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In situ stimulation of bacterial sulfate reduction in sulfate-limited freshwater lake sediments

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1. SUMMARY

By adding sulfate in the form of solid gypsum, it was possible to transform in situ a predominantly methanogenic sediment ecosystem into a sulfate-reducing one. The concentrations of sulfate, sulfide, methane, acetate, propionate, soluble iron, and manganese were determined in the porewater before and after the transition. Although sulfate was no longer limiting, acetate and propionate continued to accumulate and reached much higher concentrations than under sulfate-limited conditions. Metabolic activities of fermenting bacteria and of sulfate reducers, which belong to the group that incompletely oxidizes organic material, might be responsible for the increased production of volatile fatty acids. The elevated concentrations of soluble $Fe(II)^{2+}$ and $Mn(II)^{2+}$ observed in the porewater stem from iron and manganese compounds which may be reduced chemically by hydrogen sulfide and other microbially produced reducing agents or directly through increased activities of the iron and manganese reducing bacteria. In the horizon with high sulfate-reducing activities the methane concentrations in the porewater were lower than in non-stimulated sediment regions. The shape of the concentration depth profile indicates methane consumption through sulfate reducing processes. The in situ experiment demonstrates the response of a natural microbial ecosystem to fluctuations in the environmental conditions.

2. INTRODUCTION

The ecosystem in the top few centimeters of sediments of eutrophic freshwater lakes is characterized by a complex system of chemical gradients [23,24]. The gradients which get established in this redox transition zone are the results of diffusional fluxes and microbial activities [5,6,27,28]. Fluxes determine the availability of electron acceptors to microorganisms for the degradation

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of dead biomass. Microbial reactions influence the geochemical processes within the sediment matrix [17,18,24,38]. Electron acceptors either diffuse to deeper layers of undisturbed sediments through the sediment–water interface or they get released during dissolution and degradation of buried materials and organosulfur compounds [20] which still contain reducible elements. Lake sediments rich in organic matter are usually diffusion-limited with respect to oxidants, i.e. the rate of supply of electron acceptors is slower than their potential rate of utilization. In general, electron acceptors are used sequentially by different types of microbes according to the thermodynamic energy yield [23]. The predictable sequence in which oxidants will be consumed in oxidant-limited environments may lead to clearly stratified sediments with distinct activity horizons. Oxidant-limitation can be overcome by supplying in situ excess electron acceptors. As a consequence, the metabolism of those bacteria which are able to utilize the added oxidant is stimulated. From in situ and in vitro studies on the interaction between sulfate-reducing and methane-producing populations it has been shown that the addition of sulfate to freshwater sediments increases the metabolism of sulfate reducers and suppresses microbial methane production [12,19,35,40,41].Inhibition of methanogenic activity has been attributed to substrate competition and kinetic advantages of sulfate reducers [1,13,31,37]. It has also been suggested that methane might be oxidized anaerobically with sulfate as electron acceptor [4,29].

Here we report on in situ experiments designed to investigate the response of a natural ecosystem to environmental perturbations. Sulfate from plaster and from ground gypsum was supplied in situ to freshwater sediments to overcome the electron acceptor limitation and to redirect the electron flow locally. The changes which took place in the concomitant geochemical processes and the transitions in the microbially mediated redox sequence were followed quantitatively by determining changes in the chemical composition of the porewater. Microbial reaction rates and mass flux across the sediment–water interface were calculated with the aid of a one-dimensional diffusion model.

3. MATERIALS AND METHODS

3.1. Experimental site

All experiments described were carried out in 250 m depth in the central basin of Lake Geneva, Switzerland, outside of Lausanne. During the experimental periods the oxygen concentration never fell below approximately 4 $mg \cdot l^{-1}$ as judged from the continuous presence of a bottom dwelling fauna (fish, snails). The morphology of the surface sediments shows a characteristic pillow-like structure over many square kilometers [8,23,24,39]. The soft elevations with a diameter of about 50 cm are separated by trenches 5–15 cm deep. The sediment surface and the top 2–4 mm are oxidized and appear light brown. Combustible organic carbon content of the sediment matrix varied between 8% (dry wt) just below the sediment surface and 4% at a depth of 30 cm. Sediment porosity varied between 0.93 and 0.88 in the top 15 cm. It decreased to 0.82 in the next 5 cm and remained between 0.81 and 0.82 in the layer between 20 and 30 cm. The natural sulfate concentration of the hypolimnetic water was 0.5 mmolar.

