Biogeochemistry (2005) 76: 69–83
DOI 10.1007/s10533-005-2053-x

Ferrous iron stimulates phenol oxidase activity and organic matter decomposition in waterlogged wetlands

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Received 29 June 2004; accepted in revised form 8 February 2005

Key words: Anaerobic mineralisation, CO_2 production, Phenolics, Reduced iron, Soil organic matter

Abstract. Soil organic matter decomposition is limited at waterlogged conditions by the low activity of extracellular enzymes like phenol oxidases. In this paper, we show that ferrous iron (Fe^{2+}), which is abundant in waterlogged soils, significantly stimulates phenol oxidase activity both in pure enzyme assays and in waterlogged soil slurries from nutrient-poor dune slacks. However, the effects in soil slurries were less strong than in enzyme assays. Both the addition of Fe^{2+} and the initial presence of Fe^{2+} stimulated phenol oxidase activity at the microaerophilic conditions tested. This stimulation is attributed to the catalysis of additional OH radical production, promoting the oxidation of phenolics. Subsequently, the presence of Fe^{2+} strongly increased total decomposition rates of soil organic matter, measured as CO_2 production and Cotton strip Tensile Strength Loss. There is circumstantial evidence that this stimulation by Fe^{2+} could be important for decomposition in wetlands at field conditions, but its relevance compared to the effects of other compounds still needs to be elucidated. These results emphasise the crucial role of water quality in determining extracellular enzyme activity and decomposition in waterlogged wetlands.

Introduction

Organic carbon accumulates in most wetlands due to low organic matter decomposition (e.g. Berendse et al. 1994). Mechanisms proposed to account for the low decomposition rates in wetlands include the presence of waterlogged conditions, low nutrient supply, low chemical quality of organic matter and unfavourable water chemistry (e.g. Oomes et al. 1997). Local seepage and infiltration occur along opposite borders of groundwater driven wetlands with a net horizontal movement of water. Within such wetlands, pronounced differences exist in decomposition rates at waterlogged

conditions between borders with seepage and infiltration conditions (Sival and Grootjans 1996). Higher decomposition rates occur at sites with calcareous seepage, which is mostly attributed to pH-effects (Grootjans et al. 1998; van Dijk et al. 2004). However, ferrous iron availability may be an important, but unknown factor in determining decomposition rates at these conditions given that (i) ferrous iron (Fe^{2+})–ferric iron (Fe^{3+}) is the dominant redox couple in most freshwater environments (e.g. Inubushi et al. 1984; van Bodegom and Stams 1999), (ii) Fe^{2+} concentrations are higher at seepage than at infiltration conditions and (iii) decomposition of soil organic matter is a redox driven process.

The rate-limiting steps of decomposition processes are catalysed by extracellular enzymes produced by microorganisms. The activity of these enzymes is highly affected by waterlogged conditions. Some activities increase, whereas others are inhibited (Pulford and Tabatabai 1988). Freeman et al. (2001a) found that especially phenol oxidase – a key enzyme in carbon decomposition processes that polymerises phenolics and depolymerises lignins and phenolics – was inhibited in the absence of molecular oxygen. This leads to an accumulation of phenolics, which in themselves also inhibit microbial activities (Wetzel 1992). Fe^{2+} might also be involved in the regulation of phenol oxidase activity at waterlogged conditions.

Phenol oxidase like horseradish peroxidase (Uno et al. 1987), lignin peroxidase (Enoki et al. 1997) and other phenol oxidases (Tanaka et al. 1999) contain iron in their heme complex. In a process known as the Fenton reaction, Fe^{2+} in combination with H_2O_2 is a strong oxidising agent through the formation of OH radicals. Hydroxyl radicals play a central role in lignin decomposition through phenol oxidases at oxic conditions (Wood 1994). More recently, Gómez-Toribio et al. (2001) showed that the oxidation of Mn^{2+} produced H_2O_2 and hence strongly catalysed the oxidation of phenolics. The Mn^{3+} formed was reduced again to Mn^{2+} during subsequent reactions (Gómez-Toribio et al. 2001), resulting in a purely catalytic effect of Mn^{2+}. Given the higher reactivity of Fe^{2+} than Mn^{2+} in oxidation reactions, Fe^{2+} may stimulate phenol oxidase activity even more strongly. This catalysing effect of Fe^{2+} stimulating phenol oxidase activity may especially be important at waterlogged, microaerophilic conditions, when H_2O_2 production is limiting.

So far, the effects of Fe^{2+} on decomposition and more specifically on phenol oxidase activities have never been determined at waterlogged conditions, although the above analysis shows its potential importance. Therefore, in this paper, we determined (i) the effects of Fe^{2+} addition on phenol oxidase activities in pure enzyme assays, (ii) the effects of Fe^{2+} addition on phenol oxidase activities and phenolics concentration in waterlogged soil slurries, (iii) the effects of initial Fe^{2+} abundance on phenol oxidase activity and phenolics concentration, (iv) the influence of Fe^{2+} on decomposition rates in soil slurries. The soil samples originated from nutrient-poor calcareous dune slacks.

