nature geoscience

LETTERS

PUBLISHED ONLINE: 23 MAY 2010 | DOI: 10.1038/NGEO870

Extracellular electron transfer through microbial reduction of solid-phase humic substances

Eric E. Roden[1]*, Andreas Kappler[2], Iris Bauer[2], Jie Jiang[2], Andrea Paul[3], Reinhard Stoesser[4], Hiromi Konishi[1] and Huifang Xu[1]

The decay of soil and sedimentary organic matter yields organic compounds with a high molecular weight, termed humic substances[1]. Microorganisms can transfer electrons to dissolved humic substances[2], and reduced humic substances can rapidly reduce iron(III) oxides[3]. Thus, dissolved humic substances can serve as electron shuttles that promote iron(III) oxide reduction in sediments[2,4]. However, most humic substances in soils and sediments are in particulate, rather than dissolved, form[1]; the ability of microorganisms to reduce solid-phase humics and their capacity to shuttle electrons is thus far unknown. Here we show through incubation experiments and electron spin resonance measurements that iron(III)-oxide-reducing bacteria can transfer electrons to solid-phase humic substances in sediments sampled from wetlands. Although the electron-accepting capacity of the solid-phase humics was modest, solid-phase humics significantly accelerated iron(III) oxide reduction, by shuttling electrons from bacteria to oxide surfaces. Microbial solid-phase humics reduction represents a new mechanism for extracellular electron transfer that can facilitate reduction of iron(III) oxide and other redox reactions in sediments and soils.

Microbial reduction of solid-phase Fe and Mn oxides is an important pathway for organic matter oxidation and other biogeochemical processes in sedimentary environments[5,6]. A previous study documented changes in the relative abundance of oxidized and reduced solid-phase (0.1 M NaOH-extractable) humic substances (abbreviated hereafter as HS_{solid}) with depth in lake sediments[7]. These results provided the first evidence that HS_{solid} are subject to reduction in sediments in a manner analogous to Fe and Mn oxides. The depth distribution of HS_{solid} redox speciation was correlated with that of reactive (1 M HCl-extractable) Fe, as well as the abundance of culturable humic-acid- and Fe(III)-oxide-reducing bacteria. These results indicated a role for dissimilatory Fe(III)-reducing bacteria (FeRB) in HS_{solid} reduction in the sediment. This idea is consistent with the ability of FeRB to reduce dissolved humics[2], the suggestion that HS_{solid} can react in aqueous suspension as if their redox-active surface groups were present in solution[8] and the finding that insoluble activated carbon can serve as an electron acceptor and redox mediator for anaerobic microorganisms[9]. Experiments are required, however, to explicitly determine whether FeRB can reduce HS_{solid}.

We evaluated the ability of two well-known FeRB, *Geobacter sulfurreducens* and *Shewanella putrefaciens*, to transfer electrons to HS_{solid} in Fe-stripped sediments from a freshwater wetland in the Talladega National Forest in Alabama, USA (see the

