Differential responses of the freshwater wetland species *Juncus effusus* L. and *Caltha palustris* L. to iron supply in sulfidic environments

Marlies E.W. Van der Welle*, Karla Niggebrugge, Leon P.M. Lamers, Jan G.M. Roelofs

*Department of Aquatic Ecology and Environmental Biology, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, The Netherlands*

Received 3 March 2006; received in revised form 26 July 2006; accepted 1 August 2006

Toxicity of iron and sulfide are interacting with each other and have the potential to affect vegetation composition.

Abstract

Sulfur pollution can lead to serious problems in freshwater wetlands, including phosphorus eutrophication and sulfide toxicity. We tested the effects of anaerobic iron-rich groundwater discharge in fens, simulated by iron injection, on two characteristic species (*Juncus effusus* and *Caltha palustris*) in a sulfidic environment. Biomass production of *C. palustris* roots showed an optimum response to the combined addition of iron and sulfide, with highest values at intermediate concentrations of both substances. Iron deficiency apparently occurred at low iron concentrations, while at high iron concentrations, growth was decreased. For *J. effusus*, in contrast, no toxic effects were found of both iron and sulfide. This could be explained by larger radial oxygen loss (ROL) of *J. effusus* and could not be explained by differences in phosphorous concentrations. The results of our experiments confirm that iron-rich groundwater discharge has the potential to affect vegetation composition through toxicity modification in sulfidic environments.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Groundwater discharge; Iron; Sulfur pollution; Toxicity; Peatlands

1. Introduction

Changes in hydrological conditions, as a result of anthropogenic interference, can strongly influence freshwater wetland vegetation (Wheeler et al., 1995). Both increased and decreased water levels can cause plant species to disappear, for example as a result of changing redox conditions (anoxia) or changes in water quality (e.g. Beltman et al., 1996; Crawford, 1992; Lucassen et al., 2000; Magee and Kentula, 2005; McCartney and De la Hera, 2004; McFarlane and Williamson, 2002; Meade and Blackstock, 1988; Runhaar et al., 1996). Nutrient availability is known to be strongly influenced by changes in the redox conditions of the soil (Lamers et al., 2006), potentially leading to changes in vegetation composition.

Next to changes in nutrient availability, increased water levels may also lead to the accumulation of phytotoxins, like sulfide, iron and ammonium, which may also play an important role in determining species composition. Sulfide is a well-known phytotoxin in freshwater wetlands, especially in sulfate-polluted wetlands characterized by high sulfate reduction rates. High sulfide concentrations may lead to toxic effects to aquatic plants, such as root decay (root blackening and increased flaccidity of the roots) and mortality (Armstrong et al., 1996; Smolders and Roelofs, 1996), reduced growth (Koch and Mendelsson, 1989; Koch et al., 1990; Van der Welle et al., 2006) or even mortality (Lamers et al., 1998; Smolders et al., 1995b).
Sulfide toxicity, however, can be mitigated by the formation of highly insoluble metal sulfides like iron sulfides (FeS, FeS$_2$ or pyrite) or metal sulfide complexes (Huerta-Diaz et al., 1998; Smolders and Roelofs, 1995; Wang and Chapman, 1999), thereby reducing both sulfide and metal toxicity. In areas where iron-rich groundwater is discharged, free sulfide concentrations are usually low, as a result of iron sulfide precipitation.

We observed that in the Netherlands, vegetation types of the Calthion-type (as described by Schaminée et al., 1996) were disappearing from fen meadows after hydrological changes that led to a strong decrease in the discharge of iron-rich groundwater. Juncus effusus, in contrast, became a dominant species. This is an unwanted development, since J. effusus dominated vegetation types are much less biodiverse than those of the Calthion-type (Ervin and Wetzel, 2002) and are therefore unwanted by nature managers. To take the necessary management measures to prevent the development of J. effusus dominated vegetation types, it is very important to increase our knowledge about the underlying processes, including the role of groundwater discharge.

