Sulfate-Induced Eutrophication and Phytotoxicity in Freshwater Wetlands

Leon P. M. Lamers,* Hilde B. M. Tomassen, and Jan G. M. Rölofs

Research Group Environmental Biology, Department of Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, the Netherlands

In recent decades, sulfate concentrations in many European freshwater wetlands have increased by 10-fold or more, due mainly to the use of sulfate-polluted river water to compensate for water shortage in these areas. To test the effect of sulfate enrichment, a mesocosm experiment was set up, using waterlogged soil cores, intact with vegetation, from a mesotrophic fen meadow. During sulfate addition at environmentally relevant levels (0, 2, and 4 mmol L⁻¹), phosphate concentration and alkalinity of the pore water rapidly rose due to increased sulfate reduction rates. Free sulfide accumulated to levels toxic to several wetland plant species and biomass regrowth after harvesting was significantly lower on treated soils, especially for Carex species. Eventually, the concentrations of ammonium, phosphate, and potassium increased strongly in the treated soils due to reduced uptake by plants and extra mineralization. Sulfate availability was rate limiting, until the supply of readily decomposable organic matter became limited. It is argued that the significance of the observed changes in free sulfide concentrations and in the rate of nutrient mobilization should be recognized, and that these effects can be as important as direct eutrophication caused by the import of nutrients. The reported changes may severely influence the plant species composition of freshwater wetlands.

Introduction

Nutrient kinetics in wet soils and sediments rich in organic matter are highly influenced by the rate of microbial mineralization (1). For chemoorganotrophs, the supply of terminal electron acceptors like oxygen (during desiccation), nitrate, or sulfate (in reduced sediments) is essential, in addition to the availability of oxidizable organic compounds derived from readily decomposable organic matter (2). A high mineralization rate directly leads to a higher nutrient availability for plants. Jørgensen (3) showed that sulfate-reducing bacteria play an important role in the mineralization of organic matter in marine sediments. Sulfate reduction, however, also affects nutrient kinetics indirectly. Sulfide, produced by sulfate reduction, interferes with iron-phosphate binding in soils and sediments due to the formation of iron sulfides. In this way, phosphate is released, both in marine and in freshwater sediments (4–7). The literature shows that the amount of phosphate released depends on the availability of sulfate. In saline systems, this might be the reason why biomass production is generally not limited by phosphate (5). In freshwater systems, sulfate reduction rates are generally low, because of the modest availability of sulfate.

In many lowland regions of Europe, groundwater and surface water levels have fallen by a few decimeters up to several meters in recent decades. This is due to hydraulic operations for agricultural purposes and increased water extraction for agricultural, industrial, and domestic use. To compensate for the concomitant shortage of water, river water is used on a large scale in many freshwater wetlands, particularly in the regions of large lowland rivers. The use of such water in the restoration of groundwater tables in agricultural areas and nature reserves and for the flooding and waterlogging of desiccating natural wetlands has led to severe changes in soil and surface water quality, including an increase in the abundance of nutrients and macroions because of river pollution (6, 8–11). Initially, the concomitant eutrophication of many wetlands was blamed entirely upon the import of nutrients with river water. Recent research, however, suggests that increased import of macroions like sulfate and bicarbonate plays a major role in the observed eutrophication (6, 7, 12, 13). In recent decades, average sulfate concentrations (in incoming) polluted surface- and groundwater of freshwater wetlands have risen from less than 0.1 mmol L⁻¹ to values over 0.5–1.5 mmol L⁻¹, and even to values over 3 mmol L⁻¹. This is caused by anthropogenic sulfate input into rivers (including mining activities), increased atmospheric sulfur input, and the use of sulfate-containing fertilizers, in addition to the weathering of geological sulfur deposits (6, 14, 15). Furthermore, the desiccation of wetlands strongly promotes the oxidation of reduced sulfate compounds, leading to high sulfate concentrations in ditches and rivers receiving the drainage water (16, 17). Besides causing eutrophication, the increased supply of sulfate may also lead to sulfide toxicity to the roots of aquatic plants (13). Smolders et al. (18) demonstrated that phosphate mobilization and sulfide toxicity in sulfate-rich sediments can be prevented by iron addition, indicating the importance of free iron availability in sediments for binding of both.

