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Microbial cycling of iron and sulfur in sediments of acidic and pH-neutral mining lakes in Lusatia (Brandenburg, Germany)

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Abstract. A vast number of lakes developed in the abandoned opencast lignite mines of Lusatia (East Germany) contain acidic waters (<pH 3) with high concentrations of dissolved iron and sulfate. The elimination of iron and sulfate by microbially mediated reduction processes produces alkalinity, whereas the reoxidation of reduced sulfur compounds consumes alkalinity. In order to determine the influence of environmental factors with the prospect of biological remediation strategies, these processes were investigated in sediments of two acidic and one pH-neutral mining lake. Slightly acidic sediment underlying permanently anoxic waters showed a higher accumulation rate of reduced inorganic sulfur than the pH-neutral sediment (60 v.s. 45 $\mathrm{mmol\,S\,m^{-2}\,a^{-}}$). Potential Fe(III) reduction measured by the accumulation of Fe(II) during anoxic incubation yielded similar rates in both types of sediments, however, the responses towards the supplementation of Fe(III) and organic carbon were different. Sulfate reduction rates estimated with ^{35}S-radiotracer were much lower in the slightly acidic sediment than in the pH-neutral sediment (156 v.s. 738 $\mathrm{mmol\,SO_4^{2-}\,m^{-2}\,a^{-1}}$). However, sulfate reduction rates were increased by the addition of organic carbon. Severe limitation of sulfate-reducing bacteria under acidic conditions was also reflected by low most probable numbers (MPN). High MPN of acidophilic iron- and sulfur-oxidizing bacteria in acidic sediments indicated a high reoxidation potential. The results show that potentials for reductive processes are present in acidic sediments and that these are determined mainly by the availability of oxidants and organic matter.

Abbreviations: AVS – acid volatile sulfur; CRS – chromium reducible sulfur; FeRB – iron(III)-reducing bacteria; FeOB – iron-oxidizing bacteria; ML – mining lake; MPN – most probable number; SRB – sulfate-reducing bacteria; SOB – sulfur-oxidizing bacteria; TRIS – total reduced inorganic sulfur

Introduction

In the Lusatian opencast lignite mining area in the East of Germany a new lake district came into existence as a result of rising ground water and concomitant filling of open pits left in the abandoned mining sites (Geller et al. 1998). Whereas the greater lakes ($>10\,\mathrm{km^2}$ surface area) are flooded with pH-neutral river water, the majority of the smaller lakes contain acidic waters (pH 2–4) of high ionic strength (Klapper 2000). Primary production, that is, autochthonous production of easily degradable organic matter is very low in these acidic mining lakes (Kapfer 1998; Nixdorf et al. 1998). Acidity is generated by the weathering of overburden materials that contain pyrite and marcasite. The solution of the reaction products SO_4^{2-}, Fe^{2+}, and H^+, the oxidation of Fe^{2+}, and the consequent precipitation of Fe(III) minerals lead to the formation of acidic waters which are often referred to as acid mine drainage (AMD) (e.g., Nordstrom and Alpers 1999). If the buffer capacities of surrounding soils are low, in-lake alkalinity generation maintained by biological processes in or near the sediment plays a major role (Cook et al. 1986; Schindler et al. 1986). In AMD impacted aquatic habitats, the microbially mediated processes of sulfate reduction (e.g., Herlihy and Mills 1989) and Fe(III) reduction (e.g., Vile and Wieder 1993) coupled to the oxidation of organic material are responsible for alkalinity production, which has also been shown for acidic mining lakes of Lusatia (Peine et al. 2000). The net effect of these processes depends on the long-term storage of their endproducts as FeS or FeS_2 and the extent of reoxidation. The complete oxidation of sulfur compounds and the oxidation of Fe(II) under acidic conditions are chiefly catalyzed by microorganisms which are since long known to be involved in the formation of AMD (Ehrlich 1996). Hence the interactive play of reducing and oxidizing microorganisms is of importance, as it has been demonstrated, for example, for the mineral formation in mine tailings by Fortin et al. (1996).

Although microbiology of AMD in different environments has been intensively studied, few data are available on acidic mining lakes and few studies comprise both the reductive and the oxidative part of microbial iron- and sulfur-cycling. Acidic mining lakes are special and diverse habitats. Different mixing/stratification regimes strongly influence redox-cycling and acidity budgets. In this study we compare the sediments of two acidic mining lakes, which differ in degree of acidification, and one pH-neutral mining lake. Abundances and activities of functional bacterial groups are related to the geochemical conditions in order to assess the effect of environmental factors on neutralizing capacities of the sediments.

Methods

Site description

From 1995 to 1997 we investigated three mining lakes located in the Lusatian lignite mining district of Brandenburg, Germany (Table 1). The three lakes are relatively small and do not have surface in- or out-flows. Since their development they are left unimpaired of human activities. ML 111 and ML 107 hold acidic waters of high ionic strength, whereas the waters of ML B are of neutral pH. ML 111 consists of three basins. A water depth >7.5 m is found only in a small area (4% of total surface area) in the central basin (Büttner et al. 1998). Apart from this site the maximum depth measures 7 m. Since summer 1995, ML 111 is meromictic and anoxia prevails at a water depth >7.5 m. Additionally, summer stratification leads to an oxygen decrease at a water depth >4 m. At the deepest site, above the

Table 1. Key features of the three mining lakes (Packroff et al. 2000). Values for pH, conductivity, ion and nutrient concentrations are given for surface waters.

	ML 111 Mine Plessa	ML 107 Mine Agnes	ML B Mine Schlabendorf
Coordinates	51°29′N, 13°38′E	51′29′N, 13°30′E	51°51′N, 13°51 E
Mining activities	1929 – 1958	1827 – 1928	1963/64[a]
Surface area (km^2)	0.107	0.122	0.1
Maximum depth (m)	10.2	5.0	10
Average depth (m)	4.6	1.9	5–6
PH[b]	2.6	2.3	8.0
Conductivity (mS cm^{-1})[c]	2.4 – 2.6	2.5 – 5	1
Sulfate (mM)	13.65	27.92	4.59
Chloride (mM)	0.24	0.11	0.56
DIC[d](mM)	TE[e]	TE	1.79
Calcium (mM)	5.65	8.91	4.2
Iron (mM)	2.79	9.84	TE
Aluminum (mM)	1.41	1.91	$\ll$[f]
Magnesium (mM)	1.19	1.83	0.81
Silicon (mM)	0.61	0.66	0.027
Sodium (mM)	<0.4	<0.2	0.5
Potassium (mM)	0.1	<0.025	0.13
Manganese (mM)	0.055	0.20	TE
Ammonium (mM)	0.20	0.43	<[g]
Nitrate (mM)	0.023	0.014	<
TP[h](μM)	0.35	0.74	0.29
SRP[i](μM)	0.23	0.69	0.06

[a] Year of origin; [b] annual average for pH, ions, and nutrients; [c] range for the years 1996/1997; [d] DIC dissolved inorganic carbon; [e] TE trace element; [f] $\ll$ not detectable; [g] < minor concentrations subjected to seasonal variations; [h] TP total phosphorus; [i] SRP soluble reactive phosphorus.

sediment, the water temperature measures 5.5 °C throughout the year. In contrast, ML 107 is a shallow, polymictic lake exhibiting only weak thermal stratification during warmer periods (Leßmann et al. 1999). Ice cover and summer stratification lead to a decrease of oxygen in the water column and occasionally anoxic conditions above the sediment. The water temperatures above the sediment reach from 2 °C in winter to 16 °C in summer. The reference lake, ML B, is a mesotrophic, dimictic lake (Packroff et al. 2000). During summer stratification the oxygen content decreases in the hypolimnion (at a water depth >4 m) but it never gets completely depleted. The water temperature above the sediment at deepest site fluctuates between 4 and 9° C.

