Electron Transfer Processes between Hydrogen Sulfide and Humic Substances - Implications for Anaerobic Sulfur Cycling in Freshwater Ecosystems

Dissertation zur Erlangung des Grades
Doktor der Naturwissenschaften
(Dr. rer. nat.)
an der Fakultät Biologie/Chemie/Geowissenschaften
der Universität Bayreuth

vorgelegt von

Zhiguo Yu

Geb. am 25 Dezember 1982 in Jilin, China

Die vorliegende Dissertation wurde im Zeitraum von April 2011 bis April 2015 unter der Betreuung von Dr. Klaus-Holger Knorr an der Limnologischen Forschungsstation des Lehrstuhls für Hydrologie (Prof. Dr. Stefan Peiffer) der Universität Bayreuth angefertigt und Hydrologie Gruppe (Prof. Dr. Christian Blodau) der Universität Münster.

Vollständiger Abdruck der von der Fakultät für Chemie/Biologie/Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.).

Die Arbeiten im Rahmen dieser Dissertation wurden durch die Deutsche Forschungsgemeinschaft (DFG) gefördert im Rahmen des Projektes KN 929/2-1, einem Teilprojekt innerhalb der DFG Forschergruppe FOR 580.

Prüfungsausschuss:

PD. Dr. Werner Borken (Vorsitz)

Dr. Klaus-Holger Knorr (erster Gutachter)

Prof. Dr. Britta Planer-Friedrich (zweiter Gutachter)

Prof. Dr. Stefan Peiffer

Tag der Einreichung: 20.05.2015

Tag des wissenschaftlichen Kolloquiums: 20.07.2015

This dissertation is lovingly dedicated to my mother!

望著大河彎彎, 終於敢放膽嘻皮笑臉面對人生的難!

Difficulties in your life don't come to destroy you, but to help you realize your hidden potential.

Acknowledgements

To the end, I would like to take this opportunity to express my gratitude to a few people for their support and help during my PhD study. Sincere thanks go to Klaus-Holger Knorr for the supervision and advice during all phases of my PhD study and as well as guiding me enjoy the life in Germany!

I would like to thank those who provided scientific and technical support both in Hydrology group of Bayreuth and Münster. Though this thesis is done by me, the result would never have been the same if without the support, encouragement and assistance from the following people, thanks a million to:

Bayreuth group: Stefan Peiffer, Bettina Kuppinger, Daniela Braun, Sarah Hoffman Jutta Eckert, Silke Hammer, Martina Rohr, Heidi Zier, Bing Xia, Ruiwen Yan, Moli Wan, Kiyong Kim, Jean-lionel Payeur-Poirier, Sabine Thüns, Markus Bauer, Julia Beer, Cristian Estop, Zajac Kasia, Sven Free, Markus Schmitt, Sindy Wagner, Carolin Waldemer.

Münster group: Chritain Blodau, Andreas Malkus, Ulrike Berning-Mader, Madeleine Supper, Ines Spangenberg, Simona Bonaiuti, Sina Berger, Svenja Agethen, Frederik Bock, Peter Raabe, Yuanqiao Wu.

I would like to acknowledge German Research Foundation for the financial surpport of my PhD study and the Synchrotron Light Source ANKA for providing us with the instrumentation at their beamlines.

Special thank goes to my dear friends for bringing me a wonderful life in Germany: Jian Zhang, Kun Guo, Bo Zhang, Dong Li, Tao Li, Peng Zhao, Lei Han, Qinghai Song, Zhicheng Zheng, Yifan Chen, Zhuying Cao, Ho Nguyen, Degefie Tibebe, Malte Julian Deventer, Dirk Trompeter.

I would like to dedicate this thesis to my mother for her selfless love to me!

I especially thank my young brother for all his support during my life!

My deepest thank goes to my girlfriend Lin for all her love, support and patience even when we apart from each other over a million miles of distance between westernmost point and easternmost point of Eurasia!

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Bacterial sulfate reduction	BSR
Carbon	C
Carbon dioxide	CO_2
Chemically (H_2/Pd) reduced HA	H_2-RHA
Direct electrochemical reduction	DER
Dissolved organic carbon	DOC
Dissolved organic matter	DOM
Electrochemically reduced HA	EC-RHA
Electron accepting capacities	EAC
Electron donating capacities	EDC
Electron transfer capacities	ETC
Elemental sulfur	S^0
Energy-dispersive X-ray fluorescence spectrometry	XRF spectrometry
Equivalent	e^-
Ferric iron	Fe^{3+}
Ferrous iron	Fe^{2+}
High performance liquid chromatography	HPLC
Humic substances	HS
Hydrogen	H_2
Hydrogen sulfide	H_2S
Mediated Electrochemical Oxidation	MEO
Mediated Electrochemical Reduction	MER
Methane	CH_4
Nitrate	NO_3^-
Non-reduced humic acid	NR-HA
Organic matter	OM
Organic sulfur	S_{org}
Palladium	Pd
Potential	Eh
Reduced humic acid	RHA
Sigma Aldrich humic acid	HA
Sulfate	SO_4^{2-}
Sulfur	S
Thiosulfate	$S_2O_3^{2-}$
Total sulfide	Sulfide
Working electrode	WE
X-ray absorption near edge structure (XANES) spectroscopy	XANES spectroscopy
Zinc	Zn^0

LIST OF FIGURES

LIST OF TABLES

SUMMARY

The relevance of biogeochemical gradients for turnover of organic matter is yet poorly understood. This study aims at the identification and quantification of the interaction of different redox processes along gradients with particular emphasis on the impact of redox active humic substances. The interactions between sulfide, sulfate (SO_4^{2-}) reduction, methanogenesis, and organic matter (OM) redox processes were investigated in controlled abiotic and biotic incubation experiments.

In the first study, we investigated the abiotic transformation of sulfide upon reaction with reduced and non-reduced Sigma Aldrich humic acid (HA), under anoxic conditions. Sulfide reacted with non-reduced HA at rates comparable to sulfide oxidation by iron oxides or molecular oxygen. The main transformation products were elemental S (S^0), and thiosulfate ($S_2O_3^{2-}$), yielding electron accepting capacities (EACs) of 2.82~1.75 μmol e^- (mg C) $^{-1}$. Native iron contents in the HA explained only 6~9% of these EACs. Another important fraction of the reaction of sulfide with HA was organic S (S_{org}). For HA reduced by hydrogen (H_2) on a Palladium (Pd) catalyst, even only a formation of S_{org} was observed and no inorganic transformation products occurred. X-ray absorption near edge structure (XANES) spectroscopy supported S_{org} to be mainly about zerovalent, such as thiols, organic di- and polysulfides, or heterocycles.

In a second study, we addressed the impact of electrochemical and wet chemical (hydrogen (H_2)/Pd-catalyst) reduction of Sigma Aldrich humic acid (HA) on its reactivity towards sulfide. Moreover, we tested the impact of HA reaction with sulfide on electron transfer capacities (ETC) as detected by mediated electrochemical reduction and oxidation. The reactivity of HA towards sulfide was clearly related to the initial redox state of HA, as measured initial values of EAC of HA had a strong and positive correlation with the amount of transformed sulfide. H_2/Pd treatment of HA obviously changed HA structures and lead to a different reactivity towards sulfide, limiting a direct comparison to electrochemically reduced organic matter.

In a third study, we incubated peat samples virtually devoid of inorganic electron acceptors under anoxic conditions and monitored CO_2 and CH_4 production to estimate

EAC from organic matter. From excess CO_2 production, i.e. from CO_2:CH_4 ratios of 3.2:1, we calculated an EAC of OM of 2.36 µmol e^- cm^{-3} d^{-1}. Addition of sulfate (SO_4^{2-}) increased CO_2 production and suppressed CH_4 production as expected. However, after subtracting the EAC provided though SO_4^{2-} (0.97~2.81 µmol e^- cm^{-3} d^{-1}), OM provided even higher EAC of 3.88 to 4.85 µmol e^- cm^{-3} d^{-1}.The contribution of organic sulfur was again evaluated by sulfur K-edge XANES and using $\delta^{34}S$ natural abundance as a tracer. Bacterial sulfate reduction (BSR) presumably involved a re-oxidation of sulfide by organic matter as proposed earlier, but also a sulfurization of OM yielding reduced organic sulfur, and changes in oxidized organic sulfur species.

In conclusion, our results quantitatively demonstrated both HA and reduced HA can abiotically re-oxidize sulfide in anoxic environments at rates competitive to sulfide oxidation by molecular oxygen or iron oxides. H_2/Pd pre-treatment of HA alters redox properties and reactivity of organic matter and may therefore lead to biased results when being employed in experimental approaches. Peat incubation experiment confirmed that organic matter contributes to anaerobic respiration i) directly by EAC of redox active functional groups ii) directly by provision of EAC from oxidized organic sulfur and iii) indirectly by re-oxidation of sulfide to maintain BSR. Overall, our results indicated the importance of anaerobic sulfur cycling through organic matter and identified limitations of common approaches addressing redox properties of organic matter solely by H_2/Pd reduction or electrochemical approaches.

ZUSAMMENFASSUNG

Die Bedeutung biogeochemischer Gradienten für den Umsatz von organischer Substanz ist noch wenig untersucht. Ziel dieser Arbeit ist deshalb die Identifikation und Quantifizierung der Interaktion verschiedener Redoxprozesse entlang von Gradienten mit besondererm Fokus auf redoxaktive Huminstoffe. Hierfür wurden die Interaktionen zwischen Sulfid, bakterieller Sulfatreduktion (SO_4^{2-}), Methanogenese sowie die Redoxreaktionen von organischer Substanz in kontrollierten nasschemischen Versuchen und Inkubationsexperimenten untersucht.

In einer ersten Studie wurde die abiotische Transformation von Sulfid bei Reaktion mit reduzierter und nicht reduzierter Sigma Aldrich Huminsäure (HS) unter anoxischen Bedingungen untersucht. Die Geschwindigkeitskonstante der Reaktion von Sulfid mit nicht reduzierter Huminsäure lag dabei im Bereich der Raten der Oxidation von Sulfid mit Eisenoxiden oder molekularem Sauerstoff. Die Hauptreaktionsprodukte waren dabei elementarer Schwefel (S^0) und Thiosulfat ($S_2O_3^{2-}$). Diese Reaktionsprodukte entsprachen einer Elektronenakzeptorkapazität (EAK) von 2.82~1.75 µmol e^- (mg C) $^{-1}$. Hierbei konnte das in der HS enthaltene Eisen nur 6~9% der EAK erklären. Ein grosser Teil des Schwefels reagierte zu organischem Schwefel (S_{org}). Für mit Wasserstoff (H_2) auf Palladium (Pd) -Katalysator reduzierte HS wurde dabei ausschliesslich die Bildung von S_{org} und keine Umwandlung zu anorganischem Schwefel beobachtet. Mit synchrotronbasierter Röntgen Spektroskopie (X-ray absorption near edge structure – XANES) wurde bestätigt, dass gebildeter S_{org} fast ausschließlich nullwertig vorlag, wie etwa in Thiolen, organischen Di- und Polysulfiden oder Heterozyklen..

In einer zweiten Studie wurde der Einfluss von elektrochemischer und nasschemischer Reduktion (Wasserstoff (H_2)/Pd-Katalysator) von Sigma-Aldrich HS auf die Reaktion von HS und Sulfid untersucht. Darüber hinaus wurde der Einfluss der Reaktion von HS mit Sulfid auf die Elektronentransferkapazität (ETK) durch mediierte elektrochemische Reduktion und Oxidation ermittelt. Hierbei wurde ermittelt, dass die Reaktivität von HS mit Sulfiden mit zunehmendem Reduktionsgrad abnahm. Entsprechend wurde festgestellt, dass die ursprünglich ermittelte EAK der HS stark positiv mit der Menge an gebildetem Sulfid korrelierte. Reduktion der HS mittels H_2/Pd führte zu deutlichen Veränderungen der Huminsäurestrukturen und modifizierte die Reaktivität mit Sulfiden, was den direkten Vergleich mit elektrochemisch reduzierten HS einschränkte.

In einer dritten Studie wurden schliesslich Torfproben praktisch frei von anorganischen Elektronenakzeptoren unter anoxischen Bedingungen inkubiert, um die EAK des Torfes aus einer Elektronen-Bilanz zu berechnen. Hierbei ergab sich aus einer Produktion von CO_2 und CH_4 im Verhaeltnis 3.2:1 eine EAK der organischen Substanz von 2.36 µmol e^- cm^{-3} d^{-1}. Unter Sulfat-Zugabe erhöhte sich die CO_2 Produktion signifikant, wohingegen die CH_4 Produktion effektiv unterdrückt wurde. Abzüglich der EAK, die durch die Sulfat-Zugabe entstanden ist (0.97~2.81 µmol e^- cm^{-3} d^{-1}) war die verfügbare EAK der organischen Substanz mit 3.88 bis 4.85 µmol e^- cm^{-3} d^{-1} immer noch höher. Der Beitrag von organischem Schwefel wurde weiterführend mit XANES Spektroskopie und $\delta^{34}S$ (natürliche Häufigkeit) als Tracer untersucht. Die Ergebnisse legen nahe dass es bei bakterieller Sulfatreduktion (BSR) sowohl zur Reoxidation von Sulfid durch organische Substanz kommt, wie vorher vermutet, als auch zur Sulfurierung der organischen Substanz mit der Bildung von reduzierten organischen Schwefelgruppen und Veränderungen von oxidierten organischen Schwefelgruppen.

Zusammenfassend zeigten unsere Ergebnisse, dass sowohl nicht reduzierte HS als auch reduzierte HS abiotisch Sulfid unter anoxischen Bedingungen oxidieren können. Im Gegensatz zur elektrochemischen Reduktion führte H_2/Pd Reduktion von HS zu Veränderungen der Redoxeigenschaften und der Reaktivität von organischer Substanz. Dieser Effekt kann zu abweichenden Ergebnissen führen, wenn diese Behandlung in experimentellen Studien eingesetzt wird. Torfinkubationsstudien bestätigenden, dass organische Substanz zur anaeroben Respiration beiträgt, und zwar i) direkt, durch die EAK von redoxaktiven funktionellen Gruppen, ii) direkt über die Oxidation von S_{org}, oder iii) indirekt durch Recycling von Sulfid zur Aufrechterhaltung der BSR. Insgesamt betonen unsere Ergebnisse die bedeutende Rolle des anaeroben Schwefelkreislaufes für die Redoxeigenschaften der organischen Substanz. Wir zeigten die Limitierungen von gebräuchlichen Methoden zur Quantifizierung von Redoxeigenschaften organischer Substanz in sulfidischen Systemen, die lediglich auf H_2/Pd Reduktion oder elektrochemischen Ansätzen beruhen.

INTRODUCTION

1. Humic substances

Humic substances are a heterogeneous mixture of refractory organic macromolecules mainly originating from higher plant and microbial precursors. HS is composed of humic acid, fulvic acids and humin, while humic acid and fulvic acid represent a large portion of the DOM pool in soils, in fresh water and marine systems (Aeschbacher et al., 2010; Bauer et al., 2007; Cory and McKnight, 2005; Lovley et al., 1996; Peretyazhko and Sposito, 2006; Ratasuk and Nanny, 2007).

Concentrations of DOM are commonly in the range of miligram C per liter in fresh water system, sometimes could reach at a peak concentration of ~100 mg C L^{-1} in saturated soil solution or lake sediment (Aiken et al., 1985; Grieve, 1984; Hessen and Tranvik, 1998). DOM, as active reservoirs of organic carbon, could play important role for driving biogeochemical cycles of element in freshwater (Cory and McKnight, 2005), e.g. DOM act as redox mediator for biogeochemical redox reactions in environments with changing redox conditions, like water table fluctuations (Klüpfel et al., 2014). Previous studies found that DOM can function as recyclable electron shuttles between bacteria and Fe(III) minerals or facilitate reduction of organic pollutants (Kappler et al., 2004; Klüpfel et al., 2014; Wolf et al., 2009). However, key redox properties of DOM for biogeochemical processes remain unclear, e.g. the reversibility of electron transfer to and from HS, the function of serving as electron shuttle for facilitating electron transfer, which are important for understanding the mechanism of driving elements cycling or re-cycling in aquatic ecosystem (Nurmi and Tratnyek, 2002; Ratasuk and Nanny, 2007; Uchimiya and Stone, 2009).

2. Quinones moieties of humic substances

HS usually include a skeleton of alkyl and aromatic units with diverse functional groups, among them oxygen functional groups are considered as primary source of reactivity in humic acid (Struyk and Sposito, 2001; Sutton and Sposito, 2005). Quinones, as most important redox-active moieties, which at least partly involve in redox process of redox-active elements transformation (Aeschbacher et al., 2010; Ratasuk and Nanny, 2007; Uchimiya and Stone, 2009). It have been shown to be ubiquitous in DOM (Cory and McKnight, 2005) and known that they reversibly transfer electrons, and thus involve in both abiotic and biotic redox processes in anoxic environments (Aeschbacher et al., 2011, 2010; Bauer et al., 2007; Heitmann and Blodau, 2006; Heitmann et al., 2007; Klüpfel et al., 2014). For instance, DOM serving as electron acceptor to oxidize inorganic electron donors (Heitmann and Blodau, 2006) or reduced DOM can transfer electron to poorly accessible iron oxides (Kappler et al., 2004).

Although previous study observed quinone content account for a significant fraction of redox behavior of HS, they are not sufficiently explain the overall measured amounts of electron transfer (Bauer et al., 2007; Sutton and Sposito, 2005). Thus, other non-quinone moieties are likely involved in redox processes of biogeochemical cycles of elements (Struyk and Sposito, 2001), e.g. carboxylic acid, phenolic hydroxyl (Stevenson, 1994).

3. Determination of electron transfer capacities of humic substances

While the knowledge of the redox properties of humic substances is growing (Macalady and Walton-day, 2011; Ratasuk and Nanny, 2007; Uchimiya and Stone,

2009), it is necessary to quantitatively understand the role of HS for biogeochemical process and to set up an precise electron budget of reactions with HS. Thus, the determination of the redox properties of HS is essential, including the reversibility of electron transfer to and from HS and their electron acceptor and donor capacities, which is the moles of electrons that can be transferred to or withdrawn from HS at a given Eh (Aeschbacher et al., 2010).

Presently, methodologies for detecting redox properties of HS were well documented with variation of experimental procedures, e.g. electrochemical method, microbial assays and a diversity of chemical reactions (Bauer et al., 2007; Blodau et al., 2009; Kappler et al., 2004). Commonly, H_2S, Zn^0 and H_2 were employed as reductants (Blodau et al., 2009; Heitmann and Blodau, 2006) and their oxidation products or the H_2 consumption were analyzed for EAC calculation. In addition, indirect way of evaluating EAC was also developed accordingly, EAC value was, thus, obtained by measuring the difference in EDCs of a microbially, chemically or electrochemically pre-reduced HS and non-reduced HS (Bauer et al., 2007; Jiang and Kappler, 2008). Experimental approaches for determination of EDCs was normally made by detecting the amount of transformed Fe^{2+} from Fe^{3+} reduction by HS, e.g. Fe^{3+}-citrate or $[Fe(CN)^6]^{3-}$ (Bauer et al., 2007).

Although existing methods were frequently employed for evaluating HS redox properties, certain deficiencies of these methods appeared, e.g. error of indirect way of determination oxidation products or error due to long equilibrium time of the reaction, could led to overestimate or underestimate the measured results. Since redox properties of HS have not been sufficiently addressed with previous methods, new methodology is needed for overcome the existing limitation(Aeschbacher et al., 2010; Bauer et al., 2007; Heitmann and Blodau, 2006). Recently, a novel electrochemical

approach was developed by Aeschbacher et al., (2010) , which has the advantage to improve accuracy and shorter analysis time with quantification of both EAC and EDC for HS.

Previous measured range of DOM for EAC and EDC was varied from 0.23 to 6.1 μmol e^- (mg C) $^{-1}$, depending on DOM and used methods at environmentally relevant pH(Aeschbacher et al., 2010; Bauer et al., 2007; Ratasuk and Nanny, 2007).(details see Table 1)

Table 1. Previous published ETC range of diverse humic substances with various methods (EAC/EDC* data was transformed according to C content of humic acid)

Humic Substances	EAC	Method	EDC	Method	pH	References
Aldrich humic acid (46 % C)	0.29 ± 0.017	H_2+Pd/Al_2O_3 Fe(III) citrate	-	-	6.5	(Ratasuk and Nanny, 2007)
	0.36 ± 0.03	H_2+Pd/Al_2O_3 Fe(III) citrate	-	-	8	(Ratasuk and Nanny, 2007)
	1.96 ± 0.13	Electrochemical method	1.9±0.07	Electrochemical method	7	(Aeschbacher et al., 2010)
Suwannee River humic acid (43.8% C)	0.23 ± 0.03	H_2+Pd/Al_2O_3 Fe(III) citrate	-	-	6.5	(Ratasuk and Nanny, 2007)
	0.32 ± 0.04	H_2+Pd/Al_2O_3 Fe(III) citrate	-	-	8	(Ratasuk and Nanny, 2007)
	2.20 ± 0.06	Electrochemical method	4.20 ± 0.45	Electrochemical method	7	(Aeschbacher et al., 2010)
Pahokee Peat reference humic acid (47.3 % C)	0.60 ± 0.07	$H_2S/S_2O_3^{2-}$	0.52 ± 0.00	$[Fe(bipy)_3]^{3+}$/ $[Fe(bipy)_3]^{2+}$	6	(Bauer et al., 2007)

Humic Substances	EAC	Method	EDC	Method	pH	References
Pahokee Peat reference humic acid (47.3 % C)	6.2 ± 1.1	Zn^0/Zn^{2+}	0.33 ± 0.00	$[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$	6.5	(Bauer et al., 2007)
	3.6 ± 0.06	Electrochemical method	4.70 ± 0.07	Electrochemical method	7	(Aeschbacher et al., 2010)
	0.61 ± 0.04	H_2 + Pd/Al_2O_3 Fe(III) citrate	-	-	8	(Ratasuk and Nanny, 2007)

*Note unit for EAC and EDC is μmol e^- (mg C) $^{-1}$

4. Interaction between humic substances and terminal electron accepting processes

For anaerobic respiration, the energy yield of terminal electron accepting processes for the oxidation of a given substrate follows the sequence: NO_3^- reduction> Fe^{3+} reduction> SO_4^{2-} reduction> Methanogenesis. This sequence follows the thermodynamic theory that predicts that electron acceptors with a higher redox potential will be reduced first (Achtnich et al., 1995). For example, iron oxides are important electron acceptors in many anoxic environments (Lovley and Phillips, 1994; Nealson and Saffarini, 1994). When the availability of ferric iron is constrained by depletion of iron oxides or surface passivation is limiting accessibility, sulfate reducing conditions establish. After depletion of sulfate, methanogenesis initiates (Pester et al., 2012).

Humic substancies could serving as an electron mediator either a direct effect, if bacteria are able to directly use HS as electron acceptors, or it could shuttling electron for microorganism (Lovley et al., 1996). Since the ubiquity of HS in the environment, the HS could easily involve in many terminal electron accepting processes or competitively suppress reduction of other terminal electron acceptors (Klüpfel et al., 2014), e.g., HS could facilitate transferring of electrons from microorganisms to poorly accessible iron mineral phases for sustaining ferric iron reduction (Kappler et al., 2004); HS could serve as electron acceptor to oxidize hydrogen sulfide to sulfur intermediates (e.g. S^0, $S_2O_3^{2-}$), which would be further transformed by microorganism for regenerating SO_4^{2-} to maintain microbial sulfate reduction process under SO_4^{2-} deficiency condition (Heitmann and Blodau, 2006, Bauer et al., 2007). Recently, Klüpfel et al., (2014) confirmed that electron transfer to HS result in a suppression of hydrogenotrophic methanogenesis.

5. Interaction between humic substances and sulfur cycling in freshwater ecosystem

Bacterial sulfate reduction to H_2S is recognized as an important pathway of organic matter degradation in freshwater systems and high rates have been observed in many studies as Pester et al., (2012) reviewed. Though sulfate concentrations in peatlands, lake sediments are often in the micromolar-range and thus considered too low to sustain high sulfate reduction rates in the long-term S cycling (Pester et al., 2012). This has led to the hypothesis that unidentified electron acceptor drive abiotic reoxidation of H_2S from sulfate reduction to S^0 and $S_2O_3^{2-}$, or eventually microbially mediated to SO_4^{2-}, could sustain the observed high rates of SO_4^{2-} reduction (Heitmann and Blodau, 2006; Heitmann et al., 2007).

Humic substances, both dissolved and particulate HS, have been widely recognized as an important electron acceptor pool due to its redox active functional groups, e.g. electron transfer to quinone moieties (Klüpfel et al., 2014; Lovley et al., 1996; Roden et al., 2010; Scott et al., 1998). It is, thus, proposed that humic substance could serve as the unidentified electron acceptor for driving reoxidation of H_2S. Early study of a laboratory batch experiment found that DOM mediated re-oxidation of H_2S on a time scale of hours, however, only formation of $S_2O_3^{2-}$ was observed (Heitmann and Blodau, 2006) and measured electron transfer capacity of DOM (Pahokee Peat Reference HA (PP-HA)) from H_2S reaction was relative low, 0.6 μmol e^- (mg C) $^{-1}$. The short term and instantaneous reactivity of organic matter towards H_2S is still poorly understood and also little is known about the underlying transformation products, especially the organic sulfur species that form.

6. Terminal electron accepting processes of organic matter for anaerobic respiration

Nutrient poor freshwater ecosystems poor in inorganic electron acceptors, such as ombrotrophic peatlands, are thus important sources of CH_4 to the atmosphere (Mikaloff Fletcher et al., 2004), since CH_4 is produced under anaerobic conditions (Avery et al., 1999; Hornibrook et al., 1997). However, previous studies consistently demonstrated that a significant fraction of CO_2 was anaerobically produced through other unidentified electron acceptors (Heitmann et al., 2007; Keller et al., 2009; Lau et al., 2015). Other authors explained excess CO_2 production by BSR that is continuously sustained by recycling of sulfur upon reaction with organic matter (Heitmann et al., 2007; Vile et al., 2003a; Yavitt et al., 1987). However, this does not suffice for explaining significant fraction of anaerobic CO_2 production. It is, thus, led to the hypothesis that HS may as alternative electron acceptors are utilized for the oxidation of fermentation products and contribute to the significant suppression of methanogenesis (Klüpfel et al., 2014; Lovley et al., 1996).

OBJECTIVES AND HYPOTHESES

Study 1 Electron transfer budgets and kinetics of abiotic oxidation and incorporation of aqueous sulfide by dissolved organic matter

To quantitatively understand S cycling under anoxic conditions and to set up an electron budget of sulfide oxidation and/or incorporation upon reaction with DOM, the determination of the redox speciation of S_{org} is essential. We aimed at quantifying and characterizing the inorganic and organic products of sulfide oxidation by DOM, using a combined approach of wet chemical analysis and XANES spectroscopy. We hypothesized that the major oxidation product of sulfide would be thiosulfate, elemental S, and organic S. The latter could provide a diagenetic sink for S, but may contribute significantly to the S and electron budget. Finally, we intended to establish an electron budget including the redox state of S_{org}. We further expected that organic matter subjected to reducing conditions, i.e. with lower electron accepting capacity, would have a lower reactivity towards sulfide compared to organic matter from oxidizing environments. By calculation of reaction rates we aimed at providing time scales of the oxidation of sulfide and the formation of S_{org}.

Study 2 Electron transfer between sulfide and humic acid: electrochemical evaluation of the reactivity of Sigma Aldrich humic acid towards sulfide

Based on the fact that in electrochemically reduced HA all quinones should be reversibly reduced to hydroquinones, while in H_2/Pd reduced HA cleavage of quinone moieties and further molecular alterations may occur, the object of this study was to investigate the reactivity of reversibly, electrochemically reduced versus H_2/Pd reduced organic matter towards sulfide. We hypothesized that electrochemically

reduced quinones in DOM may not further react with sulfide. Thus it would allow identifying the contribution of non-quinone moieties to sulfide transformation.

Study 3 Contributions of organic sulfur and organic matter redox processes to electron flow in anoxic incubations of peat

The aim of the current study is based on the idea that the total electron flow of anaerobic respiration can be evaluated by measuring the end-product of CO_2. To evaluate electron accepting capacities from organic matter and contributions of sulfur cycling to anaerobic respiration, electron acceptor turnover was compared to CO_2 production and budgets were obtained in biotic incubations. To evaluate contributions of sulfur cycling, sulfate was added and the organic sulfur speciation was also analyzed prior and after incubation. To elucidate the long term effect of such recycling on the capacities we incubated peat with added additional sulfide to react with organic matter and reduce its electron accepting capacity.

METHODS AND TEHCNIQUES

Preparation of non-reduced and reduced humic acid stock solution Humic acid were purchased from Sigma-Aldrich and used as received, in addition to C/H/N contents provide by Sigma-Aldrich, other elemental composition was detected by energy-dispersive X-ray fluorescence (XRF) spectrometry (X-Lab 2000, Spectro), mixing 2 gram of milled humic acid powder with 1 g of XRF wax (Licowax C, APC Solutions SA), mixture were pressed into a 3.2-cm pellet for subsequently analysis. HA stock solution were prepared by dissolving the HA powder into Millipore water (resistivity ≥18.2 MΩ cm) at pH 9, acidified to pH 6 (HCl), filtered (0.45 μm nylon to remove non-dissolved HA fractions) and diluted to 150 mg C L^{-1}. Transferring the HA stock solution into serum bottles, tightly sealed with butyl rubber stopper and Al

crimp caps, then degassing O_2 by purged with N_2 (99.999%) under stirring for two hours. The oxygen-free HA solution was stored in glove box, N_2 atmosphere (O_2<1 ppm, Inert Lab 4GB Glovebox Systems, Innovative Technology, U.S.A.) and used as non-reduced HA stock solution and consequently further preparation for reduced HA, respectively. All DOC concentrations in the experimental solutions were detected by TOC analyzer (Shimadzu TOC-V CPN).

Wet chemically reducing the humic acid solution H_2 and Pd-catalyst (Pd 0.5% wt. on Al_2O_3, Aldrich, Germany) were employed (Benz et al., 1998; Jiang and Kappler, 2008; Visser, 1964), 4 pellets (Pd)/10 ml of solution to a 100-ml serum bottle, which was also closed with an air-tight butyl rubber stopper and crimped, the headspace was exchanged with H_2 (99.999%), which served as the reducing agent. After this, the bottles were shaking for 48 hours, tightly sealed with butyl rubber stopper and Al crimp caps, and then the solutions were filtered (0.45 μm nylon) in the anoxic glove box in order to remove traces of the Pd-pellets and used as reduced HA (RHA) stock solution. The consumption of hydrogen (headspace of serum bottles) in term of reducing the non-reduced HA was detected by H_2 analyzer TA 3000 (Trace Analytical, AMETEC, Newark, USA).

Electrochemically reducing the humic acid solution

For electrochemical reduction of HA, direct electrochemical reduction (DER) was applied (Aeschbacher et al., 2010)**.** To this end, NR- HA (150 mg C L^{-1}) stock solution was placed into a 0.1 L bulk electrolysis cell and electrochemically reduced at pH 7 (phosphate buffer) in the presence of 0.1 mol L^{-1} KCl (supporting electrolyte) and using a glassy carbon working electrode (WE), a Pt wire auxiliary electrode (separated from the main compartment by a glass frit to avoid reoxidation of reduced HA), and an Ag/AgCl reference electrode (all from Bioanalytical Systems Inc., West

Lafayette, IN). An Autolab PGSTAT101 potentiostat (Metrohm, Herisau, Switzerland) was used to record currents *I* (A) and to control potentials at the WE. Potentials were measured vs Ag/AgCl but are reported vs standard hydrogen electrode (SHE). To simulate different DOM redox properties as may adjust under natural conditions, two different potentials were set for electrochemical reduction of HA, at -0.1 and -0.4 V (redox potential of HS mostly lies in the range of +0.20 to -0.48 V) (Aeschbacher et al., 2011; Klüpfel et al., 2014).

