nature geoscience

LETTERS

PUBLISHED ONLINE: 27 FEBRUARY 2014 | DOI: 10.1038/NGEO2084

Humic substances as fully regenerable electron acceptors in recurrently anoxic environments

Laura Klüpfel[1†], Annette Piepenbrock[2†], Andreas Kappler[2*] and Michael Sander[1*]

Humic substances form through the degradation of microbial and plant precursors, and make up a significant fraction of natural organic matter in terrestrial and aquatic environments[1]. Humic substances are redox-active[2–4] and can act as terminal electron acceptors in anaerobic microbial respiration[4]. Reduced humic substances may become re-oxidized during aeration of temporarily anoxic systems, such as wetlands, sediments and many soils. If the transfer of electrons from anaerobic respiration through humic substances to oxygen is sustained over many redox cycles, it may competitively suppress electron transfer to carbon dioxide, and thereby lower the formation of methane in temporarily anoxic systems[5–8]. Here, we monitor changes in the redox states of four chemically distinct dissolved humic substances over successive cycles of reduction by the bacterium *Shewanella oneidensis* MR-1 and oxidation by oxygen, in a series of laboratory experiments. We show that electron transfer to and from these substances is fully reversible and sustainable over successive redox cycles. We suggest that redox cycling of humic substances may largely suppress methane production in temporarily anoxic systems.

Humic substances (HS) are formed during degradation of microbial and plant precursors and make up a significant fraction of natural organic matter in terrestrial and aquatic environments[1]. Under anoxic conditions, both dissolved and particulate HS (refs 4,9) may accept electrons from anaerobic microbial respiration. Microbial HS reduction involves electron transfer to quinone moieties as well as other redox-active functional groups and metals in the HS (Fig. 1)[2,3,10]. Reduced HS may serve as an electron donor in anaerobic microbial respiration[11] and can transfer electrons to poorly soluble Fe(III) (oxyhydr)oxide minerals in soils and sediments, thereby mediating dissimilatory Fe(III) reduction[4,12,13], and to a variety of organic and inorganic pollutants[14], thereby affecting their transformation and speciation. Electron transfer to HS in anoxic systems is considered to competitively suppress reduction of other terminal electron acceptors (TEAs), including CO_2 under methanogenic conditions (Fig. 1). It is reasonable to expect that this suppression is strongly enhanced in temporarily anoxic environments provided that O_2 re-oxidizes microbially reduced HS during intermittent aeration events and thereby restores the capacity of HS to accept electrons in subsequent anoxic periods. Temporarily anoxic conditions occur in sediments, soils, lakes with transitory oxyclines, and wetlands, of which the last contribute an estimated 15–40% of the global methane flux[15]. Although previous work provided evidence for largely reversible electron transfer to HS over repeated cycles of chemical and electrochemical reduction and O_2 re-oxidation[3,16,17], electron transfer over repeated microbial reductions and O_2 re-oxidations has not been studied. Clearly, these reactions warrant investigation given their expected importance to redox dynamics, carbon cycling and greenhouse gas formation in temporarily anoxic environments.

In this work we studied three fundamental aspects of HS redox cycling in temporarily anoxic laboratory systems. First, we determined whether electron transfer to and from HS was reversible over a single cycle of microbial reduction followed by O_2 re-oxidation and whether it was sustainable over consecutive redox cycles. Second, we tested whether the extent of microbial HS reduction was controlled by system thermodynamics. Third, we report the range of reduction potentials of electron-accepting moieties in different HS. We studied these aspects by quantifying the changes in the redox states of four humic acids (HAs) over three successive cycles of reduction by *S. oneidensis* MR-1 under anoxic conditions and re-oxidation by O_2 in well-controlled laboratory incubation set-ups. The HAs were isolated from Pahokee Peat (PPHA), Suwannee River (SRHA), Elliot Soil (ESHA) and Leonardite Coal (LHA) and were selected to cover a range of physicochemical properties[3,10] (Supplementary Information). From the known strains of HS-reducing bacteria, we chose the facultative anaerobe *S. oneidensis* MR-1 because it is capable of metabolizing under both anoxic and oxic conditions, which was a prerequisite for our incubation set-ups. Furthermore, *Shewanella* genera are highly abundant in temporarily anoxic systems, including minerotrophic wetlands[18], and marine and lake sediments[19,20]. The redox states of the HAs during the incubations were monitored by measuring the reduction potentials E_h of HA solutions and by quantifying the electron-accepting and -donating capacities (EAC, EDC) of the HAs (that is, the number of electrons that were accepted and donated by a given HA mass when added to electrochemical cells with well-defined reducing and oxidizing conditions, respectively; schematic in Fig. 2a,b; see Methods). The bi-directional electrochemical quantification of EAC and EDC was used to determine the stoichiometries of the conversions of electron-accepting and -donating moieties in the HAs and, hence, the reversibility and sustainability of electron transfer to and from HAs during redox cycling. This information cannot be obtained when using traditional, one-directional assays in which only the reduction of an added oxidant, commonly a Fe(III) species, is analysed[4,9].

