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Ferrihydrite-associated organic matter (OM) stimulates reduction by *Shewanella oneidensis* MR-1 and a complex microbial consortia

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Abstract. The formation of Fe(III) oxides in natural environments occurs in the presence of natural organic matter (OM), resulting in the formation of OM–mineral complexes that form through adsorption or coprecipitation processes. Thus, microbial Fe(III) reduction in natural environments most often occurs in the presence of OM–mineral complexes rather than pure Fe(III) minerals. This study investigated to what extent does the content of adsorbed or coprecipitated OM on ferrihydrite influence the rate of Fe(III) reduction by *Shewanella oneidensis* MR-1, a model Fe(III)-reducing microorganism, in comparison to a microbial consortium extracted from the acidic, Fe-rich Schlöppnerbrunnen fen. We found that increased OM content led to increased rates of microbial Fe(III) reduction by *S. oneidensis* MR-1 in contrast to earlier findings with the model organism *Geobacter bremensis*. Ferrihydrite–OM coprecipitates were reduced slightly faster than ferrihydrites with adsorbed OM. Surprisingly, the complex microbial consortia stimulated by a mixture of electrons donors (lactate, acetate, and glucose) mimics *S. oneidensis* under the same experimental Fe(III)-reducing conditions suggesting similar mechanisms of electron transfer whether or not the OM is adsorbed or coprecipitated to the mineral surfaces. We also followed potential shifts of the microbial community during the incubation via 16S rRNA gene sequence analyses to determine variations due to the presence of adsorbed or coprecipitated OM–ferrihydrite complexes in contrast to pure ferrihydrite. Community profile analyses showed no enrichment of typical model Fe(III)-reducing bacteria, such as *Shewanella* or *Geobacter* sp., but an enrichment of fermenters (e.g., *Enterobacteria*) during pure ferrihydrite incubations which are known to use Fe(III) as an electron sink. Instead, OM–mineral complexes favored the enrichment of microbes including *Desulfobacteria* and *Pelosinus* sp., both of which can utilize lactate and acetate as an electron donor under Fe(III)-reducing conditions. In summary, this study shows that increasing concentrations of OM in OM–mineral complexes determines microbial Fe(III) reduction rates and shapes the microbial community structure involved in the reductive dissolution of ferrihydrite. Similarities observed between the complex Fe(III)-reducing microbial consortia and the model Fe(III)-reducer *S. oneidensis* MR-1 suggest electron-shuttling mechanisms dominate in OM-rich environments, including soils, sediments, and fens, where natural OM interacts with Fe(III) oxides during mineral formation.

1 Introduction

Iron is the fourth most abundant element in the Earth's crust, indicating the role of Fe cycling in nature is both ubiquitous and widespread. The majority is dispersed as iron minerals in natural environments, including soils and sediments with ferrous iron, Fe(II), and ferric iron, Fe(III), as the primary redox states (Davison, 1993; Stumm and Sulzberger, 1992; Weber et al., 2006a). Microbially mediated and abiotic redox cycling of Fe play crucial roles in global biogeochemistry (e.g., preservation and degradation of organic matter, and the fate of nutrients and contaminants for living organisms; Borch et al., 2010; Melton et al., 2014). Iron minerals such as Fe(III) oxides exhibit a high reactivity towards dissolved organic matter (OM); thus Fe(III) oxides are either partially

or completely covered by OM in natural environments (Eusterhues et al., 2005; Kaiser and Zech, 2000; Lalonde et al., 2012; Torn et al., 1997). Fe(III) oxides enveloped by OM potentially lead to changes in the surface properties in comparison to non-modified Fe(III) oxides, which ultimately may influence mobility, solubility, and aggregation (Eusterhues et al., 2014; Narvekar et al., 2017; Pédrot et al., 2011; Vindedahl et al., 2016). The poorly crystalline ferrihydrite is one of the most common Fe(III) oxides and typically forms aggregates of nanometer-sized individual crystals (Bigham et al., 2002; Cornell and Schwertmann, 2003; Jambor and Dutrizac, 1998). The coprecipitation of OM with Fe results in the adsorption and occlusion of organic molecules within the interstices between individual ferrihydrite crystals, while adsorption of OM occurs on pre-existing ferrihydrite surfaces (Eusterhues et al., 2014). Moreover, the presence of dissolved OM impedes ferrihydrite growth (Cismasu et al., 2011; Eusterhues et al., 2008; Mikutta et al., 2008; Schwertmann et al., 2005); therefore, ferrihydrite with coprecipitated OM have smaller crystal sizes and more crystallographic defects. Since ferrihydrite is formed usually in OM-rich environments coprecipitation is the more common process in nature (Eusterhues et al., 2008). The different properties of pure ferrihydrite and OM–ferrihydrite coprecipitates may lead to different behaviors during microbial reduction. Due to their smaller crystal size and more defective crystal structure, coprecipitates might faster dissolve. The associated organic material will change the mineral's surface properties, e.g., the surface charge, with consequences for the accessibility of Fe(III) to microbes, redox-active shuttling compounds, or extracellular enzymes.

Not only do the mechanisms through which OM interacts with Fe(III) oxides, e.g., adsorbed or coprecipitated, influence the reactivity of the mineral–OM complex, the composition of OM may also effect its reactivity. The redox reactivity of OM is attributed mostly to quinones. Past experiments have often been performed using humic acids (e.g., alkaline extracts of natural organic material), which usually contain a higher concentration of the aforementioned redox-active functional groups than natural OM from soil (Klüpfel et al., 2014; Lehmann and Kleber, 2015; Piepenbrock et al., 2014). Humic acids should therefore be more effective in electron shuttling than natural soil organic matter. Previous studies have shown that the presence of dissolved humic acids leads to complexation of Fe(II) (Royer et al., 2002), and complexation and dissolution of Fe(III) (Jones et al., 2009), but also potentially enhance Fe(III) reduction via electron shuttling (Hansel et al., 2004; Jiang and Kappler, 2008; Lovley et al., 1996; Roden et al., 2010). Furthermore, the concentrations of dissolved humic acid or the mineral / humic acid ratios have been shown by some studies to increase Fe(III) reduction rates while other studies have not reproduced this result (Amstaetter et al., 2012; Jiang and Kappler, 2008). Despite the aforementioned studies, the influence of mineral-bound OM on Fe(III) reduction rates and mineral transformation is not fully understood in regard to the broad diversity of Fe(III)-reducing microorganisms present in nature (Weber et al., 2006a, b).

Fe(III) oxides as well as OM–mineral complexes are not able to pass through the outer membrane of Fe(III)-reducing microorganisms; therefore, different electron transfer pathways have evolved to alleviate this physiological problem (Poggenburg et al., 2016; Weber et al., 2006b). Several mechanisms used as electron transfer pathways have been characterized, including (i) direct transfer of electrons via enzymes anchored to the outer membrane of the bacterial cell (Myers and Nealson, 1988; Nevin and Lovley, 2000) or conductive nanowires (El-Naggar et al., 2010; Gorby et al., 2006; Reguera et al., 2005) and (ii) mediated electron transfer via exogenous electron-shuttling compounds found in natural OM (Lovley et al., 1996), endogenous electron-shuttling compounds, for example flavins (von Canstein et al., 2008; Kotloski and Gralnick, 2013; Marsili et al., 2008; Nevin and Lovley, 2002; Newman and Kolter, 2000), or the use of Fe(III)-chelating compounds, such as siderophores (Kraemer, 2004; Lovley et al., 1994; Nevin and Lovley, 2002).

Shewanella and *Geobacter* species represent two of the best-studied model Fe(III)-reducing microorganisms. *Shewanella* species have been shown to utilize endogenous electrons shuttles, such as flavins, rather than transferring electron via direct contact between the mineral and the microbe (von Canstein et al., 2008; Kotloski and Gralnick, 2013). Not surprisingly, microbial reduction of Fe(III) oxides like nanometer-sized lepidocrocite and ferrihydrite by *Shewanella* species can be boosted by the presence of humic acids (Adhikari et al., 2017; Amstaetter et al., 2012; Pédrot et al., 2011; Shimizu et al., 2013) and quinone moieties (Newman and Kolter, 2000) when the amount of dissolved organic carbon is greater than a threshold concentration of 5–10 $\mathrm{mg\,C\,L^{-1}}$ (Jiang and Kappler, 2008). Conversely, a study by Eusterhues et al. (2014) reported that high C / Fe ratios in coprecipitated and adsorbed ferrihydrite complexes inhibited the reduction of ferrihydrite by *G. bremensis*. Poggenburg et al. (2016) produced ferrihydrite OM complexes with different organic materials (extracted extracellular polymeric substances, water extracts of soil litter) and used *S. putrefaciens* and *G. metallireducens* to reduce them. Their results confirmed that ferrihydrite-bound OM increases reduction rates of *Shewanella*, whereas it protects the mineral surface from reduction by *Geobacter*. Furthermore, this study found that reduction rates by *Shewanella* were correlated to the aromaticity of the mineral-bound OM. *Geobacter* species have been shown to use direct contact and conductive nanowire structures for electron transfer (Boesen and Nielsen, 2013; Malvankar et al., 2011). Unlike *Shewanella*, *Geobacter* does not produce endogenous electron-shuttling or chelating compounds (Nevin and Lovley, 2000); however, in the absence of Fe(III) oxides *Geobacter* is capable of reducing both humic acid (Jiang and Kappler, 2008; Lovley et al., 1996) and extracellular quinone moieties (Scott et al., 1998). Furthermore,

the decreased rates of Fe(III) reduction by *G. bremensis* in incubations containing OM–ferrihydrite complexes compared to pure ferrihydrite minerals suggest that *Geobacter* does not use external electron shuttles in natural environments rich in OM and Fe(III) oxides. The aforementioned results suggest the effects of OM on Fe(III) reduction rates vary between the two model organisms, *Shewanella* and *Geobacter*, presumably due to the various mechanisms of electron transfer used. Taken together, these studies indicate (i) whether OM on Fe(III) oxide mineral surfaces hinders or promotes Fe(III) oxide reduction depends on the microorganisms present and their Fe respiratory pathways; (ii) when electron shuttling is the dominant electron-accepting process, a high content of redox-active groups in the mineral-associated OM speeds up reduction.