Mediated Electrochemical Reduction and Oxidation

MER and MEO of HA samples to measure EAC and EDC of samples prior to and after reaction with sulfide was conducted with the electrochemical equipment described above. The electrochemical cell was filled with 80 mL of buffer (0.1 M KCl, 0.1 M phosphate, pH 7) and the electrode was equilibrated to the desired potentials (Eh = −0.49 V in MER and Eh = +0.61 V in MEO, which were below and above the potential range reported for quinones, respectively). Subsequently the mediators DQ (Diquat dibromide monohydrate (99.5%, Supelco, St. Louis, MO)) for MER and ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (>99%, Supelco, St. Louis, MO)) for MEO were spiked. When constant background currents were again reached, defined amounts (<1 mg C) of HA samples were spiked into the cells and the transferred amount of electrons was measured by chronocoulometry (Aeschbacher et al., 2010). Values of EAC are given for reduction at -0.49 C, values of EDC for oxidation at +0.61 V vs. SHE.

Sulfur species analysis

Inorganic Sulfur sulfide was assayed by the colorimetric method (methylene blue) at 665 nm on a Varian Cary 1E spectrophotometer (Heitmann and Blodau, 2006). Elemental sulfur and thiosulfate were determined by HPLC (Beckman HPLC-system,

C18-column, 0.8 ml min^{-1} flow rate, UV detection at 215 nm for thiosulfate; PerkinElmer HPLC-system, C18-column, 0.4 ml min^{-1} flow rate, UV detection at 265 nm for elemental S). Matrix effects and reproducibility were tested using an aqueous solution of $S_2O_3^{2-}$-spiked humic acid and a laboratory standard, oxidation of retained sulfide in the aqueous during the measurement would led to overestimate the detected values, thus for thiosulfate, 50 µl, 0.1 mM Zn^{2+} was spike to precipitate the sulfide, while elemental S assay was achieved by extracting S^0 into the cyclohexane phase from the sulfide-water phase.

Organic Sulfur Measurement for the organic Sulfur species was made by attaching solid samples as thin powder films on Kapton tape (Dilutions was achieved by mixing Cellulose if it was required.).Detailed post-experimental information were obtained from XANES spectroscopy at the S K-edge under anaerobic condition with evacuated chamber, measured at the ANKA SUL-X beamline (wiggler as a source, Si(111) monocromator crystal pair collimated beam). For XANES spectroscopy at the S K-edge, the beam was calibrated to the sulfate excitation energy of Na_2SO_4 at 2481.4 eV. Due to the low X-ray energy of the S K-edge, spectra were collected under vacuum. The S *Ka* X-ray was recorded with a seven element Si(Li) solid-state detector (SGX Sensortech, former Gresham). To prevent S species from beam damage, spectra were collected in a quick scan mode with sampling step widths of 1 eV from 2432 to 2461 eV and 2502 to 2756 eV, and 0.2 eV across the S K edge from 2461 to 2501 eV. Up to 4 to 6 scans were accumulated for each spectrum. Spectra were background corrected by fitting the S K pre-edge region with a linear and the post-edge with a polynomialfunction, and normalized to an edge jump of 1.

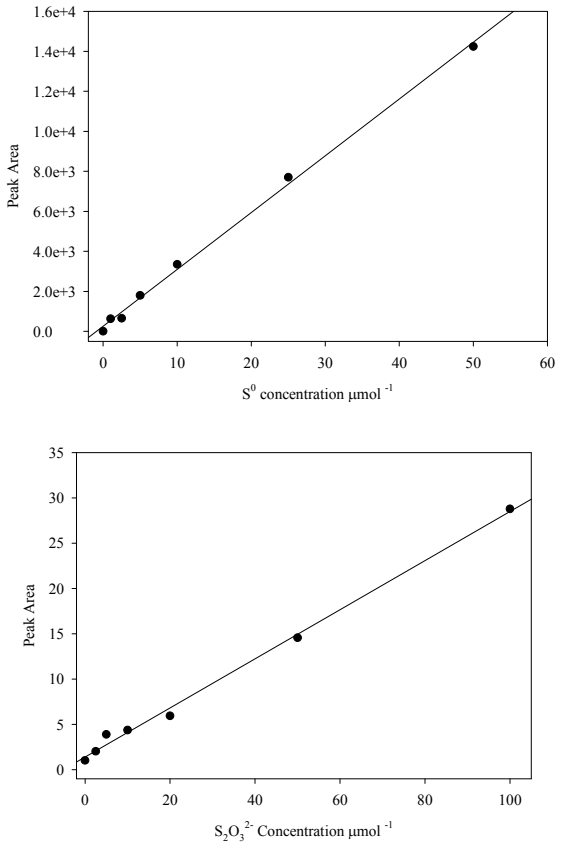


Figure1. Calibration curve for a series of elemental sulfur and thiosulfate standards

CONCLUSIONS AND PERSPECTIVES

Humic substances are abundant and ubiquitous in the freshwater ecosystems. Redox processes of DOM have strong impacts both on sulfur cycling and carbon turnover pathway under anaerobic conditions.

In laboratory studies of abiotic batch experiment of DOM and sulfide, results supported the assumption that DOM drive sulfide oxidation under anoxic condition. We identified the main oxidation products of sulfide. Elemental sulfur as predominant sulfur oxidation product, as previously observed for oxidation by iron oxides or oxygen, it indicates that elemental sulfur is probably formed in a wide range of abiotic oxidation processes of sulfide, including organic matter. Moreover, by using sulfur K-edge XANES we successfully identified organic sulfur species of short term product formation upon reaction of sulfide with DOM. Moreover, we quantified amount of transferred electron during the processes of reaction between sulfide and humic substances based on formed inorganic products and also include mostly zerovalent species organic sulfur species form (e.g. thiols, organic di- and polysulfides) in the budget calculations.

Electron transfer capacities of DOM were evaluated with mediated electrochemical method. Results demonstrate that also pre-reduced DOM (EC-RHA and H_2-RHA) further reacted with sulfide, a process that can be expected to occur in sulfidic environments, thereby competing with bacteria or other abiotic processes for the EAC of DOM. Initial values of EAC of different oxidation states of HA thereby strongly correlated to the respective sulfide transformation and may serve as a good indicator for reactivity towards sulfide. The observation that there was a higher transformation of sulfide as would be expected from electrochemically determined EAC indicated that sulfide was obviously highly efficient in ‘extracting’ electron acceptor capacity from organic matter. Furthermore, assuming an increasing degree of reduction of primarily quinones upon electrochemical reduction of HA, quinone moieties of DOM play a predominant role in the reactivity towards sulfide, also due to the observed high rate constants. Nevertheless, increasingly reduced HA presumably increases the formation of S_{org}, thereby providing a long term sink of sulfur already during early diagenesis.

Base on the frequent observation of 'unexplained' anaerobic CO_2 production mostly in organic wetland soils with low available inorganic electron acceptor, our study illustrated the importance of soil organic matter and internal sulfur cycling for electron accepting capacities for anaerobic respiration. (1) Electron acceptor budgets clearly demonstrated the predominance of soil organic matter EACs over inorganic electron acceptors. (2) Addition of sulfate induced an internal sulfur cycle, yielding even higher contributions of EACs from organic matter compared to incubations without sulfate addition. (3) Moreover, S K-edge XANES spectroscopy results demonstrated that both formation of reduced organic sulfur and transformed oxidized organic sulfur contributed to the total electron transfer during anaerobic respiration. Our results indicate that future studies do not only need to account for EACs of organic matter, but also include how an internal sulfur cycle increases EACs in a sulfidic, organic rich system.

Our results provided further support for the relevance of sulfate reduction, sulfide re-oxidation and thus sulfurs cycling in general for organic matter redox properties. Electron accepting capacities of organic matter as obtained from reduction of organic matter with sulfide seem to exceed values as obtained from electrochemical approaches or by H_2/Pd reduction. Nevertheless, through continuous formation of reduced organic sulfur species, the reaction of sulfide with organic matter reduces the amount of sulfur to be recycled on the longer term. Thus, the role of DOM-driven sulfur cycling is important, when evaluating electron transfer in temporarily or permanently water saturated freshwater ecosystem.

To further improve the understanding of redox processes between DOM and sulfur, (1) the role of thiosulfate, as an intermediate product, should be further address relate to microbial sulfate reduction. Moreover, (2) iron is also abundant and an important electron acceptor in freshwater ecosystem. There is often a interaction of between Fe and DOM, e.g. complexation of iron or shuttling of electrons to insoluble iron oxides. The role of iron associated DOM for microbial respiration should also be clearly investigated. Finally, (3) applying knowledge gained in our wet chemical and laboratory incubation experiment to evaluate the impact of competitive effects along redox gradients on overall carbon turnover is required, e.g. column experiments, or field manipulation.

REFERENCES

Achtnich, C., Bak, F., Conrad, R., 1995. Competition for electron donors among nitrate reducers , ferric iron reducers , sulfate reducers , and methanogens in anoxic paddy soil. Biol. Fertil. Soils 19, 65–72.

Aeschbacher, M., Sander, M., Schwarzenbach, R.P., 2010. Novel electrochemical approach to assess the redox properties of humic substances. Environ. Sci. Technol. 44, 87–93.

Aeschbacher, M., Vergari, D., Schwarzenbach, R.P., Sander, M., 2011. Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids. Environ. Sci. Technol. 45, 8385–94.

Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P., 1985. Humic substances in soil, sediment, and water: geochemistry, isolation and characterization. Wiley–Interscience, New York.

Avery, G.B., Shannon, R.D., White, J.R., Martens, C.S., Alperin, M.J., 1999. Effect of seasonal changes in the pathways of methanogenesis on the $\delta^{13}C$ values of pore water methane in a Michigan peatland. Global Biogeochem. Cycles 13, 475–484.

Bauer, M., Heitmann, T., Macalady, D.L., Blodau, C., 2007. Electron transfer capacities and reaction kinetics of peat dissolved organic matter. Environ. Sci. Technol. 41, 139–145.

Benz, M., Schink, B., Brune, A., 1998. Humic acid reduction by *Propionibacterium freudenreichii* and other fermenting bacteria. Appl. Environ. Microbiol. 64, 4507–4512.

Blodau, C., Bauer, M., Regenspurg, S., Macalady, D., 2009. Electron accepting capacity of dissolved organic matter as determined by reaction with metallic zinc. Chem. Geol. 260, 186–195.

Cory, R.M., McKnight, D.M., 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environ. Sci. Technol. 39, 8142–9.

Grieve, I.C., 1984. Concentrations and annual loading of dissolved organic matter in a small moorland stream. Freshw. Biol. 14, 533–537.

Heitmann, T., Blodau, C., 2006. Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. Chem. Geol. 235, 12–20.

Heitmann, T., Goldhammer, T., Beer, J., Blodau, C., 2007. Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog. Glob. Chang. Biol. 13, 1771–1785.

Hessen, D.O., Tranvik, L.J. (Eds.), 1998. Aquatic humic substances, ecological studies. Springer, Berlin, Heidelberg.

Hornibrook, E.R.C., Longstaffe, F.J., Fyfe, W.S., 1997. Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. Geochim. Cosmochim. Acta 61, 745–753.

Jiang, J., Kappler, A., 2008. Kinetics of microbial and chemical reduction of humic substances: implications for electron shuttling. Environ. Sci. Technol. 42, 3563–3569.

Kappler, A., Benz, M., Schink, B., Brune, A., 2004. Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. FEMS Microbiol. Ecol. 47, 85–92.

Keller, J.K., Weisenhorn, P.B., Megonigal, J.P., 2009. Humic acids as electron acceptors in wetland decomposition. Soil Biol. Biochem. 41, 1518–1522.

Klüpfel, L., Piepenbrock, A., Kappler, A., Sander, M., 2014. Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nat. Geosci. 7, 195–200.

Lau, M.P., Sander, M., Gelbrecht, J., Hupfer, M., 2014. Solid phases as important electron acceptors in freshwater organic sediments. Biogeochemistry.

Lovley, D.R., Coates, J.D., Blunt-Harris, E.L., Phillips, E.J.P., Woodward, J.C., 1996. Humic substances as electron acceptors for microbial respiration. Nature 382, 445–448.

Lovley, D.R., Phillips, E.J.P., 1994. Novel processes for anaerobic sulfate production from elemental sulfur by novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. Appl. Environ. Microbiol. 60, 2394–2399.

Macalady, D.L., Walton-day, K., 2011. Redox chemistry and natural organic matter (NOM): geochemists ' dream , analytical chemists ' nightmare. Aquatic Redox Chemistry. 85–111

Mikaloff Fletcher, S.E., Tans, P.P., Bruhwiler, L.M., Miller, J.B., Heimann, M., 2004. CH_4 sources estimated from atmospheric observations of CH_4 and its $^{13}C/^{12}C$ isotopic ratios: 1. Inverse modeling of source processes. Global Biogeochem. Cycles 18.

Nealson, K.H., Saffarini, D., 1994. Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. Annu. Rev. Microbiol. 48, 311–43.

Nurmi, J.T., Tratnyek, P.G., 2002. Electrochemical properties of natural organic matter (NOM), fractions of NOM, and model biogeochemical electron shuttles. Environ. Sci. Technol. 36, 617–24.

Orsetti, S., Laskov, C., Haderlein, S.B., 2013. Electron transfer between iron minerals and quinones: estimating the reduction potential of the Fe(II)-goethite surface from AQDS speciation. Environ. Sci. Technol. 47, 14161–8.

Peretyazhko, T., Sposito, G., 2006. Reducing capacity of terrestrial humic acids. Geoderma 137, 140–146.

Pester, M., Knorr, K.-H., Friedrich, M.W., Wagner, M., Loy, A., 2012. Sulfate-reducing microorganisms in wetlands - fameless actors in carbon cycling and climate change. Front. Microbiol. 3, 72.

Ratasuk, N., Nanny, M.A., 2007. Characterization and quantification of reversible redox sites in humic substances. Environ. Sci. Technol. 41, 7844–7850.

Roden, E.E., Kappler, A., Bauer, I., Jiang, J., Paul, A., Stoesser, R., Konishi, H., Xu, H., 2010. Extracellular electron transfer through microbial reduction of solid-phase humic substances. Nat. Geosci. 3, 417–421.

Scott, D.T., McKnight, D.M., Blunt-Harris, E.L., Kolesar, S.E., Lovley, D.R., 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ. Sci. Technol. 32, 2984–2989.

Stevenson, F.J., 1994. Humus chemistry: genesis, composition, reactions, 2nd ed. John Wiley & Sons, Ltd: New York.

Struyk, Z., Sposito, G., 2001. Redox properties of standard humic acids. Geoderma 102, 329–346.

Sutton, R., Sposito, G., 2005. Molecular structure in soil humic substances : the new view. Environ. Sci. Technol. 39, 9009–9015.

Uchimiya, M., Stone, A.T., 2009. Reversible redox chemistry of quinones: impact on biogeochemical cycles. Chemosphere 77, 451–458.

Vile, M.A., Bridgham, S.D., Wieder, R.K., 2003. Response of anaerobic carbon mineralization rates to sulfate amendments in a boreal peatland. Ecol. Appl. 13, 720–734.

Visser, S.A., 1964. Oxidation-reduction potentials and capillary activities of humic acids. Nature 204, 581.

Wolf, M., Kappler, A., Jiang, J., Meckenstock, R.U., 2009. Effects of humic substances and quinones at low concentrations on ferrihydrite reduction by *Geobacter metallireducens*. Environ. Sci. Technol. 43, 5679–5685.

Yavitt, J.B., Lang, G.E., Wieder, R.K., 1987. Control of carbon mineralization to CH_4 and CO_2 in anaerobic, *Sphagnum*-derived peat from Big Run Bog, West Virginia. Biogeochemistry 4, 141–157.

CONTRIBUTIONS TO DIFFERENT STUDIES

Study 1 Electron transfer budgets and kinetics of abiotic oxidation and incorporation of aqueous sulfide by dissolved organic matter

Authors: Zhi-Guo Yu, Stefan Peiffer, Jörg Göttlicher, Klaus-Holger Knorr

Zhi-Guo Yu: 70 % (concepts, laboratory work, interpretation and discussion of results, manuscript preparation)

Stefan Peiffer: 5 % (comments on manuscript)

Jörg Göttlicher: 5% (laboratory infrastructure, interpretation of results, comments on manuscript)

Klaus-Holger Knorr: 20 % (concepts, discussion of results, manuscript preparation)

Study 2 Electron transfer between sulfide and humic acid: electrochemical evaluation of the reactivity of Sigma Aldrich humic acid towards sulfide

Authors: Zhi-Guo Yu, Silvia Orsetti, Stefan B. Haderlein, Klaus-Holger Knorr

Zhi-Guo Yu: 75 % (concepts, laboratory work, interpretation and discussion of results, manuscript preparation)

Silvia Orsetti: 5 % (laboratory infrastructure, comments on manuscript)

Stefan B. Haderlein: 5 % (concepts, comments on manuscript)

Klaus-Holger Knorr: 15 % (concepts, discussion of results, manuscript preparation)

Study 3 Contributions of organic sulfur and organic matter redox processes to electron flow in anoxic incubations of peat

Authors: Zhi-Guo Yu, Jörg Göttlicher, Ralph Steininger, Klaus-Holger Knorr

Zhi-Guo Yu: 75 % (concepts, laboratory work, interpretation and discussion of results, manuscript preparation)

Jörg Göttlicher: 5% (laboratory infrastructure, interpretation of results, comments on manuscript)

Ralph Steininger: 5% (laboratory infrastructure, interpretation of results)

Klaus-Holger Knorr: 15 % (concepts, discussion of results, manuscript preparation)

STUDY 1: Electron transfer budgets and kinetics of abiotic oxidation and incorporation of aqueous sulfide by dissolved organic matter

Zhi-Guo Yu, Stefan Peiffer, Jörg Göttlicher, Klaus-Holger Knorr

Published on *Environmental Science & Technology*, 2015, 49 (9), 5441–5449

Electron transfer budgets and kinetics of abiotic oxidation and incorporation of aqueous sulfide by dissolved organic matter

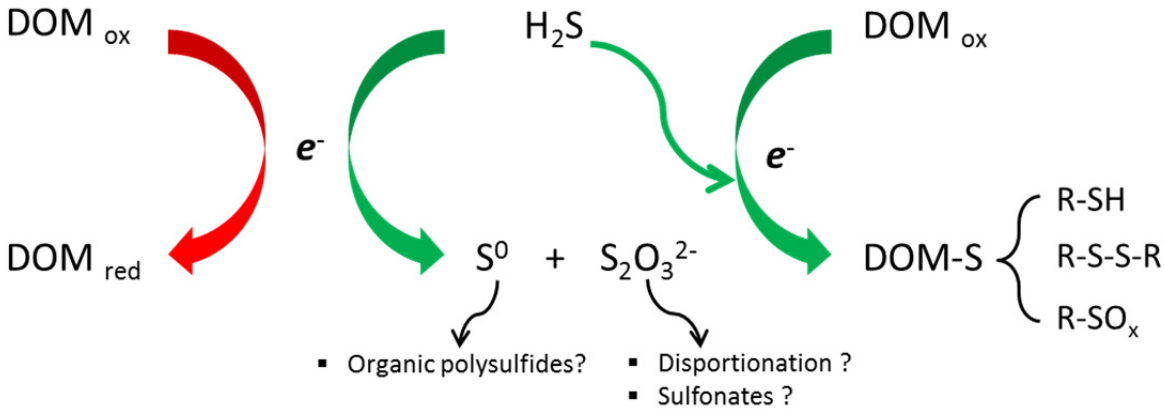
Zhi-Guo Yu[1, 2], Stefan Peiffer[1], Jörg Göttlicher[3], Klaus-Holger Knorr[1, 2,]*

[1]Limnological Research Station & Department of Hydrology, University of Bayreuth, Universitätsstrasse 30, 95447 Bayreuth, Germany

[2]present address: Hydrology Group, Institute of Landscape Ecology, University of Münster, Heisenbergstr.2, 48149 Münster, Germany

[3]ANKA Synchrotron Radiation Facility, Karlsruhe Institute of Technology, 76344 Eggenstein-Leopoldshafen, Germany

TABLE OF CONTENTS GRAPHIC



ABSTRACT

The reactivity of natural dissolved organic matter (DOM) towards sulfide and has not been well studied with regard to electron transfer, product formation and kinetics. We thus investigated the abiotic transformation of sulfide upon reaction with reduced and non-reduced Sigma Aldrich humic acid (HA), at pH 6 under anoxic conditions. Sulfide reacted with non-reduced HA at conditional rate constants of 0.227~0.325 h^{-1}. The main transformation products were elemental S (S^0), and thiosulfate ($S_2O_3^{2-}$), yielding electron accepting capacities of 2.82~1.75 µmol e^- (mg C) $^{-1}$. Native iron contents in the HA could account for only 6~9% of this electron transfer. About 22~37 % of S reacted with the HA to form organic S (S_{org}). Formation of S_{org} was observed and no inorganic transformation products occurred for reduced HA. X-ray absorption near edge structure (XANES) spectroscopy supported S_{org} to be mainly zerovalent, such as thiols, organic di- and polysulfides, or heterocycles. In conclusion, our results demonstrate that HA can abiotically re-oxidize sulfide in anoxic environments at rates competitive to sulfide oxidation by molecular oxygen or iron oxides.

KEYWORDS Sulfur cycling; Humic acid; anoxic conditions; Electron transfer; organic sulfur; Redox processes, Freshwater systems

1 INTRODUCTION

Bacterial sulfate reduction (BSR) to sulfide is recognized as an important pathway of organic matter degradation in freshwater systems. High rates of BSR have been observed in many studies(Heitmann et al., 2007; Wieder and Lang, 1988; Wieder et al., 1990). However, sulfate (SO_4^{2-}) concentrations in wetlands, lake sediments and rice paddy soils are often in the micromolar-range, and stocks are thus considered too low to sustain sulfate reduction in the long-term (Heitmann et al., 2007; Pester et al., 2012). S cycling has been intensively studied for decades (Blodau et al., 2007; Canfield et al., 1998; Ferdelman et al., 1991; Lovley and Phillips, 1994; Novák et al., 2005), also because sulfide is a highly reactive towards iron oxides and other inorganic elements, leading to partial oxidation and formation of e.g. S^0 and $S_2O_3^{2-}$ (Hellige et al., 2012; Peiffer et al., 1992). Such fast and spontaneous sulfide oxidation has led to the hypothesis that abiotic reoxidation of sulfide to S^0 and $S_2O_3^{2-}$, or potentially microbially mediated further oxidation to SO_4^{2-}, could sustain the observed high rates of sulfate reduction (Blodau et al., 2007; Heitmann et al., 2007; Lovley and Phillips, 1994).

Besides the commonly considered inorganic electron acceptors, organic matter may play a crucial role in redox processes in the environment (Lovley et al., 1996). While it is known that functional groups of organic matter, such as quinones (Aeschbacher et al., 2010), can be reversibly reduced by acting as electron acceptors in anaerobic microbial respiration (Klüpfel et al., 2014; Lovley et al., 1996; Roden et al., 2010; Uchimiya and Stone, 2009), less is known of redox reactions of reactive species, such as sulfide, with organic matter (Heitmann and Blodau, 2006; Heitmann et al., 2007; Perlinger et al., 2002).

In a laboratory batch experiment at pH 6, DOM mediated the oxidation of sulfide on a time scale of hours, leading to the formation $S_2O_3^{2-}$ and S_{org} (Heitmann and Blodau, 2006). The electron accepting capacity of DOM (Pahokee Peat Reference HA (PP-HA)) for sulfide calculated from $S_2O_3^{2-}$ formation was 0.6 µmol e^- (mg C) $^{-1}$. From studies of sediment diagenesis, it is known that upon reaction of sulfide with organic matter, also S_{org} is formed during early stages of diagenesis, e.g. by Michael additions to oxidized quinones (Perlinger et al., 2002), by addition to unsaturated C double-bonds e.g. of lipids (Adam et al., 2000; Vairavamurthy and Mopper, 1987), or by cross linkage of carbohydrates (van Dongen et al., 2003). However, the short term and instantaneous reactivity of organic matter towards sulfide is still poorly understood and little is known of the transformation products, especially the species of S_{org} that form (Heitmann and Blodau, 2006; Hoffmann et al., 2012).

Reduced organic S is identified as an important functional group in terrestrial and aquatic DOM, e.g. for influencing trace metal transport and fate in the ecosystem (Hoffmann et al., 2012). Nevertheless, it remains difficult for the organic forms of S to disentangle the different functional groups and to determine their redox states (Xia et al., 1998). In FTIR spectroscopy, spectral features of S groups are difficult to evaluate as they overlap with other non-S functional groups ubiquitous in organic matter (Heitmann and Blodau, 2006). Due to these methodological constraints there is still a need to elucidate, which organic S species typically form in sulfidic systems.

To quantitatively understand S cycling under anoxic conditions and to set up an electron budget of sulfide oxidation and/or incorporation upon reaction with DOM, the determination of the redox speciation of S_{org} is essential. While methods have been established for analysis of inorganic S specie (Heitmann and Blodau, 2006; Wan et al., 2014), only few studies applied S K-edge XANES spectroscopy to identify S_{org}

speciation in natural organic matter (Einsiedl et al., 2008; Hoffmann et al., 2012; Prietzel et al., 2007). These latter studies suggest that XANES can be used to track S_{org} speciation to understand S cycling in freshwater systems.

In our study, we aimed at quantifying and characterizing the inorganic and organic products of sulfide oxidation by DOM, using a combined approach of wet chemical analysis and XANES spectroscopy. We hypothesized that the major oxidation product of sulfide would be thiosulfate, elemental S, and organic S. The latter could provide a diagenetic sink for S, but may contribute significantly to the S and electron budget. Thus, we intended to establish an electron budget including the redox state of S_{org}. We further expected that organic matter subjected to reducing conditions, i.e. with lower electron accepting capacity, would have a lower reactivity towards sulfide compared to organic matter from oxidizing environments. By calculation of reaction rates we aimed at providing time scales of the oxidation of sulfide and the formation of S_{org}.

2 MATERIALS AND METHODS

2.1 Preparation of humic acid solutions.

As a model compound for our study, humic acid (HA) was purchased from Sigma Aldrich and used as received. The elemental composition is provided in SI, Table 5. HA stock solutions for batches were prepared by dissolving the HA powder in Millipore water (≥18.2 MΩ cm) at pH 9 for 24 hours. This solution was subsequently brought to pH 6 by addition of hydrochloric acid, filtered (0.45 µm nylon) and diluted to 150 mg C L^{-1}. For wet chemically reduction of the HA solution, we used H_2 and a Pd-catalyst (Pd 0.5% wt. on Al_2O_3, Aldrich, Germany) (Ratasuk and Nanny, 2007). H_2 consumption was experimentally measured (SI, Figure 4) to calculate an electron accepting capacity upon H_2/Pd reduction. Equilibration of the reduction was reached within 48 hours, as obtained from headspace H_2 concentrations over time.

2.2 *Setup of batch reactions*

All reagents in this study were deoxygenated by N_2-purging and stored in a glove box (O_2 < 1 ppm, N_2 atmosphere, InertLab, Innovative Technology, Amesbury, USA). All sample handling was performed in the glove box or in stoppered flasks (1 cm butyl stoppers, Glasgerätebau Ochs, Bovenden, Germany) to ensure anoxic conditions. All experiments were performed in brown serum bottles and in the dark, at 25 ±1 °C. Triplicates of reduced/ non-reduced HA solutions of 25, 50 and 75 mg C L^{-1} were set up at pH 6 ± 0.05. Subsequently, a total sulfide concentration of 250 ± 1 µmol L^{-1} was spiked (speciation at pH 6: 91.25 % H_2S, 8.75% HS^-, hereafter referred to as 'sulfide'). To avoid sulfide partitioning, the headspace of the serum bottles was kept < 0.5 ml. We are aware that these conditions may not be representative of typical freshwater environments (~10 µmol L^{-1}) (Blodau et al., 2007), nevertheless are not unrealistic for in-situ conditions and necessary to analyze the S fractions with reasonable effort. Constant pH was maintained using a bicarbonate buffer. Solutions were stirred at constant rate, three aliquots of 400 µl, 300 µl and 1ml were collected on various time points within 48 hours for determination of S^{2-}, $S_2O_3^{2-}$, and S^0, respectively.

A parallel batch for analysis of S_{org} formation was prepared to yield higher amounts of material for XANES analysis. Solutions of 150 mg C L^{-1} reduced/non-reduced HA were employed and the initial sulfide concentration was adjusted to the same molar S/C ratio as in the 25 mg C L^{-1} treatment. After 48 hours, the solutions were purged with N_2 for 45 min to remove unreacted sulfide, a 25 ml aliquot was collected and extracted for 30 min with cyclohexane (2.5:1/*v:v*) to remove S^0 which could influence detection of near-zerovalent S_{org}. The underlying water phase was transferred into air-

tight flasks, shock frozen with dry ice, freeze dried, and stored in the glove box until analysis.

2.3 ***Analytical procedures***

Dissolved organic carbon (DOC) concentrations were measured using a Shimadzu TOC V-CPN analyzer. The consumption of H_2 during reduction of non-reduced HA was measured from headspace H_2 concentrations in the serum bottles, using a H_2 trace analyzer TA 3000 (Trace Analytical, AMETEC, Newark, USA).

Inorganic S analysis: Sulfide was measured colorimetrically (methylene blue) at 665 nm on a Varian Cary 1E spectrophotometer (Cline, 1969). Elemental S and $S_2O_3^{2-}$ were determined by high-performance liquid chromatography (HPLC) (Beckman HPLC-system, C18-column, 0.8 ml min^{-1} flow rate, UV detection at 215 nm for $S_2O_3^{2-}$; PerkinElmer HPLC-system, C18-column, 0.4 ml min^{-1} flow rate, UV detection at 265 nm for S^0). Matrix effects and reproducibility were tested using an aqueous solution of either S^0 or $S_2O_3^{2-}$-spiked humic acid and a laboratory standard. To prevent interference during analysis of $S_2O_3^{2-}$, 50 µl of 0.1 mM Zn^{2+} was spiked into all samples prior to analysis to precipitate remaining sulfide.

Organic S analysis: Organic S speciation was analyzed at the Synchrotron Radiation Source ANKA, SUL-X beamline (wiggler as a source, Si(111) monocromator crystal pair, collimated beam), as powdered solid samples prepared as thin layers on Kapton tape. To adjust S contents to 1.3~2 % to avoid self-absorption, samples were diluted with cellulose (Sigmacell Type 20, 20 µm) if necessary (Prietzel et al., 2011). For XANES spectroscopy at the S K-edge, the beam was calibrated to the sulfate excitation energy of Na_2SO_4 at 2481.4 eV. Due to the low X-ray energy of the S K-edge, spectra were collected under vacuum. The S $K\alpha$ X-ray fluorescence emission was recorded with a seven element Si(Li) solid-state detector (SGX Sensortech,

former Gresham). To prevent S species from beam damage, spectra were collected in a quick scan mode with sampling step widths of 1 eV from 2432 to 2461 eV and 2502 to 2756 eV, and 0.2 eV across the S K edge from 2461 to 2501 eV. Up to 4 scans of different sample spots were accumulated for each sample spectrum. Spectra were background corrected by fitting the S K pre-edge region with a linear and the post-edge with a polynomial function, and normalized to an edge jump of 1 (Hoffmann et al., 2012; Manceau and Nagy, 2012; Prietzel et al., 2011; Ravel and Newville, 2005). Measured references are given in Table 2, spectral data is provided in the SI, Figure 5.