Based on previous, correlative research by Lucassen et al. (2006) and Van der Welle (unpublished results) in fens, we hypothesized that the distribution of vegetation types dominated by Calthion palustris (target species) and Juncus effusus (invading species) might be related to differences in iron and sulfide concentrations in the soil. For freshwater wetlands, very little is known about the effects of sulfide toxicity on vegetation composition and how this may be altered by the presence of high concentrations of iron. In the present study, we tested experimentally whether iron or sulfide toxicity could indeed be responsible for the observed differences in the distribution of C. palustris and J. effusus dominated vegetation types and how iron and sulfide interact. The results are discussed in relation to hydrological changes of freshwater wetlands.

2. Materials and methods

2.1. Experimental set-up

We used two plant species, which are common in freshwater wetlands, but have several different characteristics: Calthion palustris and Juncus effusus. J. effusus is a perennial, monocotyledonous species with an invasive character, which is often found on drained peatlands (Richards and Clapham, 1941). It generally has a high root porosity (~25–30%), which is even increased under anaerobic conditions (Visser et al., 2000). C. palustris is a perennial, dicotyledonous species, which usually grows on wet, moderately rich soil (Van der Meijden, 1997). It has a lower root porosity (~10%) than J. effusus, which is increased when growing under anaerobic conditions (Visser et al., 2000). J. effusus plants were obtained from a cultivated population, available at our department. C. palustris plants were derived from a commercial gardener, and both species were cultivated for five weeks on vermiculite and a diluted mineral medium. Six columns per treatment were filled with the sediment, and one individual of J. effusus or C. palustris was planted in each column. We carefully and homogeneously injected 7.5 ml 0.57 mol l$^{-1}$ Na$_2$S, adjusted to pH 7.5, into the upper 10 cm of the soil in all columns, resulting in free sulfide concentrations in the pore water of approximately 300 mol l$^{-1}$. Fe(H)$_2$Cl$_2$ was then injected into the upper 30 cm of the soil in four different concentrations, corresponding to 0, 3.6, 7.2 and 14.4 mol l$^{-1}$ of sediment. The actually measured concentrations in the pore water are summarized in Table 1. Sulfide had to be added every week to maintain a constant concentration of approximately 300 mol l$^{-1}$ sulfide in the pore water of columns without added iron. The soil was kept anaerobic by applying demineralized water to keep the soil water-saturated (water level 5 mm above the soil surface). Rhizon soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) were inserted into the upper 10 cm of the soil to collect pore water by vacuum extraction after 1, 4 and 6 weeks.

The plants were harvested after 7 weeks. The number of leaves, their width and length, root length, number of inflorescences and dead leaves, and fresh and dry weight of roots and shoots were determined, and the total cover of iron precipitation on the roots was estimated visually. From each plant, 0.5 g of fresh leaf material was used to determine chlorophyll concentrations (see Section 2.2). In addition, the photosynthetic yield of living C. palustris was determined by Pulse Amplitude Modulation (PAM) fluorometry (Diving PAM, Heinz Walz, Effeltrich, Germany). Unfortunately, it was not possible to measure the in situ photosynthetic capacity of J. effusus, since the leaves were too narrow for the measurements. Moreover, biomass of J. effusus did not respond to the treatments, and we therefore did not expect any effects on the photosynthetic capacity. The photosynthetic yield was calculated from the fluorescence after the leaves had been kept in the dark for at least 15 min ($F_0$), and the maximum fluorescence after a strong light pulse ($F_m$), which is saturating for photosynthesis:

$$\text{Yield} = F_0 - F_m / F_m$$

This value is assumed to reflect the vitality of the chlorophyll and its capacity to recover, and should therefore reflect the vitality of the plant (e.g. Beer and Björk, 2000; Roháček and Bartik, 1999).

2.2. Chemical analysis

Immediately after the pore water had been collected, 10.5 ml was fixed with 10.5 ml Sulfide Ani Oxidant Buffer (SAOB, Van Gemerden, 1984) to measure sulfide concentrations with an iron-specific electrode (Orion type 94-16A, Orion Research, Cambridge, MA, USA). A 10 ml subsample was used to measure pH and alkalinity. Alkalinity was estimated from the amount of HCl needed to titrate the sample to a pH of 4.2.