Anoxic incubation of HA samples inoculated with *S. oneidensis* in growth medium containing the electron donor lactate (2 mM) resulted in extensive microbial HA reduction, as evidenced from the concomitant decreases in EACs and increases in EDCs over time (Fig. 2c,e). Microbial HA reduction was fast over the first three days, followed by slower, yet continuous reduction up to three

[1]Department of Environmental Systems Science, Institute of Biogeochemistry and Pollutant Dynamics, Swiss Federal Institute of Technology (ETH) Zurich, Universitaetstrasse 16, 8092 Zurich, Switzerland, [2]Geomicrobiology, Center for Applied Geoscience, Eberhard-Karls-University Tuebingen, Sigwartstrasse 10, 72076 Tuebingen, Germany. †These authors contributed equally to this work. *e-mail: michael.sander@env.ethz.ch; andreas.kappler@uni-tuebingen.de

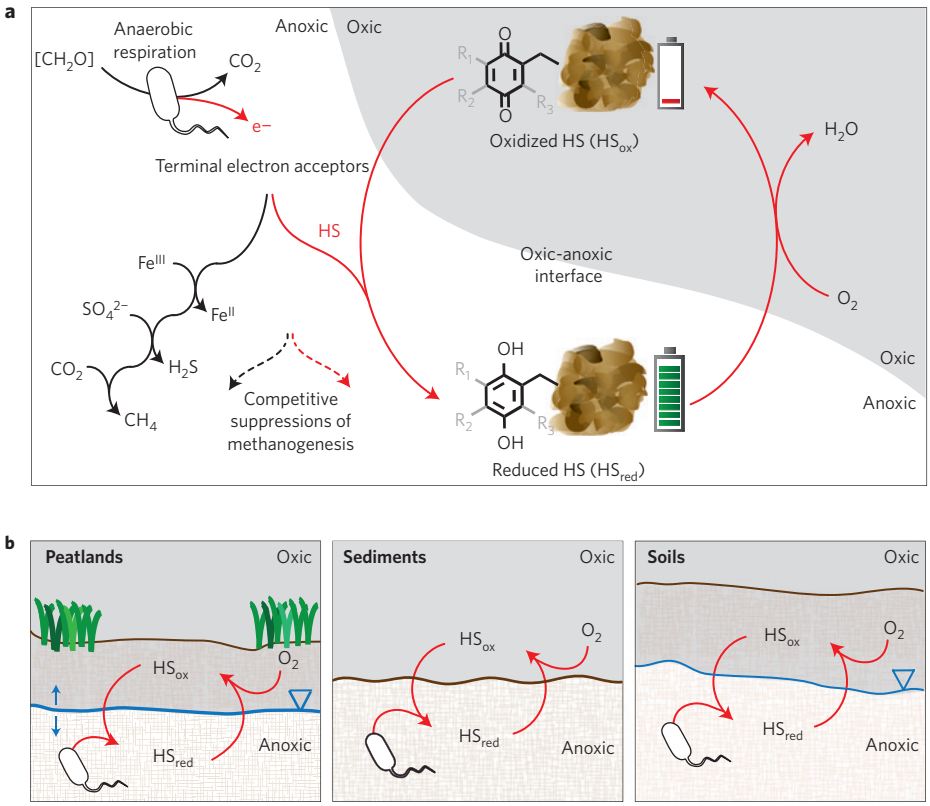

Figure 1 | Redox cycling of HS in temporarily anoxic systems. a, Microbial reduction of HS under anoxic conditions and subsequent HS re-oxidation by O_2 under oxic conditions. HA redox cycling is depicted by a quinone/hydroquinone pair along with the charging and discharging of a battery. The use of HS as a TEA decreases the number of electrons transferred to inorganic TEA. Electron transfer to HS instead of CO_2 suppresses hydrogenotrophic methanogenesis. **b**, Temporarily anoxic systems include peatlands, sediments and many soils.

