Humic-rich peat extracts inhibit sulfate reduction, methanogenesis, and anaerobic respiration but not acetogenesis in peat soils of a temperate bog

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ABSTRACT

To understand why anaerobic ombrotrophic peats can be very low in methane after drainage related afforestation, we analyzed the competition of sulfate reducing, humus reducing, and methanogenic microorganisms by incubating ombrotrophic peats of the Mer Bleue bog, Ontario. Sulfate, sulfide, and sulfate containing peat dissolved organic matter (DOM) from an afforested site were added in reduced and oxidized redox state. Sulfate and acetate concentrations were analyzed, bacterial sulfate reduction (BSR) and CO₂ and CH₄ production quantified, and results analyzed by ANOVA. DOM was characterized by Fourier transformed infrared and fluorescence spectroscopy and analyzed for trace elements. CH₄ production decreased with sulfate (16%, not significant) but addition of DOM significantly diminished BSR down to 0.4 nmol cm⁻² d⁻¹ (Kruskal Wallis test, p < 0.05). CH₄ production decreased with sulfate (16%, not significant) and sulfide addition (40%, p < 0.05) and CO₂ production increased (treatment ‘sulfate’, p < 0.05). Addition of all DOM extracts (67 mg L⁻¹) almost completely suppressed methanogenesis and CO₂ production (p < 0.05), but acetate accumulated compared to the control (p < 0.05). The DOM applied contained carboxylic, aromatic and phenolic moieties and metal contents typical for peat humic substances. We conclude that a toxic effect of the intensely humified DOM occurred on both methanogenic and sulfate reducing bacteria (SRB) but not on fermenting microorganisms. As yet it is not clear what might cause such a toxic effect of DOM on SRB and archaeb.

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1. Introduction

Owing to the importance of northern peatlands for the future of global atmospheric CO₂ and CH₄ budgets (e.g. Limpens et al., 2008), controls on the production, consumption and emission of the involved gases warrant further study. The production of CO₂ and CH₄ in peatlands broadly depends on the microbial activity in the peat, the soil temperature, plant community structure, the chemical characteristics of the peat, and the position of redox boundaries, which are roughly associated with the position of the water table (e.g. Yavitt et al., 1997; Bergman et al., 1998; Keller and Bridgman, 2007; Knorr and Blodau, 2009). Anaerobic decomposition of peat to CO₂ is generally of little importance for the greenhouse gas balance of peatlands due to the recalcitrance of buried peat, low temperatures and a number of enzymatic, geochemical, and hydrological factors that probably slow organic matter decomposition (Freeman et al., 2001; Beer et al., 2008; Limpens et al., 2008). The situation differs with respect to methanogenesis, which is a strictly anaerobic microbial process (Fetzer et al., 1993). Methanogenesis was found to be most active near and below the water table, where oxygen supply is limited and anaerobic respiration comparatively fast (Beer et al., 2008; Knorr and Blodau, 2009). Biogeochemical processes influencing methane dynamics in the zone near the water table are of critical importance for sustaining emissions of the gas.

Methanogenesis is controlled by a number of preceding and competing microbial processes. It is a syntrophic process that follows extra-cellular hydrolysis of polymers and fermentation of resulting monomers yielding acetate, hydrogen (H₂) and CO₂, which in turn serve as substrates for methanogenic archaea. Methanogenesis provides little free energy; for this reason bacteria utilizing electron acceptors other than CO₂ usually outcompete methanogens for H₂ by a thermodynamic exclusion mechanism, i.e.
by lowering substrate concentrations to levels that provide insufficient free energy for methanogenic metabolism (Conrad, 1999). Indeed, much lower production of CH4 than expected from stoichiometry has generally been observed in anaerobic peat soils (Segers, 1998; Yavitt and Seidman-Zager, 2006).

In soils of ombrotrophic bogs potential inorganic electron acceptors, i.e., nitrate, manganese, iron and sulfate, are typically scarce to non-detectable (e.g. Steinhann and Shotyk, 1997). It is hence difficult to argue for their importance in anaerobic electron flow. Somewhat surprisingly, substantial rates of bacterial sulfate reduction (BSR) have frequently been reported (e.g. Wieder et al., 1990; Vile et al., 2003). This finding implies that BSR is continuously sustained although sulfate pools typically do not support BSR for longer than a few days (Wieder et al., 1990; Vile et al., 2003). Sustained BSR and suppression of methanogenesis and methane emissions thus depend on recycling mechanisms for sulfur, i.e. repeated reduction–oxidation cycles (Blodau et al., 2007). Such reduction–oxidation cycles are of great interest due to their potential to exacerbate the impact of sulfate deposition on methane emissions (Gauci et al., 2002, 2004).

Following the publications by Lovley et al. (1996) and Scott et al. (1998), quinones and other polyphenols contained in humic substances have emerged as potential electron acceptors that may sustain internal sulfur cycling via oxidation of hydrogen sulfide (Heitmann and Blodau, 2006; Bauer et al., 2007; Heitmann et al., 2007; Keller et al., 2009), although questions regarding the capacity and kinetics of this process remain unanswered to date. One mechanism put forward by Heitmann et al. (2007) is illustrated in Fig. 1. DOM chemically oxidizes hydrosulfide (H2S) to thiosulfate, which is subsequently reduced by sulfate reducing bacteria (SRB) to H2S, or resupplies the sulfate pool after a microbial disproportionation to H2S and sulfate. Ratasuk and Nanny (2007) and Aeschbacher et al. (2010) have since demonstrated that the reduction of humic substances is fully reversible, which is a prerequisite for a repeated utilization of DOM as an electron acceptor in peat soils, for example following fluctuations in water table and soil moisture.