3.2. Sulfate supply

Sulfate from solid $CaSO_4 \cdot 2H_2O$ was supplied in situ to the sediment employing 3 different procedures: Plaster posts ($6 \times 6 \times 70$ cm) were inserted and incubated for up to 14 months to provide sulfate-depleted layers with a slowly dissolving source of sulfate. The posts were transported to the sediment surface with the manned submarine F.-A Forel [9]. They were vertically implanted in the center of sediment pillows with the aid of the hydroelectrical manipulator of the submarine. Cores taken after the incubation period at a distance of 2–8 cm from the implanted posts showed undisturbed sediment layers. Plaster posts reached a sediment depth of approximately 35 cm. Undisturbed pillows served as controls.

A plaster plate ($30 \times 30 \times 3$ cm) resting on the sediment for 14 months could supply an area of 0.09 m^2 with additional sulfate from the surface. The slowly dissolving plaster provided the surface layers with sulfate by vertical diffusion (Fig. 1). After the initial incubation period of 14 months the original plate was replaced by another which

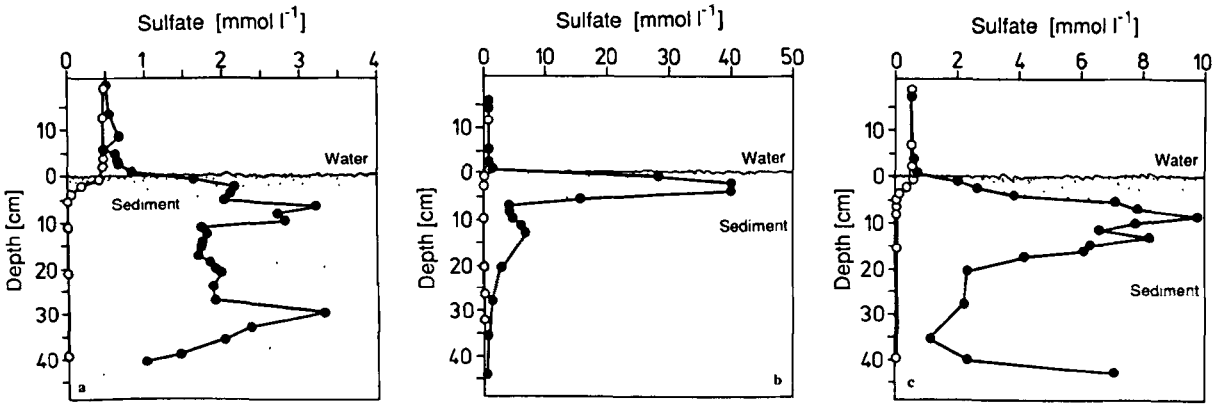

Fig. 1. Concentration–depth profiles with and without the in situ supply of sulfate from solid calcium sulfate to sediment pillows of Lake Geneva at a water depth of 250 m. ○ = undisturbed control site; ● = experimental site. Supply by plaster post (a); by plaster plate (b); by ground gypsum (c).

had a slot in the center to accommodate the porewater sampler for the collection of interstitial water from underneath the plate. A sediment area covered with a Plexiglas plate of the same size served as control. The plate prevented oxygen and sedimenting organic matter from reaching the experimental area. Due to the irregular sediment morphology (crest-trench structure), the limited load capacity of the submarine, and the structural instability of plaster plates, it was impractical to cover a larger area with this method.

However, ground gypsum (70 kg) was spread over an experimental area of about 5 m^2. The gypsum gravel (size $\leqslant 1$ cm) was homogeneously distributed over the experimental area without disturbing the surface layer of the sediment. As enclosure a broad shallow cylinder was formed with a plastic foil which was stretched between two PVC rings. The upper ring was suspended by a floating device. Lead weights kept the lower ring on the sediment surface. Lateral diffusion of the sulfate-rich water was prevented to a certain extent by the walls of the plastic cylinder. After an incubation period of 4 months porewater samples were analyzed from a region where the gypsum was not completely dissolved. An undisturbed sediment area outside the experimental area served as control site.