weeks of anoxic incubation. Subsequent aeration of the reduced HA solutions for three days resulted in rapid HA re-oxidation, as shown by pronounced increases in EACs and decreases in EDCs. Re-oxidation of the reduced HAs restored a large fraction (between 85 and 105%) of the EACs measured before microbial reduction (Supplementary Information). In agreement with the results from the first redox cycle, incubation under anoxic and oxic conditions in the second and third redox cycles resulted in microbial reduction and re-oxidation of the HAs in the inoculated samples, respectively. HA reduction during all three anoxic incubations demonstrates that *S. oneidensis*, which was added only once at the start of the incubations in single inocula, rapidly switched between anaerobic respiration using HA as a TEA and aerobic respiration. In contrast to the inoculated HA samples, there were no systematic changes in redox states of the sterile HA controls (Fig. 2d,f) and of inoculated and sterile HA-free controls (Supplementary Information) during the anoxic and oxic incubations.

The sum of the EAC and EDC values of a given sample, which we refer to as the electron exchange capacity (EEC), is a measure of the total number of redox active moieties in that sample. Redox-active moieties in the HA dominated the EEC values of inoculated and sterile HA-containing samples, given that HA-free control samples had much smaller EEC values (Supplementary Information). The EEC values of each inoculated HA changed only marginally over the course of the incubations (Fig. 2g,h) and the slopes of regression lines on the EEC data of the inoculated HAs were either statistically indistinguishable (PPHA, SRHA, ESHA) from or slightly larger (LHA) than those of the corresponding sterile HA samples (Supplementary Information). This finding has several important implications. First, the constant number of redox-active moieties in the inoculated HA samples demonstrated equimolar conversions of electron acceptors to donors during microbial HA reduction and of donors to acceptors during O_2 re-oxidation. Second, the equimolar conversions of accepting and donating moieties imply that electron transfer to the HAs was fully reversible with regard to the reference states used in electrochemical quantification. Consequently, the observation that inoculated HAs were slightly more reduced after the re-oxidation steps than at the onset of the experiment must have resulted from incomplete O_2 re-oxidation of the reduced HAs over the three days of oxic incubation and not from a loss of electron-accepting moieties during HA redox cycling. Third, the constant number of redox active moieties over successive redox cycles demonstrates that HS constitute regenerable and sustainable TEAs in temporarily anoxic environments.

We could ascribe the decreasing initial rates and final extents of electron transfer to the HAs over the three microbial reduction steps (Fig. 2) to the continuous depletion of the electron donor lactate: at day 14 of the third anoxic incubation step, we collected subsamples from the inoculated and sterile HA samples to which we added fresh lactate to nominal concentrations of 2 mM. Lactate re-addition to the inoculated HA samples stimulated HA reduction during the subsequent 16 days of anoxic incubation as compared with the original HA samples to which no further lactate was added (see arrows in Fig. 2). Lactate re-addition to the sterile HA subsamples did not affect their redox states. In a separate experiment (Supplementary Information), PPHA samples inoculated with *S. oneidensis* were incubated over three successive redox cycles in medium containing 2 mM initial lactate and in medium containing no lactate. Microbial PPHA reduction during the first anoxic incubation was faster and more extensive in treatments with than