The objective of the present study was to demonstrate a suppression of CH4 production in peat soils by the mechanism depicted in Fig. 1. We studied this issue in incubation experiments with peat samples from the Mer Bleue bog, Ontario, where very low CH4 concentrations occur in previously drained, anaerobic and intensely humified, and DOM-rich ombrogenic peats now under forest (Blodau and Siems, in press). Either sulfate, sulfide, reduced or oxidized dissolved organic matter extracts from the humified peats were added to bog peat under anaerobic conditions and the response of sulfate and acetate concentrations, anaerobic respiration, methane production, and BSR recorded. The DOM was characterized regarding its composition with spectroscopic methods, trace element and anion analysis.

Originally we expected that the addition of sulfate would enhance the activity of SRB and suppress methanogenesis. We further hypothesized that the addition of sulfide would support sulfate reduction by a reoxidation of hydrogen sulfide and lead to a suppression of methanogenic activity as well. We expected the addition of oxidized DOM to accelerate sulfate reduction and slow down CH4 production, and the addition of reduced DOM to have no effect on BSR and CH4 production compared to the control treatment.

2. Materials and methods

2.1. Site description and sampling

The Mer Bleue peatland is an open, slightly domed, ombrotrophic bog covering 25 km², situated 15 km east of Ottawa, eastern Ontario, Canada (45°25’N; 75°40’W, elevation 76 m). Mer Bleue, which started to form about 8400 years ago, has a maximum peat depth of 5–6 m thick and is underlain by continuous, marine clay deposits (Fraser et al., 2001). Mean annual temperature is 5.8 °C and the mean annual precipitation about 910 mm (Fraser et al., 2001). Vegetation is dominated by mosses (e.g. Sphagnum capillifolium, Sphagnum angustifolium, Sphagnum magellanicum and Polytrichum strictum) and shrubs (e.g. Ledum groenlandicum, Chamaedaphne calyculata, Kalmia angustifolia and Vaccinium myrtillus).

At the sampling sites, situated in the north–east of the Mer Bleue bog, a drainage ditch was dug about 1923 (Supplementary Information, Fig. S1). Subsequently, vegetation changed: on the western side the open bog (‘bog’) persisted with a thin tree margin, whereas the eastern side became wooded (‘forest’) and is currently dominated by Picea mariana, Larix laricina and Betula populus. The drainage accelerated mineralization of the peat because of groundwater table draw down, which likely led to the forest growth (Silins and Rothwell, 1998). This resulted in a subsequent lowering of the land surface at the eastern forested side. As a result of drainage and forest development, soil humification had advanced (Blodau and Siems, in press) dissolved organic carbon (DOC) concentrations were higher and, in particular, dissolved CH4 concentrations in the eastern forested bog peat were lower than under open bog vegetation (Supplementary Information, Fig. S2). This led us to the hypothesis that oxidation and reduction of the more intensively humified DOM produced in the forested bog peat would suppress methane production by a mechanism as outlined in Fig. 1. In the laboratory incubations we thus amended methanogenic peat from the open bog with humic-rich DOM extracts from non-methanogenic peat from the eastern forested site.

Three peat samples were collected from the water saturated zone at a depth of 30 cm at the bog site using a plastic cylinder on October 10th, 2008. Compaction of the peat was minimal. Subsequently, samples were transferred into gas tight glass vessels (1 L) minimizing exposure to oxygen as far as possible. Furthermore, a peat core (~740 g) was taken from the surface in the particularly DOC- and humic-rich forest site (Minderlein and Blodau, unpublished data). All samples were cooled and locally stored at the University of Ottawa at about 5 °C and subsequently transported to Germany, where they were stored and equilibrated for three months in the dark at 4 °C before being processed.

2.2. Laboratory incubations

Our experiments were conducted in six different treatments with four replicates each. (1) Experimental controls, as well as all other incubation treatments, were set up by mixing peat of ~20 g wet weight with 10 mL deaerated distilled water in rubber-stoppered crimp...
vials of 60 mL volume under a nitrogen (N₂) atmosphere in a glove box.

(II) To determine the ability of sulfate to decrease CH₄ and increase CO₂ production, vials were spiked with 150 μL 0.1 M Na₂SO₄ solution to adjust the sulfate concentration to 500 μmol L⁻¹, representing the treatment ‘sulfate’.

(III) To test whether sulfide addition had the same effect on CH₄ and CO₂ production via a reoxidation of H₂S, a ‘sulfide’ treatment was prepared with a 0.1 M Na₂S solution that was iodometrically determined at a concentration of 95 mmol L⁻¹. Vials were spiked with 150 μL of this solution adjusting the concentration to 475 μmol L⁻¹.

(IV) To determine the ability of DOM to slow CH₄ and accelerate CO₂ production by provision of electron accepting capacity, we obtained an additional peat core from the forested peatland site, mixed and aerobically incubated it with 4 L distilled water for two weeks, followed by filtration with a 3 μm membrane filter. A volume of 100 mL deaerated DOM solution with a concentration of ~201 mg L⁻¹ was then reduced under exclusion of oxygen with 5 mL Na₂S solution (95 mmol L⁻¹) for 24 h in the dark, giving a concentration of 4.75 mmol L⁻¹ (Heitmann and Blodau, 2006). Subsequently, the solution was purged with N₂ to remove remaining sulfate. In the treatment ‘reduced’ DOM 10 mL of this reduced DOM solution was added to the prepared incubation vials in the glove box. This adjusted total DOC concentrations to about 67 mg L⁻¹.