2.4 Calculations and budgets

Mass balances were calculated from measured concentrations of total sulfide addition, remaining sulfide in solution, and formation of S^0 and $S_2O_3^{2-}$, as no other inorganic oxidation products were detected. Organic sulfur could thus be calculated from a mass balance. XANES spectroscopy was not used to quantify S_{org}, but only to identify S_{org} speciation. For conversion of sulfur species transformations into electron budgets and stoichiometries, see Table 3.

3 RESULTS

3.1 Transformation of sulfide by non-reduced/reduced humic acid

As would be expected from electron accepting capacities, the amount of sulfide transformed by non-reduced humic acid (NR-HA) exceeded that of H_2/Pd pre-reduced humic acid (RHA). Sulfide transformation also increased with increasing HA concentrations from 25, 50 to 75 mg C L^{-1}, both for NR-HA and for RHA (Figure 2). The highest transformation of the initial sulfide (250 ± 1µmol L^{-1}) was thus observed for 75 mg C L^{-1} NR-HA (97.5 µmol L^{-1}, 39 %), while 50 and 25 mg C L^{-1} transformed 30.1 % and 19.8 % of the initial sulfide (Figure 2). Starting from a native S/C ratio of the employed HA of 0.0042, the calculated S/C ratio for the transformation of sulfide

was 0.022 for 25 mg C L^{-1} and 0.015 for 75 mg C L^{-1} and thus higher for lower carbon concentrations.

Since Aldrich HA contains significant amounts of iron (1.33%, SI, Table 5), we calculated the amount of the sulfide transformation which may be explained by reaction with iron (assuming all iron being present as Fe(III)). However, the calculated amount accounted for only a maximum of 6 to 9 % for transformed sulfide.

Regarding the time course of the reaction, concentrations of sulfide sharply decreased during the first 12 hours, while thereafter the reaction slowed until 48 hours of incubation (Figure 2). A sample collected immediately after addition of sulfide, already 8.7 (25 mg C L^{-1}) to 35.5 µmol L^{-1} (75 mg C L^{-1}) of sulfide had been transformed (SI, Figure 9, 10). This instantaneous transformation would again only partly be explained by native HA iron contents (25 to 34%). There was also a transformation of sulfide by RHA, i.e. by HA pre-reduced by H_2/Pd (Figure 2A). Here, 9.0, 18.0, and 22.4 % of the initial sulfide concentration were transformed in the 25, 50, and 75 mg C L^{-1} batches, respectively, equivalent to S/C ratios of 0.0036, 0.0072 and 0.0090. A sample collected immediately after the addition of sulfide to RHA, also 4 to 31.8 µmol L^{-1} of sulfide had already been transformed, although no Fe(III) would be expected here. A test of 72 hours of incubation of NR-HA and RHA yielded no further transformation of detectable quantities (not shown). Thus, all following calculations were based on the changes observed after 48 hours.

3.2 Inorganic products of sulfide transformation

In NR-HA samples, the formation of S^0 followed sulfide consumption. Within the first 10 hours, S^0 increased to 6.6~35.0 µmol L^{-1}, thereafter the formation of S^0 was significantly slower (Figure 2). After 48 h, S^0 levelled off at 20.0 to 53.6 µmol L^{-1}

(Table 4), with increasing amounts of S^0 formed at higher concentrations of C. Native iron in HA contributed at most 15 to 17% to S^0 formation.

Thiosulfate exhibited a different behavior (Figure 2 B/C/D). In the first 5 hours, $S_2O_3^{2-}$ concentrations increased sharply, reaching temporary maxima (7.5~10.7 µmol L^{-1}), but declining again thereafter. After 48 hours, only 3.8, 4.7 and 3.0 µmol L^{-1} of $S_2O_3^{2-}$ were left in the solutions of 25, 50, and 75 mg C L^{-1} (2.4 to 3.8 % of initial sulfide).

We also analyzed for sulfite and for polysulfides (not shown), which were both not detected, probably as they do not significantly form at pH < 7 (Rickard and Luther III, 2007). Based on the S mass balance after 48h, S^0 was the major terminal product, accounting for 40~55 % of the transformed sulfide, compared to 15.4~6.1 % recovered as $S_2O_3^{2-}$.

For the RHA batches, no inorganic transformation products were identified. Thus, here reacted sulfide had added to organic matter to form S_{org}.

3.3 Formation of organic S and characterization by S-K edge XANES Spectroscopy

As the inorganic fraction of S accounted for 56~61% of reacted sulfide, 39~44% (21.9~37.9 µmol L^{-1}) had thus added to NR-HA as S_{org}, yielding an S/C ratio of 0.011 to 0.006. For RHA, S_{org} formation was 22.5~56.0 µmol L^{-1}, equivalent to S/C ratios of 0.011~0.009, and therefore similar as for the NR-HA.

Investigating the species of S_{org} in NR-HA and RHA using S K-edge XANES spectroscopy on samples before and after reaction yielded two distinct peak ranges, representing two groups of S oxidation states (Figure 3 and Table 2). The range from 2471 to 2474 eV represents reduced S species, such as inorganic sulfides, elemental S, and about zerovalent organic S, such as thiols and S bridging structures involving one or more S atoms (e.g. R-SH or R-S-R, R-S-S-R). The peak range from 2475 to 2483

eV is indicative of oxidized organic and inorganic S species, e.g. organic sulfoxides, sulfones, sulfonates, and sulfate esters and inorganic sulfate S (Hoffmann et al., 2012). Despite a native S content of 0.45 % as a background and due to removal of inorganic S species prior to analysis, S K-edge XANES before and after reaction with sulfide differed clearly, enabling us to track the fate of 0.11~0.06 % of newly formed S_{org} in NR-HA and RHA. We used 5,5'-Dithiobis(2-nitrobenzoic acid) (representing organic disulfides, R-SS-R, ~2471.6 eV) and L-cysteine (representing thiols, R-SH, ~2472.6 eV) with nominal electronic oxidation states of S between 0 and 1 (Figure 3) (Prietzel et al., 2011, 2007; Xia et al., 1998), as references to reproduce predominant changes of speciation of S_{org} before and after reaction. In the NR-HA samples, peak intensities at ~2471.6 eV increased after reaction, shifting the predominant signal of reduced S_{org} from 2473.1 eV to 2471.6 eV.

We also detected changes in peaks representing oxidized S_{org} species in NR-HA, as a minor peak at 2475.1 eV (possibly sulfoxide) (Urban et al., 1999) diminished, a new peak at 2478.2 eV occurred, and the peak at ~2481 eV obviously separated into two distinct peaks at 2480.1 and 2481.2 eV. Either the oxidized S_{org} initially present in HA would be partially reduced by sulfide, or oxidized S_{org} may have formed (e.g. sulfonates). However, as these changes could not be quantified exactly from XANES spectra we did not consider them any further in the electron budgets.

RHA samples also exhibited most prominent increases of peaks at 2471.6 and 2472.6 eV (organic disulfides and thiols, respectively) after reaction with sulfide. The peak maximum of RHA at 2473.1 eV did not shift, but broadened towards lower energies, indicating a relative increase of species peaking around 2471.6 (organic disulfides). A notable increase of the peak at 2478.1 and 2480.1 eV (possibly sulfones and sulfonate, Table 2) occurred, while peaks at 2475.1 and 2481.1 eV exhibited a relative decrease.

A peak at ~2469 eV after reaction may be due to monosulfide that had not been completely extracted.

3.4 Transferred electron balance

To calculate electron budgets, all S transformation products were recalculated into electron equivalents (Table 3 B/C). For S_{org}, as XANES data supported S_{org} to be approximate zerovalent S (0~1 as nominal electronic oxidation state) (Prietzel et al., 2011, 2007; Xia et al., 1998), and as the exact proportions of the individual organic species cannot be determined exactly, we conservatively assigned an oxidation state of zero.

For the budget after 48 hours, we calculated a total electron transfer from transformation of sulfide, EACs (TSC), of 114.2~207.0 µmol e^- L^{-1} (4.57~2.76 µmol e^- (mg C) $^{-1}$) for the 25~75 mg C L^{-1} solutions (Table 4, SI, Table 7, Figure 6). Thereby, of inorganic sulfur oxidation product formation (ISF), $S_2O_3^{2-}$ accounted for 30.4~24.0 µmol e^- L^{-1} (1.22~0.32 µmol e^- (mg C) $^{-1}$), and S^0 for 40.0~107.2 µmol e^- L^{-1} (1.6~1.4 µmol e^- (mg C) $^{-1}$). This yielded an EAC_S(ISF) of 70.4 to 131.2 µmol e^- L^{-1} (1.82~1.75 µmol e^- (mg C) $^{-1}$). S_{org} added another 43.8~75.8 µmol e^- L^{-1} (1.75~1.01 µmol e^- (mg C) $^{-1}$. Measured EAC_{H2} for Aldrich humic acid was only 2.48 ± 0.13 µmol e^- (mg C) $^{-1}$, i.e. 62, 124, and186 µmol e^- L^{-1} in 25, 50, and 75 mg C L^{-1}, respectively (SI, Table 7).

After 5 hours of reaction, $S_2O_3^{2-}$ concentrations peaked at 7.5, 9.3, 10.7 µmol L^{-1} (25, 50, and 74 mg C L^{-1}, respectively). Thus, 60~85 µmol e^- L^{-1}, or 2.40~1.13 µmol e^- (mg C) $^{-1}$ of EAC was intermittently recovered as $S_2O_3^{2-}$, exceeding contributions after 48 hours by far.

Electron accepting capacities from S_{org} formation of H_2/Pd pre-reduced HA (RHA), i.e. after retrieval of EAC_{H2}, amounted to 45~112 µmol e^- L^{-1} (1.8~1.5 µmol e^- (mg

C)$^{-1}$) for 25~75 mg C L^{-1}. For NR-HA (Table 4, SI, Table 7, Figure 6), EAC_{H2} and EACs(TSC) differed by 52.2~21.0 μmol e^- L^{-1} (2.09~0.28 μmol e^- (mg C) $^{-1}$) for 25~75 mg C L^{-1}, i.e. EAC_{H2} was notably smaller than EACs(TSC).

3.5 Reaction rates of sulfide transformation by humic acid

For sulfide transformation kinetics, we applied a two pool model, as suggested earlier (Heitmann and Blodau, 2006) (SI, Eqs. S1, S2 and Table 6). For NR-HA, 13.3~19.6 % of the added sulfide (49.5~97.5 μmol L^{-1}) were transformed by a fast pool at rates of 0.2271~0.3249 h^{-1}; 9.3~11.1% of the initial total sulfide (23.3~27.8 μmol L^{-1}) were transformed by a slow pool at rates of 0.0117~0.0285 h^{-1}. About 69.6~77.5% (174.0~193.7 μmol L^{-1}) of sulfide remained in solution after 48 h. For rates of S^0 and S_{org} formation, see SI, Table 6.

For RHA, fitting resulted in a one pool model only (SI, Eq. S3 and Table 6). Obtained rates were 0.0976~0.1128 h^{-1} and thus comparable to the slow pool of NR-HA.

4 DISCUSSION

4.1 DOM-driven recycling of S

While little is known about abiotic oxidation of sulfide by DOM (Heitmann and Blodau, 2006; Heitmann et al., 2007), a number of studies elucidated abiotic oxidation pathways for sulfide by inorganic electron acceptors, e.g. oxidation by oxygen, iron, or manganese, across various environments, e.g. aquatic systems and sediments under lab and field conditions (Chen and Morris, 1972; Peiffer et al., 1992; Poulton et al., 2004). In most studies, the observed abiotic oxidation pathway of sulfide involved the formation of S^0 and possibly of polysulfides (Chen and Morris, 1972; Hellige et al., 2012; Peiffer et al., 1992; Wan et al., 2014). Direct oxidation of sulfide by oxygen in water yields S^0 as the first oxidative product under slightly acidic conditions (Chen and Morris, 1972). Existing studies of sulfide reacting with DOM reported a

formation of $S_2O_3^{2-}$, but a predominant formation of S_{org} (~95 %) (Heitmann and Blodau, 2006; Heitmann et al., 2007). Contrarily, in our study, S^0 was the main oxidative product also for the oxidation of sulfide by DOM and S_{org} made up only 39~44 %. Thiosulfate was an intermediate during this oxidation, peaking after about 5 h, but decreasing in its contribution thereafter. This difference may be due to the employment of another DOM material (PP-HA) in the previous studies (Heitmann and Blodau, 2006), as it is well recognized that commercially available humic acids span a wide range in characteristics (Malcolm and MacCarthy, 1986; Rodríguez et al., 2014). Nevertheless, a considerably larger fraction of S could be oxidized to inorganic products than previously reported. A formation of mainly S^0 also compares well to abiotic oxidation of sulfide with ferric iron and iron oxides (Evangelou and Zhang, 1995; Moses et al., 1987), while formation of $S_2O_3^{2-}$ or SO_4^{2-} would typically involve further biotic transformation (Jørgensen, 1990; Peiffer et al., 1992; Poulton et al., 2004). The elevated native iron content of Aldrich HA (SI, Table 5) could potentially explain the formation of S^0 as oxidation product (Peiffer et al., 1992; Poulton et al., 2004). However, iron contents of Aldrich HA would only yield 6~9 % of the total sulfide transformation and 15~17 % of the S^0 formation in the NR-HA solutions.

An instantaneous formation of $S_2O_3^{2-}$, as observed earlier (Heitmann and Blodau, 2006) and in our study at the beginning of the reaction, hints at a catalytic action of non-reduced DOM to form $S_2O_3^{2-}$ from sulfide oxidation. We found some experimental support that formation of high amount of $S_2O_3^{2-}$ may be triggered by traces of residual oxygen (SI, Figure 11, 12), which we could successfully exclude in this approach. Nevertheless, $S_2O_3^{2-}$ must have been further transformed. A disproportionation of $S_2O_3^{2-}$ into sulfide and sulfate was suggested to require

microbial catalysis (Jørgensen, 1990), but $S_2O_3^{2-}$ may also have reacted abiotically with organic matter (see below) (Vairavamurthy et al., 1994). We also did not detect polysulfides, presumably as they are stable only at pH > 7 (Rickard and Luther III, 2007), although they could be expected in presence of sulfide and S^0 and would be precursors to form organic polysulfides (Francois, 1987; Vairavamurthy et al., 1992).

We also observed a transformation of sulfide for RHA, but could not detect inorganic transformation products. A detection of $S_2O_3^{2-}$ is, however, constrained by a limit of detection of 0.5 μmol L^{-1} in our analytical approach. Due to mass balance considerations, in case of RHA, the major transformation product of sulfide within 48 h was thus S_{org}.

4.2 Formation of organic S

Sulfur incorporation into natural organic matter has so far mostly been considered in marine diagenetic studies, involving a formation of organic polysulfide or sulfide linkages (Brown, 1986; Brüchert and Pratt, 1996; Ferdelman et al., 1991; Vairavamurthy et al., 1992). The 'Michael addition' was suggested as a reaction pathway, yielding sulfhydryl groups in the carbonyl β-position of unsaturated carbon double bonds (Vairavamurthy and Mopper, 1987) or of quinones (Perlinger et al., 2002). These may subsequently react to thioethers or organic polysulfides by inter- or intramolecular reaction with a second electrophile (Hoffmann et al., 2012; Perlinger et al., 2002; Vairavamurthy et al., 1992). However, these studies provide little information about the time scales organic sulfur formation and the respective implications for S recycling under anoxic conditions. A rapid and irreversible addition of sulfide into the DOM structure would provide a long term sink for S, while inorganic S species could sustain a biogeochemical recycling of sulfur in anoxic systems (Heitmann and Blodau, 2006; Heitmann et al., 2007).

As proposed in recent studies (Einsiedl et al., 2008; Hoffmann et al., 2012; Manceau and Nagy, 2012; Prietzel et al., 2011, 2007; Xia et al., 1998), XANES spectroscopy was employed to evaluate the oxidation states of S in organic matter and thus the fate of sulfide upon reaction with DOM. In our study, for both NR-HA and RHA we found an increase in peak intensity in the XANES spectra at 2471~2473 eV, supporting formation of sulfhydryl groups (R-SH) thioethers or organic polysulfides ($R\text{-}S_x\text{-}R$) upon reaction with sulfide, as suggested (Francois, 1987; Vairavamurthy and Mopper, 1987).

Besides a formation of reduced S_{org} species, spectral changes at 2475.1 eV, 2478.1 eV, and the splitting of the peak at 2480~2481 eV also indicated changes in oxidized S_{org} species. A decrease of sulfoxide S (R-SO-R) may be due to reduction to thiols (Ratasuk and Nanny, 2007), an increase of sulfones could results form reduction of sulfonate S ($R\text{-}SO_3\text{-}R$) (Hoffmann et al., 2012). A transformation of sulfate esters to sulfonate S or sulfone S ($R\text{-}SO_2\text{-}R$) was suggested as biotic transformation (Kertesz, 2000), but an abiotic formation of sulfonates was reported for a reaction of thiosulfate with organic matter (Vairavamurthy et al., 1994). This may have increased the relative contribution of sulfonates and thus led to the splitting at 2480~2481 eV, and would provide an explanation for the transformation of thiosulfate.

Changes of RHA in the peak intensities at 2471.6 and 2480.1eV were most pronounced. Assuming quinones to be hydrogenated after H_2/Pd pre-reduction at pH 6, organic S formation with RHA should not involve addition to quinones (Perlinger et al., 2002; Ratasuk and Nanny, 2007). Here, addition of S to non-quinone moieties must have occurred. As S_{org} formation per carbon was almost similar for NR-HA (S/C 0.011 to 0.006) and RHA (S/C 0.011~0.009), this supports that the formation of S_{org} by addition of S to oxidized quinone moieties is rather small. S addition may thus

occur at unsaturated C-C bonds (Vairavamurthy and Mopper, 1987) that are only little affected by the H_2/Pd pretreatment, by formation of organic polysulfides in presence of S^0, or by sulfurization of carbohydrates (van Dongen et al., 2003). Moreover, the relative increase at 2480.1 eV for both NR-HA and RHA suggests that a formation of sulfonates through reaction of $S_2O_3^{2-}$ with DOM is likely (Vairavamurthy et al., 1994).

Quantification of oxidized S_{org} species from XANES spectra is difficult. Such species would result from further reaction with thiosulfate and elemental sulfur and tracers would be needed to understand pathways of formation. Thus we could not consider the oxidized S_{org} fraction separately in the electron budget, instead kept the assumption that S_{org} is zerovalent. Nevertheless, newly formed oxidized S_{org} species would mean additional EAC of DOM towards sulfide, but eventually reduce the amount of oxidized S_{org} that may be available for biotic reduction (Kertesz, 2000).

4.3 Electron accepting capacities of DOM towards sulfide

The EAC of NR-HA towards H_2/Pd (EAC_{H2}) of 2.48 µmol e^- (mg C)$^{-1}$ was comparable to electrochemically determined EACs reported for DOM in other studies: 1.7 to 2.5 µmol e^- (mg C)$^{-1}$ for Adrich HA (Aeschbacher et al., 2010; Jiang and Kappler, 2008) and 0.6 to 2.9 µmol e^- (mg C)$^{-1}$ for PP-HA (Aeschbacher et al., 2010; Heitmann et al., 2007), determined at pH 6 and 7 (Aeschbacher et al., 2010), but much higher compared to results of 0.29~0.39 µmol e^- (mg C)$^{-1}$ from wet chemical determination (Ratasuk and Nanny, 2007). From all inorganic transformation products we also obtained an EACs(ISF) in a similar range (2.88~1.75 µmol e^- (mg C)$^{-1}$). In contrast, the earlier study with a predominant formation of $S_2O_3^{2-}$ obtained lower EACs of 0.1~0.6 µmol e^- (mg C)$^{-1}$ for Pahokee-Peat HA at pH 6 (Heitmann and Blodau, 2006).

To close the budget, we included the formation of S_{org}. The nominal oxidation state of reduced S_{org} was reported to be between 0~1 (Manceau and Nagy, 2012). We thus regarded all newly formed S_{org} to be zerovalent, providing a minimum estimate of EACs of DOM towards sulfide; transformation of oxidized S would even increase EACs (Hoffmann et al., 2012). In comparison to EAC_{H2}, EACs(TSC) of NR-HA towards sulfide was considerably larger, mainly due to formation of S_{org}, although neglecting formation of oxidized species of S_{org} (Vairavamurthy et al., 1994). Also H_2/Pd pre-reduced HA still transformed significant amounts of sulfide into S_{org}, reaching 60~80 % of EAC_{H2} determined by H_2/Pd reduction of DOM and thus questioning the significance of EAC determined by H_2/Pd in sulfidic systems. The reduction of HA by H_2/Pd obviously did not affect all sites that may potentially transfer electrons to sulfide. It is in a way surprising, though, given the fact that hydrogen is a stronger reductant (Jiang and Kappler, 2008).

To estimate their contribution to the EAC, we estimated the content of quinones of Aldrich HA in our solutions, using data from Ratasuk & Nanny (0.32 µmol e^- $(mg\ C)^{-1}$ (Ratasuk and Nanny, 2007), i.e. 4~12 µmol e^- L^{-1}) and from Aeschbacher et al. (1.70 µmol e^- $(mg\ C)^{-1}$(Aeschbacher et al., 2010), assuming all reversible sites to be quinones, i.e. 21~65 µmol e^- L^{-1}). These numbers could either explain the amount of inorganic oxidation products formed (70.4~131.2 µmol e^- L^{-1}) or also the amount of S_{org} formation, if happening via Michaels addition to quinones (Perlinger et al., 2002) (21.9~56.0 µmol S_{org} L^{-1}), but not both. As S_{org} formation via Michael addition to quinones seems to be negligible, one may hypothesize that the contribution of quinones is mainly as EAC for inorganic sulfide oxidation product formation. The amount of S to be incorporated may on the other hand be limited by the number of unsaturated C-C bonds reactive towards sulfide (Adam et al., 2000).

4.4 Kinetics of sulfide transformation by DOM

Modeled rate constants for the fast pool compared well to the same pool in the study of Heitmann and Blodau (2006) (0.206 h^{-1}). The slow pool rate constant in the latter study (0.176 h^{-1}), ascribed to S_{org} formation, also compared well to S_{org} formation rates for NR-HA in our study. The one pool model for S_{org} formation with RHA yielded again comparable rate constants based on sulfide transformation (0.103 h^{-1}). As already reported(Heitmann and Blodau, 2006), these numbers support that the oxidation rates of sulfide upon reaction with DOM, both for S^0 and organic S formation, are competitive to sulfide oxidation on poorly crystalline iron oxides (Peiffer et al., 1992; Poulton et al., 2004) and faster than sulfide oxidation by more crystalline iron oxides (Poulton et al., 2004) or by molecular oxygen (Zhang and Millero, 1993).

The native iron content of Aldrich HA did not explain the fast initial rates of sulfide transformation after injection of sulfide into the HA solution, as iron contents would only explain 21~34 % of this initial transformation (SI, Figure 9, 10). This indicates that the contribution of the native iron content to sulfide oxidation cannot be separated, neither based on the obtained transformation product S^0 (see above), nor based on the kinetics. We therefore conclude that the reactivity of iron in DOM is probably to a large extent controlled by DOM properties. Natural DOM samples may contain significant amounts of iron held in strong complexes that can also prevent iron from hydrolysis (Karlsson and Persson, 2012; Karlsson et al., 2008). A separate investigation of purified humic substances and pure iron phases or complexes may thus not be appropriate.

5 Implications for S cycling in anoxic environments

To relate our results to known biogeochemical processes and rates, we sought to compare the amount of transferred electrons to in situ data from earlier studies (Heitmann et al., 2007; Roden et al., 2010). We chose data from an ombrotrophic bog, using an average peat carbon content of 40% and a peat bulk density of 0.1 g cm^{-3}at 60 cm depth (Blodau and Moore, 2002). Assuming an EAC of particulate organic matter of 5% of dissolved HA (Klüpfel et al., 2014; Roden et al., 2010) and using an EAC of HA of 2.2 µmol e^- $(mg\ C)^{-1}$ from 50 mg C L^{-1} solutions, we obtain an EAC towards sulfide of bulk peat of 4.51 mmol e^- L^{-1}, coinciding with EAC calculated by Roden et al. (7.67 mmol e^- L^{-1}) (Roden et al., 2010), but lower than the numbers obtained by Lau et al. (61.1 mmol e^- L^{-1}) (Lau et al., 2015). Assuming this capacity to be available in 48 h, this yields electron transfer rates of 2.3 µmol e^- cm^{-3} day^{-1}. Keeping in mind that many sulfate reducing bacteria can also reduce the observed intermediates S^0 and $S_2O_3^{2-}$ (Jørgensen, 1990), such capacities may help to explain high sulfate reduction rates summarized by Pester et al., (2012) although such numbers for bulk peat EAC provide only first estimates.

Abiotic oxidation of sulfide by DOM could thus explain sulfide re-cycling under anaerobic conditions within timescales of hours, regenerating more oxidized inorganic or organic forms of S. However, this mechanism would be limited due to a finite capacity of DOM, if DOM is not cycled back to an oxidized state. Such replenishment of capacities may e.g. be induced by water table fluctuations or by inflow of non-reduced DOM (Heitmann et al., 2007; Klüpfel et al., 2014). Biotic and abiotic redox transformations of S are known for different species. On the long term, S_{org} may provide a sink for S and ongoing abiotic sulfide re-cycling via S^0 and $S_2O_3^{2-}$ would depend on external sources of sulfate (e.g., atmospheric deposition) (Heitmann and Blodau, 2006; Wieder and Lang, 1988). EAC of DOM towards sulfide was

nevertheless higher than previous results from electrochemical determination, mainly due to the large contributions of S_{org}. As HA from soil/peat environments also interact with iron, and as the contributions of iron could not be separated from bulk EAC of the HA, future work should involve studying the ternary system of Fe, S and DOM.

REFERENCES

Wieder, R. K.; Lang, G. E. Cycling of inorganic and organic sulfur in peat from Big Run Bog, West Virginia. Biogeochemistry 1988, 5, 221–242.

Wieder, R. K.; Yavitt, J.; Lang, G. Methane production and sulfate reduction in two Appalachian peatlands. Biogeochemistry 1990, 10, 81–104.

Heitmann, T.; Goldhammer, T.; Beer, J.; Blodau, C. Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog. Glob. Chang. Biol. 2007, 13, 1771–1785.

Pester, M.; Knorr, K.-H.; Friedrich, M. W.; Wagner, M.; Loy, A. Sulfate-reducing microorganisms in wetlands - fameless actors in carbon cycling and climate change. Front. Microbiol. 2012, 3, 72.

Lovley, D. R.; Phillips, E. J. P. Novel processes for anaerobic sulfate production from elemental sulfur by novel processes for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. Appl. Environ. Microbiol. 1994, 60, 2394–2399.

Ferdelman, T. G.; Church, T. M.; Luther III, G. W. Sulfur enrichment of humic substances in a Delaware salt marsh sediment core. Geochim. Cosmochim. Acta 1991, 55, 979–988.

Blodau, C.; Mayer, B.; Peiffer, S.; Moore, T. R. Support for an anaerobic sulfur cycle in two Canadian peatland soils. J. Geophys. Res. 2007, 112, 1–10.

Canfield, D. E.; Boudreau, B. P.; Mucci, A.; Gundersen, J. K. The early diagenetic formation of organic sulfur in the sediments of mangrove lake, bermuda. Geochim. Cosmochim. Acta 1998, 62, 767–781.

Novák, M.; Vile, M. A.; Bottrell, S. H.; Štěpánová, M.; Jačková, I.; Buzek, F.; Přechová, E.; Newton, R. J. Isotope systematics of sulfate-oxygen and sulfate-sulfur in six European peatlands. Biogeochemistry 2005, 76, 187–213.

Peiffer, S.; dos Santos Afonso, M.; Wehrli, B.; Gaechter, R. Kinetics and mechanism of the reaction of hydrogen sulfide with lepidocrocite. Environ. Sci. Technol. 1992, 26, 2408–2413.

Hellige, K.; Pollok, K.; Larese-Casanova, P.; Behrends, T.; Peiffer, S. Pathways of ferrous iron mineral formation upon sulfidation of lepidocrocite surfaces. Geochim. Cosmochim. Acta 2012, 81, 69–81.

Lovley, D. R.; Coates, J. D.; Blunt-Harris, E. L.; Phillips, E. J. P.; Woodward, J. C. Humic substances as electron acceptors for microbial respiration. Nature 1996, 382, 445–448.

Aeschbacher, M.; Sander, M.; Schwarzenbach, R. P. Novel electrochemical approach to assess the redox properties of humic substances. Environ. Sci. Technol. 2010, 44, 87–93.

Uchimiya, M.; Stone, A. T. Reversible redox chemistry of quinones: impact on biogeochemical cycles. Chemosphere 2009, 77, 451–458.

Roden, E. E.; Kappler, A.; Bauer, I.; Jiang, J.; Paul, A.; Stoesser, R.; Konishi, H.; Xu, H. Extracellular electron transfer through microbial reduction of solid-phase humic substances. Nat. Geosci. 2010, 3, 417–421.

Klüpfel, L.; Piepenbrock, A.; Kappler, A.; Sander, M. Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nat. Geosci. 2014, 7, 195–200.

Perlinger, J. A.; Kalluri, V. M.; Venkatapathy, R.; Angst, W. Addition of hydrogen sulfide to juglone. Environ. Sci. Technol. 2002, 36, 2663–2669.

Heitmann, T.; Blodau, C. Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. Chem. Geol. 2006, 235, 12–20.

Adam, P.; Schneckenburger, P.; Schaeffer, P.; Albrecht, P. Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules. Geochim. Cosmochim. Acta 2000, 64, 3485–3503.

Vairavamurthy, A.; Mopper, K. Geochemical formation of organosulphur compounds (thiols) by addition of H_2S to sedimentary organic matter. Nature 1987, 329, 623–625.

Van Dongen, B. E.; Schouten, S.; Baas, M.; Geenevasen, J. A. J.; Sinninghe Damstéa, J. S. An experimental study of the low-temperature sulfurization of carbohydrates. Org. Geochem. 2003, 34, 1129–1144.

Hoffmann, M.; Mikutta, C.; Kretzschmar, R. Bisulfide reaction with natural organic matter enhances arsenite sorption: insights from X-ray absorption spectroscopy. Environ. Sci. Technol. 2012, 46, 11788–11797.

Xia, K.; Weesner, F.; Bleam, W. F.; Helmke, P. A.; Bloom, P. R.; Skyllberg, U. L. XANES studies of oxidation states of sulfur in aquatic and soil humic substances. Soil Sci. Soc. Am. J. 1998, 62, 1240–1246.

Wan, M.; Shchukarev, A.; Lohmayer, R.; Planer-Friedrich, B.; Peiffer, S. Occurrence of surface polysulfides during the interaction between ferric (hydr)oxides and aqueous sulfide. Environ. Sci. Technol. 2014, 48, 5076–5084.