The remaining sample was divided into two parts. One part was frozen with 0.5% HNO$_3$ until analysis for Ca, Mg, Fe, P, SO$_4^{2-}$ and Zn with ICP-AES (Spectro Analytical Instruments, Kleve, Germany). The other part was frozen with 0.125 g l$^{-1}$ citric acid and analyzed for PO$_4$, NO$_3$ (Kamphake et al., 1967), NH$_4$ (Grasshoff and Johannsen, 1977) (type AA II, Technicon Instruments, Tarrytown, NY, USA) and K (FLM3 Flame photometer, Radiometer, Copenhagen, Denmark).

Chlorophyll $a$, $b$ and carotenoid concentrations were determined by extracting 0.5 g of freshly ground (with liquid nitrogen) leaves with 96% ethanol for 24 h in the dark at 4°C and measuring the extinction of the extracted solution at different wavelengths ($\lambda = $ 470, 649 and 665 nm), using a spectrophotometer (Shimadzu UV-120-01, Duisburg, Germany). Photopigment concentrations were calculated as described by Van Dijk and Roelofs (1988). Chlorophyll $a$ and $b$ are the major photopigments, carotenoids function as accessory pigments. They were separately determined, since they were found to react differently to increased iron availability.

Approximately 200 mg of dried plant material was weighed exactly and destructed with 4 ml nitric acid (65%) and 0.9 ml 35% hydrogen peroxide, using a Milestone MLS 1200 destruction microwave (Milestone, Sorisole, Italy). The destructed sample was diluted and analyzed with ICP-AES as described above to determine plant nutrient concentrations.

2.3. Radial oxygen loss

One C. palustris or J. effusus plant was put in a cuvette made of two glass plates ($400 \times 275 \times 13$ mm), with the shoot sticking out through a hole in the
top. This hole was provided with an airtight seal of plastic gum. The roots of the plant were carefully spread out in the cuvette and their positions were fixed with small glass bars that were attached to the plate with petroleum jelly. The cuvette was given an airtight seal with clamps and was flushed with nitrogen gas. Through a small opening in the upper glass plate, the cuvette was filled with anaerobic agar solution (1 g l\(^{-1}\)) with 0.04 g l\(^{-1}\) leuco-methylene blue (method as described by Laan et al., 1989). The agar was reduced by titration with sodium dithionite until it became colorless. The shoot of the plant was placed on a lamp (Philips HPF-T, 400 W, 80 µmol m\(^{-2}\) s\(^{-1}\)), while the roots were kept in the dark. All measurements were performed at a constant temperature (15 °C).

After the cuvette had been filled, a photograph was taken of the roots (t = 0). Further photographs of the roots were taken every hour to monitor the development of oxic (blue) zones around the roots. The surface area of blue colored agar around the roots was calculated with SigmaScanPro (SPSS inc., version 5.0), providing a semi-quantitative estimation of radial oxygen loss (ROL).

2.4. Data analysis

Possible differences between treatments (e.g., nutrient concentrations in the pore water, plant nutrient concentrations, plant biomass) were analyzed with ANOVA using Tukey’s post hoc test. Data were transformed whenever necessary to obtain equal variances between treatments. Nutrient concentrations in soil moisture samples were analyzed with a repeated-measurements ANOVA to check for differences between treatments in time. Differences in ROL between C. palustris and J. effusus were analyzed using an independent sample t-test. Correlation analysis was used to test for relationships between nutrient concentrations in the pore water and plant tissue concentrations. All statistical analyses were performed with SPSS 10.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Pore water analysis

After addition of iron, iron concentrations in the pore water of J. effusus and C. palustris increased to concentrations of 157 and 380 µmol l\(^{-1}\), respectively, in the containers with the highest iron addition level (Table 1). Sulfide concentrations decreased simultaneously as a result of precipitation of iron sulfides (Table 1). When at least 7.2 mmol l\(^{-1}\) iron was added, sulfide concentrations were reduced to almost zero. Phosphate concentrations were also decreased by iron addition (Table 1), ranging from 4 (100 g m\(^{-2}\) iron) to 11 µmol l\(^{-1}\) o-PO\(_4\) (no iron) in pore water for J. effusus. For C. palustris, these concentrations were between 5 and 24 µmol l\(^{-1}\) (Table 1). Phosphate concentrations only declined when sulfide concentrations had decreased sufficiently (at least 7.2 mmol l\(^{-1}\) Fe addition). Nitrate concentrations in the soil moisture slightly decreased by iron addition (Table 1). No differences were found for ammonium concentrations in the soil moisture, except in the 7.2 mmol l\(^{-1}\) Fe treatment for Caltha, where concentrations were slightly lower than in the other treatments (Table 1).

