Redox Processes of Organic and Mineral Geochemical Phases at Aquatic Interfaces

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Summary

In this thesis, rates and extend as well as the ecological implications of electron exchange reactions that involve redox-active moieties in natural organic matter (NOM) were explored. The research builds on earlier findings that confirmed that NOM may act as terminal electron acceptor (TEA) for electrons released in microbial respiration. This property was identified to derive from quinone moieties that are ubiquitously found in NOM from terrestrial and aquatic environments and that may undergo reversible reduction to the respective hydroquinone. Previous methodological advances allowed for a rapid, direct and precise quantification of the electron accepting and donating properties of quinone moieties in dissolved NOM (DOM) by mediated electrochemical analysis.

Microbial reduction of organic matter

In this work, the previously established mediated electrochemical analysis was adapted and used in the characterization of redox properties of particulate natural samples that contain both redox active iron and organic matter (henceforth referred to as "geochemical phases"). For the first time, the reduction of geochemical phases in sediments of lakes and wetlands was directly monitored. Measurements confirmed that microorganisms transferred electrons that were released during microbial respiration to the organic and inorganic electron acceptors in the particulate phase. Particulate organic matter in the sediments was found to provide a capacity to accept or donate electrons of 650 μmol e^- gC^{-1}. This value considerably exceeds values found in many previous studies that relied on the indirect quantification of the electron accepting capacities using chelated Fe(II) as redox probe.

Electron fluxes in aquatic environments

Analysis of the combined electron-accepting properties of NOM- and Fe-bearing geochemical phases revealed pH-dependent electron fluxes between NOM and Fe species. This finding is in line with earlier studies that suggested overlapping E_h distributions of individual redox-active sites in NOM and Fe in clay minerals. It further points towards a more vivid electron exchange between conjoined redox-active species within heterogeneous matrices like sediments.

Aiming for the spatiotemporal analysis of the dynamics that organic and inorganic TEA species (i.e., nitrate, sulfate, Fe- and Mn oxyhydroxides)

in freshwater sediments are subject to, a mesocosm experiment was set up to simulate changes in oxygen availability at the sediment surface. At sediment surfaces oxygen is either consumed in aerobic respiration or when previously reduced species are oxidized. In the latter process, these species re-generate their electron-accepting capacity. The use of mediated electrochemical analysis allowed for the quantification of the redox state of the geochemical phases during their reduction and re-oxidation. Hence, the electron fluxes initiated by the oxic re-generation of the TEAs nitrate, sulfate, Fe(III), Mn(IV) and quinoid moieties in NOM were directly monitored instead of modeled from the species' one-dimensional distribution profiles in interstitial waters. With this set of direct methodologies, the redox-driven biogeochemical processes triggered by system disruptions at the sediment-water interface were closely observed.

Ecosystem dynamics initiate redox cycling

Many aquatic ecosystems undergo recurring fluctuations in oxygen availability. The associated disruptions in redox conditions can cause cyclic reduction and re-oxidation of redox-active species on different timescales. In lakes, oxygen budgets are coupled to the dynamics of benthic redox processes. In seasonally stratified lakes, extended sediment volumes are exposed to oxic conditions (dissolved $O_2 > 1$ mg L^{-1}) only upon lake overturn. A combined field and laboratory study of lake Scharmützelsee showed that this seasonal mixing events introduces a finite amount of oxygen to the hypolimnion and that about 50% of the subsequent sediment oxygen consumption is exclusively associated with the re-generation of TEA species. These reduced species previously formed in the sediment when microorganisms decomposed organic matter during anaerobia.

While lake overturn can completely de-stratify lakes and mix large quantities of epi- and hypolimnetic waters, small-scaled dynamics in temperature and oxygen availability may confine discrete parts of the water column where physicochemical conditions oscillate. In the studied lake Große Fuchskuhle, a transient thermocline cyclically introduces oxygen to hypoxic hypolimnetic waters close to the pelagic redox interface. In the said lake which is influenced by an adjacent bog area, high concentrations of DOM meet low abundance of inorganic electron accepting species. In this and comparable systems, organic TEAs may represent an important constituent of the total pelagic electron acceptor capacity. Due to the rapid and reversible redox reactions of dissolved NOM, reduced organic TEAs are re-generated upon dislocation to oxic parts of the water column. Results show that diurnal fluctuations of oxycline depth shape a micro-environment

selecting for microbial species that are released from TEA limitations by DOM in oxidized state. Pelagic microbial communities subjected to identical amounts of DOM that are in different oxidation states differed by more than 50% after one day.

Environmental implications

This work substantiates earlier findings that suggested that NOM may be an important TEA species in many aquatic and terrestrial ecosystems. NOM reduction in microbial respiration was shown to directly affect critical system parameters as bacterial activity, oxygen budgets and aquatic biodiversity.

Both the microbial reduction and subsequent abiotic oxidation of (hydro-)quinoid moieties in NOM are sufficiently fast for a relevant interaction with oxycline fluctuation. Given that organic TEAs are cyclically regenerated, a significant share of ecosystem respiration could be linked to NOM reduction.

This thesis adds to the new and important findings on the role of electron exchange reactions in NOM-rich environments. As of today, linking the chemistry of aquatic turnover processes with the microbiological and physical conditions at redox interfaces remains challenging. In conclusions, by providing several cases from aquatic environments, this thesis contributes to the mechanistic underpinning of NOM reduction in microbial respiration. The results clearly prompt for further research - especially regarding the competitive inhibition of other respiration pathways, including the reductive production of the potent greenhouse gas methane.

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1 Introduction

1.1 Freshwater sediments: reactive sites in the landscape

Sediments in aquatic environments receive material input from various sources. Depositions of organic or mineral particulate matter from autochthonous (i.e., internal) or allochthonous (external) sources that is either inert or incompletely processed during the passage through the water column settles at the sediment surface (Lundqvist 1927). At the sediment-water interface, microbial abundance is regularly three orders of magnitude larger as compared to the water column (Schmidt et al. 1998). There, transformation processes (commonly termed "early diagenesis") recycle up to 80% of the previously settled organic material (Gonsiorczyk 2002; Sobek et al. 2009).

Organic matter in freshwater ecosystems

Organic material is mainly derived from the debris of terrestrial plants and the dead biomass of aquatic organisms. From the chemical perspective, dead biomass is commonly referred to as natural organic matter (NOM) that is operationally grouped into particulate (POM) and dissolved organic matter (DOM) as the fractions that are either retained by or that pass through 0.45 μm cut–off filters, respectively. DOM is viewed as a complex mixture of small organic molecules, some of which are loosely bound in supramolecular associations (Piccolo 2001; Sutton and Sposito 2005).

In organic matter, chemical energy from primary production is stored and can be transported within and across systems. Heterotrophic microorganisms are key entities of transformation, recycling organic carbon in microbial respiration. The previously stored chemical energy is utilized for microbial activity (catabolism) and the carbon is both, used for cell growth (anabolism) and mineralized (i.e., oxidized) to carbon dioxide, the precursor for forthcoming photoautotrophic carbon fixation.

Organic matter degradation is the engine behind benthic biogeochemistry and controls the recycling of energy, nutrients and inorganic carbon, the mobility of metals, the availability of oxygen and, ultimately, the long-term storage of organic and inorganic carbon in aquatic environments (Wetzel 2001).

Carbon in freshwater systems meets several fates including transport, sequestration, transformation and evasion as the greenhouse gases carbon

dioxide and methane. Depending on the degradation pathway, NOM is completely oxidized to $C^{(+IV)}$ in CO_2, partly oxidized to compounds of intermediate C redox state or reduced to CH_4, $C^{(-IV)}$, in methanogenesis. Methane is formed in considerable amounts in the anoxic zones of sediments. Upon dislocation from the methanogenic sediment horizon, dissolved methane in interstitial pore-water or the pelagic water column represents a valuable carbon and energy source for microorganisms with the enzymatic equipment to use methane as an electron donor, the methanotrophs (Borrel et al. 2011). Ultimately, only a minor fraction of the deposited organic carbon escapes benthic degradation.

Many lakes emit more carbon to the atmosphere than they fix by autochthonous primary production, signifying a net heterotrophy (Sobek et al. 2007) and turning lentic and lotic freshwater systems to substantial contributors to global greenhouse gas balances (Battin et al. 2009; Bastviken et al. 2011). This counter-intuitive characterization as both sinks and net emitters derives from the input of allochthonous carbon, turning lakes and rivers into effective C-reactors in the landscape (Roehm et al. 2009).

Freshwater systems cover about 1% of global surface area but their collective contribution to carbon cycling is substantial (Battin et al. 2009). In the last decade, considerable attention has been directed towards the source, sink and transport functions of inland waters. Cole et al. (2007) estimated that globally freshwaters transport around 0.75 Pg C from terrestrial to marine ecosystems every year (1 Pg = 10^{15} g). Novel data suggests that this data does not accurately reflect the global inventory of lakes (including ponds), correcting the latter net value to 2.9 Pg C yr^{-1}, 0.6 Pg of which actually accumulate in sediments on a yearly basis (Tranvik et al. 2009). In general, storage of C increases with lake productivity that is inversely proportional to lake size (Kortelainen et al. 2004). Consequently, sediments of small lakes including kettle holes in agricultural landscapes belong to the major sites for C burial (Downing et al. 2008). As a result, around 820 Pg of carbon is estimated to be stored in the sediments of freshwater environments excluding wetlands and peatlands (Cole et al. 2007; Einsele et al. 2001).

Wetlands, as semi-aquatic, semi-terrestrial landscapes are especially important for carbon cycling. Globally, wetlands are estimated to store 350 to 535 Pg C (Mitra et al. 2005), compared to 720 Pg C of atmospheric CO_2 content (Falkowski et al. 2000). Peatlands alone cover only about 3% of the terrestrial earth surface, yet they contain 16 to 33 % of the soil carbon pool (Bridgham et al. 2006). The mechanisms identified to be responsible for the efficient storage of C in wetlands include low temperature, growing recalcitrance of remaining peat and strict anaerobia (Beer et al. 2008),

inhibition of enzymatic degradation (Freeman et al. 2001) and stabilization of C pools by association with other inorganic phases (Kaiser and Guggenberger 2000, Eusterhues et al. 2011, Riedel et al. 2013). Yet, interplay of mechanisms that control accumulation and loss of biomass from aquatic systems remain enigmatic (Schmidt et al. 2011).

In conclusion, the degradation of organic carbon is a key process in aquatic environments and is crucial to understand the energy flux from terrestrial to aquatic environments. Carbon biogeochemistry is characterized by the wide range of stabile C redox states (-IV to +IV). Transformation between these redox states requires the exchange of electrons with other chemical species in oxidation and reduction reactions. Hence, synthesizing early diagenetic processes prompts for the close inspection of the diversity in aquatic redox reactions that were initially triggered by NOM degradation. Global carbon dynamics are subject to ongoing research. Therefore, and especially with regard to the projections of climate change, a detailed mechanistic underpinning of the physical, chemical and biological constraints on NOM degradation in aquatic environments is of great relevance.

Effects of hydrophysical dynamics

Conservatively, long-term stability of organic matter in aquatic and terrestrial environments is assumed to be primarily a molecular property (Simpson and Simpson 2012). In a seminal work, though, the persistence of particulate organic matter is discussed to be an *ecosystem property*, including climatic, pedologic and hydrologic conditions (Schmidt et al. 2011). Those researchers propose that it is not a definite chemical structure that may compellingly explain why some thermodynamically labile NOM can persist for thousands of years while others are rapidly degenerated. Therefore, this paragraph will present physical system properties and dynamics that are expected to exercise control on biogeochemical turnover processes in aquatic ecosystems.

There is a long history of research exploring heterogeneous environments from the perspective of the interfaces between confined system components. In freshwater ecosystems, important interfaces are found between separated (e.g., stratified) water bodies but also conterminous to the terrestrial environments, the particulate sediment phase and, ultimately, the atmosphere. Interfaces feature steep gradients in physical, chemical or biological parameters and they typically exhibit the highest reaction and turnover rates in the system (McClain et al. 2003).

At the sediment-water interface, considerable species richness and disproportionally high reactivity regulates the flow of energy and material

between the adjacent environments (Urban et al. 1997; Cadenasso et al. 2003).

A diffusive boundary layer is formed between the stagnant interstitial sediment pore waters and the dynamic water column. According to Fick's law, the exchange of solutes can be estimated from the physical dimension of the layer (Berner 1980; Sweerts et al. 1991; Krause et al. 2014). The diffusive fluxes are well predicted by Fick's First Law if advective fluxes (e.g. from bioturbation or resuspension) and chemical transformations are negligible (Lavery et al. 2001). Transport of solutes is also dependent on the horizontal currents in the overlying water, increasing fluxes with increasing current velocity (Wüest and Lorke 2003). Both numerical and empirical models help to understand consumption- and transport processes of oxygen (Hutchinson 1938; Rippey and McSorley 2009; Müller et al. 2012) and nutrients (Vollenweider 1975) across the sediment-water interface.

Several non-diffusive transport mechanisms have been identified. For instance, (1) the dwelling activity of benthic macrofauna induces small-scale advection. Among others, chironomid larvae actively pump water from the water column in micrometer-sized channels up to 12 cm into the sediment so that they drastically increase both the sediment oxygen consumption and nutrient mobility (Lewandowski et al. 2007; Hölker et al. 2015). (2) The ebullition of gas bubbles greatly increases the transport velocity through the sediment-water interface to the atmosphere. The low solubility of CH_4 relative to the CO_2 gas that are both formed in sediments, signifies that methane bubbles form more readily, exhibiting typical diameters between 2 and 8 mm (McGinnis et al. 2006; Ostrovsky et al. 2008). Gas bubbles rapidly bypass potential areas of methane oxidation. (3) Plants featuring air-filled cavities (Aerenchyma), as e.g. *Typhaceae* found in many wetlands and *Potamogetonaceae* in lake littoral may facilitate vertical O_2 transport into the sediment but also expedite CH_4 fluxes to the atmosphere (Van der Nat and Middelburg 2000; Laskov et al. 2006).

Upon water drawdown, sediments are confined by a newly formed atmosphere-sediment interface. Sediments that experience dry cycles are typically found in lake littoral areas, reservoirs, and small ponds as a consequence of natural or anthropogenically derived fluctuations of the water table (Baldwin and Mitchell 2000). Causes include imbalances in precipitation-evaporation equilibria as well as drinking water supply, agriculture, waterway management and hydroelectric power generation (Pimentel et al. 1997). Influences of these water regime changes have therefore been studied in freshwater lakes, reservoirs (Baldwin 1996; Wantzen et al. 2008) and wetlands (Venterink et al. 2002; Knorr and Blodau

2009).

When the freshwater sediments are dried and exposed to atmospheric oxygen, the most frequently reported and striking consequence has been the release of nutrients (C, N, P) during subsequent rewetting (Baldwin and Mitchell 2000). Studies offered several explanations, most of which are responses of the microbial community to the changes in redox conditions. With increasing oxygen availability, facultative anaerobes are outcompeted by aerobic heterotrophs. The aerobic heterotrophs typically feature higher digestion rates and - as well as other aerobic fungi and protozoa - are commonly known to utilize lignin-rich organic substrates that are refractory to anaerobes (Baldwin and Mitchell 2000). The increased turnover rates can help to explain increased losses of carbon as CO_2 and DOM. At the end of the desiccation process (*baking*) of sediments, declining water content finally causes microbial cell lysis. A decrease of microbial abundance by three quarters was reported (Qiu and McComb 1995). Intracellular solutes, rich in labile C and N substrates, may then be released and experience subsequent mineralization, yielding a pulse of soluble nutrients upon rewetting (Fierer and Schimel 2002). The nutrient export upon episodic rewetting is especially well studied in wet/dry cycles of soil ecosystems and commonly referred to as the *birch effect* (Birch 1958).

Long-term changes of the drying and aeration patterns in wetlands were identified to affect these ecosystems with considerable consequences for carbon and nutrient sequestration. The consequences reported include top-soil loss, acidification and substantial export of DOM (Holden et al. 2004 and references therein). As mentioned earlier, enzyme inhibition under anoxic conditions was proposed to be responsible for the slow decomposition of organic substrates (Freeman et al. 2001). Fenner et al. (2011) linked the discontinuity of anoxic conditions to system hydrology and confirms that the driest (i.e., best oxygenated) sites in peatlands exhibited maximum carbon loss (as DOM and CH_4) upon episodic rewetting. During the rise of industrial agronomy, vast areas in the temperate zone have been drained in order to create fertile grassland areas for intensive agriculture. Recently, permanent rewetting of drained peatlands is intensively discussed as a possible strategy for remediation and reveals comparable patterns of nutrient release upon rewetting as in aquatic sediments (Zak and Gelbrecht 2007).

In conclusion, the hydrology in aquatic ecosystems controls microbial community structure, material transport and redox conditions and is, thus, representing a major driving force for the quality of surface water (via nutrient export) and the persistence of NOM in sediments and submerged soils (Smith et al. 2006; Schmidt et al. 2011). As laid out in this paragraph,

oxic/anoxic (i.e., redox-) interfaces are the crucial environments for abiotic as well as microbially mediated turnover processes (McClain et al. 2003). Hence, redox reaction that involves mineral and organic as well as dissolved and particulate redox species at these interfaces deserve further attention.

1.2 Aquatic redox reaction

Cycling of inorganic and organic material in aquatic ecosystems is largely driven by electron transfer reactions. Redox processes control the chemical speciation, bioavailability and mobilization of dissolved and particulate substances (Grundl et al. 2011).

Any redox reaction is the sum of two half reactions, the oxidation of the reductant and the reduction of the oxidant that are inter-aligned by the transfer of electrons. The difference in Gibb's free energy (dG) of reaction educts to products indicates whether the pathway is thermodynamically favorable. With knowledge of prevailing conditions and standard potential of reactants, feasibility of redox reactions may be predicted (Stumm and Morgan 1996).

In aquatic ecosystems, microorganisms, extracellular enzymes, nutrients, metal (oxy)hydroxides and organic substances take part in redox reactions. Due to the permanent presence of water, all natural reactions run between the redox potential of the water's reduction and oxidation endpoint-products, hydrogen and oxygen (Grundl et al. 2011). Competition within microbial communities prompts for efficient energy conversion, favoring the usage of the terminal electron acceptor with the highest standard potential available for respiratory purposes.

The diffusive transport of the respiration end products and other redox active species generates *in situ* conditions in thermodynamic disequilibrium because kinetic barriers may prevent electron exchange between species (to reach equilibration). In the next paragraphs, some of the most important redox transformation in recently deposited sediments (close to the sediment-water interface) are presented and discussed.

Microbial respiration

Heterotrophic organisms require organic carbon for cell growth. At the same time, oxidation of organic carbon releases usable energy when mineralized to CO_2 in microbial respiration. Electrons freed in that oxidation need to be passed onto a terminal electron acceptor (TEA). The energy acquired in this process is a combination of the energy freed in the electron donating half-

reaction and the electron accepting half reaction (LaRowe and Van Cappellen 2011). Whilst the energy released in the oxidation half-reaction is dependent on the substrate (i.e., carbon in low oxidation states generally releases more energy during oxidation), *d*G of the electron accepting half reaction depends on the reduction potential of the electron accepting species (LaRowe and Van Cappellen 2011). Oxygen, as being the most favorable of the TEAs in aquatic environments releases -29.9 kcal $(\text{mol } e^-)^{-1}$ (when coupled to acetate oxidation and at standard definitions defined in Megonigal et al. 2003). Then, the thermodynamic energy yield per electron transferred onto a TEA decreases with decreasing TEA reduction potential in the order of nitrate reduction, Mn(IV) and Fe(III) reduction, sulfate reduction and methanogenesis – a sequence commonly referred to as the *redox ladder* (Sposito 2008). In sediments, concentration gradients of the reduced TEA species can be found in vertical stratification according to their reduction potential and bioavailability (Figure 1). Although the spatial distributions are continuous and overlapping, the illustration of discrete redox zones with definite microbial redox reactions running is a popular concept in literature (Megonigal et al. 2003). More recently, vertical redox zonation as a paradigm of sediment geomicrobiology is increasingly questioned. Problems raised include the possible rate-limitation of preceding fermentation instead of the electron accepting reaction step (Jakobsen and Postma 1999) and the physiological constraints on the microbial consortia (Bethke et al. 2011). The role of microscopic features of particles (cracks in minerals and pores in organics) and the formation of microenvironments (along e.g. the previously discussed animal burrows and aerenchyma tissue of submerged macrophytes) as hotspots for specific turnover reactions gains increasing attention (McClain et al. 2003; Pedersen et al. 2015). Hence, understanding microbial life in subsurface relies on both - microbial ecology as well as thermodynamics.

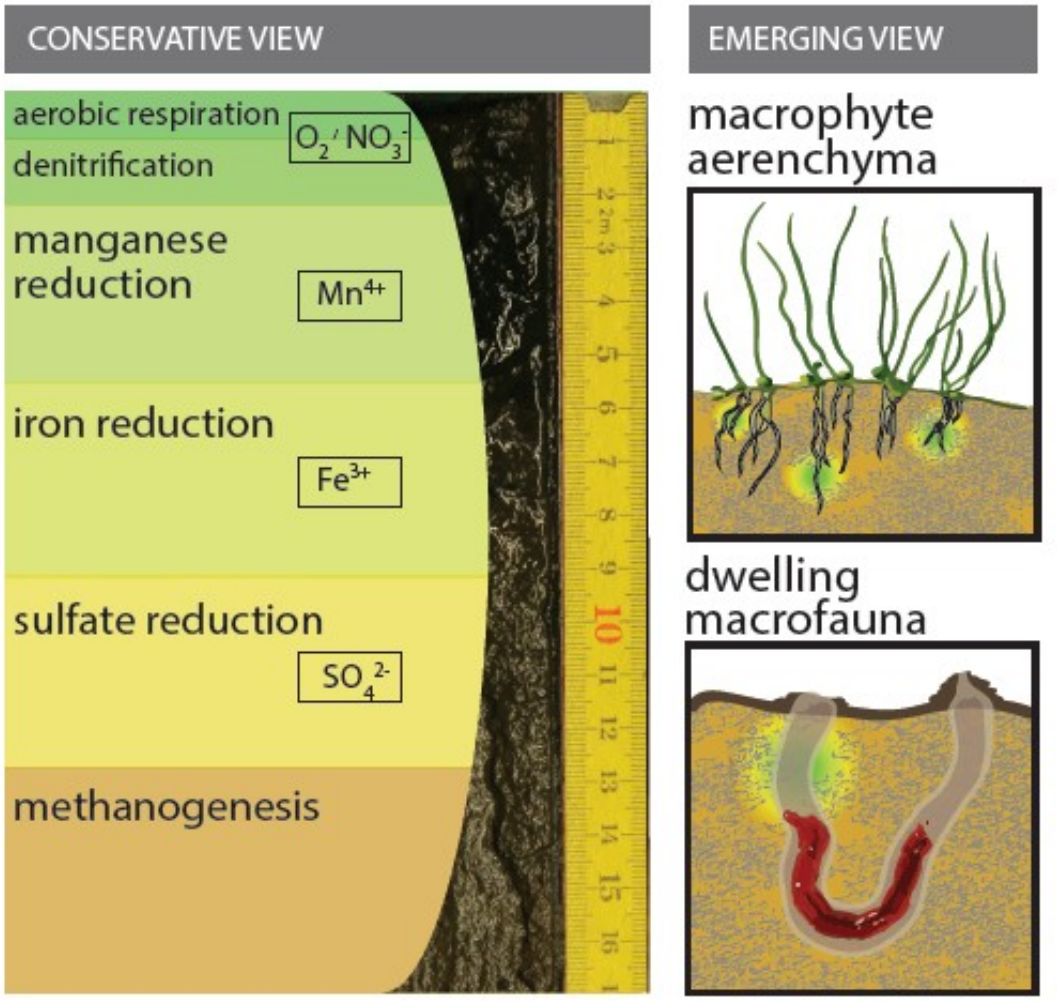

Figure 1 The conservative view on the vertical succession of microbial respiration processes according to the thermodynamic energy yield of the terminal electron accepting processes. In sediments, the distance to the oxic surface controls the availability of electron accepting species and confines redox zones in the sediment in zones where specific communities dominate. Alternatively, small scale heterogeneities in the sediment may induce the uneven distribution of different TEA processes in the three-dimensional sediment matrices. For instance, air filled cavities in submerged macrophytes (aerenchyma) or pumping activity of benthic macrofauna (e.g., chironomid larvae) introduces oxygen in previously anoxic zones of the sediment.

Thermodynamic fundamentals of microbial respiration

In sediments and waterlogged wetland soils, substantial areas deprived of oxygen can develop. Then, as mentioned earlier, a series of other, less potent electron acceptors may be the sink for electrons released in the microbial respiration of labile NOM (Megonigal et al. 2003). The series commences with the reduction of nitrate. This denitrification is initiated by facultative anaerobic communities in the sediments uppermost horizon (Bowden 1987; Pedersen et al. 1991; Straub and Buchholz-Cleven 1998). The redox transformation of the ferric to ferrous iron redox couple ($Fe^{(III)}/Fe^{(II)}$) yields

less energy per mole of electrons donated as compared to the nitrate reduction (Weber et al. 2006). However, usage of Fe as TEA is an important ecological niche inhabited by microorganisms generally referred to as *dissimilatory metal reducing bacteria* (DMRB) that are able to couple the oxidation of organic substances to the reduction of sedimentary $Fe^{(III)}$ and $Mn^{(IV)}$ oxides (Thamdrup 2000). As these (oxy)hydroxides are usually in particulate form and cannot be ingested into the cell interior, these bacteria developed specific electron transfer mechanism.

Three major pathways for this electron transfer have been identified in recent years; (1) direct contact between cell wall-bound redox co-factors and the mineral surface, (2) complexation with endogenous or exogenous chelating agents in order to solubilize $Fe^{(III)}$ and, (3), using redox-active electron shuttling compounds. Regarding (1.), micropili, also referred to as "microbial nanowires" have recently been suggested to facilitate the direct electron transfer (Reguera et al. 2005). Transmission electron imaging of *Geobacter metallireducens* revealed that conductive protein filaments produced by the microorganism connect the cells with the external electron sinks (Malvankar and Lovley 2012). Iron reduction without the direct contact with the mineral surfaces, (2.), may rely on exogenous chelating agents that facilitate the Fe(III) mobilization and increase the abundance of bioavailable metal ions at the cell surface. Many studies have demonstrated that DMRP can reduce soluble, chelated Fe(III) more rapidly than insoluble Fe(III) oxides (Nevin and Lovley 2002; Borer et al. 2005). Yet, other findings suggest that mobilized Fe(III) is rather used for microbial iron uptake than dissimilation (Boukhalfa and Crumbliss 2002). *Shewanella oneidensis* has been reported to excrete flavin compounds exclusively to bridge intracellular generated electrons and reduce insoluble extracellular electron acceptors (Marsili et al. 2008; Brutinel and Gralnick 2012). Again, no direct contact between cell surface and iron species is established, but the endogenous substance mediates redox processes while functioning as metal ligand at the same time. This systematic (but physiologically costly) synthesis of electron shuttling chelators distinguishes *Shewanella* from other metal-reducing bacteria that utilize exogenous electron shuttles for their catabolism (Lovley et al. 1998). In the latter *shuttling* process, electrons are first transferred from the cell to redox active moieties within DOM (Lovley et al. 1996; Scott et al. 1998). Quinone moieties are the primarily studied redox-active functional groups, formed from lignin precursors and ubiquitously found in NOM from a wide range of environments (Cory and McKnight 2005; Aeschbacher et al. 2010). Substituted quinone/hydroquinone redox couples in NOM feature reduction potentials in the range of +400 to -200 mV (Nurmi and Tratnyek

2011). The hydroquinone species are then - in turn - able to donate electrons to other electron accepting species with lower E_h if pH conditions permit (because the exchange of electrons is coupled to the release of one H^+) (Grundl et al. 2011). Re-formation of the original quinone upon oxidation with O_2 signifies that these electron accepting-donating processes are reversible (Ratasuk and Nanny 2007; Kluepfel et al. 2014b). This reversible electron shuttling process was identified to be especially important in freshwater and marine systems for both microbial respiration with solid-phase mineral species and remediation (that is, the redox transformation of organic pollutants) (Uchimiya and Stone 2009). Respiration with Fe(III) as TEA was shown to be significantly accelerated (7-fold) with DOM present as reversible electron shuttle (Jiang and Kappler 2008).

From a thermodynamic perspective, all previously mentioned electron accepting processes release more energy when coupled to C oxidation than the reductive formation of methane since the latter exhibits negative dG (Megonigal et al. 2003). Accordingly, methane is preferentially generated by autotrophic methanogens (from hydrogen H_2 and CO_2) or, alternatively, by acetoclastic methanogens (that convert acetate to CH_4 and CO_2) in environments that are depleted of other TEA species (Borrel et al. 2011). The presence of sulfate, ferric iron or redox-active NOM was shown to inhibit methanogens by providing slightly more thermodynamically favorable pathways (Cervantes et al. 2000; Gauci et al. 2004; Heitmann et al. 2007). Due to the high potency of methane as a greenhouse gas, the biogeochemistry of methane production and consumption in aquatic environments is well explored (Borrel et al. 2011; Bridgham et al. 2013). Reviewing the wide range of methane-related research, though, would go beyond the scope of this study.

Recent advances in subsurface biogeochemistry

An important research frontier in the biogeochemistry of microbial respiration remains the role of microenvironments in heterogeneous natural matrices like soils and sediments (Pedersen et al. 2015). Current findings indicate that wild type biofilms of *Geobacter*, for example, are shown to exhibit redox gradients on μm scales (Snider et al. 2012). In Fe(III) minerals (including clay) both coordination and structural features influence reduction potential so that the energy yield during the reduction of these phases can vary on equally small scales (Gorski et al. 2012a; Gorski et al. 2012b). Similarly, electron accepting moieties within NOM, the quinones, may exhibit different reduction potentials based on the diversity of structural analogues (Kluepfel et al. 2014b). Although mineral and organic species are found in

close spatial proximity in many environments (Kalbitz et al. 2000; Lalonde et al. 2012; Riedel et al. 2013), it remains enigmatic, to what extend these species feature inter-phase electron exchange.