Einsiedl, F.; Mayer, B.; Schäfer, T. Evidence for incorporation of H_2S in groundwater fulvic acids from stable isotope ratios and sulfur K-edge X-ray absorption near edge structure spectroscopy. Environ. Sci. Technol. 2008, 42, 2439–2444.

Prietzel, J.; Thieme, J.; Salomé, M.; Knicker, H. Sulfur K-edge XANES spectroscopy reveals differences in sulfur speciation of bulk soils, humic acid, fulvic acid, and particle size separates. Soil Biol. Biochem. 2007, 39, 877–890.

Ratasuk, N.; Nanny, M. A. Characterization and quantification of reversible redox sites in humic substances. Environ. Sci. Technol. 2007, 41, 7844–7850.

Cline, J. D. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 1969, 14, 454–458.

Prietzel, J.; Botzaki, A.; Tyufekchieva, N.; Brettholle, M.; Thieme, J.; Klysubun, W. Sulfur speciation in soil by S K-Edge XANES spectroscopy: comparison of spectral

deconvolution and linear combination fitting. Environ. Sci. Technol. 2011, 45, 2878–2886.

Manceau, A.; Nagy, K. L. Quantitative analysis of sulfur functional groups in natural organic matter by XANES spectroscopy. Geochim. Cosmochim. Acta 2012, 99, 206–223.

Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. J. Synchrotron Radiat. 2005, 12, 537–541.

Rickard, D.; Luther III, G. W. Chemistry of iron sulfides. Chem. Rev. 2007, 107, 514–562.

Urban, N. R.; Ernst, K.; Bernasconi, S. Addition of sulfur to organic matter during early diagenesis of lake sediments. Geochim. Cosmochim. Acta 1999, 63, 837–853.

Chen, K. Y.; Morris, J. C. Kinetics of oxidation of aqueous sulfide by oxygen. Environ. Sci. Technol. 1972, 6, 529–537.

Poulton, S. W.; Krom, M. D.; Raiswell, R. A revised scheme for the reactivity of iron (oxyhydr)oxide minerals towards dissolved sulfide. Geochim. Cosmochim. Acta 2004, 68, 3703–3715.

Rodríguez, F. J.; Schlenger, P.; García-Valverde, M. A comprehensive structural evaluation of humic substances using several fluorescence techniques before and after ozonation. Part I: Structural characterization of humic substances. Sci. Total Environ. 2014, 476-477, 718–730.

Malcolm, R. L.; MacCarthy, P. Limitations in the use of commercial humic acids in water and soil research. Environ. Sci. Technol. 1986, 20, 904–911.

Evangelou, V. P.; Zhang, Y. L. A review: pyrite oxidation mechanisms and acid mine drainage prevention. Crit. Rev. Environ. Sci. Technol. 1995, 25, 141–199.

Moses, C. O.; Nordstrom, D. K.; Herman, J. S.; Mills, A. L. Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. Geochim. Cosmochim. Acta 1987, 51, 1561–1571.

Jørgensen, B. B. The sulfur cycle of freshwater sediments: Role of thiosulfate. Limnol. Oceanogr. 1990, 35, 1329–1342.

Vairavamurthy, A.; Zhou, W.; Eglinton, T.; Manowitz, B. Sulfonates: a novel class of organic sulfur compounds in marine sediments. Geochim. Cosmochim. Acta 1994, 58, 4681–4687.

Francois, R. A study of sulfur enrichment in the humic fraction of marine-sediments during early diagenesis. Geochim. Cosmochim. Acta 1987, 51, 17–27.

Vairavamurthy, A.; Mopper, K.; Taylor, B. F. Occurrence of particle-bound polysulfides and significance of their reaction with organic matters in marine sediments. Geophys. Res. Lett. 1992, 19, 2043–2046.

Brown, K. A. Formation of organic sulphur in anaerobic peat. Soil Biol. Biochem. 1986, 18, 131–140.

Brüchert, V.; Pratt, L. M. Contemporaneous early diagenetic formation of organic and inorganic sulfur in estuarine sediments from St. Andrew Bay, Florida, USA. Geochim. Cosmochim. Acta 1996, 60, 2325–2332.

Kertesz, M. A. Riding the sulfur cycle-metabolism of sulfonates and sulfate esters in Gram-negative bacteria. FEMS Microbiol. Rev. 2000, 24, 135–175.

Jiang, J.; Kappler, A. Kinetics of microbial and chemical reduction of humic substances: implications for electron shuttling. Environ. Sci. Technol. 2008, 42, 3563–3569.

Zhang, J.-Z.; Millero, F. J. Environmental Geochemistry of Sulfide Oxidation; Alpers, C. N.; Blowes, D. W., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 1993; Vol. 550, pp. 393–409.

Karlsson, T.; Persson, P. Complexes with aquatic organic matter suppress hydrolysis and precipitation of Fe(III). Chem. Geol. 2012, 322-323, 19–27.

Karlsson, T.; Persson, P.; Skyllberg, U.; Mörth, C.-M.; Giesler, R. Characterization of iron(III) in organic soils using extended X-ray absorption fine structure spectroscopy. Environ. Sci. Technol. 2008, 42, 5449–5454.

Blodau, C.; Moore, T. R. Macroporosity affects water movement and pore water sampling in peat soils. Soil Sci. 2002, 98–109.

Lau, M. P.; Sander, M.; Gelbrecht, J.; Hupfer, M. Solid phases as important electron acceptors in freshwater organic sediments. Biogeochemistry 2014.

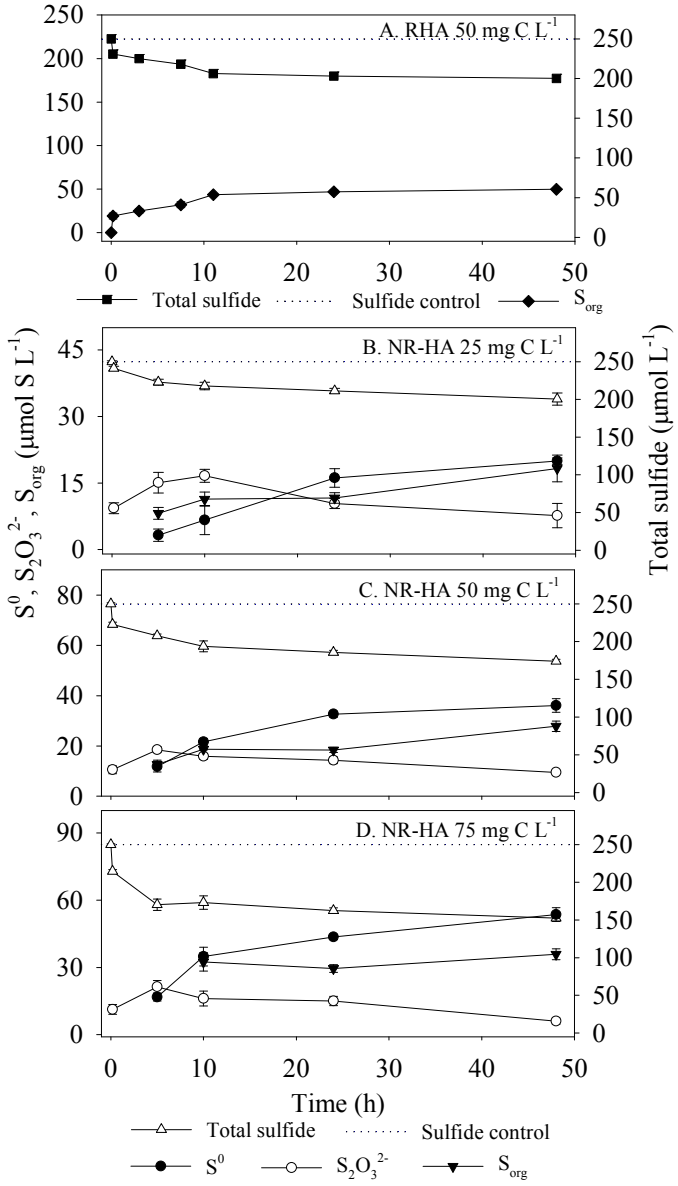


Figure 2. Transformation of sulfide over time upon reaction with Aldrich HA.

A.RHA 50 mg C L^{-1} DOC: transformation of total sulfide (left *y*-axis) into organic S (right *y*-axis) species by 50 mg C L-1 of H_2/Pd reduced Aldrich HA (see SI, Figure 9 for 25, 75 mg C L^{-1} of RHA); **B, C and D** represent sulfide (left *y*-axis) transformation of non-reduced Adrich HA solutions (NR-HA, A: 25, B: 50, and C: 75 mg C L^{-1}) into inorganic S species (S^0, $S_2O_3^{2-}$) and organic S (right axis). The abiotic incubations lasted for 48 hours at pH 6, 25° C, under anoxic conditions. Values are means ± SD (n=3).

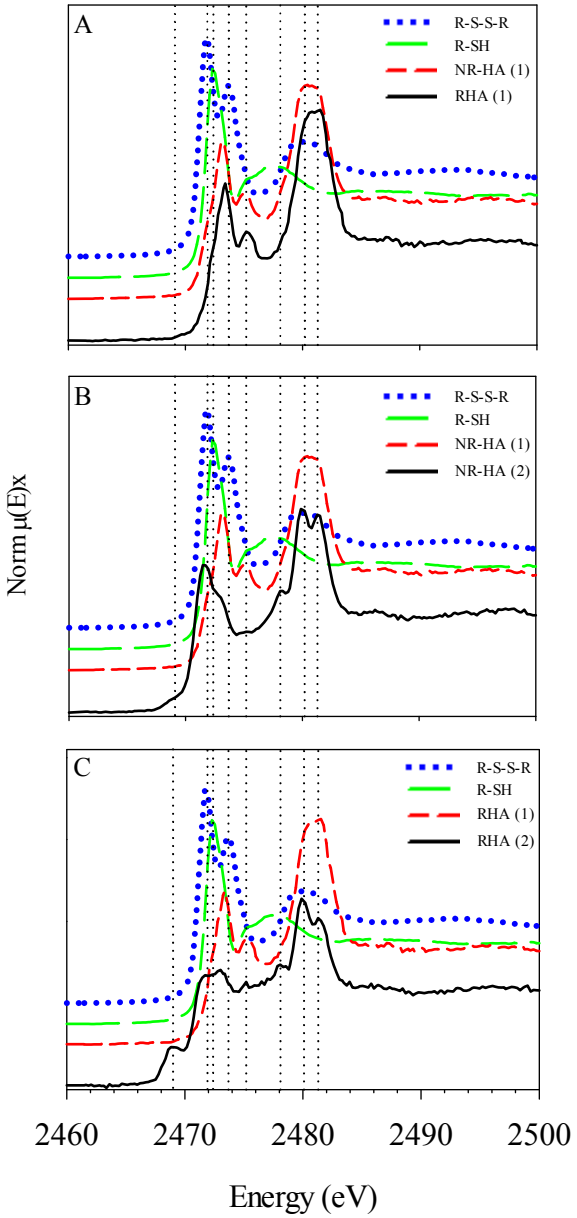


Figure 3. S-K-edge XANES spectra of non-reduced (NR-HA) and reduced Aldrich humic acid (RHA) before and after reaction upon sulfide. RHA (1) and NR-HA (1) is prior to, RHA (2) and NR-HA (2) after reaction with sulfide. The difference in the spectra only gives information about relative changes of individual electronic oxidation states. Markers (vertical dotted lines) are at 2469, 2471.6, 2472.6, 2473.7, 2475.2, 2478.1, 2480.2 and 2481.2 eV. See Table 2 for corresponding references.

Table 2. Measured sulfur model compounds with different electronic oxidation states (EOS) and predominant S-K edge XANES peaks as observed for non-reduced (NR-HA) and reduced humic acid (RHA) samples before and after reaction towards sulfide

Compounds; Molecular formula	Peak max.	EOS	Ref. ***	Peak max. (eV) Non-reduced HA		Reduced HA	
	(eV)			Before	After	Before	After
Iron mono-sulfides: FeS	2470.2	-2	[1]				
Pyrite: FeS_2	2471.1	-1	[1]				
Elemental sulfur: S^0	2471.5	0	[1]				
5,5'-Dithiobis (2-nitrobenzoic acid); ($[-SC_6H_3(NO_2)CO_2H]_2$) *	2471.6	+0~1	[2,3]		2471.6		2471.6
	2473.7			2473.1	2473.1	2473.1	2473.1
L-cysteine: $C_6H_{12}N_2O_4S_2$ **	2472.6	+0.2	[1,3]		2472.6		2472.6
Dibenzothiophene: $C_{12}H_8S$	2472.9	+0~1	[2,3]				
Methyl phenyl sulfoxide: $CH_3SOC_6H_5$	2475.2	+2	[1, 2 ,3]	2475.1		2475.1	
Dimethyl sulfone: $(CH_3)_2SO_2$	2479.1	+4	[1, 2 ,3]		2478.2		2478.2
Sodium methanesulfonate: CH_3SO_3Na	2480.2	+5	[1, 2 ,3]	(~2480)	2480.2	(~2480)	2480.2
Sulfuric acid mono(2-aminoethyl) ester: $C_2H_7NO_4S$	2481.5	+6	[1, 2 ,3]		2481.3		2481.3
Sodium sulfate: Na_2SO_4	2481.5	+6	[1, 2 ,3]				

* Representing organic disulfides (R-SS-R bridging structures); two peaks were observed; one at 2471.6 eV had a higher relative intensity than the one at 2473.7 eV.

** Representing thiols (R-SH)

*** [1] Prietzel et al., 2007; [2] Xia et al., 1998; [3] Ratasuk and Nanny, 2007;

Note: the value in set brackets may not only represent one peak.

Table 3. Calculation of the S mass balance and electron accepting capacities (EAC) of NR-HA and RHA

A: Calculation of the Sulfur mass balance	
IS(-II)	Initial S(-II) in solution
RS(-II)	Remaining S(-II) in solution after 48-h reaction time
TSC= IS(-II) - RS(-II)	Total S(-II) consumption
ISF = ISF (S^0) + ISF ($S_2O_3^{2-}$)	Inorganic sulfur product formation; identified inorganic oxidation Products S^0 and $S_2O_3^{2-}$
S_{org} = TSC - ISF	Organic sulfur formation
B: Electron accepting capacity (EAC) of NR-HA and RHA	
$EAC_S(TSC) = EAC(ISF) + EAC(S_{org})$	Total EAC of humic acid towards reaction with sulfide
$EAC_S(ISF) = ISF(S_0) \cdot 2e^- + ISF(S_2O_3^{2-}) \cdot 8e^-$	EAC from inorganic sulfur product formation
$EAC_S(S_{org}) = S_{org} \cdot 2e^-$	EAC from organic sulfur formation (assuming S_{org} to be zerovalent, see section 3.3)
$EAC_{H2}(HA) = U_{H2}$ (48 h) $\cdot 4e^-$	EAC of NR-HA as obtained from H_2 uptake (U_{H2}) after 48 hours reduction with H_2 and Pd catalyst
C: Stoichiometries and electron transfer for sulfide oxidation by HA	
1) Formation of $S_2O_3^{2-}$ and S^0	
Eq. 1 $4\ DOM\text{-}Q + 2\ H_2S + 3\ H_2O \rightarrow 4\ DOM\text{-}QH_2 + S_2O_3^{2-} + 2\ H^+$	Eq. 2 ($2H_2S \xrightarrow{-8e} S_2O_3^{2-}$)
Eq. 3 $DOM\text{-}Q + H_2S \rightarrow DOM\text{-}QH_2 + S^0$	Eq. 4 ($S^{2-} \xrightarrow{-2e} S^0$)
2) Formation of organic sulfur	
Eq. 5 $DOM\text{-}Q + S^{2-} \rightarrow S_{org}$ *	Eq. 7 ($S^{2-} \xrightarrow{-2e} S_{org}$) ***
Eq. 6 $DOM + S^{2-} \rightarrow S_{org}$ **	

[#]-Q and –Q-H_2 denote oxidized and reduced quinone moieties (most important redox active functional group in DOM), respectively

*As suggested by Perlinger et al. (Perlinger et al., 2002) for juglone and other oxidized quinones;

**Addition of sulfur to non-quinone moieties of organic matter

***see section 3.3, organic sulfur formed assumed to be zerovalent.

Table 4. Sulfur and hydrogen mass balance and electron balance for reactions of non-reduced (NR-HA) and H_2/Pd pre-reduced Aldrich humic acid (RHA) with sulfide. For terms and definitions, see methods section

	batch	H_2[(a)]	TSC	ISF(S^0)	ISF($S_2O_3^{2-}$)	S_{org}
Mass balance in µmol L^{-1}	NR-HA 25 mg C L^{-1}	15.5 ± 1.0	49.5 ± 3.5	20.0 ± 0.7	3.8 ± 0.4	21.9 ± 3.1
	NR-HA 50 mg C L^{-1}	31.0 ± 1.2	76.0 ± 5.1	36.0 ± 2.7	4.7 ± 0.2	30.6 ± 2.1
	NR-HA 75 mg C L^{-1}	46.5 ± 2.1	97.5 ± 6.9	53.6 ± 3.1	3.0 ± 0.7	37.9 ± 2.4
	RHA 25 mg C L^{-1}	-	22.5 ± 1.1	-	-	22.5 ± 1.1
	RHA 50 mg C L^{-1}	-	44.9 ± 3.4	-	-	49.9 ± 3.4
	RHA 75 mg C L^{-1}	-	56 ± 6.2	-	-	56 ± 6.2
Factor[(b)]		4	(2-2.3)[(d)]	2	8	2
Electron balance in µmol e^- L^{-1}	NR-HA 25 C mg L^{-1}	62.0 ± 3.1	114.2 ± 6.3	40.0 ± 1.3	30.4 ± 3.1	43.8 ± 5.8
	NR-HA 50 C mg L^{-1}	124.0 ± 5.2	170.8 ± 9.2	72.0 ± 5.5	37.6 ± 1.2	61.2 ± 4.3
	NR-HA 75 C mg L^{-1}	186.0 ± 8.7	207.0 ± 11.2	107.2 ± 7.0	24.0 ± 5.2	75.8 ± 4.6
	RHA 25 C mg L^{-1}	-	45.0 ± 1.9	-	-	45.0 ± 1.9
	RHA 50 C mg L^{-1}	-	99.8 ± 6.5	-	-	99.8 ± 6.5
	RHA 75 C mg L^{-1}	-	112 ± 8.3	-	-	112 ± 8.3

[(a)] determined in a 150 mg L^{-1} stock solution and recalculated for 25, 50, and 75 mg C L^{-1}

[(b)] electron transfer per species formed/consumed

[(c)] calculated from ISF(S^0), ISF($S_2O_3^{2-}$) and S_{org}

Supporting information for Study 1

1. Chemical composition of the Sigma Aldrich HA employed in th study

In addition to C/H/N contents provided by Sigma-Aldrich, elemental composition was detected by energy-dispersive X-ray fluorescence (XRF) spectrometry (X-Lab 2000, Spectro), mixing 2 gram of milled humic acid powder with 1 g of XRF wax (Licowax C, APC Solutions SA). This mixture was pressed to a 3.2-cm pellet for subsequent analysis.

Table 5. Elemental composition for used humic acid (Sigma-Adrich)

Sigma-adrich humic acid		S	Fe	C	H	N	S/C
	solid %	0.45	1.33	40.15	3.60	0.92	
25 mg C L^{-1}	*dissolved*	0.28 mg L^{-1} (8.75 µmol L^{-1})	0.83 mg L^{-1} (14.8 µmol L^{-1})	25 mg L^{-1} (2.08 mmol L^{-1})	0.9 mg L^{-1} (0.9 mmol L^{-1})	0.57 mg L^{-1} (41 µmol L^{-1})	0.0042
50 mg C L^{-1}	*dissolved*	0.56 mg L^{-1} (17.5 µmol L^{-1})	1.66 mg L^{-1} (29.6 µmol L^{-1})	50 mg L^{-1} (4.16 mmol L^{-1})	1.8 mg L^{-1} (1.8 mmol L^{-1})	1.14 mg L^{-1} (82 µmol L^{-1})	0.0042
75 mg C L^{-1}	*dissolved*	0.84 mg L^{-1} (26.3 µmol L^{-1})	2.49 mg L^{-1} (44.4 µmol L^{-1})	75 mg L^{-1} (6.24 mmol L^{-1})	2.7 mg L^{-1} (2.7 mmol L^{-1})	1.71mg L^{-1} (123 µmol L^{-1})	0.0042

Note: For HA before and after filtration through a 0.45 µm membrane, no difference for iron content was detected

2. Reduction of Aldrich HA by H_2/Pd at pH 6

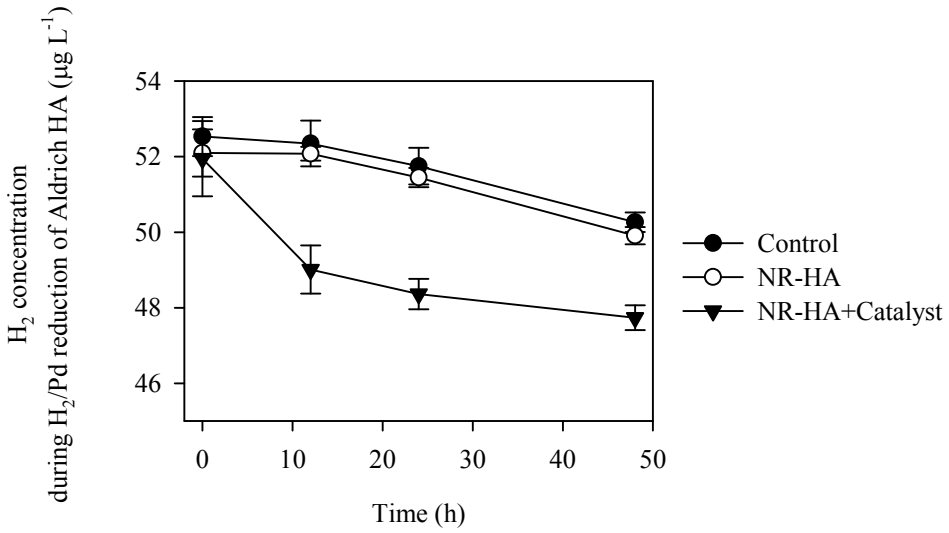


Figure 4. Consumption of H_2 over time for reduction of non-reduced humic acid stock solution (150 mg C L^{-1}). Values are means ± SD (n=3). Control: Ultra-pure water plus Pd-catalyst; NR-HA: 150 mg C L^{-1} Sigma Aldrich humic acid solution with H_2 addition, but without addition of Pd-catalyst; NR-HA+Catalyst: 150 mg C L^{-1} Sigma Aldrich humic acid solution with addition of both H_2 and Pd-catalyst

3. S-K-edge XANES spectra of reference compounds

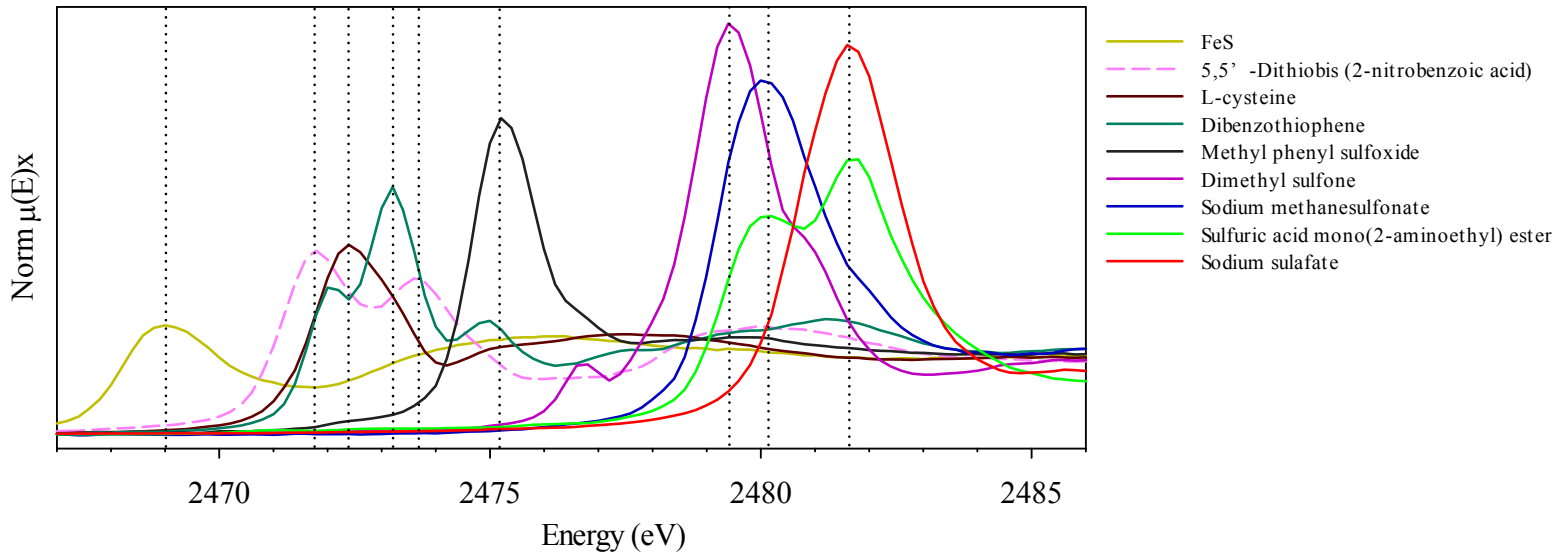


Figure 5. S-K-edge XANES spectra of measured sulfur model compounds with different electronic oxidation states. Markers (vertical dotted line) are at 2469, 2471.6, 2472.6, 2473.1, 2475.1, 2478.1, 2480.2 and 2481.2 eV

4. Sulfur and hydrogen mass balance in terms of electron equivalents

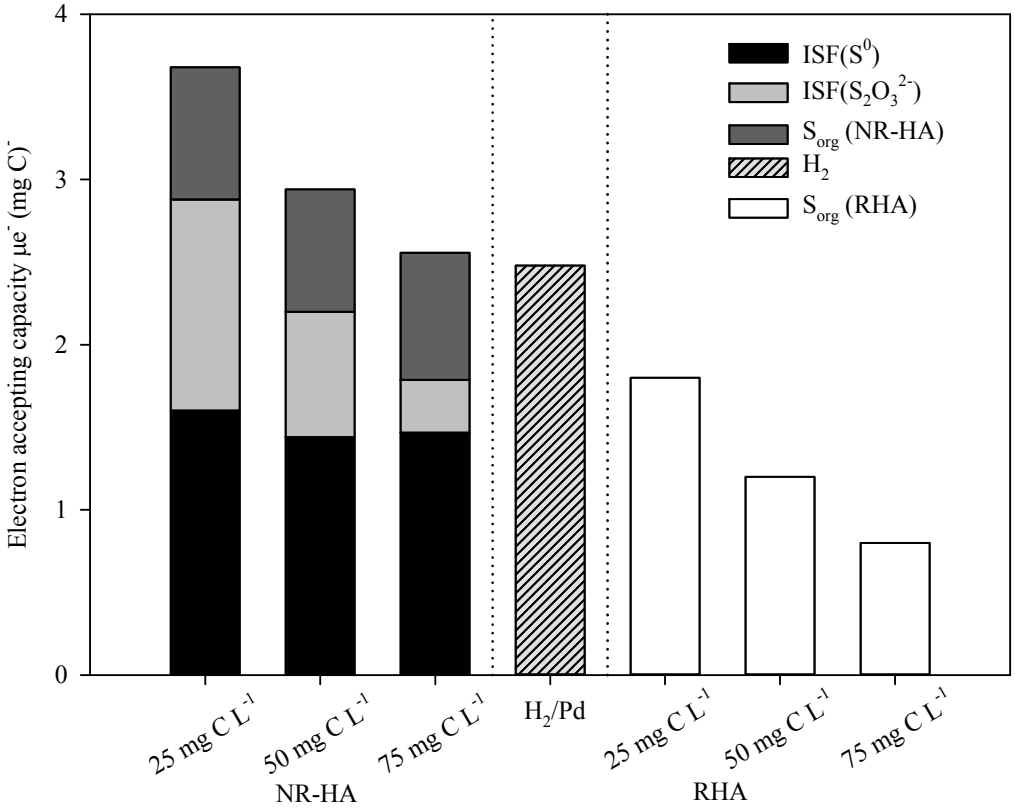


Figure 6. Sulfur and hydrogen mass balance in electron equivalents for reactions of non-reduced (NR-HA) and H_2/Pd pre-reduced Aldrich humic acid (RHA) with sulfide. For terms and definitions, see methods section.

5. Kinetic modeling of sulfide transformation

Consumption of sulfide: Based on the observation of $S_2O_3^{2-}$ and elemental S that formed quickly after the initiation of the reaction, we ascribed a reactive site *q* for the fast chemical oxidation of sulfide by non-reduced HA. For the slower ongoing processes of continuing S^0 formation and addition of sulfur into the organic structure to quinone/non-quinone moieties we ascribed another reactive site *x*, as an additional, slower sulfide consuming process. Assuming rates being pseudo-first order with respect to *q* and *x* (non

limiting supply of sulfide in solution), the overall rate equation for the consumption of sulfide comprising both oxidations by quinones and incorporation into organic matter can be described as:

$$d[H_2S] / dt = -k1 \cdot q - k2 \cdot x \qquad \text{Eq. S1}$$

This two pool model was fitted for non-reduced HA:

$$H_2S\ (t) = IS(-II) \cdot [q \cdot e^{-k1 \cdot t} + x \cdot e^{-k2 \cdot t} + (1 - q - x)] \qquad \text{Eq. S2}$$

Due to the more uniform reaction kinetics for RHA, no fast pool *q* could be identified from kinetic modeling and thus a one pool fitting resulted for reduced HA:

$$H_2S\ (t) = IS(-II) \cdot [x \cdot e^{-k3 \cdot t} + (1 - x)] \qquad \text{Eq. S3}$$

in which IS(-II) is the total initial sulfide concentration. The term *q* is the fraction of total sulfide consumption by the fast reacting pool, *x* represents the slow pool, and *(1-q-x)* is the non-reacted sulfide remaining in solution. Values of *k*i are the conditional, pseudo-first order rate constants, which were derived by nonlinear regression of sulfide concentration over time.

