Sulfate Reduction in Freshwater Peatlands

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ABSTRACT

This text consist of two parts: Part A is a literature review on microbial sulfate reduction with emphasis on freshwater peatlands, and part B presents the results from a study of the relative importance of sulfate reduction and methane formation for the anaerobic decomposition in a boreal peatland.

The relative importance of sulfate reduction and methane production for the anaerobic decomposition was studied in small raised bog situated in the boreal zone of southern Sweden. Depth distribution of sulfate reduction- and methane production rates were measured in peat sampled from three sites (A, B, and C) forming an minerotrophic-ombrotrophic gradient. SO_4^{2-} concentrations in the three profiles were of equal magnitude and ranged from 50 to 150 µM. In contrast, rates of sulfate reduction were vastly different: Maximum rates in the three profiles were obtained at a depth of ca. 20 cm below the water table. In A it was 8 µM h-1, while in B and C they were 1 and 0.05 µM h-1, respectively. Methane production rates, however, were more uniform across the three nutrient regimes. Maximum rates in A (ca. 1.5 $\mu g\ d^{-1}g^{-1}$) were found 10 cm below the water table, in B (ca. 1.0 $\mu g\ d^{-1}g^{-1}$) in the vicinity of the water table, and in C (0.75 $\mu g\ d^{-1}g^{-1}$) 20 cm below the water table. In all profiles both sulfate reduction and methane production rates were negligible above the water table. The areal estimates of methane production for the profiles were 22.4, 9.0 and 6.4 mmol $m^{-2}d^{-1}$, while the estimates for sulfate reduction were 26.4, 2.5, and 0.1 mmol $m^{-2}d^{-1}$, respectively. The calculated turnover times at the sites were 1.2, 14.2, and 198.7 days, respectively. The study shows that sulfate reducing bacteria are important for the anaerobic degradation in the studied peatland, especially in the minerotrophic sites, while methanogenic bacteria dominate in ombrotrophic sites.

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A. TAXONOMY AND ECOLOGY OF DISSIMILATORY SULFATE REDUCTION - AN OVERVIEW

INTRODUCTION

The global cycling of sulfur is a complex and dynamic process, involving several different oxidation stages and exchanges between the lithosphere, atmosphere, and biosphere. The oxidation stages range from +IV (sulfate) to -II (sulfide), and sulfur compounds can appear as gases, as well as in aerosols, liquids and minerals (Charlson et al. 1992). In comparison with other major elemental cycles (i.e. C, N, O, P) the sulfur cycle is more heavily influenced and affected by anthropogenic intervention. Estimates indicate that the amount of sulfur emitted to the atmosphere as a result of human activities (primarily from the combustion of coal and other types of fossil fuel) equal those emitted from natural sources (Andreae 1985).

Almost all microorganisms rely on sulfate (SO_4^{2-}) as sulfur source, and sulfate is taken up and converted to sulfide before being used for biosynthesis. These reactions are termed assimilatory sulfate reduction, because sulfur is assimilated into biomass after uptake. However, this text will focus on a mechanistically different type of sulfate reduction called dissimilatory sulfate reduction. This is an anaerobic process where sulfate is used as terminal electron acceptor, and the sulfide formed is excreted.

The dissimilative reduction of sulfate is an energy generating process. The sulfate reducing bacteria (SRB) is a special group of obligate anaerobic bacteria which are widely distributed in nature and can be found in marine, brackish, and freshwater sediments, as well as in anoxic and inundated soils and peats. Only a very small fraction of the sulfide formed in dissimilatory sulfate reduction is used for biosynthesis. Based on stoichiometric amounts of sulfide formed with acetate as electron donor (Widdle & Pfennig 1981, Postgate 1984), 0.2% of the sulfide formed from the reaction is incorporated into biomass, while 99.8% of the sulfide formed is excreted. Most of the knowledge concerning microbial sulfate reduction in natural environments originate from studies of marine and freshwater lake sediments. But sulfate reduction in freshwater wetlands has gained increasing attention (Brown 1985, Spratt et al. 1987, Spratt & Morgan 1990, Urban et al. 1989, Westermann & Ahring 1987, Wieder et al. 1990, Nedwell & Watson 1995). The studies in freshwater wetlands suggest that sulfate reduction can account for a considerable amount of the total anaerobic decomposition. Microbial processes resulting in methane formation, and its emission, from wetland

ecosystems is an issue of concern because it influences on the global carbon cycle, which is closely related to climatic change (IPCC 1996). Methanogenesis and sulfate reduction are competitive degradation pathways in anaerobic ecosystems, and hence, the processes controlling sulfate reduction are of importance when evaluating the effects of microbial processes on carbon cycling and global climatic systems. One hypothesis is that increased atmospheric deposition of sulfur may shift the anaerobic degradation in wetlands from methanogenesis towards sulfate reduction (Wieder et al. 1987, Nedwell & Watson 1995).

The purpose of part A of this text is to give an overview on microbial sulfate reduction in wetlands and a background to the experimental work described in part B. First I deal with the classification of sulfate reducing bacteria and biochemistry of sulfate reduction. This is followed by an overview of the biogeochemical processes involved, and of other studies on sulfate reduction in freshwater wetland ecosystems, especially *Sphagnum*-dominated peatlands. Section B presents the results from a study of the relative importance of sulfate reduction and methane formation for the anaerobic decomposition in a boreal peatland.

CLASSIFICATION AND CHARACTERISTICS OF SULFATE REDUCING BACTERIA

The sulfate reducing bacteria (SRB) is a physiologically unified group of eubacteria. Taxonomically they belong to the delta group of purple bacteria, or the proteobacteria as they are now called. The proteobacteria contains a range of different species which have evolved from the ancestral phototrophic bacteria, but have lost their phototrophic ability somewhere along the evolutionary pathway (Brock 1990). Among the eleven known genera of SRB, two distinct sub groups are recognized: non-acetate oxidizers (group I, *Desulfovibrio, Desulfomonas, Desulfoomaculum, Archaeglobus, Desulfobulbus, Thermodesulfobacterium*) and acetate oxidizers (group II, *Desulfobacter, Desulfobacterium, Desulfococus, Desulfonema, Desulfosarcina*). Group I sulfate reducers utilize ethanol, lactate, pyruvate, or other fatty acids as carbon and energy sources, while group II specialize in the oxidation of acetate.

Electron microscopy and catalogues of ribosomal 16S RNA have shown that the gram-staining behavior of sulfate reducing bacteria is unreliable as a base for classification (Widdel 1988). In the literature most SRB are described as gram-negative (Postgate

1984). *Desulfonema* species have been shown to stain irregularly but most often gram-positively, and young cultures of *Desulfotomaculum nigrificans* sometimes display a fraction of gram-positive cells. But the cell wall of the genus *Desulfonema* exhibit characteristics of gram-negative bacteria (Widdel et al. 1983), while the cell walls of *Desulfotomaculum nigrificans* displayed gram-positive characteristics (Nazina et al. 1979).

BIOCHEMISTRY OF SULFATE REDUCTION

Table 1. Substrate utilized by methanogenic and sulfate reducing bacteria (modified after Capone & Kiene 1988)

Electron donor	Methanogenes	Sulfate reducers
Pyruvate		X
Lactate		X
Propionate		X
Butyrate		X
Valerate		X
Malate		X
Succinate		X
Benzoate		X
Glucose		X
Ethanol		X
Propanol		X
Butanol		X
Alanine		X
H_2	X	X
Acetate	X	X
Methanol	X	X
Formate	X	X
Dimethyl sulfide	X	?[1]
Trimethylamine	X	?[1]
Dimethylamine	X	?[1]
Monoethylamine	X	?[1]
Ethyldimethylamine	X	?[1]
Carbon monoxide	X	?[1]

[1] Not known whether the substrate is utilized or not.