3.2. Biomass response

Increasing iron availability and concomitantly decreasing sulfide concentrations, strongly affected the growth of C. palustris roots. Root biomass showed a significant optimum (quadratic correlation, \(p = 0.014\)) at intermediate iron concentrations of \(\approx 100 \mu\text{mol l}^{-1}\). At this level of iron addition, sulfide concentrations were 1 µmol l\(^{-1}\) (Fig. 1). Shoot biomass of C. palustris was not significantly affected by iron or sulfide concentrations, although both the number and the width of the leaves showed optimum patterns similar to those found for the roots. In addition, C. palustris plants had an increasing percentage of their roots covered with iron precipitates (data not shown). In the two highest treatments, significantly more iron had precipitated on the roots than without iron addition (ANOVA, \(p < 0.05\)). No precipitates were found on J. effusus plants. For J. effusus, in contrast to C. palustris, no significant effects of iron addition were found on either shoot or root biomass.

3.3. Plant tissue nutrient concentrations

Increased iron concentrations in the pore water resulted in increased uptake of iron, which is reflected by higher iron concentrations in shoots (Table 2) and roots (data not shown) of both plant species. At the end of the experiment, shoot iron concentrations in C. palustris without iron addition were significantly lower than those at the start of the experiment (t-test: \(F = 3.26, df = 7.8, p = 0.006\) for C. palustris). This was not the case for J. effusus. J. effusus shoots had much higher iron concentrations than C. palustris shoots at similar addition levels.

Table 1

Average concentrations, ± standard error of the mean, in pore water (µmol l\(^{-1}\)) during the experiment.

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>Fe</th>
<th>o-PO(_4)</th>
<th>NH(_4)</th>
<th>NO(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. palustris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mmol l(^{-1}) Fe</td>
<td>168 ± 47(^B)</td>
<td>2 ± 0(^A)</td>
<td>24 ± 5(^B)</td>
<td>35 ± 5(^B)</td>
<td>3 ± 0.2(^B)</td>
</tr>
<tr>
<td>3.6 mmol l(^{-1}) Fe</td>
<td>70 ± 26(^AB)</td>
<td>26 ± 8(^A)</td>
<td>15 ± 5(^AB)</td>
<td>30 ± 6(^AB)</td>
<td>2 ± 0.2(^AB)</td>
</tr>
<tr>
<td>7.2 mmol l(^{-1}) Fe</td>
<td>1 ± 1(^AB)</td>
<td>106 ± 34(^AB)</td>
<td>5 ± 1(^A)</td>
<td>23 ± 4(^A)</td>
<td>2 ± 0.3(^A)</td>
</tr>
<tr>
<td>14.4 mmol l(^{-1}) Fe</td>
<td>0.2 ± 0.1(^A)</td>
<td>300 ± 151(^B)</td>
<td>12 ± 5(^AB)</td>
<td>36 ± 7(^B)</td>
<td>2 ± 0.2(^A)</td>
</tr>
</tbody>
</table>

J. effusus

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>Fe</th>
<th>o-PO(_4)</th>
<th>NH(_4)</th>
<th>NO(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mmol l(^{-1}) Fe</td>
<td>47 ± 22</td>
<td>4 ± 1(^A)</td>
<td>11 ± 1(^B)</td>
<td>26 ± 5</td>
<td>3 ± 0.3(^B)</td>
</tr>
<tr>
<td>3.6 mmol l(^{-1}) Fe</td>
<td>18 ± 29</td>
<td>17 ± 5(^A)</td>
<td>9 ± 1(^A)</td>
<td>32 ± 7</td>
<td>3 ± 0.3(^A)</td>
</tr>
<tr>
<td>7.2 mmol l(^{-1}) Fe</td>
<td>4 ± 2</td>
<td>73 ± 35(^AB)</td>
<td>5 ± 1(^A)</td>
<td>31 ± 7</td>
<td>2 ± 0.1(^A)</td>
</tr>
<tr>
<td>14.4 mmol l(^{-1}) Fe</td>
<td>1 ± 0.2</td>
<td>157 ± 54(^B)</td>
<td>4 ± 1(^A)</td>
<td>31 ± 7</td>
<td>2 ± 0.1(^A)</td>
</tr>
</tbody>
</table>