3.3. Porewater sampling

Porewater samples were collected from sediments with the dialysis equilibrium technique [10,14,26]. Dialysis samplers were positioned and retrieved by the manned submarine F-A. Forel. The samplers were incubated parallel to the plaster posts in a distance of 10–15 cm. Interstitial water from underneath the plaster plate was collected by inserting a porewater sampler through a slot in the plaster plate. Usually, the samplers were incubated in the sediment for 14–27 days. During this time period equilibrium was reached even for slowly permeating species. Membrane integrity and permeability characteristics were not altered under the incubation conditions in situ [10]. During the dive and for the transport to the laboratory the porewater samplers were kept in a protective casing which prevented contamination and minimized diffusion losses. The samples could be processed within 60–90 min after the retrieval of the porewater samples.

3.4. Analytical methods

Nitrate and sulfate were determined by ion chromatography [25], iron and manganese by flame atomic absorption spectroscopy and the composition of the plaster and the gypsum by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Head space gas chromatography with FID detection was used for the quantitative determination of methane [7,36]. Volatile fatty acids (VFA) were determined by gas chromatography [16]. Sulfide was quantified with the methylene blue spectroscopic method [22].

3.5. Calculation of diffusion fluxes and microbial reaction rates from a one-dimensional diffusion model

Diffusion fluxes were determined by numerical differentiation. Fluxes in the porewater were calculated according to the following modification of Fick's first law [32]:

$$(F_j)_{z,t,T} = (-D_j^0)_{T,\alpha} \cdot (\Phi^{m-1})_z \cdot (\Delta c_j/\Delta z)_{z,t}$$
$$= (-D_{j,\mathrm{eff}})_{T,\alpha,z} \cdot (\Delta c_j/\Delta z)_{z,t}$$
$$[\mathrm{mol} \cdot \mathrm{cm}^{-2} \cdot \mathrm{s}^{-1}]$$

where F_j is the flux of the compound j into or out of a horizon Δz at a depth z; D_j^0 is the diffusion coefficient of the chemical species j in water at a temperature T; Φ^{m-1} is the correction for tortuosity with Φ as the porosity of the sediment at depth z; m is an emperical coefficient (approximately 2.7–3 for sediment porosities > 0.7) [32]; α accounts for the viscosity of the electrolyte solution in the interstitial spaces; $\Delta c_j/\Delta z$ is the concentration change of the chemical species j in the sediment horizon Δz. Fluxes $F_j > 0$ are directed into the sediment or towards deeper layers from a production horizon within the sediment. $F_j < 0$ describes fluxes from the sediment to the water above. Diffusion coefficients used to quantify the fluxes were calculated for a temperature of 5 °C as suggested by Li and Gregory [33]. A modified mass conservation equation has been used to relate reaction rates to diffusion and advection [5]. For one-dimensional vertical diffusion processes the time-dependent concentration changes ($\mathrm{d}c_j/\mathrm{d}t$) in an undisturbed sediment horizon are expressed as:

$$\mathrm{d}c_j/\mathrm{d}t = (D_j^0)_{T,\alpha} \cdot (\Phi^{m-1})_z \cdot (\delta^2 c_j/\delta z^2) - \omega \cdot (\delta c_j/\delta z) - \sum R_j \quad (1)$$

The temperature T and viscosity α-dependent diffusion coefficient D_j^0 for species j in water was corrected for the tortuosity changes with an empirical value derived from the porosity as outlined above. This yields the effective diffusion coefficient $(D_{j,\mathrm{eff}})_z$ for j, which varies for different sediment depths z. The contribution of the advection $\omega \cdot (\delta c_j/\delta z)$ is negligible for the small input to deep sediments and relatively short observation period. Assuming steady state conditions ($\mathrm{d}c_j/\mathrm{d}t = 0$) the reaction term $\sum R_j$ may be expressed as:

$$\sum R_j = (D_{j,\mathrm{eff}})_z \cdot (\mathrm{d}^2 c_j/\mathrm{d}z^2) \; [\mathrm{mol} \cdot \mathrm{cm}^{-3} \cdot \mathrm{s}^{-1}] \quad (2)$$

$\sum R_j$ represents the sum of the rates of all consumption and production reactions involving substance j, purely chemical ones and those which are microbially mediated. If it is possible to define the kinds and the contribution of the non-biological reactions under the prevailing conditions in the sediment, a microbial reaction term $\sum R_j$ can be calculated.