Most isolates of SRB are organotrophs, and use different organic compounds as electron donors and carbon source, and sulfate as their terminal electron acceptor. However, a few species of SRB utilize hydrogen and carbon dioxide as sole energy and carbon source. In addition, some can grow fermentatively, using for example furmate as an alternative electron acceptor (Postgate 1951, Grossman & Postgate 1955, Steenkamp et al. 1981). The organic compounds used by the organotrophic species are all of low molecular weight (Table 1), and nearly all compounds are known fermentation products derived from the degradation of proteins, carbohydrates and other constituents of dead biomass (Widdel 1988).

Sulfate is an energetically unfavorable electron acceptor compared to oxygen or nitrate, but sufficient energy to produce ATP becomes available when an electron donor that yields NADH or FADH is used (Brock 1991). The reduction of sulfate involves an 8-electron reduction of sulfur through a number of intermediate stages. Figure 1 shows the pathways of sulfate reduction in *Desulfovibrio* species where lactate serves as electron donor. Overall, two moles of carbon is oxidized for every mole of sulfate reduced (Howarth & Stewart 1992), and the overall reaction yields at least 3 ATP (Gotschalk 1986).

Since the sulfate ion is fairly stable it cannot be used without first being activated. This is done by using ATP (Figure 1). The enzyme ATP sulfurylase catalyses the attachment of the sulfate ion to ATP, leading to the formation of adenosine-5'-phosphosulfate (APS). Once sulfite is formed the reduction proceeds readily catalyzed by a special siroheme protein. This protein contains two tetrapyrrol rings and iron sulfur centers, and displays sulfite reductase activity.

In order to carry out the electron transport process, sulfate reducing bacteria has a unique type of cytochrome, cytochrome c_3, which is found in the periplasmic space (Figure 1). It is this compound that enables the electrons from the energy source to be transferred to the sulfate ion in APS. Ferredoxin and flavodoxin are other electron carriers used by sulfate reducing bacteria. Sulfate-reducers in group II (the acetate oxidizers) contain a special cytochrome of the b type. This cytochrome is not present in species incapable of degrading fatty acids.

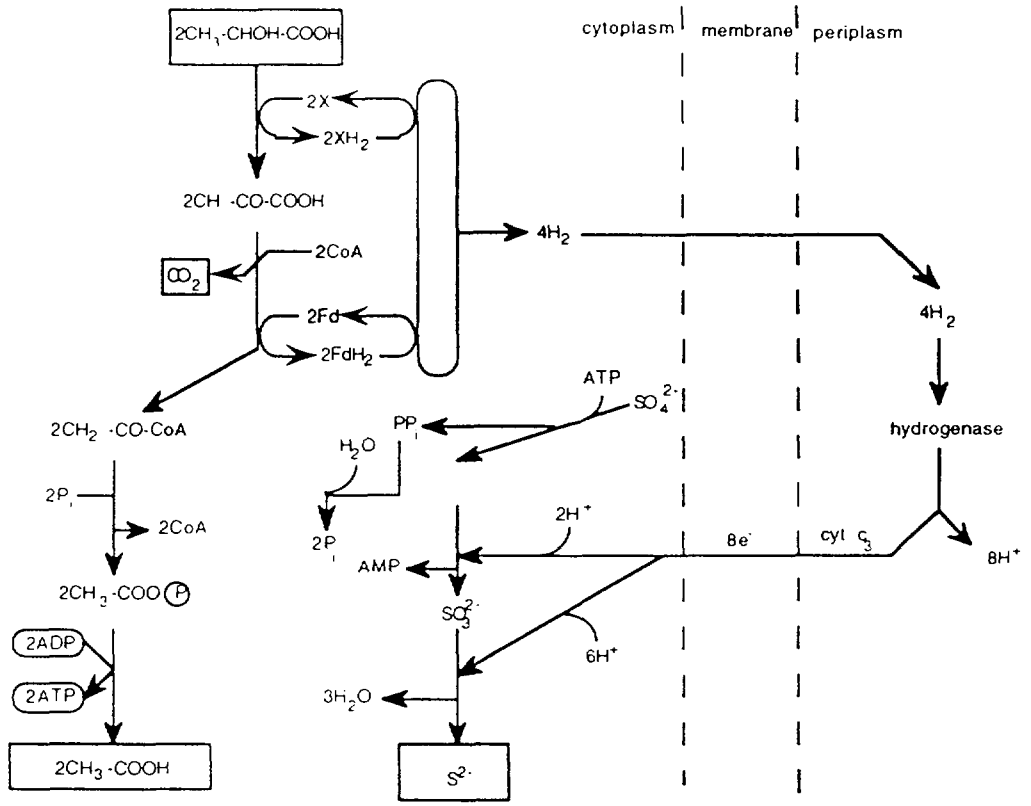

Figure 1. Pathway of dissimilatory sulfate reduction in *Desulfovibrio* species, and the hydrogen cycling hypothesis (modified after Gotschalk 1986).

Hydrogen (H_2) transfers the electrons released in the oxidation of organic substances to a hydrogenase, which is situated in the periplasmic space in close association with cytochrome c_3 (Figure 1), while the protons stay outside the membrane, the electrons are transferred back across the membrane. Hydrogen is available either directly from the environment, or via organic electron donors. At least some sulfate reducing bacteria seems capable of generating their own supply of hydrogen through hydrogen cycling (Gotschalk 1986). This hypothesis is illustrated in Figure 1, where the electrons derived from hydrogen are transferred from the cytoplasmic hydrogenase to cytochrome c_3 via the periplasmic hydrogenase and then transported through the membrane to APS and sulfite.

ECOLOGICAL ASPECTS OF SULFATE REDUCTION

Methodology for determining sulfate reduction rates in sediments and soils

Sulfate reduction rates in different ecological systems has been determined using an array of different approaches: Measurements of sulfate disappearance from sealed cores of sediment, mathematical modeling from sulfate concentration gradients, and calculations of sulfur retention from whole watershed sulfur budgets. However, the ^{35}S radiotracer technique (Jørgensen 1978, Howarth & Jørgensen 1984, Fossing & Jørgensen 1989) is the most commonly used method for quantifying microbial sulfate reduction. Tracer amounts of $^{35}SO_4^{2-}$ are added and after incubation the formation of reduced ^{35}S compounds are measured. The labeled products generated during incubation can be both acid volatile (H_2S dissolved in the porewater and iron monosulfides) and non-acid volatile (pyrite and elemental sulfur; Howarth & Teal 1979). The acid volatile compounds can easily be transformed to sulfide by acidification of the sample. The sulfide is then distilled and trapped in a solution containing zinc acetate. To volatilize the non-acid volatile ^{35}S pool, a chromium reduction technique is used, where an acid solution of Cr^{2+} reduce pyrite and elemental sulfur to sulfide (Zhabina & Volkov 1978, Jørgensen 1978, Canfield et al. 1986). An earlier technique involves the use of aqua regia to reduce the non-acid volatile pool. But aqua regia also reduce the sulfate to sulfide. The chromium reduction does not reduce remaining sulfate, and the presence of sulfate in the sample is of no concern (Wieder et al. 1985). A disadvantage is that organic forms of sulfur is not liberated by the chromium reduction method (Canfield et al. 1986, Wieder et al. 1985). Organically bound sulfur may be formed during incubation either by biotic assimilative sulfate reduction, or abiotically as sulfide reacts with organic matter via nucleophilic attack (Brown 1985). It has been suggested that freshwater environments have greater potential for formation of organically bound sulfur than marine environments (Novak & Wieder 1992, Spratt & Morgan 1990), which can lead to an underestimation of sulfate reduction rates in the former systems (Wider & Lang 1988, Rudd et al. 1986). However, Wieder and Lang (1988), showed that in short term incubations of freshwater peat with $^{35}SO_4^{2-}$, the majority of the reduced sulfate was incorporated into inorganic rather than organic forms. Further, several studies have shown that iron sulfides, such as pyrite, are major short-term products of sulfate reduction in saline wetlands and that organic forms are negligible in comparison (Howes et al. 1984, King et al. 1985).