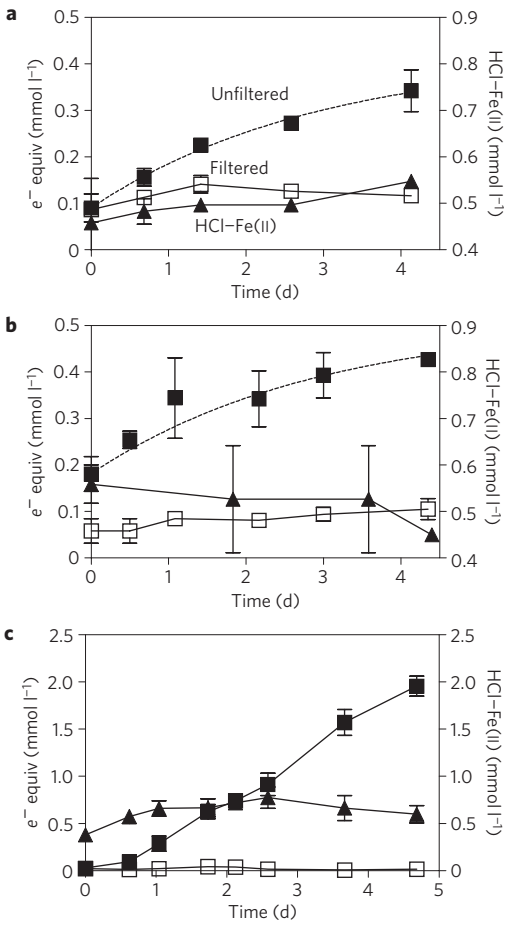

Figure 1 | Microbial and chemical reduction of Fe-stripped Talladega Wetland surface sediment. a, Reduction by acetate/fumarate-grown *G. sulfurreducens* strain PCA cells. **b**, Reduction by lactate/fumarate-grown *S. putrefaciens* strain CN32 cells. **c**, Abiotic reduction by H_2/Pd. Reducing equivalents (given in e- equiv) in unfiltered and filtered samples refer to sediment suspensions analysed by the shuttling assay (see Supplementary Fig. S1) without and with filtration, respectively. HCl-Fe(II) refers to unfiltered samples analysed by 0.5 M HCl extraction followed by determination of Fe(II) with ferrozine. The dashed lines in **a** and **b** show nonlinear least-squares regression fits to a first-order rate law (see legend for Supplementary Fig. S7).

[1]Department of Geoscience, University of Wisconsin-Madison, 1215 W. Dayton St., Madison, Wisconsin 53706, USA, [2]Geomicrobiology, Center for Applied Geoscience, Eberhard-Karls-University of Tuebingen, Sigwartstrasse 10, 72076 Tuebingen, Germany, [3]Division I.4 Process Analytical Technology, BAM Federal Institute of Materials Research and Testing, Richard-Willstaetter-Str. 11, 12489 Berlin, Germany, [4]Department of Chemistry, Humboldt University Berlin, Unter den Linden 6, 10099 Berlin, Germany. *e-mail: eroden@geology.wisc.edu.

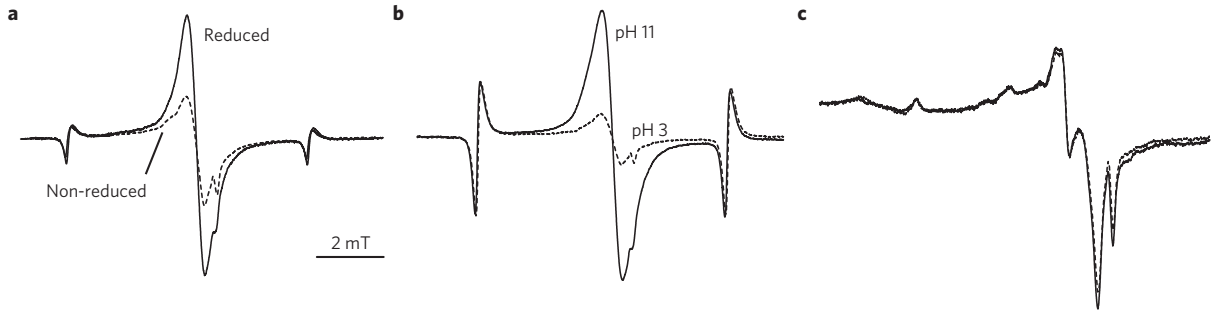

Figure 2 | ESR spectra of reduced and non-reduced Fe-stripped Talladega Wetland surface sediment. a, H_2/Pd-reduced (solid line) and non-reduced (dashed line) sediment at pH 7. **b**, Reduced sediment at pH 3 (dashed line) and 11 (solid line). **c**, The clay mineral illite before (dashed line) and after (solid line) exposure to H_2/Pd. The peak in the centre of the spectra represents an overlay of both organic radicals and paramagnetic centres originating from inorganic compounds. In **a** and **b**, the two smaller peaks to the left and right of the organic radical peak are from a manganese calibration standard.

Methods section and Fig. 1a,b). Both organisms produced solid-phase-reducing equivalents detected using an electron shuttling assay (Supplementary Fig. S1) analogous to that used in studies of dissolved humic substance reduction[2]. Only minor electron equivalents were recovered in filtered samples, suggesting direct electron transfer to solid-phase organics in the Talladega Wetland sediment. There was no significant accumulation of dilute HCl-extractable Fe(II), which indicated an absence of Fe(III) reduction activity in the Fe-stripped sediment[10]. No electron equivalents were produced in the absence of FeRB, or in sediment-free cultures (Supplementary Fig. S2). The last result proves that FeRB did not produce electron equivalents during the about 1 min shuttling assay. Linkage between FeRB activity and HS_{solid} reduction was demonstrated in 2-^{14}C-acetate experiments, where accumulation of $^{14}CO_2$ occurred only in the presence of both Fe-stripped Talladega Wetland sediment and *G. sulfurreducens* cells (Supplementary Fig. S3). There was only minor accumulation of electron equivalents during exposure of Fe-stripped sediment to fermentative metabolism by *Escherichia coli* (data not shown); thus, the mere onset of low redox potential conditions was not responsible for HS_{solid} reduction[5]. Exposure of the sediment to H_2 in the presence of Pd catalyst, however, readily reduced HS_{solid} (Fig. 1c), as observed previously for dissolved humic substances[7].