Table 6. Modeled kinetic parameters for sulfide transformation and related products formation*

		Sulfide transformation									
Fitted	mg C L^{-1}	k_1	k_2	*q*%	*x*%	*(1-q-x)*%	r^2	R_1	R_2	$t_{1/2\,(1)}$	$t_{1/2\,(2)}$
NR-HA	*25*	0.23 ± 0.06	0.012 ± 0.001	13.3	9.3	77.5	0.99	5.42	6.70	3.1	59.2
2 pools	*50*	0.29 ± 0.05	0.025 ± 0.001	14.0	10.5	76.8	0.99	5.32	6.39	2.4	28.2
	75	0.32 ± 0.14	0.029 ± 0.003	19.6	11.1	69.6	0.99	5.23	6.32	2.1	24.3
Fitted	mg C L^{-1}		k_3	*q*%	*x*%	*(1-x)*%	r^2		R_3		$t_{1/2(3)}$
RHA	*25*		0.098 ± 0.013	×	9.1	91.4	0.98		5.78		7.1
1 pool	*50*		0.099 ± 0.028	×	14.1	86.4	0.98		5.78		6.9
	75		0.113 ± 0.030	×	14.7	88.3	0.97		5.72		6.1

* R_i (mol min^{-1} mg^{-1}) = -log [k_i (S_{tot})] based on dissolved carbon (mg C L^{-1}) where k_i is the sulfide consumption rate constant (h^{-1}).Rates and reaction conditions of sulfide transformation recalculated for room temperature (297–299 K), for similar ionic strength (0.05 M), and for an initial sulfide concentration of 1 mM; Half-life for reaction i was calculated as $t_{1/2}(i) = \ln(2)/k_i$;

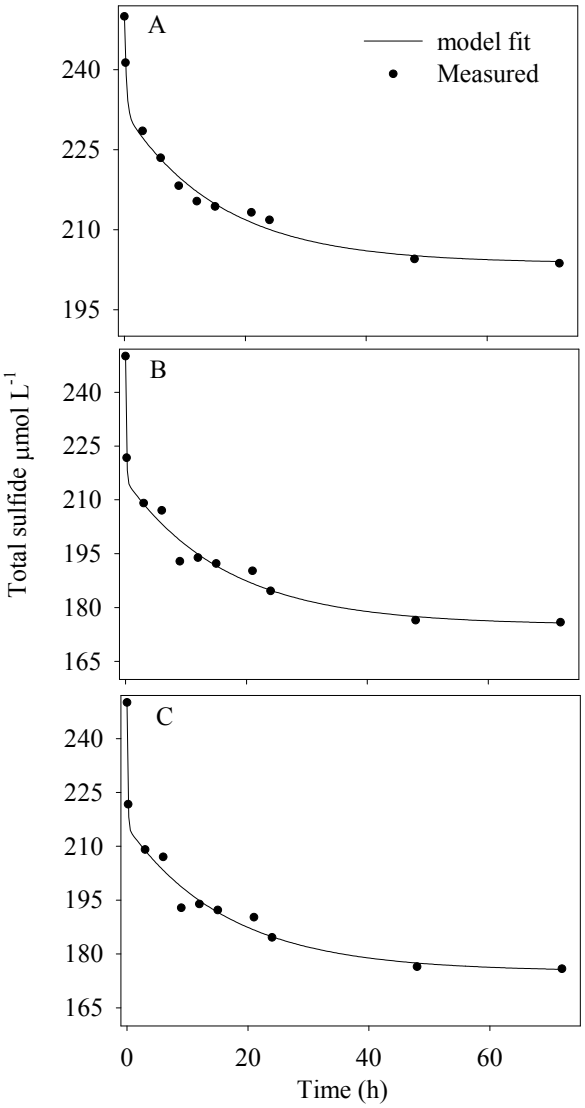


Figure 7. Measured and fitted time series of sulfide react with Non-reduced HA. A-C represents 25-75 mg C L^{-1} DOC concentration batch, respectively. A, R^2 = 0.99, SEE = 3.23; B, R^2 = 0.99, SEE = 3.61; C, R^2 = 0.99, SEE = 3.50;

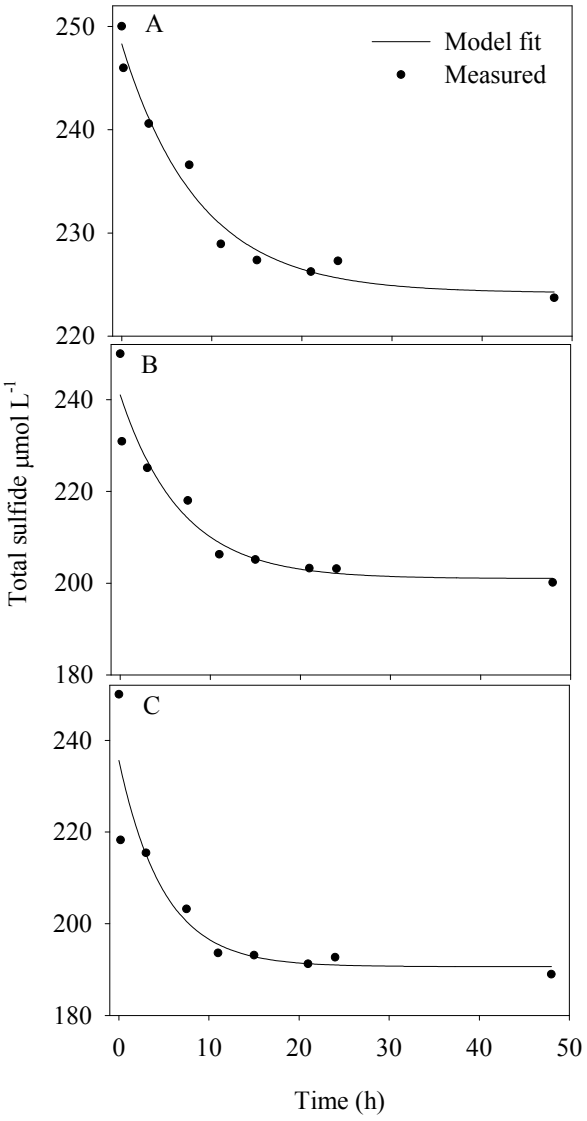


Figure 8. Measured and fitted time series of sulfide react with reduced HA. A-C represents 25-75 mg C L^{-1} DOC concentration batch, respectively. A, R^2 = 0.98, SEE = 2.44; B, R^2 = 0.98, SEE = 5.51; C, R^2 = 0.97, SEE = 6.64;

6. Initial kinetics of sulfide transformation and native iron content of Aldrich HA

To determine sulfide concentrations for kinetic modeling, we started sampling for sulfide immediately after spiking of the initial amount of sulfide into the solutions of each treatment ($t < 10$ s). Interestingly, despite immediate collection of the initial sample, we found that there was an instantaneous loss of sulfide that was related to the HA concentration of the treatments, and thus also to the HA-derived iron present in solution. However, iron explained only about 21~34 % of this initial transformation for NR-HA. As a similar effect was also observed for RHA in which we assume iron to be present in its reduced form, the initial fast kinetics of NR-HA can presumably not be explained by the native iron content of Aldrich HA.

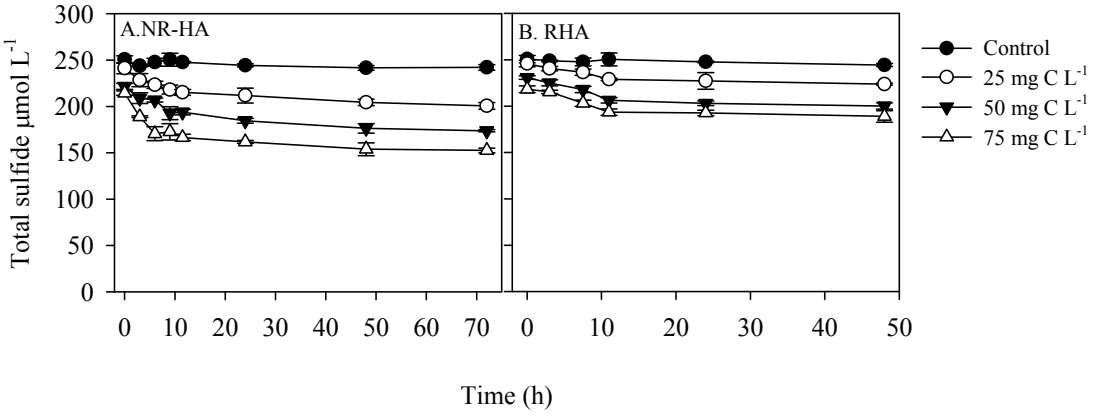


Figure 9. Transformation of sulfide over time upon reaction with non-reduced (A) and reduced humic acid (B). Values are means ± SD (n=3)

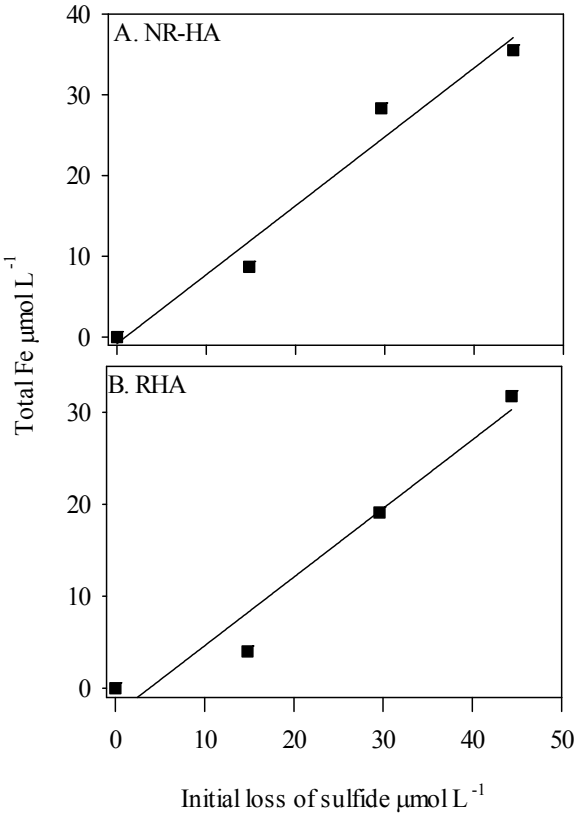


Figure 10. Correlation between total native iron content in humic acid and sulfide transformation based on sampling directly after addition of sulfide to non-reduced (A) and reduced humic acid (B). Total iron in solution here was native iron present in Sigma Aldrich humic acid; Initial sulfide loss represents the difference between initially ***added*** amount of sulfide and ***measured*** concentration at concentration t=0 **immediately *after addition***. Regressions: A. Sulfide-loss = 0.91·Fe-content - 2.63, R^2 = 0.93; B. Sulfide-loss = 0.94· Fe-content - 9.45, R^2 = 0.99)

7. Summary of electron accepting capacities

Table 7. Electron accepting capacities of Sigma Aldrich humic acid upon reaction with sulfide

Unit for all	batch	H_2	TSC	ISF(S^0)	ISF($S_2O_3^{2-}$)	S_{org}
μmol e^- (mg C)$^{-1}$	NR-HA 25 mg C L^{-1}	2.48 ± 0.13	4.57 ± 0.23	1.60 ± 0.04	1.22 ± 0.12	1.75 ± 2.1
	NR-HA 50 mg C L^{-1}	2.48 ± 0.13	3.41 ± 0.11	1.44 ± 0.11	0.75 ± 0.03	1.23 ± 0.09
	NR-HA 75 mg C L^{-1}	2.48 ± 0.13	2.76 ± 0.13	1.43 ± 0.09	0.32 ± 0.02	1.01 ± 0.07
	RHA 25 mg C L^{-1}	-	1.80 ± 0.08	-	-	1.80 ± 0.08
	RHA 50 mg C L^{-1}	-	1.99 ± 0.12	-	-	1.90 ± 0.12
	RHA 75 mg C L^{-1}	-	1.49 ± 0.11	-	-	1.49 ± 0.11

8. Impact of traces of residual oxygen on sulfide oxidation product formation

In order to verify our experimental approach, preliminary batch experiments were conducted involving similar, tightly stoppered anoxic serum bottles, but sampling the batches outside of the glove box, thus introducing traces of oxygen during sampling. In these solutions, the sulfide concentrations decreased notably faster (about 130 µmol L^{-1} within 40 h) (Figure 11), even in batches of RHA (Figure 12), and sulfide was mainly transformed into $S_2O_3^{2-}$ (concentration up to 22 µmol L^{-1} after 48 h). We thus hypothesize that small leakages due to handling outside the glovebox may stimulate formation of $S_2O_3^{2-}$ instead of S^0, but this assumption needs further support.

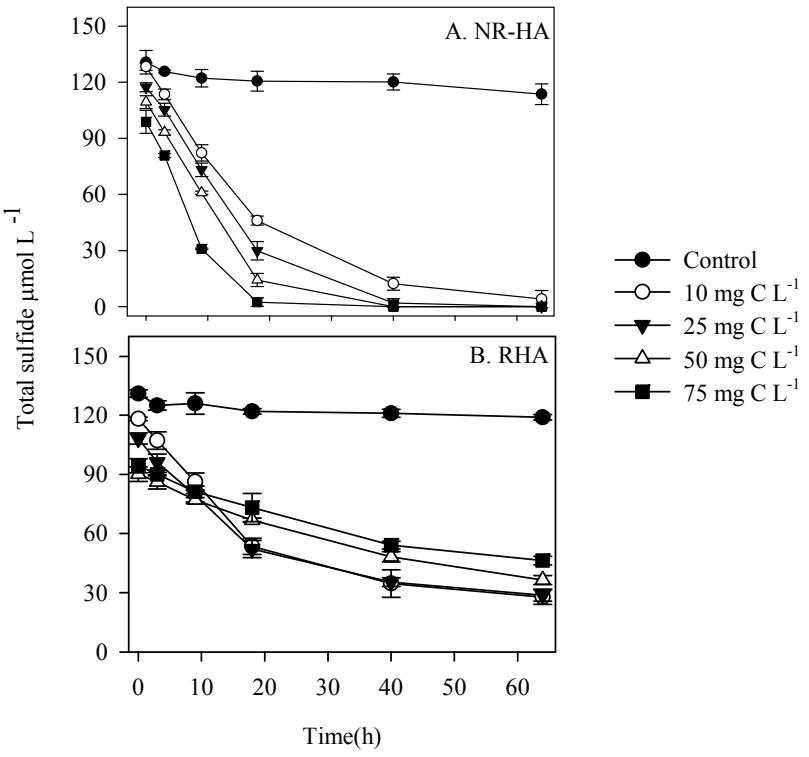


Figure 11. Transformation of sulfide over time upon reaction with reduced humic acid (A) and non-reduced humic acid (B) in an experiment where the sampling procedure was conducted outside the glove box. Values are mean ± SD (n=3)

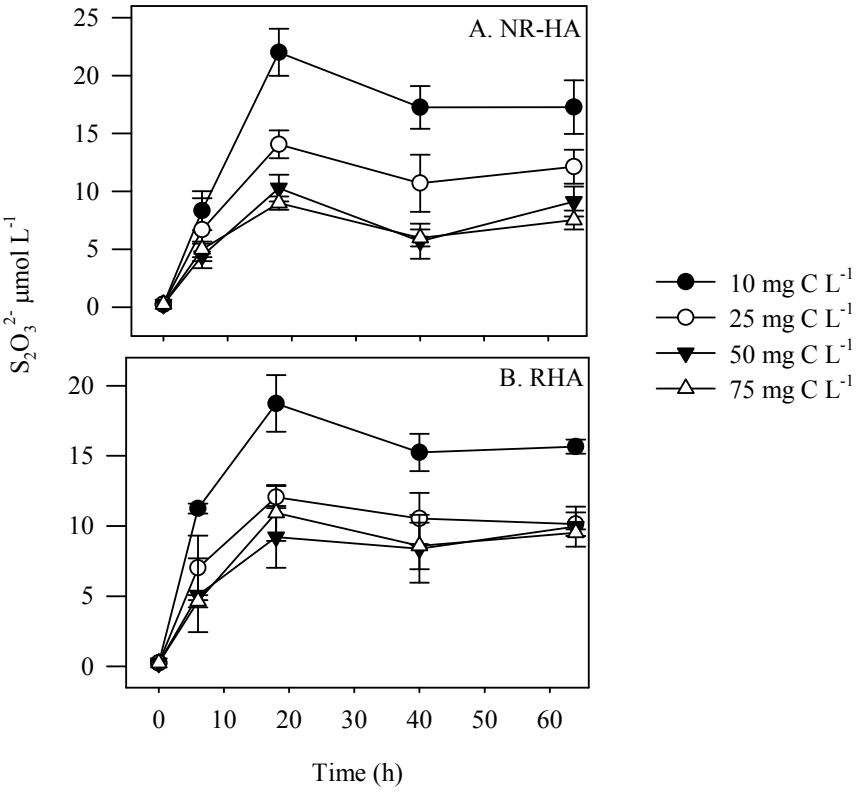


Figure 12. Concentrations of thiosulfate over time in batches of sulfide reacting with non-reduced (A) and reduced HA solution (B) in an experiment where the sampling procedure was conducted outside the glove box. Values are mean ± SD (n=3)

STUDY 2: Electron transfer between sulfide and humic acid: electrochemical evaluation of the reactivity of Sigma Aldrich humic acid towards sulfide

Zhi-Guo Yu, Silvia Orsetti, Stefan B. Haderlein, Klaus-Holger Knorr

Submitted to *Aquatic Geochemistry* on 11 May 2015

Electron transfer between sulfide and humic acid: electrochemical evaluation of the reactivity of Sigma Aldrich humic acid towards sulfide

Zhi-Guo Yu[1, 2], Silvia Orsetti[3], Stefan B. Haderlein[3], Klaus-Holger Knorr[1, 2,]*

[1]Limnological Research Station & Department of Hydrology, University of Bayreuth, Universitätsstrasse 30, 95447 Bayreuth, Germany

[2]present address: Hydrology Group, Institute of Landscape Ecology, University of Münster, 48149 Münster, Germany

[3]Eberhard Karls Universität Tübingen, Center for Applied Geosciences (ZAG), Hölderlinstrasse 12, 72074 Tübingen, Germany

*Corresponding author, kh.knorr@uni-muenster.de Tel: +49-251-8330207

ABSTRACT

Little is known of potential reactivity and redox properties of reduced dissolved organic matter (DOM), although DOM in anoxic environments, e.g. groundwaters, peat soils or lake sediments, can be expected to differ from DOM of oxidized environments. We therefore investigated the impact of electrochemical and wet chemical (hydrogen (H_2)/Pd-catalyst) reduction of Sigma Aldrich humic acid (HA) on its reactivity towards sulfide. Mediated electrochemical measurement showed that the reactivity of HA towards sulfide decreased in the order non-reduced HA > electrochemically reduced (-0.1V) HA > H_2/Pd-reduced HA > electrochemically reduced (-0.4 V) HA. Results indicated that measured initial values of electron accepting capacities (EAC) of HA had a strongly positive correlation with the sulfide transformation, except for the H_2/Pd treatment of HA. This latter treatment obviously changed HA structures and lead to a different reactivity towards sulfide, limiting a direct comparison to electrochemically reduced organic matter. Our result confirmed that reduced HA was still reactive towards sulfide, although to a lower extent compared to oxidized HA. Compared to electrochemical reduction, H_2/Pd pre-treatment of HA alters redox properties and reactivity of organic matter and may therefore lead to results that cannot be transferred to natural systems.

KEYWORDS Mediated electrochemical reduction; Fresh water; Anoxic environments; Electron transfer; Dissolved organic matter

INTRODUCTION

The main redox active functional groups in DOM is hypothesized to be quinone moieties (Lovley et al., 1996; Peretyazhko and Sposito, 2006; Scott et al., 1998). Quinones, which are ubiquitous in dissolved organic matter (Scott et al., 1998), can reversibly transfer electrons and thus interact with abiotic and biotic redox processes (Aeschbacher et al., 2011, 2010; Bauer et al., 2007; Heitmann and Blodau, 2006; Heitmann et al., 2007; Klüpfel et al., 2014). For example, DOM acts as electron shuttles between bacteria and mineral phases or mediates reduction of organic pollutants (Kappler et al., 2004; Klüpfel et al., 2014; Wolf et al., 2009). As Fe is abundant and an important electron acceptor in most freshwater ecosystem (Karlsson and Persson, 2012, 2010), there is also a close interaction of Fe (ferric/ferrous iron) and DOM, e.g. complexation (Tipping et al., 2002) or shuttling of electrons to insoluble iron oxides (Kappler et al., 2004; Karlsson and Persson, 2012; Piepenbrock et al., 2014).

Moreover, DOM can serve as direct electron acceptor for microbial respiration (Lovley et al., 1996) or as indirect electron acceptor by acting as re-oxidant for inorganic electron acceptors, such as iron or sulfur (Heitmann and Blodau, 2006; Kappler et al., 2004; Yu et al., 2015). In a laboratory study, Heitmann and Blodau, (2006) reported a DOM mediated oxidation of sulfide at pH 6 on a time scale of hours, leading to the formation thiosulfate ($S_2O_3^{2-}$) and organic sulfur (S_{org}). The electron accepting capacity of DOM (Pahokee Peat Reference HA (PP-HA)) towards sulfide calculated from $S_2O_3^{2-}$ formation was 0.6 µmol e^- (mg C) $^{-1}$, which would be available for subsequent bacterial reduction of $S_2O_3^{2-}$.

In environmental systems, DOM exists in multiple redox states and reduced DOM can also regain electron-accepting capacity if it is brought back to oxidized conditions

(Aeschbacher et al., 2010; Heitmann et al., 2007; Klüpfel et al., 2014). Results from DOM reactivity towards sulfide (Yu et al., 2015) showed that oxidized HA is more reactive towards sulfide than DOM reduced by H_2/Pd. While upon reaction with non-reduced HA predominantly inorganic sulfur oxidation products formed, for H_2/Pd-reduced HA mostly S_{org} formed upon sulfidization, even exceeding S_{org} formed with non-reduced HA(Yu et al., 2015).

The reduction of humic acids by H_2/Pd has thus increasingly come under criticism for certain limitations, such as formation of irreversibly reduced hydration products or cleavage of quinones (Aeschbacher et al., 2010; Ratasuk and Nanny, 2007), thus altering its redox properties and reactivity. From studies of sediment diagenesis, it is known that upon reaction of sulfide with organic matter, S_{org} is formed during early stages of diagenesis, e.g. by Michael additions to oxidized quinones (Perlinger et al., 2002), by addition to unsaturated C double-bonds e.g. of lipids, (Adam et al., 2000; Vairavamurthy and Mopper, 1987) or by cross linkage of carbohydrates (van Dongen et al., 2003). Assuming a complete cleavage of quinone moieties by a H_2/Pd pretreatment at pH 6 (Jiang and Kappler, 2008; Ratasuk and Nanny, 2007), formation of S_{org} by H_2/Pd pre-treated HA should thus mostly involve reactions with non-quinone moieties of DOM (Yu et al., 2015).

Therefore, a new approach has been proposed recently to evaluate truly reversible electron transfer from and to DOM by electrochemical reduction or oxidation (Aeschbacher et al., 2011, 2010; Klüpfel et al., 2014). Although several studies have investigated reversible electron transfer to DOM over repeated cycles of reduction and re-oxidation, (Aeschbacher et al., 2010; Bauer and Kappler, 2009; Ratasuk and Nanny,

2007), little is known about changes in the reactivity of reduced DOM versus oxidized DOM. As it is known that quinone addition of sulfide to DOM is limited to oxidized quinone moieties, changes in the DOM redox state can be expected to cause concomitant changes in reactivity towards sulfide (Hoffmann et al., 2012; Perlinger et al., 2002; Yu et al., 2015).

Based on the fact that in electrochemically reduced HA all quinones should be reversibly reduced to hydroquinones, while in H_2/Pd reduced HA cleavage of quinone moieties and further molecular alterations may occur, the object of this study was to investigate the reactivity of reversibly, electrochemically reduced versus H_2/Pd reduced organic matter towards sulfide. We hypothesized that electrochemically reduced quinones in DOM may not further react with sulfide. Thus it would allow identifying the contribution of non-quinone moieties to sulfide transformation.

MATERALS AND METHODS

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sigma Aldrich Humic acid (HA) was used as received. In addition to C and N contents provided, Fe and S contents were detected by energy-dispersive X-ray fluorescence (XRF) spectrometry (X-Lab 2000, Spectro) (see Table 8)

All sample handling, experiments, and electrochemical measurements were conducted in an anoxic glovebox (M. Braun, Germany) at room temperature and under exclusion of light (N_2 atmosphere at 25 ± 1 °C, O_2 < 1 ppm, Innovative Technology, Amesbury, USA) or using tightly butyl stoppered, brown flasks (1 cm thick butyl stoppers, Glasgerätebau Ochs, Bovenden, Germany). All aqueous solutions were prepared with ultrapure water (Ω ≥ 18.2, Membrapure, Hennigsdorf, Germany) and made anoxic by purging with nitrogen

for 2 h. All solutions used for electrochemical measurements contained 0.1 M KCl as supporting electrolyte and pH 7 was maintained by 0.2 M phosphate buffer (Aeschbacher et al., 2010).

Preparation of HA acid stock solutions Non-reduced HA stock solutions for batches (used 'as-is') were prepared by dissolving the HA powder at pH 9 for 24 hours. This solution was brought to pH 6 by addition of hydrochloric acid, filtered (0.45 μm nylon) and diluted to 150 mg C L^{-1}. The HA stock solution was transferred into serum bottles, sealed with butyl rubber stoppers and purged with N_2 (99.999%) to remove oxygen. This HA solution was kept in the glove box and used to prepare solutions of different concentrations of non-reduced HA and for further preparation of reduced HA.

Wet chemical reduction of HA We used H_2 and a Pd-catalyst (Jiang and Kappler, 2008; Visser, 1964). To this end, 4 pellets of 3.2 mm (0.5 wt. % Pd coated on Al) per 10 ml of solution were added into 100-ml serum bottles stoppered with 1 cm thick butyl septa. The headspace (50 ml) was exchanged with H_2 (99.999%), serving as the reducing agent. After shaking the bottles for 48 hours, the solutions were filtered (0.45 μm nylon) inside the glove box to remove traces of the Pd-pellets; this solution was used as H_2/Pd reduced HA stock solution. For simplification, this H_2/Pd reduced HA will hereafter be termed H_2-RHA.

Electrochemical reduction of HA For electrochemical reduction, 'direct electrochemical reduction' (DER) was applied (Aeschbacher et al., 2010). To this end, NR- HA (150 mg C L^{-1}) stock solution was placed into a 0.1 L bulk electrolysis cell and electrochemically reduced at pH 7 (phosphate buffer) in the presence of 0.1 M KCl (supporting electrolyte) and using a glassy carbon working electrode (WE), a Pt wire

auxiliary electrode (separated from the main compartment by a glass frit to avoid reoxidation of reduced HA), and an Ag/AgCl reference electrode (all from Bioanalytical Systems Inc., West Lafayette, IN). An Autolab PGSTAT101 potentiostat (Metrohm, Herisau, Switzerland) was used to control potentials at the WE. Potentials were adjusted vs an Ag/AgCl reference but are reported vs standard hydrogen electrode (SHE). To simulate different DOM redox properties as may adjust under natural conditions, two different potentials were set for electrochemical reduction of HA, at -0.1 and -0.4 V (The redox potential of HS mostly is in the range of +0.20 to -0.48 V) (Aeschbacher et al., 2011; Klüpfel et al., 2014). The progress of DER of HA was followed by taking MER measurements; the HA was considered at equilibrium with the DER applied potential when no further changed in MER were detected. These electrochemically reduced HA solutions will hereafter be termed EC-RHA (-0.1 V) and EC-RHA (-0.4 V).

Mediated Electrochemical Reduction (MER) and Oxidation (MEO) MER and MEO of HA samples to measure electron accepting (EAC) and donating capacities (EDC) of samples prior to reaction with sulfide was conducted with the electrochemical equipment described above, but recording the currents to calculate electron transfer. The electrochemical cell was filled with 80 mL of buffer (0.1 M KCl, 0.1 M phosphate, pH 7) and the electrode was equilibrated to the desired potentials Eh = −0.49 V in MER and Eh = +0.61 V in MEO, which were below and above the potential range reported for quinones, respectively. Thereafter, the mediators DQ (Diquat dibromide monohydrate (99.5%, Supelco, St. Louis, MO)) for MER and ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (>99%, Supelco, St. Louis, MO)) for MEO were spiked. When constant background currents were again reached, defined

amounts (<1 mg C) of HA samples were spiked into the cells and the transferred amount of electrons was measured by chronocoulometry (Aeschbacher et al., 2010). Values of EAC are given for reduction at -0.49 V, values of EDC for oxidation at +0.61 V vs. SHE.

Setup of treatments Prior to exposure towards sulfide, EACs and EDCs of all HA solutions (150 mg C L^{-1}; non-reduced HA, H_2-RHA, HA EC-RHA (-0.1 V) and EC-RHA (-0.4 V)) were determined by MEO/MER as described. Two HA concentrations of 25 and 75 mg C L^{-1} for all different HA solutions were prepared and an initial total sulfide (H_2S) concentration of 250 ± 1.2 µmol L^{-1} at pH 6 ± 0.05 was adjusted (speciation at pH 6: 91.25 % H_2S, 8.75% HS^-, hereafter referred to as 'sulfide'). A pH of 6 was chosen as previous studies on DOM reactivity towards sulfide had been conducted at similar pH and as H_2S is the reactive species, not HS^- (Heitmann and Blodau, 2006). Samples were incubated in triplicate for 48 hours, using a bicarbonate buffer (50 mM, adjusted with 1 M HCl) for constant pH, and stirred using a magnetic stirrer bar. Parallel batches were prepared as control: 1) HA without sulfide addition to monitor changes of EAC and EDC and 2) addition of sulfide only to validate the tightness of the incubation vessels. Aliquots of 400 µl were collected on various time steps within 48 hours for determination of total sulfide. Due to time constraints in using the electrochemical setup, in case of H_2-RHA, only the 25 mg C L^{-1} treatment was analyzed.

Values of EACs and EDCs before reaction with sulfide were determined as means from both controls and treatments (n=3). Mediated electrochemical measurements of HA solutions were conducted at pH 7 to facilitate comparison with existing studies, since measurements of MEO and MER were always conducted at pH 7 (Aeschbacher et al., 2012, 2010; Klüpfel et al., 2014).

Analytical methods Dissolved organic carbon (DOC) concentrations were measured as non-purgeable organic carbon (NPOC) using a TOC analyzer (Shimadzu TOC-V CPN). Sulfide was assayed colorimetrically and measured at 665 nm on a Varian Cary 1E spectrophotometer (methylene blue, Cline, 1969).

Kinetic models Based on observations from earlier studies (Heitmann and Blodau, 2006; Yu et al., 2015), we applied a one pool or two pools kinetic model for sulfide transformation kinetics.

RESULTS

Sulfide transformation by reduced humic acid

For all different treatments of HAs, i.e. NR-HA, EC-RHA, and H_2-RHA, a fast transformation of sulfide upon reaction with HA was observed in the beginning, slowing down after about 12 h. Therefore, our evaluations and calculations were based on the amount of sulfide transformed after 48 hours, when no further significant changes could be detected. The sulfide transformation in the assays significantly increased with increasing DOC concentrations from 25 to 75 mg C L^{-1}, irrespective of the treatments. Regarding the pre-treatment, the reactivity of EC-RHA (-0.4 V) towards sulfide was lower than that of EC-RHA (-0.1 V) and H_2-RHA at similar DOC concentrations.

Given the initial addition of 250 µmol L^{-1} of sulfide, sulfide transformation in batches of non-reduced HA was up to 18.2 % (45.5 µmol L^{-1}) and 38.5 % (96.3 µmol L^{-1}) at 25 and 75 mg C L^{-1}, respectively. In the pre-reduced HA batches, highest sulfide transformation of 14.3 % (35.8 µmol L^{-1}) and 26.7 % (66.7µmol L^{-1}) was observed in the 25 and 75 mg C L^{-1} batches of EC-RHA (-0.1 V), respectively. For the EC-RHA (-0.4 V) only 6.5 %

(16.1 μmol L^{-1}) to 16.2% (40.4 μmol L^{-1}) of sulfide were transformed in presence of 25 and 75 mg C L^{-1}. Interestingly, in the H_2-RHA batch, 10.5 % (26.3 μmol L^{-1}) to 24.4% (61.1 μmol L^{-1}) of the initial sulfide were transformed in presence of 25 and 75 mg C L^{-1} DOC concentration. In terms of the amount of sulfide transformed, the HA pre-treatments thus ranked in the order NR-HA > EC-RHA (-0.1 V) > H_2-RHA > EC-RHA (-0.4 V) (Figure 13).