Sediment sampling and determination of pH and redoxpotential (E_h)

The sediments were sampled at the greatest depth in all three mining lakes and additionally in ML 111 in the central basin at a waterdepth of 7 m [referred to as ML 111 (7 m) while the sampling site at greatest depth is referred to as ML (10 m)]. Undisturbed sediment cores were recovered in Plexiglas tubes by gravity coring.

Subsampling of the upper 10 cm of the sediment was done directly in the field after core retrieval. The sediment cores were extruded stepwise in 1 cm-intervals from the Plexiglas tube and then sliced. Subsamples were transferred into sterilized containers and the gasphase was flushed with oxygen-free nitrogen or argon. Subsamples for the determination of S-compounds and sulfate reduction rates were retrieved by means of tipless 5 ml-syringes. The subcores enclosed 5 cm thick sediment layers with a total volume of 6 ml. Two sets of subsamples were obtained to recover the top 10 cm of the sediment. The subcores were sealed with Parafilm® and kept in airtight boxes that were flushed with oxygen-free nitrogen. Sediment samples for the substrate incubation experiments to stimulate sulfate reduction were taken by means of a grab sampler. Temperature and O_2 concentration in the water column were measured by a Idronaut multiparameter probe (Idronaut Srl., Brugherio, Italy). Values of pH and E_h in the sediment were determined immediately after core slicing within the containers by punching the electrodes into sediment samples using commercial glass electrodes. The values of E_h were converted to standard H_2-electrode and corrected for pH.

Sediment and pore water analysis

The dry weight of the sediment was determined by weight loss at 105 °C overnight. Loss on ignition (LOI) was determined by weight loss at 550 °C for 4 h. Pore water was collected by centrifuging 20–30 ml of sediment at $9000 \times g$ for 10 min at 4 °C. The supernatant was 0.2 µm-filtered and acidified with a sterile HCl-solution to prevent precipitation of Fe(III) oxyhydroxides and stored until analysis at 4 °C. Soluble sulfate was determined by ion chromatography (one column technique) using an anion exchange column GAT Anion/S 250 mm × 4.6 mm ID (Gamma Analysentechnik GmbH, Bremerhaven-Lehe, Germany) and a conductivity detector. Dissolved organic carbon (DOC) was analyzed IR-spectrometrically by a DimaTOC 100 apparatus (Dirsus and Co, Essen, Germany).

Microbially reducible (0.25 mM hydroxylamine-reducible) Fe(III) and 0.5 mM HCl-soluble Fe(II) in bulk sediment were determined with ferrozine following the protocol of Lovley and Phillips (1987a) except that centrifugation was used for clearing photometric samples instead of filtration. Extractions were done in triplicates. Microbially reducible Fe(III) was calculated as the difference between the Fe(II) measured in the hydroxylamine- and HCl-extractions.

The amount of reduced inorganic sulfur in bulk sediment was determined on samples fixed in 20% Zn-acetate using the sequential reflux-distillation method. For the extraction of acid volatile sulfur (AVS: $H_2S + FeS$) and chromium reducible sulfur (CRS: $S^0 + FeS_2$) the protocol of Fossing and Jørgensen (1989), as modified by Meier et al. (2000) was followed. The extraction was done in duplicates. On one of the duplicate samples a separate extraction of S^0 was performed subsequently to the extraction of AVS. The sediment was retrieved by centrifugation at $3000 \times g$ for 10 min and extracted two times with 10 ml 96% ethanol at room temperature for 30 min. The supernatants were merged and solved S^0 was liberated by hot distillation with 15 ml 1 M $CrCl_2$ for 40 min. From the sediment pellet then the remaining CRS (FeS_2) was extracted. The mass of reduced sulfur was determined spectrophotometrically by the methylene blue method (Cline 1969). The total of reduced inorganic sulfur (TRIS) is referred to as the total of AVS and CRS. The AVS-S:FeS_2-S ratio was calculated from the mean value of AVS and pyritic S. Pyritic S was calculated by subtracting the percentage of S^0 (determined on one duplicate sample) from the mean value of total CRS.

Bacterial counts

Numbers of iron-oxidizing (FeOB), sulfur-oxidizing (SOB), sulfate-reducing (SRB), and iron-reducing bacteria (FeRB) were determined by the most probable number (MPN) technique. The mineral medium for FeOB, SOB, and SRB contained per liter 0.1 g KH_2PO_4, 0.1 g NH_4Cl, 0.1 g NaCl, 0.1 g KCl, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.2 g $MgCl_2 \cdot 6H_2O$, and 1 ml trace element solution (Widdel and Bak 1992). For FeOB, 22.5 g l^{-1} $FeSO_4 \cdot 7H_2O$ and 40 μg l^{-1} Vitamin B_{12} were added to the mineral medium. For SOB, 2.48 g l^{-1} $Na_2S_2O_3$, 40 μg l^{-1} Vitamin B_{12}, and 1 mg l^{-1} $FeSO_4 \cdot 7H_2O$ were added. The pH-neutral SOB-medium was carbonate-buffered (0.56 g l^{-1} $NaHCO_3$, adjusted to pH 7.0) with bromocresolpurpur as pH-indicator. The acidic SOB-medium was phosphate-buffered (0.91 g l^{-1} KH_2PO_4, adjusted to pH 4.5) with bromophenolblue as pH-indicator. For SRB, 3.69 g l^{-1} $MgSO_4 \cdot 7H_2O$, 0.05 g l^{-1} $FeSO_4 \cdot 7H_20$, 1 ml l^{-1} selenite-tungstate-solution, 1 ml l^{-1} vitamin-solution, Na-DL-lactate, and Na-acetate (each 5 mM endconcentration) were added to the mineral medium (Widdel and Bak 1992). The SRB-medium was carbonate buffered (1.68 g l^{-1} $NaHCO_3$, adjusted to pH 7.0). The medium to count FeRB was a modified *Geobacter metallireducens*-medium (DSMZ no. 579, Braunschweig, Germany) adjusted to pH 6.5. The dilution series of SRB and FeRB were incubated under CO_2-atmosphere (Anaerocult A, Merck, Darmstadt, Germany). All microplates were incubated in the dark at 28 °C for 6 weeks. MPN and approximate 95% confidence intervals were calculated with the program of Klee (1993).

Fe(III) reduction

Microbial iron reduction in ML 111 (10 m) and ML B was determined by measuring the accumulation of ferrous iron over time during anaerobic incubation (Lovley and Phillips 1986). Sediment material from five sectioned cores was pooled and homogenized. Ten milliliter sediment and 3 ml of supplements (adjusted with sterile distilled water) were combined in 25 ml flasks with airtight screw caps. Alternatively 1 ml amorphous Fe hydroxide slurry (16 mM final concentration), 1 ml carbon source-solution (10 mM acetate and 10 mM ethanol final concentration), 1 ml sodium molybdate-solution (20 mM MoO_4^{2-} final concentration), or 1 ml distilled water (no supplement) were added alone or in combinations. Treatments were prepared in duplicate, one of the no supplement flasks was autoclaved and served as abiotic control. The headspace was sparged with argon. Subsamples for the determination of HCl-soluble Fe(II) (see above) were taken immediately before addition of the supplements (to calculate initial concentrations) and after 20 days. Flasks were incubated at 20 °C in the dark. Handling took place under oxygen-free nitrogen atmosphere in glove bags.

Sulfate reduction

Depth profiles of sulfate reduction rates were estimated in duplicate applying the radiotracer technique as described in Meier et al. (2000). Forty-two microliter carrier-free $^{35}S\text{-}SO_4{}^{2-}$ (3.7 MBq ml^{-1}, Amersham Corp., Braunschweig, Germany) were injected vertically into each sediment subcore. The intact cores were subsequently incubated under oxygen-free nitrogen at ambient sediment temperature for 14–19 h. Reduced inorganic ^{35}S-sulfur was retrieved by sequential reflux-distillation (see above). Radioactivity was determined by liquid scintillation counting (LSC 1600TR, Canberra Packard, Dreieich, Germany).