Materials and methods

Enzyme assay on Fe^{2+} effects on phenol oxidase

Phenol oxidase activity of pure enzymes was determined following Pind et al. (1994) and Sinsabaugh and Findlay (1995) as a function of Fe^{2+} concentration. Triplicates of 3 ml of 0, 0.5, 1, 2, 3, 4 and 5 mmol l^{-1} Fe^{2+} in 50 mM acetate and 0.1 mg/ml peroxidase type VI-A were prepared. Fe^{2+} concentrations correspond to the range of Fe^{2+} concentrations encountered in the field and were added as $FeCl_2$. Differences in total salt concentrations were corrected for by adding $CaCl_2$. Solutions were topped with mineral oil to decrease O_2 influx to obtain microaerophilic conditions. At time zero, 1.0 ml of 5 mM L-3,4-dihydroxyphenylalanine (DOPA; a standard phenolics compound that is converted to a quinon by phenol oxidase) in 50 mM acetate was added. The tubes were shaken vigorously and incubated at 20 °C. After 10 min, the extinction was measured photospectrometrically at 460 nm against a blank with 1.0 ml of 50 mM acetate addition. Immediately afterwards, the pH was measured. Fe^{2+} and Fe^{3+} concentration measurements – by ferrozine following a 0.5 N HCl-extraction with and without 0.25 M hydroxylamine (Lovley and Phillips 1987) – showed that iron oxidation was negligible during the incubation.

The molar extinction coefficient for DOPA under the conditions of the assay was determined by mixing 1.0 ml of 1 mM DOPA in 50 mM acetate with 3.0 ml of 0.1 mg/ml peroxidase in 50 mM acetate. The mixture was shaken vigorously and the peak extinction was determined photospectrometrically at 460 nm against a blank with 1.0 ml of 50 mM acetate addition at 20 °C. The determined molar extinction coefficient was 7.5×10^3 mol^{-1}.

Effects of Fe^{2+} on carbon mineralisation and phenol oxidase activity in soil

Top soil from 0 to10 cm of waterlogged nutrient-poor calcareous coastal dunes slacks from the Kennemer dunes in the Netherlands (total C = 1.2%, total N = 0.04%, pH-KCl = 7.4) was collected, mixed and root material was removed. Immediately afterwards, six replicate suspensions of 0, 2, 4 and 6 mmol l^{-1} Fe^{2+} were prepared by adding 25 ml solution to 10 g fresh weight soil. Ferrous iron was added as $FeCl_2$. Background extractable Fe^{2+} concentrations measured by ferrozine (see above) were less than 1 mmol l^{-1} and neglected. Differences in total salt concentrations were corrected for by adding $CaCl_2$. Suspensions were closed, briefly flushed with N_2 to create microaerophilic conditions and incubated for 2 h at 20 °C while shaking at 120 rpm. Thereafter, one sample of 3 ml was withdrawn from the soil suspension and centrifuged at 2500 rpm for 3 min. The supernatant was analysed for soluble phenolics using the Folin–Ciocalteau reagents (Box 1983) with tannin as a standard. In addition, two samples of 2.0 ml soil suspension each were taken

for phenol oxidase activity analysis (Pind et al. 1994). One sample was added to 2.0 ml of 50 mM acetate (i.e. a blank) and the other to 2.0 ml of 5 mM DOPA in 50 mM acetate. The mixtures were shaken vigorously and incubated at 20 °C. After 10 min, both mixtures were centrifuged at 2500 rpm for 3 min and the extinction of the supernatant was measured photospectrometrically at 460 nm. Note that this procedure provides a measure of potential phenol oxidase activity.

After sampling, the suspensions were closed again, briefly flushed with N_2 and repositioned at 120 rpm. Immediately afterwards and after 1 h, 3 ml gas samples were withdrawn and were injected into vacuum 3-ml serum bottles until the bottles had a pressure of 10^5 Pa. Gas samples were analysed for produced CO_2 by GC on a Carboplot P7 column coupled to a TCD. GC-measurements were calibrated by CO_2 processed in the same way as the gas samples. Gamma-irradiated sterile soil slurry was incubated parallelly to correct for potential abiotically released CO_2.

Next, the suspensions were incubated at 20 °C for 5 days. Thereafter, the determinations on phenol oxidase activity, CO_2 production and phenolics concentration were repeated in the same way. In addition, the pH and Fe^{2+} concentrations (see above) were measured.