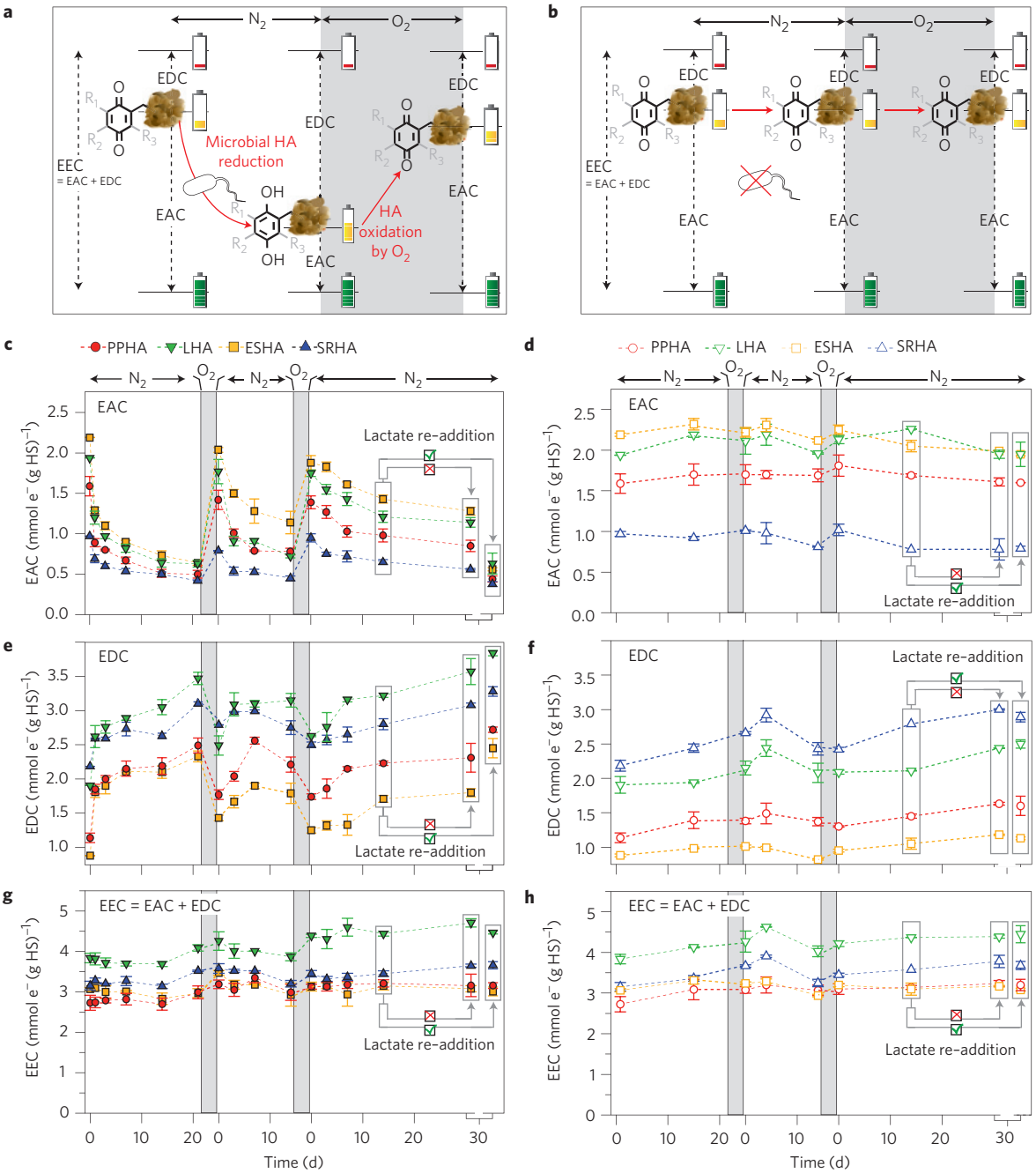

Figure 2 | Redox states of inoculated and sterile HAs during successive anoxic and oxic incubations. a, Changes in the EACs and EDCs of HAs during reduction by *S. oneidensis* MR-1 under anoxic conditions (N_2) and subsequent re-oxidation under oxic conditions (O_2). HA redox cycling is depicted by a quinone/hydroquinone pair along with the charging and discharging of a battery. **b**, Constant redox states in sterile HA controls. **c**–**h**, EACs, EDCs and EECs of inoculated (**c**,**e**,**g**) and sterile (**d**,**f**,**h**) PPHA, LHA, ESHA and SRHA over three successive anoxic–oxic incubation cycles. HA subsets were re-amended with lactate during the third anoxic incubation.

without lactate. However, the differences between the treatments became less pronounced during the second anoxic incubation and were absent during the last anoxic incubation. These findings support that lactate depletion resulted in decreasing rates and extents of PPHA reduction. PPHA reduction in the absence of lactate demonstrated that *S. oneidensis* MR-1 was capable of using alternative, energetically less favourable electron donors that were present either in the medium (such as amino acids or peptides[21]) or stored intracellularly[22].