In this study, we used the same experimental design described in the earlier study of Eusterhues et al. (2014) but replaced *G. bremensis* with *S. oneidensis* MR-1 to test the effect of increasing amounts of adsorbed and coprecipitated OM on microbial Fe(III) reduction. In a parallel set of incubation studies we used a microbial consortia as inoculum derived from an iron-rich peatland where the microbial reduction of Fe(III) is the dominant electron-accepting processes for the degradation of OM under anoxic conditions. Thus, examining the respiratory behavior of *S. oneidensis* MR-1 compared to a microbial consortia allows us to study the response of a diverse community of Fe(III) reducers living in a habitat rich in Fe(III) oxides and OM. We compared rates of Fe(III) reduction of ferrihydrite adsorbed and coprecipitated with OM with pure ferrihydrite and followed qualitative and quantitative changes in the microbial community during incubation. To our great surprise, rates observed with *Shewanella* nearly matched those observed with the complex consortia derived from Schlöppnerbrunnen fen, although *Shewanella* was not the dominant Fe(III)-reducing microorganism detected at the beginning and end of incubation.

2 Methods

2.1 Study site and sampling procedure

Peat cores were obtained from the Schlöppnerbrunnen fen (northern Bavaria, Germany; 50°07′55″ N, 11°52′52″ E) using a Pürkheimer soil corer in May 2016. This minerotrophic, slightly acidic (pH ∼ 5) fen has been previously described in detail (Blodau et al., 2004; Eusterhues et al., 2014; Hausmann et al., 2016; Küsel et al., 2008; Loy et al., 2004; Pester et al., 2012). Briefly, granite bedrock is covered by a Fibric Histosol, often referred to as peat, with a thickness between 40 and 70 cm. This fen is fed by anoxic, Fe(II)-rich groundwater (Küsel et al., 2008). The sampling site within the fen used in the current study (site "M") is positioned southwest of previously described drying–rewetting experimental plots (Knorr and Blodau, 2009; Reiche et al., 2008). Previous studies have reported that high concentrations of Fe(II) (up to 7.4 mM, mean 2001–2004 0.3 mM), dissolved organic carbon (DOC; up to 48 mM, mean 2001–2004 5.1 mM), and solid organic carbon (C_{org}; mean 37 %) are routinely found at site "M" (Küsel et al., 2008; Reiche et al., 2008). Peat cores were transported on ice under anoxic conditions (N_2) and peat samples were either processed or stored at −20 °C until use.

2.2 Microbial extraction from peat core samples

The peat microbial community was extracted using a standard protocol adapted to soil. Briefly, a 20 mL syringe with tip removed was used as a manual short core sampler to remove 20 mL fractions (∼ 25 g) from bulk peat cores and added to sterile, anoxic 125 mL serum bottles containing 100 mL of 0.85 % NaCl solution and 8 g sterile glass beads. The slurry was shaken at 4 °C overnight. Samples were transferred to 50 mL Falcon tubes and centrifuged at increasing speeds to observe sedimentation rates. The samples were initially centrifuged at 900 *g* for 5 min. The sample was decanted into a fresh Falcon tube and the supernatant was centrifuged again at 1200 *g* for 10 min. Next, the solid phase was resuspended in 10 mL anoxic 0.85 % NaCl solution. The extract, containing live organisms subsequently used for cultivation experiments, was transferred to 30 mL serum bottles and flushed with Ar to ensure anoxic conditions (Supplement Fig. S1). The presence of bacteria in the peat microbial extract was confirmed via SYTO (50 µM) staining and microscopy. The peat microbial extractions were performed in six replicates to ensure sufficient inoculum was available for subsequent incubations.

2.3 Synthesis of ferrihydrite and organic matter–mineral complexes

A water extract of the forest floor (Oa and Oe horizons) of a Podzol under spruce and two-line ferrihydrite was produced as previously described by Eusterhues et al. (2014). Briefly, the two-line ferrihydrite was produced via titration of 0.01 M $Fe(NO_3)_3$ solution with 0.1 M NaOH to pH 5 with constant stirring. Ferrihydrites with varying concentrations of adsorbed OM (AFh) were produced by mixing forest floor extract solutions containing increasing C concentrations with suspensions of freshly precipitated two-line ferrihydrite at pH 5. Molar C / Fe ratios of these initial solutions were AFhA 0.4, AFhB 1.3, and AFhD 4.2. Coprecipitated ferrihydrites (CFh) were produced by dissolving $Fe(NO_3)_3$ in forest floor extract solutions of increasing concentrations and adding 0.1 M NaOH under vigorous stirring until the solution reached pH 5. Molar C / Fe ratios of these initial solutions were CFhA 0.4, CFhB 1.3, and CFhD 4.2. The solid / solution ($g\,L^{-1}$) ratio for all synthesized minerals ranged between 0.3 and 1.6. The solid products were separated using centrifugation, washed two times with deion-

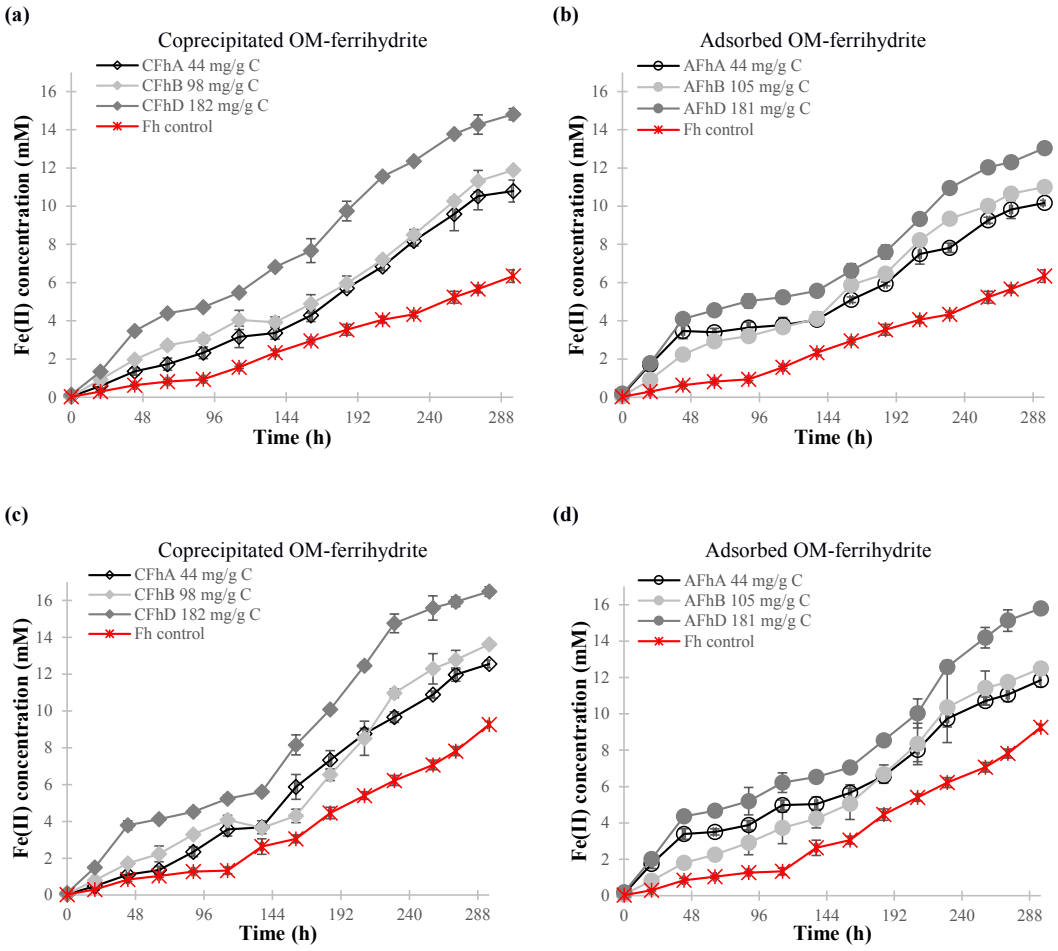

Figure 1. Microbial reduction of ferrihydrite and OM–ferrihydrite complexes by *Shewanella oneidensis* MR-1 and a microbial consortia extracted from peatland. Panels **(a)** and **(b)** depict *S. oneidensis* MR-1-mediated reduction of ferrihydrite (Fh control) and either coprecipitated OM–ferrihydrite (CFh) or adsorbed OM–ferrihydrite (AFh). Panels **(c)** and **(d)** depict reduction of ferrihydrite (Fh control) and either coprecipitated OM–ferrihydrite (CFh) or adsorbed OM–ferrihydrite (AFh) in incubations with a microbial consortia extracted from peatland. Error bars represent standard deviation in triplicate incubations.

ized H_2O, freeze-dried, and stored in Balch-type tubes under anoxic conditions (N_2). The C content was measured using a carbon–nitrogen (CN) analyzer (Vario EL, Elementar-Analysensysteme, Hanau, Germany) and the Fe content using inductively coupled plasma optical emission spectrometry (ICP-OES) after total digestion (Spectroflame, Spectro, Kleve, Germany). A characterization of the forest floor extract and ferrihydrite–OM complexes from Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) can be found in Eusterhues et al. (2014).