Effects of actual and initial Fe^{2+} concentrations on phenol oxidase activity in soils

Waterlogged top soil from 0 to 10 cm of a nutrient-poor, calcareous coastal flow-through dune slack (Grootjans et al. 1998) in the Kennemer dunes (the Netherlands) was sampled. One side was affected by seepage (pH-KCl = 7.6) and the other side by infiltration water (pH-KCl = 7.4). Each side was sampled in four replicates at the same height across a distance of 50 m. Plant biomass, plant species composition and soil nutrients were similar at all locations. Samples were stored at 4 °C until use. Samples were mixed and root material was removed. Immediately afterwards for each sample, one subsample was taken for moisture determination and one subsample was taken for the determination of extractable Fe^{2+} (Lovley and Phillips 1987).

In addition, subsamples of 10 g fresh weight were added to 25 ml of 0, 2, 4 and 6 mmol l^{-1} Fe^{2+} as $FeCl_2$. Differences in total salt concentrations were corrected for by adding $CaCl_2$. Suspensions were briefly flushed with N_2 to create microaerophilic conditions and incubated for 4 h at 20 °C while shaking at 120 rpm. Thereafter, two samples of 2.0 ml soil suspension each were taken for phenol oxidase activity analysis as described above. In addition, 3 ml soil suspension was withdrawn for dissolved phenolics analysis as described above. After sampling, the suspensions were again briefly flushed with N_2 and incubated at 20 °C. After 5 days of incubation, the determination of phenol oxidase activity and phenolics concentration were repeated in the same way. In addition, the pH and Fe^{2+} concentrations (see above) were measured.

Correlations between decomposition, phenolics and Fe^{2+} in field studies

Correlations between decomposition rates, dissolved phenolics concentrations and Fe^{2+} concentrations were determined in two field studies. The first field study was performed in the waterlogged nutrient-poor calcareous Kennemer dunes (the Netherlands) at 72 sampling locations across 8 dune slacks. Soil samples were taken, mixed and root material was removed. Soluble phenolics and extractable Fe^{2+} concentrations were determined as described above. In addition, 10 g subsamples were inserted into bottles, closed and incubated at 20 °C for 3 weeks. Gas samples were taken and CO_2 production was determined as above. Field sampling was carried out in May, June, August and September 2000, but CO_2 production was determined only in May and September 2000. In September 2000, an additional measure of decomposition rates was obtained through the Cotton Strip Tensile Strength Loss (CTSL, Maltby 1988) for a subset of 28 locations. Cotton strips of 20-cm long were inserted into the soil and one was retrieved immediately (i.e. the blank) and a 2nd was retrieved after 4 weeks. CTSL was measured pair wise against the blank by an Advanced Force Gauge AFG 1000 N.

The 2nd field study was carried out in a clayey peat land in the central part of the Netherlands. In May 2001, soil pore water was sampled randomly in 10 replicates in 5 different fields with similar groundwater levels, but affected by seepage and infiltration water, respectively. Pore water was analysed for soluble phenolics (as above) and total dissolved iron. The latter was analysed by Induced Coupled Plasma analysis. Cotton strips were incubated for analysis of CTSL, as described above.

Statistics

Prior to analysis, all data were examined for homogeneity of variances with Levene test and were log-transformed if appropriate. Differences between phenol oxidase activity with and without the addition of the pure enzyme was tested by a two-tailed paired *t*-test. Effects of Fe^{2+} on decomposition rates, total phenolics concentration and phenol oxidase activity of the pure enzymes and in soil were analysed by an analysis of variance (ANOVA). Effects of initial Fe^{2+} concentrations and incubation time on phenol oxidase activity and on total phenolics concentration were also analysed by ANOVA. Significantly different means were detected with *post-hoc* Tukey HSD multiple comparison test. Regression analysis was performed on the relation between phenol oxidase activity and Fe^{2+} concentrations in the incubated soils and on the relation between Fe^{2+} concentrations and CTSL, CO_2 production rates and phenolics in the field studies.

Results

Enzyme assay on Fe^{2+} effects on phenol oxidase

Addition of Fe^{2+} significantly ($p < 0.001$) stimulated the phenol oxidase activity of pure enzymes over orders of magnitude (Figure 1). This reaction rate was independent of light conditions (results not shown). The reaction also proceeded purely chemically, but the reaction rate was orders of magnitude slower without the enzyme independent of the Fe^{2+} concentration (results not shown) ($p < 0.001$). Assays had a pH of 4.80 ± 0.01 independent of treatment.

Effects of Fe^{2+} on carbon mineralisation and phenol oxidase activity in soil

Addition of Fe^{2+} significantly stimulated phenol oxidase activity in waterlogged soil, both initially (by maximally 50%, $p = 0.037$; Figure 2a) and after 5 days of incubation (by maximally 40%, $p = 0.032$; Figure 2c). The effects were, thus, smaller than in the pure enzyme assays. Concomitantly with the stimulation of these decomposition enzymes, total carbon mineralisation rates were significantly increased by Fe^{2+} addition, both initially (by orders of magnitude, $p < 0.001$; Figure 2b) and after 5 days of incubation (by maximally twofold, $p < 0.001$; Figure 2d). Carbon mineralisation rates and the absolute effects of Fe^{2+} on these rates were lower after 5 days. CO_2 release from the sterile control was less than 5% of the carbon mineralisation measured in any of the other treatments (results not shown). Total soluble phenolics concen-