Furthermore, in analogy to the NOM-supported Fe respiration pathway (the electron shuttling), other TEA processes are discussed to actually require the combination of abiotic and enzymatic reactions or even the co-operation between species. Regarding the latter, researchers already identified anaerobic methanotrophic communities to be close-couples of bacteria and archaea (Raghoebarsing et al. 2006). These consortia are suspected to run a "reverse methanogenesis" pathways with the archaea activating the methane and the bacteria providing the electron sink (Borrel et al. 2011). Microscopic detection confirmed the close attachment of the two species in methanotrophic yet anaerobic regions of marine sediments (Boetius et al. 2000). While co-metabolisation as e.g. in fermentation is well established (as described in the next section), the results on methanotrophic communities are especially interesting since they point towards the efficient electron transport across multiple cell walls (Kato et al. 2012). Conductive mineral nanoparticles, porin-cytochrome modules and quinoid moieties were previously proven to transport charge between extracellular redox-active species and the intracellular catabolic/respirational apparatus (Nielsen et al. 2010; Kato et al. 2012; Richardson et al. 2012). These structures and substances permit energy-conserving transfer across multiple cell walls and may also help to explain the observation of vertical electron transport over centimeter distances in marine (but recently also freshwater) sediments. There, so-called "cable bacteria" facilitate the transport across redox interfaces with yet unidentified conductive (i.e., redox active) materials (Nielsen et al. 2010; Marzocchi et al. 2014). In this rapidly developing field of biogeochemistry, current research advances the knowledge about the electron transport mechanisms that these unconventional respiration pathways rely on (Burgin et al. 2011).

Balancing respiration and the role of organic electron acceptors

From the redox perspective, balancing respiratory activity in environmental systems could be straightforward: Electrons are – catabolically - freed and require the final transfer to a terminal electron acceptor. As the stoichiometry of these electron transfer processes are generally known, changes in the spatial concentration of the TEA species corresponds to electron fluxes through environments with ongoing respiratory activity (Kelly et al. 1988).

Alternatively, microorganisms (including fungi and protozoa) may decompose macroscopic organic substances by fermentation. Here, carbon is

oxidized without the passage of surplus electrons onto external electron acceptors. Instead, carbon-carbon bonds are cleaved so that new carbon compounds with different redox state (and Gibbs free energy of formation) are formed (Lovley and Klug 1982). Fermentation end-products are found in many anaerobic and aerobic environments and can be important intermediates for the ultimate mineralization of organic compounds to carbon dioxide (Ye et al. 2014). The role of fermentation in aquatic and semi-aquatic environments is a subject of ongoing research.

Different approaches were tried to budget electron fluxes through environmental systems. The most comprehensible approaches include the quantification of the gaseous C-mineralization end-products carbon dioxide and methane to calculate CO_2:CH_4 ratios (as e.g. in Keller and Bridgham 2007). Additionally, analysis of the dynamics of other TEAs (e.g., ammonia, hydrogen sulfide, ferrous iron) in dissolved phases may provide more detailed information on electron fluxes. Combined approaches were previously used in different terrestrial and aquatic environments (Yao et al. 1999; Matzinger et al. 2010; Keller and Takagi 2013). Yet, all of these studies encounter similar methodological difficulties: The diversity of intermediate redox species formed from TEA reduction impedes the estimation of electron fluxes based solely on process stoichiometry (e.g., sulfate is not completely reduced to hydrogen sulfide). Also, Fe minerals were shown not to exhibit strict reduction-mobilization behavior (Gorski and Scherer 2011). Instead, the reduced, ferrous iron may still be integrated into the mineral matrix and not available for quantification as dissolved species in interstitial sediment solutes (Gorski et al. 2012b).

Despite these shortcomings, a number of studies on electron fluxes find similar conclusions: judged on electron balances, in freshwater sediment and wetland soils, more CO_2 is generated than TEAs are being reduced in the same process (Kelly et al. 1988; Keller et al. 2009; Keller and Takagi 2013). This electron imbalance was identified to be most important in systems especially rich in organic matter (Keller and Takagi 2013). To resolve this apparent contradiction, Matthews et al. (2008) suspected an additional electron accepting species to be responsible for the surplus electron accepting capacity: reducible moieties within organic matter itself. As laid out earlier, quinone moieties within the NOM are identified to be redox active at potentials similar to other TEA-reducing processes. The contribution of these species to the overall flux of electrons in microbial respiration has thus been tested systematically: First, it was found that a higher abundance in redox active NOM species significantly increases the respiration and thus electron transfer to Fe(oxyhydroxide) (Lovley et al. 1998; Martinez et al.

2013). Second, electron transfer to the organic species appears to be reversible over multiple cycles (Ratasuk and Nanny 2007; Kluepfel et al. 2014b) and third, NOM itself may be a significant bulk sink for electrons if in high abundance (Keller et al. 2009; Roden et al. 2010).

Recently, methodological advances permitted an improved way of quantification of electron donating and accepting capacities (in charge per mass NOM). Aeschbacher et al. (2010) introduced a novel approach where these capacities were quantified chronocoulometrically at controlled potentials and pH conditions. As a major advance, the acceleration of the electron exchange kinetics between the sample substances and the working electrode via radical redox mediators proved useful (Aeschbacher et al. 2010). As of today, this approach was used in the analysis of isolated DOM samples (Aeschbacher et al. 2012), biochar (Kluepfel et al. 2014a), clay minerals (Gorski et al. 2012a) and (in this thesis) natural soils and sediments (Lau et al. 2015; Lau et al. 2016).

In conclusion, advancements in both the conceptual understanding of respiration-induced redox processes and novel methodological approaches for the characterization of the underlying sample characteristics allow for a more in-depth analysis of the electron fluxes in aquatic environments.

Abiotic redox reactions

A fundamental reason for the complexity in assessing the redox conditions of aquatic ecosystems is that the majority of aqueous redox reactions in such systems are not in thermodynamic equilibrium due to kinetic constraints (Grundl et al. 2011). While abiotic reaction rates obey the Arrhenius law (and thus increase with temperature), enzyme catalyzed reaction may exhibit optimum conditions (such as temperature ranges, salinity) depending on the host organism (e.g., ambient are optimum temperatures for Fe-reducer *Leptothrix*, Vollrath et al. 2013). The specific set of conditions can open ecological niches for adapted microorganisms.

Despite the diversity in thermodynamically feasible redox reactions in aquatic environments, this paragraph focuses on the reactions of iron (Fe). Iron is of particular interest as it is both widely distributed in aquatic environments and Fe-bearing phases exhibit relatively wide reduction potential distribution located in the center of the range of possible aqueous redox reactions (Weber et al. 2006; Gorski et al. 2012b). Iron possesses d-electrons with π-character so reactivity towards many C, N, O and S species is given.

Abiotic Fe reduction

UV radiation can trigger the abiotic, photoinduced reduction of mineral, chelated or colloidal Fe(III). This charge-transfer (from a ligand to Fe) runs in the water column or in the uppermost millimeters of sediment (if still in the euphotic zone) and is responsible for the largest share of pelagic Fe(II) (Roy et al. 2008). In deeper sediment horizons, Fe(III) can be reduced by reaction with hydrogen sulfide diffusing upwards from the sulfate reduction zone. The first order reaction kinetics (towards H_2S and Fe(II)) is dependent on mineral surface area and pH (Yao and Millero 1996). In both reduction scenarios, Fe(oxy)hydroxides (especially from eutrophic environments) may previously have accumulated surface-bound phosphates. Hence, reductive transformation to Fe sulfides (with low surface areas) or Fe dissolution can trigger reductive mobilization of phosphates (Zak et al. 2006; Hupfer and Lewandowski 2008).

Abiotic Fe oxidation

Electron donation to ferrous iron from oxygen or other reactive oxygen species are among the most common examples for abiotic oxidation reactions. The homogeneous reaction (i.e., both reactants are in dissolved form) exhibits a first-order rate constant with the initial one-electron-transfer

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\cdot -}$$

as the rate-limiting step (King et al. 1995). Once Fe(III) precipitated from solution (as e.g. oxyhydroxide), surface processes can significantly enhance reaction rates via heterogeneous auto-catalysis (Melton et al. 2014).

Many other redox active species may react with Fe(II) in abiotic reactions. Oxidation with nitrate runs at a slow reaction rate but nitrite, NO_2^-, is a potent reductant of Fe in chemo-denitrification reactions when conditions warrant a sufficient stability of the nitrite ion (favoured by e.g. low pH and low abundance of organic matter, Sørensen and Thorling 1991). Tetravalent Mn species can also accept electrons from Fe(II) (Thamdrup 2000). Recently, stable and soluble Mn(III) species formed from abiotic redox reactions were discovered at oxic/anoxic interfaces (Trouwborst et al. 2006).

Fenton-type reactions of Fe(III) with hydrogen peroxide (H_2O_2) feature the highest abiotic reaction rates (King et al. 1995). H_2O_2 in aquatic environments may origin from multiple sources including the photodegradation of NOM (Melton et al. 2014). Page et al. (2012) also reported the dark formation of radical ·OH species during the irreversible oxidation of reduced quinones.

The same quinones within NOM may also accept or donate electrons

without irreversible decomposition. The redox active quinone moieties feature redox potential distribution that largely overlaps with reduction potentials of Fe mineral phases (Nurmi and Tratnyek 2011; Gorski et al. 2012b; Kluepfel et al. 2014b). Depending on solution pH, electron exchange reactions are thermodynamically feasible and kinetically fast. As discussed earlier, electron shuttling of electrons from microbial respiration to iron mineral typically includes an abiotic electron donation from the reduced NOM species to the mineral surface. Abiotic electron transfer was reported both from model (hydro-)quinones (Orsetti et al. 2013) as well as native NOM (Bauer and Kappler 2009). DOM was also found to exchange electrons abiotically with other redox active species in aquatic environments such as sulfides (Heitmann et al. 2007; Yu et al. 2015) and Mn (Thamdrup 2000). The reduction of quinone moieties within NOM was reported in cultures of Fe and sulfate-reducing as well as methanogenic communities (Cervantes et al. 2002).

Giving several examples, this work highlights that electron transfer reactions routinely run on both biotic and abiotic pathways. Different physicochemical processes may help to finally direct electrons in heterogeneous environments to the destination of lowest thermodynamic energetic state. Abiotic electron exchange with Fe species - including the electron delocalization in mineral matrices - may bypass electrons between otherwise unreactive species (Grundl et al. 2011). Microorganisms can profit from niches, circumventing kinetic limitations with enzymatic catalysis of redox processes. Regularly, biotic and abiotic pathways are linked as e.g. in the reduction of iron. Fast reaction rates of intermediate redox species may mask reactive species or reaction pathways. In the future, routine applications of analysis methodology with high resolution in the temporal and spatial dimension may help to shed more light on this emerging research topic (Pedersen et al. 2015).

2 Objectives

This thesis generally promotes an integrative view on abiotic and microbially mediated transformations at interfaces in aquatic environments. The presented work builds on two major novelties in biogeochemical research of the recent decade. First, several studies substantiated the important role of the electron accepting properties of NOM in microbial respiration (Lovley et al. 1996; Heitmann et al. 2007; Keller and Takagi 2013). This property of NOM was intensively studied under controlled laboratory conditions with isolated or artificial sample material (Scott et al. 1998; Aeschbacher et al. 2010). Studies tracing the biogeochemical implications *in situ* remained scarce (Matthews et al. 2008; e.g., Keller et al. 2009) yet agreed on the necessity for a more profound quantification of NOM redox characteristics. Development of an advanced quantification routine for electron accepting and donating capacities proved suitable for both dissolved organic and solid mineral phases and finally provided new opportunities to study the redox properties in heterogeneous natural samples (Aeschbacher et al. 2010; Sander et al. 2015).

Second, these and other findings promoted the increasing acknowledgment of the tremendous diversity of redox reactions in aquatic environments (Burgin et al. 2011 and references therein). While studies in that field are less coherent, they point towards a common direction: Transport of charge through natural environments involves many different species and players and includes redox-active phases of both mineral and organic origin, in dissolved and particulate state and runs on abiotic and microbial mediated reaction paths. Examples of new concepts include the reactivity-controlling E_h distribution of mineral phases (Gorski et al. 2012b), oxygen-independent CH_4 oxidation under aerobic and anaerobic conditions (Bridgham et al. 2013), spatially decoupled electron exchange through centimeter-long cable bacteria (Marzocchi et al. 2014) and the abiotic reactivity of NOM towards e.g. reduced S or Fe species (Yu et al. 2015).

Previous conceptualizations were often too simple for the accurate reproduction of these coherent processes (Paraska et al. 2014). While this work cannot – not even closely – resolve the inherent complexity of aquatic redox reactions, the primary objective of this work was to gain insight into the redox reactions that involve organic-mineral redox-active geochemical phases.

Experiments were conducted to explore the ecological relevance of these ubiquitously present but rarely considered phases in aquatic environments.

Research hypotheses

1 Reversible, redox-active moieties within natural organic matter may play a more profound role in microbial respiration than previously acknowledged (Kluepfel et al. 2014b). This work aims for a robust quantification of organic electron sinks in natural matrices. In many aquatic environments (and especially freshwater sediments), a large share of organic matter is found in particulate form (Wetzel 2001). Hence, it is crucial to understand the redox characteristics of the electron accepting moieties within particulate organic matter.
2 Recent research on the redox properties of organic and mineral phases regularly shows, on the one hand, the close spatial proximity of the two species in microscopic sites of aquatic and terrestrial environments (Kaiser and Guggenberger 2000; Lalonde et al. 2012; Riedel et al. 2013). On the other hand, researchers discuss the overlapping reduction potential distribution of the individual redox active sites within the different phases (Gorski et al. 2012a; Kluepfel et al. 2014b). Consequently, electron exchange between these phases is potentially more dynamic than previously considered. This works aims for the analysis of the electron transfer to and within these widespread combined (Fe-OM bearing) geochemical phases in natural samples. Novel electrochemical methodology, previously shown to be suitable for the quantification of redox properties of either isolated organic or mineral samples may be adopted to serve this purpose.
3 Reduced geochemical phase may act as reductants towards other redox-active species in the sediment. Hydrophysical dynamics may initiate the transport of oxidized species (most notably, O_2) to reduced environments. This work aims for the synthesis of aquatic oxygen dynamics and the oxidation-reduction cycles of redox-active moieties within submerged sediments. It is hypothesized that consumption of oxygen upon re-introduction to previously reduced environments with high quantities of reduced geochemical phases may be a significant position in the oxygen balances of stratified freshwaters.
4 Considering the rapid electron exchange reactions in aquatic environments it is plausible that organic electron acceptors may experience very fast changes in redox states. Previous work highlighted that both the microbial reduction and the abiotic re-oxidation run at comparatively fast rates (Jiang and Kappler 2008). At the same time, temporal and spatial instabilities of redox interfaces could create aquatic microenvironments characterized by rapid fluctuations in redox conditions. If these microenvironments retain

significant amounts of NOM, the NOM could cycle between reduced and oxidized state synchronous to the external redox gradient (Figure 2). Consequently, respiration with organic TEAs could be greatly amplified in these environments as its capacity to accept electrons is cyclically regenerated. Hence, it is hypothesized that external redox fluctuations open an ecological niche for specialized bacteria that may make use of this re-generable electron sinks of high reduction potential (and yet energy yield in respiration).

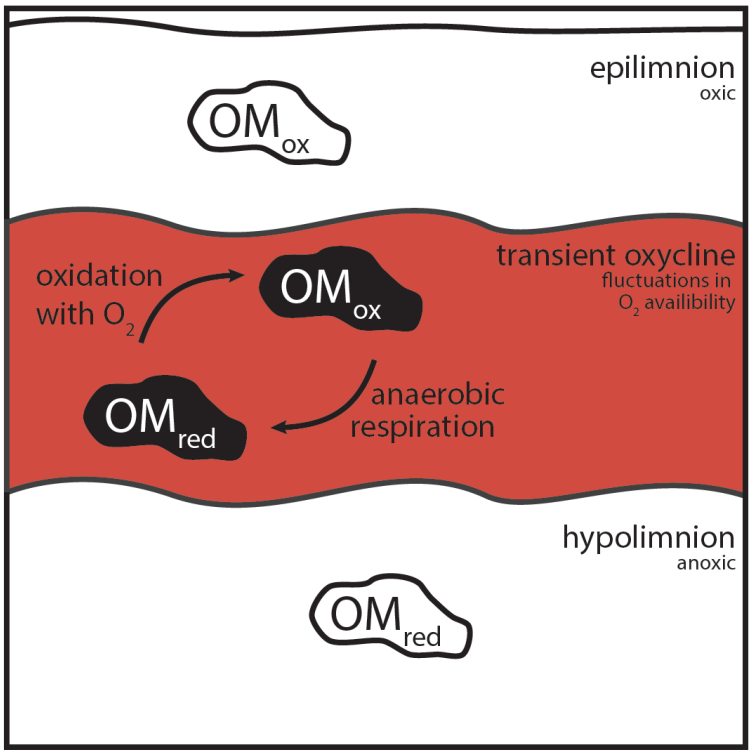

Figure 2 Cycling of electron accepting species in organic matter (OM) between reduced (red) and oxidized (ox) states at pelagic redox interfaces. Upon translocation to oxic parts of the water column, previously reduced moieties may be rapidly re-oxidized.

3 Results

This work features three articles with a focus on different aspects of the integration of redox active geochemical phases into the overall picture of aquatic redox reactions.

3.1 Solid phases as important electron acceptors in freshwater organic sediments

In the first article, we analyzed changes in the electron accepting capacity of sediment samples upon rewetting. Methodological advancements of the previously established mediated electrochemical analysis allowed for the quantification of electron fluxes to solid phase organic and mineral species in the heterogeneous sample material for the first time. The work highlighted the importance of respiration with NOM as TEA especially in environments deprived of other, inorganic TEAs as e.g. peatlands. The electron transfer capacity of NOM was quantified electrochemically and exceeded values previously reported with chelated Fe as redox probe. The results further pointed towards the fast and pH-dependent electron exchange between the Fe mineral and organic sediment constituents, stressing the necessity to analyze mixed geochemical phases in an integrative manner. With reference to the rapid re-oxidation of reduced mineral and NOM species, implications regarding the competitive mitigation of other respiration pathways were discussed.

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Solid phases as important electron acceptors in freshwater organic sediments

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Abstract Ferric iron in particulate iron (oxyhydr–) oxides and quinone moieties in dissolved organic matter (DOM) are well-established terminal electron acceptors (TEA) in heterotrophic anaerobic microbial respiration. The importance of particulate organic matter (POM) as TEA is, however, much less studied and understood despite the fact that POM is more abundant than DOM in many soils and sediments. Here, we studied the microbial reduction of POM and Fe(III)-containing phases in freshwater sediments. We present an electrochemical approach that can be used to assess the combined contributions of POM and Fe(III) to the TEA pools of soils and sediments. Following oxidation and drying of sediments from two carbonate–buffered freshwater lakes in air, wetting re–initiated anaerobic microbial respiration in the sediment samples as evidenced from electron transfer to solid–phase electron acceptors over three weeks of anoxic incubations. The microbial reduction of POM and mineral-associated ferric iron was directly quantified by mediated electrochemical analysis. We estimate that the POM from the analyzed sediments provided a capacity to accept or donate electrons of about 650 μmol e^- (g organic carbon)$^{-1}$. Our work substantiates earlier studies that suggested that the reduction of redox–active moieties in POM is an important contributor to anaerobic microbial respiration and might be responsible for the competitive suppression of methanogenesis in organic matter rich wetland soils. Our results further indicate that microbial reduction of POM must be accounted for to close respiration balances in (temporary) anoxic freshwater systems and peatlands.

Keywords Particulate organic matter · Electron acceptor · Anaerobic respiration · Electrochemistry

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Introduction

Every year, 2.9×10^{15} g of organic carbon (OC) is exported from terrestrial to freshwater ecosystems

(Tranvik et al. 2009). A significant fraction of the OC is buried as natural organic matter (NOM) in freshwater sediments. NOM includes particulate organic matter (POM) and dissolved organic matter (DOM) (Stevenson 1994), which are operationally defined as fractions that are either retained by or that pass through 0.45 μm cut–off filters, respectively. POM includes organic matter adsorbed onto and co–precipitated with minerals that are larger than the critical cutoff size (Stevenson 1994; Lalonde et al. 2012). DOM is viewed as supramolecular associations of smaller organic molecules (Piccolo 2001; Sutton and Sposito 2005). Under anoxic conditions, the NOM may serve both as electron donor and acceptor in anaerobic respiration. In general, respiration of NOM results in the formation of carbon dioxide (CO_2) and liberation of electrons (e^-) that are transferred to available TEAs other than O_2(Megonigal et al. 2003).While respiratory reactions are generally exergonic, electron transfer to some TEA is thermodynamically more favorable than to others (LaRowe and Van Cappellen 2011, more details in the online resources): based on thermodynamic estimations, inorganic electron acceptors are competitively utilized in the order of NO_3^- (nitrate reduction), Mn(IV) and Fe(III), SO_4^{2-} (sulfate reduction) and CO_2 (hydrogenothrophic methanogenesis), with exceptions from this sequence being possible (Postma and Jakobsen 1996; Bethke et al. 2011). While balancing the consumption of inorganic TEAs in the respiration of NOM has been a central part of many studies on anaerobic respiration (e.g. Yao et al. 1999; Ratering and Schnell 2000), most studies neglect that NOM may also act as TEA (Lovley et al. 1996; Scott et al. 1998). The role of NOM as TEA in anaerobic respiration is expected to be particularly important for sediments and wetland soils, which are both anoxic and rich in NOM (Keller and Takagi 2013).

Anaerobic microbial respiration using NOM as TEA was first demonstrated by Lovley et al. (1996) for DOM. The focus of subsequent studies remained on the redox properties and reactivity of DOM, including its role as electron transfer mediator in dissimilatory microbial Fe oxide reduction (see discussion on electron shuttling of DOM in Sposito 2011 and Martinez et al. 2013) and its capacities to accept and donate electrons (Scott et al. 1998; Aeschbacher et al. 2010, 2011, 2012). The redox properties of DOM were primarily attributed to a reversible electron transfer to/from quinone/hydroquinonemoieties (Lovley et al. 1996; Scott et al. 1998; Sposito 2011) that are ubiquitous in terrestrial and aquatic DOM (Aeschbacher et al. 2010). By comparison, the capacities of POM to accept and donate electrons and changes in these capacities during anaerobic respiration received only little attention (Roden et al. 2010), despite the fact that POM makes up the major part of NOM in most soils and sediments. Advances towards a better understanding of POM as TEA were impaired mainly by the lack of a reliable and direct analytical approach to quantify changes in POM redox states.

Traditional methods to determine the redox states of NOM relied on reacting these materials with added chemical oxidants, most commonly complexed Fe^{3+} species or I_2 (Kappler et al. 2004; Bauer et al. 2007; Roden et al. 2010; Struyk and Sposito 2001). The NOM redox states were then inferred from the extents of reduction of the added oxidant. While commonly used, these methods have several drawbacks including that NOM redox states are determined indirectly (i.e., via the conversion of the added oxidant) and one-directionally (i.e., the capacities of NOM to donate electrons but not to accept electrons are quantified). Furthermore, the analysis method is susceptible to kinetic artifacts due to slow electron transfer from the NOM to the added oxidants (Bauer et al. 2007). These drawbacks of the traditional analysis methods were recently overcome by a novel electrochemical approach that utilizes dissolved organic redox mediators to facilitate electron transfer between the working electrodes (WEs) of electrochemical cells and the redox–active moieties in the NOM (Aeschbacher et al. 2010). The approach comprises mediated electrochemical reduction (MER) and–oxidation (MEO) in which the number of electrons transferred from the WE to the NOM and from the NOM to the WE, are directly quantified as reductive and oxidative currents, respectively, with high sensitivity. The redox states of NOM samples are further quantified bi–directionally towards low and high E_H values applied to the WEs in MER and MEO, respectively. The electrochemical approach has been successfully applied to determine the redox properties and quantify changes in the redox states of DOM (Aeschbacher et al. 2010, 2012; Kluepfel et al. 2014b), biochar (Kluepfel et al. 2014a) and of structural Fe in clay minerals (Gorski et al. 2012a, b, 2013). Based on the unique analytical

capabilities of the electrochemical approach and its broad application domain, we intend to evaluate whether the approach allows following anaerobic respiration using POM as well as DOM and Fe(III) phases as TEAs in natural sediment samples during anoxic incubations.

The two major goals of this work were (i) to demonstrate the applicability of MER and MEO to quantify reduction of POM, DOM, and Fe(III) phases in natural sediment samples during anoxic incubations and (ii) to assess the relative importance of POM, DOM and Fe(III) phases as TEAs. To this end, we incubated sediments from two carbonate-buffered freshwater lakes for three weeks under anoxic conditions and used MER and MEO to monitor microbial reduction of POM, DOM, and Fe(III) in the sediments. The MER and MEO analyses were complemented by the analysis of $Fe^{2+}$and $Fe^{3+}$to delineate electron transfer to NOM from Fe(III) phases.

Materials and methods

Study sites and sampling

The Stangenhagen Polder (SP) is a former peatland drained for agricultural use and rewetted in the course of regional conservation efforts 20 years ago. The system has a highly decomposed upper peat layer that is now permanently inundated, forming a hypertrophic shallow lake with new limnetic sediment deposition (Zak et al. 2009). *Ceratophyllum* spp. is the dominant submerged species in this carbon–rich system surrounded by substantial reed beds (*Typha latifolia*). Lake Arendsee (LA) is a dimictic, eutrophic lowland lake with a 19.8 km^2 catchment that includes agricultural, forested and urban areas (Hupfer and Lewandowski 2005). The high natural carbonate concentrations lead to a seasonal calcite precipitation in the lake, producing a varved structure within the sediment profile (Hupfer and Lewandowski 2005).

We sampled both sites (Table 1) in late summer 2013. Sediment samples from SP were obtained by removing bulk material from the upper 20 cm of the sediment at a non-vegetated location. Non–decomposed root and leaf materials, present at low abundances, were removed from the samples. The LA samples were collected from the uppermost sediment layer (0–5 cm) from two sediment cores (N = 2) sampled at the location of largest depth of the lake (i.e., 49 m).

Sediment incubation

Following the removal of coarse particles, the sediments were oxidized by air-drying at room temperature in the dark. The organic carbon, nitrogen and sulfur contents

Table 1 Sources and selected physicochemical properties of the collected sediment samples

Study site	Stangenhagen Polder (SP)	Lake Arendsee (LA)
Type	Rewetted fen	Lowland lake
Trophic state	Polytrophic	Eutrophic
Geoloc [dec°]	**N**52.20810 **E**13.0940667	**N**52.89278 **E**11.48889
Sample composition	Mean (± SD)	Mean (± SD)
Drymass (m %)	17	7
OC[a] (mg gdw^{-1})	274	110
OC:N[a,b] (mol mol^{-1})	11.8	8.5
S (mg gdw^{-1})	15.2	9.0
Ca[c] (mg gdw^{-1})	28.0	196.5
Fe[d] (mg gdw^{-1})	33.7 (±3.6)	5.4 (±0.5)
Mn[c] (mg gdw^{-1})	1.49	0.64

SD standard deviation

[a] Organic carbon (OC), N and S contents determined by elemental analysis (N = 2)

[b] Molar ratio of OC to nitrogen (N)

[c] After digestion with aqua regia (N = 2) via ICP-OES (iCap, Thermo, USA)

[d] Spectrophotometrically after acidic mobilization (0.5 M HCl, N > 10)

were determined by elemental analysis (Vario EL, Elementar, Germany). Portions of the dried and homogenized sediment materials were transferred into 23 incubation vials (each with a volume of 10 mL), moved into an anoxic glove–box ($O_2 < 0.05$ %) and rewetted with oxygen–free, 3.4 mM NaCl solution to the same water contents that the samples had prior to the drying and homogenization steps. The vials were subsequently sealed with butyl-rubber stoppers and incubated within the glovebox in the dark at 25 ± 2 °C, as detailed in the supporting information. The samples were microbially active upon rewetting with no inoculant added, consistent with previous studies demonstrating that significant fractions of bacteria remained viable in sediments over extended drying periods (Qiu and McComb 1995). Sterile LA control samples without microbial activity were obtained by heat sterilization (N = 5; 120 °C for 90 min). Between 0 and 21 days of incubation, samples were collected and diluted with high purity (<0.01 μS cm^{-1}; Satorius) O_2-free water prior to redox analyses.

Mediated electrochemical oxidation and reduction

All electrochemical measurements were conducted inside an anoxic glovebox. Oxygen-free solutions required for the analyses were prepared by purging the solutions with nitrogen gas (N_2, grade 5.0) for 90 min prior to transferring them into the glovebox. Unless stated otherwise, all reagents were purchased from Roth (Karlsruhe, Germany). The setup for MER and MEO was adapted from Aeschbacher et al. (2010) and Kluepfel et al. (2014a) using a multichannel potentiostat (CHI1000C, CH Instruments) run in chronoamperometry mode. In brief, measurements were conducted in electrochemical cells with pH buffered solutions (pH 7.00 ± 0.05) containing 0.01 M 4–Morpholinepropanesulfonic acid as the buffering species and 0.1 M $NaClO_4$ as background electrolyte (Fig. 1a). We used glassy carbon cylinders (Volume 9 mL) as both the working electrodes (WE) and the cell reaction vessels. The WEs were polarized to $E_h = +0.61$ V for MEO or −0.49 V for MER (reported versus the standard hydrogen electrode (SHE), but experimentally measured versus Ag/AgCl reference electrodes).

Each electrochemical analysis was initiated by the addition of the electron transfer mediators 6,7–Dihydrodipyrido[1,2–a:2′,1′–c]pyraziniumdibromid monohydrate (99.5 %; $E_h° = -0.36$ V; Supelco, USA) (diquat, DQ) in MER and 2,2–azino–bis-(3–ethylbenzthiazoline–6–sulfonic acid) ammonium salt (>98 %; $E_h° = +0.7$ V; Sigma–Aldrich, MO, USA) (ABTS) in MEO to final concentrations of 250–350 μM. Both compounds are single-electron transfer mediators: DQ was reduced to the radical species $DQ^{\cdot+}$ in MER and ABTS was oxidized to the $ABTS^{\cdot+}$ radical in MEO. The resulting reductive or oxidative current responses were peak–shaped with initial high currents followed by a decrease in the currents that ultimately leveled off as the mediators approached E_h equilibria with the E_h applied to the WEs. After re–attainment of redox equilibria, small volumes of suspended samples (50–200 μL; 2–4 (g C) L^{-1}) were added to the MER and MEO cells (Fig. 1b, c). The samples were withdrawn from vigorously stirred incubation vessels. In MER, the dissolved $DQ^{\cdot+}$ transferred electrons to electron accepting moieties or species in the added sample, resulting in the formation of DQ^{2+} molecules, which were subsequently re–reduced at the WE to re–establish redox equilibrium in the cell. In MEO, $ABTS^{\cdot+}$ radicals were reduced by electron donating species or moieties in the added sample, resulting in the formation of ABTS, which was then re–oxidized to $ABTS^{\cdot+}$at the WE. The addition of samples thus resulted in reductive and oxidative current peaks. These peaks were baseline–corrected and integrated to obtain the numbers of electrons, n_{e-} (mmol e^-) transferred to and from the added sample according to Eq. 1:

$$n_{e^-} = \int \frac{I}{F}\,dt \tag{1}$$

where I(A) is the reductive or oxidative current and F(C mol^{-1}) is the Faraday constant.