The influence of different carbon sources on sulfate reduction in sediments of ML 111 (10 m) was tested in two series of batch incubations. Each batch consisted of 250 ml of sediment and 2000 ml of lake water together with 15 g of straw filled into 2 l-glass bottles that were closed with air-tight screwcaps. In the first series the substrates Na-pyruvate, glucose-D-monohydrate, and succinate, and in the second series Na-DL-lactate, ethanol, and Na-acetate were added to a final concentration of 30 mM. A sterile control with autoclaved sediment and an unsterile control without supplement were run parallel to both series. The sediments were incubated for 4 weeks in the dark at room temperature. Sulfate reduction rates were measured in duplicate for the first series and in triplicate for the second series. Four milliliter of sediment were transferred into a reaction tube and mixed with 50 µl of $^{35}S\text{-}SO_4{}^{2-}$ (3.7 MBq ml^{-1}). The headspace was flushed with oxygen-free nitrogen and the tube was closed with a rubber stopper. The sediments were incubated at room temperature for 3.5 h. The samples were analyzed as described above.

Results

The main data presented here was collected during one sampling campaign in April 1997, as from this campaign the data sets were the most complete for all parameters and sites. The results agree well with those of the other three sediment sampling campaigns (in May and July/August 1996 and in August 1997) and are therefore considered to be representative (Meier 2001). In April 1997 the temperatures of the water above the sediment were 5.5, 5.0, 7.5 and 7.1 °C in ML 111 (10 m), ML 111 (7 m), ML 107, and ML B, respectively. Bottom waters were anoxic in ML 111 (10 m), whereas 3, 11, and 11.5 mg O_2 l^{-1} were determined above the sediment in ML 111 (7 m), ML 107, and ML B, respectively.

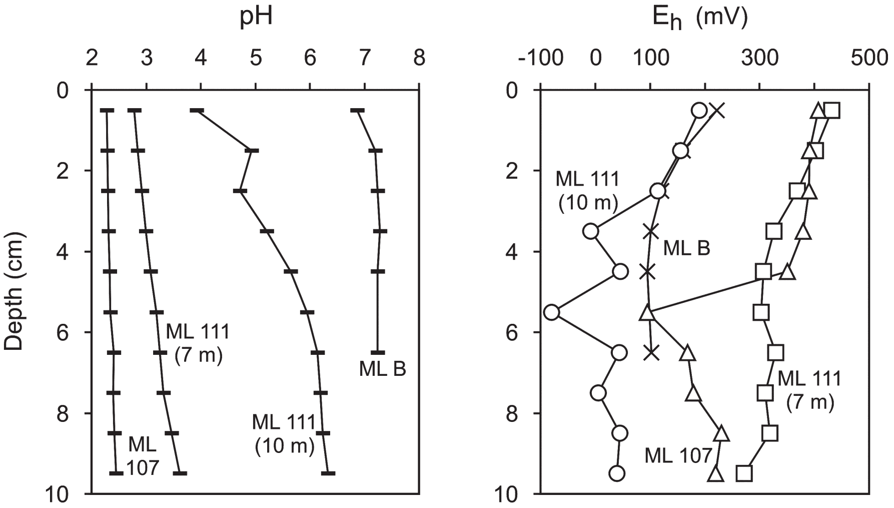

Figure 1. Depth profiles of pH (left) and E_h (right) in the upper 10 cm of sediments in April 1997. The values of E_h were converted to standard H_2-electrode and corrected for pH.

Core description, pH and redoxpotential (E_h)

In ML 111 (10 m) the top 2 cm of sediments were of brownish color. With increasing sediment depth the color turned to dark grey. Thin black layers were observed that were accompanied with the smell of hydrogen sulfide. The blackening disappeared at a depth greater than ca. 8 cm and greyish to olive colored silt appeared. LOI accounted for 25% of dry weight at sediment surface and decreased to 10% of dry weight at 10 cm depth. With increasing depth the pH rose continuously reaching pH 6.3 at 10 cm depth, while the E_h decreased displaying occasionally negative values (Figure 1). In ML 111 (7 m) the sediments were of ochre to brownish color throughout the top 10 cm. The LOI values were similar to ML 111 (10 m). The pH increased only slightly with depth and E_h values were generally much higher than in ML 111 (10 m) (Figure 1).

At the sampling site in ML 107, sediments were covered by a mat of thread-forming green algae. The top 2 cm of the sediment below this mat were of ochre to brownish color. At 2–6 cm depth the highest values of LOI (up to 43% of dry weight) were found. The blackening of this horizon resulted from small black particles that probably were plant residues or coal particles. Below this ‘organic’ horizon the sediment consisted of grey to brownish colored silt. Similar values for pH and E_h as in ML 111 (7 m) were found, except that a sudden drop of E_h was observed at 5–6 cm (Figure 1).

In pH-neutral ML B we found two distinct sediment layers. The upper layer, with a mightiness of 7–9 cm, consisted of a dark greyish silt. The layer below consisted of almost pure sand. LOI accounted for 5–10% of dry weight in the top 10 cm of the sediment. The E_h decreased with depth, though no negative values were observed (Figure 1).

Pore water sulfate and DOC

In the acidic mining lakes the sulfate concentrations of the pore water were higher than those of the lake water (Table 1). This difference was most pronounced for sediments of ML 111 (10 m) where sulfate concentrations ranged from 18 to 26 mM. In sediments of ML 111 (7 m) sulfate reached concentrations of 19–21 mM. In ML 107 the highest concentrations of pore water sulfate were determined, ranging from 25 to 32 mM. In ML B pore water sulfate reached concentrations of 4.6–5.3 mM.

A similar observation was made for DOC, which reached very high concentrations in the pore water of the acidic lakes. In sediments of ML 111 (10 m) and ML 111 (7 m) the DOC concentrations were in the range of 0.94–3.69 mM and 0.69–2.00 mM, respectively. In ML 107, at a depth of 3–4 cm, a maximum value of 4.03 mM DOC was observed. In the pore water of ML B the lowest DOC concentrations (0.33–1.13 mM) were detected. In the sandy sediment layer the DOC concentration was below detection limit.

Fe- and S-compounds

The distribution of Fe- (Figure 2) and S-compounds (Figure 3) reflected very well the either reduced or oxidized status of the sediments. In sediments of ML 111 (10 m), only small amounts of microbially reducible Fe(III) [$<4\,\mu$mol Fe(III) cm^{-3}] were observed in the top 4 cm. However, the layer below was enriched in microbially reducible Fe(III) [up to 41 μmol Fe(III) cm^{-3}]. The total amount of extracted Fe was lowest at sediment surface. In sediments of ML 111 (7 m), throughout the top 10 cm, large amounts of microbially reducible Fe(III) [13–60 μmol Fe(III) cm^{-3}] were available. Similar to sediments of ML 111 (7 m), large amounts of microbially reducible Fe(III) were present in sediments of ML 107. However, these were restricted to the top 6 cm of the sediment. Similar to sediments of ML 111 (10 m), the sediments of ML B contained only small amounts of microbially reducible Fe(III) [maximally 14 μmol Fe(III) cm^{-3}]. In contrast to ML 111 (10 m), the total amount of extracted Fe was highest in the upper centimeters of the sediment.