Zonation of biogeochemical processes in anaerobic ecosystems

Anaerobic ecosystems contain several different microbial groups and communities, which are individually controlled mainly by the availability and distribution of electron acceptors and organic substrates (Capone & Kiene 1988). Relatively undisturbed systems show a characteristic stratification in the profile based on the availability of organic substrate and the regulating redox gradient (Figure 2). The concentrations of oxygen, nitrate, ferric ion, and sulfate typically decrease across the aerobic/anaerobic interface. In some systems, the redox gradient is established vertically, but it may also occur in other dimensions in the vicinity of plant roots or pipes and burrows made by benthic animals (Conrad 1989). The bacteria in deeper layers have to rely on organic material that have already undergone considerable mineralization (Conrad 1989, Nedwell 1984, Svensson & Sundh 1992). But fresh organic matter may be introduced to the deeper layers in the profile by the bioturbation of benthic animals, or by root exudation or root decay (Holzapfel-Pschorn et al. 1986).

The aerobic/anaerobic oxidation/reduction cycle of sulfur in natural environments is shown in Figure 3. The cycle involves both aerobic and anaerobic processes carried out by several different groups of organisms and it contains a range of intermediate oxidation stages.

Dissimilatory sulfate reduction in soils may be beneficial to higher plants because it regenerates nutrients and buffers the pH. However, the product of sulfate reduction, hydrogen sulfide, is highly toxic to living organisms since it combines with iron in essential cell compounds, for example cytochromes. In anoxic soils the microbial production of sulfide may cause damage to higher plants. Additionally, SRB and methane producing bacteria (MPB) are inhibited by hydrogen sulfide (McCartney & Oleszkiewicz 1993). But its concentration is usually kept at low levels because it combines with iron, leading to the formation of FeS and pyrite (Howes et al. 1984, King et al. 1985).

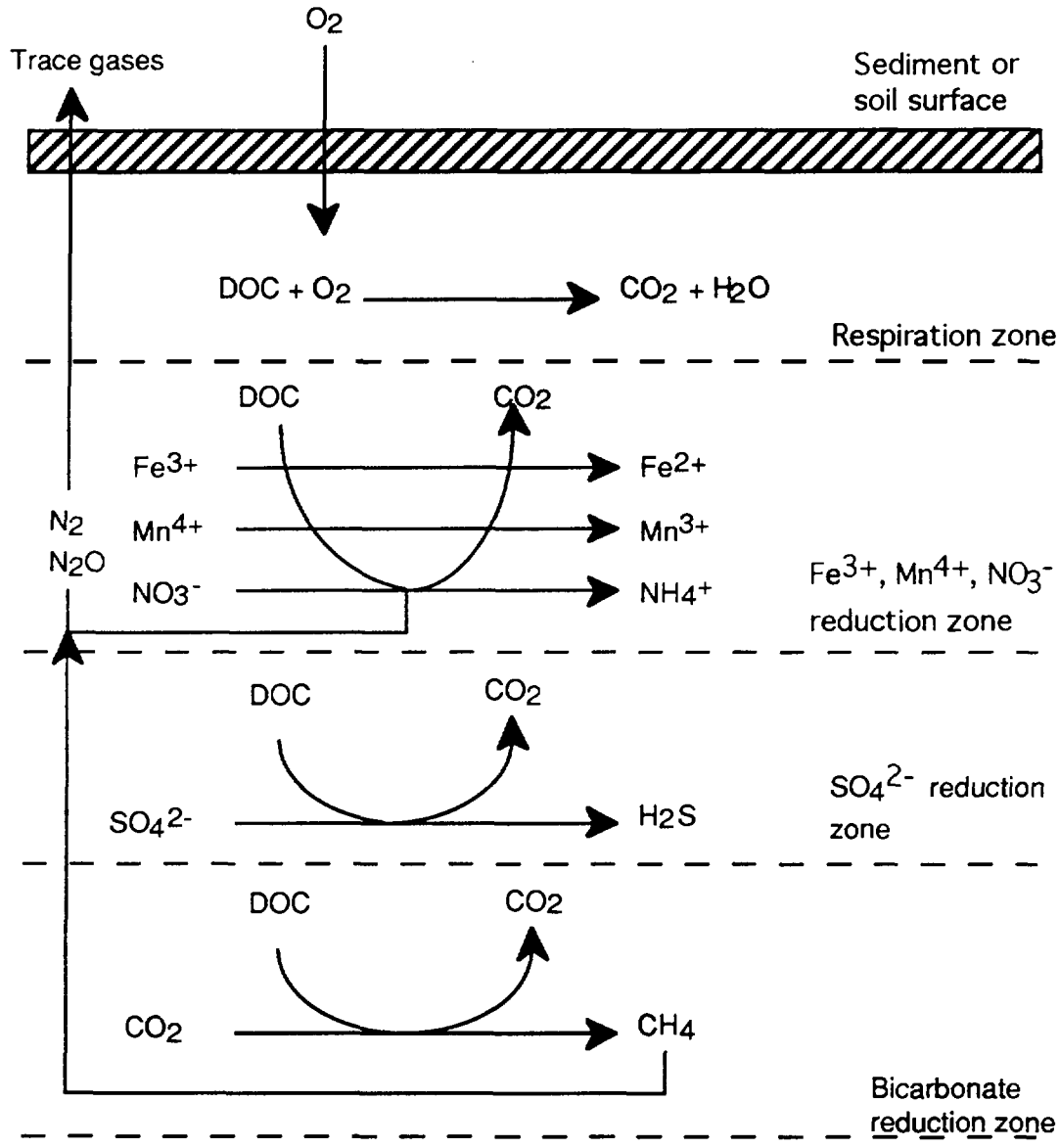

Figure 2. Zonation of microbial processes in sediments or soils with respect to redox zonation (modified after Conrad 1989).

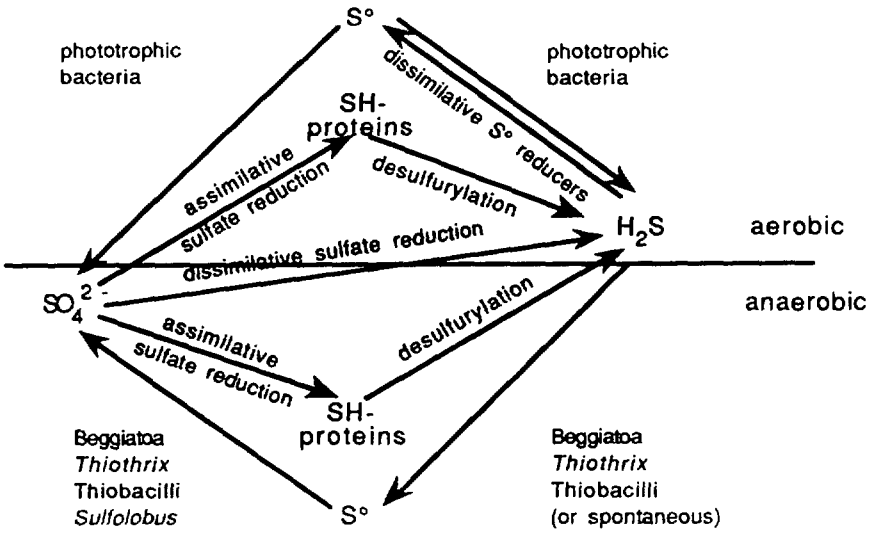

Figure 3. The aerobic/anaerobic redox cycle for sulfur in natural environments (modified after Brock 1991).