At the end of each of the three anoxic incubation steps, all inoculated HA samples still showed significant EACs (27–63% of the initial values; Supplementary Information) and therefore were not fully reduced by *S. oneidensis* MR-1. Although reduced to different EAC values, all HAs were reduced to comparable E_h at the ends of the first anoxic incubation (to $-0.15\,V < E_h < -0.2\,V$) and the third anoxic incubation for subsamples re-amended with lactate ($-0.18\,V < E_h < -0.2\,V$; Fig. 3a). These E_h ranges were narrow compared with the pronounced increases in the E_h values during O_2 re-oxidation of the reduced HAs to $0\,V < E_h < +0.1\,V$ (Fig. 3a) and to the wide E_h range over which the HAs accepted electrons (from +0.12 V to below −0.30 V at pH 7; Fig. 3b and Supplementary Information). The narrow E_h range of the most

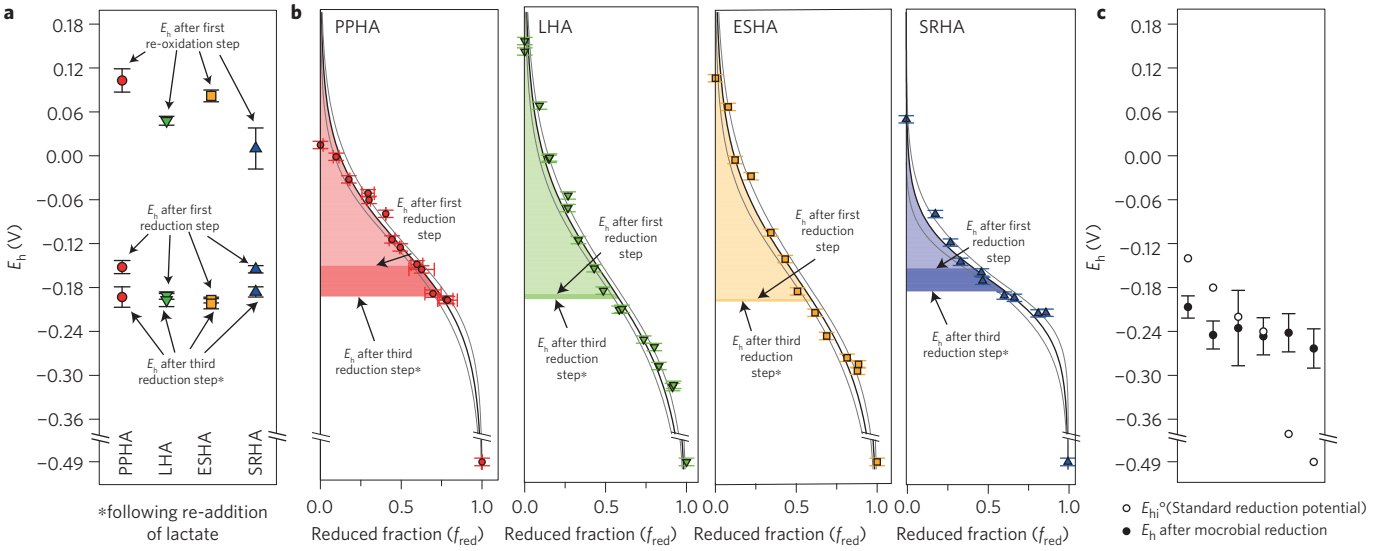

Figure 3 | Thermodynamic analyses of microbial HA reduction. a, Reduction potentials (E_h) of solutions containing PPHA, LHA, ESHA and SRHA inoculated with *S. oneidensis* MR-1 measured after the first reduction and re-oxidation steps, and the third reduction step. **b**, Dependence of solution E_h on the extents of HA reduction, expressed as the reduced fraction, f_{red} (that is, 0 (unreduced HA) $\leq f_{red} \leq 1$ (fully reduced HA); Supplementary Information). Experimental data (symbols) were fitted by a modified Nernst equation (lines represent fits and 95% confidence intervals; Supplementary Information). E_h errors were estimated (Supplementary Information) and errors in f_{red} for PPHA represent ranges of duplicate analyses. The lower limits of the light and dark shaded areas (indicated by thick horizontal lines) correspond to the E_h values obtained at the ends of the first and third microbial reduction steps, respectively (as shown in **a**). The data for PPHA are from this work and for the other HAs from ref. 10. **c**, Standard reduction potentials, $E_h^{\circ\prime}$, at pH 7 (open symbols) of model compounds and E_h of compound solutions after anoxic incubation with *S. oneidensis* MR-1 (filled symbols).