Corresponding to the Fe-species, the distribution of S-species reflected the reducing conditions prevailing in sediments of ML 111 (10 m) and ML B (Figure 3). In sediments of ML 111 (10 m) the highest amounts of TRIS (18–47 μmol S cm^{-3}) were found at 2–7 cm depth. In this horizon AVS accounted for $70 \pm 8\%$ of TRIS. Above and below this AVS-rich horizon, the amount of TRIS was much lower and was mainly recovered in the CRS-fraction. In contrast to ML 111 (10 m), only minor amounts of TRIS ($<2\,\mu$mol S cm^{-3}) were extracted from sediment of ML 111 (7 m) and these were all recovered in the CRS-fraction. In sediments of ML 107 we found similar to ML 111 (10 m) large amounts of TRIS (up to 52 μmol S cm^{-3}), however, CRS accounted for 99% of it. In sediments of ML B, TRIS increased from 3 μmol S cm^{-3} at the surface to 37 μmol S cm^{-3} at 5–6 cm depth. In this TRIS rich sediment layers (1–7 cm) AVS accounted for $62 \pm 10\%$.

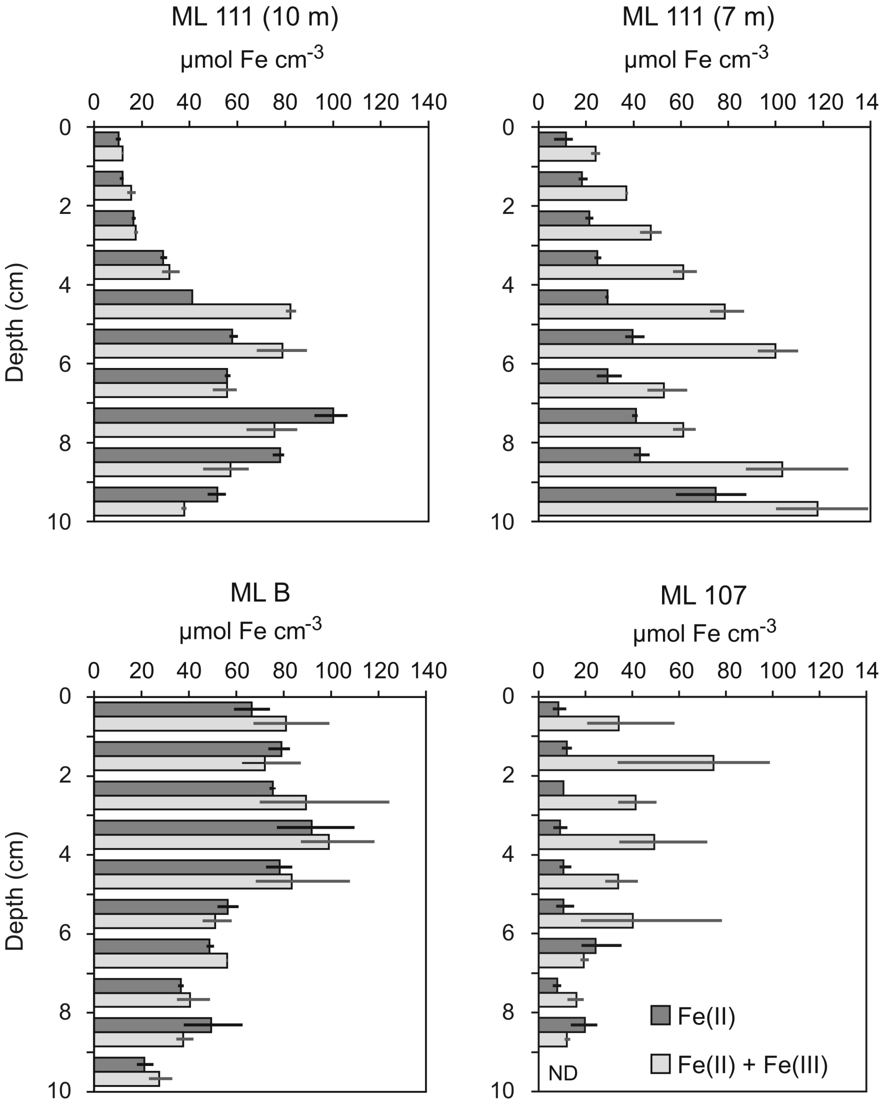

Figure 2. Depth distribution of HCl-extractable Fe(II) and HCl-extractable Fe(II) plus hydroxylamine-reducible Fe(III) in April 1997. Mean values of triplicate determinations are presented with minimum and maximum values indicated.

Bacterial counts

In the surface sediments of the acidic mining lakes high numbers of FeOB and SOB (obtained with acidic SOB-medium) were present (Figure 4). The numbers decreased from maximally 2×10^7 cells cm^{-3} at 0–1 cm to 5×10^3–3×10^4 cells cm^{-3}

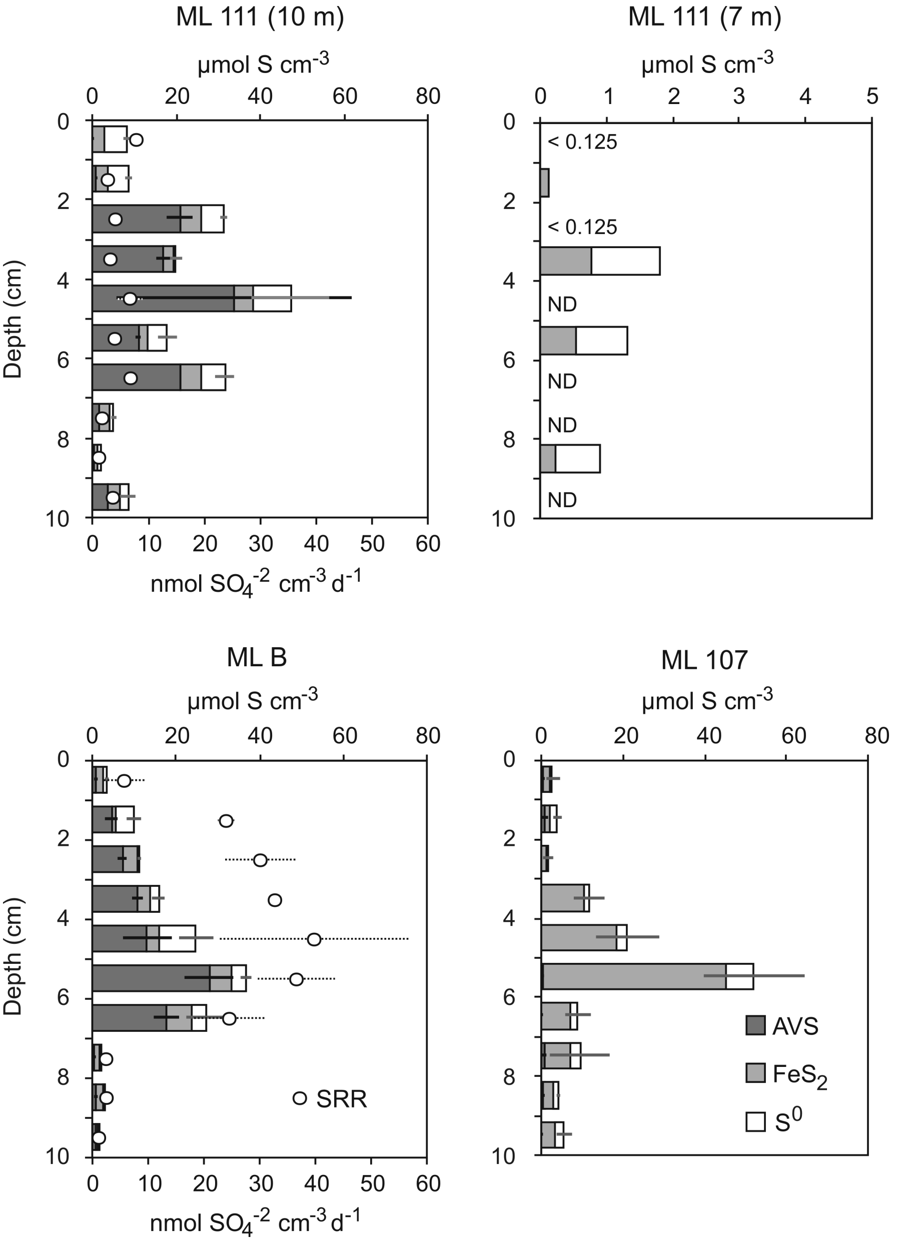

Figure 3. Depth distribution of acid volatile sulfur (AVS), pyrite [FeS_2: total of chromium reducible sulfur (CRS) minus elemental sulfur (S^0)], S^0, and sulfate reduction rates (SRR) in April 1997. AVS, total CRS and SRR were determined in duplicate, S^0 was determined on one of the duplicate samples. Mean values of AVS, total CRS and SRR are presented with minimum and maximum values indicated. S^0 is given as percentage of total CRS.