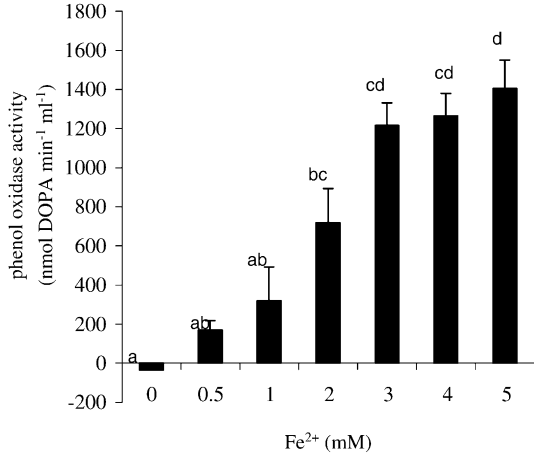

Figure 1. Phenol oxidase activity as a function of added ferrous iron measured in a laboratory experiment with purified peroxidase enzyme ($n = 3$). Different letters indicate significant differences between treatments ($p < 0.05$).

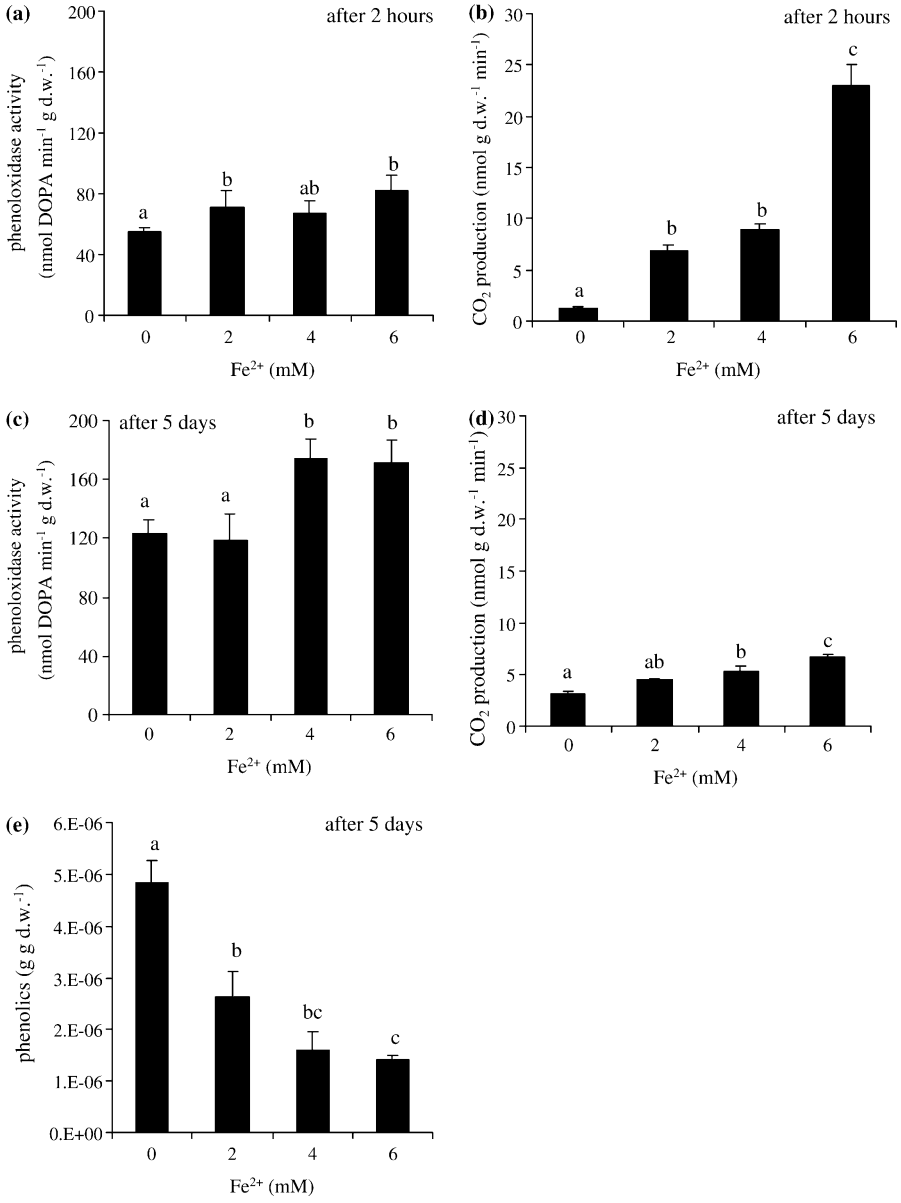

Figure 2. Effects of added ferrous iron on a dune slack soil in a laboratory incubation on (a) phenol oxidase activity after 2 h of incubation, (b) CO_2 production after 2 h of incubation, (c) phenol oxidase activity after 5 days of incubation, (d) CO_2 production after 5 days of incubation and (e) phenolic concentration after 5 days of incubation ($n = 6$). Different letters indicate significant differences ($p < 0.05$).

trations were significantly ($p < 0.001$) decreased by Fe^{2+} addition (Figure 2e), in line with the increased phenol oxidase activity by Fe^{2+}. The pH of the slurries was 7.47 ± 0.02 and was not affected by Fe^{2+} addition. This indicates, in combination with insignificant changes in Fe^{2+} during the incubation, that net Fe^{2+} oxidation was negligible.