Electron spin resonance (ESR) spectroscopy was used to evaluate whether quinone moieties were involved in HS_{solid} reduction in a manner analogous to that demonstrated for dissolved humic substances[11,12]. The ESR signal in the range of the magnetic field where organic radicals typically appear ($g \approx 2$) increased during reduction by H_2/Pd (Fig. 2a), suggesting that electrons were transferred to quinone moieties. However, paramagnetic centres originating from phyllosilicates may also be observed in this spectral region[13]. Further ESR measurements were carried out on dry sediments that had been adjusted at different pH values because semiquinone-type radicals, in contrast to phyllosilicate centres, exhibit pH dependence with an increase of signal intensity at higher pH. Sediment adjusted to pH 11 showed a pronounced increase in radical intensity compared with pH 3 sediments (Fig. 2b), indicating that quinone moieties were responsible for the observed signal increase[12,14]. In contrast, ESR measurements of purified clay mineral illite showed no changes in radical signals on exposure to H_2/Pd (Fig. 2c); the organic radical signal was thus not directly influenced by silicate phases in the sediment. Although these results are consistent with the involvement of quinones in HS_{solid} reduction, we cannot rule out contributions from other types of redox-active site (for example, thiols, disulphides or nitrogen functional groups) that would have been detected by the electron shuttling assay[15]. Several other Fe-stripped sediments contained microbially and chemically reducible HS_{solid} (Supplementary Fig. S4). In all cases most of the electron-accepting capacity was contained in the solid

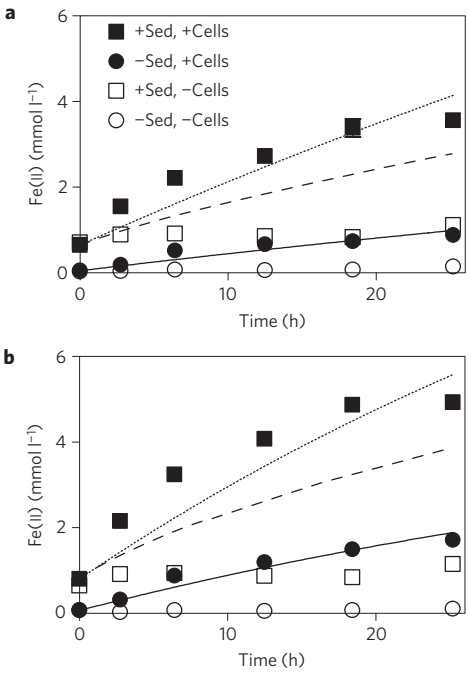

Figure 3 | Microbial reduction of synthetic Fe(III)$_{am}$ by FeRB in the presence and absence of freeze-dried Talladega Wetland surface sediment. a, Reduction by lactate/fumarate-grown *S. putrefaciens* cells. **b**, Reduction by acetate/fumarate-grown *G. sulfurreducens* cells. The open symbols show data for no-cells or no-sediment controls. The solid lines show nonlinear least-squares regression fits of the '−Sed, +Cells' data to a first-order rate law (see legend for Supplementary Fig. S7). The dotted and dashed lines show results of numerical simulations (see text and Supplementary Fig. S9).

phase (Supplementary Fig. S5). The extent of chemical reduction was about twofold higher than microbial reduction, which suggests that H_2/Pd was a more efficient reductant for HS_{solid}, in contrast to dissolved humic substances where chemical and microbial reduction lead to the same extent of reduction[16]. ESR analysis of a subset of these sediments verified that organic radical content increased during chemical reduction (Supplementary Fig. S6).

The mass-normalized electron-accepting capacity of the various HS_{solid}-containing materials was 10–50-fold lower than that of isolated humic and fulvic acids[11,15]. This makes sense given the roughly 10-fold lower organic carbon and nitrogen content of the sediments. However, even organic-rich peat from the Okefenokee Swamp and commercially available humus (40–50% organic carbon) had microbial electron-accepting capacities only