Sulfate reduction in freshwater wetlands

Only a few freshwater wetlands have been examined regarding sulfate reduction rates (Table 2). Generally, rates are somewhat lower in freshwater than in saltwater wetlands, but values tend to overlap (Giblin & Wieder 1992). However, the limited amount of data makes it premature to conclude whether these rates are typical of freshwater wetlands.

Since few rates of sulfate reduction in freshwater wetlands have been reported, there is not much information on what factors that control the process. However, the depth of the water table seems to be a major factor controlling sulfate reduction (Spratt et al. 1987), due to the fact that it to a large extent determines the distribution of aerobic and anaerobic conditions in the peat profile.

Table 2. Reported concentrations of sulfate in the porewater and sulfate reduction rates in freshwater wetlands.

Study	SO_4^{2-} (μM)	Rate of SO_4-reduction ($\mu M\ h^{-1}$)
Brown (1985)	195	NR[1]
Wieder et al (1985)	89-111	NR[1]
Spratt et al. (1987)	100-500	0.04-7.22
Westermann & Ahring(1987)	200-400	2.3-3.6
Wieder et al. (1987)	74-84	NR[1]
Wieder & Lang (1988)		
Urban et al. (1989)	2-50	NR[1]
Wieder et al. (1990)	0-350	4.4-6.1
Novak & Wieder (1992)	50-470	NR[1]
Nedwell & Watson (1995)	28-127	0.2-2.7
This study	55-176	0-11.0

[1] NR: Not reported

Wieder et al. (1990) found that sulfate reduction was positively correlated to the sulfate concentration. Based on these results they hypothesized that peatlands not receiving high sulfate loadings should have low rates of anaerobic decomposition, and a predominance of methanogenesis over sulfate reduction. However, if such peatlands were subjected to increased atmospheric deposition of sulfate, sulfate reduction rates would increase. This would result in increased total mineralization rates, and therefore have implications for the carbon balance and peat accumulation. But the published

information on the role of sulfate concentration as a control of sulfate reduction in natural freshwater environments is confusing. For example, if the sulfate concentrations controls sulfate reduction rates in freshwater systems much lower rates would be expected in freshwater than in marine systems, due to the markedly lower sulfate concentrations. But sulfate reduction in freshwater systems can be of equal magnitude as those found in marine environments (Capone & Kiene 1988, Giblin & Wieder 1992). Further, sulfate reduction can be larger than methanogensis in some freshwater systems, at least under certain conditions (Wieder et al. 1990, Nedwell & Watson 1995). These high sulfate reduction rates can only be explained by a rapid reoxidation of reduced sulfur compounds to sulfate, which can sustain high reduction rates even if the sulfate pool size is small (Wieder & Lang 1988). It has been shown that severe sulfate limitations in freshwater systems may be expected at concentrations < 30μM, while at greater concentrations it is the availability of organic substrates that regulates the process (Lovley & Klug 1986). Still, Wieder et al. (1990) speculated that increased inputs of sulfate to their examined peatlands could have increased rates of sulfate reduction (in situ sulfate concentrations ca. 70-125 μM), although an experimental augmentation of the sulfate concentration in their samples did not increase reduction rates. Obviously, the processes controlling sulfate reduction in freshwater wetlands is far from understood. Clearly, interactions between sulfate concentrations and the availability of organic matter are very important, but a range of other abiotic and biotic factors may also influence the rate of sulfate reduction in these systems. Thus, the effect of increased sulfate loading to a wetland may to a large extent depend on the inherent characteristics of that wetland. Ecological responses to perturbation of wetland ecosystems can be very site specific, because of the great heterogeneity of these systems (Öquist & Svensson 1996). Probably, wetlands rich in available organic matter, but with low sulfate concentrations are most sensitive to increased sulfate deposition.

Sulfur cycling in freshwater wetlands

Most of the information available on the sulfur cycling in freshwater wetlands come from studies of *Sphagnum*-dominated peatlands. The dominant sulfur pool in these environments is organically bound, but the cycling of sulfur involves both organic and inorganic sulfur fractions (Figure 3). In short-term incubations with $^{35}SO_4^{2-}$ the majority of the reduced sulfate is incorporated into inorganic rather than organic forms (Wieder & Lang 1988). But on the time scale of weeks most of the sulfur is converted to organic forms (Brown 1985). Apparently, the reduced inorganic sulfur pool turns over rapidly

(Figure 3). Wieder & Lang (1988) found that rates of sulfur oxidation is similar to rates of sulfate reduction. This demonstrates that sulfate reduction in freshwater wetlands is not only dependent on external sources of sulfate, but also on sulfate formed by recycling of sulfide via elemental sulfur, thus generating an internal supply of sulfate. If this is true, the sulfate concentrations in the porewater is not a good indicator of sulfate availability, and it could be questioned whether sulfate reduction in freshwater wetlands is limited by low sulfate concentrations. Oxidation of reduced sulfur compounds can proceed anaerobically involving oxidized Fe and Mn compounds (Garrels & Thompson 1960, Aller & Rude 1988, Burdige & Nealson 1986), although in such reductions oxygen would be needed at some point in order to regenerate the oxidized electron acceptors.

Interaction between sulfate reducers and methanogens

As shown in Figure 2 the redox zonations for sulfate reduction and methane production lie closely together. This, coupled to the fact that many of the substrates used by SRB and MPB are of competitive nature (Table 1), has led to an interest in the interactions between sulfate-reducers and methanogens (Oermland 1988, Howarth 1984, King 1984 Wieder et al. 1990, Nedwell & Watson 1995).

It was long suggested that in marine environments methanogenesis and sulfate reduction were mutually exclusive. However, it has been shown that low, but measurable rates of methane production do occur in sulfate containing marine sediments. But the activity of methanogens increases as salinity, and hence sulfate concentrations, decreases. Sulfate may be depleted with depth in sediments due to its consumption by sulfate reducing bacteria, which can favor methanogenesis.

In freshwater systems the sulfate concentration typically range between 50 and 400 μM (Brown 1985, Nedwell & Watson 1995, Novak & Wieder 1992, Spratt et al. 1987). Based on the difference in sulfate concentrations between freshwater and marine systems, it was for a long time believed that methanogenesis displaced sulfate reduction as the dominant terminal metabolic pathway. However, it has been shown that sulfate reduction can be of the same order of magnitude as methane production in freshwater environments (Wieder et al. 1990, Nedwell & Watson 1995, Spratt & Morgan 1990). Wieder et al. (1990) and Yavitt et al. (1987) found that carbon dioxide production from

sulfate reduction dominated anaerobic carbon mineralization, and that methanogenesis accounted for only 3-12 %. But it should be noted that these studies did not measure sulfate reduction directly but instead calculated the relative importance of SRB based on the overall production of methane and carbondioxide. It has also been reported that there may be a large seasonal variation in the relative contributions of sulfate reduction and methane production to total anaerobic mineralization. Nedwell and Watson (1995) found that in a temperate peatland sulfate reduction was the dominant pathway during winter and spring, while methanogensis was larger during the summer months.