Superscript letters indicate significant differences between treatments for each species (repeated measurements ANOVA, \(p < 0.05\), \(n = 6\)). No significant differences were found where no letters are shown.
Although iron addition decreased phosphate concentrations in the pore water, tissue phosphorus concentrations in *C. palustris* were not affected by higher iron concentrations, although they were much lower than initial values, and averaged around 75 μmol g⁻¹ dry weight (Table 2). *J. effusus*, showed only slightly decreased phosphorus concentrations at increasing iron addition levels, except at the highest level. However, all concentrations were much lower than the initial values.

Without iron addition, sulfur concentrations in the plants were 30–40% higher than in the highest iron treatment (Table 2). Sulfur concentrations were higher than the initial values, even at the highest iron addition level. In *C. palustris*, both calcium and magnesium concentrations in the plant were increased at higher iron addition levels. Potassium concentrations did not show any consistent relation with the level of iron addition (Table 2).

### 3.4. Photosynthesis

Concentrations of photosynthetic pigments were related to iron addition. Both chlorophyll *a* + *b* and carotenoid concentrations were increased in both species when more iron had been added (Fig. 2). The total concentration of photosynthetic pigments in *C. palustris* (chlorophyll *a*, chlorophyll *b* and carotenoids) was significantly correlated to the free iron concentration in the pore water (*R²* = 0.487, *p* = 0.025; data not shown). This correlation was not found for *J. effusus*, which only had significantly increased concentrations of chlorophyll *b* (*R²* = 0.462, *p* = 0.023; data not shown). Fig. 3 shows the correlations between the iron concentrations in the pore water and the concentrations of chlorophyll *a* + *b* (major photopigments) and carotenoid (accessory photopigments) concentrations in the plants. The concentration of carotenoids in *C. palustris* was positively correlated to the free iron concentration in the pore water (*R²* = 0.435, *p* = 0.049).

As *C. palustris* showed a biomass response to the iron treatments, we decided to determine the photosynthetic yield by PAM fluorometry. Photosynthetic yield was significantly lower without iron addition (Fig. 4). There was a significant negative correlation between the photosynthetic yield and the sulfur concentration in the shoot (Pearson correlation coefficient (PCC) = −0.442; *p* = 0.015) and roots (PCC = −0.547; *p* = 0.006).

### Table 2

Plant nutrient concentrations in aboveground parts of *Caltha palustris* and *Juncus effusus* at the end of the greenhouse experiment, compared with initial values

<table>
<thead>
<tr>
<th></th>
<th>P (%)</th>
<th>S (%)</th>
<th>Fe (μmol g⁻¹ dry weight)</th>
<th>K (μmol g⁻¹ dry weight)</th>
<th>Ca (μmol g⁻¹ dry weight)</th>
<th>Mg (μmol g⁻¹ dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. palustris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mmol l⁻¹ Fe</td>
<td>85 ± 9</td>
<td>156 ± 6³⁸</td>
<td>1.3 ± 0.3³³</td>
<td>425 ± 99</td>
<td>269 ± 36³³</td>
<td>114 ± 11³³</td>
</tr>
<tr>
<td>3.6 mmol l⁻¹ Fe</td>
<td>69 ± 2</td>
<td>124 ± 11³⁴</td>
<td>3.1 ± 0.5³³</td>
<td>415 ± 35</td>
<td>354 ± 13³³</td>
<td>163 ± 13³³</td>
</tr>
<tr>
<td>7.2 mmol l⁻¹ Fe</td>
<td>76 ± 3</td>
<td>141 ± 12³⁴</td>
<td>3.8 ± 0.4³³</td>
<td>495 ± 47</td>
<td>380 ± 25³³</td>
<td>164 ± 13³³</td>
</tr>
<tr>
<td>14.4 mmol l⁻¹ Fe</td>
<td>75 ± 2</td>
<td>111 ± 7³³</td>
<td>6.1 ± 2.0³³</td>
<td>404 ± 27</td>
<td>333 ± 17³³</td>
<td>142 ± 7³³</td>
</tr>
<tr>
<td>Initial</td>
<td>187 ± 10</td>
<td>76 ± 9</td>
<td>3.6 ± 0.5</td>
<td>n.d.</td>
<td>145 ± 19</td>
<td>151 ± 10</td>
</tr>
<tr>
<td><em>J. effusus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mmol l⁻¹ Fe</td>
<td>53 ± 2³³</td>
<td>89 ± 4³³</td>
<td>2.6 ± 1³³</td>
<td>319 ± 30³³</td>
<td>73 ± 6</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>3.6 mmol l⁻¹ Fe</td>
<td>46 ± 1³³</td>
<td>77 ± 5³³</td>
<td>10 ± 1³³</td>
<td>233 ± 11³³</td>
<td>85 ± 6</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>7.2 mmol l⁻¹ Fe</td>
<td>47 ± 1³³</td>
<td>68 ± 4³³</td>
<td>16 ± 2³³</td>
<td>271 ± 21³³</td>
<td>86 ± 7</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>14.4 mmol l⁻¹ Fe</td>
<td>50 ± 1³³</td>
<td>68 ± 3³³</td>
<td>16 ± 4³³</td>
<td>265 ± 17³³</td>
<td>87 ± 6</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Initial</td>
<td>80 ± 4</td>
<td>47 ± 2</td>
<td>3.7 ± 1</td>
<td>n.d.</td>
<td>44 ± 5</td>
<td>87 ± 6</td>
</tr>
</tbody>
</table>