As Aldrich HA contains significant amounts of iron (1.33%, Table 8), iron concentrations in the treatments were 5.9 and 17.8 μmol e^- L^{-1} in treatments of 25 and 75 mg C L^{-1} DOC. Assuming all iron being present as Fe(III) in the NR-HA treatments, a maximum amount of sulfide transformation that could potentially be attributed to iron would reach 6 to 9 %. For EC-RHA (-0.1 V), EC-RHA (-0.4 V) and for H_2-RHA, iron may be assumed to be present as Fe^{2+}. Therefore, in the observed transformation of sulfide, iron should play a negligible role.

The calculated S/C ratios for the transformation of sulfide upon reaction with dissolved organic carbon from 25 to 75 mg C L^{-1} were 0.0172~0.0107 for EC-RHA (-0.1 V), 0.0077~0.0065 for EC-RHA (-0.4 V), and 0.0126~0.0098 for H_2-RHA. Thus, the lowest S/C ratio for the sulfide transformation was observed for EC-RHA (-0.4 V).

Fitting a kinetic model resulted in a two pool model as best fit for NR-HA and EC-RHA, whereas a one pool model was obtained for H_2-RHA batches (Table 9). According to the fitting results, in the batches of EC-RHA (-0.1 V), sulfide was transformed with S/C ratios of 0.0108~0.0069 by a fast reacting pool at a rates of 0.1621~0.2144 h^{-1}, while the S/C ratio for the slower reacting pool was 0.0097~0.0053 at rates of 0.0394~0.0373 h^{-1}. A

fraction of 84.5~71.4% (211.3~178.5 μmol L^{-1}) of the initial sulfide did not react within the 48 h of incubation.

For EC-RHA (-0.4 V), sulfide was transformed with S/C ratio of 0.0019~0.0029 by the fast reacting pool at a rates of 0.0330~0.1159 h^{-1}, while the S/C ratio for the slower reacting pool was 0.0110~0.0029 for the slower reacting pool at rates of 0.0137~0.0348 h^{-1}. The remaining sulfide was 89.1~82.3% (223~206 μmol L^{-1}) of the initial 250 μmol L^{-1} sulfide after 48 h of incubation. The fitted conditional S transformation rate constants of the fast and slow pools in EC-RHA (-0.4 V) were both lower than the corresponding constants for the EC-RHA (0.1 V). Fitting sulfide transformation by H_2-RHA resulted in a one pool model only (Yu et al., 2015), with a conditional rate constant of only 0.0961~0.1516 h^{-1} and thus in a range between the fast pools of EC-RHA(-0.1 V) and EC-RHA(-0.4 V). Based on modeled conditional rate constants of different pre-reduced HA samples, reaction rates of the tested HA solutions toward sulfide followed the order NR-HA > EC-RHA (-0.1 V) > H_2-RHA > EC-RHA (-0.4 V).

Mediated electrochemical analysis of DOM prior to reaction with sulfide

To evaluate the relation of EAC, EDC and electron transfer capacities (ETCs: sum of EDCs and EACs) of the HA of the different treatments to the amount of sulfide transformed, the electron-accepting and -donating capacities of DOM were measured prior to exposure of DOM towards sulfide.

Mean values of EACs and EDCs of DOM ranged from 1.42 ± 0.02 to 3.35 ± 0.15 and 1.65 ± 0.07 to 2.92 ± 0.11 μmol e^- (mg C) $^{-1}$ (Figure 14). As expected, DOM solution of NR-HA had a highest EAC of 3.35 μmol e^- (mg C) $^{-1}$, while reduced HA solutions had

higher values of EDC, with the highest capacity of 2.92 µmol e^- (mg C) $^{-1}$ obtained for EC-RHA (-0.4 V). Measured EACs in different concentration of DOC solutions ranked in the order NR-HA (90.0 to 232.5 µmol e^- L^{-1}) > EC-RHA (-0.1 V) (67.3 to 205.5 µmol e^- L^{-1}> EC-RHA (-0.4 V) (40.8 to 117.8 µmol e^- L^{-1}) and were clearly related to the amount of transformed sulfide (Figure 15 A, B).

Measured values of EDC did not relate show a clear pattern along increasing degree of reduction, as observed for EAC. For NR-HA and EC-RHA (-0.1 V), EDC was 50.0 or 180.0 µmol e^- L^{-1} and 57.3 or 166.5 µmol e^- L^{-1}, respectively, in the solutions of 25 or 75 mg C L^{-1} DOC. Only for EC-RHA (-0.4 V), EDC was clearly higher, reaching 72 or 222 µmol e^- L^{-1} in solutions of 25 and 75 mg C L^{-1} DOC. Correspondingly, values of EDC did not correlate to the amount of sulfide transformed (Figure 15 C, D).

Total electron transfer capacities were comparable for NR-HA and electrochemically reduced HA. The calculated total electron transferring capacities (ETC) as the sum of EACs and EDCs were 5.56, 4.97 and 4.52 µmol e^- (mg C) $^{-1}$ for NR-HA, EC-RHA (-0.1 V), and EC-RHA (-0.4 V), respectively. For H_2-RHA, measured EACs and EDCs were lower and the ETC reached only 3.07 µmol e^- (mg C) $^{-1}$ (see Figure 14).

DISCUSSIONS

Existing studies evaluated redox properties of humic substances (mostly standard compounds) (Aeschbacher et al., 2011, 2010) and natural organic matter prior to and after incubations (Klüpfel et al., 2014) to learn about the redox state and electron transfer capacities of DOM under different preconditions. Our data clearly demonstrates that a different redox state of DOM also affects its reactivity towards sulfide. Exposure of

DOM to sulfide in turn affected its redox properties, i.e. EAC and EDC, of DOM. Moreover, DOM reactivity towards sulfide differs whether DOM is reduced by H_2/Pd or by electrochemical reduction.

Transformed amount of sulfide versus redox state of DOM

Sulfide transformation of different pre-reduced HA at 25 mg C L^{-1} decreased with increasing degree of reduction as expected, with the exception of H_2-RHA, ranking in between EC-RHA (-0.1 V) and EC-RHA (-0.4 V), although H_2/Pd could be expected to be the most strongest reductant. Also with the exception of the H_2-RHA solution, having a low value of EAC of 35.5 µmol e^- L^{-1} compared to the amount of transformed sulfide, the measured initial EACs of the other HA solutions were clearly related to the observed sulfide transformation: a strong positive correlation of the initial EAC of the solutions and the detected transformation of sulfide was observed, which approached fairly close to a 1:1 slope (Figure 15 A, B) when a conversion of sulfide to zerovalent S was assumed (Yu et al., 2015). There was no clear relation of values of EDC of the HA solutions with the amount of sulfide transformed. Therefore, in contrast to EAC, EDC does not seem to reflect a redox indicator of DOM reactivity towards sulfide. Moreover, H_2/Pd reduction of HA resulted in a very low EDC compared to the EC-RHA treatments, further supporting that H_2/Pd reduction alters DOM redox properties in a complex way.

Besides a formation of inorganic transformation products from partial oxidation of sulfide by electron accepting moieties of DOM (Heitmann and Blodau, 2006; Yu et al., 2015), such as quinones, the commonly considered reaction pathway for a formation of

organic sulfur is a Michael type addition to quinones (Perlinger et al., 2002). Electrochemical reduction is an effective way to reversibly reduce quinones to hydroquinones (Aeschbacher et al., 2010), while in H_2/Pd reduced HA cleavage of quinone moieties and further molecular alterations may occur (Ratasuk and Nanny, 2007). Thus, the latter pre-treatment can be expected to impede electron transfer from or addition to quinones. Our results therefore indicated that reactions involving oxidized quinones (electron transfer or addition reactions) play a notable role, as increasingly reduced humic acid had a lower reactivity towards sulfide in the EC-RHA samples. We, however, cannot give a detailed analysis of the contribution of quinone addition to electron transfer or organic sulfur formation, as these products were not studied separately for the different treatments applied here. Nevertheless, still a small proportion sulfide reacted with the electrochemically most strongly reduced EC-RHA (-0.4 V). Assuming quinones to be fully reduced to hydroquinones in those samples (Aeschbacher et al., 2010), here in case of organic sulfur formation an addition of S to non-quinone moieties must have occurred, such as at unsaturated C-C bonds (Adam et al., 2000; Vairavamurthy and Mopper, 1987) or by sulfurization of carbohydrates (van Dongen et al., 2003).

Yu et al., (2015) studied organic sulfur addition and speciation of H_2-RHA upon reaction with sulfide. Their results supported a substantial formation of organic sulfur, which in that case could only be non-quinones addition (Perlinger et al., 2002; Ratasuk and Nanny, 2007). Keeping in mind that upon reaction of non-reduced HA with sulfide, 56~61 % of transformed sulfide was recovered as inorganic transformation products and about 39~44 % as organic sulfur, while H_2/Pd RHA only yielded organic sulfur as transformation

product, quinones of DOM may dominate sulfide transformation into inorganic transformation products, whereas non-quinones moieties seem to be important for organic sulfur formation (Perlinger et al., 2002; Yu et al., 2015). An increasing degree of reduction of quinones should thus decrease the amount of inorganic transformation products.

Obtained rate constants and possible underlying reaction sites

As proposed earlier (Heitmann and Blodau, 2006), a two pool, inorganic S pool and organic S pool, kinetic model could be fitted to the data of sulfide transformation. The electrochemical reduction of HA should thereby reflect primarily a reduction of redox active quinone sites due to the chosen potentials (Aeschbacher et al., 2011, 2010). In NR-HA and both EC-HA solutions, fitted results of sulfide transformation rate constants exhibited a similar pattern. As the fast pool of sulfide transformation had a decreasing rate constant with increasing degree of electrochemical reduction, it may be hypothesized that this pool is mostly made up by highly redox active quinone groups reacting with sulfide (Perlinger et al., 2002) that decrease in their contribution with increasing degree of reduction. In H_2-RHA solutions, a complete cleavage of quinone moieties would be expected (Ratasuk and Nanny, 2007); here, a single pool rate constant may thus represent non-quinone addition of sulfide. Coincidently, the obtained fast pool rate constant for EC-RHA(-0.4 V), where a full but reversible reduction of quinones would be expected, was in the same range or even lower as for H_2-RHA.

Implication for sulfur biogeochemical cycling of sulfur under anaerobic condition and conclusions

In this study we could demonstrate that also pre-reduced DOM (EC-RHA and H_2-RHA) further reacted with sulfide, a process that can be expected to occur in sulfidic environments, thereby competing with bacteria or other abiotic processes for the EAC of DOM (Lovley et al., 1996; Yu et al., 2015). Initial values of EAC of different oxidation states of HA thereby strongly correlated to the respective sulfide transformation and may serve as a good indicator for reactivity towards sulfide. The electrochemical methods used in this study therefore proved to be suitable to evaluate redox properties and reactivity of DOM. The observation that there was a higher transformation of sulfide as would be expected from electrochemically determined EAC indicated that sulfide was obviously highly efficient in 'extracting' electron acceptor capacity from organic matter. Furthermore, assuming an increasing degree of reduction of primarily quinones upon electrochemical reduction of HA, quinone moieties of DOM play a predominant role in the reactivity towards sulfide, also due to the observed high rate constants. Nevertheless, increasingly reduced HA presumably increases the formation of S_{org}, thereby providing a long term sink of sulfur already during early diagenesis.

ACKNOWLEDGEMENTS

We gratefully acknowledge student assistants Daniela Braun and Sarah Hofmann for lab work support, and also thank Zhengrong Xue, Jutta Eckert, Silke Hammer for technical support. The study was funded by the German Research Foundation, research group 'electron transfer processes in anoxic aquifers' (FOR 580, KN 929/2-1).

REFERENCES

Adam, P., Schneckenburger, P., Schaeffer, P., Albrecht, P., 2000. Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules. Geochim. Cosmochim. Acta 64, 3485–3503.

Aeschbacher, M., Graf, C., Schwarzenbach, R.P., Sander, M., 2012. Antioxidant properties of humic substances. Environ. Sci. Technol. 46, 4916–4925.

Aeschbacher, M., Sander, M., Schwarzenbach, R.P., 2010. Novel electrochemical approach to assess the redox properties of humic substances. Environ. Sci. Technol. 44, 87–93.

Aeschbacher, M., Vergari, D., Schwarzenbach, R.P., Sander, M., 2011. Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids. Environ. Sci. Technol. 45, 8385–8394.

Bauer, I., Kappler, A., 2009. Rates and extent of reduction of Fe(III) compounds and O_2 by humic substances. Environ. Sci. Technol. 43, 4902–4908.

Bauer, M., Heitmann, T., Macalady, D.L., Blodau, C., 2007. Electron transfer capacities and reaction kinetics of peat dissolved organic matter. Environ. Sci. Technol. 41, 139–145.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14, 454–458.

Cory, R.M., McKnight, D.M., 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environ. Sci. Technol. 39, 8142–8149.

Heitmann, T., Blodau, C., 2006. Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. Chem. Geol. 235, 12–20.

Heitmann, T., Goldhammer, T., Beer, J., Blodau, C., 2007. Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog. Glob. Chang. Biol. 13, 1771–1785.

Hoffmann, M., Mikutta, C., Kretzschmar, R., 2012. Bisulfide reaction with natural organic matter enhances arsenite sorption: insights from X-ray absorption spectroscopy. Environ. Sci. Technol. 46, 11788–11797.

Jiang, J., Kappler, A., 2008. Kinetics of microbial and chemical reduction of humic substances: implications for electron shuttling. Environ. Sci. Technol. 42, 3563–3569.

Kappler, A., Benz, M., Schink, B., Brune, A., 2004. Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. FEMS Microbiol. Ecol. 47, 85–92.

Kappler, A., & Haderlein, B. S. 2003. Natural organic matter as reductant for chlorinated aliphatic pollutants. Environ. Sci. Technol. 37, 2714–2719.

Karlsson, T., Persson, P., 2010. Coordination chemistry and hydrolysis of Fe(III) in a peat humic acid studied by X-ray absorption spectroscopy. Geochim. Cosmochim. Acta 74, 30–40.

Karlsson, T., Persson, P., 2012. Complexes with aquatic organic matter suppress hydrolysis and precipitation of Fe(III). Chem. Geol. 322-323, 19–27.

Klüpfel, L., Piepenbrock, A., Kappler, A., Sander, M., 2014. Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nat. Geosci. 7, 195–200.

Lovley, D.R., Coates, J.D., Blunt-Harris, E.L., Phillips, E.J.P., Woodward, J.C., 1996. Humic substances as electron acceptors for microbial respiration. Nature 382, 445–448.

Peretyazhko, T., Sposito, G., 2006. Reducing capacity of terrestrial humic acids. Geoderma 137, 140–146.

Perlinger, J.A., Kalluri, V.M., Venkatapathy, R., Angst, W., 2002. Addition of hydrogen sulfide to juglone. Environ. Sci. Technol. 36, 2663–2669.

Piepenbrock, A., Schröder, C., Kappler, A., 2014. Electron transfer from humic substances to biogenic and abiogenic Fe(III) oxyhydroxide minerals. Environ. Sci. Technol. 48, 1656–1264.

Ratasuk, N., Nanny, M.A., 2007. Characterization and quantification of reversible redox sites in humic substances. Environ. Sci. Technol. 41, 7844–7850.

Scott, D.T., McKnight, D.M., Blunt-Harris, E.L., Kolesar, S.E., Lovley, D.R., 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ. Sci. Technol. 32, 2984–2989.

Tipping, E., Rey-Castro, C., Bryan, S. E., Hamilton-Taylor, J. 2002. Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation. Geochim. Cosmochim. Acta 66, 3211–3224.

Urban, N.R., Ernst, K., Bernasconi, S., 1999. Addition of sulfur to organic matter during early diagenesis of lake sediments. Geochim. Cosmochim. Acta 63, 837–853.

Vairavamurthy, A., Mopper, K., 1987. Geochemical formation of organosulphur compounds (thiols) by addition of H_2S to sedimentary organic matter. Nature 329, 623–625.

Van Dongen, B.E., Schouten, S., Baas, M., Geenevasen, J.A.J., Sinninghe Damstéa, J.S., 2003. An experimental study of the low-temperature sulfurization of carbohydrates. Org. Geochem. 34, 1129–1144.

Visser, S.A., 1964. Oxidation-reduction potentials and capillary activities of humic acids. Nature 204, 581.

Wolf, M., Kappler, A., Jiang, J., Meckenstock, R.U., 2009. Effects of humic substances and quinones at low concentrations on ferrihydrite reduction by *Geobacter metallireducens*. Environ. Sci. Technol. 43, 5679–5685.

Yu, Z., Peiffer, S., Goettlicher, J., Knorr, K.-H., 2015. Electron transfer budgets and kinetics of abiotic oxidation and incorporation of aqueous hydrogen sufide by dissolved organic matter. Environ. Sci. Technol., 2015, 49 (9), 5441–5449

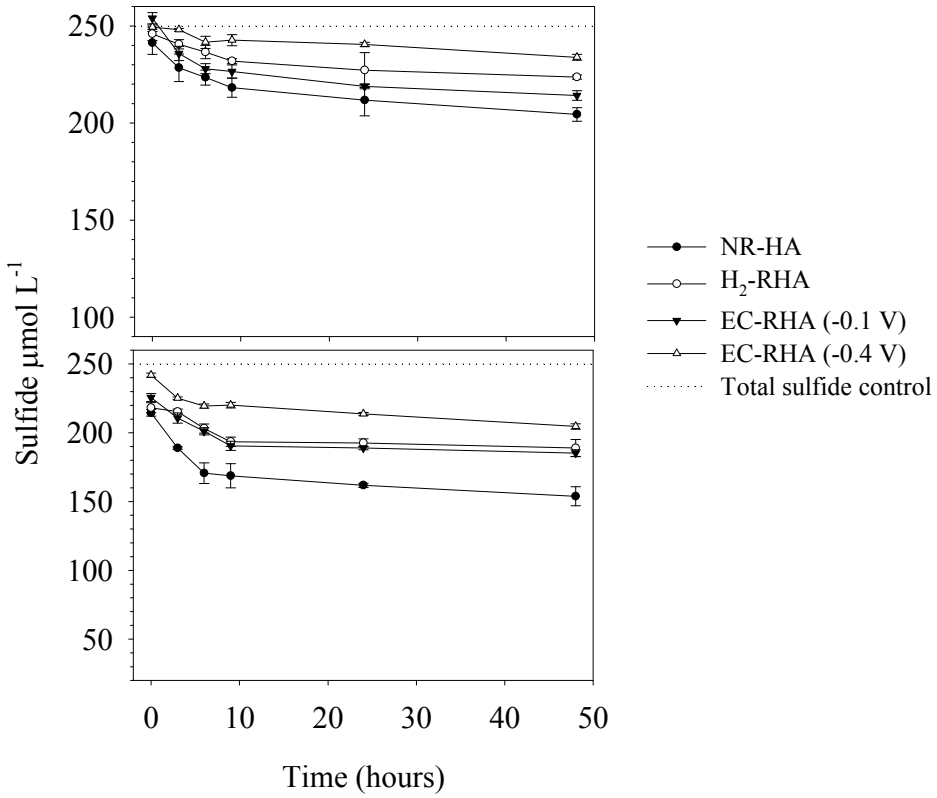


Figure 13 Transformation of sulfide upon reaction with Sigma Aldrich humic acid at a concentration of 25 (top) and 75 mg C L^{-1} DOC (bottom). NR-HA: non-reduced Sigma Aldrich (SA) humic acid; H_2-RHA: SA humic acid reduced by H_2/Pd, EC-RHA (-0.1 V) and EC-RHA (-0.4 V): SA humic acid electrochemically reduced at -0.1 and -0.4 V, respectively.

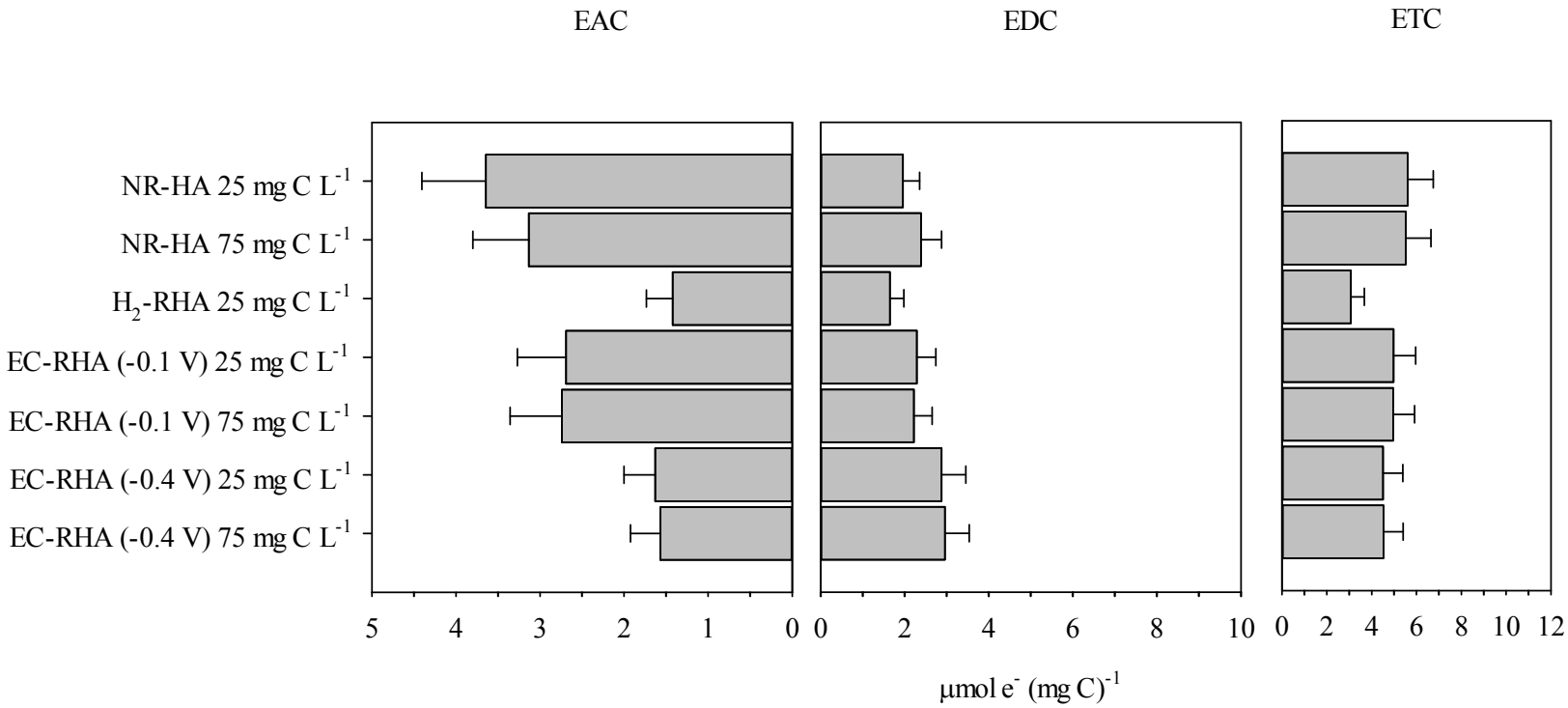


Figure 14 Electron accepting capacity (EAC) and electron donating capacity (EDC) of non-reduced and reduced Sigma Aldrich humic acids solutions of 25 and 75 mg C L^{-1} DOC prior to reaction with sulfide. EAC and EDC were determined by mediated electrochemical reduction (MER) and Oxidation (MEO). ETC, electron transfer capacity, represents the sum of EAC and EDC.

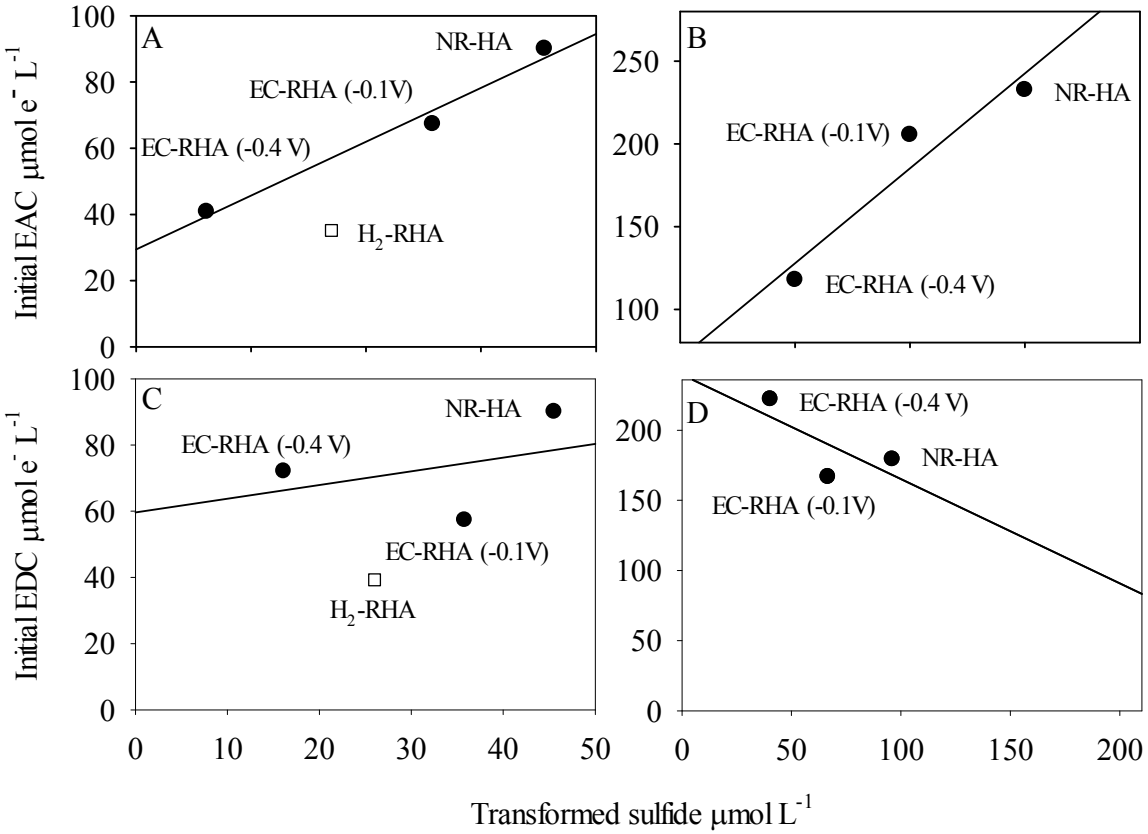


Figure 15 Correlation between initial EAC or EDC of humic acid and sulfide transformation. (**A, C**) 25 mg C L^{-1} DOC and (**B, D**) 75 mg C L^{-1} DOC; **EAC-Regressions**: **A**. Sulfide $_{transformed}$ = 0.6·EAC $_{initial}$ – 7.2, R^2 = 0.98; **B**. Sulfide $_{transformed}$ = 0.5·EAC $_{initial}$ - 13. 7, R^2 = 0.90; **EDC-Regressions**: **C**. Sulfide $_{transformed}$ = 0.4·EDC $_{initial}$ + 7.2, R^2 = 0.14; **D**. Sulfide $_{transformed}$ = -0. 7·EDC $_{initial}$ + 197.3, R^2 = 0.51; Note: H_2-RHA was not included in regressions.

Table 8 Elemental composition for used humic acid (Sigma-Aldrich) as supplied by the manufacturer upon purchase (S, C, N) or as determined by X-ray fluorescence spectroscopic analysis (Fe)

Sigma-adrich humic acid		S	Fe	C	N
	solid %	0.45	1.33	40.15	0.92
25 mg C L^{-1}	*dissolved* *	0.28 mg L^{-1} (8.75 µmol L^{-1})	0.83 mg L^{-1} (14.8 µmol L^{-1})	25 mg L^{-1} (2.08 mmol L^{-1})	0.57 mg L^{-1} (41 µmol L^{-1})
75 mg C L^{-1}	*dissolved*	0.84 mg L^{-1} (26.3 µmol L^{-1})	2.49 mg L^{-1} (44.4 µmol L^{-1})	75 mg L^{-1} (6.24 mmol L^{-1})	1.71 mg L^{-1} (123 µmol L^{-1})

* 'dissolved' was recalculated results, it was based on elemental composition of solid powder of Sigma-Aldrich humic acid

Table 9 Modeled kinetic parameters for sulfide transformation by pre-reduced HA solutions

Sulfide transformation reacted with H_2 reduced HA												
Fitted	mg C L^{-1}	$k1$ (h^{-1})	$k2$(h^{-1})	q%	x%	$(1-x)$%	r^2	R^1	R^2	$t_{1/2}(3)$		*Sulfide%*
H_2-RHA	*25*	×	0.0961	×	9.1	91.4	0.98	×	5.78	7.1		89.5
	75	×	0.1516	×	14.7	88.3	0.97	×	5.72	6.1		75.6
Sulfide transformation reacted with EC reduced HA												
Fitted	mg C L^{-1}	$k1$ (h^{-1})	$k2$(h^{-1})	q%	x%	$(1-q-x)$%	r^2	R^1	R^2	$t_{1/2}(1)$	$t_{1/2}(2)$	*Sulfide%*
EC-RHA	25 (-0.1 V)	0.1621	0.0394	9.0	8.1	84.5	0.99	5.6	6.2	4.3	17.6	84.9
2 pool	75 (-0.1 V)	0.2144	0.0373	17.3	13.2	71.4	0.99	5.4	6.2	3.2	18.6	72.4
	25 (-0.4 V)	0.0330	0.0137	1.6	9.16	89.1	0.98	6.3	6.5	21.0	40.1	92.7
	75 (-0.4 V)	0.1159	0.0348	7.2	7.3	82.3	0.99	5.7	6.2	6.0	19.9	80.9

* R **(**mol min^{-1} mg^{-1}) = -log [k_i (S_{tot})] in terms of dissolved carbon weigh (mg C L^{-1}) where k_i is the sulfide consumption rate constant(h^{-1}), Rates and reaction conditions of sulfide consuming processes at room temperature (294–299 K) and similar ionic strength (0.05 M), calculated for an initial H_2S concentration of 1 mM. We ascribed a reactive site q for the fast chemical oxidation of sulfide by quinone moieties. For the slower ongoing processes of addition of sulfur into the organic structure to non- quinone moieties we ascribed another reactive site x, as an additional, slower sulfide transformation process. Assuming rates being pseudo-first order with respect to q and x (non limiting supply of sulfide in solution),

** Measured retained sulfide percentage of initial concentration at the 48-h end point of kinetic process

STUDY 3: Contributions of organic sulfur and organic matter redox processes to electron flow in anoxic incubations of peat

Zhi-Guo Yu, Jörg Göttlicher, Ralph Steininger, Klaus-Holger Knorr

Submitted to *Environmental Chemistry* on 06 May 2015

Contributions of organic sulfur and organic matter redox processes to electron flow in anoxic incubations of peat

Zhi-Guo Yu[A] *, Jörg Göttlicher*[B]*, Ralph Steininger*[B]*, Klaus-Holger Knorr*[A,*]

[A]Hydrology Group, University of Münster, Heisenbergstr. 2, 48149 Münster, Germany

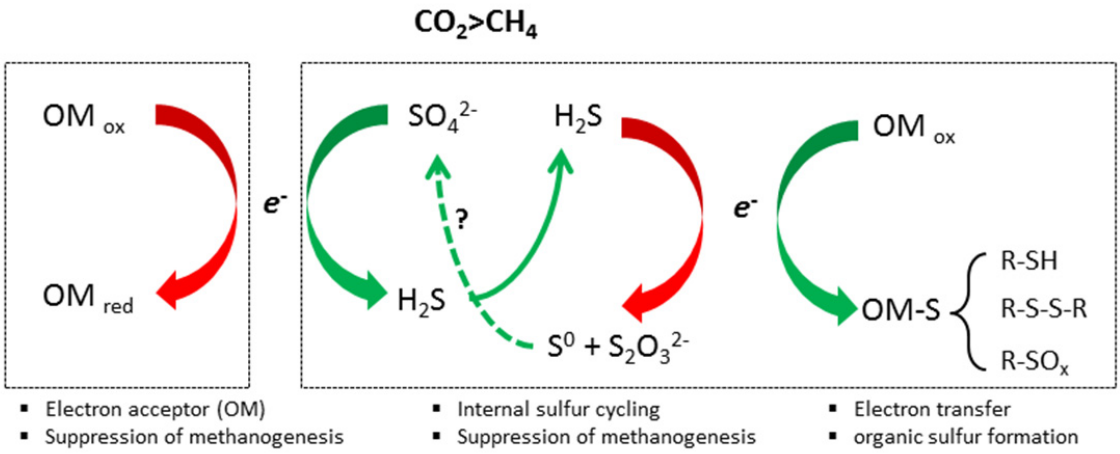
[B]ANKA Synchrotron Radiation Facility, KIT, 76344 Eggenstein-Leopoldshafen, Germany

[*]Corresponding author, kh.knorr@uni-muenster.de Tel: +49-251-8330207

Environmental context

Organic matter decomposition generates carbon dioxide (CO_2) or methane (CH_4) in anaerobic ecosystems is determined by the presence or absence of electron acceptors. Evaluating CO_2 and CH_4 production in anaerobic incubation of peat, we found a predominance of organic matter as electron acceptor over considered inorganic electron acceptors and a high relevance of internal cycling of sulfur. Our results highlight the importance of organic matter as electron acceptor in anaerobic systems via a support of an internal sulfur cycle.