The aim of the present study was to analyze the effects of increased sulfate pollution on the biogeochemistry of anoxic peaty soils and the consequences of biogeochemical changes for growth and survival of characteristic plant species. A long-term mesocosm experiment was set up using intact soil cores, including the vegetation, from a mesotrophic wetland meadow. We hypothesized that increased sulfate concentrations, at levels similar to those in polluted freshwater wetlands, would induce the mobilization of nutrients and the accumulation of free sulfide, due to enhanced sulfate reduction rates. Moreover, it was also hypothesized that these changes would influence vegetation growth.

Experimental Section

Experimental Design and Treatments. The experiment was carried out between November, 1994, and May, 1995. Sods were collected from a mesotrophic wetland meadow in the nature reserve “De Bruuk” near Nijmegen, the Netherlands (51°45’ N, 5°58’ E). The soil at this location was classified as Rhodic Humaqual. The upper 12 cm, used for the experiment, included moderately decomposed peat containing loam and living roots. The grassland is annually mown for hay making in late summer, and the vegetation zone is dominated by Carex nigra. The vegetation can be considered Caricion nigrae (19). In contrast, the adjacent zone is flooded...
with sulfate-rich ditch water for a large part of the year and is dominated by *Glyceria maxima*, indicating a higher trophic status.

In total, 18 sods (diameter 18 cm, depth 12 cm) were cut to fit tightly into plastic containers. Perforations in the bottom of the containers were covered from inside with plastic gauze to prevent loss of soil. A few hours after putting the sods into the containers, they were transported to a climate room where each container was suspended in a larger plastic container (12 L). The climate room had a light level of 110 μeinsteins m⁻² s⁻¹ with a daily photoperiod of 12 h, an ambient air temperature of 20 °C, and air humidity of 50-60% saturation. Each experimental flow-through unit received water from its own stock through black silicone tubes, at a flow speed of 10 L/week maintained by peristaltic pumps. The water level in the outer container could be manipulated by changing the overflow level (Figure 1).

During the acclimatization period of 3 weeks, the water level was maintained at 8 cm below surface level (overflow 1), corresponding to the field level recorded when the sods were collected. The basic composition of the surface water used previous to treatment was representative of moderately alkaline surface water, however, without nitrogen, phosphorus, and sulfate (Table 1). During acclimatization, inflow tubes were placed at the bottom of the outer containers. After this period, water level was raised to 1 cm above the soil surface over 3 days by setting a higher overflow level. Replicate redox potential measurements showed that com-

**FIGURE 1.** Experimental setup for one sod including vegetation. The water level in the soil could be manipulated by using different overflow levels.

**TABLE 1. Chemical Composition of the Basic Medium Used (in micromoles per liter)**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂</td>
<td>2000</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>400</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2000</td>
</tr>
<tr>
<td>KCl</td>
<td>5</td>
</tr>
<tr>
<td>Mn</td>
<td>0.9</td>
</tr>
<tr>
<td>Zn</td>
<td>0.7</td>
</tr>
<tr>
<td>Cu</td>
<td>0.2</td>
</tr>
<tr>
<td>B</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*For the sulfate treatments, either 2000 or 4000 μmol L⁻¹ Na₂SO₄ was added.*
plete and uniform water saturation existed in the soils. From this time (0 weeks), three different sulfate concentrations (added as Na2SO4) were used in addition to the basic composition: 0, 2, and 4 mmol L−1 (indicated as [O], [S2], and [S4]). Each experimental group consisted of six replicates, randomly distributed over the 18 units. During waterlogging, the inflow tubes were placed in the center of the top of the inner container, enabling a vertical water flow through the soil (Figure 1).