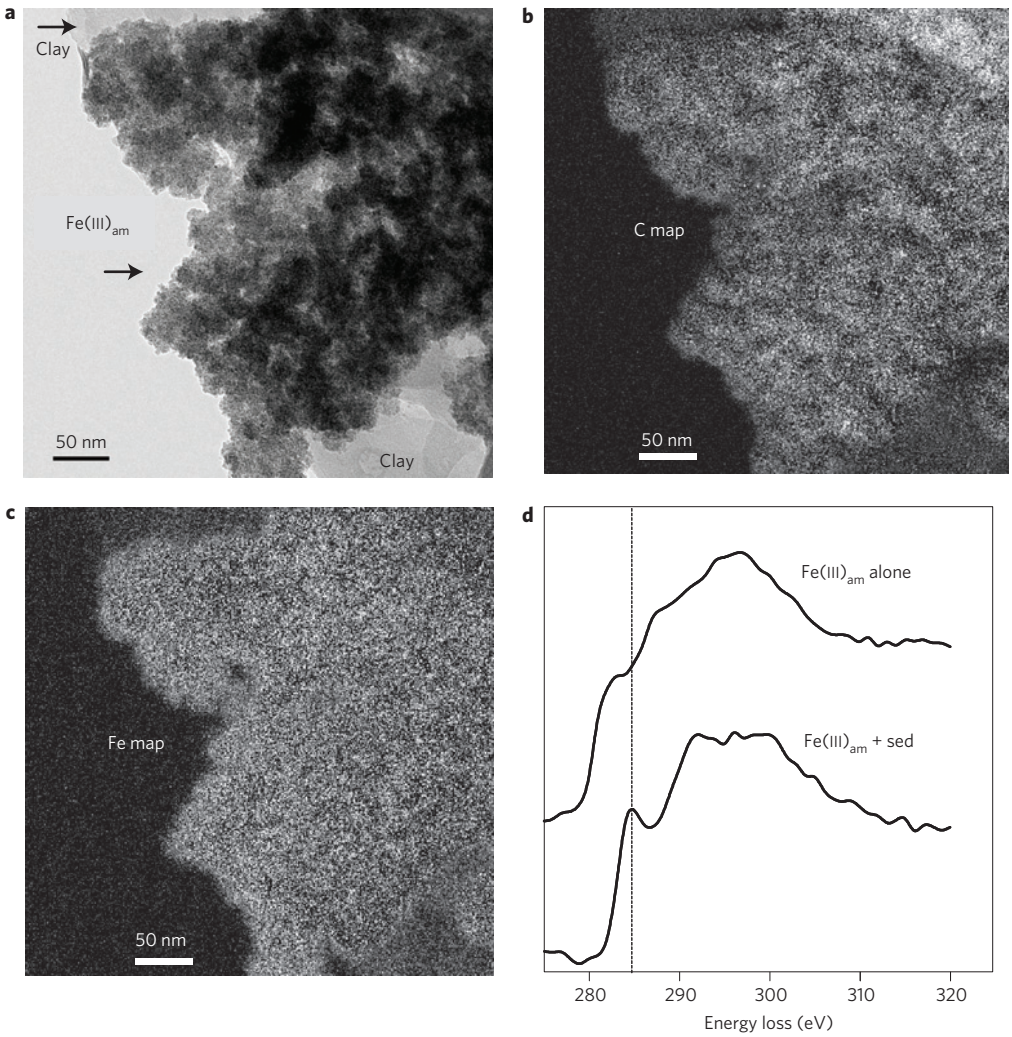

Figure 4 | TEM analysis of Fe(III)$_{am}$/Fe-stripped Talladega Wetland sediment mixture. a, Zero-energy-loss TEM image. **b**,**c**, EFTEM carbon and iron maps. **d**, EELS spectra of Fe(III)$_{am}$/sediment mixture versus Fe(III)$_{am}$ alone. **b** and **c** demonstrate the association of carbon with Fe(III)$_{am}$ on smectite clay plates. Note the absence of carbon on clay plates without associated Fe(III) oxide (upper left and lower right). The carbon film used to support the colloidal sample is visible in the upper right hand corner of the carbon map. The peak at 284.8 eV in **d** indicates the presence of 1s-π^*C = C bonds in aromatic moieties.

2–3-fold higher than most of the sediments examined here (see Supplementary Information). These findings indicate that most organic carbon in organic-rich soils and sediments (that is, humins[1]) is not redox active (see Supplementary Information for calculations), and signal caution regarding speculations that HS$_{solid}$ could play a major role in mediating (either directly or indirectly through inorganic sulphur cycling[17]) anaerobic carbon flow in deeply buried sediments[18,19].

Microbial HS$_{solid}$ reduction does, however, have major implications for transformations of inorganic and organic natural compounds and contaminants that are reactive towards reduced quinones[14,20,21]. Our results showed that the abundance of redox-active HS$_{solid}$ was much higher than dissolved humic substances in equilibrium with the sediments in aqueous suspension (Supplementary Fig. S5). Recent data suggest that even organic-rich peat sediments with a relatively high dissolved organic carbon concentration having the maximum electron-accepting capacity demonstrated so far for dissolved humics[17] would be expected to have only a few tenths of a millimole per litre of bulk dissolved electron-accepting capacity, about 10-fold lower than bulk solid-phase electron-accepting capacities estimated from the data in Supplementary Fig. S5 (see Supplementary Information for calculations). If reduced HS$_{solid}$ has intrinsic reactivity comparable to dissolved humic substances (see below), then HS$_{solid}$ may play a key but thus far unrecognized role in electron transfer reactions in soils and sediments. This conclusion is consistent with the suggested role of HS$_{solid}$ compounds in promoting reductive dechlorination of carbon tetrachloride by FeRB (ref. 22).