Concentrations are in μmol g⁻¹ dry material, showing averages ± standard error of the mean. Concentrations in italics are statistically different from the initial value (*t*-test, *p* < 0.05). Superscript letters indicate significant differences between treatments for each species. No significant differences were found where no letters are shown. n.d. means not determined (ANOVA, *p* < 0.05, *n* = 6).
and with the P concentration in the shoot ($PCC = 0.440; p = 0.016$). The photosynthetic yield showed no significant correlation with tissue iron concentrations.

### 3.5. Radial oxygen loss

*J. effusus* plants showed a higher ROL than *C. palustris* plants (Fig. 5). Oxic blue zones developed faster and the coloring was more intense for *J. effusus* (Fig. 5). *C. palustris* plants were slower to develop blue zones and oxygen loss was spread evenly over the entire length of the roots. As a result, the surface area of blue colored agar solution, which we used as a semi-quantitative measurement, was larger for *J. effusus* than for *C. palustris*. For *J. effusus*, we found a ROL of $0.168/C60.043$ cm$^2$/g$^1$/min$^1$, while the area of $0.031/C60.009$ cm$^2$/g$^1$/min$^1$ for *C. palustris* was significantly lower (independent samples $t$-test, $p = 0.022$, $n = 4$). *J. effusus* plants had the highest oxygen loss along young (white) roots, at the tips of the root and along older roots, which were visually damaged.

### 4. Discussion

#### 4.1. Effects on vegetation composition

The results of our experiments confirm that iron-rich groundwater discharge has the potential to affect vegetation composition in sulfidic environments by modifying toxicity. *C. palustris* is much more sensitive to high sulfide concentrations than *J. effusus*, since *J. effusus* can decrease sulfide concentrations in its rhizosphere by its much higher radial oxygen loss. Toxicity modification provides a competitive advantage for this vigorous species if nutrient availability is sufficient.

Iron addition, mimicking groundwater discharge at environmentally relevant concentrations, strongly reduced the concentration of dissolved sulfide, thereby immobilizing both iron and sulfide through precipitation of iron sulfides. The present study showed clearly that growth of *C. palustris* was much higher when sulfide concentrations were decreased as a result of iron addition. Previous experiments by Smolders et al. (1995a) and Lucassen et al. (2000) also showed that iron-rich groundwater discharge can strongly influence the water quality and reduce sulfide toxicity.

Increased sulfide concentrations in *Calthion* vegetation types will lead to the decrease or even the disappearance of *C. palustris* and will probably lead to the decrease of other sulfide-sensitive species like *Carex* species (Lamers et al., 1998; Lucassen, 2004). In a study by Lamers et al. (1998), *Carex nigra* was unable to recover from cutting as a result of sulfide toxicity at sulfide concentrations comparable to those in the treatment without iron addition. The decrease of sulfide-sensitive species and the co-occurring increase of...
more tolerant species could lead to an unwanted loss of biodiversity at higher nutrient availability (e.g., Havill et al., 1985; Ingold and Havill, 1984; Lucassen, 2004).