Three soil moisture samplers (Rhizion SM 5-10 cm; Eijkelkamp Agrisearch Equipment) were placed in each container, in order to collect a representative pore water sample and to prevent the extraction of water from the outer compartment. Soil moisture was collected by connecting vacuum infusion flasks (30 mL) to each sampler. The first 5 mL collected was discarded to exclude the internal stagnant sampler water. The three subsamples were pooled after the bottles had been almost completely filled. Variation among the subsamples was moderate (standard deviation, at most, 10–15% of the means for all parameters measured), showing that sulfate was evenly distributed throughout the soil.

In order to quantify vegetation regrowth, the vegetation was cut 4 times during the experiment, at 2 cm above the soil surface.

**Analysis of Soil Pore Water.** Soil redox potential measurements were carried out in triplicate in each pot at 5 cm depth, using a platinum wire electrode and a Ag/AgCl (3 mol L−1 KCl) reference electrode. Values were converted to the potential relative to the normal hydrogen reference electrode (Eh). The pH was determined with a standard KCl pH-electrode, and alkalinity was estimated by titrating part of the sample down to pH 4.2 using 0.01 mol L−1 HCl. After adding a few grains of citric acid to prevent precipitation of metal ions, color at 450 nm was measured for colorimetric backround correction. The samples were stored (maximal 3 weeks) in isodated polyethylene bottles (100 mL) at −28 °C until further analysis.

The concentration of free sulfide was determined in a 10 mL subsample fixed immediately after collection with sulfide antioxidant buffer containing sodium hydroxide, sodium EDTA, and ascorbic acid (20). A sulfide ion-specific Ag electrode and a double junction calomel reference electrode were used (6).

The concentrations of o-phosphate, nitrate (and nitrite), ammonium, and chloride in the pore water samples were measured colorimetrically with Technicon AA II systems, using ammonium-molybdate (21), hydrazinesulfate (22), salicylate (23), and ferriammoniumsulfate (22), respectively. The data were corrected for color caused by humic acids. Sodium and potassium were determined by flame photometry (Technicon FlamePhotometer IV). Total concentrations of calcium, magnesium, iron, aluminium, manganese, zinc, sulfur, and silicon in the samples were determined by inductively coupled plasma emission spectrometry (Jarrell Ash IL Plasma-200). At the high sulfate concentrations used, total sulfur gives a good estimate of sulfate, because only a small percentage of sulfur is present in organic form.

**Analysis of Plant Biomass.** After cutting, the vegetation was sorted into six groups: Carex sp. (in particular C. nigra; also some C. disticha), Juncus acutiflorus, Galium palustre, Ranunculus flammula, Gramineae (Anthoxanthum odorum, Holcus lanatus, G. maxima, Festuca rubra), and a rest group (Equisetum palustre, Cardamine pratensis, Myosotis palustris, and others). After drying at 70 °C until constant weight was reached, samples were weighed. Nitrogen content was determined in dry samples, ground in liquid nitrogen, using a C-N-S analyzer (Carlo Erloc Instruments NA1500). Phosphorus content was determined in diluted destruates by ICP-emission spectrometry (Jarrell Ash IL Plasma-200). For the latter, samples were ground, mixed with concentrated H2SO4 and incubated at room temperature for 24 h, and subsequently heated to 150 °C and digested by slowly adding 30% H2O2.

**Data Analysis.** As samples were collected several times from the same units, a repeated measures analysis was used to examine the response to treatments, the time effect (overall changes during the experiment), and the interaction (differences in time effects among treatments) (24). The results were analyzed using the SAS procedure GLM, model one-way ANOVA, for repeated measures (25). To compensate for uneven time intervals, an orthogonal polynomial transformation was used, after log-transformation of the data to make the variances less dependent on the sample means and to make the data fit better to the normal distribution. Differences at a given time were analyzed by a Tukey post test at the 0.05 confidence limit (25). For clarity of presentation, the means and standard errors presented in the figures represent the nontransformed data.