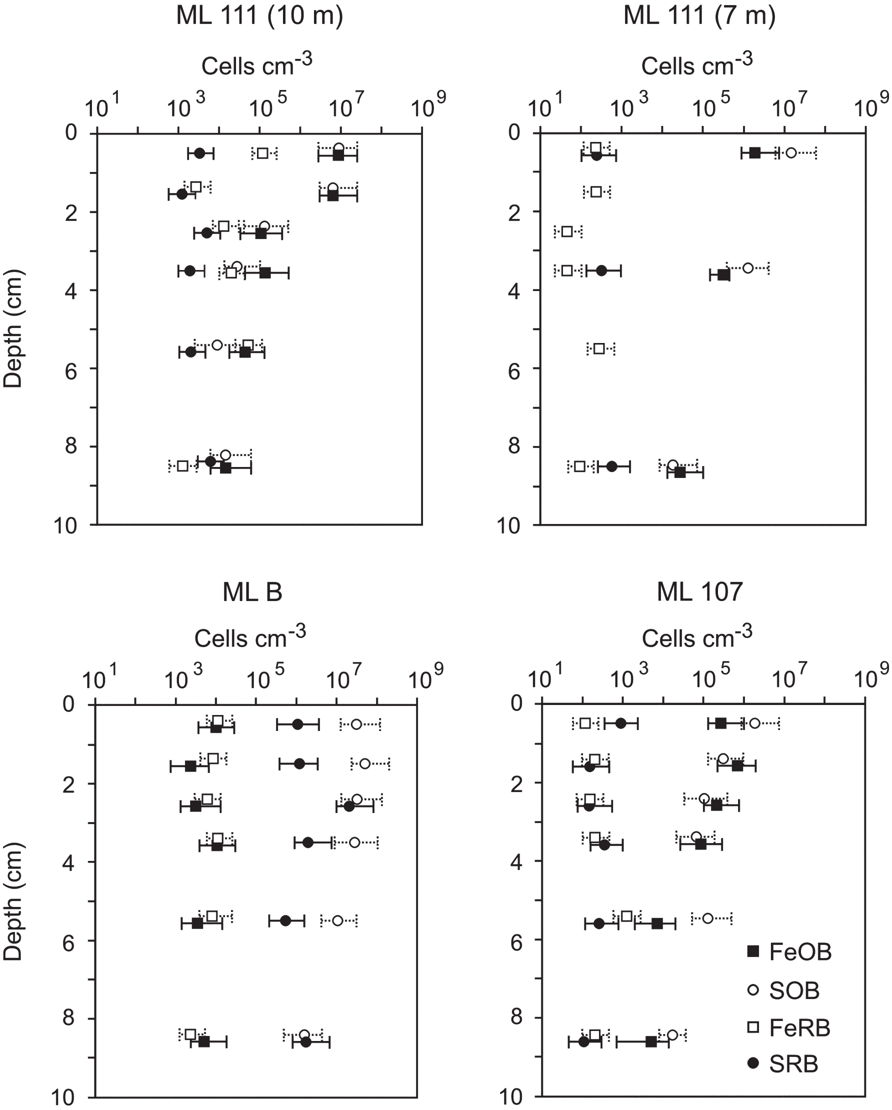

Figure 4. MPN of FeOB, SOB, FeRB, and SRB determined in six sediment horizons [with the exception of ML 111 (7 m) where FeOB, SOB, and SRB were determined in only three horizons] in April 1997. For ML B sediment samples, pH-neutral SOB-media was used. Error bars indicate 95%-confidence intervals.

at 8–9 cm. In sediments of ML B the numbers of FeOB were low (2×10^3–10^4 cells cm^{-3}) throughout the top 10 cm, however, up to 5×10^7 cells SOB cm^{-3} were determined with the pH-neutral SOB-medium at sediment surface. The numbers of FeRB and SRB were generally low in the sediments of the acidic lakes,

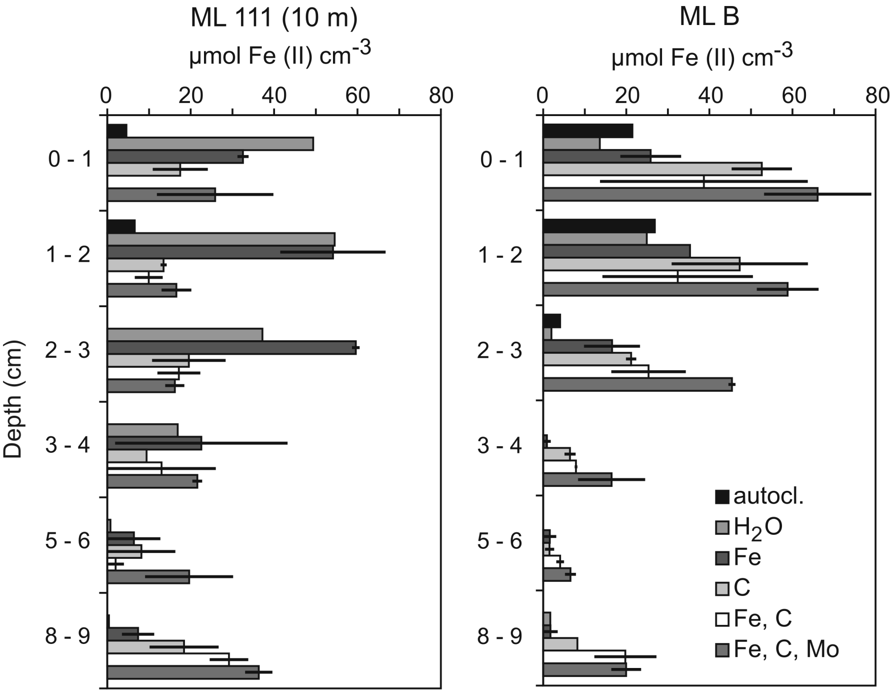

Figure 5. Accumulation of HCl-extractable Fe(II) after a 20-days incubation of ML 111 (10 m) (left) and ML B sediments (right) with different supplements at room temperature. Sediment samples were retrieved in July 1996. Data are presented as mean values with minimum and maximum values of duplicate measurements indicated. autocl. – autoclaved control; H_2O – control, no amendments; Fe – amorphous Fe hydroxide slurry (16 mM); C – acetate (10 mM) plus ethanol (10 mM); MO – molybdate (20 mM).

ranging from 10^2 to 10^3 cells cm^{-3} in ML 111 (7 m) and ML 107, and from 10^3 to 10^5 cells cm^{-3} in ML 111 (10 m). In ML 111 (10 m) MPN of FeRB were slightly higher than those of SRB. In ML B the highest numbers of SRB were obtained reaching a maximum of 2×10^7 cells cm^{-3} at 2–3 cm. They outnumbered the FeRB by a factor of $60–3 \times 10^3$.