Table of contents graphic



Abstract

An often observed excess of CO_2 production over CH_4 production in freshwater ecosystems presumably results from a direct or indirect role of organic matter (OM) as electron acceptor, possibly supported by a cycling of oxidized and reduced sulfur species. To test the contribution of OM electron accepting capacities (EAC) to anaerobic microbial respiration, peat soil virtually devoid of inorganic electron acceptors was incubated under anaerobic conditions for 6 weeks at 30 ^{o}C. Thereby, a production of CO_2 and CH_4 at a ratio of 3.2:1 was observed. From excess CO_2 production, we calculated an EAC of OM of 2.36 μmol e^- cm $^{-3}$ d^{-1}. Addition of sulfate (SO_4^{2-}) increased CO_2 production and suppressed CH_4 production as expected. However, after subtracting the EAC provided though SO_4^{2-} (0.97~2.81 μmol e^- cm^{-3} d^{-1}), OM provided even higher EAC of 3.88 to 4.85 μmol e^- cm^{-3} d^{-1}.The contribution of organic sulfur was evaluated by sulfur K-edge X-ray absorption near edge structure (XANES) spectroscopy and using $\delta^{34}S$ natural abundance as a tracer. Bacterial sulfate reduction (BSR) presumably involved a re-oxidation of sulfide by organic matter as proposed earlier, a sulfurization of OM yielding reduced organic sulfur, and also changes in oxidized organic sulfur species. Organic matter thus contributes to anaerobic respiration i) directly by EAC of redox active functional groups ii) directly by oxidized organic sulfur and iii) indirectly by re-oxidation of sulfide to maintain BSR.

Keywords: Methanogenesis; Electron transfer; organic sulfur; Redox processes; Freshwater systems;

INTRODUCTION

Redox processes of inorganic terminal electron acceptors (TEAs), e.g. nitrate, ferric iron and sulfate, have been studied over decades in anoxic freshwater environments (Klueber and Conrad, 1998; Lovley, 1991; Lovley et al., 1982). TEAs provide the electron-accepting capacity for anaerobic degradation of organic matter to CO_2 and only after depletion of these electron acceptors, methanogenesis is a competitive process (Achtnich et al., 1995; Lovley and Phillips, 1986; Lovley, 1991; Lovley et al., 1982; Roden and Urrutia, 2002).

Nutrient-poor freshwater ecosystems with low availability of inorganic electron acceptors, such as ombrotrophic peatlands, are thus important sources of CH_4 to the atmosphere (Mikaloff Fletcher et al., 2004), since CH_4 is produced on depletion of inorganic electron acceptor under anaerobic conditions (Avery et al., 1999; Hornibrook et al., 1997). However, previous studies consistently demonstrated that there is an excess of CO_2 over CH_4 and known inorganic electron acceptors, thus a significant fraction of CO_2 is produced anaerobically through other unidentified electron acceptors (Heitmann et al., 2007; Segers and Kengen, 1998). Humic substances (HS), both dissolved and particulate HS, have been recognized as important electron acceptors due to its redox active functional groups, e.g. quinone moieties (Klüpfel et al., 2014; Lovley et al., 1996; Roden et al., 2010; Scott et al., 1998). Especially, in organic-rich peat soil, measured concentrations of dissolved organic matter (DOM) are high in porewaters (Bauer et al., 2007; Heitmann et al., 2007), which led to the hypothesis that HS here are especially important as electron acceptors for anaerobic microbial respiration with CO_2 production,

thereby partly suppressing methanongenic activity (Keller and Takagi, 2013; Klüpfel et al., 2014; Lovley et al., 1996).

Moreover, it was proposed that there is an effective recycling process of sulfide from BSR, being re-oxidized by HS to replenish the oxidized sulfur pool for BSR (Heitmann and Blodau, 2006; Heitmann et al., 2007; Yu et al., 2015) . Thus, the excess CO_2 production may in part be due to ongoing bacterial sulfate reduction by recycling of sulfur upon reaction with organic matter (Heitmann and Blodau, 2006; Heitmann et al., 2007; Vile et al., 2003b; Yavitt et al., 1987). Existing results also indicate that upon reaction of H_2S with DOM, more electron accepting capacities can be retrieved from HS compared to electrochemical or wet chemical (H_2/Pd-reduction) approaches, presumably due to formation of zerovalent organic sulfur (Yu et al., 2015). Such recycling of sulfur seems therefore effective in extracting electron accepting capacities from DOM, but seems in part also leaky due to ongoing irreversible formation of organic sulfur impeding a long term cycling (Canfield et al., 1998; Solomon et al., 2011, 2003; Urban et al., 1999; Wakeham et al., 1995).

The aim of the current study is based on the idea that the total electron flow of anaerobic respiration can be evaluated by measuring the end-product of CO_2. To evaluate electron accepting capacities from organic matter and contributions of sulfur cycling to anaerobic respiration, electron acceptor turnover was compared to CO_2 production and budgets were obtained in biotic incubations. To evaluate contributions of sulfur cycling, sulfate was added and the organic sulfur speciation was also analyzed prior and after incubation. To elucidate the long term effect of such recycling on the capacities we incubated peat

wiht added additional sulfur to react with organic matter and reduce its electron accepting capacity.

MATERALS AND METHOD

All reagents in this study were deoxygenated by nitrogen (N_2)-purging and storage in the glove box; all sample handling was performed in the glove box (N_2 atmosphere, O_2< 1 ppm, Innovative Technology, Amesbury, MA, USA). All incubation experiments were performed in tightly butyl stoppered flasks (2 cm thick butyl stoppers, Glasgerätebau Ochs, Bovenden, Germany).

Peat incubation setup

For incubations, we used commercial bog peat, inoculated with a small amount of peat from a restored peat harvesting site to introduce an active microbial community; the peat elemental composition is provided in Table10.

The incubations were set up inside the glove box by filling 100-ml flasks with 10 g (2 mm-sieved) wet weight peat and adding 30-ml deoxygenated water. Thereafter, the flasks were tightly stoppered. Prior to eventual dosing of electron acceptors or donors for the incubations, a 3 days pre-incubation was carried out to validate microbial activity and adjust reducing conditions by measuring CO_2 and CH_4 production as an indicator. Thereafter, the head space was exchanged with N_2 and the pre-incubated samples were subjected to different treatments.

Treatment set up

Five different treatments with three replicates each were incubated in the dark at a constant temperature of 30 ^{o}C for 40 days. This elevated incubation temperature was chosen to stimulate microbial turnover to be able to detect changes also in the peat solid phase during reasonable time scales.

Treatments One pre-incubated set was incubated anoxically for further 40 days to serve as a control (**Control $_{A}$**) to determine electron accepting capacities from organic matter without amendments. Another set was incubated under aerobic conditions (outside the glove box) as an oxic control, covering the flasks by a 0.2 µm filter membrane and maintaining soil moisture by adding ultra-pure water over time (**Control $_{O}$**). The original peat material prior to incubation (**Original $_{O}$**) was washed up to 48 hours in ultra-pure water on a horizontal shaker at 150 r min^{-1}, 25 ± 1 °C, to remove SO_4^{2-} prior to analysis by XANES spectroscopy.

To test whether addition of sulfide to organic matter reduces its electron accepting capacities and thus limits excess CO_2 production and the recycling of sulfur from BSR to be recycled a third set of samples was amended by weekly dosing of sodium sulfide (Na_2S) equivalent to a concentration of 250 ± 1 µmol L^{-1} (hereafter termed **Sulfide $_{A}$**). A fourth set was incubated with weekly spiking of SO_4^{2-} equivalent to 5000 ± 10 µmol L^{-1} (Na_2SO_4) (hereafter termed **SO_4 $_{A}$**). Finally, we set up a treatment with weekly dosing of equal concentrations of sodium acetate ($NaCH_3COO$) and Na_2SO_4, 5000 ± 19µmol L^{-1} for 40 days (**Ac-SO_4 $_{A}$**).

We are aware that these conditions of spiking supplements may not represent typical freshwater environments, but were chosen in order to be able to analyze the S fractions in the solid phase with reasonable effort.

Sampling and analysis

Porewater was extracted once every two weeks from the peat suspension with a needle and subsequently filtered (0.45 µm, nylon); concentrations of SO_4^{2-} and CH_3COO^- were determined by ion chromatography (Metrohm 883 Basic IC plus, METROSEP A-Supp 4 column, chemical suppression). Total H_2S was determined photometrically (Cline, 1969). Iron in the solid-phase was extracted for 48 hour by 0.5 mol L^{-1} HCl(Lau et al., 2015). Dissolved ferrous iron (Fe^{2+}) and 0.5 mol L^{-1} HCl extractable ferric iron (Fe^{3+}) were quantified by the phenanthroline assay (Harvey et al., 1955). Dissolved organic carbon (DOC) (Elementar Liqui-TOC, Hanau, Germany) and pH were measured both prior to and at the end of the incubation for all treatments.

Gas samples were collected once a week from the headspace of the incubation flasks to measure CO_2 and CH_4 production by gas chromatography (SRI gas chromatograph 8610 GC-FID with methanizier). Dissolved concentrations were recalculated from the measured headspace concentrations in the vial, volume of the headspace and the water phase, and Henry's law constant ($K_H(CO_2) = 2.97 \cdot 10^{-2}$ mol L^{-1} atm^{-1} and $K_H(CH_4) = 1.28 \cdot 10^{-3}$ mol L^{-1} atm^{-1}) corrected to the temperature of the sample (Stumm and Morgan, 1995).

Prior to analysis of the solid samples, the peat suspension was purged with N_2 to remove remaining H_2S from solution and subsequently washed three times with 0.1 mol L^{-1} NaCl to remove remaining SO_4^{2-}. Finally, a subsample of peat soil from each incubation treatment was freeze-dried, milled (µ<50 µm) and analyzed for total element concentrations by wavelength-dispersive X-ray fluorescence spectroscopy (WD-XRF ZSX Primus II, Rigaku, Tokyo, Japan). Total C, S, and N contents and $\delta^{34}S$ stable

isotopic signatures were analyzed using an isotope ratio mass spectrometer (EA-IRMS, Horizon, Nu instruments, Wrexham, UK, and EA 3000, Eurovector/Hekatech GmbH, Wegberg, Germany). Values of $\delta^{34}S$ are reported in the conventional delta notation versus V-CDT (calibrated using a ^{34}S value of -0.3‰ of the IAEA-S-1 reference). Reproducibility of the measurements was better than 0.2‰ for standards and better than 0.4‰ for selected replicate samples. Na_2S and Na_2SO_4 employed for the incubations were also analyzed for $\delta^{34}S$.

Speciation of organic S in the peat soil was investigated by S K-edge XANES spectroscopy at the SUL-X beamline of the ANKA Synchrotron Radiation Facility (KIT) (Yu et al., 2015). The energy was calibrated to the sulfate excitation energy of sodium sulfate at 2481.4 eV. Spectra were collected under vacuum by recording the S *Kα* X-ray fluorescence emission with a seven element Si(Li) solid-state detector (SGX Sensortech, former Gresham). To prevent S species from beam damage, spectra were collected in a quick scan mode with sampling step widths of 1 eV from 2432 to 2461 eV and 2502 to 2756 eV, and 0.2 eV across the S K edge from 2461 to 2501 eV. Up to 4 scans of different sample spots were accumulated for each sample spectrum. Measured references are given in Figure 16 and Table 11. Spectral data is provided in the supporting information. Data were energy calibrated, background corrected, and normalized with the ATHENA program of the IFFEFIT package (Ravel et al., 2005). Also the linear combination fits were carried out with ATHENA.

Turnover calculation

Net turnover of CO_2, CH_4, $SO_4^{2-}$and Fe^{3+}during the incubation period were calculated from their change in concentration over time.

For electron budget calculations, we assumed a reduction of SO_4^{2-} to H_2S, equivalent to a transfer of 8 electrons, Fe^{3+} reduction to Fe^{2+}, equivalent to a transfer of one electron, an oxidation of organic matter with an average oxidation state of 0 to CO_2 with a carbon oxidation state of +IV. Eventual production of CH_4 (carbon oxidation state of -IV) was subtracted from CO_2 production for budgets. All CO_2 produced in excess of inorganic electron acceptors or CH_4 was ascribed to electron transfer to organic matter and converted into electron equivalents. We are aware that this calculation neglects intermediates of e.g. sulfur, such as thiosulfate ($S_2O_3^{2-}$) that was not analyzed. However, according to early studies, the concentration of $S_2O_3^{2-}$ is mostly lower than 10 μmol L^{-1} in pore water of ombrotropic peat soil and an accumulation of this intermediate seems unlikely (Heitmann et al., 2007; Yu et al., 2015). To analyze for CO_2 production from fermentation, which would not be accounted for in the outlined budgets, both CH_3COO^- and hydrogen (H_2) concentration were evaluated during incubation. As these fermentation products did not accumulate (CH_3COO^- mostly < 1 μmol L^{-1}, H_2 mostly < 0.05 μg L^{-1}), CO_2 production from fermentation is accounted for by the assumption of an oxidation state of zero for the bulk organic matter (data not shown).

RESULTS

1 Evolution of solute concentrations

The initial pH of all treatment was about 3.6~3.8. At the end of the incubation, values of pH had increased to 4.5~4.7, except of the Ac-SO_{4A} treatment with both SO_4^{2-} and CH_3COO^- addition, where the pH reached 5.7. DOC concentrations of all treatments ranged from 359.2 to 1467.5 mg C L^{-1}. The calculated highest dissolved organic carbon

(DOC) release rates were 2.5 nmol cm^{-3} peat day^{-1} also occurred in the Ac-SO_{4A} treatment (after subtraction of the added CH_3COO^-), whereas for all other treatments it ranged from 0.60 to 0.84 nmol cm^{-3} peat day^{-1} (Table 12).

Mean SO_4^{2-} concentrations at the end of the incubation were 3.92 µmol L^{-1} with no detectable dissolved total sulfide (detection limit of method is ca. 2.5 µmol L^{-1}) in the Control $_A$ treatments without SO_4^{2-} addition. In the treatments with sulfur addition, final concentrations of SO_4^{2-} in were in the order $SO_{4\ A}$ (15.14 mmol L^{-1}) > Ac-$SO_{4\ A}$ (8.29 mmol L^{-1}) > Sulfide $_A$ (1.49 µmol L^{-1}), and final concentrations of dissolved sulfide were 26.5, 237.5 and 3.9 µmol L^{-1}, respectively (Figure 17).

Dissolved Fe^{2+} concentration increased gradually and approached an apparent equilibration at the end of the incubation (Figure 17). For all treatments, the finial dissolved concentration was in the range of 87.5 to 94.8 µmol L^{-1}, indicating a similar availability of iron in all treatments as derived from the parent peat material.

2 Net rates of CO_2 and CH_4 production, net rates of SO_4^{2-} , Fe^{3+} reduction

Production of CO_2 initiated quickly, while there was the typically observed delay in CH_4 production in all treatments. In Control $_A$ treatment, the mean amount of CO_2 in the flasks increased to 45.90 µmol cm^{-3} and of CH_4 to 8.44 µmol cm^{-3} after 40 days (Figure 18). The mean production rate of CO_2 was thus 845.3 nmol cm^{-3} peat d^{-1} and of CH_4 263.9 nmol cm^{-3} peat d^{-1} (Table 13). The observed ratio of CO_2 to CH_4 production (CO_2/CH_4) was 5.0:1.

Addition of SO_4^{2-} enhanced CO_2 production and suppressed CH_4 production as expected. In the $SO_{4\ A}$ treatment, CO_2 production rates were 1200.1 nmol cm^{-3} peat d^{-1} and in the

Ac-SO_4 $_A$ treatment 1884.9 nmol cm^{-3} peat d^{-1} (Table 13). Additions of SO_4^{2-} and 'SO_4^{2-} + CH_3COO^-' kept CH_4 production rates as little as 0.13 and 0.17 nmol cm^{-3} peat d^{-1}.

Since there were no detectable concentration change of SO_4^{2-}, net SBR rates were calculated to about zero for the treatments with no external SO_4^{2-} addition (Control $_A$; Sulfide $_A$). In the SO_4 $_A$ treatment, net sulfate reduction was on average 121.1 nmol cm^{-3} peat d^{-1}. Compared to the SO_4 $_A$ treatment, net sulfate reduction rates were higher after both SO_4^{2-} and CH_3COO^- addition, with a mean rate of 351.2 nmol cm^{-3} peat d^{-1} in the Ac-SO_4 $_A$ treatment.

Net ferric iron reduction rates ranged from 2.5 to 2.8 nmol cm^{-3} peat d^{-1} for all treatments; iron was thus less important for respiration. To account for solid phase iron contributions to anaerobic respiration, we quantified 0.5M HCl extractable Fe^{3+} from the peat soil, ranging from 2.1 to 2.2 µmol gdw^{-1}in all treatments. As there was no significant difference between the peat prior to incubation and at end of incubation in all treatments, solid phase iron did obviously not net contribute to CO_2 production.

3 Electron flow budgets

The electron budgets were not closed for the incubation period when considering inorganic electron acceptors only, and also differed among the treatments. The Control $_A$ treatment, with no inorganic electron acceptor addition, had an electron acceptor deficit of 103.9 µmol e^- cm^{-3} over the 40-days incubation period, as calculated from CO_2 production subtracted by CH_4 production. For the Sulfide $_A$ treatment, the electron acceptor deficit was 105.1 µmol e^- cm^{-3} and thus comparable to Control $_A$ (Table 13). These capacities thus had been provided by the organic matter.

In the SO_4 $_A$ and the Ac-SO_4 $_A$ treatments, the overall electron acceptor deficit, considering inorganic electron acceptors only, was 170.9 and 213.6 µmol e^- cm^{-3} for the time course of incubation, respectively. When compared to Control $_A$, after subtraction of the added SO_4^{2-} as electron acceptor, still more CO_2 was produced in these treatments, i.e. the electron accepting capacity provided by organic matter was higher under addition of SO_4^{2-}, probably due to internal H_2S re-oxidation to fuel SO_4^{2-}.

4 Characterization of sulfur in organic matter by XANES spectroscopy

To analyze predominant organic sulfur species, to identify changes of organic sulfur under sulfidic conditions, and to characterize the contribution of organic sulfur redox processes for anaerobic respiration, S K-edge XANES spectroscopy was used to determine the changes of major organic sulfur species prior to and after the incubation (Figure 16 and Table 11). In general, in all treatments we observed two distinct peak ranges, from 2471 to 2474 eV representing mainly reduced organic S species, such as thiols and S bridging structures involving one or more S atoms (e.g. R-S-H or R-S-R, R-S-S-R), and from 2475 to 2483 eV indicative of oxidized organic S species, e.g. organic sulfoxides, sulfones, sulfonates, and sulfate esters. However, due to a high sensitivity for sulfate, in the latter region also traces of inorganic sulfate S may contribute to the signal (Table 12and Figure16).

When compared to Control $_A$, in both SO_4^{2-} addition treatments, peak intensities mainly increased around 2471.6 eV after the incubation, especially in Ac-SO_4 $_A$ treatment. This shifted the predominant signal of reduced organic S from 2473.1 eV, indicative of thiols, to 2471.6 eV, comparable to references of organic disulfide. This indicated that H_2S from

BSR attached to the peat organic matter as reduced organic sulfur, presumably forming S bridging structures involving more two or more S atoms (R-S-S-R; R-S_x-R, x > 2). In addition, we also observed slight changes of a minor peak at 2475.1 eV (possibly sulfoxide) diminishing, and a new peak at 2481.2 eV (sulfate or sulfate ester) forming after incubation. Changes of organic sulfur in the Sulfide $_A$ treatment were small, presumably due to the comparably low amount of sulfide added, not to impede microbial activities. Changes only involved a broadening of the peak of reduced organic sulfur towards lower energies, indicating a relative increase also here of sulfur bridging structures involving two or more S atoms.

Linear combination fittings (LCF) of S-K-edge XANES spectra with reference spectra of known compounds was applied to ge approximate contributions of different organic sulfur species to peat bulk samples prior to and after incubation (Manceau and Nagy, 2012). To allow for relative comparison we also calculated a sulfur oxidation index (OXI), dividing the sum of oxidizes organic S species by the sum or reduced species (see Table 14). The original $_O$ prior to incubation, with an OXI of 0.44, contained 68.3% reduced organic S (R-S-H, R-S-R, R-S-S-R), and 31.5% oxidized S (R-SO_3, R-SO_2-R, R-SO_4-R), whereas the treatment with H_2S or 'SO_4^{2-} + CH_3COO^-' addition comprised 73.9 and 81.3% reduced organic S, and only 18.7 and 26.1 % oxidized S, respectively. OXI was thus 0.23 for Ac-$SO_{4\ A}$ and 0.35 for Sulfide $_A$. Surprisingly, in the $SO_{4\ A}$ treatment amended with only SO_4^{2-}, a notable increase of organic sulfur (39 %) versus reduced organic sulfur (61 %) was observed, possibly due to a formation of sulfate esters, yielding an OXI of 0.61. However, due to the high amount of sulfate added, we cannot exclude

that this high content of oxidized organic sulfur species may be biased by traces of inorganic SO_4^{2-} which were not completely removed during the washing step.

5 Isotopic composition of incubated peat

The $\delta^{34}S$ values of the original peat in Control $_O$ and Control $_A$ were +6.62 and +6.30 ‰, respectively (Table 10 and Table 14). In the Sulfide $_A$ treatment, $\delta^{34}S$ values increased to +8.11‰, as expected from addition of another 0.03 (elemental analysis) to 0.04 % S (isotope budget) from sulfide with a $\delta^{34}S$ of +17.26 ± 0.58 ‰. The Na_2SO_4 used for the sulfate amendments had a $\delta^{34}S$ of 4.30 ± 0.57 ‰. Due to a discrimination of the ^{34}S isotope during BSR and therefore low $\delta^{34}S$ values in the biogenic sulfide that added to the organic matter, $\delta^{34}S$ in the sulfate amended treatments decreased to +2.39 ‰ and +3.57 ‰ in the SO_4 $_A$ and Ac-SO_4 $_A$ treatment, respectively. Assuming the change in S contents from elemental analysis as a basis (increase from 0.18 % to 0.33~0.34 % S), $\delta^{34}S$ of the biogenic sulfide would have been -2.5 ‰ and +0.1 ‰ in the SO_4 $_A$ and Ac-SO_4 $_A$ treatments, respectively.

DISSCUSSION

We examined the hypothesis that organic matter provides electron accepting capacity for anaerobic respiration and sustains internal sulfur cycling to maintain high rates of BSR. This would lead to both more CO_2 production as expected from inorganic electron acceptors and continuous suppression of methanogenesis. Our results support that the direct or indirect contribution of organic matter to electron accepting capacities in anaerobic respiration cannot be neglected, as studied in a peat soil at 30 °C and 40 d incubation.

1 Contribution of organic matter to electron accepting capacities

Under methanogenic conditions, CO_2 and CH_4 should be produced in equal amounts resulting in a theoretical 1:1 ratio of CO_2:CH_4 (Conrad, 1999; Heitmann et al., 2007). A deviation from this ratio towards higher numbers would indicate the presence of an alternative electron acceptor. In terms of possible inorganic electron acceptors, the calculated net Fe^{3+} and SO_4^{2-} reduction rates were negligible (only little or no significant changes of concentrations, <1 µmol e^- cm^{-3}). As Fe (III) oxides are highly abundant in freshwaters (Lovley, 1991; Roden and Wetzel, 1996), a contribution of solid-phase inorganic TEAs could be important (Lovley, 1991; Roden and Urrutia, 2002; Roden and Wetzel, 1996). Although a formation of dissolved Fe(II) was often monitored, it was suggested that Fe(III) reduction may be underestimated if Fe (II) was adsorbed onto solid-phase compounds, e.g. clays or organic matter in early study (Lovley and Woodward, 1996; Lovley, 1991). However, analysis of HCl-extractable Fe (III) and Fe(II) in our study showed that total EAC from solid-phase Fe(III) reduction should be less than 1 µmol e^- cm^{-3}, which was far too less to close the electron accepting budgets in the different treatments. Thus, almost the entire non-methanogenic CO_2 production, equivalent to 103.9~105.1 µmol e^- cm^{-3}, could not be explained by the microbial reduction of inorganic terminal electron acceptors (TEA) in our incubations.

In the Control $_A$ treatment, incubated peat material produced CO_2 and CH_4 at a ratio of 3.2, equivalent to a consumption rate of EAC from organic matter of 2.36 µmol e^- cm^{-3} d^{-1}. A similar CO_2:CH_4 ratio of 3.4 and a similar rate of EAC of 2.39 µmol e^- cm^{-3} d^{-1} consumption from organic matter were observed for the Sulfide $_A$ treatment. This was surprising, as sulfide was supposed to pre-reduce organic matter, thereby decreasing

electron accepting capacities (Yu et al., 2015) and resulting in a lower CO_2 production and compared to the Control $_A$, which was not observed. Thus it may be hypothesized that rapid reaction of sulfide with organic matter did not affect the total electron accepting capacities in the system, as it was operating at or below the redox potential of sulfide oxidation. As a net effect, the sulfide added to the Sulfide $_A$ treatment must have remained in an oxidation state of –II, thus not changing the EAC of the organic matter, or sulfide added to organic matter at sites that did not significantly contribute to EAC. Of course, right after addition, sulfide may have in part been oxidized (Heitmann and Blodau, 2006) but these oxidized sulfur species must subsequently have been reduced by BSR. Such rapid cycling or an addition of sulfide to organic matter is supported by the observation that in this treatment hardly any sulfide was detectable soon after addition.

Many existing studies similarly observed that freshwater peat soils reach high CO_2:CH_4 ratios clearly deviating from 1 (Blodau, 2002; Bridgham et al., 1998; Vile et al., 2003a, 2003b; Yavitt et al., 1987) (van Hulzen et al., 1999) despite low amounts of inorganic TEAs (e.g., NO_3^-, Fe^{3+}, SO_4^{2-}). Thus, our study again confirms the importance of organic matter as an electron acceptor(Klüpfel et al., 2014). In our case, the incubated peat provided an EAC of 103.9~105.1 µmol e^- cm^{-3}(30 °C). Converting results of earlier studies to the same unit, reported EACs are in a range of 5.2~13.1 µmol e^- cm^{-3} at 25 °C (Roden et al., 2010) or 9.3 µmol e^- cm^{-3} at 20 °C (Keller and Takagi, 2013). Our results exceeded these values by a factor of 13~ 19 or by a factor of 11, respectively. In a recent study of Lau et al., (2014), electrochemically measured EACs of peat reached 61.1 µmol e^- cm^{-3} for a fen soil, which was much closer to our current observation. Thus, Lau et al., (2014) suggested previously reported lower EACs may result from methodological

limitations, as in these studies EACs were not measured electrochemically (Aeschbacher et al., 2010), but by quantify the amount of electrons transferred to Fe-stripped sediments during microbial or chemical reduction (Lovley et al., 1996; Roden et al., 2010). Moreover, our incubation was performed at 30°C to increase microbial activity to be able to detect changes in solid peat sulfur speciation for analysis by S-K-edge XANES spectroscopy.

2 Contribution of organic matter to electron accepting capacities under sulfidic conditions

Previous studies by Heitmann and Blodau, (2006) and Yu et al., (2015) suggested that organic matter can support an internal cycling of inorganic electron acceptors, e.g. a re-oxidation of sulfide to more oxidized sulfur species. Such a recycling could sustain high rates of TEA reduction despite small pool sizes.

Since BSR is only slightly thermodynamically superior to methanogenesis (Zehnder and Stumm, 1988), we dosed a significant amount of SO_4^{2-} to completely suppress CH_4 production. The SO_4^{2-} addition in the treatments $SO_{4\ A}$ and Ac-$SO_{4\ A}$ stimulated CO_2 production and inhibited CH_4 production as expected from previous studies (Achtnich et al., 1995; Knorr and Blodau, 2009; Segers and Kengen, 1998), resulting in a much higher final CO_2:CH_4 ratio. Interestingly, sulfur isotope fractionation of BSR in the treatments $SO_{4\ A}$ and Ac-$SO_{4\ A}$ was low with a $\Delta = \delta^{34}S_{sulfate} - \delta^{34}S_{sulfide}$ of 4.2~6.8 ‰ (α = 1.0042~1.0068) compared to a Δ of 10~40 ‰ reported by (Canfield, 2001), keeping in mind the uncertainties in calculating $\delta^{34}S$ of biogenic sulfide from a mass balance. Nevertheless, also here under addition of sulfate, most of the CO_2 production could not

be explained by the use of inorganic TEAs. These results coincide with e.g. the study of Neubauer et al. (2005), who found only 20% of CO_2 production originating from reduction of inorganic TEAs; or with a study of Segers and Kengen, (1998), where total CO_2 production was also mainly from unknown electron acceptors, i.e. organic matter. In our case, EAC provided though SO_4^{2-} addition explained 10~37 % of the CO_2 produced; EAC supplied by organic matter reached 3.88 to 4.85 μmol $e^- cm^{-3} d^{-1}$ (with and without addition of CH_3COO^-, respectively).

Interestingly, calculated EACs supplied by organic matter for the SO_4^{2-} addition treatments were 1.65-2 folds the EAC supplied by organic matter of the Control $_A$ treatment. This indicates that recycling of sulfur by organic matter provided more electron accepting capacity than organic matter could provide without addition of sulfate. Thus, organic matter presumably served as an additional indirect electron via oxidation of hydrogen sulfide (Bauer et al., 2007; Heitmann et al., 2007; Keller et al., 2009). A higher yield of EAC from organic matter by addition of sulfur into the system coincided with our previous studies of Sigma Aldrich humic acid, where we could also retrieve higher EAC through sulfide oxidation than by wet chemical approaches (Heitmann and Blodau, 2006; Heitmann et al., 2007; Keller and Bridgham, 2007; Keller et al., 2009; Yu et al., 2015). These results demonstrate that an evaluation of simplified systems, such as quantifying EACs solely of organic matter, may be too simplistic and cannot be easily transferred to more complex natural systems.