The mass–normalized electron accepting capacities (EAC, in mmol e^- gdw^{-1}) and electron donating capacities (EDC, in mmol e^- gdw^{-1}) of each sample were calculated by normalizing the measured n_{e-} to the added samples mass (in grams dry weight, gdw).

The sum of the EAC and EDC values is referred to as the electron exchange capacity (EEC, in mmol e^- gdw^{-1}) (Eq. 2):

$$\text{EAC} + \text{EDC} = \text{EEC} \tag{2}$$

The EEC value corresponds to the total number of electrons transferred to and from redox–active species in a given sample. Systems with equimolar conversions of electron accepting to donating moieties (or vice

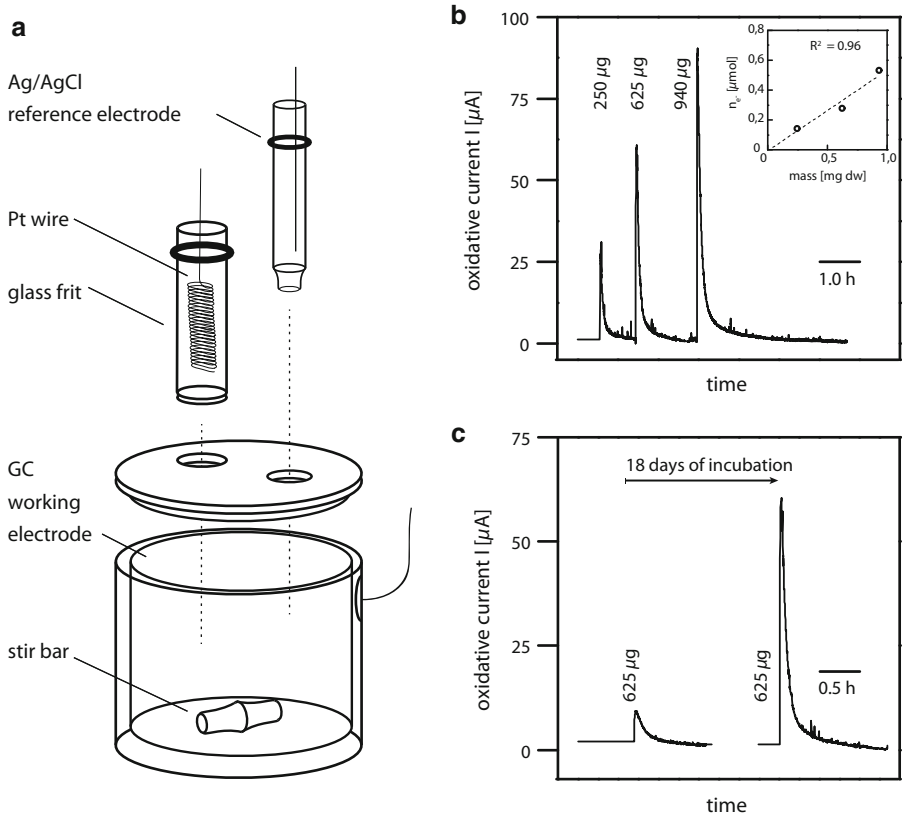

Fig. 1 **a** Scheme of the electrochemical cell used for mediated electrochemical reduction (MER) and oxidation (MEO). Each cell consisted of a cylindrical glassy carbon working electrode (WE), a platinum wire counter electrode that was separated from the main compartment by a porous glass frit and an Ag/AgCl reference electrode. The solution in the electrochemical cell was continuously stirred. Constant reduction potentials of $E_h = -0.49$ V (MER) and +0.61 V (MEO) (both reported against the Standard Hydrogen Electrode) were applied to the WEs over the entire analysis time (>5 h). The reductive or oxidative currents were continuously measured in 5 s intervals. **b** Baseline-corrected oxidative current peaks in MEO in response to 3 successive additions of suspended sediment into the electrochemical cell. Integration of the current peaks yielded the number of transferred electrons (n_{e-}). *Inset* Linear dependence of n_{e-} on the mass of sample added to the cell. **c** Oxidative current response to the additions of 625 μg of sediment aliquots (Stangenhagen polder) obtained from samples incubated under anoxic conditions for either 1 day (first peak) or 18 days (second peak). The increase in the current response reflects the reduction of the sediment during incubation

versa) have constant EEC values. Conversely, increasing or decreasing EEC values during incubations would signify that redox–active constituents are lost or formed over time, respectively.

Iron redox state analysis

The iron in solid-phase (oxyhydr–) oxides was quantified for each sample following acidic dissolution: 3 mL suspension were withdrawn from each sample and acidified with 3 M hydrochloric acid (HCl) to a final concentration of 0.5 M HCl. Unless stated otherwise, the acid treatment was carried out for at least 4 days in the dark inside the anoxic glovebox. Following filtration through 0.45 μm syringe filters, ferrous iron (Fe^{2+}) concentrations in the filtrate were quantified spectrophotometrically via the 1,10–phenanthroline method (Tamura et al. 1974). Ferric iron (Fe^{3+}) contents in the sample were determined by fully reducing the Fe^{3+} in the same sample with excess ascorbic acid (10 % solution) followed by the subtraction of the prior measured Fe^{2+} content.

Statistical analysis

Response variables (EAC, EDC, Fe^{2+}, Fe^{3+}) were quantified repeatedly in independent replicates of the two sample groups (SP and LA) over the course of the

incubations. Time-independent variables (e.g. EEC, Fe_{tot}) were compared using two–way analysis of variance (ANOVA) to test for differences between the two sample groups. Data integrations and non–linear data fitting were performed with Origin (Origin 8.5.OG, Origin Lab Corporation, USA). Statistical tests were performed with SPSS (SPSS 20, IBM, USA).

Results

Over the course of 21 days of anoxic incubation, the SP and LA sediments showed pronounced decreases in their total electron accepting capacities (EAC_{tot}) to about 25 % of their initial values, and, at the same time, increases in their total electron donating capacities (EDC_{tot}) (Fig. 2a, b). The comparatively low EAC values of the SP samples at the onset of the anoxic incubation (i.e., 0 days of incubation, Fig. 2a) likely resulted from incomplete wetting of the sediment prior to the MER analysis. For this reason, these initial values were not included in the statistical analysis of the SP samples. While the EAC_{tot} and EDC_{tot} values changed systematically during the incubations, there were no distinct trends in the total electron exchange capacities (i.e., $EEC_{tot} = EAC_{tot} + EDC_{tot}$) of both the SP and LA samples over time (Fig. 2e, f). When comparing the two sediments, the SP samples exhibited about 9–fold

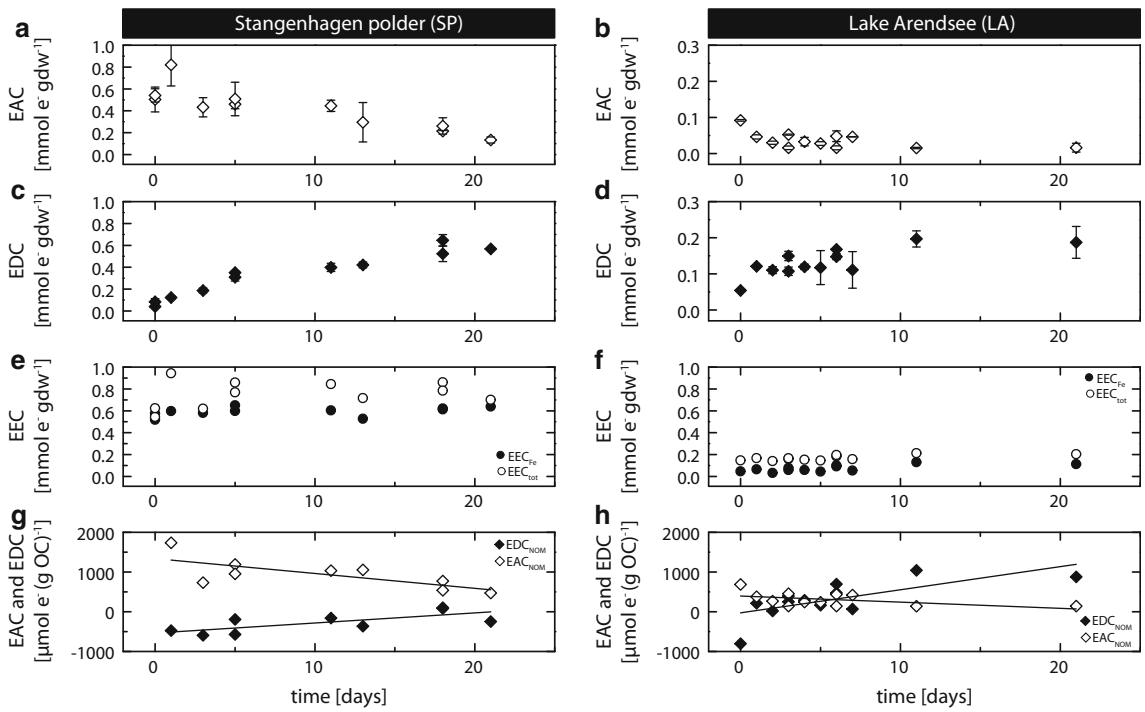

Fig. 2 **a–d** Changes in the total electron accepting and donating capacities (EAC_{tot} and EDC_{tot}) of sediment samples from the Stangenhagen polder (SP, *left*) and Lake Arendsee (LA, *right*) over the course of three weeks of anoxic incubations. Prior to the onset of the anoxic incubation, the SP and LA samples were oxidized by air–drying. Note the different scales on the ordinates of the *panels* a/c and b/d. At each sampling timepoint, one independent incubation flask was sacrificed for the electrochemical analysis of the total redox state of the sample and for spectrophotometric analysis of the Fe redox states. The *error bars* on the EAC and EDC data correspond to standard deviations of replicate ($N \geq 3$) measurements. (**e**, **f**) Comparisons of the total electron exchange capacities ($EEC_{tot} = EAC_{tot} + EDC_{tot}$, *open circles*) and the total iron contents (0.5 HCl extracted; expressed as EEC_{Fe}, *filled circles*) of the SP and LA samples. (**g**, **h**) Decreases in the electron accepting capacities (*open symbols*) of the NOM (EAC_{NOM}) and concomitant increases in the electron donating capacities (*filled symbols*) of the NOM (EDC_{NOM}) during anoxic incubations, as calculated from Eqs. 4 and 5. The EAC_{NOM} and EDC_{NOM} values are normalized to the organic carbon (OC) contents of the respective sediments. The *lines* are linear regression fits to the experimental data

higher EEC_{tot} values per gram dry weight (gdw) than the LA samples. The heat–sterilized control samples (120 °C, 90 min) showed no systematic trends in either EAC or EDC values during anoxic incubations, as shown in the online resource material.

The EAC_{tot} and EDC_{tot} values reflected the number of electrons transferred to and from redox-active NOM and metals in both sediments. Iron dominated the pools of redox-active metals in both sediments, given that the contents of Mn were comparatively small (< 0.15 m %) in both sediments. To determine the relative contribution of Fe to the measured EAC_{tot} and the EDC_{tot} values, we separately quantified changes in the redox states of Fe in all samples from both sediments over the course of the incubations by spectrophotometric Fe^{2+} and Fe^{3+} quantification (the experimental data is provided in the online resource material). Each mole of Fe atoms in the sediment samples contributed one mole of electrons to the total EEC_{tot}, irrespective of the redox state of the Fe (i.e., each ferric iron contributed one electron to the EAC_{tot} and each ferrous iron one electron to the EDC_{tot}). Both SP and LA samples had higher EEC_{tot} values than Fe contents (noted as EEC_{Fe}, Fig. 2e, f) and therefore must have contained redox–active constituents other than Fe. Because of the small Mn contents of the sediments and given that nitrate and sulfate are electro–inactive in MER and MEO (data not shown), we ascribe the difference between EEC_{tot} and EEC_{Fe} to redox–active NOM (Eq. 3):

$$EEC_{NOM} = EEC_{tot} - EEC_{Fe} \quad (3)$$

Using Eq. 3, the calculated EEC_{NOM} values were 177 ± 91 μmol e^- gdw^{-1} (mean ± SD; N = 9) for the SP and 72 ± 30 μmol e^- gdw^{-1} (N = 12) for the LA samples. We note that these EEC_{NOM} values were not significantly different between the two sediments when normalized to the organic carbon contents (and not to dry masses) of the sediments (N = 21, P = 0.05): 645 and 659 μmol e^- $(g\ OC)^{-1}$) for the SP and the LA sediment samples, respectively.

In addition to delineating the contributions of total Fe and NOM to the overall EEC_{tot} values, it is possible to determine the relative contributions of oxidized, electron accepting NOM and Fe^{3+} to the measured EAC_{tot} and of reduced, electron–donating NOM and Fe^{2+} to the measured EDC_{tot} (Eqs. 4 and 5):

$$EAC_{NOM} = EAC_{tot} - EAC_{Fe^{3+}} \quad (4)$$

$$EDC_{NOM} = EDC_{tot} - EDC_{Fe^{2+}} \quad (5)$$

where $EAC_{Fe^{3+}}$ and $EDC_{Fe^{2+}}$ (mmol e^- gdw^{-1}) are the electron accepting and donating capacities associated with ferric iron and ferrous iron in the sediment samples that were quantified separately. Analogously, the EAC_{NOM} and EDC_{NOM} are the electron accepting and donating capacities of reducible and oxidizable organic moieties in the SP and LA samples.

The EAC_{NOM} values of the SP and LA samples values showed pronounced decreases over the 21 days of anoxic incubations (Fig. 2g, h). Fitting of the data to a linear regression function resulted in EAC_{NOM} consumption rates of 21 μmol e^- d^{-1} $(g\ OC)^{-1}$ for the SP and 13 μmol e^- d^{-1} $(g\ OC)^{-1}$ for the LA samples. At the same time, the EDC_{NOM} values increased for the SP and the LA samples. For both sediments, the increases in the EDC_{NOM} values and the decreases in the EAC_{NOM} values were comparable in absolute values (regression data is provided in the online resource material).

Figure 2g shows that most of the EDC_{NOM} values of the SP samples were negative over the course of the incubation. The negative EDC_{NOM} values resulted from the fact that the EDC_{tot} values of the samples, quantified by MEO, were smaller than the $EDC_{Fe^{2+}}$ values, quantified after acidic extraction of the SP samples using the phenanthroline method (see Eq. 5). To assess the causation of negative EDC_{NOM} values, we conducted independent experiments to investigate the stability of the redox states of Fe in the samples during the acidic extraction. In these experiments, LA samples were incubated under anoxic conditions for 1 day. In a second step, the samples were acidified (final concentration of 0.5 M HCl) and subsequently analyzed for Fe redox speciation at different time points over several days. Figure 3 shows that at least 4 days were required for the complete mobilization of the particulate Fe^{n+} in the samples (i.e. >95 % of dissolvable Fe measured after 8 days of acid treatment). However, during this time, the Fe^{3+} concentrations in the acidified samples decreased from approximately 40 to 10 μmol $Fe^{3+}gdw^{-1}$. The redox state of the Fe^{3+} was therefore not stable during acid extraction. The low pH (i.e. pH < 1) of the acidified samples implies that the reduction of Fe^{3+} to Fe^{2+}

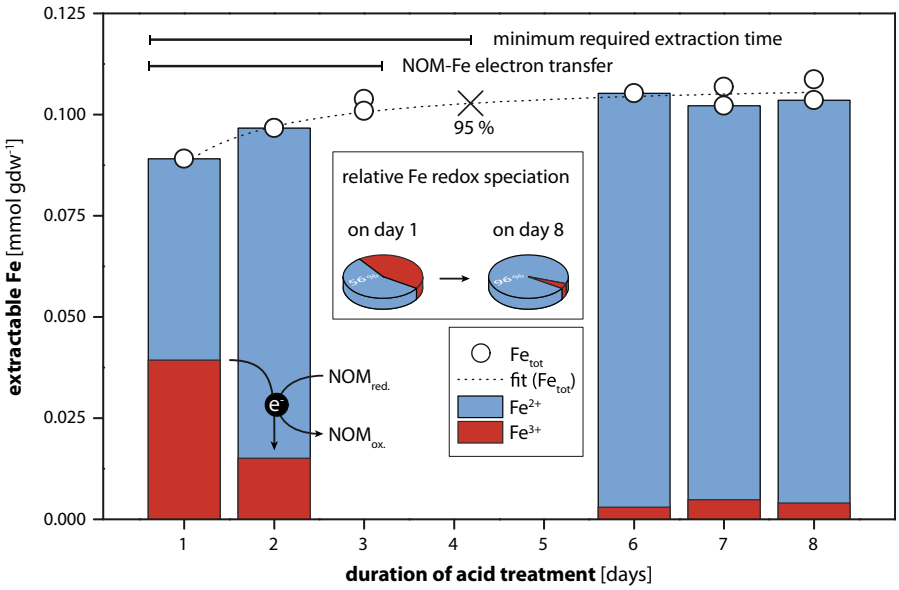

Fig. 3 Time dependent changes in the total amounts and the redox speciation (i.e. Fe^{3+} vs. Fe^{2+}) of iron extracted from Lake Arendsee (LA) samples during continuous extraction with hydrochloric acid (0.5 M). The *open circles* correspond to the total extractable Fe and the *dashed line* is a non–linear fit to the Fe extraction data (see online resources). Based on the data fitting, at least 4 days of acid treatment were required to extract all iron from the sample (i.e., extraction of >95 % of the extracted amount of Fe after 8 days of continuous acid treatment). The redox speciation of Fe^{n+} is shown in absolute terms (Fe^{3+}: *lower bar* and Fe^{2+}: *upper bar*) and relative terms (pie charts referenced to the total sediment Fe content, Fe_{tot}) for a set of samples that were incubated under anoxic conditions for 1 day prior to initiating the acid extraction of Fe. The *arrows* depict the proposed electron transfer from reduced organic matter (NOM_{red}) to Fe^{3+} under the formation of oxidized organic matter (NOM_{ox}) and Fe^{2+}. We propose that this reaction may give rise to artifacts in the Fe redox analysis, as detailed in the discussion section

must have resulted from abiotic rather than biotic reactions.

Discussion

The first goal of this study was to demonstrate the applicability of MER and MEO to quantify electron transfer to NOM and Fe phases in different freshwater sediments during anaerobic microbial respiration. The decreases in EAC values and the concomitant increases in EDC values, quantified by MER and MEO, of the SP and LA samples (Fig. 2) demonstrated that the sediments became increasingly reduced during the anoxic incubations. Sediment reduction resulted from anaerobic microbial respiration because no external chemical reductant was added to the samples and, more importantly, because sterilized control samples exhibited constant EAC and EDC values under the same incubation conditions. Microbial reduction of the SP and LA sediment samples upon rewetting demonstrated that anaerobic bacteria in the sediments survived the temporarily oxic conditions during sample drying and respired onto NOM and Fe in the sediments. We note that no exogenous electron donor was added after rewetting to initiate microbial respiration. Similar findings have been reported previously (Qiu and McComb 1995; Fierer and Schimel 2002; Wilson and Baldwin 2008). The experimental design including MER and MEO was therefore viable to assess the contributions of solid–state organic and inorganic TEAs to overall anaerobic respiration. This study is the first to successfully apply MER and MEO to analyze the redox dynamics of complex, natural samples.

Particulate organic matter and Fe phases as TEAs

The second goal of this work was to assess the relative importance of POM and of Fe phases as TEAsin anaerobic microbial respiration. We note that the

measured EAC_{tot} and EDC_{tot} values correspond to the number of electrons transferred to and from all reducible and oxidizable species in the sediment samples. These species did not include nitrate and sulfate given that they are not electro-active in MER (data not shown). The approximately constant EEC_{tot} values of the two sediments during anoxic incubations (Fig. 2e, f) further suggest that sulfate either was not a major TEA in the incubation experiments and/or that anaerobic microbial respiration of sulfate did not result in significant amounts of electro-active organic or inorganic sulfur species. If the latter were the case, the EEC_{tot} values would have increased over time as new electro-active sulfur species had been formed. Sulfate reduction may have been competitively inhibited in the sediments by microbial electron transfer to thermodynamically more favorable TEAs including Fe and NOM (Megonigal et al. 2003; Kluepfel et al. 2014b). Furthermore, it is possible that if reduced sulfur species were formed, they acted as abiotic reductants towards Fe(III) and oxidized moieties in the NOM (Zak et al. 2006; Heitmann et al. 2007). We therefore conclude that the contributions of sulfur species to the measured EAC_{tot} and EDC_{tot} were small. Given the small contents of Mn in both the SP and LA samples, as detailed above, NOM and Fe phases are expected to be the major contributors to the measured EAC_{tot} and EDC_{tot}.

We previously demonstrated that MER and MEO are capable of detecting all structural Fe(III) and Fe(II) in selected iron-bearing smectites (Gorski et al., 2012a; 2012b), which directly supports that Fe phases were electro-active in the sediment samples. We assessed the relative contributions of Fe(III) to EAC_{tot} and Fe(II) to EDC_{tot} in the sediment samples by independent quantification of changes in the redox states of the iron in the sediments during anoxic incubations using the phenanthroline method. This approach allowed determining EEC_{NOM} according to Eq. 3 and hence the importance of NOM as TEA in anaerobic respiration in the sediments.

The organic carbon–normalized EEC_{NOM} values of the SP and LA sediments were statistically indistinguishable. This finding suggests that the differences in the mass-normalized EEC_{NOM} values of the sediments were due to their different NOM contents rather than different capacities of the NOMs to exchange electrons. Comparable carbon–normalized EEC_{NOM} values indirectly support that NOM, in addition to iron, was the major redox–active species in MER and MEO. Because of the much larger POM than DOM pools in the two sediments (i.e., mass ratios of C_{POM} to C_{DOM} larger than 2,000, as detailed in the online resources), the EEC_{NOM} values of 645–659 μmol e^- (g OC)$^{-1}$ primarily reflected electron transfer to and from redox–active moieties in the POM (i.e., $EEC_{NOM} \approx EEC_{POM}$). The EEC_{NOM} values of the sediments were smaller than values previously reported for dissolved aquatic humic substances of 1,900–6,900 μmol e^- (g C)$^{-1}$(Aeschbacher et al. 2012). This difference can be ascribed to the lower oxygen contents and hence likely quinone/hydroquinone moieties in POM than DOM. At the same time, the EEC_{POM} values determined herein were about six-fold higher than values reported for POM-rich soils of an ombrotrophic bog (Keller and Takagi 2013) and 25–fold larger than for POM in wetland sediments (Roden et al. 2010). The lower EEC values of POM reported previously likely resulted from determining the values indirectly by monitoring the reduction of added complexed Fe^{3+} to Fe^{2+}. This approach was previously shown to underestimate EEC values of DOM due to slow electron transfer to the added Fe^{3+} (Bauer et al. 2007; Aeschbacher et al. 2010). Similar or even larger kinetic artifacts are expected when using this assay to determine the redox state of POM. Conversely, MER and MEO of DOM were shown not to be susceptible to kinetic artifacts (Aeschbacher et al. 2010), supporting the higher EEC_{POM} values determined herein. A direct comparison of EEC_{POM} values determined by the traditional and the novel electrochemical approaches will be the focus of future studies. We conclude that our results largely substantiate that NOM is an important and significant TEA in anaerobic microbial respiration, particularly in anoxic systems rich in NOM (Scott et al. 1998; Heitmann et al. 2007).

The data in Fig. 2 shows that microbes transferred electrons to both NOM and Fe phases during the anoxic incubations of the sediments. This finding suggests that the reduction potential ranges of the NOM and the Fe phases overlapped at circumneutral pH, consistent with Kluepfel et al. (2014b). Reduced and oxidized NOM and Fe phases therefore co-existed in the sediments during the anoxic incubations. This co-existence helps to rationalize the reduction of Fe^{3+} to Fe^{2+} in the acidified sediment samples prepared for total iron extraction (Fig. 3) and, therefore, the finding of negative EDC_{NOM} values (i.e., larger $EDC_{Fe^{2+}}$ than

EDC_{tot} values) in the SP samples (Fig. 2g). Decreasing the pH in the sediment is expected to have increased the reduction potentials (E_h) of both the NOM and the Fe phases (Stumm and Morgan 1996; Uchimiya and Stone 2010; Aeschbacher et al. 2011; Gorski et al. 2012a). However, this pH-induced increases in E_h were likely larger for the Fe phases than for the NOM, due to the fact that a one electron reduction of Fe(III) in an (oxyhydr–)oxide to dissolved Fe(II) is coupled to the transfer of more than one proton and hence is expected to result in an E_h increase of >59 mV per decrease in one pH unit. Conversely, the transfer of two electrons to/from quinone/hydroquinone moieties is coupled to the transfer of two protons at neutral to acidic pH, and hence results in an increase of about 59 mV per decrease in the pH by one unit (as shown in Aeschbacher et al. (2011) and in the online resources). Because of these different E_h–pH dependencies of NOM and Fe(III) minerals, the acidification of the samples likely caused redox dis–equilibria between the NOM and Fe(III)/Fe(II) couples that ultimately triggered electron transfer from reduced NOM moieties to Fe(III) (Fig. 3). We note that Fe(III) reduction by NOM upon sample acidification was also observed in heat sterilized samples (data not shown). We conclude that sediment acidification resulted in artificial Fe(III) reduction to Fe(II). As a consequence, Fe^{2+} quantification in acidified samples leads to an overestimation of the importance of Fe and an underestimation of the importance of NOM as TEAs in anaerobic respiration (as by Eq. 5). This artifact can not be eliminated by shorter acid treatments as the abiotic reduction of Fe(III) occurred on shorter timescales than required for the complete acid-induced solubilization of Fe(III) phases in the sediments (Fig. 3). Our results suggest that previous studies that reported Fe redox states in natural samples based on the amounts of Fe^{2+} quantified in acid extracts of the samples need to be carefully re–evaluated.

Environmental implications

Rates and extents of carbon mineralization in anoxic environments can be estimated by measuring the formation of carbon dioxide (CO_2), the most oxidized end–product of respiration. During heterotrophic respiration of organic substrates, electrons are transferred onto one or more TEAs (LaRowe and Van Cappellen 2011). Mineralization may thus also be analyzed by monitoring the depletion of TEAs during anaerobic respiration (as e.g. in Yao et al. 1999). A closed respiration stoichiometry can, however, only be achieved if all electrons transferred from organic donors to TEAs are accounted for. In general, the contribution of NOM to the total TEA pool was not included in most previous studies of TEAs. Yet, our work shows that NOM can provide a significant share of the solid-state electron accepting capacity in many aquatic environments, even those that are rich in iron (Fig. 4).

Previous work has shown that reduced moieties in DOM formed in anoxic environments are rapidly re-oxidized upon aeration (Ratasuk and Nanny 2007; Aeschbacher et al. 2010; Kluepfel et al. 2014b), thereby restoring the capacity of DOM to accept electrons under subsequent anoxic periods. The importance of NOM as TEAs is thus expected to be particularly large in temporarily anoxic systems that cycle between reducing and oxidizing conditions.

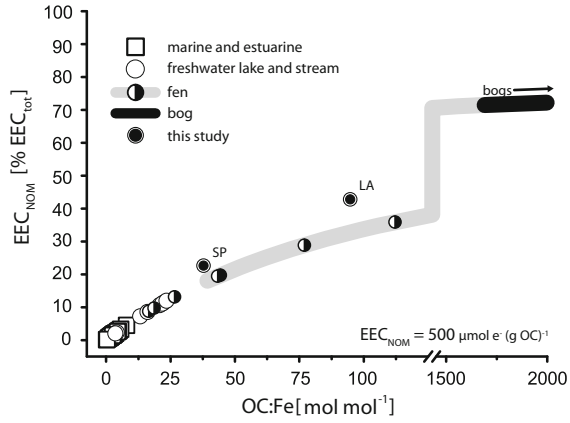

Fig. 4 The relative contributions of natural organic matter (NOM) (EEC_{NOM}) to the total pool of solid–phase electron acceptors (EEC_{tot}) in soils and sediments from different aquatic environments. Particulate organic matter (POM) dominates the NOM pools in these systems. The depicted contributions are based on the assumption that contributions from Mn and other redox-active trace metals can be neglected due to their generally small contents in relation to Fe. Relative EEC_{NOM} values were calculated using a conservative estimate of $EEC_{NOM} = 500\ \mu mol\ e^-\ (g\ OC)^{-1}$, which was below the values measured herein for the SP and LA samples (depicted in the figure based on quantified EEC_{NOM} values). The *lines* display OC: Fe ranges for fens and peat bogs taken from Shotyk (1988). All other symbols represent independent systems (Roden and Wetzel 1996; Knösche 2006; Zak et al. 2006, 2009; Kjaergaard et al. 2012; Lalonde et al. 2012)

Redox cycling of NOM may help to explain CO_2 emission patterns from wetlands after drought (Fenner and Freeman 2011). In this study, we subjected the sediment samples to a drastic oxidation–reduction sequence by airdrying followed by re-wetting of the samples. Analogous changes in redox conditions may occur in natural systems that undergo drying and wetting cycles (i.e., temporary rivers, lake littoral). Oxygen can also enter permanently waterlogged micro-environments that are separated from the macroscopic oxic/anoxic interface via advective or diffusive oxygen transport facilitated by air–filled cavities in plant tissue (*Aerenchyma*), benthic bioturbation (Lewandowski et al. 2007) and/or preferential water flow through heterogeneous peat soils (Kjaergaard et al. 2012). In all of these systems, the redox cycling of NOM may 'short–circuit' electron transfer from anaerobic respiration to O_2 (Kluepfel et al. 2014b). Assuming that reduced POM is re–oxidized by dissolved O_2 (similar to DOM) then POM is expected to play an important role as TEA in anaerobic microbial respiration and hence carbon dynamics in many aquatic ecosystems.

This work largely substantiates that POM is, in and of itself, an important TEA in anaerobic respiration (Heitmann et al. 2007) that needs to be considered when trying to close respiration balances (Keller and Takagi 2013; Bridgham et al. 2013). While consistent with a previous study that has shown that anaerobic bacteria respire onto POM in wetland soils (Roden et al. 2010), our results suggest much higher capacities of POM to exchange electrons than previously reported. The reduction of POM may explain why 29–85 % of CO_2 formation along hydrogeomorphic gradients remained unexplained when considering only the reduction of conventional (i.e. inorganic) TEAs (Keller and Bridgham 2007). Furthermore Keller and Takagi (2013) showed that NOM accepted a significant fraction (33–61 %) of the electrons that were released during anaerobic respiration in a bog soil. Electron transfer to POM therefore may competitively suppress hydrogenothrophic methanogenesis in wetlands (Cervantes et al. 2000; Blodau and Deppe 2012; Bridgham et al. 2013) akin to the effect previously proposed for inorganic TEAs (Roden and Wetzel 1996; Gauci et al. 2004, Dowrick et al. 2006). We propose that NOM (and primarily POM) is a key redox-active phase in (temporarily) anoxic systems rich in organic matter. As a consequence, the redox dynamics of POM need to be accounted for to close microbial respiration balances and to link anaerobic respiration to the formation of greenhouse gases (CH_4 and CO_2) in these systems on a mechanistic level.