**Results**

**Soil Response to Waterlogging.** The soil response to waterlogging is illustrated by the control [O] treatment (Figure 2). After raising the water table, soil redox potential (Eh) decreased from 100 mV to values of about 0 mV. There was a 4-fold increase in iron concentration of soil pore water after 2 weeks, after which the concentration reverted to the original value. Alkalinity rose to 1500 μequiv L−1, which was less than the value of the experimental medium, containing 2000 μequiv L−1. o-Phosphate concentration increased 10-fold from approximately 0.5 to 5 μmol L−1, while ammonium levels showed little change. Potassium rose initially, but dropped again to low levels after the first week. Calcium and magnesium concentrations increased in 2 weeks to the concentrations of the medium used.

**Soil Response to Sulfate Addition.** Soil redox potential (Eh) dropped to levels below −50 mV after 4 weeks due to sulfate treatment (Figure 2, Table 2). Eh further decreased to −150 mV after 10 weeks for the 4 mmol L−1 [S4] treatment and after 19 weeks for the 2 mmol L−1 [S2] treatment, both significantly lower than the [O] treatment. Sulfate concentrations in soil pore water were half the amount added to the media of both sulfate treatments during the first 20 weeks. After this time, sulfate concentration increased to 3500 μmol L−1 in the [S4] treatment, while it remained at 1000 μmol L−1 in the [S2] treatment. After 7 weeks, alkalinity significantly increased by about 1000 μequiv L−1 for [S2] and 2500 μequiv L−1 for [S4], as compared to the control treatment. As a result, pH was slightly higher in both sulfate treatments, as compared to [O]. Iron concentrations were lower as a result of sulfate addition, with almost no detectable iron levels being reached after 27 weeks [S4] treatment. The values for [S4] were significantly lower than for [O] after 10 weeks, and those for [S2] after 27 weeks.

Phosphate concentrations increased markedly in both [S2] and [S4], as compared to the control treatment (Figure 2, Table 2). Differences with [O] were significant after 7 weeks for [S4], and after 27 weeks for [S2]. Both ammonium and potassium concentrations began to increase after 10 weeks of sulfate treatment. For ammonium, there was a significant difference with [O] after 10 weeks for [S4] and after 29 weeks for [S2]. For potassium, the differences with [O] were significant after 10 weeks for [S4] and after 27 weeks for [S2]. Nitrate concentrations were low, approximately 5 μmol L−1 for all treatments. Free sulfide concentrations in soil pore water rose to 10 μmol L−1 for [S2] and to as much as 20 μmol L−1 for [S4] (both significant after 2 weeks). In the [O] soils, the sulfide concentration remained below 5 μmol L−1.

Magnesium concentrations were lower due to sulfate treatment, while calcium concentrations did not change significantly (Figure 2, Table 2). Zinc concentrations were...
FIGURE 2. Soil pore water characteristics during 32 weeks of waterlogging with either 0, 2, or 4 mmol L$^{-1}$ sulfate (indicated 0, 2, 4; white, grey, and black markers, respectively). Means are given, with their standard error ($n = 6$).
lowered by the treatment, but for aluminium, manganese, and silicon, no differences were detected between the treatments (Table 2).

Response of the Vegetation. After cutting, regrowth of above ground biomass at the end of the 10 week intervals corresponded well to the original biomass for the [0] treatment, although the contribution of the various species corresponded well to the original biomass for the [0] treatment. Carex biomass, however, remained constant. Biomass production was significantly lowered for sulfate-treated soils (Table 3). At weeks 21 and 32, total biomass regrowth was lower in the [S4] treatment, although the contribution of the various species corresponded well to the original biomass for the [0] treatment. Carex regrowth was negligible.