2.4 Microbial OM ferrihydrite reduction experiments

We used *S. oneidensis* MR-1 as model Fe(III)-reducer since it is well characterized and able to utilize a variety of carbon substrates as electron donors (Myers and Nealson, 1988, 1990; Venkateswaran et al., 1999). Liquid aerobic pre-cultures were grown in 50 mL of lysogeny broth (Luria Bertani medium; LB medium) containing 10 g of tryptone, 10 g of NaCl, and 5 g of yeast extract. These cultures were incubated on a rotary shaker at 150 rpm and the cell concentration was determined with optical density (OD) measurements at 600 nm. For the Fe(III) reduction experiments 2 mL of LB-grown pre-culture was harvested after 14 h at late exponential growth phase and centrifuged at 10 000 *g* for 5 min. Cells were washed twice with a defined medium, resuspended in 2 mL of medium, and diluted to a final concentration of 2×10^5 cells mL^{-1} in culture tubes, containing a defined medium. For all incubations a defined freshwater medium (pH 6.8; *Geobacter* medium ATCC 1957) containing 1.5 g L^{-1} NH_4Cl and 0.1 g L^{-1} KCl was used. After autoclaving and cooling under anoxic conditions (N_2 / CO_2, 80 / 20 v/v, atmosphere), 30 mL L^{-1} of 1 M $NaHCO_3$ (autoclaved, CO_2), 10 mL L^{-1} Wolfe's vitamin solution (ATCC, 1957), and 10 mL L^{-1} modified Wolfe's minerals (ATCC,

1957) were added. A combination of filter-sterilized (0.2 µm) electron donors (sodium acetate, 2 mM; sodium lactate, 2 mM; and glucose, 2 mM) were added as carbon sources to all incubations to ensure standardization. NaH_2PO_4 was not added to circumvent interaction of PO_4^{3-} with ferrihydrite.

In the current study the microbial community extracted from peat cores collected in the Schlöppnerbrunnen fen as well as *S. oneidensis* MR-1 were used as the inoculum in incubations containing either ferrihydrite, OM-adsorbed ferrihydrite, or OM-coprecipitated ferrihydrite. For OM-adsorbed ferrihydrite incubations, three different concentrations of organic matter were used: 44, 105, and 181 mg g^{-1} total organic carbon (TOC). Similarly, three different concentrations of organic matter were used in OM-coprecipitated ferrihydrite incubations: 44, 98, and 182 mg g^{-1} TOC.

10 mL medium was added to pre-sterilized (6 h, 180 °C) 21 mL hungate tubes containing pre-weighed ferrihydrite, OM-adsorbed ferrihydrite (TOC: 44, 105, 181 mg g^{-1} C), or OM-coprecipitated ferrihydrite (TOC: 44, 98, 182 mg g^{-1} C) with constant flushing (N_2). The concentration of Fe per tube ranged from 0.63 to 0.85 mmol. The hungate tubes were sealed with butyl rubber stoppers and flushed with sterile N_2 / CO_2 (80 % / 20 % v/v) for ∼ 2 h to ensure anoxic conditions. Either peat microbial extract (5 % v/v) or *S. oneidensis* MR-1 were used as inoculum in separate incubations containing ferrihydrite, OM-adsorbed ferrihydrite (TOC: 44, 98, 182 mg g^{-1}), or OM-coprecipitated ferrihydrite (TOC: 44, 105, 181 mg g^{-1}). All treatments were performed in triplicate and incubated horizontally at room temperature in the dark and shaken periodically. Fe(III) reduction was monitored approximately every 24 h using the phenanthroline assay (Tamura et al., 1974; data not shown). Fe(II) formation kinetics were used as analogues for Fe(III) reduction. Apparent initial reaction rates were estimated by fitting linear regression lines to Fe(II) (mM) versus time (in hours) for all data points. The slope of the line represents the initial reaction rate. At the end of incubation (∼ 12 days), solid remnants were freeze-dried and stored under N_2 until X-ray diffraction (XRD) measurements were performed (see description below).

2.5 DNA extraction

Genomic DNA was obtained from the peat microbial extract and from the incubation studies using the PowerSoil DNA isolation kit (MoBio Laboratories, Catalog # 12888-50, Carlsbad, CA, USA) according to manufacturer's instructions and stored at −20 °C. The DNA was extracted from three biological replicates at three different time points (T_0, T_{middle} – 136 h, T_{end} – 296 h) from all incubations inoculated with either peat microbial extract or *S. oneidensis* MR-1. DNA extracts were subsequently used as a template for quantitative PCR (qPCR) reactions targeting bacterial 16S rRNA genes to quantify abundance of bacteria over time or microbial community analysis via Illumina sequencing. Genomic DNA from samples taken at T_0 and T_{end} (296 h) were used for Illumina sequencing, while DNA from all three time points were used as the template for qPCR.

2.6 Quantitative PCR

Bacterial 16S rRNA gene copy and transcript numbers of the total peat microbial community were determined by quantitative PCR using an Mx3000P instrument (Agilent) with Maxima SYBR Green Master Mix (Fermentas). Bacterial 16S rRNA genes were amplified using primer pairs Bac8Fmod/Bac338Rabc (Daims et al., 1999; Loy et al., 2002) according to cycling conditions and standards previously described (Herrmann et al., 2012). *S. oneidensis* MR-1 16S rRNA gene copy numbers were determined with primer pairs She12F/She220R (Himmelheber et al., 2009). Two to 20 ng of genomic DNA was used as template. Standard curves were produced using serial dilutions of representative plasmid mixtures and were linear for both primer sets from 5×10^8 to 5×10^2 copies with R^2 values of 0.999–1.000, and the qPCR performed with efficiencies between 80 and 90 %. To monitor temporal transcriptional activity on subsamples from T_0, T_{middle} (136 h), and T_{end} (296 h), transcript numbers were normalized to copy numbers of the corresponding gene copy number per genome: 4.02 for bacterial 16S rRNA and 9 for *S. oneidensis* 16S rRNA genes (https://rrndb.umms.med.umich.edu, as retrieved on 13 June 2017). One-way analysis of variance (ANOVA) was used to compare treatments using the calculated quantification results and determine statistical significance ($p < 0.05$).

2.7 Illumina MiSeq Amplicon sequencing

16S rRNA gene-targeting amplicon sequencing was done using a bacteria-specific primer pair (341f [S-D-Bact-0341-b-S]/805r [S-D-Bact-0785-a-A]; Klindworth et al., 2013). Amplicon sequencing was performed by LGC Genomics GmbH (Berlin, Germany). PCR products were generated starting from about 5 ng of extracted genomic DNA extract, 15 pmol of forward and reverse primer in 20 µL of 1× MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline, Luckenwalde, Germany), and 2 µL of BioStabII PCR Enhancer (Sigma-Aldrich, Munich, Germany). For each sample, the forward and reverse primers had the same 10 nt barcode sequence. PCRs were carried out for 30 cycles using the following parameters: 96 °C for 2 min (pre-denaturation), 96 °C for 15 s, 50 °C for 30 s, and 70 °C for 90 s. DNA concentrations of amplicons of interest were quantified semi-quantitatively via gel electrophoresis. ∼ 20 ng amplicon DNA of each sample was pooled for up to 48 samples carrying different barcodes. If necessary PCRs showing low yields were further amplified for an additional five cycles. The amplicon pools were purified with one volume AMPure XP beads (Beckman Coulter, Krefeld, Germany) to remove

primer dimer and other small mispriming products, followed by an additional purification on MinElute columns (Qiagen, Düsseldorf, Germany). About 100 ng of each purified amplicon pool DNA was used to construct sequencing libraries using the Ovation Rapid DR Multiplex System 1–96 (NuGEN, Leek, Netherlands). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 chemistry (Illumina) in paired-end mode (2×300 bp). Amplicon sequencing was performed with genomic DNA samples taken at T_0 and T_{end} (296 h).

2.8 Microbiome profiling

Sequence characteristics were checked using prinseq (v. 0.20.4; Schmieder and Edwards, 2011). Adaptor sequences still present were subsequently removed with cutadapt (v. 1.7; Martin, 2011). Paired-end assembly and subsequent quality control were done using vsearch (v. 1.10.2; Rognes et al., 2016) using the implemented -fastq_mergepairs (-fastq_minmergelen = expected amplicon size) and -fastq_filter (-fastq_maxee = 0.5) functions. Chimera removal was done with UCHIME (v. 4.2.40; Edgar et al., 2011). Operational taxonomic unit (OTU) clustering with a defined threshold was omitted, instead sequences were dereplicated to streamline the data set. Deduced dereplicated sequences were treated as representative for sequence populations featuring an identity of 100 % (in the following referred to as OTU). After alpha diversity analyses, the data set was stripped by removing OTUs that were represented by less than three sequences. Relative abundances were calculated and standardized based on median sequencing depth across all samples. Data sets were not rarefied to prevent a loss of sensitivity in downstream sample-wise comparisons (McMurdie and Holmes, 2014). Sequences from this simplified data set were taxonomically assigned against SILVA (release 123, as of September 2016) using the UCLUST algorithm (Edgar, 2010) implemented in vsearch applying a minimum similarity of 90 %. An OTU table including determined taxonomic affiliations and available metadata was assembled for downstream analysis in QIIME (v. 1.9.2; Caporaso et al., 2010) and phyloseq (v. 1.10; McMurdie et al., 2013). Statistical analyses were carried out using the R statistical software framework (R Development Core Team, 2008) and the following packages: respective dependencies, phyloseq, vegan (v. 2.4-3; Oksanen et al., 2017), and DESeq2 (v. 1.10.1; Love et al., 2014). Differentially abundant taxonomic groups were identified in sample-wise comparisons assuming negative binomial distributions for taxa read count data and using Wald significance tests, and parametric fitting for determined dispersions. These reflect biological variation better than, for instance, Poisson distributions (Love et al., 2014) and thus prevent overestimation due to false positives.