Effects of actual and initial Fe^{2+} concentrations on phenol oxidase activity in soils

The effect of different initial Fe^{2+} concentrations on the abovementioned relationships was tested with soil samples obtained from seepage sites (with 4–6 mmol l^{-1} Fe^{2+}) and from infiltration sites (with 0–2 mmol l^{-1} Fe^{2+}) with otherwise similar soil chemical properties. The effects of initial Fe^{2+} concentrations on phenol oxidase activity changed with time of incubation, as indicated by a significant interaction effect ($p = 0.049$). At the short-term, initial Fe^{2+} concentrations ($p = 0.047$) affected phenol oxidase activity and was 4 times higher in samples from seepage sites (Figure 3a) in line with the higher Fe^{2+} concentrations. After 5 days of incubation, these effects had become insignificant (Figure 3a). However after 5 days of incubation, the actual Fe^{2+} concentration (combining initial Fe^{2+} concentrations and Fe^{2+} additions) was significantly ($p = 0.006$; $r^2 = 0.42$) related to phenol oxidase activity, again over orders of magnitude (Figure 3c). Phenol oxidase activity increased significantly ($p < 0.001$) over time.

Initial Fe^{2+} concentration also had a significant effect ($p < 0.001$) on soluble phenolics concentration after incubation of 4 h and 5 days (Figure 3b). At both moments, soil samples obtained from seepage sites had 30–45% lower phenolics concentrations than those from infiltration sites. The effects of actual Fe^{2+} concentration on soluble phenolics concentration were insignificant. The soluble phenolics concentration increased significantly over time by about threefold ($p < 0.001$). The pH of the slurries was 7.64 ± 0.08 and was not affected by Fe^{2+} additions. This indicates again, in combination with insignificant changes in Fe^{2+} during the incubation, that net Fe^{2+} oxidation was negligible in these incubations.

Correlations between decomposition, phenolics and Fe^{2+} in field studies

There was a highly significant ($p < 0.001$) positive relation between Fe^{2+} and CTSL, both in the Kennemer dunes ($R = 0.64$; Figure 4a) and in the clayey peat land ($R = 0.46$; Figure 4b). Similar strong relations ($p < 0.001$) were found between Fe^{2+} and CO_2 production rates in the Kennemer dunes ($R = 0.60$; Figure 4e). Contrary to the incubation studies, a highly significant ($p < 0.001$) positive relationship was found between Fe^{2+} and soluble phenolics