(V) To identify whether effects of DOM could be attributed to the provision of electron accepting capacity rather than other properties of the DOM, we carried out the same procedure as in (IV) but reoxidized the reduced and N₂-purged DOM solution prior to addition with an air stream for about an hour. Subsequently, we purged it again with N₂ to remove remaining O₂.

(VI) To test the impact of a natural percolate containing both DOM and O₂, we carried out again the same procedure as in (V) but did not purge the oxidized DOM solution with N₂ before adding it to the incubation vials.

Each crimp vial was immediately evacuated after these preparations and flushed with N₂ 10 times to homogenize the atmosphere and incubated for 18 d (19 d for treatment ‘oxidized DOM’) at a mean temperature of 20–21 °C.

2.3. Analytical procedures

Gas samples of all solutions were taken at the beginning and after 1, 2, 4, 6, 10, 14 and 18 d, or after 19 d (‘oxidized DOM’), and CH₄ and CO₂ immediately analyzed using a gas chromatograph (GC, SRI 8610C Instruments, USA) with flame ionization detector (FID) and CO₂ methanizer. Quantity was recalculated from the measured headspace concentration in the vial, the ratio of headspace to water phase volume and Henry’s law constant (Kₘ(CO₂) = 3.78 × 10⁻² mol L⁻¹ atm⁻¹ and Kₘ(CH₄) = 1.51 × 10⁻³ mol L⁻¹ atm⁻¹) corrected to the temperature of the sample (Heitmann et al., 2007). Anaerobic potential production rates of CO₂ and CH₄ were determined from the volume-corrected linear regression of concentration over time at the end of the incubation. Additional measurements of sulfate, both prior to and at the end of the incubation, and acetate, at the end of the incubation, were carried out with 1–2 mL pore water of the vials, taken under a N₂ atmosphere in a glove box, except for the ‘DOM/O₂’ treatment. Concentrations were quantified with ion chromatography (Metrohm IC-System, Metrosep Anion Dual 3 column at 0.8 mL min⁻¹ flow rate, with chemical suppression) after filtration (0.2 μm, nylon, Rottlabö®). Detection limit of acetate was 4 μmol L⁻¹, of sulfate was 4.2 μmol L⁻¹ prior to the incubation and 2.1 μmol L⁻¹ at the end of the incubation.

DOC concentration of the DOM extract was determined after dilution and acidification on a Shimadzu Total Organic Carbon analyzer (TOC-V CPN with ASI-V). Concentrations of dissolved metals contained in the DOM extract were determined on an inductively coupled plasma-optical emission spectrometry (ICP-OES).

The chemical nature of the peat and DOM extract added was studied by Fourier transformed infrared spectroscopy (FTIR), which provides information about characteristic chemical bonds in natural DOM that can be assigned to carboxylic, aromatic, phenolic, and polysaccharide structures (Niemeyer et al., 1992). The technique is employed for the study of peat humification and allows for comparisons between added DOM with material found in situ and at other sites (e.g. Beer et al., 2008). FTIR spectra of the DOM and freeze-dried peat samples of both sampling sites were recorded on a Bruker Vector 22 FTIR spectrometer on KBr pellets (200 mg KBr + 2 mg freeze-dried sample) in the absorption mode with subsequent baseline subtraction. The KBr was dried at 60 °C for 4 h prior to use. In the frequency region 3100–900 cm⁻¹, 30 scans were accumulated with a resolution of 2 cm⁻¹. Peaks were assigned according to Niemeyer et al. (1992) and Senesi et al. (1989), and relative changes in intensity ratios of major peaks used to evaluate structural changes with respect to polysaccharides (reference peak 1090 cm⁻¹).

The DOM extract was further characterized by fluorescence spectroscopy revealing the molecular condensation and fluorescent aromatic structures of peat humic substances. We calculated a fluorescence index FI, the ratio of emission at 450 nm—500 nm at excitation wavelength of 370 nm, which is used to trace the origin of humic substances (McKnight et al., 2001). The fluorescence emission—excitation spectrum was recorded on a Perkin Elmer LS 55 on 1:100 diluted DOM in the excitation range of 240—440 nm, in 10 nm steps, and emission range of 350—550 nm, in 0.5 nm steps. A Milli-Q water EEM was subtracted from the sample.

2.4. ³⁵S-radiotracer sulfate reduction rates

Sulfate reduction rates were determined using the ³⁵S-sulfate radiotracer method (Jorgensen, 1978). 60 μL of a diluted ³⁵S-sulfate solution were injected through the rubber stopper of each crimp vial with a microsyringe (Hamilton), amounting to an activity of 162 kBq, or 182 kBq in the ‘oxidized DOM’ treatment. The label was deposited in a line through the peat slurries by slowly withdrawing the syringe during injection (Jorgensen, 1978). The samples were incubated at a temperature of 19 °C and 20.5 °C (‘oxidized DOM’) for 1.5–2 h. Subsequently, sulfate reduction was stopped by adding 15 mL 1 M zinc (Zn) acetate solution. All samples were analyzed within a week.