2.9 Sequence data deposition

Generated amplicon sequencing data was deposited as raw data at NCBI SRA and are available under the following BioProject accession number: PRJNA391563.

2.10 X-ray diffraction (XRD) measurements

X-ray diffraction measurements were performed with a D8 Advance DaVinci diffractometer (Bruker AXS, Karlsruhe, Germany) using Cu-Kα radiation at 40 kV and 40 mA, scanning from 5 to 80°2Θ with increments of 0.02°2Θ. Mineral identification was based on the International Centre for Diffraction Data (ICDD) powder diffraction file (PDF) database of 2011 (ICDD, 2011). XRD measurements were performed on both the starting material and the end products from all incubation experiments.

3 Results

3.1 Microbial Fe(III) reduction by *Shewanella oneidensis* MR-1 and by the microbial consortia extracted from peat

We compared the Fe(III) reduction capacity of the model organism *S. oneidensis* MR-1 with a peat microbial consortia extracted from an anoxic iron-rich peatland for incubations containing pure ferrihydrite (Fh control), coprecipitated OM–ferrihydrite complexes (CFhA, 44 mg g^{-1} C; CFhB, 98 mg g^{-1} C; CFhD, 182 mg g^{-1} C), or adsorbed OM–ferrihydrite complexes (AFhA, 44 mg g^{-1} C; AFhB, 105 mg g^{-1} C; AFhD, 181 mg g^{-1} C). The Fe(III) reduction patterns of *S. oneidensis* MR-1 and the peat microbial consortia were nearly identical. Rates of Fe(III) reduction in incubations with the peat microbial consortia and *S. oneidensis* MR-1 increased with increasing TOC concentrations (and C / Fe ratios) regardless of whether adsorbed OM–ferrihydrite complexes or coprecipitated OM–ferrihydrite complexes were used as the terminal electron acceptor (Fig. 1, Table 1). The lowest Fe(III) reduction rate was observed in *S. oneidensis* MR-1 incubations with pure ferrihydrite with a reduction rate of 0.022 mmol h^{-1} compared to 0.031 mmol h^{-1} for incubations with our peat microbial consortia (Fig. 1, Table 1). The highest Fe(III) reduction rates were observed in incubations containing coprecipitated OM–ferrihydrite complexes, with increasing rates of reduction corresponding to increased C / Fe ratios (from 44 to 182 mg g^{-1} C). Similar patterns occurred in incubations with adsorbed OM–ferrihydrite complexes such that the higher the C content the higher the rate of Fe(III) reduction (from 44 to 181 mg g^{-1} C). For samples with the same C content, rates of Fe(III) reduction were slightly higher in incubations with peat microbial extract compared to *S. oneidensis* MR-1 (0.037–0.058 and 0.032–0.051 mmol h^{-1}, respectively; Fig. 1, Table 1). At the same time, rates of Fe(III) reduc-

tion were slightly higher for OM–ferrihydrite coprecipitates compared to OM–ferrihydrite adsorption complexes (0.038–0.058 mmol h^{-1} compared to 0.032–0.050 mmol h^{-1}) when all incubations are considered (Fig. 1, Table 1).

3.2 Quantitative changes of 16S rRNA genes in incubation experiments

We followed the abundance of bacterial 16S rRNA gene copies over time in incubations inoculated with either *S. oneidensis* MR-1 or the microbial consortia extracted from peat to determine potential effects of coprecipitated or adsorbed OM–ferrihydrite complexes on microbial abundances. In general, bacterial 16S rRNA copy numbers increased over time in all incubations with noticeable increases in gene abundances at the end of incubation and slight variations dependent on the TOC concentration of the OM coprecipitated or adsorbed to the surface of ferrihydrite (Fig. 2). In incubations with pure ferrihydrite, *S. oneidensis* MR-1 16S rRNA gene copy numbers increased 4 orders of magnitude from 7.90×10^7 cells mL^{-1} at T_0 to 1.99×10^{11} cells mL^{-1} at T_{endq} (296 h). *S. oneidensis* MR-1 16S rRNA gene copy numbers increased approximately 5 orders of magnitude in incubations with coprecipitated or adsorbed OM–ferrihydrite complexes with the highest C / Fe ratios (8.10×10^7 cells mL^{-1} at T_0 to 8.47×10^{12} cells mL^{-1} at T_{end} and 8.10×10^7 cells mL^{-1} at T_0 to 1.24×10^{12} cells mL^{-1} at T_{end}, respectively). In incubations with pure ferrihydrite the peat microbial consortia, bacterial 16S rRNA gene copy numbers only increased 2 orders of magnitude from 6.23×10^{10} cells mL^{-1} at T_0 to 2.02×10^{12} cells mL^{-1} at T_{end}. Bacterial 16S rRNA gene copy numbers increased roughly 3 orders of magnitude in incubations using the peat microbial consortia as inoculum and adsorbed OM–ferrihydrite complexes with the highest C / Fe ratios (6.23×10^{10} cells mL^{-1} at T_0 to 2.58×10^{13} cells mL^{-1} at T_{end}); however, the gene copy numbers increased approximately 4 orders of magnitude in incubations with coprecipitated OM–ferrihydrite with the highest C / Fe ratios (6.23×10^{10} cells mL^{-1} at T_0 to 1.36×10^{14} cells mL^{-1} at T_{end}, respectively). Thus, increases in gene copy numbers correlate to the increased Fe(III) reduction rates observed across all incubations with OM–ferrihydrite complexes and pure ferrihydrite.

3.3 Microbial community profiling of the microbial consortia in incubation experiments

Incubations with absorbed and coprecipitated OM–ferrihydrite and varying C / Fe ratios led to distinct shifts in microbial community structure when compared to pure ferrihydrite incubations and the initial microbial consortia extracted from peat (Figs. 3 and 4). Alpha diversity indices addressed intra-sample diversity either based on the observed number of taxa according to the applied OTU definition

Table 1. C and Fe concentrations and molar C / Fe ratios of ferrihydrite–organic matter associations and results of microbial reduction experiments (reduction rate k and Fe(II) concentration after 296.5 h of incubation as percent of total Fe).

					S. oneidensis MR-1			Microbial consortia			*G. bremensis**		
					Linear fit			Linear fit			Linear fit		
		C	Fe	C / Fe	k	r^2	Fe(II) at T_{end}	k	r^2	Fe(II) at T_{end}	k	r^2	Fe(II) at T_{end}
		$mg\,g^{-1}$	$mg\,g^{-1}$	$mol\,mol^{-1}$	$mM\,h^{-1}$		% of Fe	$mM\,h^{-1}$		% of Fe	$mM\,h^{-1}$		% of Fe
Control	Fh	–	560	–	0.022	0.98	10	0.031	0.95	15	0.119	0.96	59
Adsorbed OM	AFhA	44	527	0.39	0.032	0.96	15	0.037	0.97	17	0.148	0.96	55
	AFhB	105	468	1.04	0.038	0.98	14	0.044	0.97	16	0.096	0.96	35
	AFhD	181	342	2.46	0.041	0.97	16	0.050	0.96	19	0.088	0.94	32
Coprecip. OM	CFhA	44	498	0.41	0.038	0.97	17	0.046	0.98	19	0.142	0.93	66
	CFhB	98	428	1.06	0.039	0.97	17	0.048	0.95	20	0.142	0.97	49
	CFhD	182	299	2.83	0.051	0.99	20	0.058	0.97	22	0.091	0.95	39

* Data from Eusterhues et al. (2014).