Experiments with acetate-oxidizing FeRB from Talladega Wetland surface sediment (see the Methods section) showed that HS$_{solid}$ in Talladega Wetland sediments accelerated synthetic amorphous Fe(III) oxide (Fe(III)$_{am}$) reduction as much as the known electron shuttling compound[2] anthraquinone-2,6-disulphonate, and more than surface sediment pore water (dissolved organic carbon about 30 mg C l^{-1}; Supplementary Fig. S7). Freeze-dried surface sediment that had been washed twice with distilled water stimulated reduction as much as unwashed material, but the wash water (which contained about 100 mg C l^{-1}) had no effect. Combustion of freeze-dried surface Talladega Wetland sediment completely eliminated its stimulatory effect on Fe(III)$_{am}$ reduction. Collectively, these results suggested that HS$_{solid}$ in the sediment was responsible for accelerating Fe(III)$_{am}$ reduction in a manner analogous to dissolved humic substances[2]. These findings agree with the report that addition of soil can accelerate synthetic Fe(III)$_{am}$ reduction[23].

Experiments with *G. sulfurreducens* and *S. putrefaciens* confirmed that modest amounts (50 g l^{-1}) of Fe-stripped Talladega Wetland sediment greatly increased the rate of $Fe(III)_{am}$ reduction (Fig. 3). Comparable results were obtained with the wild-type strain of *S. oneidensis* strain MR-1 with a lower concentration (0.5 mg ml^{-1}) of Talladega Wetland sediment. Parallel experiments were conducted with two *S. oneidensis* MR-1 mutants (*mtrC* and *mtrB*) defective in outer-membrane proteins known to be involved in reduction of metal oxides[24]. The relative decrease in $Fe(III)_{am}$ reduction rate in the presence of HS_{solid} for the two mutants compared with the wild type was similar to the relative decrease in $Fe(III)_{am}$ reduction rate in the absence of HS_{solid} (Supplementary Fig. S8). These findings indicate that $Fe(III)_{am}$ and HS_{solid} were reduced by the same extracellular electron transfer system.

The above results suggest that HS_{solid} accelerated $Fe(III)_{am}$ reduction by serving as a solid-state electron shuttle between bacteria and oxide surfaces. This conclusion is consistent with the lack of significant accumulation of dissolved reducing equivalents in the Fe-stripped sediment microbial reduction experiments (Fig. 1a,b), and the inability of dissolved humic substances released from freeze-dried Talladega Wetland surface sediments to stimulate $Fe(III)_{am}$ reduction (Supplementary Fig. S7). Some of the acceleration could have resulted from reduction of HS_{solid} independent of Fe(III), followed by rapid reaction of the reduced humic substances with $Fe(III)_{am}$ on mixing of the solids during sampling. Separate experiments verified that $Fe(III)_{am}$ reduction by microbially reduced Fe-stripped Talladega Wetland sediment took place within seconds. Numerical simulations (Supplementary Fig. S9) were conducted to evaluate the significance of this pathway. Reduction of HS_{solid} and $Fe(III)_{am}$ followed first-order kinetics using rate constants obtained from data in Figs 1 and 3. Reaction of $Fe(III)_{am}$ by reduced HS_{solid} was assumed to follow second-order kinetics, using a rate constant of 600 $(mmol\,l^{-1})^{-1}\,h^{-1}$. Simulations in which $Fe(III)_{am}$ reduction, HS_{solid} reduction and reaction of $Fe(III)_{am}$ with reduced HS_{solid} proceeded in parallel approximately reproduced the observed Fe(II) versus time data (Fig. 3, dotted lines). Lower amounts of Fe(II) were produced in simulations in which the reaction of $Fe(III)_{am}$ with reduced HS_{solid} took place only at the times of sampling (Fig. 3, dashed lines). These results indicate that HS_{solid} shuttled electrons from FeRB to $Fe(III)_{am}$ in the suspensions of Fe-stripped Talladega Wetland sediment and synthetic $Fe(III)_{am}$.