The peat slurries were transferred with ~0.1 M zinc acetate solution into three-necked flasks, which then were flushed with N₂ for 20 min. For TRIS (total reduced inorganic sulfur compounds: FeS₂, FeS, S²⁺, H₂S) distillation (Fig. 53, Supplementary Information), 3 mL ethanol (C₂H₅OH p.a.), 5 mL of 5 N HCl and 15 mL reduced chromium solution (CrCl₃ · H₂O, 1 M) were added sequential to each of the three-necked flasks. Beforehand, the chrome solution (1 M CrCl₃ · H₂O and 0.5 M HCl) was reduced with a Jones-Reducer. Afterwards, the sample mixture was boiled for 1/2 h. With the N₂ carrier gas the released H₂S was trapped in 50 mL of 0.15 N NaOH. Both 1 mL of the NaOH solution and 100 μL of the remaining digest supernatant was added to 19 mL of a scintillation cocktail (Aquasafe 300 Plus, Zinser Analytic) and radioactivity was counted on a scintillation counter (Beckman LS 6500). A quench curve was measured separately to obtain the counting efficiency of the instrument.

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The sulfate reduction rate (SRR) was calculated per unit peat volume and time from Equation (1):

\[
SRR = \frac{\left[SO_4^{2-}\right]}{\left(\left[35S - \text{TRIS}\right] + \left[35S - \text{digest}\right]\right)t} \quad (\text{nmol SO}_4^{2-} \text{ cm}^{-3} \text{ d}^{-1})
\]

where \([SO_4^{2-}]\) is the sulfate concentration measured at the end of the incubation (nmol cm\(^{-3}\), \(35S\)-TRIS is the radioactivity of TRIS (departures per minute, DPM), measured from the NaOH solution, \(35S\)-digest is the radioactivity of the remaining digest (i.e. DPM), \(\alpha\) is the isotope fractionation factor of bacterial sulfate reduction (1.03) and \(t\) is the incubation time (d). The counting efficiency was determined as 0.5 (DPM = CPM/0.5).

Equation (1) was derived from Jorgensen (1978) and modified, basing it on the recovery of spiked radioactivity instead of the total radioactivity added. This was done as we did not measure the amount of tracer incorporated into the organic material.

### 2.5. Statistics

The aim of the statistical analysis was to ascertain the effects of treatments on concentrations of acetate and sulfate, rates of CO\(_2\) and CH\(_4\) production and sulfate reduction. Normality of CO\(_2\) and CH\(_4\) production and sulfate reduction. Normality of CO\(_2\) and CH\(_4\) production and sulfate reduction, and rates of CO\(_2\) and CH\(_4\) production in the incubation (nmol cm\(^{-3}\)) was assessed by the Shapiro Wilk test. The post hoc test Tukey’s HSD (Honest Significant Difference) (Faraway, 2005, pp. 179–186).

As Box Cox transformed data of the sulfate reduction rates indicated neither normality for all samples nor homoscedasticity, an ANOVA could not be conducted. Therefore, we applied the Kruskal Wallis test to SRR data and determined if differences exist among the means (Kruskal and Wallis, 1952). Subsequently, a multiple comparison test after Kruskal Wallis (\(\alpha = 0.05\) and \(\alpha = 0.1\)) was carried out using the statistics software R.

### 3. Results

#### 3.1. Chemical characterization of the added DOM extract

Fluorescence intensity in the emission–excitation spectrum peaked at a high emission wavelength of 450–470 nm and was generally characterized by strong intensities at combinations of high excitation-emission wavelengths (Fig. 2, left panel), which is typical for terrestrial humic substances (McKnight et al., 2001). The fluorescence index, calculated as the ratio of emission intensity at 450–500 nm, at an excitation wavelength of 370 nm, was 1.26. The DOM extract was characterized by strong IR-absorption (Fig. 2, right panel) at characteristic wave number near 1725 cm\(^{-1}\) (C=O stretch), 1610 cm\(^{-1}\) (aromatic C=C stretch/asymmetric –COO\(^-\) stretch), and 1420 cm\(^{-1}\) (–OH deformations and C–O stretch of phenols or C–H deformation of CH\(_2\) or CH\(_3\) groups). An absorption peak occurred at 1270 cm\(^{-1}\) (C–OH stretch of phenolic –OH), and weak absorption in the range of 1050–1200 cm\(^{-1}\) (mostly aliphatic –OH typical for polysaccharides; Niemeyer et al., 1992). Absorbance was also relatively strong at about 1510 cm\(^{-1}\) which is typically ascribed to aromatic C=C or to CO of amide groups. The carboxylic-, aromatic-, and phenolic-rich nature of the DOM extract is apparent by comparing characteristic peak ratios of FTIR spectra (1725/1090, 1630/1090, 1420/1090 and 1510/1090) to bulk peat from the sampling sites (Supplementary Information, Table S1). Dissolved metal concentrations in the DOM extract decreased in the order Na (652 \(\mu\)mol L\(^{-1}\)), K (99.7 \(\mu\)mol L\(^{-1}\)), Ca (88.3 \(\mu\)mol L\(^{-1}\)), Mg (63.4 \(\mu\)mol L\(^{-1}\)), Al (52.6 \(\mu\)mol L\(^{-1}\)), Fe (43.0 \(\mu\)mol L\(^{-1}\)), Zn (3.37 \(\mu\)mol L\(^{-1}\)), Cu (0.209 \(\mu\)mol L\(^{-1}\)), Pb (0.152 \(\mu\)mol L\(^{-1}\)), Ni (0.088 \(\mu\)mol L\(^{-1}\)) and Co (0.014 \(\mu\)mol L\(^{-1}\)).