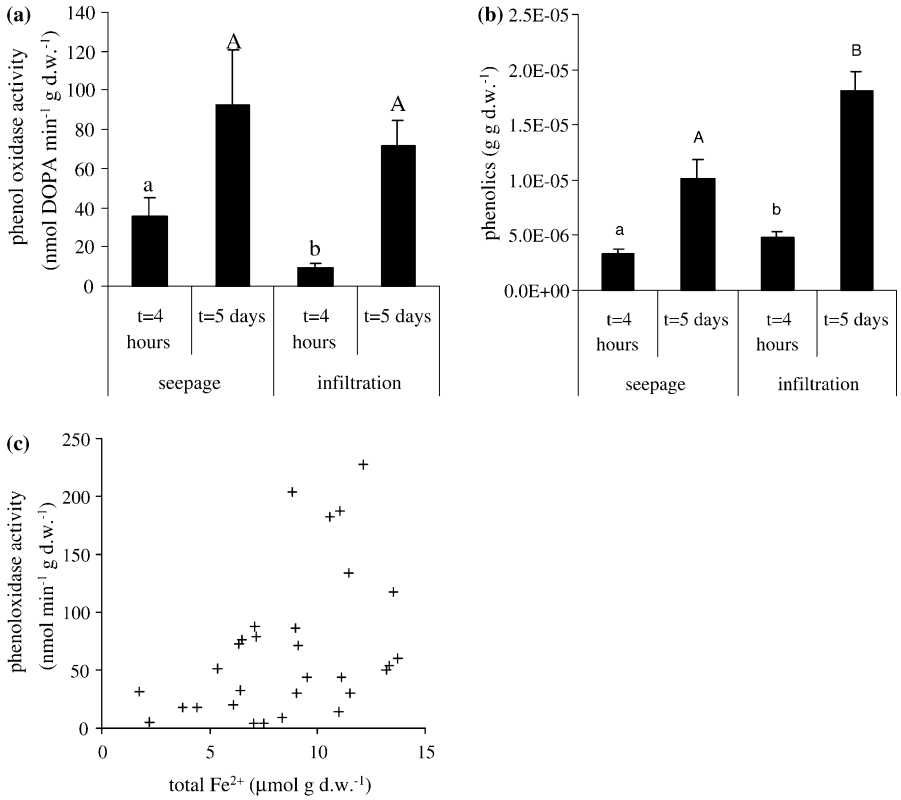

Figure 3. Effects of added and initial Fe^{2+} concentrations in a laboratory experiment with dune slack soils. (a) Phenol oxidase activity and (b) phenolics concentrations as a function of time of incubation and site with seepage sites having high initial Fe^{2+} concentrations and infiltration sites low initial Fe^{2+} concentrations, combining the various Fe^{2+} addition treatments ($n = 16$). Different letters indicate significant differences ($p < 0.05$). (c) Relation between total ferrous iron concentration and phenol oxidase activity after 5 days of incubation.

concentrations, both in the Kennemer dunes ($R = 0.38$; Figure 4c) and in the clayey peat land ($R = 0.92$; Figure 4d).

Discussion

Ferrous iron stimulates phenol oxidase activity

Lignin is one of the most abundant and most recalcitrant soil organic compounds (Hammel 1997). Its decomposition is limited to a few extracellular enzymes. These extracellular enzymes include laccases and different peroxidases including lignin peroxidase, manganese peroxidase and horseradish per-

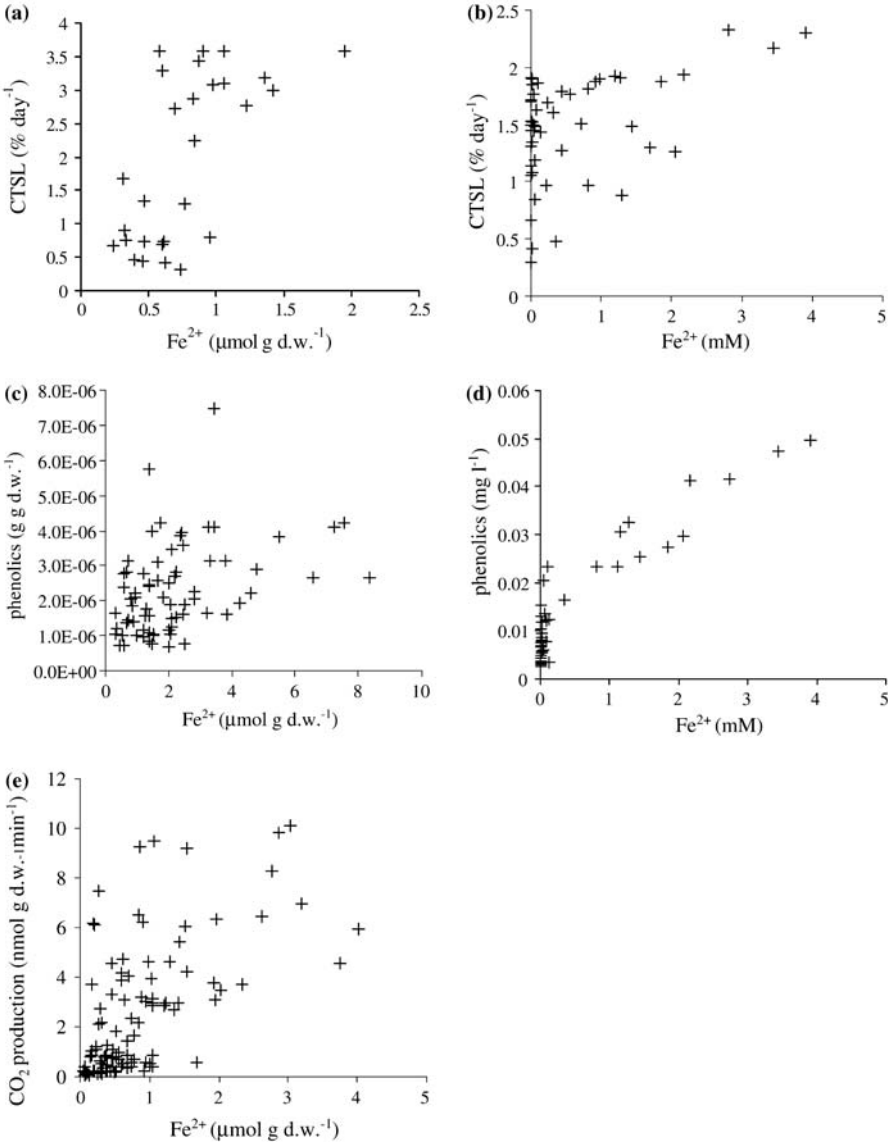

Figure 4. Correlations between Fe^{2+} concentrations and (a, b) the Cotton strip Tensile Strength Loss (CTSL), (c, d) the soluble phenolics concentrations and (e) CO_2 production rates measured in coastal dune slacks (a, c, e) and clayey peat lands (b, d), respectively. Note the different measurement units along the axes.