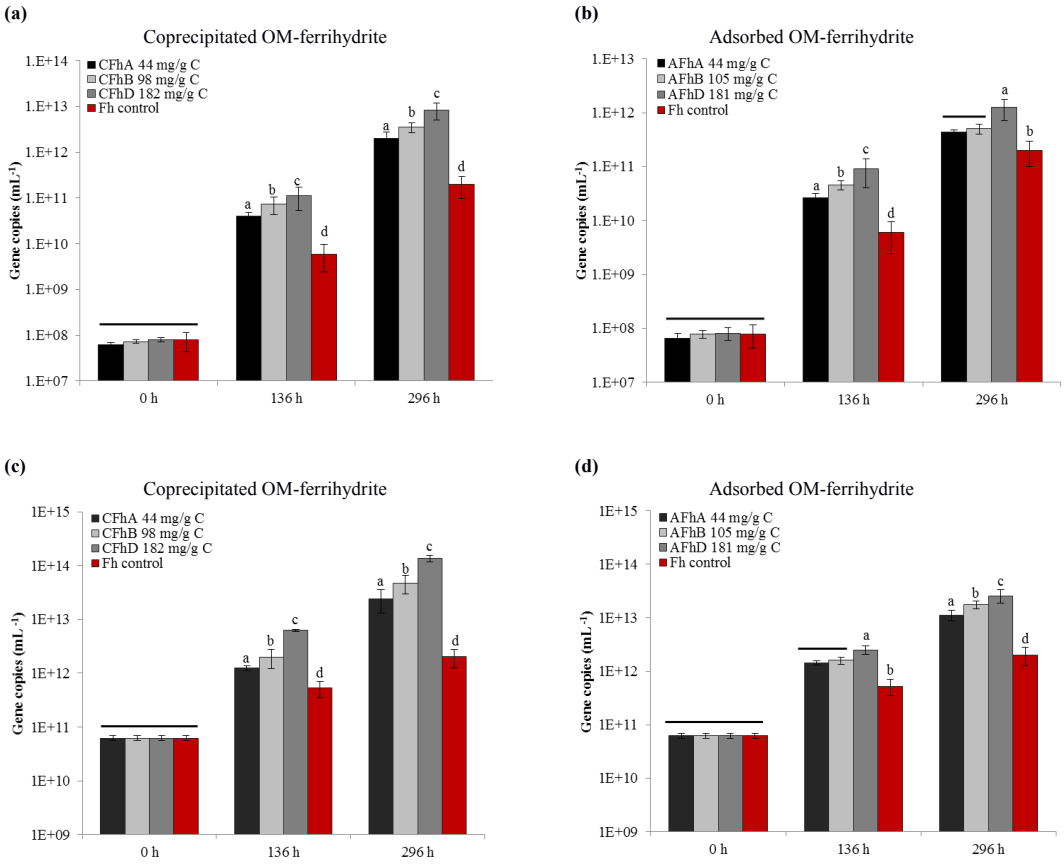

Figure 2. Abundances of bacterial 16S rRNA gene in subsamples taken from ferrihydrite incubations containing either *S. oneidensis* MR-1 or peat microbial consortia extracted from peat at T_0 (0 h), T_{middle} (136 h), and T_{endq} (296 h). Bars represent mean abundances of triplicate samples from *S. oneidensis* MR-1 incubations with coprecipitated OM–ferrihydrite (CFh) **(a)** or adsorbed OM–ferrihydrite (AFh) **(b)** and peat microbial consortia incubations with coprecipitated OM–ferrihydrite (CFh) **(c)** or adsorbed OM–ferrihydrite (AFh) **(d)**. Each sample was measured in triplicate qPCR reactions with applicable standard deviations. One-way ANOVA was applied to compare individual data sets for statistically significant differences between ferrihydrite incubations. Different letters (a, b, c, d) indicate statistically significant differences, while horizontal black lines indicate non-significant differences.

(observed OTUs) or based on extrapolations of observed taxa numbers putting special emphasis on rare taxa (Chao1). Comparing the different incubations with the initial peat microbial extract (observed OTUs: 52064, Chao1: 801599; except AFhB) revealed decreased diversities in all cases (observed OTUs: 23120-46770, Chao1: 334438-545083, ACE: 266190-455099) except AFhB (Fig. 4a). Intra-sample diversity measures were complemented by assessing the evenness of data sets by determining the entropy of respective data sets (Shannon, 1948). Respective values matched observed intra-sample diversities. Calculated Shannon indices showed strongly increased evenness for the initial peat microbial extract (Shannon: 9.47) against all incubations (Shannon: 6.77–7.48), excluding AFhB. Beta diversity analysis is a common approach for gaining insights into inter-sample diversity when dealing with complex data sets such as microbial community profiles (Fig. 4b). Corresponding analyses based on Jensen–Shannon divergence highlighted a minor clustering of microbial communities linked to coprecipitated OM–ferrihydrite incubations in the case of low and medium C / Fe ratios (CFhA, CFhB). Community profiles originating from incubations supplemented with OM–ferrihydrite having a high C / Fe ratio revealed a clustering independent of ferrihydrite complexation (AFhD, CFhD). The observation of community profiles from incubations clustered distinctly from communities present in the initial peat microbial extract matched the abovementioned differences with respect to intra-sample diversity and evenness (Fig. 4a).

Looking at underlying community profiles from a taxonomic perspective revealed a dominance of few phyla when considering determined community profiles as a whole (Fig. 3a). Dominant phyla included γ-Proteobacteria (57.9–95.4 % relative abundance), Firmicutes (3.1–41.6 %), and to a lesser extent Bacteroidetes (up to 0.6 %l AFhD, CFhD) and Acidobacteria (4.5 %; peat microbial extract). Looking at the taxonomic composition at a more resolved level (family-

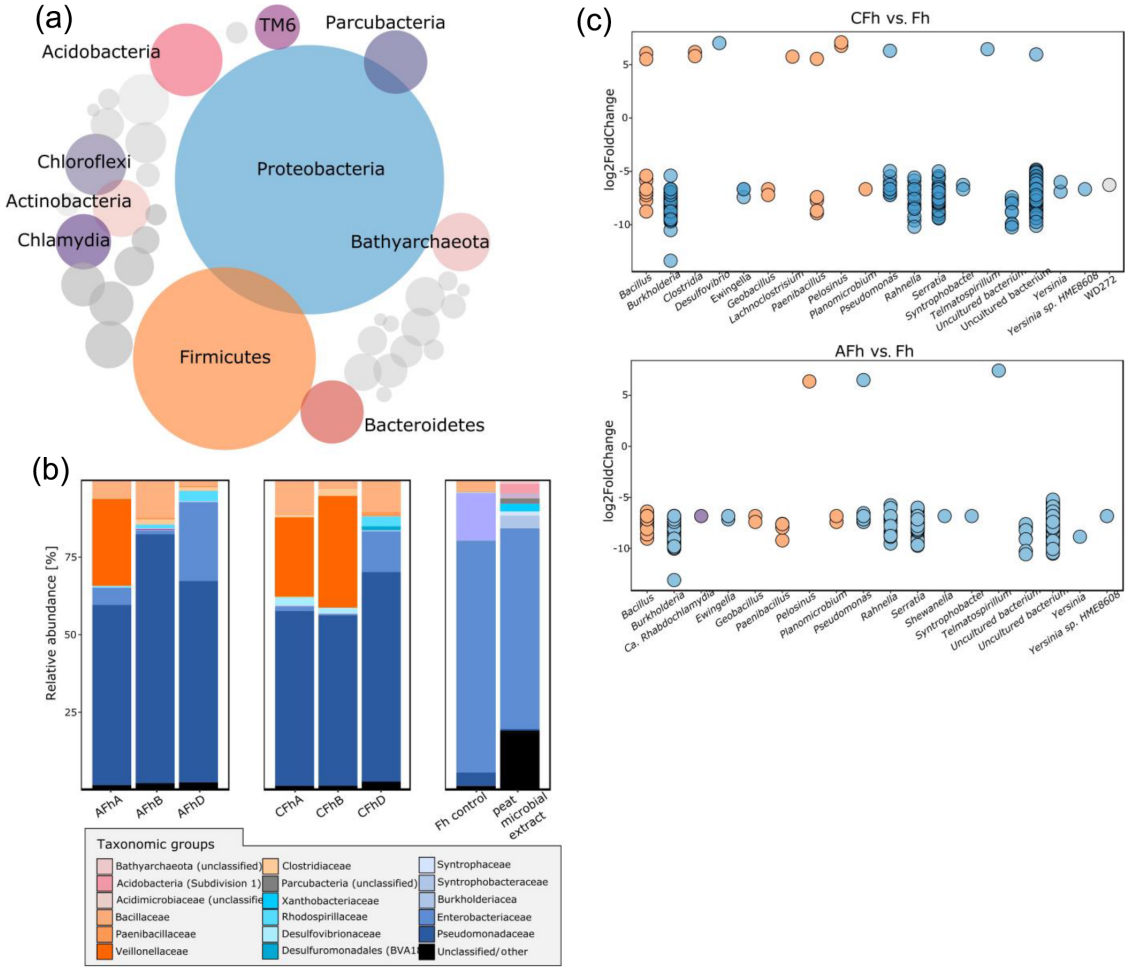

Figure 3. Microbial community profiling from incubations with coprecipitated OM–ferrihydrite (CFh) or adsorbed OM–ferrihydrite (AFh) compared to microbial communities with pure ferrihydrite (Fh control) at the end of incubation and microbial consortia extracted from peatland at beginning of incubation (peatland microbial extract). Bubble plot indicating the relative abundance of prokaryotic phyla when individual data sets are pooled **(a)**. Bubbles are sized according to respective relative abundances. The 10 most abundant phyla are highlighted by color. Grey bubbles refer to less abundant phyla. Family-level taxonomic profiles are given for individual data sets. Family-level groups showing abundances less than 1 %, and unclassified OTUs are summarized as "Unclassified/Other" **(b)**. Differentially abundant genus-level groups in sample-wise comparisons were identified assuming negative binomial distributions for read count data and applying Wald significance tests, and parametric fitting for determined dispersions. Tests were carried out using ferrihydrite complexation as decisive parameter. Multiple inference correction was done according to Benjamini-Hochberg (1995). Values of p less than 0.05 were considered significant **(c)**. The color scheme in panels **(b)** and **(c)** is according to panel **(a)**. Family-level groups belonging to the Firmicutes are for instance shown in shades of orange in the case of panel **(b)**. In panel **(b)**, AFhA-C refers to increasing TOC concentrations (44, 105, 181 mg g^{-1} C) in adsorbed OM–ferrihydrite incubations and CFhA-C refers to increasing TOC concentrations (TOC: 44, 98, 182 mg g^{-1} C) in incubations with coprecipitated ferrihydrite.