Under boundary conditions mentioned above, a simpler model may also be used to derive reaction rates from concentration-depth profiles. According to Fick's first law, diffusion fluxes F_j are proportional to concentration changes:

$$F_j = -(D_{j,\mathrm{eff}})_z \cdot (\delta c_j/\delta z) \quad (3)$$

The first derivative of Eqn. (3) under time-dependent steady-state conditions

$$\mathrm{d}F_j/\mathrm{d}z = -(D_{j,\mathrm{eff}})_z \cdot (\mathrm{d}^2 c_j/\mathrm{d}z^2)$$

is proportional to the microbial reaction term derived from Eqn. (2)

$$\sum R_j = -\mathrm{d}F_j/\mathrm{d}z$$

Integration between depth z_1 and z_2 yields the consumption or production rates of species j in the horizon between z_1 and z_2. From

$$\int_{z_1}^{z_2} \sum R_j \, \mathrm{d}z = -\int_{z_1}^{z_2} \mathrm{d}F_j$$

we obtain under the assumption that $(D_{j,\mathrm{eff}})_z$ remains constant over the depth interval $\Delta z = z_2 - z_1$:

$$\sum R_j (z_2 - z_1) = -[F_j(z_2) - F_j(z_1)].$$

For practical purposes we divided the sediment column into horizons of equal thickness Δz and calculated fluxes into and out of each horizon

through numerical differentiation from the concentration-depth profile.

$$-\sum R_j \cdot \Delta z = F_{j,\text{in}} - F_{j,\text{out}}$$

$$\sum R_j = -1/\Delta z \cdot \Delta F_j = \left(D_{j,\text{eff}}\right)_z \cdot \left(\Delta^2 c_j/(\Delta z)^2\right)$$

$$[\text{mol} \cdot \text{cm}^{-3} \cdot \text{s}^{-1}] \qquad (4)$$

The maximal diffusion flux changes are thus proportional to the maximal conversion rate. Eqn. (4) is equal to Eqn. (2). Both give a first approximation of the location and the extent of microbial activities in the sediments. $\sum R_j$ represents a consumption rate for $\Delta F_j > 0$ and a production rate for $\Delta F_j < 0$. According to this notation a positive $\sum R_j$ denotes a production rate whereas a negative $\sum R_j$ denotes a consumption rate.

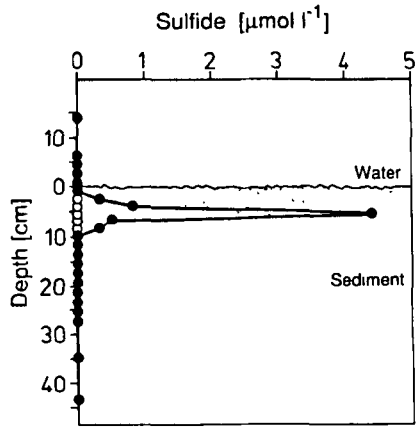

Fig. 2. Sulfide concentrations in pillow-like sediments of Lake Geneva with and without in situ addition of sulfate by a plaster post which had been exposed in the sediment for 4 months. ○ = undisturbed control site; ● = experimental site. Sulfide = sum of H_2S, HS^-, and S^{2-}.

4. RESULTS AND DISCUSSION

4.1. Sulfate stimulation

The major components of the plaster and gypsum used for in situ addition of sulfate were calcium and sulfate (Table 1). Only magnesium was also present in significant quantities. A small amount of sodium and only trace amounts of other cations were observed in the gypsum. Besides sulfate, small quantities of other anions such as chloride, silicate and carbonate were present. They did not harm the ecosystem since they are also present in fairly large amounts in the sediment matrix.

Table 1

Composition of the sulfate sources for in situ stimulation of sulfate reduction [a]

	Plaster (%d.w.)	Gypsum (%d.w.)
Calcium	29.8	28.8
Magnesium	0.7	1.1
Potassium	0.02	0.03
Sodium	0.05	0.1
Aluminum	0 03	0.04
Iron	0.02	0.03
Sulfate	61.5	63.3
Other [b]	7.8	6.4

[a] Analyses were made after dissolution of rock powder in 5% (v/v) nitric acid. The material dissolved completely.

[b] Other anions besides sulfate were not determined quantitatively; the numbers given represent the remaining weight difference. In both preparations $CaSO_4$ accounts for 89% (w/w). The rest are magnesium and calcium carbonates.