extensively reduced HAs suggests that microbial HA reduction was controlled by system thermodynamics with lactate as the electron donor. Reduction to comparable E_h of −0.21 V was also reported for anaerobic microbial fuel cells with *S. oneidensis* MR-1 and lactate as electron donor[23]. A thermodynamic constraint on HA reduction was further experimentally supported by the finding that *S. oneidensis* MR-1 reduced six low-molecular-weight quinones and riboflavin to comparable E_h of −0.2 V to −0.24 V in separate, HA-free anoxic incubations with lactate as electron donor (Fig. 3c). The set of model compounds was chosen to cover a wide range of standard reduction potentials from $E_h^{\circ\prime} = -0.14$ V to −0.49 V at pH 7 (Supplementary Information) and included quinones as models for quinone moieties in the HA[2,3], as well as riboflavin, which is the predominant extracellular electron shuttle produced by *S. oneidensis* MR-1 for respiring to insoluble TEAs (refs 24,25). We note that we detected only small concentrations of riboflavin (<50 nM; Supplementary Information) in the inoculated HA samples at the end of the experiments, suggesting that direct (non-mediated) respiration of soluble HAs predominated. Furthermore, *S. oneidensis* MR-1 did not release redox-active substances, such as riboflavin, in inoculated HA-free controls in amounts that were detectable by mediated electrochemical analysis (Supplementary Information).

This work shows that HAs are regenerable and sustainable TEAs in temporarily anoxic systems, that the extent of microbial HA reduction is controlled by system thermodynamics, and that HAs may accept electrons from anaerobic microbial respiration over a wide E_h range. These findings largely substantiate the importance of HS redox dynamics in biogeochemical cycles in temporarily anoxic systems. As an illustrative example, we estimated that HS redox cycling in peatlands may result in a suppression of hydrogenotrophic methanogenesis of approximately 190,000 mol $CH_4\,km^{-2}\,yr^{-1}$, assuming an annual water table fluctuation of 0.2 m, and EACs of particulate and dissolved HS of 0.05 mmol e^- (g $HS_{particulate}$)$^{-1}$ and 1 mmol e^- (g $HS_{dissolved}$)$^{-1}$ reported previously[9] and in this work (Supplementary Information). This suppressed amount of methane corresponds to between 10 and 166% of reported average CH_4 fluxes from northern peatlands of 114,000 to 1,825,000 mol $CH_4\,km^{-2}\,yr^{-1}$ (ref. 26). Although simplistic, this calculation highlights the potentially large impacts of HS redox dynamics on carbon cycling in and CH_4 emissions from temporarily anoxic environments. The wide E_h range of reducible moieties in HS reported in this work substantiates previous qualitative findings of HS as a versatile TEA for anaerobic microbial respiration under widely differing redox conditions[8,12,26], including iron- and sulphate-reducing, and methanogenic conditions (Fig. 4).

Methods

Electron acceptors. Humic acid standards PPHA, LHA, SRHA and ESHA were purchased from the International Humic Substances Society and used as received. The tested model compounds were 2-hydroxy-1,4-naphthoquinone (lawsone), 9,10-anthraquinone-2,6-disulphate (AQDS), 9,10-anthraquinone-2-sulphate (AQS), alizarin, carminic acid and riboflavin. Standard reduction potentials of the model compounds, $E_h^{\circ\prime}$, were determined by cyclic voltammetry (Supplementary Information).

Microorganisms. *S. oneidensis* strain MR-1 from a frozen stock was streaked out oxically on lysogeny broth agar plates (10 g peptone l^{-1}, 5 g yeast extract l^{-1}, 10 g NaCl l^{-1} and 15 g agar l^{-1}) and then transferred twice on anoxic freshwater medium[29] containing 20 mM lactate and 40 mM fumarate until reaching a density of 2×10^7 cells ml^{-1}. Aliquots of these cultures were used to prepare inoculated HA samples and inoculated HA-free controls.

Incubation experiments. Experiments were carried out in LML medium[30] (pH 7, 50 mM phosphate). Twenty five millilitres of sterile and anoxic LML medium were aliquoted into 58 ml serum bottles and sealed with butyl rubber stoppers, followed by exchange of the headspace with N_2. The LML medium did not contain reducible components detectable by mediated electrochemical reduction (MER; Supplementary Information).