Solid-phase electron shuttling must require intimate physical association of organics with Fe(III) oxide. Previous studies have demonstrated coassociation of organic carbon with clays and Fe(III) oxides in natural soils and sediments[25,26]. Energy-filtering transmission electron microscopy (EFTEM) and electron energy-loss spectroscopy (EELS) were conducted to evaluate whether this was the case in the Fe-stripped sediment/$Fe(III)_{am}$ suspensions. Zero-energy-loss transmission electron microscopy (TEM) showed aggregates of $Fe(III)_{am}$ nanocrystals associated with smectite clay plates (Fig. 4a). EFTEM C and Fe elemental maps indicated direct association of C with $Fe(III)_{am}$ nanocrystals, whereas clay plates alone did not show significant associated C (Fig. 4b,c). Examination of $Fe(III)_{am}$ not exposed to Fe-stripped sediment showed only minor C–Fe association (data not shown). EELS spectra of the sediment/$Fe(III)_{am}$ mixture showed a peak at 284.8 eV indicating $1s\text{-}\pi^{*}C=C$ bonds associated with aromatic moieties[27] (Fig. 4d), which were not present in suspensions of $Fe(III)_{am}$ alone in bicarbonate buffer. These data suggest that humic substances in the Talladega Wetland sediment were coordinated with $Fe(III)_{am}$ at the nanometre scale in the sediment-oxide suspensions.

The ability of FeRB to transfer electrons to HS_{solid} and of HS_{solid} to serve as an electron shuttle has important implications for the redox biogeochemistry of sediments. A recent study[28] provided evidence for electrical currents in sediments linked to extracellular electron transport through a conductive network composed of bacterial nanowires[29,30] combined with dissolved electron shuttles and outer-membrane cytochromes. Our results suggest that electron shuttling by HS_{solid} could play a role in the operation of such a network. In addition, if redox-active moieties in HS_{solid} are associated with one another (for example, in coordination with sediment mineral phases; Fig. 4) at the nanometre scale, it seems possible that HS_{solid} could be an integral part of the network.

Methods

Solid-phase humic substance reduction. Sediments were stripped of their reactive Fe content with dithionite-citrate-bicarbonate to avoid interference with the electron shuttling assay (see below) for reduced humic substances. The Fe-stripped sediments were washed extensively under oxic conditions to remove the extractant and to reoxidize any redox-active solid-phase moieties that may have been reduced during Fe extraction.

For microbial reduction assays, Fe-stripped surface sediment was suspended (1 g/20 ml) in 30 mM $NaHCO_3$ buffer and inoculated with about 10^8 cells ml^{-1} of washed (twice in $NaHCO_3$ buffer) acetate/fumarate-grown *Geobacter sulfurreducens* strain PCA or lactate/fumarate-grown *Shewanella putrefaciens* strain CN32 with acetate or lactate as the energy source. For chemical reduction assays, Fe-stripped sediment was suspended in either $NaHCO_3$ (pH 6.8) or 10 mM PIPES buffer (pH 6.8) and incubated for 24 h with shaking (100 r.p.m.) under a 100% H_2 atmosphere in the presence of five Pd-coated aluminium oxide pellets.

An electron shuttling assay analogous to that previously described[2] was used to quantify the amount of electrons transferred to Fe-stripped sediments during microbial or chemical reduction (Supplementary Fig. S3). For unfiltered samples, 0.25 ml of sediment suspension was reacted with 2 ml of 5 mM ferric iron complexed with nitriloacetic acid (Fe(III)-NTA) for 1 min. This reaction mixture was then filtered (0.2 μm) and 0.5 ml of filtrate was added to 5 ml of 50 mM HEPES-buffered ferrozine (1 g l^{-1}) solution[2]. Absorbance was measured immediately at 562 nm. For filtered samples, 0.5 ml of sediment suspension was filtered (0.2 μm), and 0.25 ml of filtrate was reacted with 2 ml of 5 mM Fe(III)-NTA for 1 min. A 0.5 ml portion of the reaction mixture was then added to 5 ml of buffered ferrozine solution and the absorbance at 562 nm was immediately measured. The electron-accepting capacity (in μmol e^{-} equiv/g dry sediment) of the filtered (dissolved) and unfiltered (solid plus dissolved) component was calculated by subtracting the initial (before reduction) from the final (after reduction) amount of electrons transferred to Fe(III).

ESR spectroscopy. Continuous-wave X-band ESR spectra were recorded at 25 °C with a MiniScope MS 300 (Magnettech GmbH) at 0.1 mW. Dry samples of H_2/Pd-reduced and unreduced sediment were measured in quartz glass tubes with an inner diameter of 4 mm closed with plastic caps (Magnettech GmbH) and parafilm to maintain anoxic conditions throughout the measurements.