B. THE RELATIVE IMPORTANCE OF SULFATE REDUCTION AND METHANE PRODUCTION FOR THE ANAEROBIC DECOMPOSITION IN A MIRE ECOSYSTEM

INTRODUCTION

Northern boreal and sub-arctic peatlands play an important part in the global carbon cycle (Armentano & Menges 1986, Gorham 1991,1994,1995, Sjörs 1980). Generally, peatland ecosystems are carbon sinks because they sequester carbon from the atmosphere through photosynthesis, and accumulate it as peat. Estimated accumulation rates for boreal and subarctic peatlands are 0.05-0.11 Pg C yr^{-1} (Armentano & Menges 1986, Silvola 1986). However, biogeochmical processes in peatlands also results in emissions of greenhouse gases, such as CH_4 (Svensson 1980, Matthews & Fung 1987, Roulet et al. 1994).

The global sulfur cycle has become a major public and scientific topic, because anthropogenic activities such as the combustion of fossil fuels has resulted in large emissions of sulfur compounds to the atmosphere. This has lead to a significant perturbation of the global sulfur cycle (Andreae 1985), affecting ecosystems through deposition of sulfur through precipitation (H_2SO_4 in acid rain).

Sulfate reduction and methanogenesis are regarded as alternative degradation pathways in biogeochemical processes, because both sulfate reducing and methane producing bacteria (SRB and MPB) are obligate anaerobes, and compete for common substrates, such as acetate and hydrogen. While rates of methane production in anaerobic peat and rates of net methane emission from peatlands are fairly well studied few measurements of sulfate reduction in freshwater wetlands have been conducted, and the regulating factors of the process is poorly understood. For a long time, it was suggested that anaerobic carbon mineralization in freshwater systems is dominated by MPB (Gorham 1986), and that sulfate reduction would be limited by low sulfate concentrations. However, Lovley and Klug (1986) found that severe limitations of sulfate reduction in the sediments of a freshwater lake can only be expected at sulfate concentrations of <30μM. This is below the sulfate concentrations typically found in many freshwater systems, including peatlands (Wieder et al. 1985, Westermann & Ahring 1987, Urban et al. 1985, Brown 1985). Further, recent studies suggest that sulfate reduction can

account for a substantial part of the anaerobic carbon mineralization in freshwater peatlands (Wieder et al. 1990, Nedwell & Watson 1995).

The object of this study was to examine the relative importance of sulfate reduction and methane production for anaerobic carbon mineralization at different sites of a *Sphagnum* dominated peatland, and to evaluate what factors that control the rates of the two processes.

MATERIALS AND METHODS

Site decription

Peat was collected from Ryggmossen, a small raised bog in the boreal zone of southern Sweden. The bog is situated in a geological depression, has a distinct dome-shaped ombrotrophic centre, and a wet, minerotrophic, lagg surrounding the plateau. The vegetation of the lagg consists mainly of *Sphagnum* mosses, sedges (*Carex lasiocarpa, C. rostrata, Eriophorum vaginatum*), ericaceous shrubs (*Oxycocus quadripetalus, Andromeda politia*), and some small individuals of *Pinus sylvestris*. In the center, the vegetation is dominated by *Sphagnum* species and scattered individuals of *E. vaginatum*, *Drosea rotundifolia, A. polifolia*, and some low sedges. Between the lagg and the center is a sloping section with denser populations of *Pinus sylvestris, E. vaginatum* and ericaceous shrubs (mainly *Ledum palustre, Calluna vulgaris, Myrica gale*) over the *Sphagnum*-dominated ground layer.

Field sampling and peat treatment

One peat profile was collected from each of three sites: the lagg (profile A), the forested section (profile B), and from the mire center (profile C). The water table position at each sampling site was measured in perforated plastic tubes, after equilibration of the water surface. The water table in the three profiles were at 10, 17, and 25 cm below the moss surface, respectively. The profiles were withdrawn with a peat auger, and care was taken to minimize compaction of the peat during insertion of the auger. In each profile, samples were taken from five depths: 2.5-7.5, 10-15, 19-26, 30-35, and 40-45 cm in profile A, and from 5-10, 15-20, 25-30, 35-40, and 45-50 cm in profiles B and C.

Peat from each profile was transferred into double plastic bags and transported to the laboratory, where every sample was manually homogenized under a continuos flow of N_2. Three replicate 5g samples were transferred to 130-ml bottles that had been amended with 20 ml distilled water, autoclaved, and evacuated and refilled with N_2 to a slight overpressure. The bottles were then sealed with a gastight lid equipped with a butyl rubber septum, and evacuated and refilled with N_2 in three subsequent cycles.

In the laboratory, prior to the peat sampling, the bottles to be used for the CH_4 production measurements (118 ml bottles sealed with a butyl rubber septum), were pre-treated in the same manner as those used in the sulfate reduction measurements. Immediately after sampling of each profile, triplicate samples were transferred to opened bottles under a constant flow of N_2.

Sulfate concentrations in the pore water

5g of peat from each depth were transferred to a 130-ml bottle filled with 20 ml distilled water. The bottles were left overnight at 15°C after which the slurry was filtered The filtrates were frozen until analysis. Sulfate was analyzed by ion-chromatography (Dionex 40000I), with a AS4A Donex Column, using 1.7mM NaHCO3/1.8 mM Na2CO3 as eluent and detected by a Conductivity Detector. The detection limit is 0.010 ppm (3* S.D. for repeated analysis of 0.5 ppm).

Sulfate reduction rates

In order to decide an adequate incubation time for the sulfate reduction analysis, peat from the same area as profile A (depth 15-25 cm) was sampled 9 months prior to the experiment. 18 replicates were treated and analyzed in the same manner as described below, and three each were incubated for 0, 1, 3, 8, 16, and 24 h, respectively. In order to test if sulfate reduction observed in the samples were biotic, duplicate peat samples were autoclaved or amended with formaldehyde before incubation. Neither treatment resulted in detectable sulfate reduction rates.

Sulfate reduction rates were measured using a radio tracer technique described by Jørgensen (1978, cf. Zhabina & Volkov 1978, Canfield et al. 1986, Fossing &

Jørgensen 1989). This method has been evaluated for use in peatlands (Wieder et al. 1985). The bottles with added peat were left at 15°C for ca. 10 h, after which 0.5 ml $Na2^{35}SO_4$ (ca. 2μ Ci ml^{-1}) was added to each sample using a syringe. The flasks were shaken vigorously in order to mix the tracer amounts of ^{35}S with the peat slurry, and then incubated without agitation at 15°C for 5 h. The incubation was terminated by addition of 1 ml of 20% (w/v) Zn-acetate, followed by immediate freezing of the samples (-20°C). The Zn-acetate precipitate free H_2S that may have been formed during the incubation. The samples were kept frozen until the distillation.

Before distillation the flasks were amended with an additional 1ml of 20% (w/v) Zn-acetate, and allowed to thaw at room temperature. The slurries were transferred to a reaction vessel fitted with a cooler (200 mm) and connected to a gas trap containing a solution of Zn-acetate (20ml, 2% w/v). 15 ml of ethanol and 20 ml of HCl (12N) was added to the reaction vessel, and the system was flushed with N_2 for 0.5 h in order to deprive it of oxygen. This acidification liberates the acid volatile pools of sulfur (H_2S and FeS). A chromium (II) reduction procedure was used to reduce the pyrite and elemental sulfur fractions: 20 ml of a Cr^{2+} solution (2M) was added to the sample using a syringe. The $CrCl_2$ solution had been prepared by letting a 2M solution of $CrCl_3 \cdot 6H_2O$ in 0.5 M HCl react with "mossy zinc" in a flask flushed with N_2. After the addition of the Cr^{2+}-solution, the slurries then were boiled for 1h with N_2 flushing, allowing the inorganic sulfur compounds in the sample to be reduced to H_2S, transported through the cooler, and precipitated in the traps containing Zn-acetate (Zahbina and Volkov 1978). After distillation 5 ml was removed from the trap, mixed with 10 ml of scintillation fluid (Aquasafe), and shaken vigorously. The sample was stored overnight in the dark in order to avoid chemiluminescence. Radioactivity was measured with a Beckman LS6000TA scintillation counter. The exact amount of added ^{35}S was measured by diluting the stock solution (ca. 2μ Ci ml^{-1}) 400 times, after which 5 ml was added to 10 ml of scintillation fluid (Aquasafe) and measured as above.