Humic acids. HA stock solutions (10 $g_{HA}\,l^{-1}$) were prepared in 50 mM phosphate buffer (pH 7), sterile filtered (cellulose acetate, 0.22 μm) and deoxygenated. A lactate stock solution (0.1 M) was prepared in Millipore water, deoxygenated and autoclaved. HA and lactate were added to the serum bottles containing LML medium to final concentrations of 2 mM lactate and

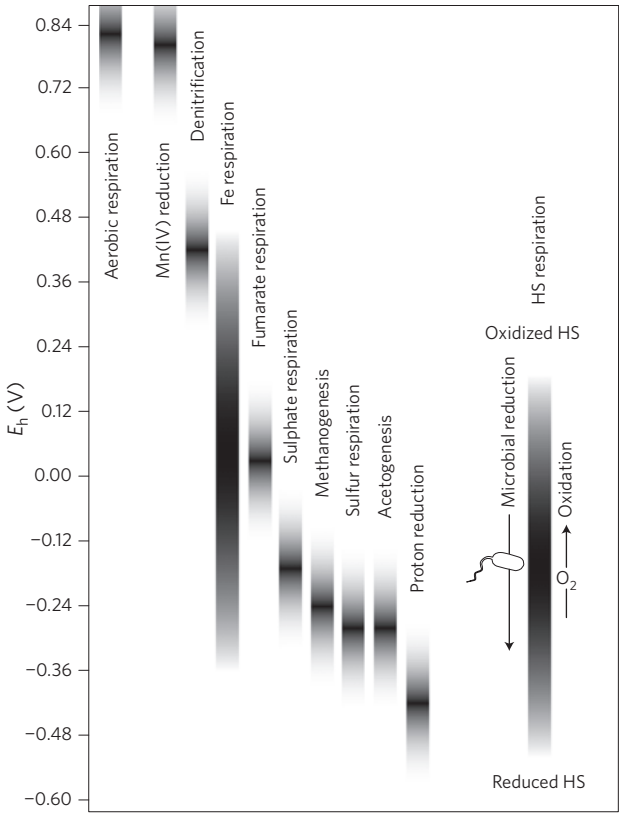

Figure 4 | Reduction potential ranges of aerobic and anaerobic microbial respiration using different TEAs, including HS. HS may be reduced under varying redox conditions, including iron-reducing, sulphate-reducing and methanogenic conditions. Microbially reduced HS are re-oxidized on aeration, restoring the capacity of HS to accept electrons. Potential ranges are given for pH 7 (refs 27,28).

0.42–0.97 g HA l^{-1}. The HA concentrations were determined by spectrophotometry (Supplementary Information).

Inoculated HA samples (run in triplicates) and sterile HA controls (run in duplicates) were prepared as detailed in the Supplementary Information. Each inoculated HA sample received a single inoculum of 2×10^5 cells ml^{-1} at the beginning of the incubation experiments. All samples were incubated on a horizontal shaker (125 rpm, 25 °C) in the dark. Over the course of the experiment, aliquots for electrochemical and pH measurements were withdrawn in a N_2-atmosphere glovebox ($O_2 < 0.1$ ppm) using sterile needles.

Following anoxic incubation, the samples were aerated by exchanging the sample bottle headspaces for 5 min with air for a total of three times on three successive days, which was previously shown to result in extensive re-oxidation of electrochemically reduced HS[3]. During oxic incubations, the bottles were stored on a horizontal shaker in the dark. On the fourth day, the bottles were purged with N_2 for 15 min to re-establish anoxic conditions. The samples were incubated three times under anoxic conditions and twice under oxic conditions. *S. oneidensis* was added once as a single inoculum at the beginning of the experiment. On day 14 of the third reduction step, 5 ml subsamples from each bottle were transferred into sterile vials inside the anoxic glovebox and re-amended with 2 mM lactate.

The pH of all HA solutions was measured with a combined pH electrode (Metrohm) in aliquots taken immediately before and after the first re-oxidation step and at the end of the third reduction step (both original HA and subsamples with added lactate). The pH was stable over the entire experiment.

Model quinones and riboflavin. LML medium containing 2 mM lactate and one of five model compounds (lawsone (46 μM); AQDS (50 μM), AQS (43 μM); alizarin (1.4 μM); carminic acid (37 μM); riboflavin (4.8 μM)) were inoculated with 2×10^5 cells *S. oneidensis* MR-1 ml^{-1}. The reported E_h values were measured in aliquots sampled after 11–20 days of anoxic incubation.

Electrochemical analysis. All electrochemical measurements were conducted in an anoxic glovebox (N_2, atmosphere at 25 ± 1 °C, $O_2 < 0.1$ ppm).