Influence of HS_{solid} on microbial $Fe(III)_{am}$ reduction. Dilute HCl-extractable Fe(II) production was monitored after addition of washed acetate/fumarate-grown *G. sulfurreducens* PCA or lactate/fumarate-grown *S. putrefaciens* CN32 cells (about 10^8 cells ml^{-1}) to a suspension (about 20 mmol l^{-1}) of $Fe(III)_{am}$ with or without 50 g l^{-1} of Fe-stripped Talladega Wetland sediment. Analogous experiments were conducted with about 10^9 cells ml^{-1} of Luria-Broth medium-grown *Shewanella oneidensis* MR-1 cells incubated with 20 mM lactate and 1 mmol l^{-1} synthetic $Fe(III)_{am}$. Both wild type and the outer-membrane mutants, *mtrB* and *mtrC* (ref. 24), were studied.

FeRB enrichment culture experiments. An acetate-oxidizing, $Fe(III)_{am}$-reducing enrichment culture was obtained by adding portions of Talladega Wetland surface sediment (0–1 cm depth interval) to anoxic, $NaHCO_3$-buffered (pH 6.8) growth medium containing acetate as the electron donor and synthetic $Fe(III)_{am}$ as the electron acceptor. The $Fe(III)_{am}$ was synthesized as previously described[10]. The medium contained KH_2PO_4 (0.44 mM), NH_4Cl (4.4 mM) and 1 ml l^{-1} of trace element and vitamin stock solutions[10], and was rendered anoxic by bubbling with 80% N_2/20% CO_2. The effect of various amendments on synthetic $Fe(III)_{am}$ reduction (Supplementary Fig. S7) was evaluated with the first 20 transfers of one of three initial enrichments. All experiments were conducted in duplicate or triplicate and included parallel non-amended controls. Each culture was inoculated (10% vol/vol) with a previous generation of a non-amended control culture. This approach ensured that a common suite of organisms was present in the various treatments designed to examine the influence of the amendments on $Fe(III)_{am}$ reduction. A 16S ribosomal RNA gene clone library revealed that the enrichment culture was dominated by an organism 99% similar in 16S rRNA gene sequence to *Geobacter argillaceus*.

The 0.5 M HCl-extractable Fe(II) and Fe(III) content of the enrichment cultures was determined using ferrozine as previously described[10]. The dissolved organic carbon content of pore water and culture medium amended with

solid-phase sediment materials was determined by high-temperature combustion with a Shimadzu TOC-5000 analyser following filtration through combusted glass fibre (Whatman GF/F) filters. Sediment organic carbon and nitrogen content was determined with a LECO CNS-2000 Elemental Analyser.

TEM. See Supplementary Information.

