of the aquatic vegetation (uptake of carbon dioxide and bicarbonate; Prins et al. 1979; Søndergaard 1988). PO_4^{3-} binding is reduced by this rise in pH (Patrick et al. 1973). Although this does not explain differences between treatment (equal pHs), it might intensify the effects of sulfide production on PO_4^{3-} mobilization. In WR, however, a similar increase in pH due to bicarbonate addition did not induce a PO_4^{3-} response. In contrast to poorly buffered lakes, where alkalinization is suggested to be the primary mechanism for sulfate-enhanced P mobilization (Curtis 1989), direct interaction of sulfide with Fe ~ P complexes appears to be largely responsible for the P mobilization observed in the moderately buffered waters in the present study.

The relatively high free Fe concentrations in the TH sediment did not prevent P eutrophication. Fe did, however, sequester all free sulfide produced in sediment pore water and kept its concentration below 5 μmol L^{-1} (Fig. 4). The higher Fe/S ratio in the sediments at this location indicate a higher Fe availability for FeS_x formation and concomitant sulfide immobilization. In WR, in contrast, extremely high free sulfide concentrations were reached as a result of SO_4^{2-} addition. Sulfide has been shown to be toxic for many aquatic macrophytes (Koch and Mendelssohn 1989; Koch et al. 1990; Armstrong et al. 1996). For *Stratiotes aloides,* an aquatic macrophyte characteristic for mesotrophic freshwater marshes, it has been shown that levels as low as 10 μmol L^{-1} are toxic to the roots (Smolders and Roelofs 1996). Root parts growing into the peaty sediment clearly show sulfide toxicity, becoming black, slimy, and unfit for nutrient uptake from the sediment. Only the roots in the surface water will survive, provided there is no sulfide accumulation in (deeper) water layers. In the present experiment, free sulfide could not be detected in the surface water (results not shown). These records of sulfide toxicity were supported by the observation that seedlings of the rooting species *Nuphar lutea,* found in all controls treatments, did not occur in the SO_4^{2-} treated WR enclosures. However, the sensitivity of a wetland plant species to free sulfide not only depends on the actual sulfide levels in the rhizosphere but also on detoxification mechanisms like radial oxygen loss. In addition to generating toxic effects, high sulfide concentrations in freshwater sediments may also lead to higher fluxes of volatile organic sulfur compounds to the atmosphere due to the microbial methylation of hydrogen sulfide (Lomans et al. 1997).

The present experiment shows that increased SRRs in marshes as a result of increased SO_4^{2-} input from river water compensating for desiccation, FeS_x oxidation during desiccation, FeS_x oxidation due to nitrate pollution, and atmospheric S pollution form a strong threat to freshwater wetlands by the induction of P eutrophication and sulfide accumulation. The extent to which both problems may occur may, however, varies between wetlands. It was found that the level of P eutrophication was mainly determined by P concentration of the sediment and not by dissolved Fe in the pore water (as stated in our hypothesis). As P eutrophication leads to a strong and undesired vegetation shift, knowledge about the role of SO_4^{2-} in eutrophication is highly desirable. The occurrence of sulfide accumulation was, in contrast, completely determined by Fe. Phytotoxicity to rooting species due to SO_4^{2-} pollution is therefore regulated by Fe availability in the sediment. The present experiment clearly shows that the elucidation of key factors controlling the response to SO_4^{2-} pollution makes it possible to understand and predict the differences observed between freshwater wetlands.

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