An activity horizon of sulfate utilization was observed in a depth below 10 cm (Fig. 1a). The decrease of the interstitial sulfate concentration below 35 cm is due to the limited penetration depth of the plaster post. Addition of sulfate to surface layers from a plaster plate increased the in situ sulfate concentration up to 80-fold in the top 5 cm (Fig. 1b). With this procedure deeper sediment layers were supplied with sulfate by diffusion from the sediment surface with a flux of 71 $\text{mmol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. In these sediments microbial sulfate reduction was stimulated to such an extent that sediments below the plate turned black from metal sulfide precipitates. A 20-fold increase of sulfate compared to the overlaying water was detected in the horizon 8 cm below the surface when sulfate was added as ground gypsum (Fig. 1c). By the in situ addition of sulfate, conditions could be created where the electron acceptor (sulfate) was no longer the limiting factor for the degradation of organic matter by sulfate-reducing bacteria. Due to the stimulation of sulfate-reducing bacteria, increased amounts of free sulfide were detected in the porewater (Fig. 2). At undisturbed experimen-

tal sites interstitial sulfide concentrations were usually below the detection limit (< 0.2 μM). A large portion of the sulfide was possibly precipitated as iron or manganese sulfide minerals, it might also have reacted to form organic thiols, or be used as a reducing agent for compounds containing iron(III) and manganese(IV). The distinct sulfide production horizon at a depth of 5 cm localized a site where bacterial sulfidogenesis exceeded biological and geochemical sulfide consumption. More organic material was now mineralized by bacterial sulfate reduction. In addition, a possible sulfur limitation for the growth of fermenting bacteria was overcome by the production of sulfide. The two effects might have led to a general in situ stimulation of bacterial metabolism of various populations.

4.2. Metabolic consequences

Much higher concentrations of organic intermediates characteristic for anaerobic mineralization processes, i.e. volatile fatty acids, were detected in the porewater as a consequence of stimulation. More organic matter was oxidized, probably on routes which led to an elevated interstitial concentration of acetate and propionate (Fig. 3). A steady pH decrease from 7.6 at the sediment-water interface to 7.1 in 10 to 20 cm depth was associated with a marked increase in dissolved CO_2 (data not presented). Fermenting microorganisms and sulfate reducing bacteria belonging to the group that incompletely oxidize organic matter to acetate, propionate, and CO_2 might have been responsible for the production of large amounts of VFA observed. The horizon with high acetate and propionate concentrations coincided with the sediment layer where the supplied sulfate was utilized most actively (Fig. 1a). Also the appearance of free hydrogen sulfide demonstrated active sulfate reducing activities (Fig. 2). This suggests a correlation between sulfate utilization and VFA production. A similar phenomenon was observed in evaporation ponds of salt works by Klug et al. [30]. However, our experiments could not distinguish between sulfate-reducing and fermenting processes. It is reasonable to assume that acetate and propionate are metabolites of sulfate reducing bacteria.