Received 25 February 2010; accepted 22 April 2010; published online 23 May 2010

References

1. Stevenson, F. J. *Humus Chemistry: Genesis, Composition, Reactions* (John Wiley, 1994).
2. Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P. & Woodward, J. C. Humic substances as electron acceptors for microbial respiration. *Nature* **382,** 445–448 (1996).
3. Skogerboe, R. K. & Wilson, S. A. Reduction of ionic species by fulvic acid. *Anal. Chem.* **53,** 228–232 (1981).
4. Nevin, K. P. & Lovley, D. R. Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. *Environ. Sci. Technol.* **34,** 2472–2478 (2000).
5. Lovley, D. R. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* **55,** 259–287 (1991).
6. Thamdrup, B. Bacterial manganese and iron reduction in aquatic sediments. *Adv. Microb. Ecol.* **16,** 41–84 (2000).
7. Kappler, A., Benz, M., Schink, B. & Brune, A. Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol. Ecol.* **47,** 85–92 (2004).
8. Szilagyi, M. Redox properties and determination of normal potential of peat-water system. *Soil Sci. Am. J.* **115,** 434–437 (1973).
9. Van der Zee, F. P., Bisschops, I. A. E., Lettinga, G. & Field, J. A. Activated carbon as an electron acceptor and redox mediator during the anaerobic biotransformation of azo dyes. *Environ. Sci. Technol.* **37,** 402–408 (2003).
10. Lovley, D. R. & Phillips, E. J. P. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* **51,** 683–689 (1986).
11. Scott, D. T., McKnight, D. M., Blunt-Harris, E. L., Kolesar, S. E. & Lovley, D. R. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. *Environ. Sci. Technol.* **2894,** 2984–2989 (1998).
12. Paul, A. *et al.* Nature and abundance of organic radicals in natural organic matter: Effect of pH and irradiation. *Environ. Sci. Technol.* **40,** 5897–5903 (2006).
13. Stoesser, R., Bartoll, J., Schirrmeister, L., Ernst, R. & Lueck, R. ESR of trapped holes and electrons in natural and synthetic carbonates, silicates and aluminosilicates. *Appl. Radiat. Isot.* **47,** 1489–1496 (1996).
14. Jiang, J., Bauer, I., Paul, A. & Kappler, A. Arsenic redox changes by microbially and chemically formed semiquinone radicals and hydroquinones in a humic substance model quinone. *Environ. Sci. Technol.* **43,** 3639–3645 (2009).
15. Ratasuk, N. & Nanny, M. A. Characterization and quantification of reversible redox sites in humic substances. *Environ. Sci. Technol.* **41,** 7844–7850 (2007).
16. Jiang, J. & Kappler, A. Kinetics of microbial and chemical reduction of humic substances: Implications for electron shuttling. *Environ. Sci. Technol.* **42,** 3563–3569 (2008).
17. Heitmann, T., Goldhammer, T., Beer, J. & Blodau, C. Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog. *Global. Change Biol.* **13,** 1771–1785 (2007).
18. Segers, R. & Kengen, S. W. M. Soil methane production as a function of anaerobic carbon mineralization: A process model. *Soil Biol. Biochem.* **30,** 1107–1117 (1998).
19. Neubauer, S. C., Givler, K., Valentine, S. K. & Megonigal, J. P. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. *Ecology* **86,** 3334–3344 (2005).
20. Van der Zee, F. R. & Cervantes, F. J. Impact and application of electron shuttles on the redox (bio)transformation of contaminants: A review. *Biotechnol. Adv.* **27,** 256–277 (2009).
21. Gu, B. & Chen, J. Enhanced microbial reduction of Cr(VI) and U(VI) by different natural organic matter fractions. *Geochim. Cosmochim. Acta* **67,** 3575–3582 (2003).
22. Collins, R. & Picardal, F. Enhanced anaerobic transformations of carbon tetrachloride by soil organic matter. *Environ. Toxicol. Chem.* **18,** 2703–2710 (1999).
23. Luu, Y., Ramsay, B. A. & Ramsay, J. A. Nitrilotriacetate stimulation of anaerobic Fe(III) respiration by mobilization of humic materials in soil. *Appl. Environ. Microbiol.* **69,** 5255–5262 (2003).
24. Myers, J. M. & Myers, C. R. Role for outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. *Appl. Environ. Microbiol.* **67,** 260–269 (2001).
25. Poulton, S. W. & Raiswell, R. Chemical and physical characteristics of iron oxides in riverine and glacial meltwater sediments. *Chem. Geol.* **218,** 203–221 (2005).
26. Wagai, R. & Mayer, L. M. Sorptive stabilization of organic matter in soils by hydrous iron oxides. *Geochim. Cosmochim. Acta* **71,** 25–35 (2007).
27. Scheinost, A. C., Kretzschmar, R., Christl, I. & Jacobsen, C. in *Humic Substances Structures, Models, and Functions* (eds Ghabbour, E. A. & Davies, G.) 39–50 (The Royal Society of Chemistry, 2001).
28. Nielsen, L. P., Risgaard-Petersen, N., Fossing, H., Christensen, P. B. & Sayama, M. Electric currents couple spatially separated biogeochemical processes in marine sediment. *Nature* **463,** 1071–1074 (2010).
29. Reguera, G. *et al.* Extracellular electron transfer via microbial nanowires. *Nature* **435,** 1098–1101 (2005).
30. Gorby, Y. A. *et al.* Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* MR-1 and other microorganisms. *Proc. Natl Acad. Sci. USA* **103,** 11358–11363 (2006).

Acknowledgements

This research was financially supported by grants from the US Department of Energy (Office of Biological and Environmental Research) and the US National Science Foundation (Directorates for Biological Sciences and Geosciences) to E.E.R.; by an IPSWaT fellowship from the German Federal Ministry for Science and Education to J.J.; by financial support from the German Research Foundation to A.K. and A.P. and financial support from the Stifterverband der Wissenschaft to A.K. We thank G. Scott, B. Golson and B. Kollah for technical assistance, and the late R. G. Wetzel for fruitful early discussions of microbial humics transformation. This letter is dedicated to the memory of R. G. Wetzel.

Author contributions

The concept of HS_{solid} reduction was developed together by E.E.R. and A.K. E.E.R. coordinated the overall project, conducted or directed microbial and chemical reduction assays, analysed data and wrote the paper. A.K. directed the *S. oneidensis* reduction assays, coordinated with A.P. and R.S. on the ESR analyses and wrote the paper. I.B. and J.J. conducted the *S. oneidensis* microbial reduction assays, and I.B. helped to coordinate the ESR analyses. A.P. and R.S. conducted and interpreted the ESR analyses; H.K. and H.X. conducted and interpreted the TEM analyses.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/naturegeoscience. Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions. Correspondence and requests for materials should be addressed to E.E.R.