Conclusion

This study is the first to successfully employ mediated electrochemical analysis to quantify electron transfer to particulate NOM and Fe pools in freshwater sediments during anaerobic microbial respiration under anoxic incubation conditions. Through combined electrochemical analyses and spectrophotometric determination of Fe redox states we were able to demonstrate microbial reduction of POM as a TEA as an important respiration pathway. We propose the use of mediated electrochemical analyses of solid phases in future studies directed towards closing electron balances of anaerobic respiration in natural systems.

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Supplementary Information

Electronic Supplementary Material (ESM).

Thermodynamic parameters

ESM Table 1 Electron transfer stoichiometry, thermodynamic energy yield (ΔG) and reduction potentials (E_h) for respiration of selected terminal electron acceptors

Reaction	TEA	electron stochiometry [mol e^- (mol TEA)$^{-1}$]	E_h [a] [mV]	ΔG [b] [kJ mol^{-1} e^{-1}]
Oxic respiration	O_2	4	812	-125
Denitrification	NO_3^-	6	747	-118
Manganese reduction	MnO_2 [c]	2	526	-98
Iron reduction	$Fe(OH)_3$ [c]	1	-47	-42
Sulfate reduction	SO_4^{2-}	8	-221	-24
Methanogenesis	CO_2	8	-244	-23
Humic acids (after re-oxidation) [d]			E_h [d] [mV]	ΔG [kJ mol^{-1} e^{-1}]
Pahokee Peat HA	DOM	n	103	-56
Suwannee River HA	DOM	n	-10	-46

a Potentials are given for pH 7 (25°C) (Megonigal et al. 2003). b Reaction free energies for the reduction of the TEAs coupled to the oxidation of organic matter as electron donor. The ΔG=-RT ln(K) values were calculated assuming that the reduction of the TEAs is coupled to the oxidation reaction ½ CH2O + ½ H2O → ½ CO2 + H+ + e- (Megonigal et al. 2003). c Values are considered approximations as the reduction potentials and associated reaction free energies are dependent on the type and crystallinity of the mineral phases. d Reduction potentials measured in solutions of the respective oxidized humic acids (HAs) (pH 7), determined using Pt-ring electrodes (Kluepfel et al. 2014)

ESM Table 2 Standard reduction potentials ($E_h°$) of the electron transfer mediators

electrochemical mediators	$E_h°$ [mV]
ABTS [a] 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) ammonium salt	700
DQ [b] 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyraziniumdibromid monohydrate	-360

[a] used in mediated electrochemical oxidation [b] used in mediated electrochemical reduction

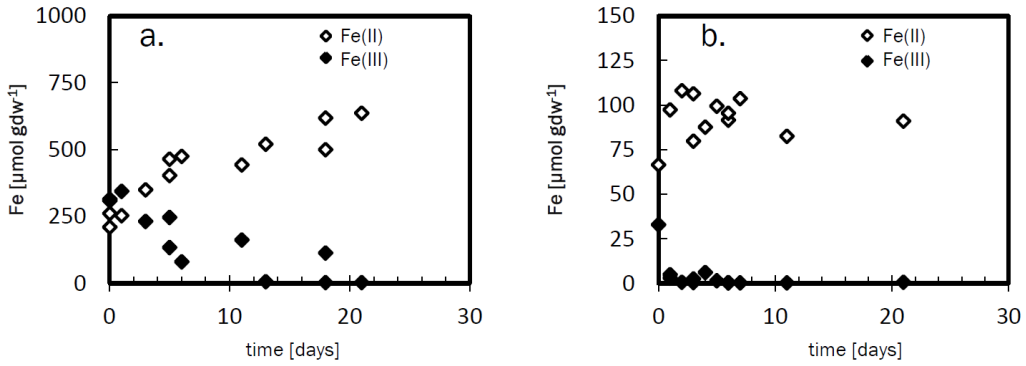
Incubation temperature

The SP and LA samples were incubated under anoxic conditions inside the glove box at temperatures that were higher than the *in situ* sediment temperatures in the natural sediments. According to the Arrhenius law, the higher temperatures during incubation likely enhanced microbial activity over the activity in the natural sediments. We therefore acknowledge the possibility that the sediment reduction rates in the incubation experiments were higher than observed in the natural sediments. Previous studies have, however, shown that increased temperatures may not alter which terminal electron acceptors (TEAs) are used in anaerobic respiration (Roden and Wetzel 1996). We therefore consider the experimental design viable to assess the relative contributions of solid state TEAs to overall anaerobic microbial

respiration.

Iron redox dynamics

Ferric and ferrous iron concentrations were measured in all solutions following acidic extraction. The concentration values are given in ESM Fig. 1. These measurements were necessary to determine the relative contributions of the electron exchange capacities of Fe in Fe minerals (EEC_{Fe}) and from the electron exchange capacities of the natural organic matter (NOM) (EEC_{NOM}) to the total electron exchange capacities measured for the samples (EEC_{tot}).



ESM Figure 1 Ferric iron (i.e., Fe(III); filled symbols) and ferrous iron (i.e., Fe(II); open symbols) concentrations in samples from the Stangenhagen polder (a.) and Lake Arendsee (b.). Fe(III) concentrations were determined in solution following acidic dissolution of Fe minerals (i.e., treatment with 0.5 M HCl; > 4 days extraction duration).

Linear and non-linear data fitting

Over the course of the incubation, the NOM in both sediments became increasingly reduced as a result of microbial respiration and the use of POM as TEA. Consumption rates can be estimated by linear fits to the data. ESM Table 3 provides the linear regression parameters for the NOM-associated electron donating capacities (i.e., EDC_{NOM}) and accepting capacities (i.e., EAC_{NOM}) from both sediments (Fig. 2g and 2h).

ESM Table 3 Linear regression parameters obtained from fitting the experimental data of EAC_{NOM} and EDC_{NOM} versus time

	Stangenhagen polder (SP)			Lake Arendsee (LA)		
	Intercept [µmol e⁻ (g OC)⁻¹]	Slope [µmol e⁻ (g OC)⁻¹ d⁻¹]	Pearson correlation coefficient	Intercept [µmol e⁻ (g OC)⁻¹]	Slope [µmol e⁻ (g OC)⁻¹ d⁻¹]	Pearson correlation coefficient
EAC_{NOM}	1337	-37	0.72	370	-13	0.75
EDC_{NOM}	-577	28	0.78	95	47	0.51

The determination of the minimum required extraction time required a non-linear fit to the data of Fig. 3. For evaluation of the Fe extraction yields (Fe_{extr}) as a function of time, the data was fitted to a Hill-function (OriginLabs 8.5)

$$Fe_{extr}(t) = Fe_{max}\frac{t}{k+t}$$

where t is the duration of acid treatment (days), Fe_{max} corresponds to the highest measured Fe_{tot} value (0.11 mmol gdw^{-1}) and k to a fitting parameter. The r^2 was 0.85. According to the fit, 95% of all Fe was extracted when the extraction time was longer than 4.1 days.

Assessment of the relative pool sizes of DOM and POM

To compare the pool of dissolved organic matter (DOC) to that of particulate organic matter (POM) in sediments from SP and LA, we compared the organic carbon contents of the sediments with the DOC concentrations in the pore waters of the respective sediments, collected by *in situ* porewater samplers.

ESM Table 4 Organic carbon (OC) contents in the solid and dissolved phases of the sediments

	OC		
	Solid phase (POC) [(g OC) L⁻¹]	Dissolved (DOC) [(g OC) L⁻¹]	POC : DOC [ ]
Stangenhagen Polder	396	0.179 [a]	2 200
Lake Arendsee	130	0.007 [a]	18 406

[a] Median values obtained from passive pore water samplers as reported in other studies (Zak, pers. comm.)

Sulfur content of the sediment samples

The dried sediments were analyzed for their sulfur contents. Total sulfur was determined by elemental analysis of the sediment samples. Sulfate was measured after re-suspension of the sediment in the filtered water (filtered through 0.45 µm). All values in ESM Table 5 are normalized to the dry masses of the sediments.

ESM Table 5 Total sulfur and sulfate contents of the sediment samples

	Sulfur	
	total S [a] [mg gdw^{-1}]	sulfate-S [mg gdw^{-1}]
Stangenhagen Polder	15.2	4.1
Lake Arendsee	9.0	3.1

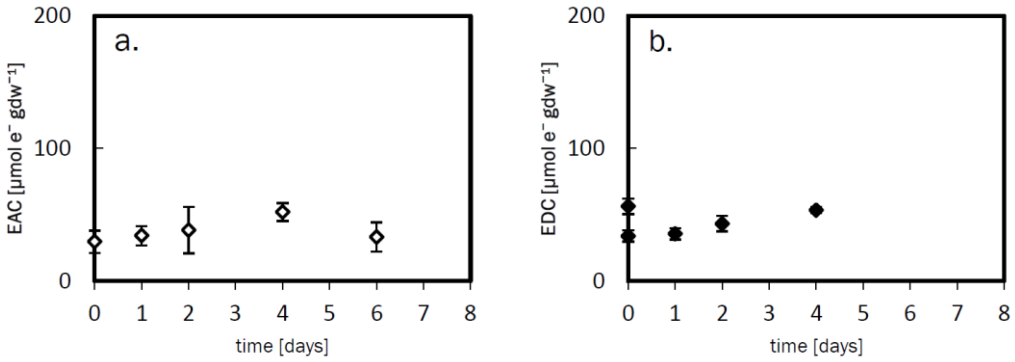
[a] Includes sulfate-S

Sulfur that was not mobilized as sulfate (i.e., the difference between total S and sulfate-S) may have been associated with sulfur-bearing minerals (e.g., pyrites, gypsum), sulfur-containing moieties within the NOM (i.e., amino acids, sulfonates) or with end- or intermediate products of anaerobic S-cycling (i.e., thiosulfate, elemental sulfur and polysulfides).

Thiosulfate and elemental sulfur can be used as terminal electron acceptors in anaerobic microbial respiration and be reduced to sulfide or abiotically disproportionate to sulfate and sulfide (Pester et al. 2012 and references therein). If the sediment contained electro-active redox couples with fully reversible electron transfers, these couples may have contributed to the electrochemically determined EEC_{NOM}. We note that sulfur (S^0) is electro-inactive in both MER and MEO and hence did not interfere with the determinations of EEC_{NOM}.

Sterile controls

Sterile control samples of Lake Arendsee were obtained by heat-sterilization (120°C, 90 min) of sediment material, prior to re-suspension and subsequent anoxic incubation. Electron accepting and donating capacities (EAC and EDC) were repeatedly determined in independent replicates over a six days incubation period. Absolute EAC and EDC values were lower than in non-sterilized samples, presumably due to larger particle aggregation in the sterilized samples (i.e., the sterilized samples showed larger suspended particles). The sterile controls showed no clear trends in the EAC (slope 0.1 ± 0.1 μmol e^- gdw^{-1} d^{-1}) and EDC (slope: 2 ± 3 μmol e^- gdw^{-1} d^{-1}) values with time over the course of the incubation period (ESM Fig. 2).



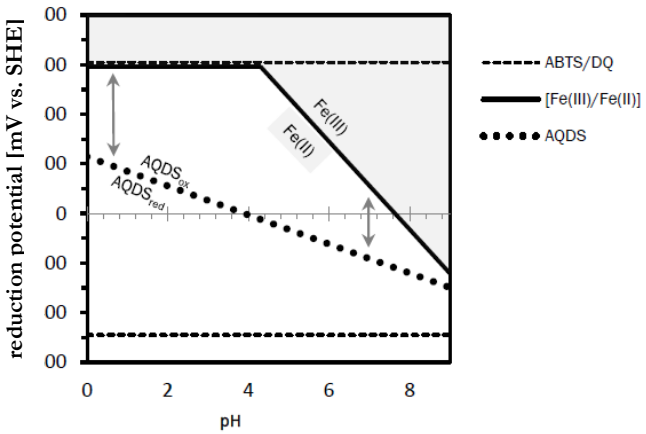
ESM Figure 2 Total electron accepting capacity (EAC, panel a.) and electron donating capacity (EDC, panel b) of sterile control samples of Lake Arendsee over the course of the anoxic incubations. Sterilization was performed in an autoclav (120°C; 90 min) on air dried sediment material, followed by resuspension of the sediment in de-ionized water. At day 6 of the incubation, the samples were analyzed only by mediated electrochemical reduction (MER).

Electron transfer from reduced NOM to Fe^{3+} upon sample acidification

Based on experimental evidence, we propose that sample acidification for Fe extraction resulted in electron transfer from reduced NOM to Fe(III). This electron transfer can be rationalized given the different E_h-pH dependencies of NOM and Fe(III)-containing phases (ESM Fig. 3). 9,10-Anthraquinone-2,6-disulfonate (AQDS) is a common model for quinone moieties in NOM. The reduction of $AQDS_{ox}$ to $AQDS_{red}$ at circumneutral pH involves two electrons and two protons, resulting in an increase in the E_h of +59 mV per decrease in pH. Conversely, many Fe(III) species have a stronger pH dependency of +180 mV per decrease in pH, e.g.,

$$Fe(OH)_3 + 3\ H^+ + 1e^- \rightarrow Fe^{2+} + 3H_2O$$

Decreasing the pH thus makes electron transfer from reduced NOM to Fe(III) thermodynamically more favorable.



ESM Figure 3 Change in the apparent reduction potential E_h with pH for redox couples Fe^{3+}/Fe^{2+} (black line) and oxidized to reduced 9,10-Anthraquinone-2,6-disulfonate (AQDS, dotted line). Dashed horizontal lines depict the applied potential E_h during mediated electrochemical redution (MER, E_h= -0.49 V) and oxidation (MEO, E_h= +0.61 V). The arrows indicate the difference during MER/MEO (pH 7) and Fe extraction (apprx. pH 1). AQDS data was calculated with formulas given in Aeschbacher (2011).

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3.2 Spatiotemporal redox dynamics in a freshwater lake sediment under alternating oxygen availabilities: combined analyses of dissolved and particulate electron acceptors

In the second article, we then traced the fate of the most relevant electron accepting species in freshwater sediment cores. The studied lake typically exhibits seasonal redox fluctuations at the sediment surface. In fine scale resolution, we measured how the external redox gradient influences the electron transport to and from dissolved and particulate redox species close to the oxic-anoxic interface. Re-oxidation of reduced species upon lake overturn (i.e., bottom-water oxygenation) could then be quantified based on the species' redox stoichiometry. A major advancement was the inclusion of particulate organic-mineral TEA species into the electron flux assessment. Orthogonal to previous approaches (that relied on distribution profiles), we were able to quantify O_2 consumption by directly tracking the re-oxidation dynamics of reduced sediment species. The resulting O_2 flux was found to be a significant position in the lakes oxygen budget which we modeled from the dynamics in water-column O_2 content.

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Spatiotemporal redox dynamics in a freshwater lake sediment under alternating oxygen availabilities: combined analyses of dissolved and particulate electron acceptors.
Environmental Chemistry, 13, 826–837, http://dx.doi.org/10.1071/EN15217

The published version of the manuscript can be obtained from
http://www.publish.csiro.au/paper/EN15217.htm

Abstract

Benthic mineralization in lakes largely controls the availability of oxygen in the water column above the sediment. In stratified lakes with anoxic hypolimnetic waters, mineralization proceeds by anaerobic respiration using terminal electron acceptors (TEAs) other than O_2. In past work, hypolimnetic oxygen consumption has been estimated from vertical concentration profiles of redox-active dissolved species in the water column and the underlying sediment. Electron transfer to and from particulate mineral and organic phases in the sediments was, however, not accounted for, mainly because of methodological constraints. In this work we use anelectrochemical approach, mediated electrochemical analysis, to directly quantify changes in the redox states of particulate geochemical phases in a lake sediment. In mesocosm incubations, sediments were subjected to shifting oxygen availability similar to conditions during and after lake overturn events. The temporal redox dynamics of both dissolved and particulate phases in sediments were monitored at a high spatial resolution. We used a combination of experimental and modeling approaches to couple the observed changes in the redox state of dissolved and particulate species in the sediment to the oxygen turnover in the overlying water column.

For the studied freshwater sediment, the amount of O_2 consumed during the re-oxidation of these phases in the top 21 mm of the sediment after switching from hypoxic to oxic conditions corresponded to approximately 50% of the total sediment oxygen consumption that was estimated from in-lake measurements after the onset of summer stratification. We found that solid phases in the sediments play a more profound role in electron accepting processes than previously considered. Based on these results, we propose that the herein presented analytical method offers the possibility to constrain parameters in theoretical models that simulate benthic redox dynamics including the electron transfer to and from geochemical phases in the sediments.

Introduction

Redox processes in the sediments of thermally stratified lakes largely control the biogeochemical conditions in the hypolimnion which is decoupled from the oxygen-rich water in the epilimnion.[1] Benthic oxygen consumption models were developed that use lake morphometry and pelagic oxygen profiles as input parameters.[2, 3] Newer oxygen consumption models are capable of accounting for fine-scale dissolved oxygen (DO) dynamics at the sediment-water interface.[4-6] Modeling advances were also complemented by conceptual advancements to provide a more fundamental understanding of benthic redox processes: Matzinger et al. [7] recently introduced the concept of 'areal hypolimnetic mineralization' (AHM) which explicitly accounts for the transfer of electrons (e^-) from electron donating organic substrates to O_2 in aerobic respiration and to inorganic TEAs in anaerobic respiration [8] based on measured vertical concentration profiles of dissolved reduced species (i.e., $Fe^{(II)}$, S^{2-}, $Mn^{(II)}$, NH_4^+, NO_2^-) and methane in the sediment pore water.[9-11] Although integrating redox reactions of these inorganic species into oxygen consumption models was new, these reactions are routinely included in reactive transport models that simulate early diagenesis with a focus on organic carbon consumption in sediments.[12, 13] Oxygen consumption in the latter model is viewed as a "secondary redox reaction" that re-oxidizes formerly reduced species. [14]

Both model types help to understand the benthic-pelagic coupling of redox processes. Yet, the model predictions highly depend on parameter constraints and implemented model components including the set of redox reactions of species that are dissolved in interstitial waters and associated with the solid phases in the sediment.[14]

Solid phase electron accepting species include iron (oxyhydr-)oxides, Fe(III)-containing clay minerals, as well as natural organic matter.[15-21] Under anoxic conditions in lake sediments, redox-active solid phases may be reduced in anaerobic microbial respiration. In the case of mineral Fe(III) reduction, it is important to note that the formed Fe(II) may remain associated with the sediment particulate fraction either through Fe(II) adsorption to mineral surfaces or because the Fe(II) is part of the mineral structure (e.g., structural Fe(II) in iron-containing clay minerals and mixed Fe(III)/Fe(II) oxides).[22, 23] Furthermore, it is possible that the formed Fe(II) initially dissolves but subsequently forms precipitates (e.g., Fe(II) sulfides).[24] In all of these cases, microbial reduction of particulate Fe(III) is not accounted for if solely dissolved Fe(II) in interstitial waters is incorporated in modeled electron fluxes in the sediment pore water (as in the AHM concept).

Furthermore, neither of the model approaches accounts for the anaerobic respiration with NOM as TEA. In this metabolic process, electrons are transferred from labile forms of the NOM to reducible moieties that are part of the more recalcitrant NOM pool. The reducible moieties include quinones,[16] which are ubiquitous in NOM[25] and can be reduced to the corresponding hydroquinones over a wide range of reduction potentials E_h.[26, 27] Several lines of evidence suggest that anaerobic respiration with particulate mineral and organic phases as TEAs is a major respiration pathway in anoxic freshwater systems and gives rise to larger amounts of carbon dioxide formed in these systems than can be explained by the reduction of dissolved inorganic TEAs, including nitrate and sulfate.[28-30]

Upon re-aeration of the deep lake water through lake turnover in spring and autumn, oxygen diffuses into the reduced lake sediments. Among the processes that consume oxygen in the sediments is the abiotic electron transfer from the reduced particulate phases in the sediment to the O_2. The oxidation of particulate Fe(II) species (as well as dissolved Fe(II) species) will form particulate Fe(III)-phases. Similarly, hydroquinone moieties in reduced NOM will be re-oxidized by O_2 to the corresponding quinone moieties. A time-resolved experimental assessment of the relative contribution of the abiotic reduction of O_2 by reduced particulate phases to the total O_2 consumption in lake sediments remained missing. The inclusion of solid-phase redox dynamics in electron budgets of lake sediments has previously been impaired by the lack of analytical techniques to quantify changes in the redox states of solid phases.

This work aimed at assessing the relative importance of electron transfer to and from redox-active particulate phases to overall redox dynamics of lake sediments when the redox conditons at the sediment suface are disrupted. To this end, we conducted two sediment mesocosm incubations in which we determined the spatiotemporal redox dynamics of both dissolved inorganic species (i.e., O_2, NO_3^-, SO_4^{2-}) and of particulate mineral and organic phases in the top 21 mm of sediments that resulted from changing the conditions in the water overlying the sediments from either hypoxic to oxic (first mesocosm) or from oxic to hypoxic (second mesocosm). The sediments used were obtained from Lake Scharmützelsee, a dimictic eutrophic lake.[31] The redox dynamics of organic and mineral phases were determined by quantifying the changes in the electron accepting capacities (EAC) and donating capacities (EDC) (i.e., the moles of electrons that can be transferred to and from one gram of the specific phase) of sediment subsamples using mediated electrochemical reduction (MER) and oxidation (MEO). These electrochemical techniques were successfully

employed in past studies to quantify the EAC and EDC values of dissolved NOM,[25, 32] Fe-bearing clay minerals [33], biochar [34] and particulate NOM and iron minerals in freshwater sediments.[30] In combination, we aim for the spatial resolution of the major dissolved and particulate redox active species in the sediment and quantify the electron fluxes to and from these phases across the sediment-water interface. We further assess the extents to which geochemical phases are re-generable as electron-acceptors in heterogeneous natural samples.

Since oxygen availability controls many facets of lacustrine ecosystem health, the implications of the quantified redox dynamics in the sediment to O_2 dynamics in lakes are discussed. Based on in-lake data, a simple empirical model was used to allocate O_2 consumption to either pelagic or benthic processes in Lake Scharmützelsee and compared to the experimentally determined sediment re-oxidation dynamic.[3, 35]

Materials and methods

Sediments and experimental setup

Sediments were obtained from Lake Scharmützelsee, a eutrophic, carbonate-rich lowland lake in northeast Germany (area 12.1 km^2, mean depth (z_{mean}) 8.8 m, maximum depth (z_{max}) 29.5 m; 52.25°N, 14.05°E). The lake is dimictic with complete mixing in late autumn and in spring.[31] The sedimentation rate of organic detritus is 3.6 – 4.2 g dry weight (dw) m^{-2} d^{-1}.[36] Two undisturbed sediment cores (circular surface areas of 80 cm^2 and lengths of 65 cm) were collected at 27 m depth in October 2013 before the autumn overturn (Uwitec corer, Mondsee, AT). At this time, no oxygen was detected in the water above the sediment surface. The core liners were composed of acrylic glass with a wall thickness of 5 mm. After retrieval, the cores were immediately capped, transferred to the lab and stored in the dark at 4 °C.

In the laboratory, one core was pre-incubated under hypoxic conditions (7 weeks), which was achieved by bubbling the water overlying the sediment with N_2 (0.1 vol-% CO_2) (Figure 1). We note that attainment of hypoxic conditions suggested that some oxygen still entered the water during N_2 bubbling, presumably by diffusion through the core liners or via the water surface at the top of the core. While more rigorous bubbling with N_2 may have decreased the oxygen content in the water, we decided against this measure as it would have increased the risk of suspending the top sediment layer. At the same time, we cannot exclude that sparging the water column with gas decreases the thickness of the diffusive boundary layer at the sediment surface. If the boundary layer was indeed thinner as compared to less turbulent (in situ) conditions in lakes, the transport of solutes as well as

the re-oxidation kinetics may have been accelerated in our experimental setup.

The second core was pre-incubated under oxic conditions (3 weeks), which was achieved by bubbling the water overlaying the sediment with compressed air. The dissolved oxygen concentrations in the water above the sediments were quantified using a DO probe (WTW, Weilheim, Germany). The DO profiles at the sediment-water interface were collected at a 100 μm depth resolution using an optometric O_2 sensor (PreSens, Regensburg, Germany) attached to a micromanipulator. The exact position of the sediment-water interface was determined visually using an endoscope.

Following the pre-incubations, the redox regimes in both core mesocosms were inverted. To this end, the water of the mesocosm pre-incubated hypoxically was bubbled with air, thereby establishing oxic incubation conditions. This mesocosm simulated changes in the boundary conditions at the sediment-water interface in lakes during autumn overturn and is subsequently referred to as the 'hypoxic-oxic' mesocosm. The water of the second mesocosm that was pre-incubated oxically was bubbled with N_2, resulting in hypoxic conditions. This core simulated changes in the redox boundary conditions in a lake at the onset of winter stagnation after autumn mixing. This mesocosm is subsequently referred to as the 'oxic-hypoxic' mesocosm.

Immediately following the changes in the redox boundary conditions and during the subsequent incubations, subsamples were repeatedly collected from both mesocosms and analyzed for the spatiotemporal dynamics of dissolved and particulate redox-active phases (Figure 1). Subsamples were collected by lowering two separate small sub-cores (diameter of 7 mm, glass) into the sediment of each mesocosm up to a depth of approximately 10 cm. The smaller subcores were then carefully retrieved from the sediment, capped under water and immediately transferred into an anoxic glove box (N_2; O_2 < 0.05 %). Over the entire course of the incubations, the sub-sampling procedure removed approximately 7% of the original sediment surface area. A sampling jig was used to ensure sufficient distance of the subcores from the (oxygen-permeable) liner of the larger sediment cores and a maximum distance between the individual sampling locations.

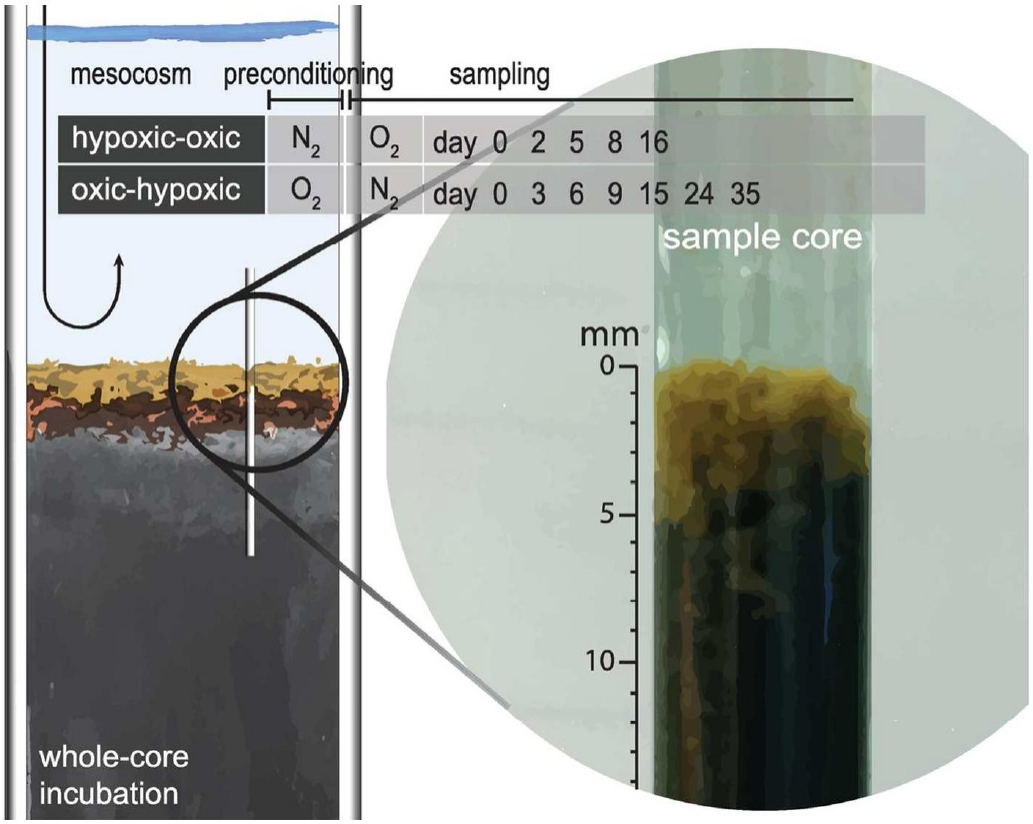

Figure 1 Scheme of the experimental setup consisting of two whole core mesocosms with contrasting redox fluctuations. The first mesocosm was pre-incubated under hypoxic conditions and subsequently subjected to oxic conditions by purging the water above the sediment surface with air (i.e., redox fluctuation subsequently termed 'hypoxic-oxic'). The second mesocosms was pre-incubated under oxic conditions with dissolved O2 in the water above the sediment surface. This core was subsequently subjected to hypoxic conditions by sparging the water above the sediment with N_2 (i.e., redox fluctuation subsequently termed 'oxic-hypoxic'). Immediately following sparging (i.e., starting on day 0) and in regular time intervals thereafter, sediment material was collected from both mesocosm cores using smaller sampling cores (diameters of 7 mm).

In the anoxic glove box, the sub-cores were sliced into smaller cylinders (diameter: 7 mm; height: 7 mm) from the top. A total of three cylinders were obtained per sub-core (i.e., depths of 0-7, 7-14 and 14-21 mm). The two cylinders of the same depth increment from the replicate sub-cores were combined and mixed with 10 mL of O_2-free water (< 0.01 μS cm^{-1}; Sartorius, Goettingen, Germany). This mixing compensated for potential spatial heterogeneity in the larger mesocosms and provided a sufficiently large suspension volume for all subsequent analyses.