When there is little iron available, increased sulfide concentrations will lead to phosphate mobilization (Böström et al., 1982; Caraco et al., 1989; Roelofs, 1991; Smolders et al., 1995), which may also alter vegetation composition (e.g., Gough et al., 2000; Güsewell et al., 2005; Shaver and Chapin, 1995). However, our experiment shows that the effect of sulfide toxicity on C. palustris overrules any effects of increased phosphate concentrations, as higher phosphate concentrations in the pore water along with high sulfide concentrations did not result in increased growth of this species. In field situations, however, increased phosphate concentrations will favor the growth of fast-growing species, like J. effusus (A.J.P. Smolders, personal communication) or other graminoids (Lucassen, 2004; Van der Hoek et al., 2004).

Ammonium toxicity is not likely to have occurred, as no large differences were found between treatments, and concentrations in the soil moisture were low compared to those found in a previous study on ammonium toxicity (Lucassen et al., 2003) and compared to values measured in the field (concentrations up to 65 μmol l⁻¹, Van der Welle, unpublished data).

Lucassen et al. (2003) found that ammonium was toxic at concentrations of 250 μmol l⁻¹, at least at low pH (pH 4).

4.2. Radial oxygen loss and toxicity

The rhizosphere of J. effusus featured strongly different conditions than that of C. palustris. The concentrations of iron and especially of sulfide were much lower in the columns with J. effusus, although equal amounts of sulfide and iron had been added. The major decrease in iron concentrations in the pore water that we found could not be entirely explained by a greater iron uptake by J. effusus, as this uptake was much smaller (~17 times lower) than the decrease in iron concentrations in the pore water. Sulfur concentrations in the plants were lower for J. effusus. Therefore, the lower sulfide concentrations must have resulted from oxidation in the rhizosphere, which also may lead to immobilization of iron through the formation of iron hydroxides and iron oxides.

Previous research has shown that J. effusus is often found in areas with higher sulfide concentrations in soil moisture than C. palustris (Lucassen, 2004; Lucassen et al., 2006). Sulfide is a strong phytotoxin that can damage plant roots (Armstrong et al., 1996; Smolders and Roelofs, 1996). Plants can reduce the toxic effect of sulfide by preventing high oxygen loss along most of the root, especially at high sulfide concentrations, and allowing oxygen leakage only at the root tips and young roots, which are the most important parts for growth and nutrient uptake (Armstrong, 1971, 1979; Armstrong and Armstrong, 1988; Colmer et al., 1998; Connell et al., 1999; Končalová, 1990). Oxygen loss from J. effusus roots was approximately five times higher than that from C. palustris roots, which likely resulted in the oxidation of sulfide (and iron) and decreased concentrations of the toxin. Moreover, as C. palustris has its oxygen loss evenly spread over the whole length of its roots, oxygen transported through the roots is most probably depleted in the upper few centimeters of the soil when the soil is highly reduced and provides a strong oxygen sink. As a result the apical parts of the roots cannot be reached, which results in root blackening and root flaccidity as we observed. Since J. effusus has a mechanism to prevent unnecessary oxygen loss along most of its roots by forming a layer of
compact cells with thickened, lignified cell walls (Končalová, 1990; Kutschera and Lichtenegger, 1982), it should be able to transport oxygen to roots at greater depths. The differences in ROL may therefore explain why the biomass of *C. palustris* roots was affected more strongly by increased sulfide (and iron) concentrations than that of *J. effusus*. These differences in ROL are slightly higher than those found by Visser et al. (2000), who found that the ROL of *C. palustris* can be approximately 2 times lower than that of *J. effusus* and who also showed that ROL is more evenly spread over the whole root length in *C. palustris*.

4.3. Plant tissue nutrient concentrations and photosynthesis

The results of the plant tissue analysis suggest that toxic effects of sulfide may be caused by sulfide-induced iron deficiency rather than direct toxicity of sulfide itself, as the uptake of other nutrients was not noticeably decreased. Without iron addition, iron concentrations in *C. palustris* were approximately 65% lower than the initial values and field values. Under the original field conditions (at the same location where soil was collected), the pore water iron concentrations were in between those of 0 and 3.6 mmol l\(^{-1}\) iron addition (\(\sim 6 \mu\text{mol l}^{-1}\); data not shown). *C. palustris* plants growing under these conditions in the field had tissue iron concentrations of 3 \(\mu\text{mol g}^{-1}\), which is comparable to the initial values measured (Table 2).