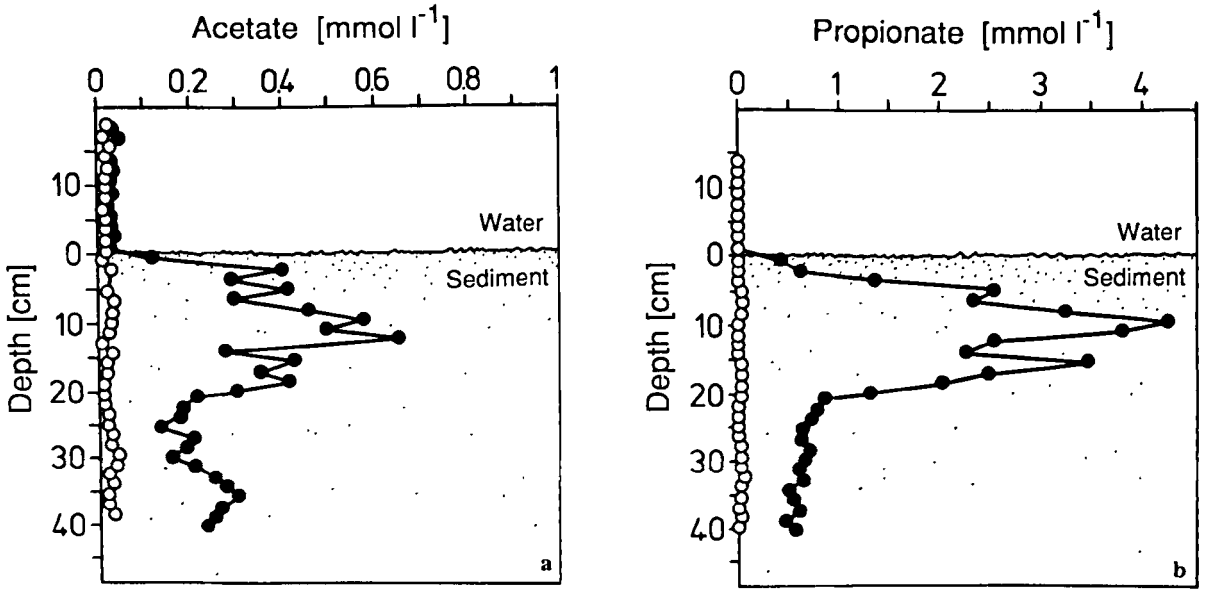

Fig. 3. Acetate (a) and propionate (b) gradients in pillow-like sediments of Lake Geneva with and without in situ addition of sulfate by a plaster post. ○ = undisturbed control site; ● = experimental site.

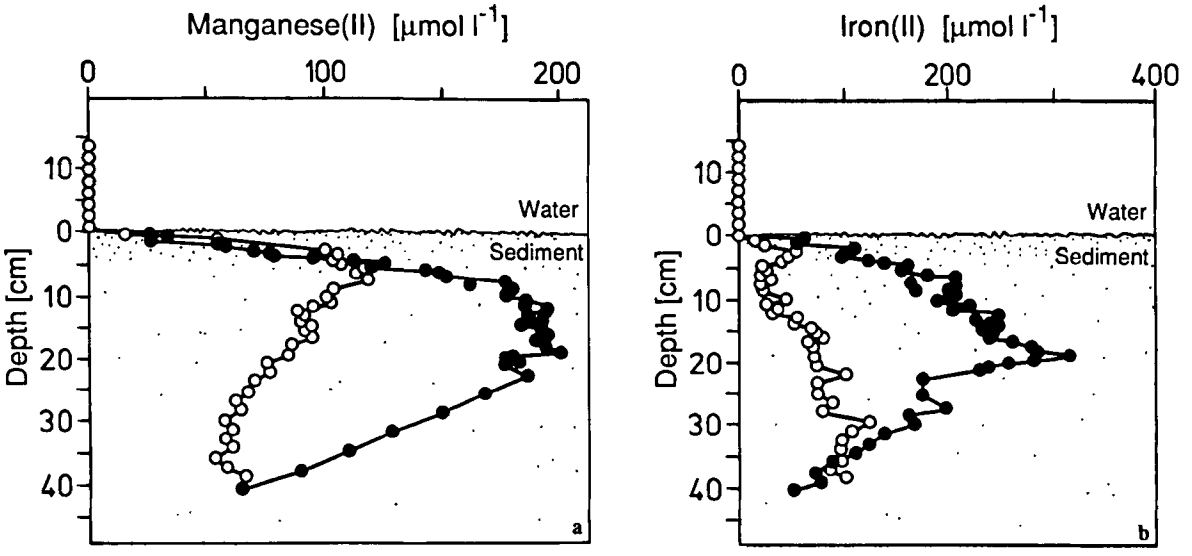

Fig. 4. Manganese(II) (a) and iron(II) (b) gradients in pillow-like sediments of Lake Geneva with and without in situ addition of sulfate by a plaster post. ○ = undisturbed control site; ● = experimental site. Manganese(II) = sum of mobile Mn(II) species; iron(II) = sum of mobile Fe(II) species.

4.3. Geochemical consequences

Increased bacterial activities also produced higher concentrations of soluble Fe(II) and Mn(II) in the porewater (Fig. 4). Concentration depth profiles of both Mn(II) and Fe(II) revealed reduction horizons 5–20 cm deep in the sediment. This correlated well with the horizons of sulfate utilization (Fig. 1a). Iron(III) and manganese(IV) were reduced in these sediment layers either directly by bacteria possessing specific reductase systems [21] or indirectly through chemical reactions with sulfide as the electron donor [2,11].