The concentration of dissolved sulfate in the oxidized DOM extract was 1616 \(\mu\)mol L\(^{-1}\), resulting in 538 \(\mu\)mol L\(^{-1}\) after addition to the incubation flasks, if zero background of sulfate in the pre-reduced peat is assumed. In the reduced DOM extract this concentration was 686 \(\mu\)mol L\(^{-1}\) resulting in 229 \(\mu\)mol L\(^{-1}\) in the incubation solute under the same assumptions. Acetate could only be detected in the reduced DOM solution at <20 \(\mu\)mol L\(^{-1}\).

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**Fig. 2.** Emission–excitation fluorescence spectrum (left) and FTIR spectrum (right) of the DOM extracts added in incubation experiments. For reference the FTIR spectra of total peat from bog and forest locations are shown as well.
3.2. Acetate and sulfate concentrations

Mean acetate concentration at the end of the incubation was 646 μmol L⁻¹ in treatment ‘control’. In the other treatments, final concentration increased in the order ‘sulfate’ (18 μmol L⁻¹) < ‘sulfide’ (275 μmol L⁻¹) < ‘oxidized DOM’ (438 μmol L⁻¹) < ‘reduced DOM’, which contained almost the same concentration (990 μmol L⁻¹) as the ‘DOM/O2’ treatment (1010 μmol L⁻¹) (Fig. 3). All treatments had a statistically significant influence with the exception of the treatment ‘oxidized DOM’ (Fig. 3; ANOVA, p < 0.05, see also Supplementary Information for details). Acetate concentrations in the ‘oxidized DOM’ at the start of the incubation experiment was 10.9 μmol L⁻¹ ± 2.7 μmol L⁻¹ (s.d., n = 4). Since identical DOM extract was added it can be excluded that differences in acetate concentrations at the end of the incubation were caused by acetate contained in the DOM extract.

In treatment ‘control’ mean sulfate concentration was initially below detection and around 7 μmol L⁻¹ at the end. Sulfate addition in treatment ‘sulfate’ strongly raised the mean concentration to an initial level of 559 μmol L⁻¹ from where it decreased to 4 μmol L⁻¹, whereas sulfide addition only raised concentration to 54 μmol L⁻¹, decreasing to 9 μmol L⁻¹. In treatment ‘oxidized DOM’ sulfate concentration was raised to 531 μmol L⁻¹, but subsequently only slightly consumed to a level of 503 μmol L⁻¹. Concentration in treatments ‘reduced DOM’ and ‘DOM/O2’ decreased from 148 μmol L⁻¹ to 25 μmol L⁻¹ and from 234 μmol L⁻¹ to 58 μmol L⁻¹, respectively (Fig. 3). Addition of DOM had a statistically significant influence on final sulfate concentrations (ANOVA, p < 0.05, see Supplementary Information for details).

3.3. CO₂ and CH₄ production

Production of CO₂ production was faster in the first couple of days. We thus differentiated between a ‘settling-down’ phase I (days 0–2), and a phase II (days 2–18) that we used to analyze the impact of the treatments (Figs. 4 and 5). In controls, the concentration of CO₂ increased to 1000 μmol L⁻¹ and of CH₄ to 1300 μmol L⁻¹ at day 18 (Fig. 4). The average production of CO₂ was 55 nmol cm⁻³ day⁻¹ and of CH₄ 116 nmol cm⁻³ day⁻¹ (Fig. 5). The control produced most CH₄ (Fig. 4) and had the smallest CO₂/CH₄ ratio, which decreased from 4.8 to 0.8 over time (Supplementary Information, Fig. S4).

Addition of sulfate increased CO₂ production significantly to 103 nmol cm⁻³ day⁻¹, twice the production of the control (p = 0.002; Fig. 5), and decreased CH₄ production (not significant) by 16% to 84 nmol cm⁻³ day⁻¹ (Fig. 5). The CO₂/CH₄ ratio decreased with time as well from 5.7 to 1.3 (Supplementary Information, Fig. S4). Addition of sulfide significantly inhibited CH₄ production compared to the control (Fig. 5) and enhanced CO₂ production to 75 nmol cm⁻³ day⁻¹ (Figs. 4 and 5), albeit not significantly (p > 0.05). The CO₂/CH₄ ratio in the sulfide treatment initially declined more strongly than in the sulfate treatment before both ratios converged (Supplementary Information, Fig. S4).

Addition of DOM generally diminished CO₂ and CH₄ production. In treatment ‘oxidized DOM’, CO₂ production significantly decreased to only 6 nmol cm⁻³ day⁻¹ and in ‘reduced DOM’ to 33 nmol cm⁻³ day⁻¹ (Fig. 5). After two days, the addition of oxidized and reduced forest extracts inhibited CH₄ production already by 65–75% (p = 0.000) followed by nearly complete inhibition at 0.1 nmol cm⁻³ day⁻¹ in the ‘oxidized DOM’ treatment (p = 0.000). In the treatment ‘reduced DOM’, methanogenesis slowed less from 23 to 16 nmol cm⁻³ day⁻¹, which was still significantly lower than in the ‘control’ (p = 0.000; Fig. 5). Addition

Fig. 3. Mean acetate concentrations (top) at the end of the incubation and mean sulfate concentrations (bottom) both prior to and at the end of the incubation. Concentrations are given in μmol L⁻¹. Note the different scale for concentrations. Error bars represent the standard deviation of four replicates. Treatments with no letters in common are significantly different at p < 0.05 (ANOVA).