Discussion

Effects of Increased Sulfate Concentrations. Nutrient kinetics were examined by pore water analysis, because nutrient concentrations in pore water are a good reflection of nutrient exchange processes in soils and benthic sediments (26). The experimental design made it possible to distinguish between the effects of raising the water table and of increased sulfate availability. It is well-known that waterlogging leads to mobilization of iron and phosphate in soils. Due to limited access of oxygen to the soil and the concomitant shift to other electron acceptors available for soil microorganisms, redox potential decreases and alkalinity is generated (27). Simultaneously, the concentration of free iron increases, and phosphate is mobilized (28).

An increase in sulfate availability clearly accelerated microbial sulfate reduction rates in the waterlogged soils, as indicated by the loss of sulfate, the increase in alkalinity (29–31) and the production of sulfide. Concentration stoichiometry in soil pore water in both sulfate treatments corresponded well to the reaction for sulfate reduction (32):

$$SO_4^{2-} + 2CH_2O \rightarrow HS^- + HCO_3^- + CO_2 + H_2O \text{ (pH 6.4)}$$

Bicarbonate, which accounts for the observed alkalinity changes, is generated at a 1:1 ratio to sulfate consumption at the carbon dioxide equilibrium for pH 6.4. At the 4 mmol L$^{-1}$ SO$_4^{2-}$ [S4] treatment, sulfate reduction rates doubled as compared to the 2 mmol L$^{-1}$ treatment [S2], showing that sulfate availability was the limiting factor. After about 20 weeks, however, sulfate concentrations for [S4] began to rise again so that, at the end of the experiment, there was no longer any difference between the alkalinitities of both sulfate groups (Figure 2). The rate of sulfate reduction decreased, indicating either increased competition between sulfate reducers and microorganisms using other electron acceptors, sulfide toxicity, or reduced availability of oxidizable organic substrates. As the availability of oxygen, iron, and nitrate was very limited, the first alternative is not plausible. Toxicity of the sulfide produced is also not very likely, as much higher sulfide concentrations, in the millimole per liter range, have been reported for wetland soils (33). It is therefore most likely that another factor, such as reduced availability of organic substrates (like acetate, pyruvate, fatty acids), HS$^-$, or inorganic essential nutrients, may be responsible. This clearly shows the importance of decomposition rate and the extent to which organic matter has already been degraded, as the availability of low-molecular organic compounds largely depends on both.

Phosphate concentrations in the interstitial water increased considerably as a result of sulfate treatment (Figure 2). Phosphate is bound in soils and sediments to iron, aluminum, and calcium, and to organic matter and clay (32, 34, 35). Several studies showed that phosphate sorption to iron and iron (hydr)oxides is of major importance in the phosphate kinetics of waterlogged soils and underwater sediments (4, 36, 37). As anion binding capacity is very low at circumneutral pH and phosphate binds more strongly to anion binding sites than sulfate (34), direct replacement of phosphates from anion binding sites is very unlikely. Sulfide production is known to interfere with the 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 phosphate is released (4, 7, 12, 35–38). Moreover, sulfide reacts with iron, iron hydroxides, and iron–phosphate complexes, forming iron sulfides like FeS and FeS$_2$ (pyrite), thereby reducing the availability of iron for phosphate binding (4–7, 39). The low iron concentrations observed in pore water of the sulfate-treated soils, as compared to the [0] treatment in the present experiment, suggest that iron sulfide formation is taking place and points to the interaction of sulfide with the iron–phosphate cycle, as stated above. The observed decrease in redox potential, due to sulfate reduction and FeS$_2$ formation, probably stimulated the reduction of Fe(III) and concomitant sulfate mobilization. As sulfate is removed by the sulfate treatment, a negative effect of a pH increase on phosphate binding to metals (40) or to metal oxides (41) cannot be the explanation here. Calcium concentrations were not significantly lowered by sulfate treatment (Figure 2, Table 2). Therefore, the precipitation of calcium sulfide, leading to the release of phosphate, is not very likely. The decrease in magnesium concentrations must be attributed to precipitation with sulfide, as the solubility product for magnesium sulfate was not reached.