Since iron plays an important role in the synthesis of chlorophyll (Marschner, 1998), this may explain the reduced photosynthetic capacity of *C. palustris* in the sulfide only treatment (with no iron added). All available iron appeared to be bound to sulfide, possibly leading to iron deficiency. Carotenoid concentrations tended to increase with increasing iron availability. It is known that carotenoid concentrations decrease as a result of iron deficiency (Marshner, 1998). Apparently, carotenoids play an important role in photosynthesis, as despite equal chlorophyll \(a\) and \(b\) concentrations, the photosynthetic activity decreased in the treatment without iron addition. It appears that the negative effect of concomitant high sulfide and low iron concentrations is related to the lower concentrations of carotenoids, resulting in reduced photosynthetic activity, which may indicate iron deficiency (Marshner, 1998).

Potassium, magnesium and calcium deficiencies, which may also lead to chlorosis, do not appear to have occurred during the experiment, as most values were within the range of values measured in presumably healthy plants in the field or even higher (Lucassen et al., 2004 and unpublished data).

4.4. Iron toxicity and nutrient deficiency

Unexpectedly, the highest iron concentrations appeared to decrease the growth of *C. palustris*. This is remarkable, as this species naturally occurs in areas with similar iron concentrations (Lucassen, 2004). This suggests that either the sensitivity of the plants used was higher than in the field or that even *C. palustris* performed less well at high iron concentrations. Iron toxicity may be related to the formation of iron plaques on the roots (e.g. Cook, 1990; Lucassen et al., 2004; Smolders and Roelofs, 1996; Snowden and Wheeler, 1993), which prevent nutrient uptake. However, although we did indeed observe iron plaque formation on roots of *C. palustris*, nutrient concentrations were not affected.

Although high iron concentrations might lead to phosphorus deficiency as a result of lower phosphate concentrations in the pore water, as iron is able to immobilize phosphate by forming iron phosphates, this does not seem to have been the case in our experiments. Phosphorus concentrations in *C. palustris* were hardly altered by iron addition. A study by Wheeler et al. (1985) similarly found no effects of iron on phosphorus concentrations in *Epilobium hirsutum* or *Juncus subnodulosus*.

5. Conclusion

Our results show that in wetlands with high concentrations of free sulfide as a result of high water tables and sulfate pollution, vegetation types dominated by *C. palustris* can be replaced by vegetation types dominated by *J. effusus* as a result of interactions between iron and sulfide. The high radial oxygen loss of *J. effusus* decreased the concentrations of sulfide and other reduced substances in the rhizosphere, which would otherwise be toxic. *C. palustris*, on the other hand, is unable to effectively oxidize large amounts of sulfide in its rhizosphere, since it loses most of the oxygen in the upper few centimeters without reaching the deeper roots. This may even lead to reduced performance of this species at high iron concentration. In addition, increased sulfide concentrations under low iron conditions will lead to increased phosphate mobilization, of which fast growing species like *J. effusus* can profit. This will give *J. effusus* a double advantage under sulfide-rich conditions: it does not suffer from sulfide toxicity since it can oxidize potentially harmful reduced compounds in its rhizosphere and it can effectively profit from increased phosphate availability and overgrow or out-shadow other species. Under such conditions, sulfide-sensitive species like *C. palustris* will therefore not be able to survive. This implies that next to nutrient availability, changes in the accumulation of phytotoxins may provide an additional explanation for vegetation changes observed after hydrological changes.

Acknowledgments

We would like to thank Jelle Eygenstein, Liesbeth Pierson and Rien van der Gaag for their assistance with chemical analyses, Jan Klerkx for correcting the English, and Joost Vogels, Martin Veststeeg, Roy Peters and Germa Verheggen for technical assistance. This study was funded by the Netherlands Organization for Scientific Research (NWO), through its stimulation programme on system-oriented ecotoxicological research (SSEO).

References