level) showed that the incubations carried out led in all cases to a strong increase of Pseudomonadaceae in relative abundance within the γ-Proteobacteria (e.g., AFhA 58.1 %, in comparison to peat microbial extract 5.3 %; Fig. 3b). Pronounced increases were also seen for Veillonellaceae (AFhA, CFhA, CFhB, 24.6–35.5 % in comparison to peat microbial extract < 0.00001 % and Fh, < 0.00001%), Bacillaceae (AFhA, AFhB, CFhA, 5.4–12.0 % in comparison to peat microbial extract < 0.00001 % and Fh, 3.6 %), and Desulfovibrionaceae (CFhA, CfhB, 1.8–2.5 % in comparison to peat microbial extract < 0.001% and Fh, < 0.00001%). Increased abundances of Veillonellaceae correlated with low and medium C / Fe ratios, while incubations with high C / Fe ratios favored elevated relative abundances of Enterobacteriaceae, when compared with low and medium C / Fe ratios. Groups that appeared to be enriched in the incubation with pure ferrihydrite, for example Burkholderiaceae, were found to show only minor abundances in all of the other setups.

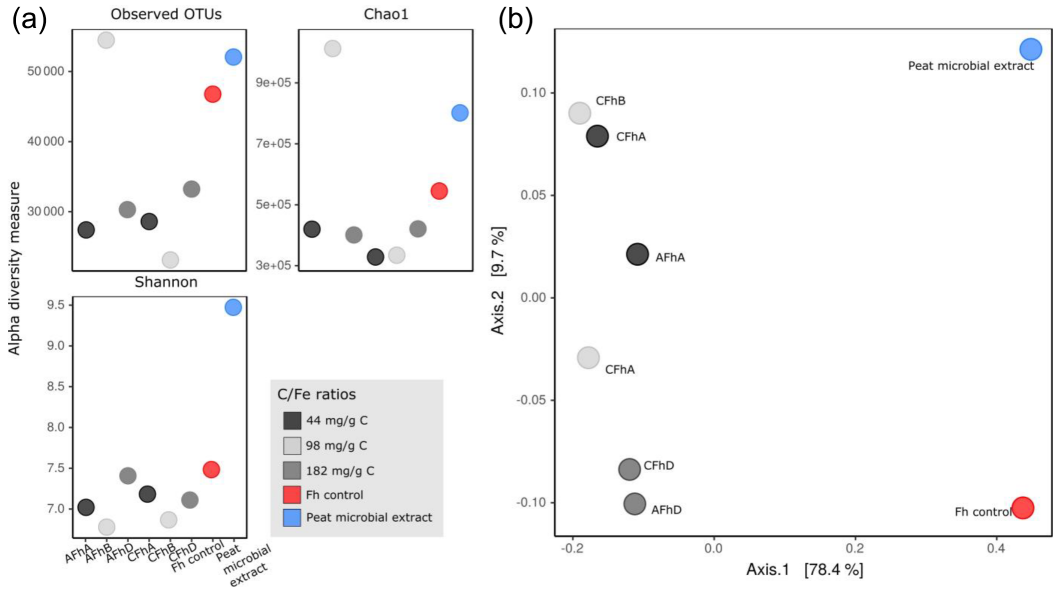

Figure 4. Alpha and beta diversity analyses. Alpha diversity analysis was done using various indices indicative for diversity (observed OTUs, Chao1) and evenness (Shannon) **(a)**. Alpha diversity in general describes within-data set diversity. Observed OTUs refers to OTU counts based on the OTU definition given in the materials and methods. Chao1 (Chao, 1984) is an index that puts special emphasis on rare taxa to extrapolate the real number of OTUs based on observed OTUs. The Shannon index (Shannon, 1948) describes the entropy in a community profile, and reaches its maximum when taxonomic groups are evenly distributed in a data set. Beta diversity (differences in diversity between samples) analysis was based on Jensen–Shannon divergences (Koren et al., 2013) **(b)**.

Considering the results from beta diversity analysis and community profiling, we were interested in assessing the differential abundance of genus-level taxonomic groups to check for the potential enrichment of taxonomic groups that are linked to iron cycling when comparing the pure ferrihydrite incubation and OM–ferrihydrite incubations. Differential abundances were examined by assuming negative binomial distributions for taxa read counts (Fig. 3c). It was apparent that numerous genus-level taxonomic groups were underrepresented when CFh and AFh are contrasted against Fh. This was consistent with reduced intra-sample diversities for adsorbed and coprecipitated incubations in comparison to the pure ferrihydrite incubation (Fig. 4a). Most underrepresented genus-level taxonomic groups were linked to Proteobacteria and especially Enterobacteriales/Enterobacteriaceae including *Ewingella*, *Rahnella*, *Serratia*, and *Yersinia*. This pattern was similar for CFh against Fh as well as AFh against Fh. In most cases log2 fold changes ranged between -5 and -10 for underrepresented groups. The number of over-represented taxonomic groups was in general lower than the number of underrepresented groups. This finding was much more pronounced for AFh vs. Fh than CFh vs. Fh. Only three over-represented genus-level taxonomic groups were identified for AFh vs. Fh, namely *Pelosinus*, *Pseudomonas*, and *Telmatospirillum* with log2 fold changes above 5. The same groups were also identified to be enriched in the case of CFh vs. Fh. However, overall the number of enriched taxonomic groups was higher including various Firmicutes such as *Bacillus*, *Clostridium*, *Lachnoclostridium*, and *Paenibacillus*. Few genus-level groups comprised over- and underrepresented OTUs. This was the case for *Bacillus*, *Paenibacillus*, and *Pseudomonas*. In the case of *Pseudomonas*, one particular OTU was found to be enriched. Consequently, this indicates that pronounced increases in the abundance of Pseudomonadaceae in adsorbed and coprecipitated OM–ferrihydrite are apparently mediated by a well-defined subpopulation of *Pseudomonas*.

3.4 XRD measurements

X-ray diffraction patterns of the starting material showed the two very broad peaks of ferrihydrite ($Fe_{10}O_{14}(OH)_2$). After reduction, diffractograms of the solid remnants still revealed the presence of ferrihydrite but additionally showed sharp peaks of different salts such as halite (NaCl), nahcolite ($NaHCO_3$), and salammoniac (NH_4Cl), which derived from the medium. Goethite (α-FeOOH), other Fe(III) oxides, or Fe(II) minerals were not found. The newly formed Fe(II) was either effectively chelated by the present organic material or dissolved Fe concentrations were too low to exceed the solubility product of Fe oxides or other common secondary Fe(II) minerals. The latter seems likely as the reduction rates of the present experiment were in fact low (0.022–0.058 mM h^{-1}; Table 1). In previous experiments, using the same OM–ferrihydrites, the same medium, but *Geobacter bremensis* as the model Fe(III) reducer, we detected goethite

and siderite for samples without OM or only low OM contents (Fh, AFhA, CFhA, CFhB), where reduction rates where high (0.119–0.148 mM h^{-1}). Similar reduction experiments reported no mineral transformation for OM ferrihydrites (Henneberry et al., 2012) or concluded that secondary mineral formation is depended on the OM content of the coprecipitates: Shimizu et al. (2013) observed goethite formation from pure ferrihydrite, while HA-ferrihydrites transformed into green rust and magnetite (Fe_3O_4) after reduction by *Shewanella* (Shimizu et al., 2013). Abiotic reduction of OM–ferrihydrite coprecipitates by aqueous Fe(II). Chen et al. (2015) found that OM hindered transformation into goethite and magnetite but favored transformation into lepidocrocite (γ-FeOOH). Likewise, Adhikari et al. (2017) reduced HA-ferrihydrite coprecipitates by *Shewanella* and found complete transformation into goethite and magnetite for pure ferrihydrite, while the amount of secondary minerals, goethite, magnetite. lepidocrocite, siderite ($FeCO_3$), and akaganeite (β-FeO(OH, Cl)), decreased with the content of humic acids in the coprecipitates.

4 Discussion

The ability of OM to serve as a terminal electron acceptor for microbial respiration in anoxic environments is of special importance and has been well characterized (Lovley et al., 1996). However, most experiments utilizing OM as a terminal electron acceptor have been performed with alkaline extracts: reduced humic acids have been shown to transfer electrons abiotically to terminal electron acceptors, such as metals, thereby functioning as electron shuttles between microbes and electron acceptors (Kappler et al., 2004). Humic acids therefore possess a redox-mediating ability which positively affects the cycling of redox-active minerals, including Fe(III) and Mn(IV) / Mn(III), in conjunction with transformation of organic and inorganic compounds. Subsequent studies have shown that reduced forms of humic substances can serve as electron donors for anaerobic organisms growing on a variety of terminal electron acceptors, including nitrate and fumarate (Coates et al., 2002). Under these conditions, microorganisms utilize the reduced humic substances as an energy source, while using a readily degradable carbon source, such as acetate, for growth. This unique ability to use humic substances as an electron donor provides a possible competitive advantage over other heterotrophs in the environment which are only capable of utilizing a single organic compound as both the carbon and energy source. Previous studies have revealed that some Fe(III)-reducing microorganisms, such as *S. alga*, *G. metallireducens*, and *Geothrix fermentans*, have the ability to undergo dissimilatory humic reduction and to couple reduced humic oxidation with nitrate reduction. Moreover, *Paracoccus denitrificans*, a known denitrifier, is unable to utilize humics or Fe(III) as a terminal electrons acceptors; however, these microorganisms are capable of using reduced humics as an electron donor for denitrification (Coates et al., 2002; Lovley et al., 1999).