After 10 weeks of sulfate treatment and an increase in the phosphate concentration, the concentrations of ammonium and potassium also began to rise. This might be explained by increased mineralization due to alkalinity generation and concomitant internal pH buffering of organic matter (42–44), or by the observed decrease in vegetation regrowth. Bicarbonate concentrations were, however, already higher after 4 weeks treatment, which makes the first option less plausible. Moreover, Kok et al. (45) and Kok and Van de Laar (44) showed that mineralization was stimulated in the range between 0.1 and 0.5 mmol L$^{-1}$, without any further effect.

#### Table 2. Repeated Measures Analysis of Variance (General Linear Models) of the Effects of Sulfate Addition on Several Pore Water Characteristics, as Shown in Figure 2

<table>
<thead>
<tr>
<th>dependent variable</th>
<th>504-treatment</th>
<th>time</th>
<th>interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkalinity</td>
<td>$51.90^e$</td>
<td>$83.07^d$</td>
<td>$8.56^d$</td>
</tr>
<tr>
<td>pH</td>
<td>$16.29^e$</td>
<td>$89.09^d$</td>
<td>$6.73^d$</td>
</tr>
<tr>
<td>SO4</td>
<td>$142.37^a$</td>
<td>$34.97^a$</td>
<td>$27.78^a$</td>
</tr>
<tr>
<td>Eh</td>
<td>$22.67^a$</td>
<td>$29.03^a$</td>
<td>$2.20^a$</td>
</tr>
<tr>
<td>HS</td>
<td>$37.10^a$</td>
<td>$16.71^a$</td>
<td>$11.87^a$</td>
</tr>
<tr>
<td>PO4</td>
<td>$6.70^b$</td>
<td>$59.92^d$</td>
<td>$2.99^b$</td>
</tr>
<tr>
<td>NH4</td>
<td>$18.99^a$</td>
<td>$28.35^d$</td>
<td>$7.04^d$</td>
</tr>
<tr>
<td>NO3</td>
<td>$1.45NS$</td>
<td>$14.12^d$</td>
<td>$1.40NS$</td>
</tr>
<tr>
<td>K</td>
<td>$28.34^a$</td>
<td>$7.83^a$</td>
<td>$3.08^a$</td>
</tr>
<tr>
<td>Fe</td>
<td>$15.62^a$</td>
<td>$113.74^a$</td>
<td>$13.76^a$</td>
</tr>
<tr>
<td>Ca</td>
<td>$0.97NS$</td>
<td>$1.88^a$</td>
<td>$0.97NS$</td>
</tr>
<tr>
<td>Mg</td>
<td>$6.20^a$</td>
<td>$14.12^a$</td>
<td>$1.40NS$</td>
</tr>
<tr>
<td>Zn</td>
<td>$12.26^a$</td>
<td>$20.63^d$</td>
<td>$1.11NS$</td>
</tr>
<tr>
<td>Mn</td>
<td>$0.53NS$</td>
<td>$47.74^d$</td>
<td>$1.43NS$</td>
</tr>
<tr>
<td>Al</td>
<td>$2.34NS$</td>
<td>$12.35^d$</td>
<td>$1.33NS$</td>
</tr>
<tr>
<td>Si</td>
<td>$2.75NS$</td>
<td>$3.75^b$</td>
<td>$1.11NS$</td>
</tr>
</tbody>
</table>

*For treatment, d.f. = 2, for time, d.f. = 13, except for Eh and HS, for which d.f. = 7 and 12, respectively. F values are given, with their level of significance: $P < 0.05$. $P \leq 0.01$. $P < 0.001$. NS, not significant.
beyond this range. The production of humic acids during decomposition probably explains the lower alkalinity in the soils compared to the bicarbonate concentration in the medium.