The sulfate reduction rates were calculated according to the formula (Jørgensen 1978):

$$\text{Rate} = [SO_4^{2-}] \cdot H_2{}^{35}S \cdot k / {}^{35}SO_4^{2-} \cdot t \text{ mol } SO_4^{2-} \text{ cm}^{-3} \text{h}^{-1}$$

Where $[SO_4^{2-}]$ is the sulfate concentration in the pore water, $H_2{}^{35}S$ and ${}^{35}SO_4^{2-}$ are the total sulfide and sulfate radioactivities, t is the incubation time, and k is the isotope

fractionation factor. It has been shown that the lighter $^{32}SO_4^{2-}$ isotope is metabolized approximately 2-4% faster than the $^{34}SO_4^{2-}$ -isotope, and the relative discrimination against $^{35}SO_4^{2-}$ is 50% larger than against $^{34}SO_4^{2-}$ (Goldhaber & Kaplan 1974: Jørgensen 1978). Thus, in this study k is approximated to 1.06.

Methane production rates

After adding peat to the bottles they were re-sealed and transported to the laboratory. There they were evacuated and refilled with N_2 for five subsequent cycles. The peat slurries were then incubated without agitation at 15°C. CH_4 concentrations in the gas phase were measured at the start of the incubation, and at intervals, ranging from hours to days, for a total incubation period of 15 days. CH_4 was measured with gas chromatography according to Örlygson et al. (1993). The increase in CH_4 concentrations were close to linear in most samples, and the production rates were calculated from the linear least-square fit to all measurements between 0.5 and 15 days.

Substrate/inhibitor amendments

To determine what factors that control sulfate reduction and methane production in the peat, selected substrates of potential importance for these processes, or inhibitors of either process were added to samples from profile A, 19-26 cm depth. The samples were treated and analyzed in the same way as described above. The substrates added were: acetate, sulfate, or a mixture of pyruvate, lactate, methanol, and ethanol, while molybdate and BES were used as inhibitors of SRB and MPB, respectively. 1mM of each compounds were added, except for the mixture where 0.5 mM of each compound was added. Further, in order to test the sensitivity of sulfate reduction to shaking, duplicate samples were incubated on a rotary shaker.

Calculations and statistical analyses

Areal estimates of sulfate reduction and CH_4-production in each profile were calculated assuming a density of 1.00 g cm^{-3} water logged peat, and a mean gas-filled porosity above the water table of 0.4 (Sundh et al. 1994, Fechner & Hemond 1992).Total

mineralization through sulfate reduction and methane production was estimated assuming that two moles of carbon is oxidized for every mole of sulfate reduced, and that one mole of carbon is oxidized for every mole of methane produced.

To determine the effects of the added substrates and inhibitors, these samples were compared to the control using the Students t-test. The significance level of 0.05 was used as a minimum for statistically significant differences.

RESULTS

Time series

The amount of reduced $^{35}SO_4^{2-}$ was almost linear in relation to time up to 24h (Figure 4). Based on this the incubation time in the experiment was set to 5h.

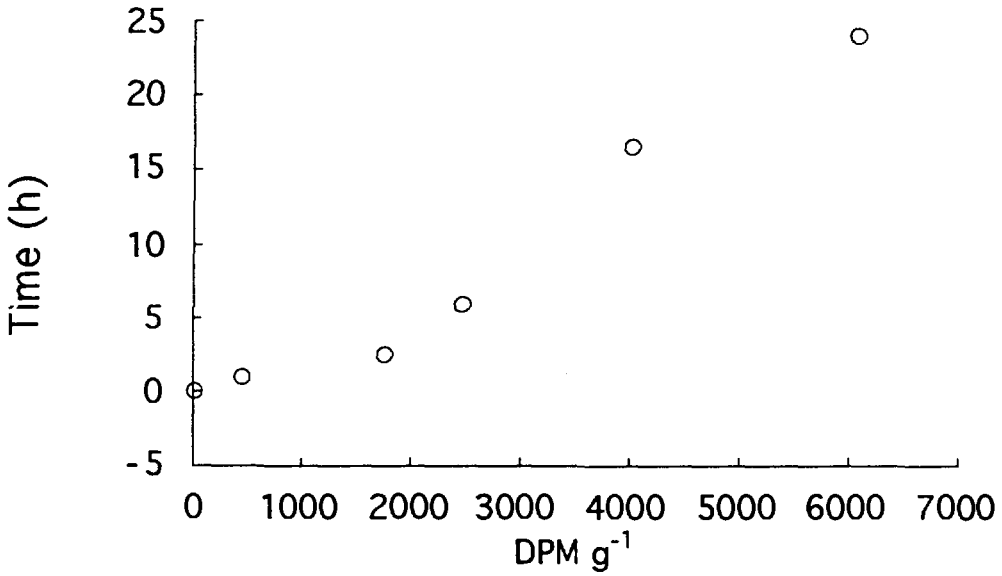

Figure 4. Reduction expressed as desintegrations per minut of $^{35}SO_4$ over time in peat from profile A (depth 15-25 cm).

Pore-water sulfate concentrations

The concentration of SO_4^{2-} in the pore water ranged from ca. 50 to 150 µM (Figure 5). No clear concentration patterns were apparent among or over depth within the three profiles.

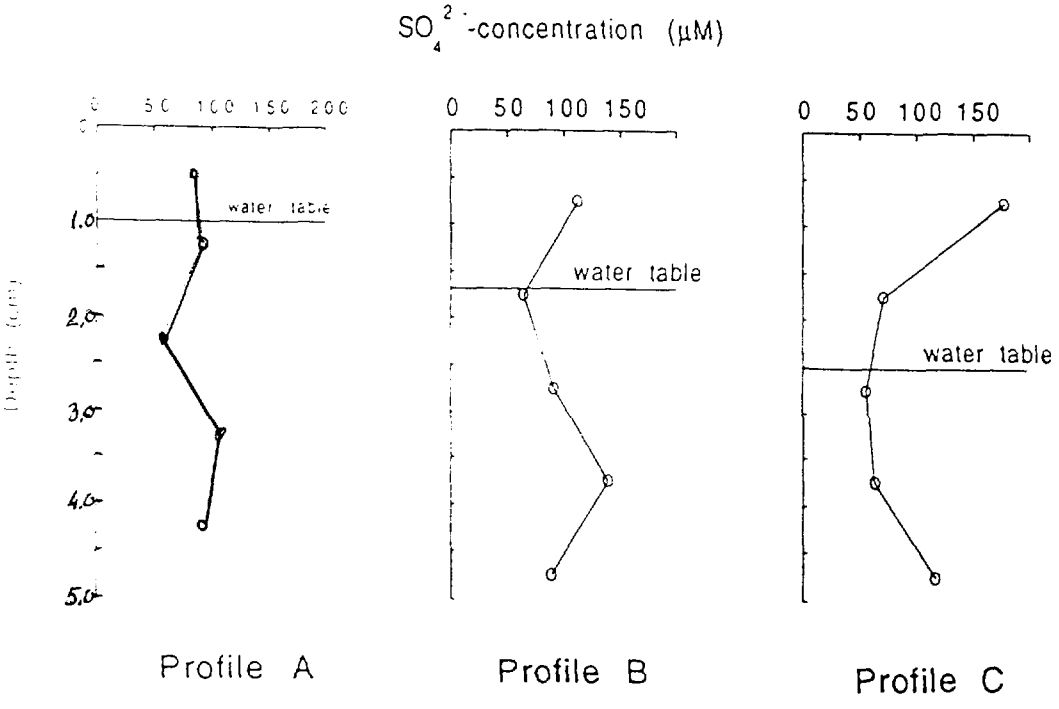

Figure 5. Concentrations of sulfate in the peat profiles at sites A, B, and C at Ryggmossen.