Fe(III) reduction

In most treatments of ML 111 (10 m) and ML B sediments, Fe(II) had accumulated after 20 days (Figure 5). Little or no Fe(III) reduction or even a decrease in Fe(II) occurred in autoclaved flasks, except in the upper 2 cm of ML B sediment. However, live treatments had higher Fe(II) contents showing that the Fe(III) reduction potential was essentially biogenic. Both ML 111 (10 m) and ML B sediments showed highest Fe(III) reduction in the upper 3 cm. Assuming linear Fe(III)

Table 2. Sulfate reduction rates (SRR) and amounts of acid volatile sulfur (AVS) after a 4-week incubation of ML 111 (10 m) sediments amended with different substrates at room temperature. Incubations without supplements served as control. SRR were determined in duplicate during the first series (samples from September 1996) and in triplicate during the second series (sediment samples from October 1996). AVS was always determined in triplicate. Mean values are given with minimum and maximum values in brackets.

	Substrate	Sulfate reduction rate (nmol SO_4^{2-} cm^{-3} d^{-1})	Acid volatile sulfur (μmol S cm^{-3})
Series 1	30 mM pyruvate	1219 (889/1443)	6.25 (3.63/10.03)
	30 mM glucose	1.2 (0.0/3.6)	1.28 (0.28/2.09)
	30 mM succinate	1.7 (0.7/3.5)	0.25 (0.06/0.41)
	Control	69.0 (61.3/82.6)	1.59 (1.38/1.78)
Series 2	30 mM lactate	69.8 (19.2/120.4)	3.13 (1.94/3.78)
	30 mM ethanol	12.1 (6.8/17.4)	1.41 (1.00/1.81)
	30 mM acetate	234 (197/270)	2.59 (1.59/3.50)
	Control	27.7 (15.7/39.7)	0.04 (0.00/0.06)

reduction rates, maximum rates of 3.0 and 3.3 μmol Fe cm^{-3} d^{-1} were reached in ML 111 (10 m) and ML B sediments, respectively. In the upper 3 cm of ML 111 (10 m) sediment highest Fe(III) reduction occurred in unsupplemented flasks and in those where only Fe(III) was added. Carbon-supplemented flasks showed lower values (except 8–9 cm), indicating an inhibitory effect. In ML B sediment, Fe(III) reduction appeared to be stimulated by carbon supplements in all depths. Addition of Fe(III) had only a minor effect which was restricted to the upper 3 cm. Molybdate did not inhibit Fe(III) reduction in any of the sediment samples.

Sulfate reduction

In sediments of ML 111 (10 m) sulfate reduction rates were almost by a factor of 10 lower than in ML B (Figure 3). Rates ranged from 1.6 to 7.2 nmol SO_4^{2-} cm^{-3} d^{-1} showing little variation with depth. In sediments of ML 111 (7 m) and ML 107 sulfate reduction rates were below detection limit (<0.3 nmol SO_4^{2-} cm^{-3} d^{-1}). In ML B the highest rate of 40 nmol SO_4^{2-} cm^{-3} d^{-1} was observed at 4–5 cm sediment depth. Rates below 10 nmol SO_4^{2-} cm^{-3} d^{-1} were obtained at sediment surface and in sandy layers (>7 cm). The effects of supplemented carbon sources on sulfate reduction in sediments of ML 111 (10 m) are summarized in Table 2. The amendments of pyruvate, lactate and acetate resulted in higher sulfate reduction rates and larger amounts of AVS compared to the unamended control. Sediments amended with ethanol showed lower rates, however, the amounts of AVS were higher than in the control. The addition of glucose and succinate lead to both lower rates and lower amounts of AVS than in the control.

Discussion

A scheme of Fe and S transformations which, inferred from our data, may take place in the sediments of the four sites is proposed in Figure 6. The overall presence and the coexistence of the functional bacterial groups, as reflected by MPN estimates, suggest that capacities for mediating both reduction and oxidation processes are available. The intensities of bacterial activities and the net outcome, that is, the burial of iron sulfides, however, depend on the nature of the microenvironment (e.g., acidity, availability of oxidants or organic matter). In this study we found out that meromixis and permanent anoxic conditions lead to the accumulation of reduced sulfur compounds as a result of iron and sulfate reduction. Furthermore, our comprehensive investigation on sediments of different mining lakes confirms that acidic mining lakes are unique aqueous habitats and that their biogeochemistry is distinct from that of marine and freshwater habitats including those impacted by AMD or artificially acidified with sulfuric acid (Geller et al. 1998).

Fe(III) minerals and sulfate transport

The sediments of the mining lakes were rich in reactive Fe and sulfate. Microbially reducible or reactive iron accounted for 1.4–5.1% of dry weight. Amounts of total Fe reached up to 8 and 40% of dry weight in sediments of ML 111 (Friese et al. 1998) and ML 107 (Morgenstern et al. 2001), respectively. In comparison, total Fe (and reactive Fe) amounts to 1.7–5.6% (0.06–3.7%) in marine sediments (Canfield et al. 1992; Gagnon et al. 1995; Haese et al. 1997) and 3.6–6.7% in lake sediments (Cook et al. 1986). Cummings et al. (2000) reported up to 5% reactive Fe for sediments of AMD-receiving Lake Coeur d' Alene. The concentrations of pore water sulfate in the acidic mining lakes were in the range of those found in marine environments (e.g., Haese et al. 1997). Even pore water sulfate concentrations in pH-neutral ML B were much higher than those reported from lakes receiving AMD (6–1270 μM, Herlihy and Mills 1985) and from artificially acidified lakes (>70 M, Rudd et al. 1986; 2–45 μM, Urban et al. 1994).

The high contents of Fe and pore water sulfate result from the precipitation of Fe(III) hydroxides and hydroxosulfates. The formation of goethite (FeOOH) and jarosite [$KFE_3(SO_4)_2(OH)_6$] has been shown for ML 111 (Göttlicher and Gasharova 2000). Besides the formation of sulfate minerals, the adsorption of sulfate to Fe(III) minerals is a major form of transport of sulfate from the water body to the sediment (Herlihy and Mills 1989). In the sediments, the reductive dissolution of Fe(III) minerals, the dissolution of Fe(III) hydroxosulfates at elevated pH values, and the conversion of amorphic Fe(III) minerals to more crystalline forms lead to a release of sulfate (Rose and Ghazi 1997). Hence, in acidic mining lakes the concentrations of pore water sulfate is determined primarily by other processes rather than sulfate reduction and reoxidation of reduced sulfur compounds.