This Schlöppnerbrunnen fen has been studied in great detail during the last 10 years; however, the impact of OM on the microbially mediated Fe(III) reduction processes in this site is not fully understood. Earlier incubation studies with peat soil from the Schlöppnerbrunnen fen demonstrated that amendment with each of these individual substrates (lactate, acetate, glucose) stimulates Fe(III) reduction, with glucose showing the most rapid increase of Fe(II) production (Küsel et al., 2008). Glucose was completely consumed within 3 days of incubation, yielding acetate and CO_2 as end products, and H_2 and ethanol as transient products. Supplemental lactate yielded acetate as a main product (Küsel et al., 2008). Both acetate and H_2 are preferred substrates for many Fe(III)-reducing microorganisms, including *Shewanella* and *Geobacter* species. Thus, amendment of incubations containing a microbial consortia extracted from Schlöppnerbrunnen fen with a substrate mixture comprised of the carbon sources used in the aforementioned study (i.e., lactate, acetate, glucose) and ferrihydrite as the terminal electron acceptor should augment growth of many peat microorganisms involved in different steps of anaerobic organic matter degradation using Fe(III) as terminal electron acceptor.

The current study provides clear evidence that the microbial community found in the fen not only has the potential to reduce Fe(III) oxides complexed with organic matter to the same extent as the model Fe(III) reducer, *S. oneidensis* MR-1, but that the microbes also increase in abundance under the experimental growth conditions used. We utilized a mixture of electron donors (glucose, lactate, and acetate) to stimulate a great variety of peat microorganisms capable of dissimilatory Fe(III) reduction including those that couple Fe(III) reduction with growth, but also fermenting microorganisms and others that just use Fe(III) oxides as electron sink (Hori et al., 2015; Lentini et al., 2012). Differences in the gene copy numbers at the end of the incubation suggested that not only did the supplemented substrates support growth but also that OM adsorbed or coprecipitated to the surface of the ferrihydrite minerals. Based on these results, we can infer that the mineral-associated OM was at least partially degradable and not totally protected by its association to minerals or by its molecular structure and size. Furthermore, it is likely that a portion of the ferrihydrite-associated OM became re-dissolved over the course of the incubation period, which may have facilitated its utilization by the microbes. Thus, the enhanced growth observed in both the *S. oneidensis* MR-1 and the microbial consortia can be attributed to the presence of additional complex carbon sources in the form of OM–ferrihydrite complexes compared to pure ferrihydrite.

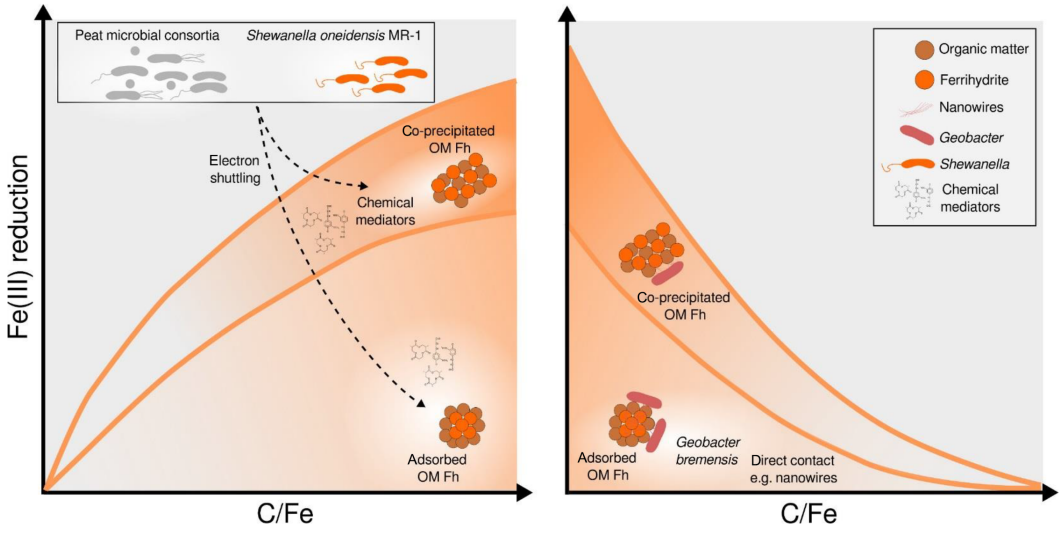

Figure 5. Microbial reduction of ferrihydrite with both adsorbed and coprecipitated OM by Shewanella oneidensis MR-1 and the microbial community consortia extracted from Schlöppnerbrunnen peat samples. For comparison, microbial reduction of ferrihydrite with both adsorbed and coprecipitated OM by *Geobacter bremensis* as described in Eusterhues et al. (2014) is depicted in the right panel.

4.1 Mechanisms of OM–ferrihydrite reduction by *S. oneidensis* MR-1 and the microbial consortia

The results of this study demonstrate that OM stimulates growth and Fe(III) reduction by *S. oneidensis* MR-1 and the microbial consortia. Fe(II) production increased as the C / Fe ratios increased in all incubations. Both the reduction rates and the total amount of Fe(II) produced at the end of incubation ($\sim$ 12 days) increased in the order Fh < adsorbed OM–ferrihydrite < coprecipitated ferrihydrite (Figs. 1 and 5 and Table 1). Samples containing coprecipitated OM–ferrihydrite complexes were more reactive than samples containing adsorbed OM–ferrihydrite complexes with similar OM loadings. The latter can be explained by a generally smaller crystal size of OM–ferrihydrite coprecipitates compared to pure ferrihydrite (Mikutta et al., 2008; Eusterhues et al., 2008), and was also observed during reduction by *Geobacter bremensis* (Eusterhues et al., 2014). The finding that mineral-associated OM may enhance ferrihydrite reduction supports the results presented in previous studies examining the role of humic acids as potential electron shuttles by *S. putrefaciens* under Fe(III)-reducing conditions, which showed that *S. putrefaciens* was capable of using humic acids as an electron shuttle to enhance Fe(III) reduction (Amstaetter et al., 2012; Shimizu et al., 2013). However, enhanced Fe(III) reduction was only observed when the concentration of organic material was high enough to overcome any slowing in the reduction processes caused by OM sorption to the surface reduction sites of the mineral (Amstaetter et al., 2012; Shimizu et al., 2013). Our study revealed that a water extract of natural soil organic matter, which has lower contents of aromatic moieties and usually lower electron-shuttling capacity to humic acids (Piepenbrock et al., 2014), can also provide enough electron-shuttling compounds to stimulate ferrihydrite reduction by *S. oneidensis* as well as by the peat microbial consortium.

Furthermore, since the *S. oneidensis* MR-1 pre-culture used in the present study was grown aerobically and the peat microbial consortia was resuspended in salt solution, we can assume no electron-shuttling molecules were secreted by the microorganisms themselves prior to inoculating the incubation experiments. Thus, the absence of any electron shuttles in our pure ferrihydrite incubations might explain the lowest Fe(III) reduction rates out of all the experiments conducted. However, we suggest the presence of electron-shuttling compounds, such as quinones, in the incubations with coprecipitated or adsorbed OM. Quinone moieties have been described as electron-shuttling compounds in previous studies (Scott et al., 1998). In our experiments even small contents of OM on ferrihydrite (44 mg g^{-1}, i.e., small molar C / Fe ratios of 0.4) led to increased reduction rates. This is in contrast to Shimizu et al. (2013) and Adhikari et al. (2017), who reported surface passivation at low C / Fe ratios, but increased reduction rates at molar ratios C / Fe > 1.8. A study by Poggenburg et al. (2016) reported enhanced Fe(III) reduction rates in incubations containing OM–ferrihydrite complexes with molar C / Fe ratios of 0.9–2.1 and using *S. putrefaciens* as model reducer. Here reduction rates differed mostly due to OM-composition, i.e., aromaticity. Lowest reduction rates were found for ferrihydrite coprecipitates produced with extracellular polymeric substances, while highest reduction rates were found for ferrihydrite coprecipitates produced with a water extract from Podzol litter. The latter is likely comparable in composition to the OM of our experiment. However, absolute reduction rates cannot be compared directly because

Poggenburg et al. (2016) attributed their high rates of Fe(III) reduction partly to the desorption of NOM from coprecipitates due to large concentration of phosphate in their nutrient solutions. In the present study, our C / Fe molar ratios ranged from 0.4 to 4.2, yet we observed an increase in Fe(III) reduction rates for all incubations containing either coprecipitated or adsorbed OM–ferrihydrite complexes compared to incubations with pure ferrihydrite.