Sulfate reduction rates

In all profiles reduction rates were very low in the upper, aerated, parts of the profile, but considerably higher below the water table (Figure 6). The rates differed markedly between the three sites. On average, A had the highest rates of reduction and C the lowest, while B displayed intermediate rates. The areal estimates of sulfate reduction for the three profiles were 26.4, 2.5, and 0.1 mmol m^{-2} d^{-1}, respectively. The theoretical turnover times of the sulfate pool at the three sites were estimated by dividing the total

depth-integrated amount of sulfate by the depth-integrated rate of sulfate reduction. The turnover rates at sites A, B, and C were 1.2, 14.2, and 198.7 days, respectively.

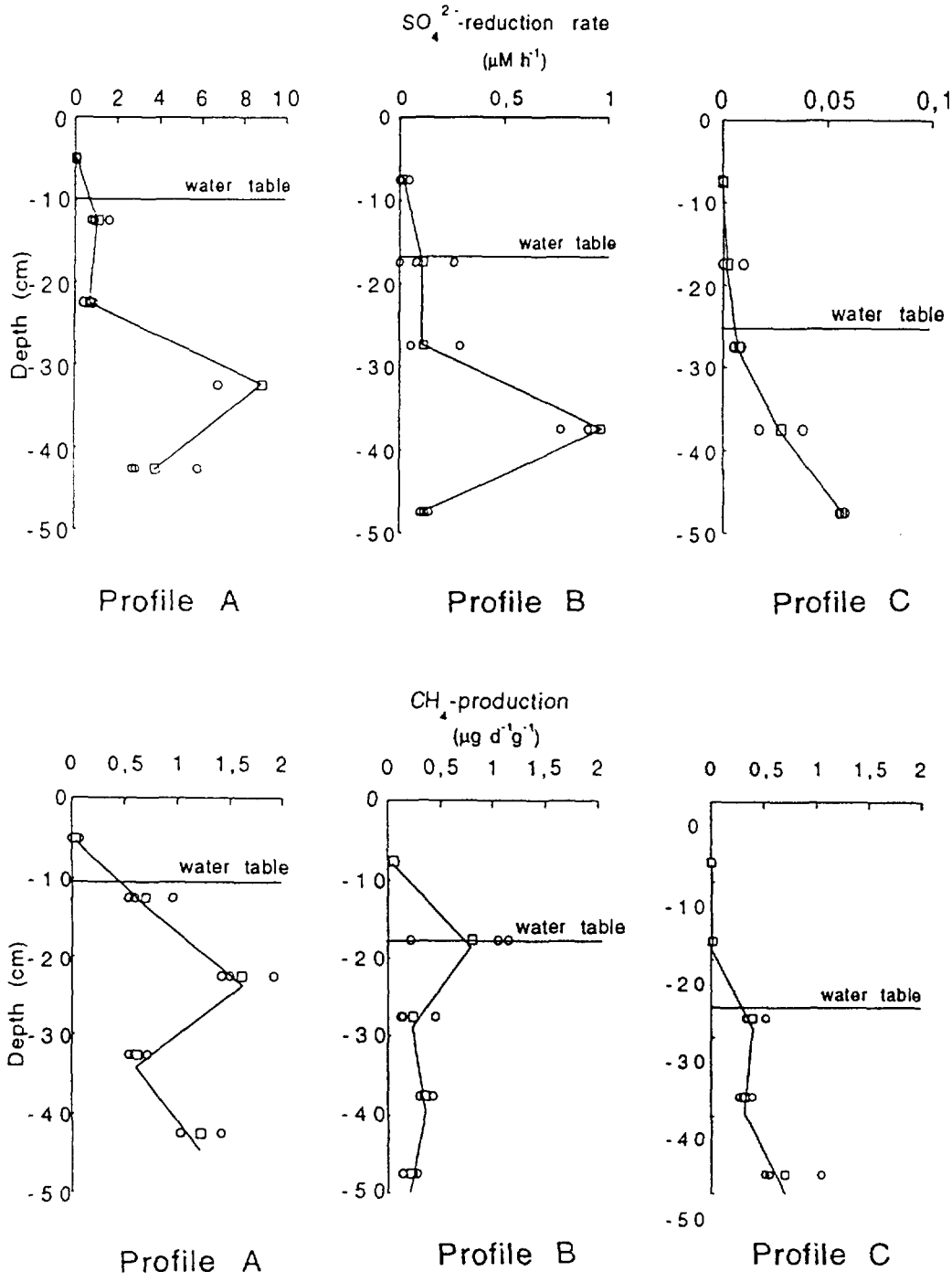

Figure 6. Sulfate reduction rates (top) and methane production rates (bottom) in peat profiles from sites A, B, and C at Ryggmossen (□ mean of replicates, O replicates)

Methane production rates

The estimated areal CH_4 production rates were 22.4, 9.0 and 6.4 mmol m^{-2} d^{-1}, respectively. The depth distribution of CH_4-production in the profiles are shown in Figure 6. Like sulfate reduction, CH_4 production rates were low above the water table in comparison with deeper parts of the profiles. Profile A had highest rates ca. 20 cm below the water table, while profile B displayed a peak (at only one depth, however) in production close to the water table. In profile C, the CH_4-production increased with depth in the whole interval down to 50 cm.

The difference in CH_4 production among the sites was much smaller than the difference in sulfate reduction rates. In comparison with profile A, the production rates were 2-3 times lower in profile B, and 3-4 times lower in profile C.

Effects of amendments on sulfate reduction and methane production rates

Table 3. Effects of amendments on sulfate reduction and methane production rates (standard deviation shown in brackets).

Amendment	SO_4^{2-} reduction rate ($\mu M\ h^{-1}$)	CH_4-production rate ($\mu g\ d^{-1}\ g^{-1}$)
Control	0.69 (0.25)	1.61 (0.27)
Molybdate	0.28 (0.04)	0.58 (0.07)
BES	0.27 (0.13)	0.43 (0.11)
SO_4^{2-}	0.96 (0.31)	0.72 (0.22)
Acetate	0.10 (0.01)*	0.84 (0.02)
Ethanol/methanol/ Pyruvate/lactate	0.02 (0.02)	1.40 (0.29)
Incubated on rotary shaker	0.09 (0.08)	NM[1]

[1] Not measured
*significant on the 0.05 level

The effects of the selected substrates and inhibitors are shown in Table 4. All amendments of potential substrates as well as inhibitors, resulted in lower sulfate reduction rates. Only acetate, however, had a statistically significant effect (0.05 significance level). Similarly methane production was negatively affected by the various substrates and inhibitors, but no amendment inhibited production rate significantly on the 0.05 level.