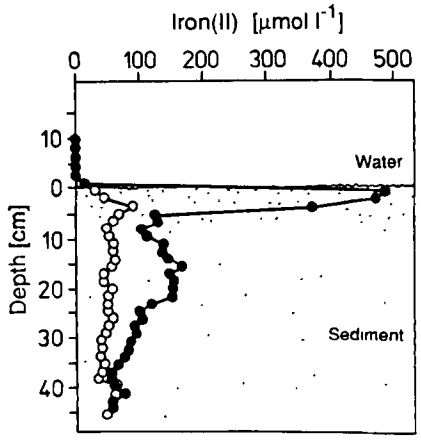

Fig. 5. Iron(II) gradients in pillow like sediments of Lake Geneva with and without in situ addition of sulfate by a plaster plate. ○ = undisturbed control site covered with a Plexiglas plate; ● experimental site. Iron(II) = sum of mobile Fe(II) species.

Supplying sediment layers with sulfate by means of a plaster plate or ground gypsum showed similar effects on the microbial reaction patterns. Covering the sediment surface cut-off supply of organic material to the sediment and also prevented the exchange of substances through the sediment–water interface. Since the supply of sulfate to deeper layers depended exclusively on diffusion from the sediment surface, all reactions which could be stimulated with this procedure were shifted towards the sediment-water interface. The data in Fig. 5 demonstrate the influence of a sulfate supply from the sediment surface on the interstitial content of soluble iron(II). Therefore, the horizon of active iron(III) reduction was located directly below the sediment surface.

4.4. Shift in community composition

Freshwater sediments are characterized by the coexistence of sulfate-reducing and methane-producing bacteria. Under naturally low sulfate con-

centrations the methanogens constitute the final populations in the electron transfer web. The domains of the two groups within the sediment are spatially separated [15,18,34]. In the presence of elevated sulfate concentrations, however, the sulfate reducers compete with methanogenic organisms in the common habitat. It has been proposed that either the higher concentrations of sulfate, the toxicity of sulfide to other organisms, the more effective utilization of available substrates by the sulfate reducers or environmental changes such as elevated pH could explain the dominance of sulfate reducers [13,31,37,41].

The interstitial methane concentrations decreased in sulfate-stimulated sediments indicating either an inhibition of methane production or a stimulated oxidation under anaerobic conditions (Fig. 6). Recently, it was demonstrated with experiments in vitro that the addition of sulfate to sulfate-depleted sediments can inhibit bacterial methane formation by 95% [35]. The concave shape of the methane concentration profile which we always observed in the stimulated sediments is more likely due to methane utilization in the zones between 5 and 30 cm sediment depths [3]. These observations lend support to the proposal that methane is oxidized under anoxic conditions. Neither the dihydrogensulfide content nor the hydrogen ion activity (pH from 7.3 to 7.8) reach toxic levels which could inhibit sulfate reduction.

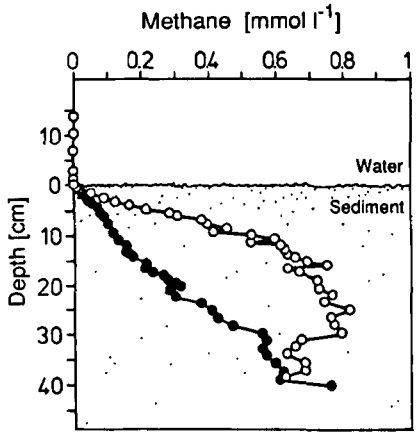

Fig. 6. Gradient of methane in pillow-like sediments of Lake Geneva with and without in situ addition of sulfate by a plaster post. The values are corrected for diffusion losses which occurred between the time of the retrieval of the porewater sampler from the sediment and the time of sampling. ○ = undisturbed control site; ● = experimental site.

In general, sulfate reducers outcompete methane producers in the presence of substrates such as hydrogen and acetate [35,37]. In our sediments the large amount of acetate produced by the sulfate reducers and the fermenting organisms was still available to the acetoclastic methanogens, however. Methanogenic substrates were not limiting and methanogenesis could proceed.

Table 2
Ranges of maximal diffusion fluxes of different chemical species within the sediment redox transition zone

Sulfate feeding method	Maximal diffusion fluxes [a] F_j(μmol·m^{-2}·d^{-1})				
	Nitrate $+F_j$	ΣManganese(II) [b] $-F_j$	ΣIron(II) [c] $-F_j$	Sulfate $+F_j$	Methane $-F_j$
Undisturbed control site	56–178 (4)	80–230 (5)	77–112 (5)	578–751 (5)	222–1250 (5)
Plaster post	95 (1)	376–506 (3)	224–266 (3)	[d]	138–145 (2)
Ground gypsum	n.d.	376 (1)	212 (1)	[d]	216 (1)
Plaster plate [e]	158 (1)	341 (1)	940 (1)	[d]	198 (1)
Control site [e] (Plexiglas plate)	74 (1)	196 (1)	88 (1)	699 (1)	424 (1)