Fig. 4. Mean concentration of CO₂ (top) and CH₄ (bottom) in μmol L⁻¹ against the incubation time in days of the different treatments. Error bars represent the standard deviation of four replicates. Distinguish between phase I: days 0–2 and phase II: days 4–18 or 4–19 for the treatment ‘DOM/O2’.

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of oxidized DOM even caused a slight loss of CH₄ from the incubation flask and a constant CO₂ concentration after 10 days (Fig. 4).

In the ‘DOM/O₂’ treatment CO₂ production significantly decreased to 35 nmol cm⁻³ peat d⁻¹, and CH₄ production to 7 nmol cm⁻³ peat d⁻¹ (p = 0.000; Fig. 5).

3.4. Sulfate reduction

Sulfate was on average reduced at a rate of 102 nmol cm⁻³ peat d⁻¹ in controls and 73 cm⁻³ peat d⁻¹ following sulfate addition (Kruskal Wallis, p > 0.1; Fig. 6). Sulfate reduction was fastest after sulfide addition with a maximum rate of 118 nmol cm⁻³ peat d⁻¹, but this difference was not statistically significant either (Kruskal Wallis, p > 0.1). Compared to the ‘control’ incubation, DOM addition slowed sulfate reduction to 1.7 nmol cm⁻³ peat d⁻¹ (oxidized DOM, p < 0.1) and 0.4 nmol cm⁻³ peat d⁻¹ (reduced DOM, p < 0.05). In treatments ‘control’, ‘sulfate’ and ‘sulfide’ 71–92% of the tracer were recovered in the TRIS pool of the peat (Fig. 6). This transfer within 1.5 h of labeling corresponds to a sulfate turnover frequency of 11–15 times per day. In the DOM treatments, the fraction of tracer recovered as TRIS was only <0.5%, which implies sulfate turnover times of several days to weeks.

4. Discussion

4.1. Effects of sulfur addition

We examined the hypothesis that internal sulfur cycling maintains high rates of activity of SRB and thus leads to suppression of methanogenesis (Blodau et al., 2007) by adding Na₂SO₄ and Na₂S to incubation flasks. The CO₂/CH₄ production ratio of the control was 0.8 after 18 d (Fig. 5) and similar to the ratio of 0.7 of an untreated control of a wetland soil at the end of a 7 d incubation by Keller et al. (2009). CO₂/CH₄ production ratios <1 imply methanogenic conditions and a depletion of electron acceptors, which was a prerequisite for identifying effects of electron acceptor addition on methanogenesis. It has to be noted that this conclusion is not in agreement with a BSR rate of 102 nmol cm⁻³ peat d⁻¹, thus almost reaching the average CH₄ production of 116 nmol cm⁻³ peat d⁻¹. BSR was thus faster in terms of electron flow equivalents and potential CO₂ production than methanogenesis (Figs. 5 and 6). Even if it is assumed that methane was exclusively produced by the hydrogenotrophic pathway, i.e. by CO₂ consumption, BSR would have produced 204 nmol cm⁻³ peat d⁻¹ CO₂, which compares to a maximum CO₂ production of 171 nmol cm⁻³ peat d⁻¹ from combined CO₂ and CH₄ release. It is thus likely that BSR was overestimated by the ³⁵S radiotracer method applied or that additional sinks for CO₂, such as homoacetogenesis, existed (Conrad, 1999). Regardless of this inconsistency, methane production was substantial enough to identify the effects of the treatments on microbial processes.

Mean sulfate and sulfide concentration in the Na₂SO₄ and Na₂S treatments was adjusted to 500 μmol L⁻¹ and 475 μmol L⁻¹, respectively, which is within the range of sulfate saturation of 300–3000 μmol L⁻¹ reported for SRB (Odom and Singleton, 1993). Such concentrations are higher than typically measured in situ at the Mer Bleue bog (Blodau et al., 2007; Goldhammer et al., 2008), but not unrealistically large, as concentrations in oligotrophic peatlands can temporarily increase to such levels (Shannon and White, 1996). Although, at the end of the incubation sulfate concentrations in treatments ‘control’, ‘sulfate’ and ‘sulfide’ were small, ranging from 4 to 9 μmol L⁻¹ (Fig. 3), bacterial sulfate reduction (BSR) was active, indicating an ongoing internal supply of sulfate. This is in agreement with other studies by Vile et al. (2003) and Blodau et al. (2007).

The results of both treatments were mostly in agreement with our hypothesis. The addition of sulfate did not induce significantly higher sulfate reduction rates compared to the control at the end of the incubation period, as we had expected (Fig. 6). Likely this finding was due to the small sulfate concentration of 4 μmol L⁻¹ remaining at this time (Fig. 3). Since the sulfate concentration in the ‘sulfate’ treatment was initially much higher at 559 μmol L⁻¹ than the control (Fig. 3), we assume that sulfate was initially also reduced much faster. The assumption is supported by the depletion of acetate in the treatment (Fig. 3), which may, at 18 μmol L⁻¹, have entailed a kinetic limitation of sulfate reducers by electron donor concentration towards the end of the incubation. These considerations are in agreement with a moderate overall decrease in CH₄ production by 16%. BSR thus partly inhibited methanogenesis in the peat, as reported in many previous studies employing different approaches (Yavitt et al., 1987; Shannon and White, 1996; Gauci et al., 2002; Vile et al., 2003). In addition, CO₂ was produced at 103 nmol cm⁻³ peat d⁻¹, twice the rate as in the control (Fig. 5),
suggested that utilization of sulfate can accelerate anaerobic CO₂ production in principle, as proposed earlier (Wieder et al., 1990; Vile et al., 2003).