Chemical analyses of subsamples

For all solutes, except sulfide, subsamples of the sediment suspensions were filtered through pre-rinsed 0.45 μm syringe filters (Whatman, Maidstone, UK) and the filtered solutions were immediately frozen. Sulfate and chloride concentrations were quantified in thawed solutions by liquid chromatography

coupled to a photometric detection unit (Shimadzu, Kyotot, Japan). Ammonium and nitrate were measured photometrically by continuous flow analysis (Skalar, Breda, The Netherlands) using Berthelot's reagent and reaction with N-(1-Naphthyl)ethylenediamine dihydrochloride, respectively.[37] Hydrogen sulfide was directly quantified in aliquots withdrawn from the suspended sediments without filtration to avoid potential degassing of H_2S. The aliquots were reacted with a 0.2 M zinc acetate solution to fix the hydrogen sulfide (0.25 ml (ml sample)$^{-1}$), followed by its photometric quantification with methylene blue.[38]

Manganese (Mn^{n+}) contents in the sediment suspensions were quantified by treating aliquots with aqua regia followed by analysis with inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Scientific, Waltham, USA). To estimate the iron content of the sediments that readily participated in redox reactions (i.e., iron that was part of amorphous (oxyhydr-)oxides, adsorbed to particle surfaces, as well as freely dissolved in the sediment pore water), 3 mL aliquots of the sediment were acidified in the dark and under anoxic conditions for at least 4 days with 3 M hydrochloric acid at a final HCl concentration of 0.5 M.[39] The acidified samples were subsequently filtered (0.45 µm syringe filters). The ferrous iron (Fe(II) concentrations in the filtrate were quantified spectrophotometrically (Agilent, Santa Clara, USA) using the phenanthroline method.[40] Total iron (Fe(II) and Fe(III)) concentrations in the filtrate were determined using the same method after complete reduction of Fe(III) by added ascorbic acid (in excess; 10% solution).

The dry mass and the carbon and nitrogen contents of each sediment suspension were determined in duplicate analyses. The dry mass was determined gravimetrically from the increase in dry mass of a calcinated glass-fiber filter (Whatman GF/F) that resulted from passing a defined volume of the sediment suspension through the filter. The organic carbon and total nitrogen contents of the filtered sediment were determined by elemental analysis (Vario EL, Elementar, Hanau, Germany) after removal of carbonate by acidification with 0.2 M HCl. If not stated otherwise, the concentrations of all determined species are reported relative to the dry mass of the sediment (i.e., per gram dry sediment weight, gdw) to allow for a direct comparison of dissolved and solid phase species. The dilution of the sediment suspensions for analysis were accounted for.

Mediated electrochemical oxidation and reduction

Mediated electrochemical reduction (MER) and oxidation (MEO) were carried out inside an anoxic glove box using an electrochemical cell setup adapted from Aeschbacher et al.[25], Kluepfel et al. [27] and Lau et al..[30] In

brief, each cell contained a glassy carbon cylinder (Sigradur G, HTW Carbon, Tierhaupten, Germany) that served as both working electrode (WE) and reaction vessel, an Ag/AgCl reference electrode (ALS, Japan), and a Pt wire counter electrode (CE) in a compartment seperated from the working electrode by a porous glass frit. The WE cylinder and the CE compartment contained five and one mL of buffered solutions (pH 7; 0.01 M 3-(N-morpholino)propanesulfonic acid, 0.1 M $NaClO_4$), respectively. The WEs were polarized to reduction potentials of E_h = -0.49 V in MER and +0.61 V in MEO respectively (referenced *v.* the standard hydrogen electrode). The electrochemical measurements were run in chronoamperometry mode using a CHI (1000C) potentiostat (CH Instruments, Austin, USA). Following the addition of the dissolved electron transfer mediators Diquat (MER) and ABTS (MEO) to the electrochemical cells and re-attainment of background current readings in the cells, suspended sediment samples were pipette transferred into the electrochemical cells. Electron transfer to and from the samples in MER and MEO resulted in reductive and oxidative current peaks, respectively. The current peaks were baseline-corrected and integrated to yield the total charge transferred to or from the added sample. The charges were converted into numbers of electrons transferred using the Faraday constant and normalized to the added mass of the sample to obtain EAC and EDC values (both in μmol e^- gdw^{-1}) of the added samples (see Supplementary material). The sum of the EAC and EDC values is referred to as the total electron exchange capacity (EEC, derived from $EAC + EDC = EEC$) and is a measure of the number of electro-active (organic and mineral) species for a given sample.

The lower and upper potentials in MER and MEO were chosen because the majority of biotic and abiotic redox reactions involving geochemical phases in aqueous systems fall into the potential range from -0.49 to + 0.61 V.[27, 41, 42] Changes in the redox states of the geochemical phases during the incubations were thus quantified as changes in the EAC and EDC values of the samples relative to the conditions used in MER and MEO.

The EAC and EDC values integratively capture the redox state of all (dissolved and solid phase-associated) electro-active species within the sediment samples. Given that electron accepting minerals (i.e., Fe(III) and Mn(IV) oxides) and sediment NOM are predominantly particulate (i.e., non-dissolved), we refer to 'solid phase TEAs' when describing and interpreting the results of MER and MEO. Inorganic dissolved species that accept more than two electrons and involve changes in the coordination of the central redox-active element (e.g., the reduction of nitrate and sulfate) require

(enzymatic) catalysis and are electro-inactive in the electrochemical cell (data not shown). Therefore, nitrate and sulfate in the sediment suspensions did not contribute to the measured EAC. More details on the electrochemical analyses are provided in the Supplementary material.

Statistics

Curve integration, and linear and non-linear data fitting were performed with Origin (Origin 8.5.OG, OriginLab Corporation, Northampton, USA), statistical tests were conducted with SPSS (SPSS 20, IBM, Armonk, USA).

Calculation of areal TEA consumption and formation rates

The numbers of electrons that can be accepted by sulfate and nitrate in the three sediment layers (i.e., 0-7, 7-14, and 14-21 mm depths) during incubations were calculated based on the measured concentrations in the sediment pore waters and the reduction stoichiometries of these species (i.e., eight and five electrons for the SO_4^{2-}/S^{2-} and NO_3^-/NH_4^+ couples, respectively).[8] The EAC values during hypoxic incubations were used to calculate the number of electrons that were transferable to sediment NOM and Fe and Mn (oxyhydr-)oxides. The cumulated number of electrons that the dissolved and solid-phase TEA species can accept were summed up for each individual sampling point and sediment layer (∑TEAs, in electron equivalents). Linear fits on the temporal changes in ∑TEAs correspond to the electron flux rates to or from all TEAs. The total areal TEA consumption and formation was then determined by weighing the rates by the sediment porosity of the respective sediment depth increment (see Supplementary material for more details).

Calculation of areal hypolimnetic mineralization

The sediment oxygen consumption in Lake Scharmützelsee was estimated using the approach of Livingstone & Imboden.[3] To this end, we first determined the hypolimnetic oxygen consumption rates for different layers of the hypolimnetic water column (10-14 m, 14-18 m,…) based on biweekly O_2 profiles that were collected in 2014 in the water body of Lake Scharmützelsee. Based on the bathymetry of the lake, the ratio of sediment area to water volume (α, in units of $m^2\ m^{-3}$) was determined for each layer. Neglecting diffusive transport of DO, the oxygen consumption rate associated with the sediment was obtained from a linear regression on the oxygen depletion rates for all layers plotted versus their corresponding α values. The intercept and the slope of the regression line correspond to the oxygen consumption rates in the water column and in the sediment (in $gO_2\ m^{-2}\ d^{-1}$), respectively. The slope also corresponds to the sediment

component of AHM as long as oxygen is present in the hypolimnion (i.e., DO > 0 mg L^{-1}). As oxygen was present in most of the hypolimnion only from April to June 2014, we used data that were collected over this time period in the calculations. As mineralization rates are not influenced by seasonal DO fluctuations in lakes,[7, 43] values determined herein also apply for episodes of anoxia.

Results

Dynamics of dissolved, inorganic electron acceptors

Following the hypoxic and oxic sediment pre-incubation phases of the two mesocosms, the DO concentrations in the water bodies above the sediments were perturbed by sparging with air and N_2, respectively. Figure 2a/b show the resulting changes in the DO concentrations in the water bodies above the sediments. These DO concentrations defined the redox boundary conditions for the underlying sediments over the course of the subsequent incubations. In the hypoxic-oxic mesocosm, the DO concentrations increased from approximately 1 mg O_2 L^{-1} during hypoxic pre-incubation to about 8 mg O_2 L^{-1} upon aeration. The oxygen slowly penetrated into the uppermost millimeters of the underlying sediment, as shown by high-resolution DO micro-profiling in the sediment (Figure 3). Within one day of the onset of aeration, the DO pore water concentrations in the uppermost two millimeters of the sediment increased from zero during hypoxic pre-incubation to values well above zero (i.e., between 0.8 and 3.6 mg O_2 L^{-1} in the top 1 mm). Within 20 days of aeration, nonzero DO concentrations were measured approximately 3 mm into the sediment. Switching from hypoxic to oxic conditions also resulted in increases in the concentrations of nitrate and sulfate in the sediment pore water (Figure 2c/e). The concentration increases of nitrate were confined to the uppermost sediment layer (from 0 to 7 mm depth) and were detected only 8 days after the onset of aeration. Sulfate concentrations increased in all depth layers within a few days of the onset of aeration. We note that nitrate concentrations in the top sediment layer increased only after the increase in sulfate concentrations had leveled off. Reduced N and S species were analyzed in all three layers (data can be found in the Supplementary material). The concentrations of ammonium decreased over time during the aeration period and hence showed temporal trends opposite to those of nitrate. Dissolved sulfide concentrations were either small or, in most samples, remained below the limit of quantitation.

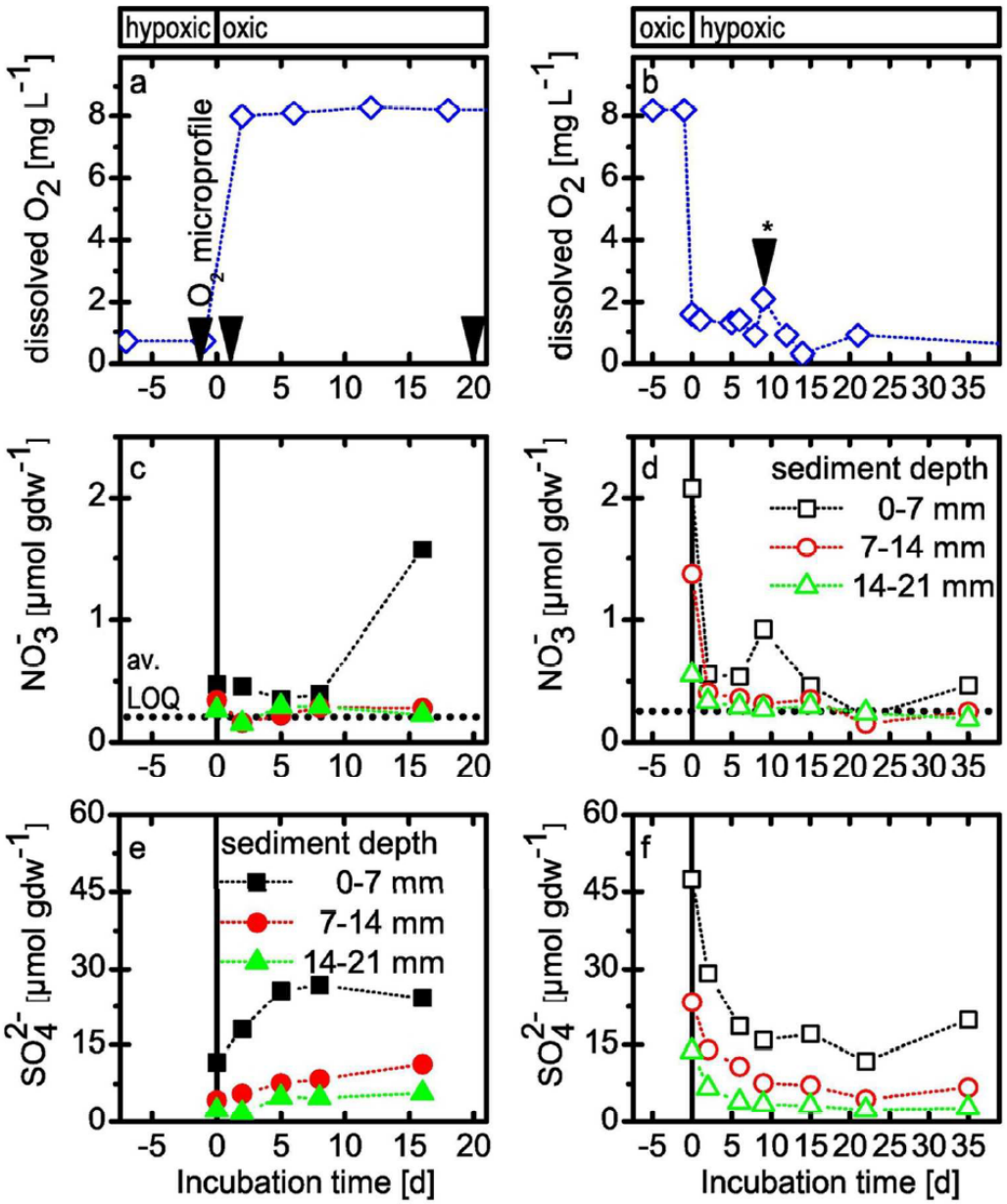

Figure 2 Temporal and spatial dynamics of the inorganic terminal electron acceptors (TEAs) oxygen (panels a and b), nitrate (panels c and d) and sulfate (panels e and f) in two sediment core mesocosms collected from Lake Scharmützelsee. The dissolved oxygen concentrations in the water overlying the sediments were perturbed from low to high (i.e., hypoxic-oxic mesocosm, panels a, c, and e) and from high to low (i.e., oxic-hypoxic mesocosm, panels b, d, and f) to mimic changes in the redox regimes at the sediment-water interface following overturn and following the formation of hypolimnetic anoxia, respectively. The subsequent dynamics in the concentrations of the TEAs were monitored over time. The three sediment depths are represented by different symbols (i.e., squares for 0-7 mm, circles for 7-14 mm and triangles for 14-21 mm below the sediment-water interface). For the hypoxic-oxic mesocosm, vertical profiles of dissolved oxygen concentrations in the uppermost sediment layers were measured one day prior to and one day and 20 days after sparging the water with O_2 (time points are indicated by arrows in panel a). Insufficient N_2 sparging of the water in the oxic-hypoxic mesocosm resulted in a temporary increase in the oxygen concentration at around day nine of the incubation (marked by arrow and asterisk in panel b). The concentrations of nitrate and sulfate are

expressed in μmol of the respective species per gram dry weight (gdw) of the sediment to facilitate comparisons with the dynamics of solid phase TEAs (see supplementary material).

In the oxic-hypoxic mesocosm, sparging of the water with N_2 resulted in a pronounced decrease in the DO concentration above the sediment from approximately 8 to between 0.3 and 1.4 mg O_2 L^{-1} (Figure 2b). Within one day of the onset of N_2 sparging, the concentrations of nitrate (Figure 2d) and sulfate (Figure 2f) had markedly decreased. The concentration decreases were most pronounced in the uppermost sediment layer (i.e., 0-7 mm depth). In this layer, the initial concentrations of nitrate and sulfate were 2-4 fold higher than in the two deeper sediment layers (i.e., from 7-14 and 14-21 mm depths). A short period of insufficient N_2 sparging after day nine of the incubation resulted in a slight increase in the DO concentration in the water overlaying the sediment core from around 1 to 2.1 mg O_2 L^{-1} (Figure 2b). This intermittent introduction of O_2 resulted in a temporary increase in the nitrate concentration in the top sediment layers.

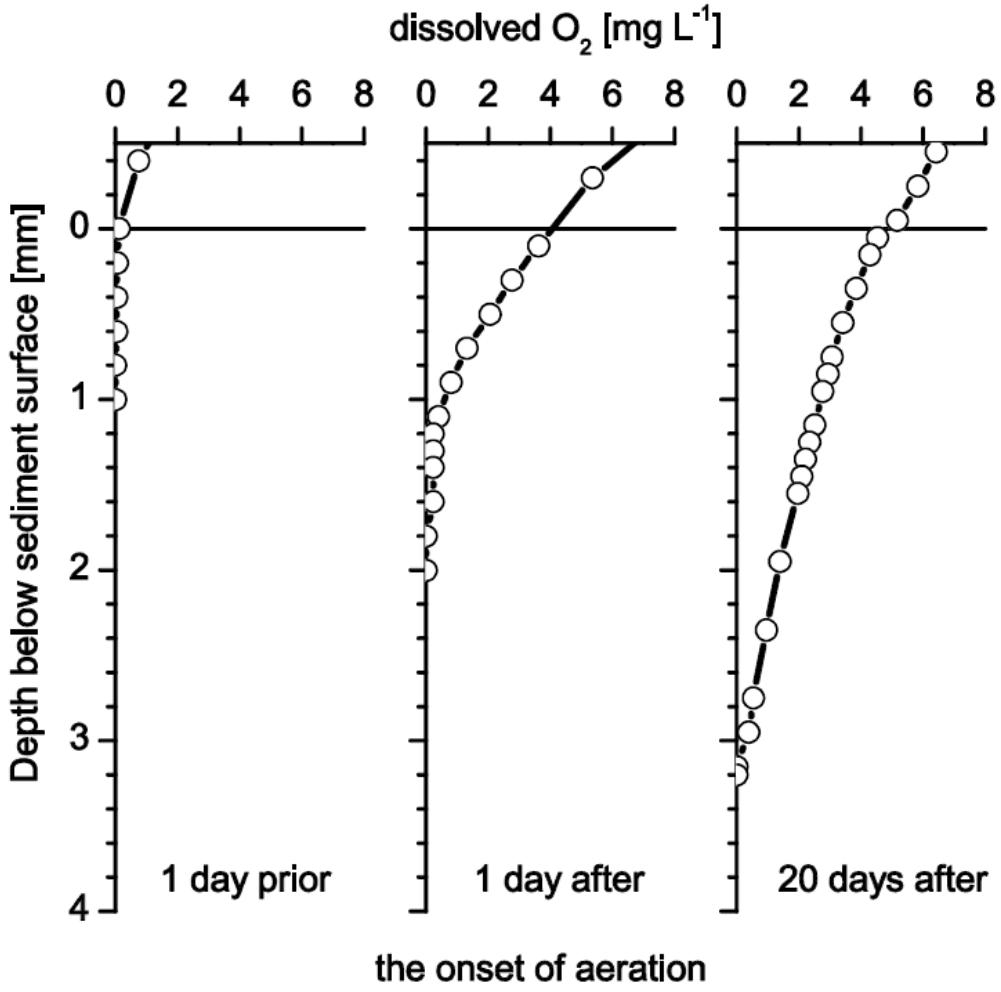

Figure 3 Microprofiles of the dissolved oxygen concentrations in the sediment pore water of the hypoxic-oxic mesocosms measured 1 days prior to and 1 and 20 days following sparging of the water with air.

Dynamics of solid phase inorganic and organic electron acceptors

The electron accepting capacity (EAC) of a sediment sample is an integrative measure for the number of electrons that can be transferred to electron accepting (i.e., reducible) geochemical phases in the sample during MER analysis. In principle, these phases include Fe(III) and Mn(IV)–containing minerals and dissolved and particulate NOM. Similarly, the electron donating capacity (EDC) is a measure of the number of electrons that can be withdrawn from electron donating (i.e., oxidizable) species in the sediment during MEO analysis. These species include Fe(II)-bearing minerals, reduced DOM and POM, as well as dissolved Fe(II) and Mn(II) species.

Figure 4a shows that aeration of the water in the hypoxic-oxic mesocosm resulted in increasing EAC values of the sediment over time. At the same time that EAC values increased, the EDC values decreased over time (Figure 4c). Aeration therefore resulted in increasing concentrations of reducible geochemical phases and decreasing concentrations of reduced species that donate electrons. The most pronounced increase in the EAC values occurred between 5 and 8 days after the onset of aeration in the uppermost sediment layer in direct contact with the aerated water (i.e., 0-7 mm depth) (Figure 4a). The EAC values thus increased after the increase in the sulfate concentrations in the same sediment had leveled and prior to the increase in nitrate concentrations (see Figure 2c,e).

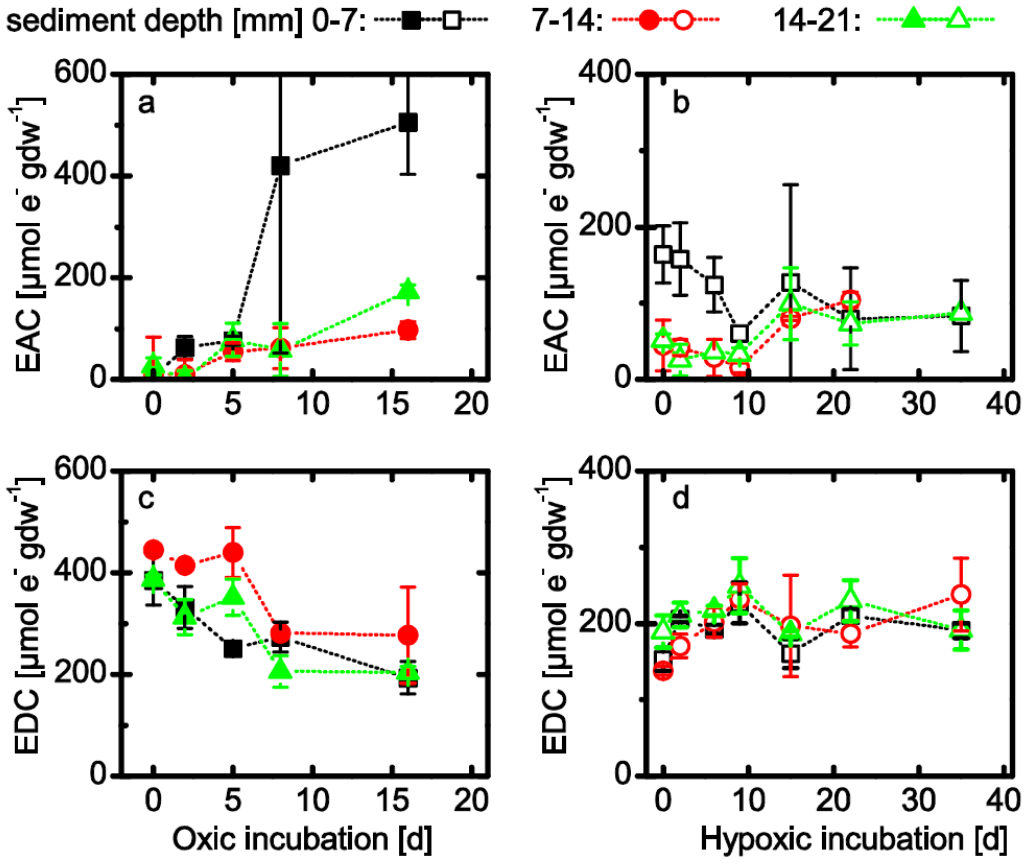

Figure 4 Temporal and spatial dynamics in the total electron accepting and donating capacities, EAC and EDC, of the sediments collected from Lake Scharmützelsee in two mesocosms. After a period of pre-equilibration the dissolved oxygen concentrations in the water overlying the sediments were perturbed (at day t = 0 d) from low to high (i.e., hypoxic-oxic mesocosm, panels a and c) and from high to low (i.e., oxic-hypoxic mesocosm, panels b and d) to mimic changes in the redox regimes at the sediment-water interface following mixis and following the formation of hypolimnetic anoxia, respectively. The error bars indicate standard deviations from at least triplicate measurements (≥ 3). The three sediment depths are represented by different symbols (i.e., squares for 0-7 mm, circles for 7-14 mm, and triangles for 14-21 mm below the sediment surface).

Switching the redox boundary conditions from oxic to hypoxic in the second mesocosm resulted in an initial decrease in the EAC values in all sediment layers (Figure 4b). In the uppermost sediment layer (i.e., 0-7 mm depth) the EAC values decreased from 160 to 60 μmol e^- gdw^{-1} over the course of nine days of hypoxic incubation. The intermittent increase in the DO concentration nine days after the onset of N_2 sparging resulted in an increase in the EAC values of the sediment between 9 and 15 days of hypoxic incubation. Following this event, EAC values decreased again, albeit not to levels as low as measured before the event. Figure 4d shows that EDC values increased during hypoxic incubation. The opposing trends in EDC to EAC values were also observed following the temporary oxidation event after which EDC values decreased and EAC values increased.

For an integrative assessment of the redox dynamics of the sediment in the hypoxic-oxic mesocosm caused by aeration, we re-plotted the EAC and EDC data from Figure 4a,c as changes in their relative contributions to the EEC of the sediment, averaged over all depth layers (i.e., over the entire 21 mm depth of the sediment). The averaged EAC and EDC values were calculated accounting for the decrease in the sediment porosity and hence sediment water contents with increasing depth, as detailed in the Supplementary material. Figure 5a shows that the aeration resulted in a systematic and continuous decrease in the relative contribution of electron donating species and an increase in the contribution of electron accepting species to the total pool of electroactive species.

In an attempt to determine which electroactive species in the sediment gave rise to the EAC and EDC dynamics in the hypoxic-oxic mesocosms, we complemented the MER and MEO analyses of the sediment subsamples by the analysis of acid extractable iron in the samples using 0.5 M HCl. Although the used extraction and quantification procedure does not allow determination of the redox state of Fe (i.e., not delineation of Fe(II) and Fe(III)), the redox stoichiometry of Fe implies that each mole of extracted Fe contributed one mole of electrons to the determined EEC values. We

therefore denoted the concentration of extractable Fe (in μmol Fe gdw^{-1}) as EEC_{Fe} (in μmol e$^-$ gdw^{-1}). Figures 5a and 5b show that the concentrations of HCl-extractable Fe and hence the contributions of Fe to the overall sediment EEC values decreased (from approximately 110 to 70 µmol e$^-$ gdw^{-1}) over 16 days of oxic incubation. A similar approach was taken to assess the relative contributions of Mn species to the measured EEC values. For both mesocosms, the total Mn concentrations in the sediment were determined at the onset of incubations and corresponded to approximately 25 µmol Mn gdw^{-1}. A second set of whole-core incubation experiments with three replicate cores showed that total Mn concentrations in the sediments changed only slightly upon subjecting the sediments to alternating redox conditions, as detailed in the Supplementary material. While the Mn analysis was not redox sensitive (i.e., did not delineate between Mn(IV) and Mn(II) species; similar to the Fe analysis), we assumed that the Mn(IV)/Mn(II) was the predominant Mn redox couple, resulting in a contribution of Mn to the total EEC of approximately EEC_{Mn} = 50 µmol e$^-$ gdw^{-1} throughout the incubations (Figure 5a).

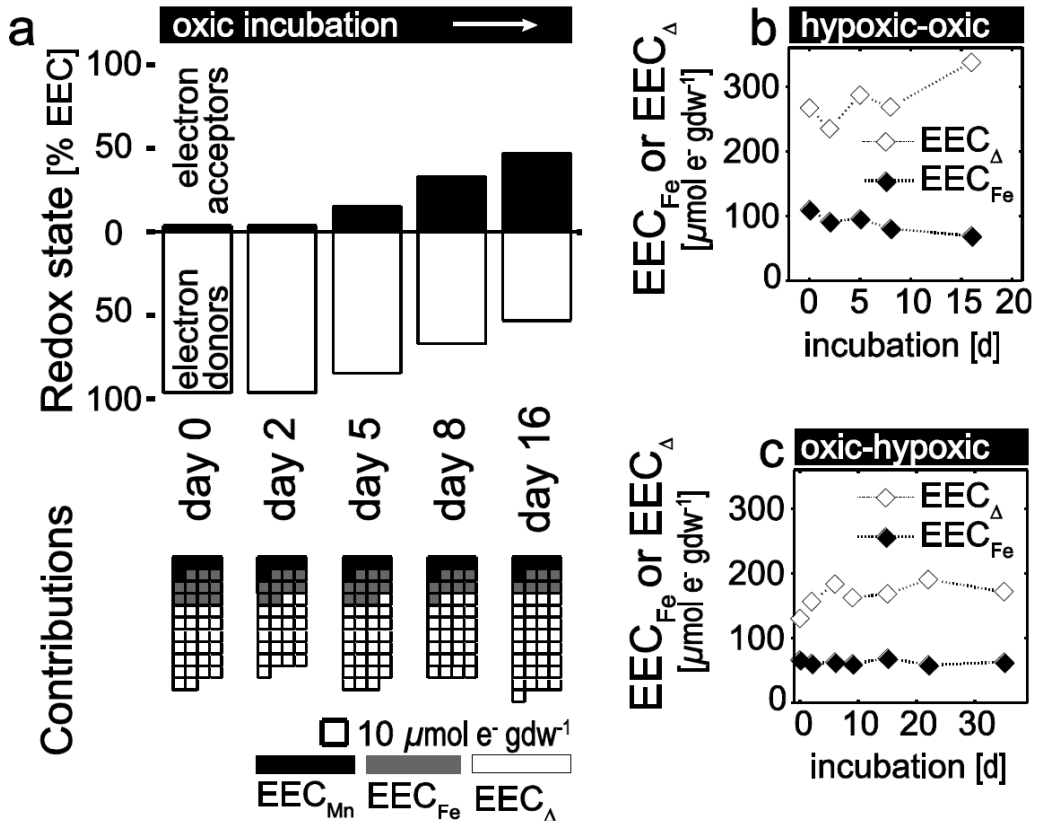

Figure 5 Shift in the redox state of the upper sediment (i.e., averaged over the depth of 0 to 21 mm below the sediment surface) during oxic incubation of the hypoxic-oxic mesocosm. (a) The shift is depicted in terms of percent contribution of electron acceptors (black bars) and electron donors (open bars). The contributions of iron and manganese (Fe and Mn) containing species and phases to the electron exchange capacity (EEC) is broken

down into increments of 10 μmol e^- gdw^{-1}. The contributions to the EEC values from iron (EEC_{Fe}, grey), manganese (EEC_{Mn}, black) and unidentified redox-active sediment constituents (EEC_{Δ}, white) are shown. (b) EEC_{Fe} and EEC_{Δ} values in the hypoxic-oxic mesocosm after switching the incubation conditions to oxic and (c) EEC_{Fe} and EEC_{Δ} values in the oxic-hypoxic mesocosm after switching the incubation conditions to hypoxic.

Figure 5a shows that the total sediment EEC values (depicted by the squares) were much larger than the estimated contributions from acid extractable Fe and the Mn species in the sediment (i.e., $EEC > EEC_{Fe} + EEC_{Mn}$). The relatively large unallocated fraction of the EEC (denoted as EEC_{Δ} in Figure 5) implies that the sediment contained one or more electroactive species that contributed to the EEC besides the acid-extractable Fe and Mn. We note that the EEC_{Δ} values increased from approximately 250 to 340 μmol e^- gdw^{-1} during oxic incubation in the hypoxic-oxic mesocosm (Figure 5b). In contrast, the contribution remained approximately constant over the course of the hypoxic incubation in the oxic-hypoxic core mesocosm (Figure 5c).

Oxidation and reduction rates

To provide an integrative assessment of the dynamics of all TEA species in the sediments (i.e., nitrate and sulfate as well as dissolved and particulate geochemical phases quantified by MER and MEO), we calculated the total number of electrons that had to be exchanged to result in the measured concentration changes of these TEA species. This number is denoted as ∑TEAs in Figure 6 and has units of μmol e^- per g of dry sediment.