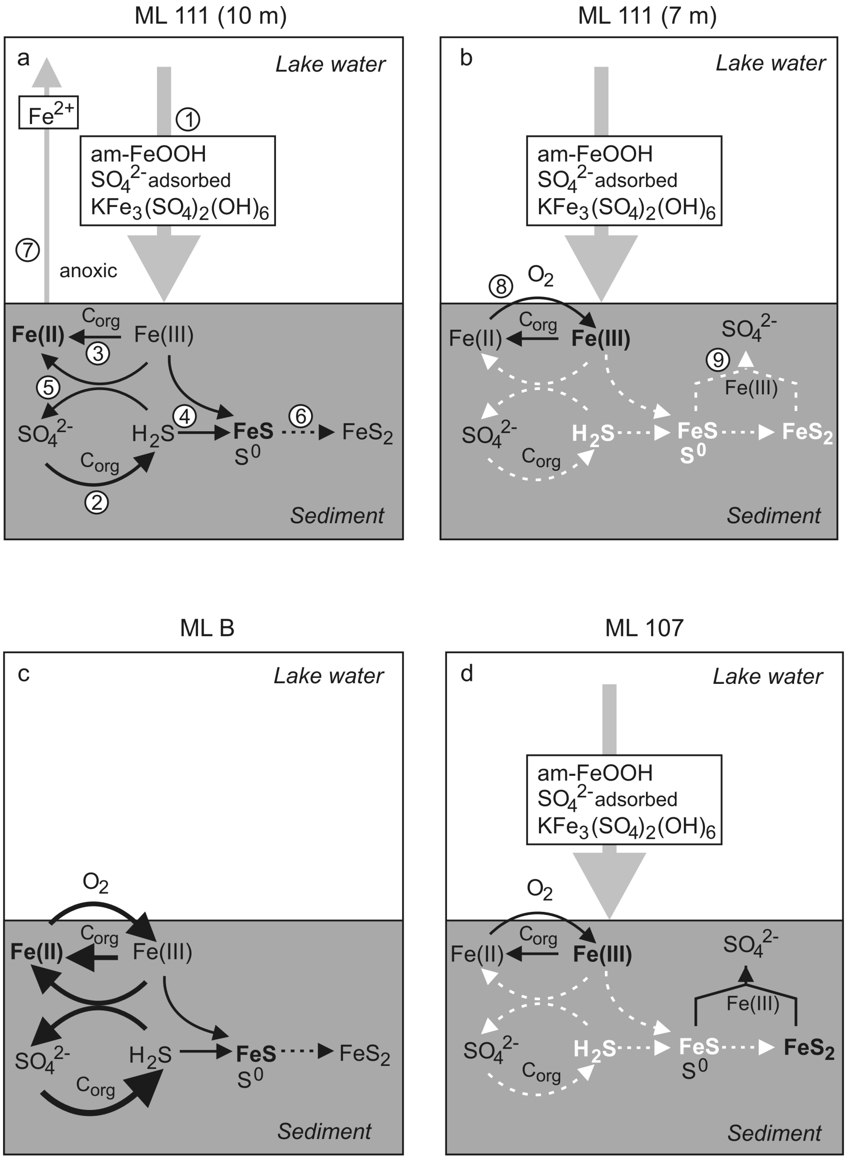

Figure 6. Proposed reaction scheme for Fe- and S-cycling in sediments of the four sites investigated. (1) Precipitation of Fe(III) hydroxides and hydroxosulfates. (2) Sulfate reduction coupled to the oxidation of organic matter. (3) Microbial Fe(III) reduction coupled to the oxidation of organic matter. (4) Chemical Fe(III) reduction with H_2S. (5) Microbial Fe(III) reduction coupled to the oxidation of reduced S-compounds. (6) Conversion of FeS to FeS_2. (7) Diffusion of Fe^{2+} from the sediment into the water column. (8) Reoxidation of Fe(II) with O_2 (microbially or chemically). (9) Reoxidation of reduced S-compounds (combined chemically and microbially mediated reactions). Please note that reactions (4)–(9) are only hypothesized. Black dotted lines mean limited reactions, white dotted lines mean that there was no indication for this reaction. Arrow thickness symbolizes reaction intensities.

Fe(III) reduction

The presence of Fe(II) in the surface sediments of the mining lakes indicates a reduction of Fe(III). The reduction of Fe(III) by H_2S is a possible mechanism in sediments of ML 111 (10 m) and ML B, where sulfate reduction was observed. This is supported by the high amounts of S^0 found in the sediments as the formation of FeS by the reaction of hydrous Fe oxide (e.g., goethite) with sulfide leads to S^0 as the main sulfide oxidation product (Pyzik and Sommer 1981). The results from the anaerobic incubation experiments in order to determine Fe(III) reduction, however, gave no direct evidence for chemical Fe(III) reduction by H_2S. Molybdate specifically inhibits sulfate reduction. If Fe(III) reduction was mediated by H_2S, a decrease of Fe(III) reduction would have been expected in the molybdate treatment, which, however, did not occur.

Microbially mediated reduction of Fe(III) was demonstrated by anaerobic incubation experiments in sediments of ML 111 (10 m) and ML B. Besides enzymatic Fe(III) reduction, Fe(III) reduction via electron shuttling (Nevin and Lovley 2000) may have also contributed to the overall reaction. The increase of Fe(II) in autoclaved controls may be the effect of abiotic Fe(III) reduction. The reductants may have been produced during the autoclaving process or result from earlier microbial activities. The reduction of Fe(III) by free sulfide, however, appears to be unlikely, as the amount of Fe(II) produced exceeded by far the amount of AVS determined in all sediment samples. Insufficient inactivation of spore-forming Fe(III)-reducing microorganisms, such as *Sulfobacillus* ssp. in acidic (Bridge and Johnson 1998) and fermentative organisms in pH-neutral environments (e.g., Dobbin et al. 1999), can also be responsible for Fe(III) reduction in autoclaved controls.

Comparison of Fe(III) reduction in ML 111 (10 m) and ML B yielded similar rates, but the reaction towards different supplements implicate that physiologically different microbial populations were responsible for this activity. Both ML B and ML 111 (10 m) sediment showed highest Fe(III) reduction potentials near the sediment surface and can be explained by the regular supply of fresh, easily degradable organic matter. This may also hold for ML B sediment, where organic carbon stimulated Fe(III) reduction. However, in ML 111 (10 m), where organic carbon inhibited Fe(III) reduction in upper layers, different mechanisms may apply. Sedimenting material in ML 111 consists predominantly of Fe(III) hydroxides and contains little organic carbon (own unpublished observations), so the availability of freshly precipitated Fe(III) is more likely to be the key factor promoting microbial Fe(III) reduction in ML 111 sediments *in situ*. This is supported by the observation that the highest Fe(III) reduction potentials were found in the upper sediment layers which were incubated with supplemented amorphous Fe(III) hydroxide slurry. In addition, the chosen carbon sources might have been inappropriate, since acetate can be toxic at low pH because it acts as an uncoupler. Inhibition of Fe(III) reduction by acetate has also been observed in surface sediments of acidic ML 77 from where the acidophilic Fe(III)-reducing strain *Acidiphilium cryptum* JF-5 was isolated (Küsel et al. 1999; Küsel and Dorsch 2000). *Acidiphilium*-like acidophilic heterotrophic bacteria may also be responsible for Fe(III) reduction in sediments of

ML 111 (and ML 107). Alternatively, *Acidithiobacillus ferrooxidans*-like organisms may have been involved in Fe(III) reduction since this species can reduce Fe(III) with S^0 as electron donor (Brock and Gustafson 1976). S^0 was available in ML 111 (10 m) and also in ML 107 throughout the top 10 cm. Acidophilic FeRB are generally facultative anaerobes able to respire on both O_2 and Fe(III) (Johnson 2003). This enables them to thrive in anoxic sediments such as in ML 111 (10 m) and in sediments where oxic and anoxic conditions alternate such as in ML 111 (7 m) and ML 107.

The numbers of neutrophilic acetate-utilizing FeRB found in ML 111 (10 m) and ML B are comparable to those reported for mining-impacted freshwater sediment (Cummings et al. 2000), however, they were significantly higher than the numbers of lactate-utilizing FeRB in acidic ML 77 sediments (Küsel and Dorsch 2000). The very low numbers yielded in the acidic sediments of ML 111 (7 m) and ML 107 suggest that this group of bacteria may be of minor importance at these acidic sites.

The dissolved Fe^{2+} formed during reduction either reacts further to form Fe(II) minerals or diffuses upwards to the sediment surface. Assuming that all sulfide of the AVS fraction reacted with iron stoichiometrically to FeS, the comparison of HCl-extractable Fe(II) (comprising amorphic FeS) and AVS shows that 19–100% of Fe(II) was present as FeS in the AVS-rich layer of ML 111 (10 m) sediments. In ML B sediments maximally 50% of Fe(II) were present as FeS. Hence, other forms of Fe(II) such as siderite ($FeCO_3$) must have been present. In contrast to ML B, we found low concentrations of Fe(II) at the sediment surface in ML 111 (10 m). This is probably due to the diffusive loss of dissolved Fe^{2+} to the anoxic and acidic water body which leads to an increased concentration of dissolved Fe(II) in the monimolimnion (Büttner et al. 1998, Friese et al. 1998, Herzsprung et al. 1998). In ML B, diffusive loss of dissolved Fe(II) is limited because of oxic (and pH-neutral) conditions at the sediment–water interface and consequently rapid reoxidation. Internal Fe cycling occurring in sediments underlaying oxic waters has been described for acidic ML 77 (Peine et al. 2000) and may also be applied to ML 111 (7 m) and ML 107.

