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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 CO_2 and CH_4 production quantified, and results analyzed by ANOVA. DOM was characterized by Fourier transformed infrared and fluorescence spectroscopy and analyzed for trace elements. CH_4 production (116 nmol cm^{-3} d^{-1}) and BSR rate (102 nmol cm^{-3} d^{-1}) were similar in 'controls'. BSR in treatments 'sulfate' (73 nmol cm^{-3} d^{-1}) and 'sulfide' (118 nmol cm^{-3} d^{-1}) did not significantly differ from 'controls' but addition of DOM significantly diminished BSR down to 0.4 nmol cm^{-3} d^{-1} (Kruskal Wallis test, $p < 0.05$). CH_4 production decreased with sulfate (16%, not significant) and sulfide addition (40%, $p < 0.05$) and CO_2 production increased (treatment 'sulfate', $p < 0.05$). Addition of all DOM extracts (67 mg L^{-1}) almost completely suppressed methanogenesis and CO_2 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 *archaea*.

1. Introduction

Owing to the importance of northern peatlands for the future of global atmospheric CO_2 and CH_4 budgets (e.g. Limpens et al., 2008), controls on the production, consumption and emission of the involved gases warrant further study. The production of CO_2 and CH_4 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 Bridgham, 2007; Knorr and Blodau, 2009). Anaerobic decomposition of peat to CO_2 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 (H_2) and CO_2, which in turn serve as substrates for methanogenic *archaea*. Methanogenesis provides little free energy; for this reason bacteria utilizing electron acceptors other than CO_2 usually outcompete methanogens for H_2 by a thermodynamic exclusion mechanism, i.e.

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by lowering substrate concentrations to levels that provide insufficient free energy for methanogenic metabolism (Conrad, 1999). Indeed, much lower production of CH_4 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. Steinmann 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 (H_2S) to thiosulfate, which is subsequently reduced by sulfate reducing bacteria (SRB) to H_2S, or resupplies the sulfate pool after a microbial disproportionation to H_2S 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 CH_4 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 CH_4 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.

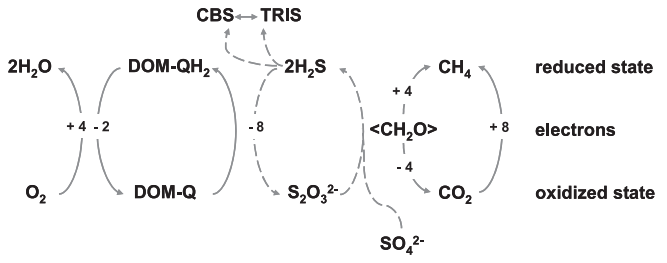

Fig. 1. Conceptual model of anaerobic carbon and sulfur transformations involving an organic quinone moiety DOM-Q (reduced form DOM-QH_2), modified after Heitmann et al. (2007). The reduction of DOM may either be directly coupled to the heterotrophic respiration of substrate, or indirectly (optional, dashed cycle) via a partial reoxidation of sulfide to thiosulfate. TRIS signifies total reduced inorganic sulfur and CBS stands for carbon-bonded sulfur.

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 CH_4 production, and the addition of reduced DOM to have no effect on BSR and CH_4 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 km^2, 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 myrtilloides*).

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 populifolia*. 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 CH_4 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.

(I) 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