During oxic incubation of the hypoxic-oxic mesocosm, the ∑TEAs values increased approximately linearly over time in all three sediment layers (Figure 6a). Conversely, during hypoxic incubation in the oxic-hypoxic mesocosm, ∑TEAs values decreased linearly during the first nine days of incubation (data shown in the Supplementary material). From the changes in the ∑TEAs values in both mesocosms over time, net electron fluxes for the three sediment layers were calculated. These electron fluxes are shown as rates of TEA consumption (oxic-hypoxic mesocom) and formation (hypoxic-oxic mesocosm in Figure 6b). Within the uncertainties of this approach, the TEA formation rates during aeration are equally high as their depletion during hypoxia.

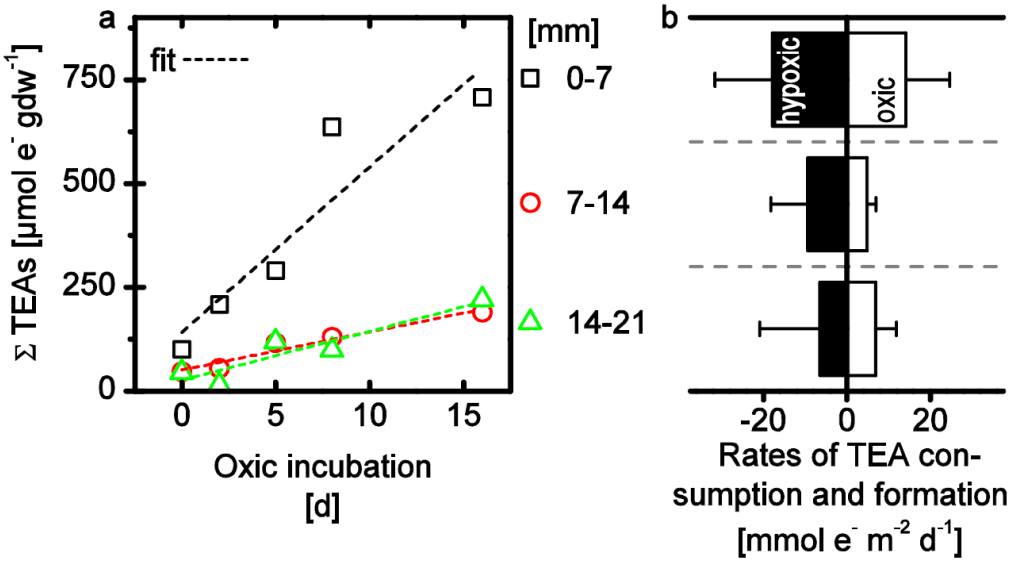

Figure 6 (a) Changes in the concentrations of all TEAs (i.e., ∑TEAs in µmol e^- gdw^{-1}) over the course of oxic incubation in the hypoxic-oxic mesocosm. The ∑TEAs values were calculated from concentrations of nitrate and sulfate and sediment EAC values. The dashed lines correspond to linear fits to the experimental data. (b) Rates of TEA formation during oxic incubation in the hypoxic-oxic mesocosm and TEA consumption during hypoxic incubation in the oxic-hypoxic mesocosm. These rates were estimated from the slopes of linear fits to the changes in ∑TEAs values, followed by normalization to the sediment surface area. Error bars were calculated from the 95% confidence intervals on the slopes of the linear fits (not shown).

Finally, for the hypoxic-oxic mesocosm, we estimated the total electron flux that was required to result in the measured TEA formation in the top 21 mm of the sediment. The total flux was obtained by integrating over all ∑TEAs values determined for the three sediment layers (weighted according to their porosity) and amounts to 26 ± 5 mmol e^- m^{-2} d^{-1}. This value corresponds to an oxygen flux into the sediment, assuming that oxygen was the predominant oxidant for reduced species in the sediment and that it was reduced to water with a stoichiometry of 4 moles of e^- per mole O_2 reduced (Figure 6b). Assuming that the diffusive O_2 flux into the mesocosm sediment does not significantly deviate from in situ conditions, this value can be compared to the total sediment oxygen consumption that was determined from DO profiles measured in Lake Scharmützelsee on a biweekly basis between April and June 2014. The sediment oxygen consumption was approximately 0.41 g O_2 m^{-2} d^{-1}, (i.e., 51 mmol e^- m^{-2} d^{-1}) as determined from the slope of a linear regression of in-lake oxygen depletion rates in horizontal layers of the lake plotted versus the α values of each layer (R^2 = 0.77, see Supplementary material). By addition of the oxygen consumption in the water column (the regression intercept), this value yields the AHM, estimated to be 62.5 mmol e^- m^{-2} d^{-1}.

Discussion

This work aimed at providing an integrative assessment of the spatiotemporal redox dynamics in the sediments of two mesocosms triggered by redox perturbations in the water columns above the sediments. We assessed how oxygen budgets in the hypolimnion are affected by the generation of TEAs from reduced species in the sediment upon introduction of O_2. Each separate step involved in these overall assessments are subsequently addressed.

Consumption and formation of TEA species

Over the course of the oxic incubation in the hypoxic-oxic mesocosm, the dynamics of the major dissolved inorganic TEAs (i.e., NO_3^-, SO_4^{2-}) and of geochemical TEAs (i.e., mineral associated Fe(III) and Mn(IV), and of NOM) exhibited expected trends. Aeration of the water above the sediment resulted in immediate increases in the sediment sulfate concentrations, consistent with the biotic (and abiotic) oxidation of reduced sulfur species with O_2 and the low reduction potential of sulfate/sufide redox couples. Preferential oxidation of reduced sulfur species to sulfate is supported by the finding that nitrate concentrations increased in the topmost sediment layer only after the increase in sulfate concentrations in the same layer had leveled off (Figure 2c/e). The diffusion of O_2 into the sediment and the consumption of O_2 in the sediment due to oxidation reactions is supported by the temporal changes in the high-resolution DO microprofiles in the sediment pore waters (Figure 3). The increasing O_2 penetration depth in the sediment upon continuous aeration of the overlying water is consistent with other studies that reported non-zero O_2 values between 1 to 5 mm depth in lake sediments that are in contact with oxic waters.[9, 44]

The changes in the redox states of geochemical phases in the sediment were determined by quantifying the EAC and EDC values of sediment aliquots over the course of the incubation (Figure 5). Immediately following the aeration of the water in the hypoxic-oxic mesocosm, reduced geochemical phases in the upper sediment layers were oxidized, as evidenced from increases in the EAC and decreases in the EDC values (Figure 4a/c). This oxidation is particularly clear from the continuous increase in the contribution of EAC and the concomitant decrease in the contribution of EDC to the EEC values of the sediment samples (Figure 5a). We note that the largest EAC increase in the topmost sediment layer between 5 and 9 days of oxic incubation (Figure 3a) occurred after the increase in sulfate concentrations had leveled off (after about 5 days of oxic incubation; Figure 2e) and prior to the increase in the nitrate concentrations (between 8 and 16 days; Figure 2c) in the same layer. This finding suggests a sequential oxidation

of reduced sulfur species, followed by reduced geochemical phases, and finally ammonium. Interestingly, this order matches the reduction potential ranges of the redox couples with intermediate reduction potential ranges from -0.4 V to +0.2 V covered by iron-containing minerals and NOM.[27, 45, 46]

Sparging with N_2 in the oxic-hypoxic mesocosm resulted in hypoxic conditions in the water that led to decreasing concentrations of nitrate and sulfate in the sediment (Figure 2d,f). The consumption of these TEA species was expected and is well-documented for anaerobic microbial respiration in both soils and sediments in previous studies.[17, 47] The nitrate concentrations decreased to levels that were close to or below the detection limit of the analytical method used (depicted as dashed line in Figure 2d). The temporary increase in nitrate concentrations in the top sediment layer at around day nine of the hypoxic incubation can be ascribed to the oxidation of reduced nitrogen species (presumably ammonium) by O_2 during the short phase of higher DO concentrations in the water above the sediment (Figure 2b).

Decreasing EAC values in the sediment during hypoxic incubation (Figure 4b) demonstrated that electron accepting geochemical phases were reduced, presumably by anaerobic microbial respiration. A comparable decrease in the EAC was previously reported for sediments after oxidation by drying these materials in air.[30]

The rates of reduction of the dissolved inorganic TEAs and the geochemical phases were initially high but started to decrease after a few days of hypoxic incubation to final, non-zero values. Two explanations for these dynamics are conceivable. First, it is possible that anaerobic respiration may have slowed down significantly as the availability of easily oxidizable electron donors in the mesocosm sediment decreased. We note that the sediments in the mesocosms, in contrast to the natural lake sediments, did not receive any input of precipitated organic detritus as electron donor over the course of the incubation. The missing input of fresh organic matter to the sediment may thus have slowed down respiration activity in the mesocosms as compared to the lake. Second, it is possible that the reduction potential in the sediment had decreased to values that were sufficiently low to slow down anaerobic respiration. In this case, the limitation would have lied in the thermodynamics of the system (i.e., decrease in the free energy obtained by electron transfer from an organic donor to the TEA) rather than in the availability of a labile carbon source. These two possibilities were, however, not further investigated in the present work.

Identification of geochemical TEAs

This work shows that MER and MEO can be used to directly quantify the

redox states of geochemical phases in sediments (Figure 4). In order to detect all relevant redox active sediment constituents, the reduction potentials used in MER and MEO were deliberately set to low and high values to define a potential range that entails the reduction potentials of benthic redox processes (i.e., from O_2 reduction to water at the upper potential end to H^+ reduction to H_2 at the lower limit). Figure 5 shows that between 25 and 37 % of the sediment EEC values could be allocated to Fe and Mn that was acid extractable from the sediments. We deliberately used a mild extraction protocol (0.5 M HCl) to be relatively selective for the pool of readily available Fe, including amorphous iron (oxyhydr-)oxide phases and adsorbed and complexed Fe. We assume that this pool was also accessible as TEA in microbial respiration. It is, however, possible that the mild extraction underestimated the pool of mineral-associated Fe that contributed to the EEC. This possibility is supported by previous studies that have shown that Fe in crystalline (oxyhdyr)-oxides and clay minerals can be reduced without it being extractable by mild acid treatment.[48]

Besides the possibility that the contribution of Fe to the EEC was greatly underestimated, a significant fraction of the EEC was likely associated with redox-active moieties in the organic matter pool of the sediment. Significant contributions of NOM to the EEC have recently been demonstrated for two lake sediments.[30] To critically evaluate the observation of NOM as a previously disregarded TEA species in sediments, we compared this finding to other publications on electron fluxes in benthic systems. In pioneer work, Kelly et al. determined the individual contributions of different respiration pathways in the anoxic decomposition of organic carbon in Canadian lake sediments.[49] This work and several follow-up studies showed that there was a mismatch between the numbers of electrons that were transferred from electron donors to inorganic TEAs and the carbon dioxide (CO_2) that was generated during respiration: between 11 and 14% of the produced CO_2 could not be explained on the basis of the measured reduction of traditional TEAs.[49, 50] The authors suggested that anaerobic respiration (and not fermentation) using particulate NOM as TEA may have contributed to CO_2 formation.[50] This explanation is also supported by numerous studies that showed that mineral and organic geochemical phases are redox-active [17, 30, 51] and that these phases may accept electrons from anaerobic respiration of diverse groups of bacteria and archaea.[21, 52]

In a plausibility assessment, we normalized the EEC_Δ values to the sediment organic carbon content. The resulting EEC values of 1.7 ± 0.2 mmol e^- $(g\ C)^{-1}$ falls into the range of EEC values previously reported by Lau et al. [30] for sediment particulate organic matter (i.e.,

0.65 mmol e^- (g C)$^{-1}$) and by Aeschbacher et al. [32] for dissolved humic substances (i.e., range of 1.9 – 6.9 mmol e^- (g C)$^{-1}$). This assessment therefore supports that NOM contributed to the EEC of the sediment.

The EEC_Δ values of sediment subsamples from the oxic-hypoxic mesocosm changed only slightly during hypoxic incubation, demonstrating that the reduction of electron accepting species resulted in an equimolar formation of electron donating species. Equimolar conversions of electron accepting to donating species are expected both for the Fe(III)/Fe(II) couple as well as for the reduction of quinone moieties in NOM to the respective hydroquinones.[16, 27, 30] In contrast to the oxic-hypoxic mesocosm, EEC_Δ values increased during oxic incubation in the hypoxic-oxic mesocosm (Figure 6c). One possible explanation is that the increase originated from the measured decrease in EEC_{Fe}. While remaining electro-active, Fe may have transitioned from acid-extractable amorphous Fe phases into more crystalline, poorly extractable Fe phases (Figure 6b).

Electron fluxes at the sediment-water interface

We directly quantified the spatiotemporal dynamics of all TEAs, including inorganic dissolved species and electron accepting geochemical phases, in the upper 21 mm of the sediment when changing the incubation conditions in the mesocosms from hypoxic to oxic or vice versa (Figure 7). The determined electron flux from this sediment layer to O_2 during oxic incubation was 26 ± 5 mmol e^- m^{-2} d^{-1}. Since the deepest layer (14 to 21 mm depth) contributed <20% to that areal flux, we suggest that the inclusion of even deeper layers would only marginally change this value. This value can be compared to the AHM value of Lake Scharmützelsee that were converted from oxygen to electron fluxes. AHM associated with the sediment generally reflects the consumption of O_2 by both aerobic mineralization of labile organic carbon and the re-oxidation of reduced species in the sediment (F_{red}). These reduced species are formed by anaerobic respiration during preceding anoxic episodes and, in addition to the reduced inorganic dissolved species, include reduced geochemical phases in the sediment. In general, the reduced species readily re-oxidize on both abioic and biotic pathways upon the introduction of O_2 to the sediment.[21, 27]

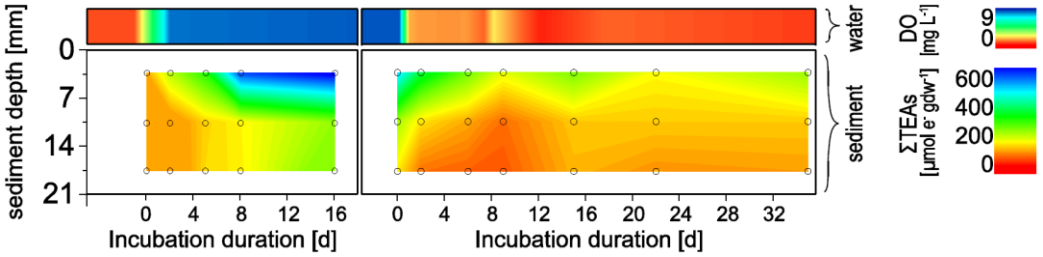

Figure 7 Spatiotemporal distribution of terminal electron acceptors (TEAs) in sediment of Lake Scharmützelsee (lower bars) as a result of changes in the dissolved oxygen (DO) regime in the water column (upper bars). The data on the left and right are modeled based on the redox dynamics determined in the hypoxic-oxic and the oxic-hypoxic mesocosms, respectively. The colors indicate the concentrations of oxidized species (red: low, blue: high). Circles indicate spatial and temporal measurement resolution.

Using the model by Livingstone & Imboden [3] we calculated an AHM of 62.5 mmol e^- m^{-2} d^{-1} (sediment: 51) from DO data in Lake Scharmützelsee measured in 2014. This value is in fairly good agreement with an estimated AHM values of 73 mmol e^- m^{-2} d^{-1} (i.e., 0.58 g O_2 m^{-2} d^{-1}) for Lake Scharmützelsee (average hypolimnion thickness z_H = 5.5 m) based on the model for eutrophic lakes proposed by Müller et al..[11] An even higher AHM value of 128 mmol e^- m^{-2} d^{-1} reported by Nixdorf et al. [53] for the same lake reflected its highly eutrophic state in that year (1994).

In the model by Müller et al., the F_{red} fraction of the AHM is estimated to be about 45 mmol e^- m^{-2} d^{-1} based on data from different eutrophic lakes.[11] In general, the oxidation of methane is considered to have the largest contributions to F_{red}. Reported contributions of methane oxidation range from between 57 to 76% in two Swiss lakes,[7] to 70% in Lake St. George,[54] to 50-80% in six eutrophic lakes,[11] and to >95% in Lake Zug.[10] Similar values were reported by Kreling et al. for Lake Scharmützelsee: based on eddy flux measurements in the thermocline, the authors estimated that methane oxidation accounted for 52 % of the oxygen that was consumed in the oxidation of all reduced species that were transported across the anoxic-oxic interface.[55]

In all of these studies, F_{red} was determined based on the oxygen-consuming fluxes of reduced species (i.e., NH_4^+, S(-II), Fe(II), Mn(II) and CH_4) derived from their pore water concentrations and one-dimensional modeling of formation, consumption and molecular diffusion. More sophisticated reactive transport modeling revealed that 30-80% of oxygen flux through the sediment water interface in marine environments can be attributed to the re-oxidation of these species.[12, 13, 56] The finding in this work that a large fraction of the AHM can be explained by the formation of sulfate, nitrate and EAC upon exposure of the sediment to O_2 seemingly

contradicts with previous studies that ascribed most of the AHM to the oxidation of methane in the sediment. These seemingly contrasting results may, however, be rationalized if the oxidation of methane occurred anaerobically instead of aerobically: electrons from the oxidation of methane would then be transferred to at least one (and possibly more) species that we monitored (i.e., nitrate, sulfate and electron accepting geochemical phases). The formed reduced species would subsequently react with O_2 at the sediment-water-interface. In this case, the reducing equivalents from methane and their contributions to overall O_2 consumption could either be determined based on methane concentration profiles (as done in previous work) or from the formation rate of nitrate, sulfate and EAC. In the latter case, the overall measured formation rate would be the net rate resulting from the simultaneous reduction of nitrate, sulfate and electron accepting geochemical phases (i.e., EAC) and the re-oxidation of the formed reduced species by O_2.

The notion that methane oxidation may have occurred abiotically is substantiated by recent studies. Although anaerobic methane oxidation with nitrate has been shown,[57] it is presently debated whether methane oxidation may also be coupled to the reduction of sulfate, Fe(III)-containing phases or NOM.[58, 59] Future studies are warranted to investigate this possibility by simultaneously monitoring all redox active species involved (i.e., CH_4, nitrate, sulfate, EAC and O_2).

In conclusion, the combination of electrochemical approaches with traditional methods to analyze redox dynamics of dissolved inorganic species, allows for the quantification of the electron fluxes from electron donors to dissolved and solid-phase TEAs in lake sediments upon changes in the redox conditions in the water above the sediment. Such measurements provide a direct measure of the benthic oxygen consumption that results from introducing oxygen to freshwater sediments following preceding anoxic episodes with anaerobic microbial respiration. In the case of recently deposited freshwater sediment (top 21 mm) from Lake Scharmützelsee studied herein, the amount of oxygen required to regenerate dissolved and solid-phase TEAs when switching from hypoxic to oxic conditions corresponded to approximately 50% of the modeled oxygen consumption within the sediment after the onset of stratification. Besides substantiating the view that the high microbial activity at the sediment-water interfaces controls the redox conditions in the adjacent water column, this work highlights the importance of considering redox-active geochemical phases as TEAs in freshwater sediments when balancing electron flows in benthic respirational systems.[49, 60] We described the benthic-pelagic coupling of redox processes

by accounting for changes in the redox states of all currently recognized (organic and inorganic) TEA species in aquatic environments. In future studies, the herein presented analytical method offers the possibility to constrain parameters in theoretical models that simulate the complex redox dynamics at oxic-anoxic interfaces in sediments as well as other systems.

Acknowledgements

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Supplementary material

In this appendix, additional information on methods, mathematical operations and data is presented.

Mediated electrochemical reduction and mediated electrochemical oxidation

The setup for MER and MEO was adapted from Aeschbacher et al..[1] In brief, measurements were conducted in electrochemical cells with pH buffered solutions (pH 7.00 ± 0.05) containing 0.1 M $NaClO_4$ as background electrolyte and 0.01 M 4-Morpholinepropanesulfonic acid as the buffering species. We used glassy carbon cylinders (Volume 9 mL; Sigradur G, HTW Carbon, Germany) that served as both the cell reaction vessels and working electrode (WE). The WEs were polarized to reduction potentials of E_h = -0.49 V for MER or +0.61 V for MEO (reported versus the standard hydrogen electrode, but experimentally measured versus Ag/AgCl reference electrodes). Each electrochemical analysis was initiated by the addition of the dissolved electron transfer mediators 6,7-Dihydrodipyrido [1,2-a:2',1'-c]pyraziniumdibromid monohydrate (99.5 %; $E_h°$ = -0.36 V; Supelco, USA) (Diquat, DQ) to MER cells and 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) ammonium salt (>98 %; $E_h°$ = +0.7 V; Sigma-Aldrich, MO, USA) (ABTS) to MEO cells to final concentrations of 250 - 350 µM.

Both ABTS and DQ are single-electron transfer mediators: DQ was reduced to the radical species $DQ^{\bullet+}$ in MER and ABTS was oxidized to the $ABTS^{\bullet+}$ radical in MEO. The resulting current responses were peak-shaped with initial high currents followed by a decrease in the currents that ultimately leveled off as the mediators approached E_h equilibria with the E_h applied to the WEs. We subsequently pipette-transferred small volumetric aliquots of 50 - 200 µl from vigorously stirred sediment suspensions to the MER and MEO cells. In MER, the dissolved $DQ^{\bullet+}$ transferred electrons to electron accepting species in the added sample, resulting in the formation of DQ^{2+} molecules. The formed DQ^{2+} were subsequently re-reduced to $DQ^{\bullet+}$ at the WE to re-establish E_h equilibrium in the MER cell. In MEO, $ABTS^{\bullet+}$ radicals were reduced by electron donating species in the added sample, resulting in the formation of ABTS, which was then re-oxidized to $ABTS^{\bullet+}$ at the WE. The addition of redox-active samples to MER and MEO thus resulted in reductive and oxidative current peaks, respectively. These peaks were baseline-corrected and integrated to obtain the numbers of electrons, n_e (mmol e^-) transferred to and from the added sample according to:

$$n_e = \int \frac{I}{F} dt$$

where I (A) is the reductive or oxidative current and F (C mol^{-1}) is the Faraday constant. The method detects particulate species that are electroactive in MER/MEO. These species do not include nitrate and sulfate, which we quantified separately.

Sediment porosity

In this work, the concentrations of dissolved and particulate terminal electron acceptors (TEAs) in the sediment suspensions are referenced to the dry mass of the respective sediment sample. We chose this approach to equally account for contributions of dissolved and particulate TEAs to the total TEA pool. The concentrations could have alternatively been referenced to the concentration of the conservative tracer chloride, which showed no variations over time. We verified that the conclusions drawn in the manuscript were unaffected when using chloride concentrations instead of dry sediment masses as a reference.

The sediment porosity (volumetric water content) generally decreases with increasing sediment depths (Fig. S1a). These changes with depth were accounted for when calculating concentration values that integrated over the three separate sediment layers (i.e., 0-7 mm, 7-14 mm and 14-21 mm) by weighing the averages on the basis of the dry mass of each of the three layers. Also, porosity was considered when calculating area-dependend fluxes (as e.g. in Figure 5).

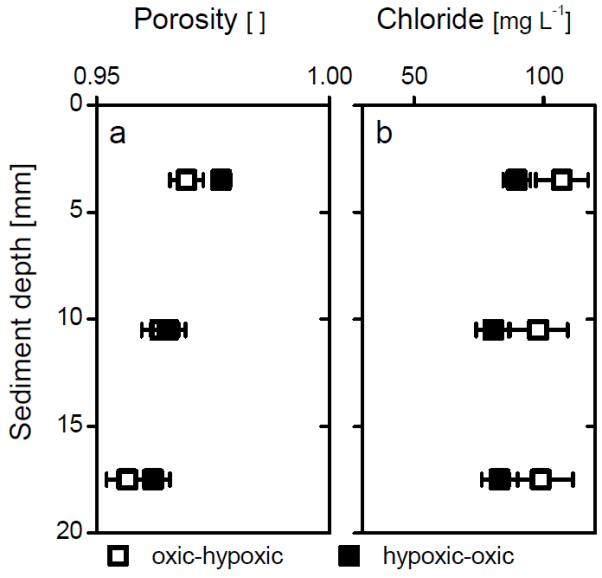

Figure S1 Sediment porosity (left) and dissolved chloride concentrations in the sediment porewater (right) for each of the three sediment layers analyzed (i.e., 0-7 mm, 7-14 mm, and 14-21 mm). Values are averages from all samples analyzed over the course of the incubation

periods (n=7 for the oxic-hypoxic mesocosm and n=5 for the hypoxic-oxic mesocosm experiments). Bars represent standard deviations.

The porosity ϕ was calculated from

$$\phi = \frac{\frac{water\ content\ (mass\ fraction\ wet\ sediment)}{water\ density\ (4\ °C)}}{\frac{water\ content}{water\ density} + \frac{1 - water\ content}{dry\ mass\ desity}}$$

where the density of the dry mass was assessed with regard to its content of organic (specific density ϱ = 1.4 g cm^{-3}) and mineral (ϱ = 2.6 g cm^{-3}) constituents.

Reduced TEA species

In this work, the spatiotemporal dynamics of the reduction of nitrate and sulfate were monitored as changes in the concentrations of both the oxidized TEA species and of the reduction end-products ammonium and sulfide. The measured sulfide concentrations are presented in Figure S2. Sulfide concentrations were below the limit of quantitation (LOQ, 30 µg L^{-1}) for most samples. The concentrations of dissolved ammonium in the sediment pore water of the hypoxic-oxic mesocosm are provided in Figure S3.

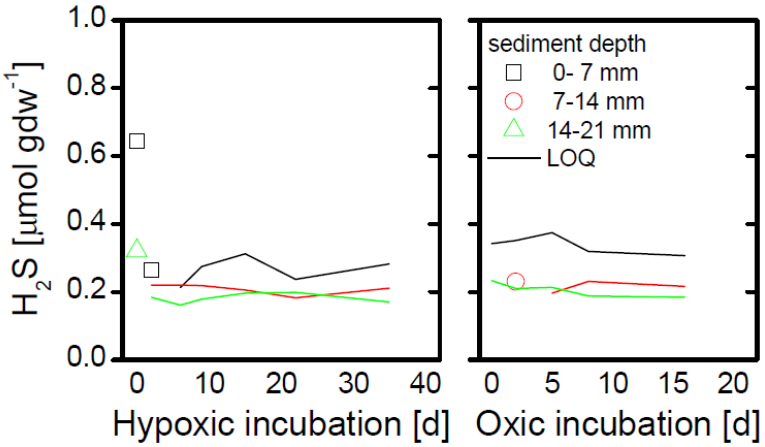

Figure S2 Hydrogen sulfide (H_2S) concentrations in the wet sediment samples. The oxygen regimes in the water overlying the sediments were changed on day 0 from oxic to hypoxic (left panel) and from hypoxic to oxic (right panel), respectively. Lines indicate the limit of quantitation (LOQ) for the different sediment depths. The symbols represent measurement data where values were above LOQ

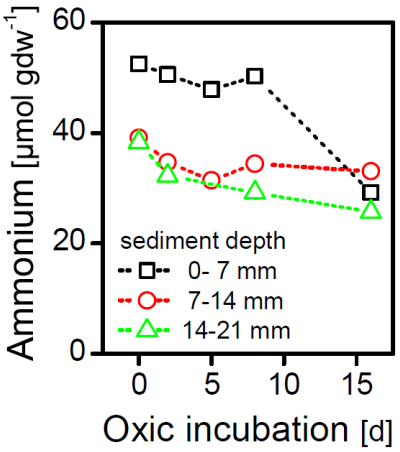

Figure S3 Ammonium concentrations in the wet sediment samples. The oxygen regime in the water overlying the sediment was changed from hypoxic to oxic on day zero.

Manganese

The total content of manganese (Mn) in the sediment samples was quantified without determination of the oxidation state of the Mn (i.e., Mn(II) or Mn(IV)). The Mn was quantified by inductively coupled plasma atomic emission spectrometry after dissolving the sediment samples in aqua regia. The Mn concentrations in both mesocosms were determined only at the onsets of the incubations. However, in a separate control experiment with six sediment cores from Lake Scharmützelsee that were subjected to similar incubation cycles, no significant changes in Mn concentrations in the uppermost 21 mm of sediment were detected (Fig. S4). Therefore, we assumed that manganese had a constant contribution of EEC_{Mn} of 46 µmol e^- gdw^{-1} to the EEC_{tot}. This value could overestimate the contribution of Mn to the EEC_{tot} if a significant amount of the sediment-associated, aqua-regia extractable Mn is not accessible in electron transfer reactions at milder conditions (as e.g. in situ or during the electrochemical analysis).

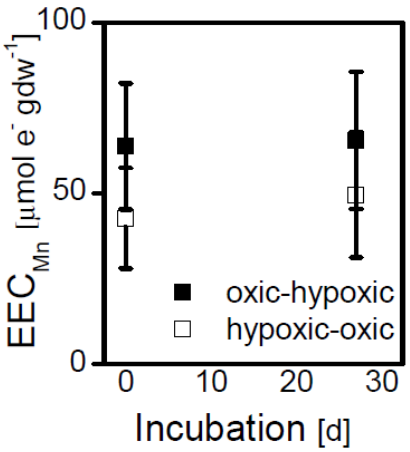

Figure S4 Manganese (Mn) content in the lake sediments determined after digestion of the sediment with aqua regia. The contents are expressed as electron exchange capacity EEC_{Mn},

based on the assumption that each Mn contributed two electrons to the EEC (i.e., assuming that the redox couple Mn(IV)/Mn(II) predominated). The oxygen regime in the water overlying the sediment was altered on day zero from oxic to hypoxic (filled symbols) and from hypoxic to oxic (open symbols). Error bars represent standard deviations between triplicate cores.

TEA consumption during hypoxic incubation

The dynamics of all oxidized TEA species was determined in the oxic-hypoxic mesocosm (Fig. S5) similar to the dynamics of the same species in the hypoxic-oxic mesocosm (Fig. 5a in the manuscript). The resulting reduction rates in the three sediment layers are provided in the results section of the manuscript (Fig. 5b).