Sulfate reduction and reoxidation of S compounds

In sediments of ML 111 (7 m) and ML 107 where acidic and oxic conditions predominate, no sulfate-reducing activity could be detected. In general, SRB are neutrophilic or at the most acidotolerant (e.g., Fauque 1995), however, the isolation of acidophilic sulfate reducers has been reported (Johnson 2003). During anoxic conditions like in ML 111 (10 m) sulfate reduction took place. However, the sulfate reduction rates and the MPN of SRB stayed far below the values estimated for ML B. The rate integrated over the upper 10 cm was 0.4 mmol SO_4^{2-} m^{-2} d^{-1} in ML 111 (10 m), being within the range estimated for mining lakes (0.8 mmol SO_4^{2-} m^{-2} d^{-1} at pH 3.0–6.0 in ML 77, Peine et al. 2000; 0.2–1.5 mmol SO_4^{2-} m^{-2} d^{-1} at pH 3.8 in Reservoir 29, Gyure et al. 1990). Higher sulfate reduction rates were observed in freshwater habitats receiving AMD (up to

266 mmol SO_4^{2-} m^{-2} d^{-1} in Lake Anna, Herlihy and Mills 1985) and in artificially acidified lakes (0.7–5.2 mmol SO_4^{2-} m^{-2} d^{-1} in Little Rock Lake, Urban et al. 1994), however, pH values in these sediments range from pH 5.8–6.7. A rate of 2.04 mmol SO_4^{2-} m^{-2} d^{-1} at pH 2.6–3.4 has been reported from a naturally acidified glacial Lake Caviahue, Argentina, suggesting that in such a natural and much older system a well-adapted microflora evolved (Koschorreck et al. 2003).

Sulfate reduction was stimulated in ML 111 (10 m) sediments by the addition of carbon sources and electron donors which are used by a wide range of SRB. This may indicate substrate limitation of present bacteria or be the effect of selective enrichment of bacteria during the incubation of 4 weeks. In acidic mining lakes of Lusatia the production of easily degradable organic matter is low. High values of LOI, which can serve as indicator of organic carbon content, may result from lignite residues, and high DOC concentrations probably result from lignite dissolution products consisting mainly of aromatic polycarbonic acids (Laskov et al. 2002). The concentrations of short chain fatty acids in pore water of ML 111 (10 m) were below the detection limit (K. Küsel, personal communication). Blodau et al. (2000) demonstrated that carbon oxidation via Fe(III) and sulfate reduction was not positively correlated to pore water DOC in acidic mining lakes of Lusatia, concluding that the majority of DOC did not serve as a substrate.

Substrate limitation would be expected to be even more severe in the presence of amorphous Fe(III). The reduction of amorphous Fe(III) is always the thermodynamically favored process towards sulfate reduction (Postma and Jakobsen 1996). In addition, FeRB may outcompete SRB for organic substrates (Lovley and Phillips 1987b). However, in this study we observed that sulfate reduction took place at the sediment surface of ML 111 (10 m) where also amorphous Fe(III) and potentials for Fe(III) reduction were found. Sulfate reduction was stimulated primarily by short chain fatty acids and ethanol. These substrates (except ethanol by *A. cryptum*) are not used by acidophilic FeRB (see above). Therefore, competitive inhibition of SRB by FeRB may not play an important role in sediments of acidic mining lakes in contrast to pH-neutral environments (e.g., Roden and Wetzel 1996; Lovley and Phillips 1987b).

The large amounts of reduced S compounds in the sediments could result from diagenetic processes or alternatively originate from the mine tailings. Sulfate reducing activities in ML 111 (10 m) and ML B sediments suggest that at least the AVS-fraction was of authigenic origin. Isotope analysis by Friese et al. (1998) revealed heavier $\delta^{34}S$ values for the CRS fraction (pyrite) than those of the AVS fraction in sediments of ML 111. The same authors proposed that sedimentary pyrite is composed from varying proportions of primary pyrite (from the mine tailings) and authigenically formed pyrite (by early diagenetic processes). Minor amounts of AVS indicates that sulfate reduction may take place temporally in ML 107. Despite its shallow depth, anoxic conditions established at the sediment–water interface during thermal stratification in summer and ice cover in winter. In addition, the dense mat of filamentous algae might provide a temporal supply of organic substrates during decay. Therefore, it is suggested that reduced sulfur compounds in ML 107 are likewise a mixture of primary and authigenically formed S-compounds.

The rate of reoxidation was estimated for ML 111 (10 m) and ML B sediments by relating the integrated sulfate reduction rates to the accumulation rates of reduced S. Friese et al. (1998) suggested that the upper 14 cm of the sediment have been deposited in the 30–40 years since mining was stopped in ML 111. If we postulate an age of 30 years for the upper 10 cm the accumulation rate amounts to 60 mmol S m^{-2} a^{-1}. Compared to an integrated reduction rate of 156 mmol $SO_4{}^{2-}$ m^{-2} a^{-1} this gives a reoxidation of 61% in ML 111 (10 m). For the upper 10 cm of sediments in ML B we also estimated an age of 30 years as we found sandy overburden material at approximately 10 cm depth. The accumulation rate of reduced S is then 45 mmol S m^{-2} a^{-1} and compared to an integrated reduction rate of 738 mmol $SO_4{}^{2-}$ m^{-2} a^{-1} this gives a reoxidation of 94% in ML B. The estimated accumulation rates of S are higher than those reported from freshwater Lake Scharmützelsee (16 mmol S m^{-2} a^{-1}, Kleeberg 1998) and artificially acidified Little Rock Lake (16–28 mmol S m^{-2} a^{-1}, Urban et al. 2001). Reoxidation of reduced S is low in ML 111 (10 m) due to a limited supply of oxidants, which consists of freshly precipitated Fe(III) minerals. However, we still found high numbers of FeOB and SOB which may be explained by the presence of acidophilic FeRB oxidizing reduced S coupled to Fe(III) reduction under anoxic conditions. In general, the high abundance of FeOB and SOB indicates a high reoxidation potential in sediments.

Pyrite formation

The sediments of ML 111 (10 m) and ML B were characterized by small amounts of CRS (S^0 and FeS_2) compared to large amounts of AVS. The AVS-S/FeS_2-S ratios were 3.5 (0.1–8.4) and 3.3 (0.4–5.7) in ML 111 (10 m) and ML B, respectively (with minimum and maximum values given in brackets). For this calculation we assumed that all reduced S was of authigenic origin. These values are in the upper range of AVS-S/FeS_2-S ratios (0.02–7.2) determined for coastal marine sediments (Gagnon et al. 1995 and references therein). High AVS-S/FeS_2-S ratios indicate a limited conversion of AVS to pyrite. The amount of sulfidic bound Fe is controlled by the availability of reactive Fe, while the ratio of FeS to FeS_2 is determined by the availability of free sulfide (Boesen and Postma 1988; Canfield et al. 1992). An excess of Fe prevents the diffusion of free sulfide to the redox boundary and the consequent formation of intermediate reduced S (Gagnon et al. 1995). This mechanism may also apply to ML 111 (10 m) and ML B, though high concentrations of S^0 seem to contradict this at first sight. However, S^0 needs to react further with HS^- to form polysulfides which then react with FeS to FeS_2 (Luther 1991). With respect to a sustainable neutralization process the burial of pyrite as the more stable form of ironsulfides is of great importance. A higher proportion of pyrite may be achieved by means of higher sulfate reduction rates and a lower supply of reactive Fe.

Even though an alkalinity generating potential is present in the acidic mining lakes, neutralization of these waterbodies is not expected within the near future. The availability of organic matter appears to be one of the limiting factors.

Therefore, abatement strategies on the basis of controlled addition of organic substrates were developed and are currently being tested in ML 111 (e.g., Wendt-Potthoff et al. 2002).

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