3 Contribution of organic sulfur for electron flow budget

Under sulfidic conditions, also an incorporation of sulfur into natural organic matter, e.g. by formation of organic polysulfides or H_2S linkages, has been widely observed in studies of sediment diagenesis, both in freshwater or marine systems (Brown, 1986; Brüchert and Pratt, 1996; Ferdelman et al., 1991). In the current study, organic sulfur in incubated peat samples with sulfur amendment (sulfide or sulfate) comprised of 10~14, 46, or 47 % of newly formed organic sulfur in the treatments Sulfide $_A$, SO_4 ^{A}, and Ac-SO_4 $_A$, respectively (Table 14). Such high contribution of newly formed organic sulfur strengthens the role of organic sulfur in sulfur cycling and partly exceeded organic sulfur formation reported in (Yu et al., 2015), where after exposure to 250 µmol L^{-1} of sulfide 11~20 % of organic sulfur originated from newly added sulfur.

Moreover, our experimental design allowed for sufficient formation of organic sulfur to detect changes in speciation using S K-edge XANES spectroscopy, which had been proven to yield additional insights into the organic matter/sulfur system (Einsiedl et al., 2008; Hoffmann et al., 2012; Manceau and Nagy, 2012; Prietzel et al., 2011, 2007; Xia et al., 1998). Following up an earlier study of Heitmann and Blodau, (2006), Yu et al., (2015) recently provided detailed information about the time scales and species of organic sulfur formation. Sulfur K-edge XANES spectroscopy results of the current study confirmed observations in abiotic experiments of Yu et al., (2015): as observed for addition of sulfide to organic matter under abiotic conditions, also sulfide produced from BSR mainly added to organic matter as reduced organic sulfur, presumably forming about zerovalent organic sulfur species, such as thiols and organic di- and polysulfides. These organic sulfur species may be regarded to provide additional electron transfer capacities, e.g. of 1.01~1.80 µmol e^- $(mg\ C)^{-1}$ determined in Yu et al., (2015). It may be

hypothesized that formation of organic polysulfides could provide a source of elemental sulfur for sulfate reducing bacteria, as suggested for inorganic polysulfides (Schauder and Müller, 1993).

Besides the contribution of formed reduced organic sulfur for the electron acceptor budget during the anaerobic incubations, also slight changes of oxidized organic sulfur were observed as reported previously for abiotic incubation of humic acid and sulfide (Yu et al., 2015). A decrease of the peak indicative of sulfoxide S (R-SO-R), at 2475.1 eV, was found after incubation of SO_4^{2-} addition treatments, possibly due to a reduction of sulfoxides by H_2S (Ratasuk and Nanny, 2007). However, this decrease of sulfoxides was not observed for the treatment with sulfide addition only; possibly the added amount of sulfide was too low, as only 1000 μmol L^{-1} sulfide were dosed compared to a reductive consumption of 4863 and 11709 μmol L^{-1} of sulfate in the sulfate addition treatments. Moreover, slight decreases of sulfate esters may have occurred in the treatments with no sulfate addition, suggesting a possible reduction of sulfate esters during incubation (Kertesz, 2000). In presence of added sulfate, the S K-edge XANES signal indicative of sulfate esters was presumably biased by inorganic sulfate due to incomplete washing of the samples. On the basis of our data, we cannot consider changes of these fractions separately in the mass balance. However, it is interesting to note that measurable changes of both reduced and oxidized organic sulfur in solid peat organic matter occurred within such short timescales of incubation. Moreover, such changes in oxidized organic S species would provide additional electron transfer capacity of soil organic matter towards sulfide.

CONCLUSIONS

Base on the frequent observation of 'unexplained' anaerobic CO_2 production mostly in organic wetland soils with low available inorganic electron acceptor, the current study illustrates the importance of soil organic matter and internal sulfur cycling for electron accepting capacities for anaerobic respiration. (1) Electron acceptor budgets clearly demonstrated the predominance of soil organic matter EACs over inorganic electron acceptors. (2) Addition of sulfate induced an internal sulfur cycle, yielding even higher contributions of EACs from organic matter compared to incubations without sulfate addition. (3) Moreover, S K-edge XANES spectroscopy results demonstrated that both formation of reduced organic sulfur and transformed oxidized organic sulfur contributed to the total electron transfer during anaerobic respiration. Our results indicate that future studies do not only need to account for EACs of organic matter, but also include how an internal sulfur cycle increases EACs in a sulfidic, organic rich system.

ACKNOWLEDGEMENTS

We thank Ines Spangenberg, Markus Schmitt and Simona Bonaiuti for lab work support, and Ulrike Berning-Mader and Madeleine Supper for technical support. The study was funded by the German Research Foundation, research group 'electron transfer processes in anoxic aquifers' (FOR 580, KN 929/2-1). We acknowledge the Synchrotron Light Source ANKA for providing us with the instrumentation at their beamline SUL-X within proposals ENV-236 and A2013-022-004203.

REFERENCES

Achtnich, C., Bak, F., Conrad, R., 1995. Competition for electron donors among nitrate reducers , ferric iron reducers , sulfate reducers , and methanogens in anoxic paddy soil. Biology and Fertility of Soils 19, 65–72.

Aeschbacher, M., Sander, M., Schwarzenbach, R.P., 2010. Novel electrochemical approach to assess the redox properties of humic substances. Environmental science and technology 44, 87–93.

Avery, G.B., Shannon, R.D., White, J.R., Martens, C.S., Alperin, M.J., 1999. Effect of seasonal changes in the pathways of methanogenesis on the $\delta^{13}C$ values of pore water methane in a Michigan peatland. Global Biogeochemical Cycles 13, 475–484.

Bauer, M., Heitmann, T., Macalady, D.L., Blodau, C., 2007. Electron transfer capacities and reaction kinetics of peat dissolved organic matter. Environmental Science and Technology 41, 139–145.

Blodau, C., 2002. Carbon cycling in peatlands-A review of processes and controls. Environmental Research 10, 111–134.

Bridgham, S.D., Updegraff, K., Pastor, J., 1998. Carbon, nitrogen and phosphorus mineralization in northern wetlands. Ecology 79, 1545–1561.

Brown, K.A., 1986. Formation of organic sulphur in anaerobic peat. Soil Biology and Biochemistry 18, 131–140.

Brüchert, V., Pratt, L.M., 1996. Contemporaneous early diagenetic formation of organic and inorganic sulfur in estuarine sediments from St. Andrew Bay, Florida, USA. Geochimica et Cosmochimica Acta 60, 2325–2332.

Canfield, D.E., 2001. Isotope fractionation by natural populations of sulfate-reducing bacteria. Geochimica et Cosmochimica Acta 65, 1117–1124.

Canfield, D.E., Boudreau, B.P., Mucci, A., Gundersen, J.K., 1998. The early diagenetic formation of organic sulfur in the sediments of mangrove lake, bermuda. Geochimica et Cosmochimica Acta 62, 767–781.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnology and Oceanography 14, 454–458.

Conrad, R., 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiology Ecology 28, 193–202.

Einsiedl, F., Mayer, B., Schäfer, T., 2008. Evidence for incorporation of H_2S in groundwater fulvic acids from stable isotope ratios and sulfur K-edge X-ray absorption near edge structure spectroscopy. Environmental Science and Technology 42, 2439–2444.

Ferdelman, T.G., Church, T.M., Luther III, G.W., 1991. Sulfur enrichment of humic substances in a Delaware salt marsh sediment core. Geochimica et Cosmochimica Acta 55, 979–988.

Goldhaber, M.B., Kaplan, I.R., 1980. Mechanisms of sulfur incorporation and isotope fractionation during early diagenesis in sediments of the gulf of California. Marine Chemistry 9, 95–143.

Harvey, A.E., Smart, J.A., Amis, E.S., 1955. Simultaneous spectrophotometric determination of iron(II) and total iron with 1,10-phenanthroline. Analytical Chemistry 27, 26–29.

Heitmann, T., Blodau, C., 2006. Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. Chemical Geology 235, 12–20.

Heitmann, T., Goldhammer, T., Beer, J., Blodau, C., 2007. Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog. Global Change Biology 13, 1771–1785.

Hoffmann, M., Mikutta, C., Kretzschmar, R., 2012. Bisulfide reaction with natural organic matter enhances arsenite sorption: insights from X-ray absorption spectroscopy. Environmental Science and Technology 46, 11788–11797.

Hornibrook, E.R.C., Longstaffe, F.J., Fyfe, W.S., 1997. Spatial distribution of microbial methane production pathways in temperate zone wetland soils: stable carbon and hydrogen isotope evidence. Geochimica et Cosmochimica Acta 61, 745–753.

Keller, J.K., Bridgham, S.D., 2007. Pathways of anaerobic carbon cycling across an ombrotrophic-minerotrophic peatland gradient. Limnology and Oceanography 52, 96–107.

Keller, J.K., Takagi, K.K., 2013. Solid-phase organic matter reduction regulates anaerobic decomposition in bog soil. Ecosphere 4.

Keller, J.K., Weisenhorn, P.B., Megonigal, J.P., 2009. Humic acids as electron acceptors in wetland decomposition. Soil Biology and Biochemistry 41, 1518–1522.

Kertesz, M.A., 2000. Riding the sulfur cycle-metabolism of sulfonates and sulfate esters in Gram-negative bacteria. FEMS Microbiology Reviews 24, 135–175.

Klueber, H.D., Conrad, R., 1998. Effects of nitrate, nitrite, NO and N_2O on methanogenesis and other redox processes in anoxic rice field soil. FEMS Microbiology Ecology 25, 301–318.

Klüpfel, L., Piepenbrock, A., Kappler, A., Sander, M., 2014. Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nature Geoscience 7, 195–200.

Knorr, K.-H., Blodau, C., 2009. Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil. Soil Biology and Biochemistry 41, 1187–1198.

Lau, M.P., Sander, M., Gelbrecht, J., Hupfer, M., 2014. Solid phases as important electron acceptors in freshwater organic sediments. Biogeochemistry.

Lovley, D.R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiological Reviews 55, 259–287.

Lovley, D.R., Coates, J.D., Blunt-Harris, E.L., Phillips, E.J.P., Woodward, J.C., 1996. Humic substances as electron acceptors for microbial respiration. Nature 382, 445–448.

Lovley, D.R., Dwyer, D.F., Klug, M.J., 1982. Competition between sulfate for hydrogen in sediments kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sedimentst. Applied and Environmental Microbiology 43, 1373–1379.

Lovley, D.R., Phillips, E.J., 1986. Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal potomac river. Applied and Environmental Microbiology 52, 751–757.

Lovley, D.R., Woodward, J.C., 1996. Mechanisms for chelator stimulation of microbial Fe(III)-oxide reduction. Chemical Geology 132, 19–24.

Manceau, A., Nagy, K.L., 2012. Quantitative analysis of sulfur functional groups in natural organic matter by XANES spectroscopy. Geochimica et Cosmochimica Acta 99, 206–223.

Mikaloff Fletcher, S.E., Tans, P.P., Bruhwiler, L.M., Miller, J.B., Heimann, M., 2004. CH4 sources estimated from atmospheric observations of CH_4 and its $^{13}C/^{12}C$ isotopic ratios: 1. Inverse modeling of source processes. Global Biogeochemical Cycles 18.

Neubauer, S.C., Givler, K., Valentine, S., Megonigal, J.P., 2005. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86, 3334–3344.

Phillips, D.L., Koch, P.L., 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia 130, 114–125.

Prietzel, J., Botzaki, A., Tyufekchieva, N., Brettholle, M., Thieme, J., Klysubun, W., 2011. Sulfur speciation in soil by S K-Edge XANES spectroscopy: comparison of spectral deconvolution and linear combination fitting. Environmental Science and Technology 45, 2878–2886.

Prietzel, J., Thieme, J., Salomé, M., Knicker, H., 2007. Sulfur K-edge XANES spectroscopy reveals differences in sulfur speciation of bulk soils, humic acid, fulvic acid, and particle size separates. Soil Biology and Biochemistry 39, 877–890.

Ratasuk, N., Nanny, M.A., 2007. Characterization and quantification of reversible redox sites in humic substances. Environmental Science and Technology 41, 7844–7850.

Ravel, B. Newville, M., 2005. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. Journal of Synchrotron Radiation, 12,537–541.

Roden, E.E., Kappler, A., Bauer, I., Jiang, J., Paul, A., Stoesser, R., Konishi, H., Xu, H., 2010. Extracellular electron transfer through microbial reduction of solid-phase humic substances. Nature Geoscience 3, 417–421.

Roden, E.E., Urrutia, M.M., 2002. Influence of biogenic Fe(II) on bacterial crystalline Fe(III) oxide reduction. Geomicrobiology Journal 19, 209–251.

Roden, E.E., Wetzel, R.G., 1996. Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnology and Oceanography 41, 1733–1748.

Schauder, R., Müller, E., 1993. Polysulfide as a possible substrate for sulfur-reducing bacteria. Archives of Microbiology 160, 377–382.

Scott, D.T., McKnight, D.M., Blunt-Harris, E.L., Kolesar, S.E., Lovley, D.R., 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environmental Science and Technology 32, 2984–2989.

Segers, R., Kengen, S.W.M., 1998. Methane production as a function of anaerobic carbon mineralization: A process model. Soil Biology and Biochemistry 30, 1107–1117.

Solomon, D., Lehmann, J., de Zarruk, K.K., Dathe, J., Kinyangi, J., Liang, B., Machado, S., 2011. Speciation and long- and short-term molecular-level dynamics of soil organic sulfur studied by X-ray absorption near-edge structure spectroscopy. Journal of Environmental Quality 40, 704–718.

Solomon, D., Lehmann, J., Martínez, C.E., 2003. Sulfur K-edge XANES spectroscopy as a tool for understanding sulfur dynamics in soil organic matter. Soil Science Society of America Journal 67, 1721–1731.

Stumm, W., Morgan, J.J. (Eds.), 1995. Aquatic chemistry: chemical equilibria and rates in natural waters, 3rd ed. Wiley, New York.

Urban, N.R., Ernst, K., Bernasconi, S., 1999. Addition of sulfur to organic matter during early diagenesis of lake sediments. Geochimica et Cosmochimica Acta 63, 837–853.

Van Hulzen, J.B., Segers, R., van Bodegom, P.M., Leffelaar, P.A., 1999. Temperature effects on soil methane production: an explanation for observed variability. Soil Biology and Biochemistry 31, 1919–1929.

Vile, M.A., Bridgham, S.D., Wieder, R.K., 2003a. Response of anaerobic carbon mineralization rates to sulfate amendments in a boreal peatland. Ecological Applications 13, 720–734.

Vile, M.A., Bridgham, S.D., Wieder, R.K., Novák, M., 2003b. Atmospheric sulfur deposition alters pathways of gaseous carbon production in peatlands. Global Biogeochemical Cycles 17.

Wakeham, S.G., Sinninghe Damsté, J.S., Kohnen, M.E.L., De Leeuw, J.W., 1995. Organic sulfur compounds formed during early diagenesis in Black Sea sediments. Geochimica et Cosmochimica Acta 59, 521–533.

Xia, K., Weesner, F., Bleam, W.F., Helmke, P.A., Bloom, P.R., Skyllberg, U.L., 1998. XANES studies of oxidation states of sulfur in aquatic and soil humic substances. Soil Science Society of America Journal 62, 1240–1246.

Yavitt, J.B., Lang, G.E., Wieder, R.K., 1987. Control of carbon mineralization to CH_4 and CO_2 in anaerobic,Sphagnum-derived peat from Big Run Bog, West Virginia. Biogeochemistry 4, 141–157.

Yu, Z., Peiffer, S., Goettlicher, J., Knorr, K.-H., 2015. Electron transfer budgets and kinetics of abiotic oxidation and incorporation of aqueous hydrogen sufide by dissolved organic matter. Environmental Science and Technology 9, 5441–5449.

Zehnder, A.J.B., Stumm, W., 1988. Geochemistry and biogeochemistry of anaerobic habitats, in: A.J.B., Z. (Ed.), Biology of Anaerobic Habitats. John Wiley & Sons, Ltd: New York, pp. 1–38.

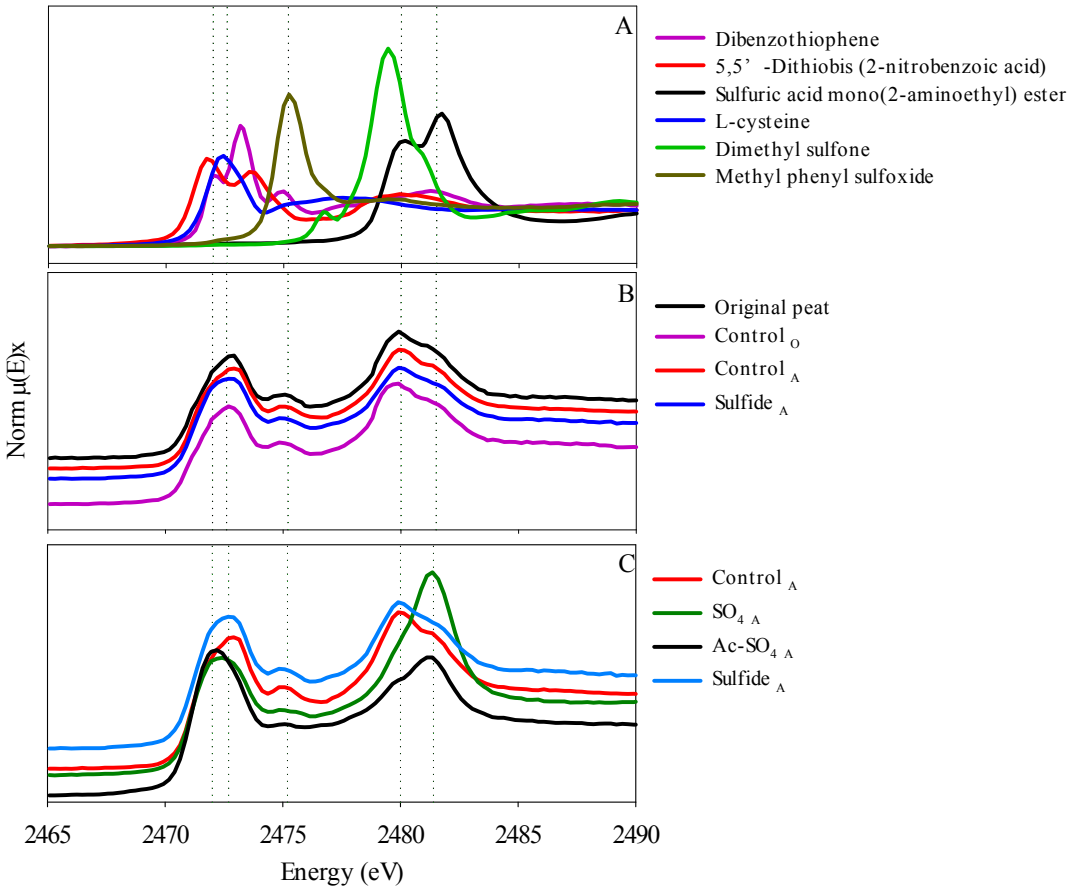


Figure 16. Difference in S-K-edge XANES spectra of organic sulfur references (A) and incubated peat samples (B, C). The difference in the spectra only gives information about relative changes of individual electronic oxidation states. Markers (vertical dotted line) are at 2471.6, 2472.6, 2475.2, 2480.2 and 2481.2 eV.

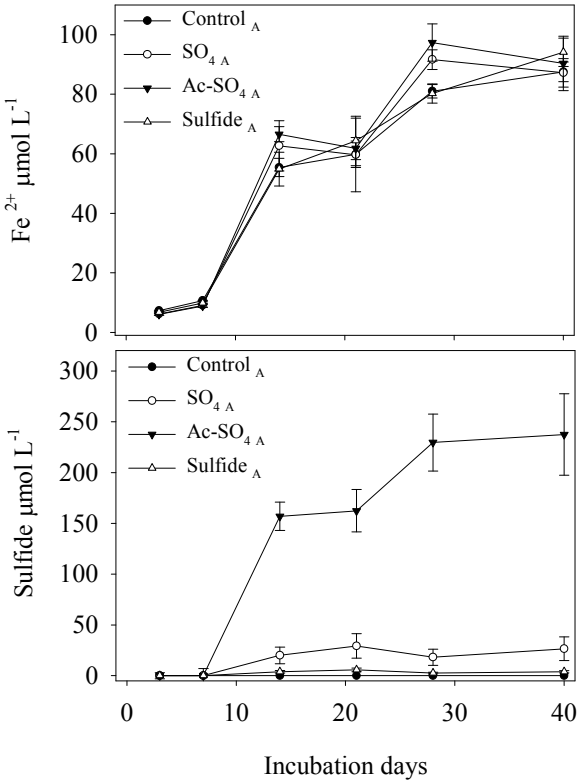


Figure 17. Measured dissolved Fe^{2+} and sulfide concentration over 40 days peat incubation of different treatment

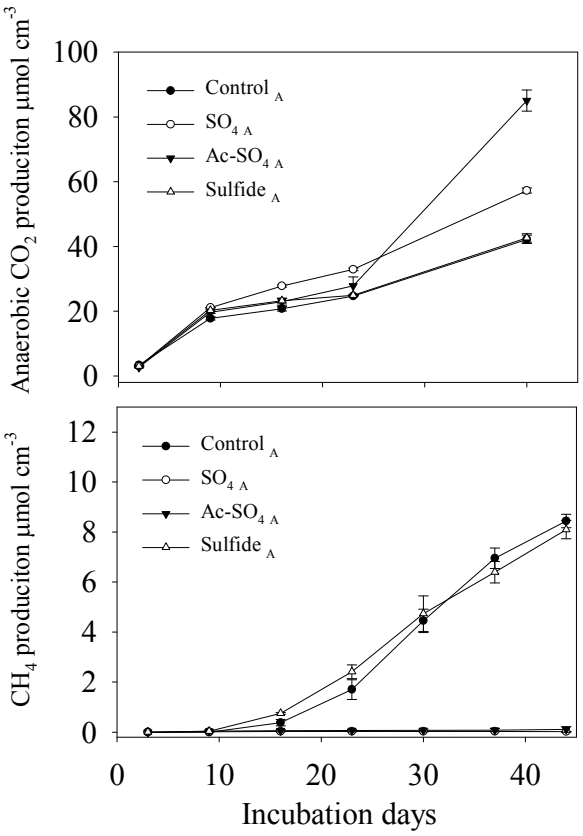


Figure 18. Anaerobic CO_2 and CH_4 production in µmol cm^{-3} against the incubation time in days of the different treatment

Table 10. Elemental composition and $\delta^{34}S$ signature of incubated peat in different treatments

%	Original $_O$	Control $_O$	Control $_A$	Sulfide $_A$	$SO_{4\ A}$	Ac-$SO_{4\ A}$
Fe	0.44 ± 0.01 a	0.42± 0.02 a	0.33± 0.00 b	0.31 ±0.01 b	0.30 ± 0.01 b	0.36 ±0.01 c
C	50.59 ± 1.51 a	54.18 ± 0.95 a	51.55 ±1.05 a	49.58 ± 0.49 a	51.13 ± 1.62 a	51.59 ± 1.31 a
S	0.18 ± 0.01 a	0.17 ± 0.00 a	0.20 ± 0.01 a	0.20 ± 0.00 a	0.33 ± 0.02 b	0.34 ± 0.01 b
N	1.29 ± 0.04 a	1.32 ± 0.02 a	1.24 ± 0.02 a	1.22 ± 0.01 a	1.26 ±0.04 a	1.25 ± 0.03 a
$\delta^{34}S$	6.62 ± 0.23 a	6.98 ± 0.29 a	6.30 ± 0.13 a	8.11 ± 0.30 b	2.39 ± 0.12 c	3.57 ± 0.16 d

Note measured $\delta^{34}S$ signature for Na_2S and Na_2SO_4, chemicals used as supplements for peat incubation, was 17.26 ±0.58 ‰ and 4.30 ± 0.57 ‰, respectively. Values are mean ± S.D (n = 3). Different letters indicates significant difference between the two treatments ($p < 0.05$)

Table 11. Measured sulfur model compounds with different electronic oxidation states (EOS) and predominant S-K edge XANES peaks as observed for peat samples after incubation

Compounds; Molecular formula	Peak max.	EOS	Ref.[#]	Peak max. (eV) Incubated peat					
	(eV)			Original $_O$	Control $_O$	Control $_A$	Sulfide $_A$	SO_4 $_A$	Ac-SO_4 $_A$
Iron mono-sulfides: FeS	2470.2	-2	[1]						
Pyrite: FeS_2	2471.1	-1	[1]						
Elemental sulfur: S^0	2471.5	0	[1]						
5,5'-Dithiobis (2-nitrobenzoic acid); ([-$SC_6H_3(NO_2)CO_2H]_2$) *	2471.6			L***	L	L	2471.8	2471.8	2471.8
	2473.7	+0∼1	[2, 3]						
L-cysteine: $C_6H_{12}N_2O_4S_2$ **	2472.6	+0.2	[1, 3]	2472.7	2472.7	2472.7	2472.7	2472.7	2472.7
Dibenzothiophene: $C_{12}H_8S$	2472.9	+0∼1	[2, 3]						
Methyl phenyl sulfoxide: $CH_3SOC_6H_5$	2475.2	+2	[1, 2, 3]	2475.2	2475.2	2475.2	2475.2	L	L
Dimethyl sulfone: $(CH_3)_2SO_2$	2479.1	+4	[1, 2, 3]						
Sodium methanesulfonate: CH_3SO_3Na	2480.2	+5	[1, 2, 3]	2480.2	2480.2	2480.2	2480.2	2480.2	2480.2
Sulfuric acid mono(2-aminoethyl) ester: $C_2H_7NO_4S$	2481.5	+6	[1, 2, 3]	L	L	L	2481.2	2481.2	2481.2
Sodium sulfate: Na_2SO_4	2481.5	+6	[1, 2, 3]						

* Representing organic disulfides (R-SS-R); two peaks were observed, one at 2471.6 eV had a higher relative intensity than the one at 2473.7 eV.

** Representing thiols (R-SH)

*** Representing low intensity of the peak

[#] [1] Prietzel et al., 2007; [2] Xia et al., 1998; [3] Ratasuk and Nanny et al., 2007

Table 12. Calculated DOC release rate during 40 days peat incubation

	DOC release rate	pH	
	nmol C cm^{-3} day^{-1}	pre-incubation	end of incubation
Control $_A$	0.60 ± 0.02 a	3.60 ± 0.10 a	4.50 ± 0.04 a
Sulfide $_A$	0.65 ± 0.02 a	3.80 ± 0.01 a	4.52 ± 0.01 a
SO_4 $_A$	0.64 ± 0.05 a	3.60 ± 0.05 a	4.46 ± 0.08 a
Ac-SO_4 $_A$	2.47 ± 0.11 b	3.70 ± 0.09 a	5.65 ± 0.10 b

Values are mean ± S.D (n = 3). Different letters indicates significant difference between the two treatments ($p < 0.05$)

Table 13. Anaerobic CO_2 and CH_4 production rates, sulfate reduction rates and Fe^{3+} reduction rates and calculated electron accepting capacities (EAC) from organic matter (OM)

Treatments	**Anaerobic CO_2 Production rates**	**CH_4 Production rates**	**SO_4^{2-} reduction rates**	**Fe^{3+} reduction rates**	**EAC from OM***
	nmol cm^{-3} d^{-}	nmol cm^{-3} d^{-}	nmol cm^{-3} d^{-}	nmol cm^{-3} d^{-}	μmol e^{-} cm^{-3}
Control $_A$	855.3 ± 3.5 a	263.9 ± 9.8 a	0.0 ± 0.0 a	2.5 ± 0.0 a	103.86 ± 8.2 a
Sulfide $_A$	841.3 ± 4.5 a	243.1 ± 4.8 a	0.0 ± 0.0 a	2.7 ± 0.0 a	105.07 ± 5.3 a
SO_4 $_A$	1214.1 ± 2.8 c	0.13± 0.01 c	121.1 ± 7.0 b	2.5 ± 0.0 a	170.88 ± 11.7 b
Ac-SO_4 $_A$	1917.2 ± 16.5 d	0.17 ± 0.01 c	352.2 ± 1.7 c	2.8 ± 0.0 a	214.1 ± 9.6 c

*EAC from OM = ((CO_2 production rate × 4 e^{-}) - (CH_4 production rate × 4 e^{-}) - (Sulfate reduction rates × 8 e^{-}) - (Fe^{3+} reduction rates × e^{-})) × 40 days

EAC was a sum of transferred electron for 40 days incubation.Values are mean ± S.D (n = 3). Different letters indicates significant difference between the two treatments ($p < 0.05$)

Table 14. Overview the organic sulfur oxidation index of organic sulfur of incubated different treatments.

Treatment	% S*	S_{org}** oxidation index	$\delta^{34}S$ ‰	Percentage of newly formed S_{org} (% of total)
Original $_O$	0.18 ± 0.01 a	0.43	6.62 ± 0.23 a	-
Control $_O$	0.17 ± 0.01 a	0.46	6.98 ± 0.29 a	-
Control $_A$	0.19 ± 0.01 a	0.42	6.30 ± 0.13 a	-
Sulfide $_A$	0.20 ± 0.01 b	0.35	8.11 ± 0.30 b	10 ± 2 a*** / 14 ± 6 a****
SO_4 $_A$	0.33 ± 0.01 c	0.61	2.39 ± 0.12 c	46 ± 2 b ****
Ac-SO_4 $_A$	0.34 ± 0.02 c	0.23	3.57 ± 0.16 d	47 ± 3 b ****

* Measured sulfur content at the end of incubation.

** Organic sulfur oxidation index was calculated by dividing the sum of oxidized organic sulfur species (sulfate esters, sulfones, sulfoxides) by the sum of reduced organic S species (R-S-R, R-SS-R bridging structures, thiols and sulfur heterocycles), as obtained from Linear Combination Fitting (Hoffmann et al., 2013, 2012)

*** Newly formed organic S calculated from difference in elemental analysis

**** Newly formed organic S calculated from isotope data.

Different letters indicates significant difference between the two treatments ($p < 0.05$)

Versicherungen und Erklärungen

(§ 5 Nr. 4 PromO)

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

(§ 8 S. 2 Nr. 5 PromO)

Hiermit erkläre ich mich damit einverstanden, dass die elektronische Fassung meiner Dissertation unter Wahrung meiner Urheberrechte und des Datenschutzes einer gesonderten Überprüfung hinsichtlich der eigenständigen Anfertigung der Dissertation unterzogen werden kann.

(§ 8 S. 2 Nr. 7 PromO)

Hiermit erkläre ich eidesstattlich, dass ich die Dissertation selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

(§ 8 S. 2 Nr. 8 PromO)

Ich habe die Dissertation nicht bereits zur Erlangung eines akademischen Grades anderweitig eingereicht und habe auch nicht bereits diese oder eine gleichartige Doktorprüfung endgültig nicht bestanden.

(§ 8 S. 2 Nr. 9 PromO)

Hiermit erkläre ich, dass ich keine Hilfe von gewerbliche Promotionsberatern bzw. -vermittlern in Anspruch genommen habe und auch künftig nicht nehmen werde.

Bayreuth,
……
Ort, Datum, Unterschrift