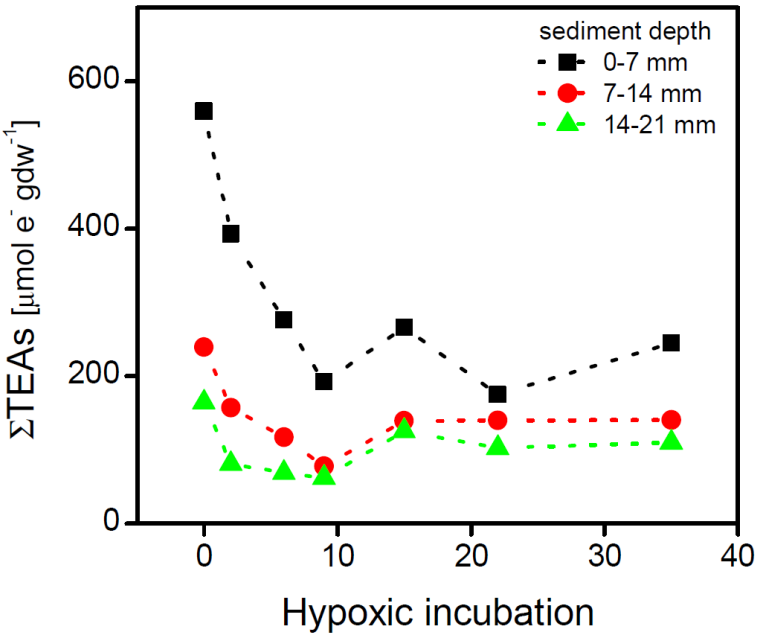

Figure S5 Dynamics of the dissolved and particulate TEAs determined from changes in the concentration of NO_3^- and SO_4^{2-} and the changes in the electron accepting capacity of the sediments (EAC), respectively. The incubation conditions were changed from oxic to hypoxic on day zero. Values are expressed in terms of transferred electron (e^-) equivalents.

Calculation of the areal hypolimnetic mineralization (AHM) from oxygen profiles

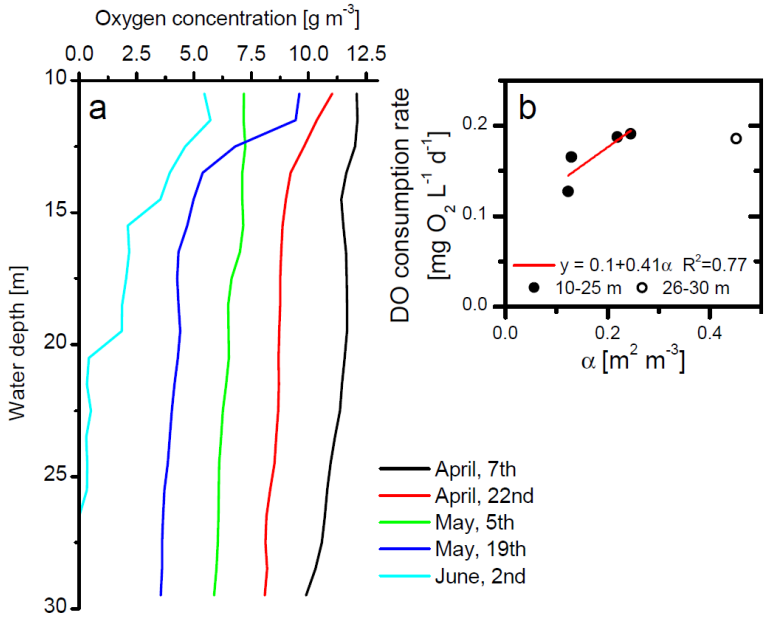

Figure S6 Dissolved oxygen (DO) concentrations in the hypolimnion of Lake Scharmützelsee as a function of depth, measured at different dates (represented by lines of different colors) b. DO consumption rate as a function of the ratio of sediment area to water volume, α (circles, calculated for hypolimnion cross-sections of 4 m depth, see methods section). The line is the result of a linear regression fit on the data. The DO consumption rate at α=0.45 was omitted from the fit: this value corresponds to the hypolimnion cross-section at the depth of 26-30 m (empty circle) and no oxygen was detected in this layer at the end of the measurement period.

In the deepest layer of the hypolimnion (26-30 m), we measured a lower oxygen consumption than what was expected based on its α (i.e., the sediment area to water volume ratio). These lower-than-average values remain, even when only sampling points were considered when DO was still >0 mg L^{-1}. Hence, we assume that no disproportionate share of fine sediments moved to deeper parts of the lake ('sediment focussing') where this sediment fraction could have additionally accelerated oxygen consumption.

Supplementary References

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3.3 Reduction-Oxidation cycles of organic matter increase bacterial activity in the pelagic oxycline

The third and final article of this work deals with the question whether faster – diurnal – fluctuations of redox conditions trigger NOM redox-dynamics and hence the bacterial community structure at redox interfaces. In the studied lake, cyclic de-stratification as a result of night-time cooling gives rise to spatially confined environments with rapidly changing oxic/anoxic conditions. In a unique approach, isolation of the lakes DOM and the subsequent electrochemical adjustment of its redox state allowed for a systematic assessment of the bacterial community response to changes in organic electron accepting capacity. While other DOM properties (chain length distribution and aromaticity) remained unaffected by the treatment, results showed that amendment with DOM in higher oxidation state resulted in higher bacterial abundance and biomass production. The results suggest that oxidized DOM may release the microbial community from TEA limitation in the system. Based on that, the community structure was shown to be significantly altered when oxidized DOM as TEA was provided. Ultimately, changes in DOM redox state may happen in such short cycles that their relevance may have been overlooked in the bigger pictures of geochemical and microbial turnover reaction.

This following section was submitted as manuscript and is currently under review.

Lau MP, Hupfer M, Grossart HP;
Reduction-Oxidation cycles of organic matter increase bacterial activity in the pelagic oxycline
(**in review**)

Abstract

Dissolved organic matter (DOM) in aquatic ecosystems contains redox-active moieties, which are prone to oxidation and reduction reactions. Oxidized moieties feature reduction potentials E_h, so that the moieties may be used as terminal electron acceptor (TEA) in microbial respiration with a thermodynamic energy yield between nitrate and sulfate reduction. Here, we study the response of pelagic freshwater bacteria to exposure to native DOM with varying availabilities of oxidized moieties and hence redox state. Our results show that the prevalence of oxidized DOM favors microbial production and growth in anoxic waters. Reduced DOM in stratified lakes is oxidized when redox cline fluctuations expose previously anoxic water to epilimnetic oxygen. The resulting oxidized DOM may be used as TEA in microbial respiration during subsequent periods of anoxia. Our results reveal that the DOM traversing transient redox interfaces controls the pelagic microbial communities by sorting for species that adapt well to such spatially confined and cyclically restored TEA reservoirs.

Introduction

Humic substances in aquatic systems comprise the largest proportion (50 to 80%) of DOM and consist of complex organic molecules, including polyphenolic, quinoid and semiquinoid units (Aeschbacher et al, 2010). The quinoid units can serve as terminal acceptors of electrons that are freed during microbial respiration (Lovley et al, 1996; Kluepfel et al, 2014). This (extracellular) electron transport was first shown to be important for iron-reducing bacteria, where the DOM facilitates electron flow to the solid mineral phases (Lovley et al., 1996). However, the DOM can also be reduced by other microbial groups, such as sulfate reducers and methanogens (Cervantes et al, 2002). Mounting evidence substantiates the relevance of DOM as TEA species in microbial respiration in aquatic ecosystems. DOM isolated from anaerobic sediments was found to induce the abiotic reduction of chelated iron (Kappler et al, 2004). Semiquinones in the microbially reduced DOM were identified using electron spin spectroscopy (Roden et al, 2010). Later, the direct tracking of microbial organic matter (OM) reduction was realized using electrochemical quantification of electron-accepting and electron-donating capacities (EAC and EDC) (Aeschbacher et al., 2010; Lau et al, 2015). The OM reduction helped to rationalize imbalanced emissions of CO_2 and CH_4 from OM-rich wetlands because inorganic TEAs (including CO_2 in hydrogenothrophic methanogenesis) were not sufficient to accept all electrons released in the respiratory oxidation of labile organic matter to CO_2 (Keller et al, 2009). Hence, the imbalanced emission pattern was assumed to originate from the single (or cyclic) reduction of electron-accepting OM moieties (Keller et al., 2009; Keller and Takagi, 2013; Miller et al, 2015). This assumption was recently substantiated by a seminal paper (Kluepfel et al., 2014) reporting that the EAC of these moieties is depleted during the respiration of *Shewanella* lab cultures but fully and rapidly restored when the moieties are oxidized with O_2. Therefore, we hypothesize that systems providing both cyclic changes in oxygen availability and DOM as TEA are favorable, but unrecognized environments for DOM-reducing bacteria.

In the acidic peat bog Lake Grosse Fuchskuhle (Fuku), DOM is exposed to diurnal fluctuations in the redox environments because of a transient redox cline (Figure 1). There, we studied two fundamental aspects of microbial reduction of aquatic DOM. Using a electrochemical procedure to reduce and re-oxidize natural DOM, which was isolated by reverse osmosis in its ambient oxidation state, we generated DOM samples in different redox states in a controlled manner. We then exposed the ambient microbial assemblages to the resulting range of DOM redox states and tracked the changes in microbial abundance and activity. Finally, we assessed

whether the composition of the ambient bacterial consortia was affected by the exposure to DOM of different oxidation states.

Methods

Study site and sampling

Lake Grosse Fuchskuhle (Fuku) and Lake Barschsee are two adjacent peat bog lakes in a forested, lowland area of NE Germany (53°06' N; 12°59' E). Lake Fuku has been divided with plastic curtains into four compartments for >20 years; only the most dystrophic compartment (SW) was sampled (Burkert et al, 2005). The acidic SW basin (average pH of 4.7) is strongly affected by the input of DOM from the adjacent bog area (Hutalle-Schmelzer et al, 2010). Lake Barschsee is equally dystrophic. Water samples for incubation were obtained from the surface of Lake Barschsee because of ice coverage of Lake Fuku. DOM was extracted from water of Lake Fuku with a mobile reverse osmosis unit (Sachse et al, 2001). Concentrated lake water (ca. 1 g C L^{-1}) was freeze-dried to obtain DOM in solid state.

Electrochemical analyses

All electrochemical analyses were performed inside an anoxic glovebox (O_2 < 0.5 ppm). Mediated electrochemical reduction (MER) and oxidation (MEO) of the DOM samples were conducted to analyze the DOM redox state (Aeschbacher et al., 2010; Kluepfel et al., 2014; Lau et al., 2015). The electrochemical cell consisted of a glassy carbon cylinder, which acted as both the cell vessel and working electrode, an Ag/AgCl reference electrode and a platinum wire auxiliary. The electrochemical cell was equilibrated to the desired potentials (i.e., E_h=-0.49 V in MER and E_h=+0.61 V in MEO, which were below and above the reported potential range for quinones, respectively). The redox mediators were spiked, which resulted in reductive and oxidative currents, respectively. After re-attainment of constant background currents, small amounts (< 0.5 mg) of the samples were spiked to the cells, and the amount of electrons transferred was measured using chronocoulometry.

DOM reduction and oxidation

DOM was electrochemically reduced in a 60 mL bulk electrolysis cell (Aeschbacher et al., 2010; Aeschbacher et al, 2011). The same electrochemical equipment as described above was used. This direct electrochemical reduction was performed at E_h= -0.59 V (vs. SHE). The amount of electrons transferred was obtained by chronocoulometry (i.e., integration of the reductive current over time, see Supplementary Figure S4). The solutions

contained 0.1 M phosphate buffer (pH 7).

The electrochemically reduced DOM samples were oxidized with diluted hydrogen peroxide (H_2O_2) solutions in an oxygen-free atmosphere. Aside from the oxidation with O_2, oxidation states that feature a high EAC may be formed via the enzymatic oxidation of aromatic moieties or the reaction of aromatic and phenolic structures with hydroxyl radicals (Uchimiya and Stone, 2009; Kluepfel et al., 2014). The radicals can be generated during DOM photolysis or via enzymatic Fenton-chemistry (Aeschbacher et al., 2011; King et al, 1995).

The H_2O_2 solution was capped with butyl stoppers and sparged with N_2 for 2 h before being transferred to an anoxic glove box. There, small amounts of the H_2O_2 solution were added to the reduced DOM samples. The samples were capped and stored in the dark until they were used in the incubation experiment.

Incubation

The water samples were cooled (4°C) after sampling. The incubation solution was prepared from sterilized lake water (filtered through 0.2 µL). Small amounts of NH_4Cl were added to avoid N limitation (1.4 mg N L^{-1}). Oxygen was removed from the water by sparging with N_2 for 2 h in sealed glass bottles which were then placed in an anoxic glove box. The transfer to incubation vials and mixing with anoxic DOM solution (final concentration 81 mg DOC L^{-1}) in different oxidation states was also performed in the anoxic glove box. Then, the Lake Barschsee inoculum (unfiltered) was added (final volume of 20 mL). The vials were sealed with washed and sterilized butyl rubber stoppers and incubated at 22°C in the dark outside the glove box.

^{14}C Leucine uptake and bacterial cell abundance

The rates of bacterial carbon production were determined using ^{14}[C]-leucine incorporation (Simon and Azam, 1989). The anoxic ^{14}C-radiolabeled leucine solution (1.15×10^{10} Bq mmol, Amersham, Amersham, England) was added to a 20 mL incubation sample at a final concentration of 50 nmol L^{-1}, which ensured the saturation of the uptake systems. The samples were further incubated for 1 h at ambient conditions in the dark in quadruplicates plus four prefixed (2% formalin) controls. Experimental details can be found in the Supplementary Information.

The total bacterial numbers were determined by epifluorescence microscopy (DR-MB, Leica, Wetzlar, Germany) at 1,000× magnification (Allgaier et al, 2008). The triplicate subsamples were fixed with neutralized formaldehyde (pH 7.4) at a final concentration of 4%. A 1 mL water sample was filtered

onto a black 0.2 μm pore size Nucleopore membrane. The filter was stained with SYBR Gold (Invitrogen, Carlsbad, Ca, USA) and stored frozen at –20°C until counting.

DNA extraction and PCR amplification of 16S rRNA gene fragments

First, we filtered 50 mL water samples onto 0.2 μm polycarbonate filters (Nuclepore, Whatman, Maidstone, UK). The filters were transferred into sterile Eppendorf tubes and maintained frozen at -20°C until further processing. The genomic DNA and rRNA were extracted as previously described (Allgaier and Grossart, 2006).

For the denaturing gradient gel electrophoresis (DGGE) a 550 bp fragment of the 16S rRNA gene was amplified using the primer pair 341f and 907r (5' – CCT ACG GGA GGC AGC AG – 3' and 5' – CCG TCA ATT CMT TTG AGT TT – 3') (Muyzer and Smalla, 1998). At the 5' -end of the primer 341f, an additional 40 bp GC-rich nucleotide sequence (GCclamp) was added to stabilize the migration of the DNA fragment in the DGGE (Muyzer et al, 1993). The PCR reaction mixture contained 2 to 5 μL of template DNA, each primer at a concentration of 200 nM, each deoxyribonucleoside triphosphate at a concentration of 250 μM, 2 mM MgCl, 10× PCR reaction buffer, and 0.5 U of BIOTAQ Red DNA Polymerase (Bioline, Luckenwalde, Germany) in a total volume of 50 μL. PCR amplification was performed with a Gradient Cycler PT-200 (MJ Research, St. Bruno, Canada) using the conditions provided by Allgaier et al. (2008).

Bacterial communities in incubations with DOM in different oxidation state were analyzed on different DGGE gels. Two replicates and negative controls with no substrate additions were analyzed. We used a 7% v/v polyacrylamide gel with a denaturing gradient of urea and formamide of 40% to 70%. The amplified DNA was quantified on agarose gels using the Low DNA Mass Ladder (Invitrogen, Carlsbad, Ca, USA). Then, the banding patterns were compared.

Sequencing of DGGE bands

For the sequencing, several DGGE bands were excised and incubated overnight at 35°C in an elution buffer (0.5 M ammonia acetate, 1 mM EDTA). The eluted DNA fragments were precipitated (purified NH_4COOH-isopropanol solution). The DNA fragments were sequenced as previously described using primers 341f and 907r (Allgaier and Grossart, 2006). Phylogenetic analyses of the partial 16S rRNA gene sequences were performed as described in the Supplementary Information.

Results & Discussion

Transient redox clines act as a DOM redox cycler

The studied Lake Fuku undergoes regular cycles of formation and dissolution of a thermocline (Figure 1a). As observed from the high-resolution oxygen profiles in the lake, a transient thermocline induces the convective mixing of oxygen-depleted hypolimnetic water with oxygen-rich epilimnetic water as a result of diurnal night time cooling (Figure 1b). During the day, (semi-)stable stratified conditions re-establish, and the respiration activity consumes O_2 which was introduced into deeper waters. However, in the epilimnion, phototrophic activity and diffusion from the atmosphere replenish the oxygen consumed by microbial respiration. At and below the redox cline, microbial respiration must proceed via anaerobic pathways when oxygen is absent.

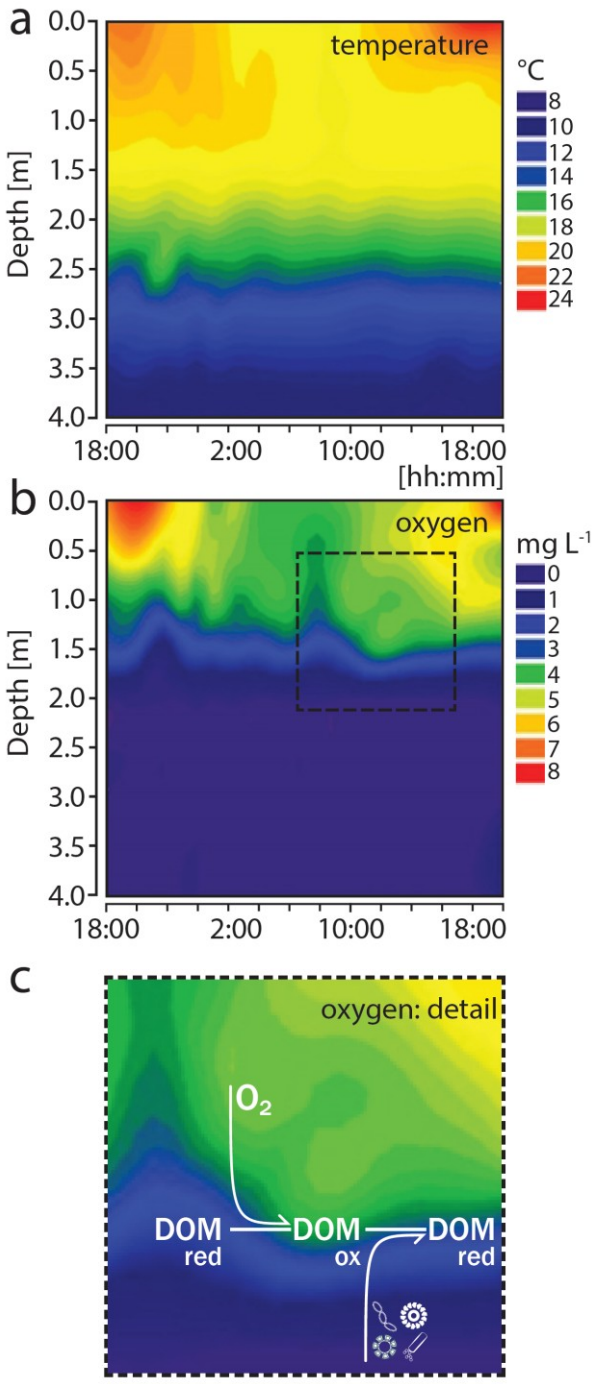

Figure 1 Water temperature (a) and dissolved oxygen concentration (b) in the water column of Lake Fuchskuhle (color scale) over time (x-axis) and depth (y-axis). Samples were taken every 30 minutes and interpolated to visualize the diurnal variations. (c) Detailed illustration of the proposed passage of dissolved DOM through the transient redox cline. The reduced DOM in the oxygen-depleted hypolimnion was subjected to (abiotic) re-oxidation upon night time mixing with oxygen-rich epilimnetic waters. Hypoxic conditions were re-established after subsequent stratification. However, the pelagic community near the interface was provided with newly formed oxidized DOM as TEA reservoir.

Lake Fuku features reported DOM concentrations of above 70 mg L^{-1} (Wurzbacher et al, 2014). At the same time, inorganic substances that can serve as TEAs under oxygen depletion (e.g., nitrate and sulfate) are only

present at low concentrations (Supplementary Table S1).

We manipulated the oxidation state of the redox-active moieties in the extracted DOM using direct electrochemical reduction. This methodology enables a quantifiable reduction of redox-active moieties in the DOM at a controlled potential without a reducing agent (Aeschbacher et al., 2011). The redox state of the analyzed system was determined by separately quantifying of the number of electrons that can be added in a reductive reaction (i.e., its EAC) or withdrawn during an oxidative reaction (its EDC). The transferred charge during the electrochemical reduction matched the change in EAC (Figure S4). Re-oxidation was performed with either O_2 or an H_2O_2 solution and could restore the DOM's capacity to accept electrons. According to the measured EDC values, 58±7% (±SD) of the H_2O_2 directly reacted with the redox active constituents of the DOM and generated electron-donating species. The full reduction and re-oxidation procedure was designed to mimic the passage of DOM through the diurnal redox gradient in Lake Fuku, which is prevalent near the oxycline (Figure 1c).

Table 1 Selected chemical properties of DOM from Lake Fuku

Fuchskuhle DOM						
					$\frac{Red}{Ox}$ 0.2	$\frac{Red}{Ox}$ 1.0
C	mg g^{-1} (±SD)	344.9 ± 0.4	LMWS[a]	%	15	13
H		35.0 ± 0.4	HMWS[b]		3.1	2.6
N		12.2 ± 0.2	BB[c]		82	85
S		35.1 ± 0.1	$SUVA_{254}$[d]	L mg^{-1} m^{-1}	3.6	3.5
obtained 2005						

[a] low- and [b] high-molecular-weight substances [c] building blocks, all of which were quantified using size-exclusion liquid chromatography (see supplementary information); [d] Specific UV absorbance at 254 nm

We successfully prepared DOM samples that featured different redox states (denoted according to their EAC:EDC ratio, $\frac{red}{ox}1-0$, Figure 2). The oxidation with H_2O_2 might have induced decreases in chain length or ring cleavage, but neither size distribution nor aromaticity changed significantly (Table 1, Figure S1). Coherent with our results, the stability of redox-active moieties within isolated DOM was previously confirmed using nuclear magnet resonance and fluorescence spectroscopy (Fimmen et al, 2007). Thus, we are confident that the oxidation treatment did not change any of the key DOM properties, except the ability to donate and accept electrons.

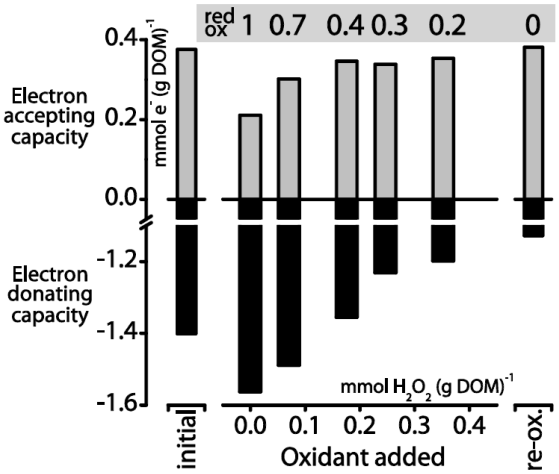

Figure 2 Redox properties of previously identical DOM samples from Lake Fuku after electrochemical reduction and re-oxidation. DOM was reduced and either non- ($^{re}_{ox}1.0$) or partly re-oxidized ($^{red}_{ox}0.7 - 0.2$) with H_2O_2, which was quantified in terms of the electron-accepting and -donating capacity (both in mmol electrons per g DOM). The re-oxidation with excess O_2 yielded the most oxidized DOM sample ($^{red}_{ox}0$).

Effect of the DOC redox state on bacterial abundance and dark carbon fixation

The incubation of natural bacterial communities from the peat bog lake with natural DOM of different oxidation states reveals significant differences in both activity and abundance during the first hours of incubation (Figures 3a and b). Our measurements show consistent patterns in the community's response to differences in oxidation state of the carbon source: increases in microbial activity in the samples with oxidized DOM, which were measured via ^{14}C-leucine uptake, were particularly pronounced at the beginning of the incubation (at 18 and 25 h) but less pronounced with further incubation (Figure 3a). Accordingly, we observed a higher bacterial abundance in samples with higher proportions of oxidized to reduced DOM moieties throughout 50 h of incubation (Figure 3b). More specifically, we found that the bacterial carbon production in the samples that received DOM with the highest amount in reducible moieties were statistically significantly different from all other treatments up to 25 h after the start of incubation ($p < .05$, see Supplementary Information). The incubation conditions (DOM concentration, light regime, temperature, inoculum, pH) were identical in all treatments.

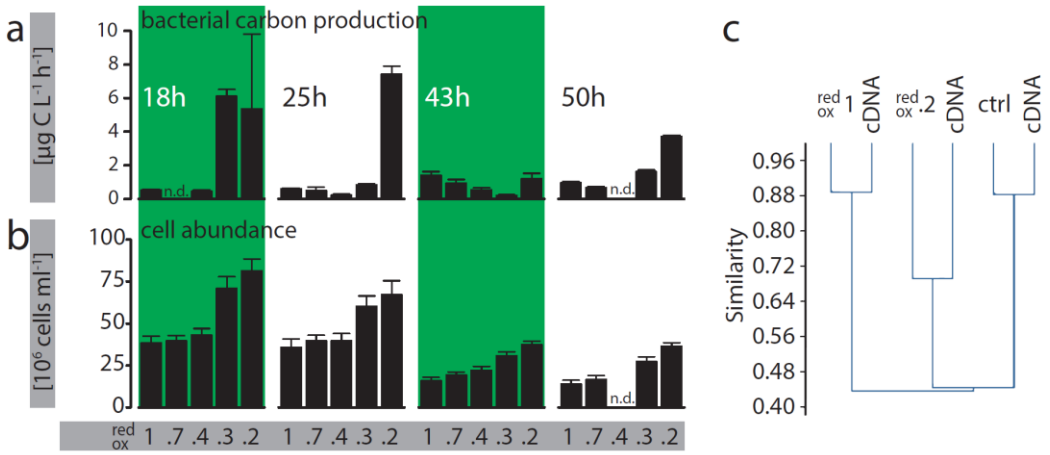

Figure 3 (a) Bacterial carbon production and **(b)** abundance of the microbial community from a peat bog lake (Lake Barschsee) incubated with DOM in different oxidation states ($^{red}_{ox}1$ to $^{red}_{ox}.2$). The replicate incubations were stopped after 18, 25, 43 and 50 h by adding formalin. The error bars indicate the standard deviation of quadruplicate measurements. Statistical details can be found in the Supplementary Information. **(c)** Cluster diagram indicating the percentage of similarity in bacterial community composition based on the amplified 16S rDNA and cDNA PCR products, which were obtained from samples that received reduced $^{red}_{ox}1$ DOM, oxidized $^{red}_{ox}0.2$ DOM and only the inoculum sample (ctrl). Incubation and sequencing were conducted in duplicates with an additional sterile control (not shown). The phylogenetic affiliations of the obtained sequences are presented and discussed in the Supplementary Information (Table S2).

Consequently, the higher degree of activity of the microbial community is related to the availability of oxidized DOM moieties. Although the added DOM represents a reservoir of electron donors, it also represents a substantial pool of EAC when in the oxidized state. Previous studies have shown that the redox-active moieties of the DOM become increasingly reduced during periods of anoxia, as evidenced by a decrease in the EAC and an increase in the EDC (Kluepfel et al., 2014; Lau et al., 2015). Although we did not follow the EAC changes, our results indicate that bacteria in samples with (partially) oxidized DOM may have been released from limited TEA availability. Thermodynamically, the electron transfer to quinone moieties in the DOM is considered more energetically favorable than other anaerobic respiration pathways, e.g., the electron transport to DOM under fermentative or methanogenic conditions (LaRowe and Van Cappellen, 2011).

As alternative explanations, the initially increased cell abundance and activity/growth can result from increased bioavailability of labile DOM after the H_2O_2 treatment (Pullin et al, 2004). However, a control experiment confirms that DOM that experienced successive reduction-oxidation cycles behaves in the same manner as the singularly reduced DOM (Figure S2). Some phenolic substances may hamper bacterial growth and are, because of their antioxidant properties, more abundant in the less re-oxidized DOM

(Aeschbacher et al., 2010; Aeschbacher et al, 2012). Phenols may cause enzyme inhibition which affects the DOM degradation (Freeman et al, 2004). However, the cell abundance increased in all samples that were amended with DOM regardless of its oxidation state compared to the abundance directly after inoculation. The absence of inhibitory effects was previously substantiated by showing that a large fraction of pelagic bacteria is not inhibited by addition of phenol (Hutalle-Schmelzer et al., 2010).

In conclusion, we suggest that microbial respiration (and hence growth) is limited by the availability of both labile DOM (Kortelainen et al, 2006) and TEAs. The data demonstrates that the amendment of oxidized DOM releases the natural microbial community from TEA limitation. Hence, C oxidation with redox-active DOM as a TEA may represent a viable respiration pathway in pelagic systems.

Effect of DOM redox state on the bacterial community composition

In a second experiment, we analyzed the bacterial community composition of a peat bog lake (Lake Barschsee, adjacent to Lake Fuku) after 25 h of incubation with DOM in both reduced $^{red}_{ox}\mathbf{1.0}$ and oxidized $^{red}_{ox}\mathbf{0.2}$ redox states. Community fingerprints using 16S rDNA and 16S rRNA (cDNA) revealed differences between the entire and active bacterial communities (Figure 3c), which indicates that not all present bacteria in the incubations grew continuously. Instead, over the course of the incubation, the transient occurrence of microorganisms was indicated by the occurrence of DNA sequences that were not continuously expressed. We observed significant differences in bacterial communities between DOM-amended and DOM-free control samples when the samples were incubated under similar conditions. More interestingly, the samples that were amended with oxidized, EAC-rich DOM were significantly different from those with reduced, EAC-depleted DOM (Figure 3c).

In general, the bacterial community composition in the $^{red}_{ox}\mathbf{0.2}$ DOM treatment differed from both the control and $^{red}_{ox}\mathbf{1.0}$ DOM treatment by over 50 %. This drastic change in community composition after the addition of DOM with high EAC indicates a metabolic niche that can readily be occupied by bacterial genera using redox-active DOM as a TEA.

Conclusion

When oxidized DOM was added to samples of a peat bog lake, we observed increases in microbial activity and abundance and drastic changes in bacterial community composition. Although the EAC associated with this oxidized DOM may decrease rapidly, the repeated passages of DOM through an

aerobic-anaerobic interface (e.g., across the transient redox-cline) can cyclically regenerate the oxidized redox state (Keller et al., 2009; Kluepfel et al., 2014; Lau et al, 2016).