The addition of sulfide raised sulfate reduction rates to 118 nmol cm⁻³ peat d⁻¹, which was the highest level recorded overall and slightly higher, yet not significant at the 90% significance level, than in the control (Fig. 6). This increase occurred despite the fact that the final sulfate concentration of 9 μmol L⁻¹ was not much greater than in the ‘sulfate’ treatment (Fig. 3). It has to be considered, however, that the concentration of acetate remained higher in this treatment by the end of the experiment, which may have assisted in maintaining high sulfate reducing activity (Fig. 3). In line with the results of the sulfate addition, in this treatment CH₄ production significantly decreased compared to the control (Fig. 5), and the ratio of sulfate reduction to CH₄ production even reversed from <1 in the control to 1.5. We estimate that sulfate must have been recycled frequently, i.e. 11 times a day in this treatment, to maintain the sulfate reduction rate measured with the ³⁵S tracer. This finding suggest that internal recycling of sulfide, most likely by oxidation of sulfide to thiosulfate or sulfate, occurred and that it has the potential to reduce CH₄ production. The observation has been made before (Wieder and Lang, 1988; Vile et al., 2003) and is in agreement with ³²S data obtained along solute flow paths in mechanisms experiments with these peats (Blodau et al., 2007). The only electron acceptor potentially available for the anaerobic oxidation process in these peats we are aware of is humic substances, whose reduction by H₂S we have previously demonstrated (Heitmann and Blodau, 2006). Based on the obtained data and our current lack of knowledge about the role of solid-phase humic substances (Heitmann et al., 2007) we cannot assess if capacity and kinetics of organic electron acceptors would suffice for maintaining BSR. If humic substances fueled sulfur recycling it is also an open question why their effect would differ so strongly from that of the added humic-rich DOM extract.

Apart from a reoxidation of sulfide, the sulfate pool may have been to some degree replenished by sulfonates and sulfate esters, which can hydrolyze under sulfate-poor conditions (Kertész, 2000; Mandernack et al., 2000). The decrease in sulfate concentration over time suggests that the reduced products of sulfate reduction were not fully reoxidized and that S was removed over time from internal cycling, which is in agreement with earlier results obtained in these peats (Blodau et al., 2007). It is known that H₂S can be incorporated into humic substances (Heitmann and Blodau, 2006), for example by Michael’s addition reaction with quinone moieties (Perlinger et al., 2002), and produce stable carbon-bonded sulfur (CBS) in peats with a residence time on the order of years to decades (Fig. 1) (Wieder and Lang, 1988).

4.2. Effects of DOM addition

The added DOM extract was characterized by spectroscopic properties and metal contents that are typical for bog waters and peat humic substances. The fluorescence pattern has been linked to polycondensation and large relative quantities of conjugated aromatic π-electron systems (Chen et al., 2003). The low fluorescence index of 1.26 is characteristic for strongly humic terrestrial DOM (McKnight et al., 2001), and falls in the range reported from Mer Bleue bog pore water by Fraser et al. (2001). The carboxylic-, aromatic-, and phenolic-rich nature of the DOM extract is evident when compared to FTIR spectra of bulk peat from the sampling sites (Fig. 2) and when characteristic peak ratios of these organic matter pools are compared (Supplementary Information, Table S1). The chemical nature of the DOM was not unusual for bog peats; the obtained peak ratios are similar to those reported for DOM that was sampled in Mer Bleue bog pore water in depths of 75 cm and in peat leachates obtained from depths of 200 cm (Beer et al., 2008). The metal and trace metal concentrations also resembled those reported from pore water of the bog (Beer et al., 2008), with concentrations of Co, Zn, and Al being in the low range. Based on the inconspicuous chemistry of the DOM extract we did not expect surprising effects of the DOM addition.

Contrary to our expectation, addition of DOM, in combination with the sulfate contained in the extract, had a detrimental effect on all aspects of anaerobic respiration, particularly when added in the oxidized state. Methanogenesis decreased, as predicted, from 116 nmol cm⁻³ peat d⁻¹ (control) to 0.1 nmol cm⁻³ peat d⁻¹ (‘oxidized DOM’) (Fig. 6). Unfortunately, we cannot affirm that this inhibition of methanogenesis was caused by consumption of electron acceptors contained in humic substances, because a similar, albeit weaker, decrease to 16 nmol cm⁻³ peat d⁻¹ also occurred when we added reduced DOM (Fig. 6). Furthermore, sulfate reduction rates diminished in the ‘oxidized DOM’ treatment by a factor of 60–1.7 nmol cm⁻³ peat d⁻¹ as well (Fig. 6), sulfate concentrations remained almost stable (Fig. 3), and CO₂ production decreased by a similar factor to 6 nmol cm⁻³ peat d⁻¹ compared to the control (Fig. 5). As both acetate and sulfate remained available for sulfate reduction (Fig. 3), we must conclude that methanogenesis, BSR and possibly other terminal respiration processes were suppressed by the ‘oxidized DOM’ treatment, probably due to a toxic effect on bacteria and archaea. These effects furthermore did not occur with sulfate addition alone (Figs. 3, 5 and 6) and, for this reason, cannot be attributed to the sulfate contained in the DOM extracts.