We assume that the observed vegetation response, due to toxicity, was responsible for the increase in the N and K concentrations and the further increase in the P concentration. It is unlikely that sulfate itself was toxic at the concentrations measured in the soils (1 and 2 mmol L\(^{-1}\)), because these values are often found in similar vegetations and in other soft water wetland soils, in combination with higher iron concentrations and low sulfide concentrations (15, and unpublished data of the authors). The fact that biomass regrowth was hampered by the sulfate treatment therefore indicated a toxic effect by accumulating reduced substances like sulfide or ammonium. Both sulfide and ammonium toxicity are known to severely reduce the viability of (semi)aquatic plant species, also at the concentration range measured in this study (6, 14, 46). Experiments involving iron addition to underwater sediments, which caused sulfide binding, indicated that sulfide toxicity was the main reason for plant die-back due to increased sulfate reduction (18). Sulfate and ammonium concentrations remained high in the iron treated sediments, indicating that the concentrations of these ions were not toxic, in accordance with the assumption mentioned above. In the present vegetation, Carex species in particular, were very sensitive to reduced compounds, presumably free sulfide (Figure 3). The observed increase of ammonium and potassium in soil pore water was most probably related to a combined effect of reduced nutrient uptake and increased availability of readily decomposable organic matter for mineralization, due to root die-back.

**Implications for Wetland Conservation.** An increase in sulfate availability in freshwater wetlands will stimulate sulfate reduction in soils and sediments. The concomitant mobilization of extra phosphate and ammonium (so-called indirect or internal eutrophication) and the induction of phytotoxicity are likely to induce major changes in vegetation composition. In rich fens, both phosphorus and nitrogen limitation have been reported (47). The N:P ratio in Carex sp. and Juncus acutiflorus in the present vegetation was between 5 and 8 g g\(^{-1}\) (results not shown), suggesting nitrogen limitation (48). Regardless of the type of nutrient limitation, however, fast-growing species resistant to reduced compounds like sulfide will be able to outcompete others if N, P, and K are all increased, as in the present experiment. The system will eventually gain a higher trophic status with increased nutrient cycling.

The effects of an increased supply of sulfate largely depend on the availability of organic substrates for sulfate reducers.
As these molecules are provided by organisms that degrade larger organic compounds, the availability of readily decomposable organic matter is very important. Therefore, the sensitivity to sulfate pollution may vary between different locations due to differences in humus characteristics (49). Besides the availability of easily decomposable organic matter, the presence of more favorable electron acceptors like oxygen and nitrate obviously influences sulfate reduction rates.

Considering the findings of this study, wetland conservation and management must not only allow for the nutrient loads of the intruding ground- and surface water (direct eutrophication), but also the effects of sulfate enrichment. A reduction of phosphorus and nitrogen levels in the incoming water, for instance by means of technical nutrient stripping, may not be sufficient to prevent the eutrophication of certain wetlands. Given the problems caused by sulfate pollution, restoration of the original hydrology and hydrochemistry is probably the only way to conserve endangered wetlands.

Acknowledgments
The authors wish to thank Mrs. S. Van Roozendael for her assistance during the experiment, Mr. J. Eijgensteyn for his help with chemical analyzes, and Mrs. J. Van Westreenen for drawing the first figure. We are also indebted to Professor Dr. J. M. Van Groenendael, Dr. C. Van der Drift, and Mr. B. Kelleher for critically reading the manuscript and to Mr. M. Bolten for his kind permission to collect the sods from the nature reserve “De Bruuk”.

Literature Cited
(20) Van gemerden, H. Arch. Mikrobiol. 1984, 139, 289.
(21) Henriksen, A. Analyt. 1965, 90, 29.
(22) Technicon, Technicon Auto Analyzer Methodology, Industrial method 11 (Cl), 33-69w(NO3-NO2); Technicon Corporation: New York, 1968/1969.
(45) Kemmers, R. H. Landschap 1996, 3, 157 (In Dutch; Engl. abstr.).

Received for review April 23, 1997. Revised manuscript received October 14, 1997. Accepted October 20, 1997.©

ES970362F