Previous studies of DOM reduction and oxidation reactions reveal fast turnover rates under ambient conditions: DOM reduction rates in *Geobacter* culture solutions up to 185 μmol e^- min^{-1} L^{-1} were measured, which is 27 times faster than the rate at which the same genus reduces Fe(III) (Jiang and Kappler, 2008). Oxygen consumption in the oxidation of reduced DOM was reported to reach maximum rates within only 3 min (Bauer et al, 2007). Further kinetic analyses reveal that the rates of *in situ* DOM oxidation is sufficiently high to scavenge oxidized species from other relevant aquatic redox reactions (e.g., H_2S or Fe(II) oxidation) (Bauer and Kappler, 2009).

Considering these rapid turnover rates, redox fluctuations on a similar temporal scale can further increase the significance of DOM as dissolved TEA species for natural bacterial communities. Compared to the convection-driven diurnal fluctuations in oxygen availability, small-scale turbulences and water movements in the pelagic thermocline may induce redox fluctuations in confined water bodies on considerably shorter timescales (Preusse et al, 2010). In fact, additional high-resolution spatiotemporal O_2 measurements in Lake Fuku show that the diurnal cycles are superimposed with momentary redox cline fluctuations (Figure S3). Consequently, faster depletion and regeneration cycles correspond to higher proportion of aquatic carbon that is potentially respired with DOM as a TEA.

We expect that this respiration pathway is most important in stratified lakes with anoxic water layers that feature high concentrations of organic matter, e.g., peat bog lakes. Globally, 55% of lakes surpass the threshold value of 5 mg DOC L^{-1}, which indicates net heterotrophy (Sobek et al, 2007). The same concentration was also identified as a critical value, above which microbial DOM reduction is prevalent (Jiang and Kappler, 2008).

The reduction of quinoid moieties within the DOM induces changes in DOM fluorescence (Fulton et al, 2004). Periodic changes in the fluorescence of DOM-rich lake waters are suspected to be a metabolism (rather than light)-driven phenomenon and widespread across temperate freshwater ecosystems (Watras et al, 2016). While the underlying mechanism remains unresolved, DOM traversing redox gradients may help to rationalize the diel cycles in DOM optical properties that were observed in Canadian peat bog lakes (Watras et al, 2015).

We conclude that carbon oxidation with recurrently oxidized DOM as TEA has the potential to be an important component of lake metabolism and functioning. The relatively high thermodynamic energy yield of carbon

oxidation coupled to the reduction of the putative redox couples in DOM mitigates less favorable respiration pathways, e.g., methanogenesis (Cervantes et al, 2000; Keller et al., 2009). We expect the competitive inhibition of water-column methanogenesis to be most pronounced in eutrophic lakes of the temperate and boreal zone because their water bodies are typically stratified and generally exhibit pelagic oxic/anoxic interfaces. Hence, the carbon oxidation with DOM as TEA may significantly alter projections of greenhouse gas emissions from these environments. However, further research on DOM redox cycling in pelagic and benthic redox gradients is warranted to estimate its global relevance.

Acknowledgments

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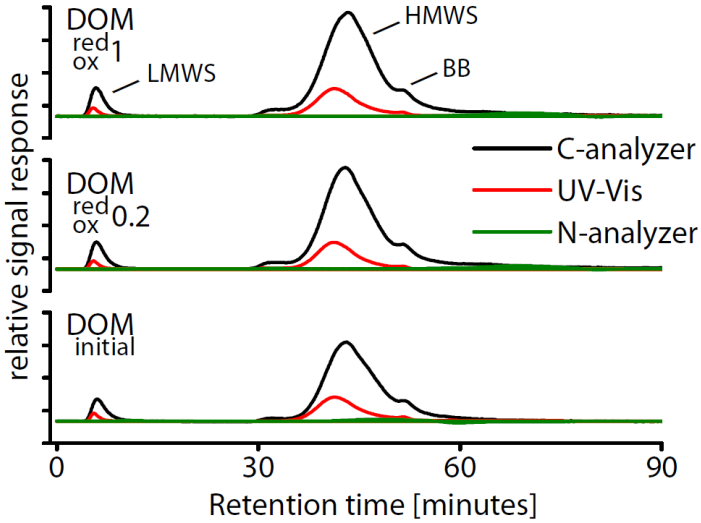
Supplementary Information

Lake characteristics and chemistry

Supplementary Table S1 Selected physicochemical properties of Lake Fuku

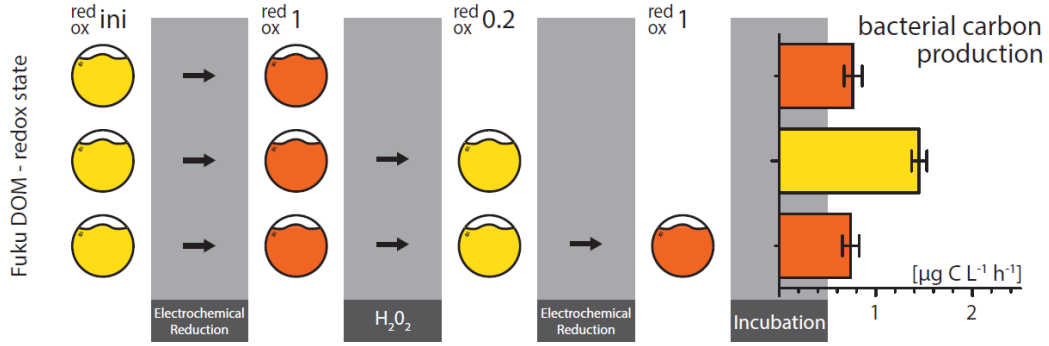
Lake Fuchskuhle			
	low	high	References
Nitrate	0 µg L^{-1}	17 µg L^{-1}	[c]
Nitrite	0 µg L^{-1}	10 µg L^{-1}	[c]
Sulfate		3.3 µg (mL sediment)$^{-1}$ [d]	(Casper et al, 2003)
DOC [a]	21 mg C L^{-1} [c]	70 mg C L^{-1}	(Wurzbacher et al, 2014)
Fe [b]		41 µmol (mL sediment)$^{-1}$ [d]	(Kanaparthi, 2013)

[a] dissolved organic carbon [b] ferrous and ferric iron [c] unpublished data from long-term data sets 2014/2015 [d] in the surface sediment (0-5 cm depth)



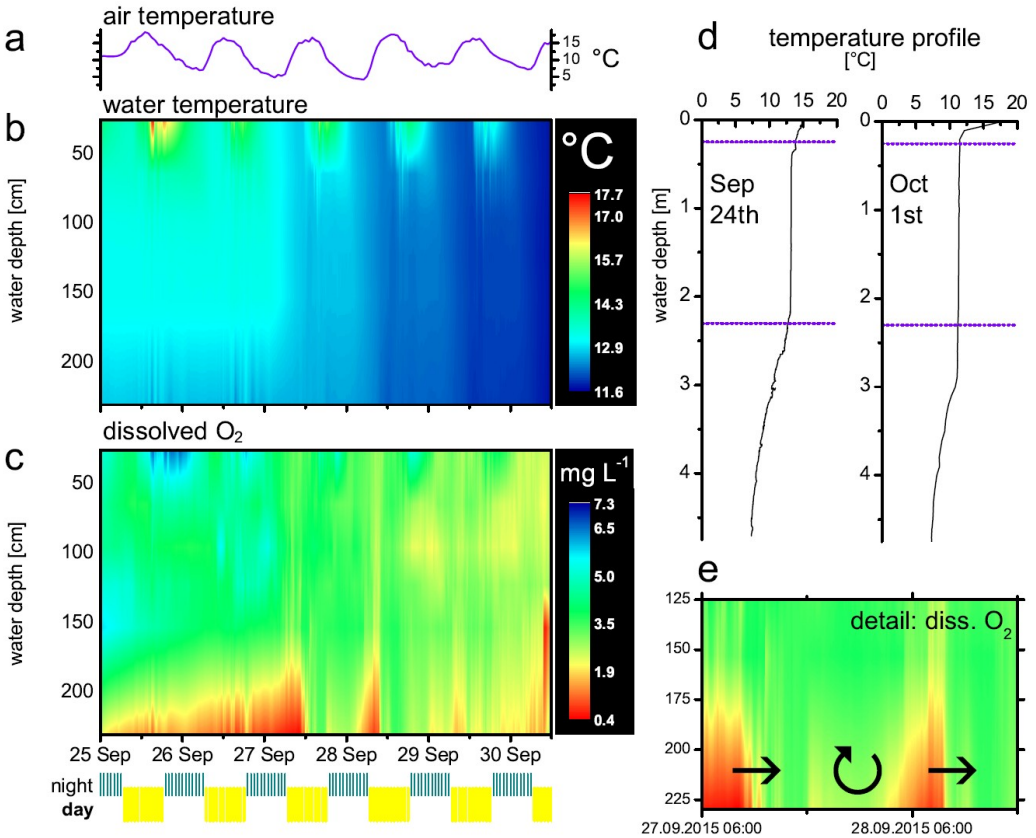
Supplementary Information Figure S1 Initial, reduced ($\frac{red}{ox}1$) and re-oxidized ($\frac{red}{ox}0.2$) DOM samples, which were analyzed using size-exclusion liquid chromatography. The relative signal responses of three on-line detector units: organic carbon analyzer, organic nitrogen analyzer and UV-Vis spectrometer. The peaks were classified to represent low- and high-molecular-weight substances (LMWS and HMWS, respectively) and building blocks (BB) based on their retention time (Sachse et al, 2001).

Repeated reduction-oxidation treatment of DOM from Lake Fuku



Supplementary Information Figure S2 Similar to the initial experiment, we amended water samples (taken in Oct. 2015) with reduced ($\frac{Red}{Ox}$ 1) and H_2O_2 oxidized ($\frac{red}{ox}$ 0.2) DOM from Lake Fuku and quantified the bacterial production after 24 h of incubation. Additionally, we prepared reduced ($\frac{red}{ox}$ 1) DOM from the previously oxidized DOM by repeating the electrochemical reduction treatment (last row). The samples that were amended with this redox-cycled DOM did not show significantly different bacterial carbon production compared to samples that were amended with singularly reduced DOM. Hence, the combination of electrochemical redox treatment and subsequent oxidation did not cause irreversible changes in the DOM (redox) properties.

Thermo- and oxycline fluctuations in Lake Fuku



Supplementary Information Figure S3 Hydrophysical conditions in the SW basin of Lake Fuku. (a) Regional air temperature (source: Deutscher Wetterdienst) (b) water temperature and (c) dissolved oxygen, which were measured with high temporal resolution (1 min) in a cross section of the water column. (d) Temperature profile in Lake Fuku at the

beginning and the end of the O2 measurement in September 2015. The horizontal lines confine the upper and lower limits of the position of six O2 probes. (e) Proposed passage of solutes in a confined water body through a temporal oxygen gradient. Stratification and mixing periods alternate in diurnal cycles (arrows).

Statistics

Statistical tests were conducted with SPSS (SPSS 20, IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) was applied to explore differences between treatments at different incubation durations in either bacterial carbon production or cell abundance. Homogeneity of variances was tested using the Levene's F test. Datasets that failed Levene's F test were explored using the more robust Brown & Forsythe Test (Brown and Forsythe, 1974). Homogeneity of variances was violated in only one case, bacterial production measured after 18 h of incubation.

At all other time points and in both parameters determined we found statistically significant differences between treatments as explored by one-way ANOVA ($p < 0.05$).

More specifically, a Tukey PostHoc Test revealed that microbial abundance in treatments $^{red}_{ox}\mathbf{0.3}$ and $^{red}_{ox}\mathbf{0.2}$ was significantly different from treatments that received less oxidized DOM both after 18 and 25 h at $p < .05$. Moreover, carbon production for the most oxidized sample $^{red}_{ox}\mathbf{0.2}$ was statistically significantly different from treatments that received DOM in more reduced state at 25 h (no statistically significance could be determined using ANOVA at 18 h of incubation due to violation of the homogeneity of variances).

Phylogenetic affiliations

The incubated $^{red}_{ox}\mathbf{0.2}$ samples, which had high organic TEA capacities, had a high abundance of Actinobacteria. These ubiquitous organisms are distributed throughout various limnetic systems (including boreal lakes) and are the prevalent fraction of heterotrophic bacterial plankton (Allgaier and Grossart, 2006; Lew et al, 2011). The microbiome in the EAC-depleted samples was dominated by *Bacillus* species and Betaproteobacteria. These genera constitute a significant part of the bacterial population in the superficial layer of eutrophic and humic lakes (Taipale et al, 2009; Newton et al, 2011). The metabolic versatile *Brevundimonas* sp. was the only species that clearly showed metabolic activity in all samples regardless of the DOM amendment based on both 16S rDNA and cDNA.

Supplementary Table S2 Phylogenetic identification of DGGE bands

#	**Description**		**Ctrl**[a]	**cDNA**		$\frac{Red}{Ox}$ 0.2	**cDNA**		$\frac{Red}{Ox}$ 1.0	**cDNA**
1	Brevundimonas sp.	partial sequence								
2	Brevundimonas sp.	partial sequence								
3	Actinobacterium clone BR1E3	partial sequence								
4	Microbacterium sp.	partial sequence								
5	Actinobacterium	partial sequence								
6	Actinobacterium	partial sequence								
7	Brevundimonas sp. clone 6TB05	partial sequence								
8	Brevundimonas sp.	partial sequence								
9	Actinobacterium clone ZS-2-25	partial gene								
10	Brevundimonas aurantiaca, strain PrJ	partial gene								
11	Ralstonia sp.	partial sequence								
12	Beta proteobacterium SHNN385	partial sequence								
13	Pseudomonas pickettii									
14	Microbacterium sp.	partial sequence								
15	Brevundimonas sp.	partial sequence								
16	Brevundimonas sp.	partial sequence								
17	Bacillus siralis strain 171544	partial sequence								
18	Bacillus siralis strain 171544	partial sequence								
19	Burkholderia ambifaria strain ZH2	partial sequence								
20	Bacillus firmus strain N12-3	partial sequence								
21	Bacillus siralis strain 171544	partial sequence								

[a] The control treatment (Ctrl) did not received DOM but was incubated under similar conditions as the samples with DOM amendments.

	band
n.d.	
visible	
thin	
wide	
very wide	

Methods

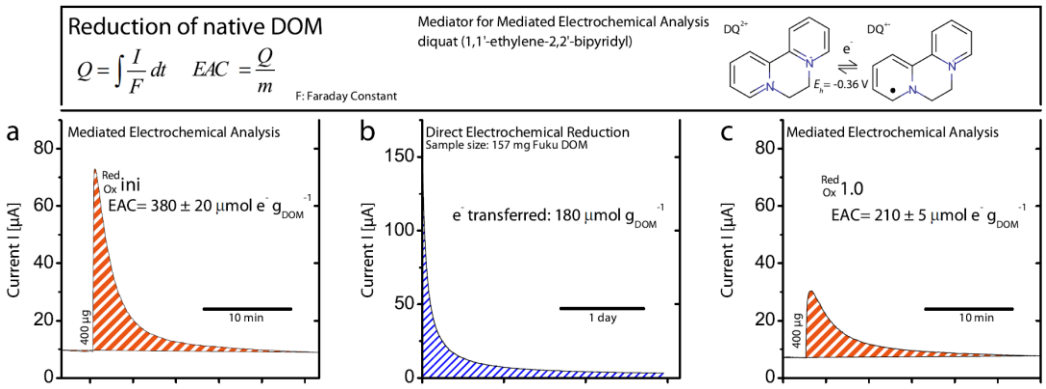
Mediated electrochemical reduction and mediated electrochemical oxidation

Measurements were conducted in electrochemical cells with pH buffered (pH 7.00 ± 0.05) solutions, which contained 0.01 M 4-Morpholinepropanesulfonic acid as the buffering species and 0.1 M $NaClO_4$ as the background electrolyte. We used glassy carbon cylinders (Volume 9 mL; HTW Carbon, Thierhaupten, Germany), which served as both the working electrode (WE) and cell reaction vessels, an Ag/AgCl reference electrode and a platinum wire auxiliary electrode (both from Bioanalytical Systems Inc., West Lafayette, IN, USA). The auxiliary electrode was placed in a glass frit to minimize the re-oxidation of the reduced DOM.

A potentiostat (CHI 1000C, Bee Cave, TX, USA) was used to measure the currents and control the potentials at the WE. The WEs were polarized to reduction potentials of E_h = -0.49 V for MER or +0.61 V for MEO (reported versus the standard hydrogen electrode, but experimentally measured versus the Ag/AgCl reference electrodes). Each electrochemical analysis was initiated by adding the dissolved electron transfer mediators 6,7-dihydrodipyrido [1,2-a:2',1'-c]pyraziniumdibromid monohydrate (99.5%; $E_h°$ = -0.36 V; Supelco, Bellefonte, PA, USA) (Diquat, DQ) to MER cells and 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) ammonium salt (>98%; $E_h°$ = +0.7 V; Sigma-Aldrich, St. Louise, MO, USA) (ABTS) to MEO cells to final concentrations of 250 - 350 μM.

Both ABTS and DQ are single-electron transfer mediators: DQ was reduced to the radical species $DQ^{\bullet+}$ in MER (Figure S4), and ABTS was oxidized to the $ABTS^{\bullet+}$ radical in MEO. The resulting current responses were peak-shaped with initial high currents and a subsequent decrease in currents, which ultimately leveled off when the mediators approached E_h equilibria with the E_h that was applied to the WEs (Figures S4a and S4c). Small volumetric aliquots of 50-200 μL from vigorously stirred sediment suspensions were transferred to the MER and MEO cells via a pipette. In MER, the dissolved $DQ^{\bullet+}$ transferred electrons to the electron-accepting species in the added sample, which resulted in the formation of DQ^{2+} molecules. The formed DQ^{2+} were subsequently re-reduced to $DQ^{\bullet+}$ at the WE to re-establish E_h equilibrium in the MER cell. In MEO, $ABTS^{\bullet+}$ radicals were reduced by electron-donating species in the added sample and resulted in the formation of ABTS, which was then re-oxidized to $ABTS^{\bullet+}$ at the WE. Thus, the addition of redox-active samples to MER and MEO resulted in reductive and oxidative current peaks, respectively. These peaks were baseline-corrected and integrated to obtain the numbers of electrons, Q

(mmol e^-), that were transferred to and from the added sample according to Figure S4. The electron-accepting capacity (EAC) and the electron-donating capacity (EDC) were calculated by normalization to the added sample mass. With the potentials used in MER and MEO, the EAC and EDC are quantified between the upper and lower ends of the reduction potentials of the quinone/hydroquinone moieties in the DOM.



Supplementary Information Figure S4 Electrochemical techniques used to measure and manipulate the oxidation state of DOM. (a) Current response over time to the addition of a DOM sample in its initial redox state ($^{Red}_{ox}$ini) to the polarized electrochemical cell. (b) Reduction of the DOM in the initial redox state in direct electrochemical reduction. The area under the curve (blue) corresponds to the charge transferred to the sample. (c) Current response of the DOM after electrochemical reduction ($^{red}_{ox}$1.0). Transferred charge (Q) was determined from the integrals of the I-t curves (red) and Faraday constant (F). The electron-accepting capacity (EAC) was calculated relative to the added sample mass (m, in this case, 400 µg of DOM) in mediated electrochemical analysis. The redox mediator Diquat (DQ) was added to enhance the electron transfer kinetics between the electrodes and sample. The ΔEAC from the measured values before and after the treatment matches the transferred Q in the reduction treatment (on a mass basis).

^{14}C Leucine uptake

The rates of bacterial protein production (BPP) were determined based on the incorporation of ^{14}[C]-leucine (^{14}C-Leu) according to Simon & Azam (1989). The ^{14}C-Leu-amended samples were incubated for 1 h at ambient conditions in the dark in quadruplicates plus four prefixed (2% formalin) controls. After fixation with 2% formalin, samples were filtered onto 0.2 µm nitrocellulose filters (Sartorius, Goettingen, Germany) and extracted with ice-cold 5% trichloroacetic acid (TCA) for 5 min. Thereafter, the filters were rinsed twice with ice-cold 5% TCA, rinsed once with ethanol (96% v/v), and dissolved with ethylacetate for measurement using liquid scintillation counting. The standard deviation of the replicate measurements was typically <15%. The amount of incorporated ^{14}C-Leu was converted into BPP using an intracellular isotope dilution factor of 2. A conversion factor of 0.86 was used to convert the produced protein into bacterial carbon production (Simon and Azam, 1989).

DGGE Evaluation

Banding patterns were compared among the gels using the software GelCompar II version 3.5 (Applied Maths, Sint-Martens-Latem, Belgium). The external standard was used to standardize the DGGE profiles of single gels and perform correct and reliable band matching among different gels. We applied a 5-15% background subtraction depending on the signal-to-noise ratio of the gels. Band-based binary presence/absence tables were calculated, and single bands of each DGGE profile were scored using the minimum profiling tool of GelCompar II with a cut-off value of 5%. Thus, all bands with at least 5% of the density of the darkest band were counted as present. This presence/absence table was imported into the software PRIMER 5 used in the hierarchical clustering analyses.

Sequencing

Phylogenetic analyses of the partial 16S rRNA gene sequences were performed using the ARB software package (http://www.arb-home.de). The retrieved sequences were imported into an ARB database of 52,000 reference sequences, which included the closest related sequences that were determined by BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The sequences were first automatically aligned using the integrated alignment module in the ARB package and subsequently manually corrected.

Supplementary Literature

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3.4 Erklärung zu Publikationen

Artikel 1

Lau MP, Sander M, Gelbrecht J, Hupfer M. (**2015**). Solid phases as important electron acceptors in freshwater organic sediments. *Biogeochemistry*, **123**, 49-61.

Dieses Manuskript ist in Zusammenarbeit mit Dr. Michael Sander, Dr. Michael Hupfer und Dr. Jörg Gelbrecht entstanden und veröffentlicht.

Maximilian Lau war wesentlich an der Konzeption und Durchführung der Studie beteiligt. Maximilian Lau hat die Probenahmen geplant und durchgeführt. Alle nasschemischen Analysen wurden von Maximilian Lau geplant, durchgeführt und ausgewertet, ein Teil wurde von Mitarbeitern des Labors übernommen (Ionenchomatographie). Alle elektrochemischen Analysen wurden von Maximilian Lau ausgeführt und ausgewertet. Darüber hinaus hat Maximilian Lau das Manuskript verfasst und durch den Begutachtungsprozess geführt. Maximilian Lau ist der korrespondierende Autor.
Michael Sander hat Maximilian Lau in die elektrochemischen Methoden eingeführt und war beratend bei der Anfertigung des Manuskripts beteiligt. Jörg Gelbrecht war bei der Planung der Probenahme maßgeblich beteiligt. Michael Hupfer hat alle finanziellen Mittel eingeworben und war beratend bei der Konzeption der Analysestrategie beteiligt. Alle Autoren haben vorläufige Versionen des Manuskripts kommentiert.

Artikel 2

Lau MP, Sander M, Gelbrecht J, Hupfer M. (**2016**). Spatiotemporal redox dynamics in a freshwater lake sediment under alternating oxygen availabilities: combined analyses of dissolved and particulate electron acceptors. *Environmental Chemistry*, 13, 826–837 http://dx.doi.org/10.1071/EN15217

Dieses Manuskript ist in Zusammenarbeit mit Dr. Michael Sander, Dr. Michael Hupfer und Dr. Jörg Gelbrecht entstanden und veröffentlicht.

Maximilian Lau war wesentlich an der Konzeption und Durchführung der Studie beteiligt. Maximilian Lau hat die initiale Probenahmen geplant und durchgeführt. Der Experimentaufbauten wurden von Maximilian Lau vorgenommen und betreut. Alle nasschemischen Analysen wurden von Maximilian Lau geplant, durchgeführt und ausgewertet, ein Teil wurde von Mitarbeitern des Labors übernommen (Ionenchomatographie, Nitratanalyse). Bodenkundliche Parameter (Dichte, Kohlenstoff und Stickstoffgehalt der Sedimente) wurden von Maximilian Lau bestimmt. Alle elektrochemischen Analysen wurden von Maximilian Lau ausgeführt und ausgewertet. Darüber hinaus hat Maximilian Lau das Manuskript verfasst und durch den Begutachtungsprozess geführt. Maximilian Lau ist der korrespondierende Autor.
Michael Sander hat Maximilian Lau in die elektrochemischen Methoden eingeführt und war beratend bei der Anfertigung des Manuskripts beteiligt. Jörg Gelbrecht und Michael Hupfer haben alle finanziellen Mittel eingeworben, Analyseinstrumente zur Verfügung gestellt (Glovebox) und waren beratend bei der Konzeption der Analysestrategie beteiligt. Michael Hupfer hat Maximilian Lau in das Sauerstoffmodell nach Livingston eingeführt und die dafür notwendigen Daten bei Kooperationspartnern eingeholt. Alle Autoren haben vorläufige Versionen des Manuskripts kommentiert.

Artikel 3

Lau MP, Hupfer M, Grossart HP; Reduction-Oxidation cycles of organic matter increase bacterial activity in the pelagic oxycline, (**in review**)

Dieses Manuskript ist in Zusammenarbeit mit Dr. Michael Hupfer und Prof. Dr. Hans-Peter Grossart entstanden und ist zur Veröffentlichung eingereicht worden.

Maximilian Lau war wesentlich an der Konzeption und Durchführung des Manuskripts beteiligt. Alle elektrochemischen Manipulationen der DOC Präparate wurden von Maximilian Lau vorgenommen. Alle Inkubationsexperimente wurden von Maximilian Lau vorbereitet. Die mikrobiologischen Experimente wurden, zum Teil von Maximilian Lau durchgeführt (bakterielle Aktivität) und alle Ergebnisse von ihm ausgewertet. Die Hälfte der Sauerstoff Feldmessungen sind von Maximilian Lau durchgeführt und ausgewertet worden. Darüber hinaus hat Maximilian Lau das Manuskript verfasst.

Hans Peter Grossart hat die zweite Hälfte der Sauerstoff Daten und die Infrastruktur für die Bestimmung der mikrobiologischen Parameter bereitgestellt. Michael Hupfer und Hans Peter Grossart haben die notwendigen Finanzmittel eingeworben, waren an der initialen Konzeption beratend beteiligt und haben Einschätzungen zu einzelnen kritischen Aspekte von Kollegen eingeholt (Hydrodynamik, Stil). Alle Autoren haben vorläufige Versionen des Manuskripts kommentiert.

Alle weiteren Teile (Kapitel 1,2 und 4) der Dissertation sind ausschließlich von Maximilian Lau angefertigt worden.

4 Synthesis

In conclusion, these works present an overview on how organic electron acceptors, redox species that were only recently acknowledged to play a significant role in microbial respiration, respond to the biogeochemical and ecological conditions at oxic/anoxic interfaces.

First, it was shown that organic and Fe-bearing constituents in mixed environmental phases change redox states in anaerobic conditions and that their redox states are ideally quantified in an integrative manner. We highlighted that Fe-bearing mineral phases do not exhibit strict reduction-dissolution correlation. The use of mediated electrochemical analysis as a tool proved beneficial since it does not distinguish between dissolved and particulate redox species. Consequently, this approach permitted the first electrochemical quantification the electron-accepting properties of organic-mineral (*geochemical*) phases in natural sediments and peat soils.

According to the initial finding that organic-rich geochemical phases are likely to be found ubiquitously in natural matrices, this work explored the hydrodynamic and biogeochemical conditions under which the redox dynamics of these geochemical phases may be important for ecosystem functioning.

Second, this work examined how the re-oxidation of microbially reduced organic and mineral sediment constituents affected the oxygen regime in a stratified lake. It was found, that upon the (bi-)yearly lake overturn events, significant amounts of the oxygen that was then introduced to bottom waters of the lake was consumed by the re-oxidation of geochemical phases that originated from the previous microbial activity when conditions were anoxic.

In further work, it was shown that fluctuations in oxygen availabilities may occur not only bi-yearly but also on considerably shorter timescales (minutes to hours) at redox interfaces in aquatic environments. This specific work is novel in that it demonstrated how short fluctuations in confined parts of the pelagial may have drastic consequences for the microbial communities in these water bodies. Dissolved organic matter may both serve as organic electron accepting species reducible by aquatic microorganisms, and rapidly re-generate its electron-accepting property upon dislocation to oxic parts of the water column.

As significant parts of ecosystem respiration could possibly be directed through this pathway, the proposed redox cycling of organic matter at interfaces may be an important part of lake functioning that was previously

disregarded.

In conclusion, the joined findings promote the notion that natural organic matter comprises a pool of reversible redox active species and, beyond that, these species are linked to other redox active species (Fe^{n+}, O_2) on both abiotic and microbially mediated pathways. As of today, connecting the chemistry of aquatic turnover processes with the microbiological and physical conditions at redox interfaces remains challenging. Future work should be directed towards the mechanisms and ecology of NOM redox chemistry:

1 Phenolic moieties in NOM are redox active. These moieties may partly origin from microbial reduction of quinone moities and are prone to oxidation. Depending on the transformation route, this oxidation can be irreversible. Hence, with respect to the proposed cyclic regeneration of electron-accepting moieties in NOM, it is crucial to assess the conditions and structures that result from oxidation reactions which are not fully reversible. Ultimately, the reduction may be a pre-condition for the final mineralization of quinone moieties in NOM.

2 Ample evidence is given that NOM may represent a considerable reservoir of electron accepting capacity in a range of carbon-rich aquatic environments. Its most striking implication is the use of this reservoir in an additional respiration pathway (i.e., "humics reducing" microorganisms) and the possibility of a competitive inhibition (mitigation) of the thermodynamically less-favorable methanogenesis. Linking respiration with NOM as TEA in spaces that represent cold-spots of methanogenesis would be a promising approach to help rationalize patterns of greenhouse gas emissions from aquatic environments.

3 Quinonid moieties in NOM have been shown to interact with redox co-factors of cells. Small structural heterogeneities may facilitate an effective electron transport cascade from intracellular C oxidation, bridging redox gradients and finally reduce extracellular redox species. It is likely that many organisms possess an enzymatic apparatus for the electron transfer to redox active, extracellular NOM (Cervantes et al. 2002). Thus, it is deemed promising to assess whether methanogenic or chemolithotrophic microorganisms (those that

acquire energy from the oxidation of extracellular substances as e.g. Fe(II)) may also possess the enzymatic precondition to receive electron from reduced organic species. If so, it would extend the role of redox active NOM in microbial respiration to yet another class of microbiota.

4 And last, this work encourages further examination of fermenting processes. While fermentation is a ubiquitous process, description of its mechanism and even terminology are found to be blurry across a wide area of contexts. Fermentation products as H_2, acetate and other oxygen-rich organic compounds are important substances in many aquatic and terrestrial environments with high microbial activity. It is legitimate to put more effort into the research of the mechanistic underpinnings of these processes.

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6 Declaration of Originality

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst, keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Greifswald, den 07. April 2016 Maximilian Peter Lau

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