The basic pattern was similar with respect to the ‘reduced DOM’ and DOM/O₂ treatments. Final sulfate concentrations of 25 and 58 μmol L⁻¹, respectively, remained higher than in the control (Fig. 3) and sulfate reduction rates were only 0.4 and 3.5 nmol cm⁻³ peat d⁻¹ (Fig. 6). Acetate accumulated to concentrations of 990 and 1010 μmol L⁻¹, respectively, which suggests that acetogenesis was not affected to the same degree (Fig. 3). The accumulation of acetate occurred in synchrony with low CO₂ production rates (Figs. 3 and 5), yet was not significant at the 95% level. As to be expected, we recorded the strongest decrease in CH₄ production in the treatment ‘DOM/O₂’ and the highest initial CO₂/CH₄ ratio of 91.2 (Supplementary Information, Fig. S4). The inhibitory effect of the added DOM on CH₄ production was thus compounded by the toxic effect of oxygen on the obligate anaerobic archaea (e.g. Hao et al., 1996).

With regard to possible explanations for these surprising results a toxic effect of acetic acid on methanogens at low pH, such as described by Russell (1991) and Bergman et al. (1998), can be ruled out because acetate concentrations were also high in controls (Fig. 3). Other possible causes include toxicity of heavy metals with regard to SRB and methanogens (e.g. Poulsen et al., 1997; Ram et al., 2000; Karri et al., 2006). Trace metals are bioavailable in simple ionic form, weak complexes, or as a lipid soluble species, which could have been associated with the added fulvic and humic acids, along with organic compounds derived from microbial and plant activities (e.g. Hao et al., 1996; Campbell et al., 2000; Li and Fang, 2007). We do not believe that a toxic effect of this kind occurred because measured concentrations of heavy metals, contained in the DOM extract, remained far beneath toxic concentration levels to SRB and to methanogens (e.g. Bhattacharya et al., 1995; Poulsen et al., 1997; Ram et al., 2000; Utljar et al., 2003; Karri et al., 2006; Alts, 2009). Alternatively, a limitation by micronutrients may also affect methanogenesis in peatland soils. In particular, all methanogens require nickel, cobalt, and iron for growth, and some have a high requirement for sodium (Kortylewski and Yavitt, 1992). Information about micro-nutrient requirements of SRB does not seem available; it has only been reported that a dependence of hydrogenase synthesis on nickel and iron ions existed for the enzymes of SRB and methanogens.
(Schneider et al., 1984). Hydrogenase synthesis is, however, also required for acetogenesis, which appeared to be less or not affected by the treatments ‘reduced DOM’ and ‘DOM/O2’ (Fig. 3) (Madigan et al., 2003, pp. 690–692).

The results are the more puzzling because we have previously recorded substantial activity of SRB and methanogenesis at DOC concentrations between 80 and 90 mg L−1 at a depth of 60 cm at the site of peat extraction, and also at the site where material for the incubations was sampled (Minderlein and Blodau, 2008, and Supporting Information, Fig. S2). Obviously, specific chemical properties of the DOM extract that are difficult to capture must have played a decisive role rather than concentrations of humics in these DOM-rich peat soils. A strong inhibition of methanogenesis by moderate concentrations of humic-rich DOM has rarely been reported and would be of significance for CH4 production and emission. Keller et al. (2009) observed a near complete inhibition of CH4 production along with a relatively minor stimulation of CO2 production, which is much larger than expected from mass balancing of concentrations between 80 and 90 mg L−1. The study confirms that anaerobic sulfur cycling assists in maintaining high rates of activity of SRB and can assist in the suppression of methanogenesis by BSR. To our knowledge this is the first time that a stimulation of sulfate reduction and suppression of methanogenesis by sulfide addition has been reported. Such results provide mechanistic support for the detrimental effect of sulfate deposition on CH4 emissions from peatlands, which is much larger than expected from mass balancing of sulfate input and CH4 emissions (Gauci et al., 2004). Furthermore, our results are in partly agreement with Cervantes et al.’s (2008) observation that DOM extracts rich in humic substances can have a toxic effect on methanogenic archaea and, in addition, on SRB. As yet, it is not clear what might cause such a toxic effect on anaerobic microorganisms and further investigations are necessary to elucidate it. Regardless of the reasons for this effect it is obvious that changes in DOM concentrations and quality potentially impact methanogenic activity in peat soils, and thus the greenhouse gas balance of peatlands. This is consistent with the study of Keller et al. (2009) who suggested that solid-phase humic acids in wetland soils can play a significant role in anaerobic decomposition, with important implications for wetland carbon cycling.

5. Conclusions

The study confirms that anaerobic sulfur cycling assists in maintaining high rates of activity of SRB in peat soils and can assist in the suppression of methanogenesis by BSR. To our knowledge this is the first time that a stimulation of sulfate reduction and suppression of methanogenesis by sulfide addition has been reported. Results provide mechanistic support for the detrimental effect of sulfate deposition on CH4 emissions from peatlands, which is much larger than expected from mass balancing of sulfate input and CH4 emissions (Gauci et al., 2004). Furthermore, our results are in partly agreement with Cervantes et al.’s (2008) observation that DOM extracts rich in humic substances can have a toxic effect on methanogenic archaea and, in addition, on SRB. As yet, it is not clear what might cause such a toxic effect on anaerobic microorganisms and further investigations are necessary to elucidate it. Regardless of the reasons for this effect it is obvious that changes in DOM concentrations and quality potentially impact methanogenic activity in peat soils, and thus the greenhouse gas balance of peatlands. This is consistent with the study of Keller et al. (2009) who suggested that solid-phase humic acids in wetland soils can play a significant role in anaerobic decomposition, with important implications for wetland carbon cycling.