[a] $+F_j$ is a flux directed into the sediment, $-F_j$ is flux towards the overlaying water.
[b] ΣManganese(II) = sum of mobile Mn(II)-species at the prevailing pH- and pe-conditions.
[c] ΣIron(II) = sum of mobile Fe(II)-species at the prevailing pH- and pe-conditions.
[d] Sulfate is present in excess.
[e] Free diffusion exchange between water and sediment was hindered in these experiments by the plate which covered the sediment surface.
n.d., not determined.
Average sediment porosities at the depth of flux determination were 0.9. Values in parentheses represent the number of experiments.

Table 3
Ranges of average microbial conversion rates in situ at 4.8°C

Sulfate feeding method	average rates [a] R_j (μmol·dm^{-3}·d^{-1})				
	Nitrate $-\Sigma R_j$	ΣManganese(II) [b] $+\Sigma R_j$	ΣIron(II) [c] $+\Sigma R_j$	Sulfate $-\Sigma R_j$	Methane $+\Sigma R_j$ [f]
Undisturbed control site	3.7–11.9 (4)	5.3–15.3 (5)	0.2–1.8 (5)	19.3–25.0 (5)	4.7–24.6 (5)
mean ± SD	8.3 ± 3.4	11.5 ± 5.8	0.7 ± 0.5	21.8 ± 2.2	12.5 ± 7.4
Plaster post	6.3 (1)	25.0–33.7 (3)	3.7–6.1 (3)	[d]	−1.2– −6.2 (2)
mean ± SD	n.d.	29.2 ± 3.5	5.2 ± 1.0	[d]	−3.7 ± 2.5
Ground gypsum	n.d.	25.0 (1)	5.7 (1)	[d]	−3.6 (1)
Plaster plate [e]	10.5 (1)	22.7 (1)	31.3 (1)	[d]	−6.2 (1)
Site covered with Plexiglas plate [e]	4.9 (1)	13.0 (1)	2.9 (1)	23.3 (1)	13.6 (1)

[a] $+\Sigma R_j$ is an overall production rate, $-\Sigma R_j$ an overall consumption rate.
[b–e] As in legend to Table 2.
[f] A negative value in this column indicates a consumption rate.
n.d., not determined.
Number of experiments in parentheses.

4.5. Changes in mass flux and microbial production and consumption rates

Rates of microbial substrate utilization and product formation ($\sum R_j$) were estimated from changes in the maximal conversion fluxes (ΔF_j) of specific compounds. Provided a gradient is stable for the time period of the experiment, one may calculate diffusion fluxes in defined sediment horizons. Maximal fluxes are summarized in Table 2. Generally, fluxes have been calculated for the top 20 cm of the sediment. Addition of sulfate led to an increase in the fluxes of reduced manganese and iron and to a decrease in the overall methane flux towards the anoxic interface. In horizons with maximal diffusion flux changes we determined microbial reaction rates according to Eqn. 4 (Table 3).

A comparison of conversion fluxes calculated at different depths of the methane concentration profile (Fig. 6) indicates methane consumption horizons between 8 and 30 cm sediment depth (Table 3). An inhibition of methanogenesis or a competition between sulfate reducers and methanogens for the same electron source would lead to a ΔF for methane equal to zero between a deep production horizon and a location of the methane sink. Our observations clearly indicate methane consumption. Therefore, the hypothesis of methane oxidation under sulfate-reducing conditions is supported [4,29].

We did not observe significant changes in the fluxes of nitrate at the sulfate-stimulated sites. It is consumed in the upper horizon at similar rates independent of sulfate concentration. Nitrate-reducing bacteria must not be affected significantly. The priorities set according to thermodynamic considerations for the utilization of nitrate and sulfate as oxidants still hold even in the presence of an excess of another oxidant [23].

The conversion rates (Table 3) demonstrate distinct changes in the bacterial reaction patterns to the specific perturbations in situ. A freshwater sediment in which methane-producing organisms naturally prevail could be converted in situ into a predominantly sulfate-reducing ecosystem. The stimulated microbial activities also led to predictable geochemical consequences. The changes observed in the biogeochemical patterns demonstrate the large potential of microbial abilities present in these sediments and their adaptability to environmental fluctuations.

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