oxidase (de Jong et al. 1994). All these phenol oxidase enzymes display a fundamental similarity; they depend on the generation of lignin free radicals through OH radicals (Tanaka et al. 1999). At oxic conditions, these radicals are

generated via O_2. As a consequence, phenol oxidase activity is seriously hampered at oxygen-limited conditions as occurs in many wetlands (Freeman et al. 2001b). Our pure enzyme assays show that Fe^{2+} strongly stimulates phenol oxidase activity in concentrations normally encountered in the field at those microaerophilic conditions (with still traces of O_2 present). There is thus a direct stimulating effect of Fe^{2+} addition on phenol oxidase activity, independent of microbial growth, induction of enzymes or microbial competition for common substrates.

We hypothesise that this stimulating effect of Fe^{2+} is either due to the production of additional $O_2^{-\cdot}$, that subsequently dismutates spontaneously to H_2O_2 (as proposed by Enoki et al. 1997; Tanaka et al. 1999), or via the reaction of $O_2^{-\cdot}$ produced through auto oxidation of phenolics with Fe^{2+} in presence of the enzyme to H_2O_2 and Fe^{3+} (Gómez-Toribio et al. 2001). The latter reaction would energetically stimulate further production of $O_2^{-\cdot}$, thus catalysing the total phenol oxidation rate (cf. Wood 1994). Unfortunately, the high turnover rates of the intermediates did not allow us to measure these intermediates to test these hypotheses. In either case, however, Fe^{2+} catalyses additional OH radical production and promotes the oxidation of phenolics. The stimulation through catalysis explains why no net oxidation of Fe^{2+} occurred in any of the incubations. This hypothesised mechanisms may also explain why the response of phenol oxidase activity to Fe^{2+} is linear for such a wide range of concentrations, which would be improbable if direct binding of Fe^{2+} to the enzyme would have been involved. At high Fe^{2+} concentrations, the rate of H_2O_2 generation is probably limited by O_2 input rather than Fe^{2+} abundance, causing an asymptotic relationship between phenol oxidase activity and Fe^{2+} concentrations.

The effects of Fe^{2+} on phenol oxidase activity were less strong in the soil incubations than for pure enzymes. This may indicate chemical or physical interactions, e.g. sorption of phenol oxidase to the complex soil matrix, precipitation of iron or stronger oxygen limitations induced by soil oxygen consumption. However, also the soil incubations showed that Fe^{2+} stimulates phenol oxidase activity. This effect occurred both under influence of high actual Fe^{2+} concentrations by Fe^{2+} addition and by high initial Fe^{2+} concentrations, induced by the presence of seepage with significantly higher iron and calcium concentrations. The latter stimulating effect indicates that the weaker effects of Fe^{2+} in soils is probably not due to adverse effects of Fe^{2+} on the induction of these extracellular enzymes or on microbial growth of soil microorganisms (as Fe^{2+} is potentially toxic to microorganisms (e.g. Braun 1998)) involved in the production of phenol oxidase.

Previously, the effects of other compounds than Fe^{2+} on phenol oxidase activity were described. The effects of pH are the most apparent, causing an increase in activity from pH 2 to 8 (Pind et al. 1994). In this study, differences in pH cannot explain the observed effects of Fe^{2+} as the enzyme assays were carried out in pH-buffered media and the soil incubations had a natural buffering by calcium carbonates. Indeed, no significant pH effects were present in

any of the experiments. Effects of other compounds were ruled out by the controlled set-up of the experiments. So, all studies indicate a direct stimulation of phenol oxidase activity by Fe^{2+}. This stimulation is potentially important at waterlogged conditions in wetlands.

Effects of ferrous iron on decomposition

Given the importance of lignin degradation for soil organic matter decomposition, its limited degradation at waterlogged conditions and the subsequent stimulation of phenol oxidase by Fe^{2+}, the presence of Fe^{2+} may well stimulate decomposition at waterlogged conditions. Phenol oxidase may enhance decomposition by depolymerisation of lignin and phenolics, although it may also lead to polymerisation of lignin at specific conditions (Ander 1994). Indeed, addition of Fe^{2+} in concentrations normally encountered in the field also led to a very strong, up to an order of magnitude, increase in CO_2 production. This is consistent with the stimulation of phenol oxidase activity, although also other ligninolytic enzymes that thrive on H_2O_2 (e.g. Wood 1994) may have been stimulated through Fe^{2+}. It supports the results of Freeman et al. (2001a) on the importance of phenol oxidase activity for determining decomposition rates at waterlogged conditions. Again, these results cannot be explained from either direct pH effects as indicated by the insignificant pH changes or from indirect pH effects through the abiotic release of CO_2 as indicated by the small contribution of abiotic CO_2 emission compared to the total CO_2 release.