In *Geobacter* sp., direct contact with oxide surfaces is required for Fe(III) reduction (Nevin and Lovley, 2000). *Geobacter* produces nanowires, typically described as conductive extracellular appendages, which facilitate electron transfer by serving as an electrical conduit to the Fe(III) oxide surface (Alves et al., 2016; Reguera et al., 2005). Conversely, *Shewanella* species are capable of using three primary strategies to allow for electron transfer between themselves and solid Fe(III) oxide surfaces via direct contact with the oxide surfaces, production of a complexing ligand (i.e., siderophore), and the utilization of endogenously or exogenously produced electron shuttles (Cooper et al., 2016; Melton et al., 2014; Shi et al., 2012). A previous study by Eusterhues et al. (2014) showed the rates of Fe(III) reduction by *G. bremensis* decreased as the concentrations of OM adsorbed or coprecipitated to the surface of ferrihydrite increased (Eusterhues et al., 2014). However, under identical reduction experiments and the same ferrihydrite material, Fe(III) reduction by *G. bremensis* resulted in considerably higher reduction rates (0.088–0.148 mM h^{-1}) compared to *S. oneidensis* (0.022–0.051 mM h^{-1}) and the microbial consortia (0.031–0.058 mM h^{-1}; Table 1, Eusterhues et al., 2014). Although favored at high concentrations of ferrihydrite-associated OM, electron shuttling is slower than direct electron transfer via nanowires. Similar results were reported in a study by Poggenburg et al. (2016), in which the rates of Fe(III) reduction by *G. metallireducens* were greatest with pure ferrihydrite compared to ferrihydrite coprecipitates produced with different natural organic materials. These findings suggest that the reduction rates are not only influenced by organic matter composition and the C / Fe molar ratios; instead, they also reflect species-dependent capabilities. It was hypothesized that OM may passivate Fe(III) oxide surfaces for direct contact requiring reducers such as *Geobacter*, whereas it may promote reduction by providing exogenous electron shuttles for non-direct contact requiring reducers such as *Shewanella* sp. (Eusterhues et al., 2014). In the current study, we observed a similar behavior for *S. oneidensis* and the complex microbial consortia extracted from peatland suggesting that microorganisms not requiring direct contact dominate reduction in the anoxic, Fe-rich fen, making use of the exogenous electron-shuttling compounds of the natural OM (Fig. 5). The similarity of the patterns observed point to the suitability of *S. oneidensis* as model organism to study the effect of OM on ferrihydrite reduction in natural environments.

4.2 Potential key players of OM–ferrihydrite reduction

Though the microbial consortia behaves similarly to *S. oneidensis* MR-1 with regard to Fe(III) reduction capacities and growth over time, the community profiles at the end of incubation did not reveal an enrichment of *Shewanella* species. Differential abundance analyses between adsorbed OM–ferrihydrite incubations and the pure ferrihydrite incubation even revealed reduced abundances for *Shewanella* in the case of adsorbed OM–ferrihydrite incubations. The enriched community is indeed comprised of microorganisms capable of reducing Fe(III) oxides. However, *Shewanella* sp. and *Geobacter* sp. did not respond to these growth conditions and have noticeably low abundance. But the lack of enrichment of typical dissimilatory Fe(III)-reducer cannot be explained by the lack of suitable electron donors. Previous work using ^{13}C-labeled acetate in paddy soils revealed that *Geobacter*-related organisms, in particular, respond to acetate amendment (Hori et al., 2010).

We saw distinct changes in microbial community structure over time as reflected by alpha and beta diversity analyses which appeared to be linked to several main drivers shaping the microbial communities in the incubations. The unexpectedly high alpha diversity observed for the adsorbed OM–ferrihydrite incubation featuring a medium C / Fe ratio (AFhB) is a result of overestimating diversity, due to a high frequency of singletons (OTUs that are only represented by single sequences). The overall anaerobic incubation conditions with ferrihydrite offered as terminal electron acceptor and a mixture of the carbon substrates glucose, lactate, and acetate activated only a subset of the original community extracted from peat. Fermentative Enterobacteriaceae (*Ewingella*, *Rahnella*, *Serratia*) were over-represented in the pure ferrihydrite incubation which is reflective of their ability to use glucose as carbon source and available Fe(III) as electron sink. Enterobacteriaceae are known for their mixed-acid fermentation profile similar to the products observed in earlier peat soil incubation studies amended with glucose (Reiche et al., 2008). The question of whether Fe(III) can be also used for energy conservation by these bacteria remains unanswered. Early work by Ottow (1970) suggested *Serratia* to be involved in hematite reduction. The strong reduction in their relative abundance in OM–ferrihydrite incubations is presumably a result of Fe(III) being less accessible.

Community profiles of OM–ferrihydrite incubations differed tremendously from the one of the pure ferrihydrite incubation. Pseudomonadaceae were highly enriched in all OM–ferrihydrite incubations. The enrichment of Veillonellaceae in adsorbed and coprecipitated OM–ferrihydrite incubations with low C concentrations could be mostly attributed to *Pelosinus*. *Pelosinus* spp. have been characterized as fermentative bacteria which utilize Fe(III) as an electron sink (Shelobolina et al., 2007). These bacteria can use H_2 as an electron donor and acetate as a carbon source, and are also capable of fermenting citrate, lactate, and organic impurities.

Pelosinus has also been shown to reduce small concentrations of anthraquinone-2.6-disulfonate (AQDS), a synthetic quinone, in the presence of citrate or lactate (Shelobolina et al., 2007). The ability of these microorganisms to use AQDS as an electron sink under reducing conditions might explain their enrichment during our incubations. Quinone moieties are ubiquitous in soils and peat, thus providing the perfect breeding ground for these microorganisms.

Interestingly, Fe(III) reduction was previously found to be correlated with the presence of Desulfovibrionaceae, when lactate is available as carbon source (Lentini et al., 2012). This partially matches our observations for incubations with coprecipitated ferrihydrite where *Desulfovibrio* was enriched at low and medium C / Fe ratios. However, *Desulfovibrio* was nearly absent in adsorbed OM–ferrihydrite incubations. Differences in Fe(III) accessibility in adsorbed and coprecipitated OM–ferrihydrite or the higher solubility of coprecipitates might explain this difference in community structure. The presence and ecological role of sulfate-reducing bacteria in the Schlöppnerbrunnen fen has been documented several times (Loy et al., 2004; Pester et al., 2010). This acidic (pH 4), low-sulfate (20–200 µM) peatland shows significant sulfate-reducing capacities (Loy et al., 2004) which is explained by a rapid recycling mechanism (Küsel et al., 2008). Recycling of sulfate can proceed through the aerobic oxidation of sulfide, e.g., in regions where oxygen penetration and anoxic micro-niches overlap (Knorr and Blodau, 2009). The sulfate-reducing activity is mainly attributed to a member of the "rare biosphere" in the peat, a *Desulfosporosinus* species (Pester et al., 2010). Our study suggests that other members of the sulfate reducers are involved in Fe(III) reduction in this fen to overcome the temporal and/or spatial limitation of the terminal electron acceptor sulfate.

5 Summary and conclusions

In the present study mineral-bound soil OM is shown to increase microbial Fe(III) reduction by *S. oneidensis* MR-1 and by a microbial consortia extracted from Schlöppnerbrunnen fen peat cores. The reactivity of ferrihydrites with adsorbed OM differed from ferrihydrites with coprecipitated OM such that higher extents of Fe(III) reduction were observed for coprecipitated ferrihydrites in comparison to adsorbed ferrihydrites. This can be explained by the smaller crystal size and more defective crystal structure of coprecipitated ferrihydrites compared to normal ferrihydrites. The fact that reduction rates for both *S. oneidensis* MR-1 and the microbial consortia increased systematically with increasing contents of OM in the OM–ferrihydrite complexes tempts us to speculate how reduction rates are related to electron transfer mechanisms. *Shewanella* spp. are capable of reducing Fe(III) oxides using one of three mechanisms known for reductions: direct contact, electron shuttles, or by production of complexing ligands. Thus, the enhanced Fe(III) reduction rates led us to the conclusion that electron shuttling using exogenous moieties of the mineral-bound OM is likely the preferred mechanism used for reduction (Fig. 5). Since coprecipitation of OM to mineral surfaces is a common process in nature, we thus hypothesize that electron-shuttling mechanisms are the dominant mechanism for microbial Fe(III) reduction in environments with high concentration of OM, such as soils, sediments, and peat.

Experiments with the microbial consortia extracted from peat showed that the presence of mineral-associated OM influenced the microbial community composition: whereas Enterobacteriaceae and Burkholderiaceae dominated in incubations with pure ferrihydrite, Pseudomonadaceae were the dominant microbes present when ferrihydrites were (partly) covered by OM. When ferrihydrites had low organic matter loadings Veillonellaceae occurred in relatively high concentrations; conversely, Rhodospirillaceae are found only in samples with high OM contents. In incubations with OM–mineral complexes, dominant microbes enriched included *Desulfobacteria* and *Pelosinus* sp., both of which can utilize lactate and acetate as an electron donor under Fe(III)-reducing conditions.

Thus, our observations imply OM coverage does not protect the underlying Fe mineral from microbial reduction in soils dominated by microorganisms that predominantly use electron shuttles or ligands for Fe(III) reduction. This study also revealed that natural soil OM on the surface of ferrihydrites provides enough redox-active groups to maintain microbial Fe(III) reduction processes, instead of passivating the surface. At the same time, the OM enhanced overall microbial growth and shaped the microbial community structure.

Data availability. Generated amplicon sequencing data can be found as raw data at NCBI SRA and are available under the following BioProject accession number: PRJNA391563.

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Author contributions. KE, KUT, and KK designed the experiments. REC performed the experiments. KE synthesized the pure ferrihydrite and OM–ferrihydrite complexes. CEW carried out sequence analyses. REC prepared the manuscript with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

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