DISCUSSION

Methodological aspects

Ideally, the incubation time used for sulfate reduction measurements must be long enough to let measurable amounts of sulfide to be produced, but as short as possible to minimize secondary effects. Secondary effects can include re-oxidation of reduced sulfur compounds to sulfate, and incorporation of radio-labeled ^{35}S into organic matter (which is not reduced to H_2S in the Cr^{2+}-reduction). Oxidation of reduced sulfur, such as hydrogen sulfide and iron sulfides can proceed anaerobically through the reduction of oxidized Fe and Mn compounds (Garrels & Thompson 1960, Aller & Rude 1988, Burdige & Nealson 1986) However, in these experiments excess amounts of the metal oxides were added, and natural peatlands are unlikely that to have such concentrations. Although earlier studies show that freshwater environments have a large potential for the formation of organically bound sulfur (Brown 1985, Novak & Wieder 1992, Spratt & Morgan 1990), reports from both freshwater and saline wetlands indicate that the major products of sulfate reduction in short term incubations are inorganic rather than organic (Wieder & Lang 1988, Howes et al. 1984 King et al. 1985). The fact that the sulfate reduction rate was approximately linear throughout 24 h of incubation in our experiments indicates that no secondary effects interfered with the sulfate reduction within that time. Based on this I conclude that with the incubation time chosen for the experiment (5h) the underestimation of sulfate reduction due to these factors must be very small. However, sulfate reduction rates might still be slightly underestimated because of isotopic exchange and sulfur formation from sulfide during the distillation (Howarth & Jørgensen 1984).

Rates of sulfate reduction and methane production

In all three profiles the sulfate reduction and methane production rates displayed similar depth distributions. Thus, rates of both were low in the upper, aerated, parts of the profile, but much higher below the water table. This observation is in line with the physiological requirements of these organisms. Both SRB and MPB are obligate anaerobes. Although SRB are only moderately sensitive to oxygen (Capone & Kiene 1988), they are metabolically inhibited by it. In the same manner, the fact that methanogenesis did occur in the aerated parts of the profiles, although at low rates, is not surprising. It has been shown that MPB are capable of surviving exposure to oxygen for several weeks (Öquist & Sundh submitted). In both profile A and B sulfate reduction rates were maximal ca. 20 cm below the water table. A peak ca. 20 cm below the water table may be present in profile C as well, but I did not sample at sufficient depth to detect this. In profiles A and B methane production was highest just below or in the vicinity of the water table, and this pattern is consistent with earlier studies in boreal peatlands (Sundh et al. 1994). The decreased activity deeper in the profile is a well known pattern for microbial activity in soils The main reason for this pattern is that the availability of substrates and redox potentials typically decreases with depth (Conrad 1989, Svensson & Sundh 1992), leading to an unfavorable environment for biological activity. It is evident from my results that on the scale of centimeters SRB and MPB occupy the same vertical strata. The redox zonation of the two processes in sediments or soils lie closely together (Conrad 1989). This, coupled to the fact that many of the substrates utilized by the two microbial groups are of a competitive nature (McCartney & Oleszkiewicz 1993, King 1984) suggest that in these types of peatlands there is a strong competition for substrates between these two microbial groups.

Sulfate reduction rates in profile A were 10 times higher than in B, and close to 200 times higher in C. The same pattern was seen for methane production, but it was less pronounced: Production in profile A was 2-3 times higher than in B, and 3-4 times higher than in C. The explanation to different rates among the sites can perhaps be related to differences in supply of available organic substrates. Profile A is minerotrophic, and receives a substantial input of inorganic as well as organic nutrients from the surrounding areas. Another factor influencing the availability of organic substrates in peat is the exudation of fresh organic matter through the roots of the vascular plants. This stimulation of activity would be most pronounced in profile A where the populations of vascular plants is most dense. Thus, the high availability of nutrients in this part of the peatland can be expected to favor biological activity,

including sulfate reduction and methane production. In profile C in the ombrotrophic centre of the bog, nutrient availability is constrained to the input through precipitation. The nutrient poor environment is reflected in both lower biological activity and in the composition of the vegetation at this site.

The theoretical sulfate turnover time in the profiles were 1.2, 14.1, and 198.7 days, respectively. In profiles A and B turnover of sulfate was comparatively rapid, and the values are similar to estimates from other freshwater peatlands (Nedwell & Watson 1995, Wieder et al. 1990). But in profile C turnover time was much longer than in earlier studies. However, in the results of Nedwell & Watson (1995) the turnover time seems to be linked to the water table position, and the turnover was faster in hollows compared to hummocks (water table levels of 0 and 17 cm, respectively). My results follow the same pattern as those of Nedwell & Watson (1995) and sulfate turnover rates decreases as the depth of the water tale increases.

The sulfate concentrations in the porewater of the profiles ranged from 50 to 150 μM, which is in the lower range of concentrations typical of freshwater peatlands (Brown 1985, Novak & Wieder 1990, Spratt et al. 1987). But despite that there were no major differences in sulfate concentrations among the profiles, the sulfate reduction rates differed greatly. This strongly imply that sulfate reduction rates in this peatland is not limited by the availability of sulfate, but more likely by other factors such as supply of organic substrates or perhaps other nutrients. This result contradicts those of Wieder et al. (1990) who found a positive correlation between sulfate reduction rate and sulfate pool size. However, all studies I am aware of has reported that sulfate reduction rates in peat are not stimulated by sulfate additions (Nedwell & Watson 1995, Westermann & Ahring 1987, Wieder et al. 1990), once again indicating that sulfate reduction is not limited by low sulfate supplies in these systems.

Estimated total areal mineralization

There is a vast difference among the three profiles in total areal mineralization rates through sulfate reduction and methane production. I estimated that the ratio of carbon flow through sulfate reduction to that through methane production was 2.4:1 (profile A), 0.6:1 (profile B), and 0.04:1 (profile C). Thus, sulfate reduction is higher in profile A, while methanogenesis is higher in profiles B and C. Other studies on bog ecosystems have similarly reported that carbon flow through sulfate reduction can exceed that of

methane production (Wieder et al. 1990, Sinke et al. 1992). Additionally, Nedwell & Watson (1995) found a substantial seasonal variation in the ratios between these processes, where sulfate reduction dominated during winter and spring, but methanogenesis dominated during summer (cf. Spratt et al. 1987). Nedwell and Watson (1995) suggested that the decline in sulfate reduction rates in summer was caused by limitations of sulfate when concentrations decreased to ca. 40μM. My results contradict this to some degree, because sulfate concentrations are not likely to have controlled reduction rates. Thus, reduction rates in profile A were almost 200 times higher than in profile C, even though sulfate concentrations were as low as ca. 50 μM at both sites. This further supports the hypothesis that it is the availability of organic substrates rather than sulfate concentrations that control sulfate reduction in these types of peatlands.

Effect of substrate/inhibitor amendments

The fact that I only found one significant effect of the amendments on sulfate reduction or methane production is surprising. Earlier studies have reported significant effects in peat samples amended with different substrates and/or inhibitors (Westermann & Ahring 1987, Nedwell & Watson 1995). For example, acetate amendments have stimulated both sulfate reduction rates and methane production rates. Molybdate is a well known inhibitor of sulfate reduction, but in my samples it had no significant effects. There are several possible reasons for the absence of responses. First, the amendments were made by adding 2 ml of water in which the sodium salts of the various compounds were dissolved. By adding the dissolved sodium salts of the various compounds, ion exchange between the positive Na^+ ion and adsorbed cations in the peat sample may have released for example H^+, which could have influenced the microbial activity in the samples by lowering the pH. No similar sodium solution was added to control samples as a control of the possible effects of the water addition. Indeed, the negative influence of all additions on both SRB and MPB suggests that the water addition in itself may have inhibited rates. Second, during the manual homogenization of the peat prior to incubation sufficient "extra" substrates may have been released to sustain close to maximum activities in the samples, which could have lead to no effects of the amendments. Third, perhaps responses would have been greater in samples from profiles B or C, where the initial levels of available organic substrates were probably lower. Due to these ambiguities, the results of the amendment experiments are probably not reliable, and they are therefore not discussed further.

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