Other studies have also shown the crucial importance of water quality for CO_2 production in wetlands, particularly in relation to directly (Wright and Reddy 2001) or indirectly (e.g. Lamers et al. 1998; Beltman et al. 2000) enhanced phosphate availability that increased decomposition rates and the activity of some extracellular enzymes. The stimulation of decomposition rates by Fe^{2+} has not been shown before. In fact, given that iron may precipitate with phosphate or adsorb with phosphate to the soil matrix, a decrease in decomposition rates based on analogies with the internal eutrophication mechanisms described above would have been expected. The opposite occurred in our study. The complex interactions between water quality – and thus hydrological regimes – and decomposition in freshwater ecosystems merit further quantification to understand and predict changes in CO_2 emission patterns.

Importance of ferrous iron at field conditions

Quantifying the relevance of the abovementioned stimulation of phenol oxidase activity and decomposition by Fe^{2+} in comparison to other processes is difficult at field conditions due to collinearity problems: In agreement with

the observations of our experiments, we found highly significant positive relationships between Fe^{2+} and decomposition rates – measured as CO_2 production rates and CTSL – across a broad range of field conditions in two highly different wetland ecosystems. Unfortunately, in both wetland systems there were strong correlations between Fe^{2+} and pH and O_2 concentrations as occurs frequently in wetland systems. This collinearity hampers the quantification of the relevance of the individual parameters from these field observations. Thus, these results on the importance of Fe^{2+} for decomposition at field conditions should be treated cautiously although it underlines the importance of water quality in determining carbon decomposition in waterlogged wetlands.

Similarly, it is difficult to assess the stimulation of phenol oxidase activity by Fe^{2+} at field conditions from soluble phenolics. In this study, phenol oxidase activity was assayed from the depolymerisation of soluble phenolics, a pathway dominant in Freeman et al. (2001a). However, phenol oxidase also depolymerises lignin leading to the production and accumulation of soluble phenolics (Freeman et al. 2001b). In addition, phenol oxidases can polymerise phenolics (Sulfita and Bollag 1981; Sinsabaugh and Linkins 1988) following a similar pathway as discussed above (Mai et al. 2001). In soils, this pathway seems partly prevented by cellobiose-oxidising enzymes (Ander 1994). Given the involvement of phenol oxidase at these different stages of phenolics turnover and the effects of substrate limitation on phenol oxidase activity on top of the problems of collinearity, proof of the involvement of stimulation by Fe^{2+} at field conditions is difficult to assess through soluble phenolics concentrations.

Indeed, contrary to the decrease in soluble phenolics concentrations in the experiments of this study, we found that the phenolics concentration was significantly positively correlated to Fe^{2+} concentrations in both field studies. In conformity, the phenolics concentration was significantly ($p < 0.001$) positively correlated to decomposition rates (results not shown). This implies that the inhibiting effect of phenolics on microbial activities (Wetzel 1992) cannot explain the mineralisation patterns in the field studies. These results provide circumstantial evidence that, similar to the results obtained for a broad range of wetlands in Freeman et al. (2001b), phenol oxidase was more active as a depolymeriser of lignin, which also releases CO_2 (Dec et al. 2001), than as an oxidiser of soluble phenolics across the wide range of wetlands studied and that Fe^{2+} stimulated this process. Further circumstantial evidence for this is given by the highly significant ($p < 0.001$) positive correlation between Fe^{2+} concentrations and the accumulation of soluble phenolics in incubations of field samples from the clayey peat land (results not shown).

An alternative explanation for the positive correlation between Fe^{2+} and phenolics concentrations is the possible co-dissolution of Fe^{2+} and phenolics from Fe(hydr)oxides/humic complexes during microbial Fe^{3+} reduction (e.g. Tipping and Woof 1983). On the other hand, several studies demonstrated that low pH-values favour the formation of Fe(hydr)oxides/ humic complexes (e.g.

Tipping 1981; Avena and Koopal 1999) and that these complexes disappear at pH > 7.0 (Schnitzer 1978), the pH present in the field studies. Clearly, more research is needed to elucidate the importance of the proven stimulation of phenol oxidase activity and decomposition by Fe^{2+} at field conditions.

In conclusion, we have shown that Fe^{2+} promotes and catalyses phenol oxidase activity of pure enzymes and in waterlogged soils. Consequently, CO_2 production rates are increased at these microaerophilic conditions. The importance of this stimulation by Fe^{2+} for decomposition in wetlands at field conditions compared to the effects of other compounds still needs to be elucidated, but it emphasises the crucial role of water quality in determining decomposition in wetlands.

Acknowledgements

This research was supported by the Technology Foundation STW, applied science division of NWO and the technology programme of the Ministry of Economic Affairs (STW-NWO Grant Nr. VBI.4840).

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