HA samples were sterile filtered immediately after sampling and the number of electrons transferred to and from the HA was quantified by integration of reductive and oxidative current responses in MER (at $E_h = -0.49$ V) and mediated electrochemical oxidation (MEO; at $E_h = +0.61$ V) with 1,1'-ethylene-2,2'-bipyridyldiylium dibromide (DQ) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as electron transfer mediators, respectively[3]. The integrated current responses were normalized to the analysed HA masses, m_{HA} [g HA], to obtain EAC and EDC values (Eqs 1, 2):

$$\mathrm{EAC} = \frac{\int \frac{I_{red}}{F}\,dt}{m_{HA}} \quad (1)$$

$$\mathrm{EDC} = \frac{\int \frac{I_{ox}}{F}\,dt}{m_{HA}} \quad (2)$$

where I_{red} and I_{ox} (both [A]) are baseline-corrected reductive and oxidative currents in MER and MEO, respectively, and F (= 96,485 sA/mol_{e^-}) is the Faraday constant.

Potentiometric E_h measurements of HA and model compound solutions were conducted with combined Pt-ring and Au-ring electrodes (Metrohm) in the presence of small amounts of the redox mediators DQ and 1,1'-bis(cyanomethyl)-4,4'-bipyridinium dibromide (total concentration added <4% of sample EAC) to facilitate E_h equilibration[10]. Attainment of E_h equilibrium was inferred from stable and comparable E_h readings on Pt (data reported) and Au (Supplementary Information) redox electrodes.

Received 18 October 2013; accepted 9 January 2014; published online 27 February 2014

References

1. Stevenson, F. J. *Humus Chemistry: Genesis, Composition, Reactions* (John Wiley, 1994).
2. 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. Tech.* **32,** 2984–2989 (1998).
3. Aeschbacher, M., Sander, M. & Schwarzenbach, R. P. Novel electrochemical approach to assess the redox properties of humic substances. *Environ. Sci. Tech.* **44,** 87–93 (2010).
4. Lovley, D. R., Coates, J. D., BluntHarris, E. L., Phillips, E. J. P. & Woodward, J. C. Humic substances as electron acceptors for microbial respiration. *Nature* **382,** 445–448 (1996).
5. Keller, J. K., Weisenhorn, P. B. & Megonigal, J. P. Humic acids as electron acceptors in wetland decomposition. *Soil Biol. Biochem.* **41,** 1518–1522 (2009).
6. Blodau, C. & Deppe, M. Humic acid addition lowers methane release in peats of the Mer Bleue bog, Canada. *Soil Biol. Biochem.* **52,** 96–98 (2012).
7. Keller, J. K. & Takagi, K. K. Solid-phase organic matter reduction regulates anaerobic decomposition in bog soil. *Ecosphere* **4,** 1–10 (2013).
8. Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K. & Zhuang, Q. L. Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. *Glob. Change Biol.* **19,** 1325–1346 (2013).
9. Roden, E. E. *et al.* Extracellular electron transfer through microbial reduction of solid-phase humic substances. *Nature Geosci.* **3,** 417–421 (2010).
10. Aeschbacher, M., Vergari, D., Schwarzenbach, R. P. & Sander, M. Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids. *Environ. Sci. Tech.* **45,** 8385–8394 (2011).
11. Lovley, D. R., Fraga, J. L., Coates, J. D. & Blunt-Harris, E. L. Humics as an electron donor for anaerobic respiration. *Environ. Microbiol.* **1,** 89–98 (1999).
12. 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).
13. Wolf, M., Kappler, A., Jiang, J. & Meckenstock, R. U. Effects of humic substances and quinones at low concentrations on ferrihydrite reduction by *Geobacter metallireducens*. *Environ. Sci. Tech.* **43,** 5679–5685 (2009).
14. Borch, T. *et al.* Biogeochemical redox processes and their impact on contaminant dynamics. *Environ. Sci. Tech.* **44,** 15–23 (2010).
15. Denman, K. L. *et al.* in *Climate Change 2007: The Physical Science Basis* (eds Solomon, S. *et al.*) (Cambridge Univ. Press, 2007).
16. Ratasuk, N. & Nanny, M. A. Characterization and quantification of reversible redox sites in humic substances. *Environ. Sci. Tech.* **41,** 7844–7850 (2007).
17. Bauer, I. & Kappler, A. Rates and extent of reduction of Fe(III) compounds and O_2 by humic substances. *Environ. Sci. Tech.* **43,** 4902–4908 (2009).

18. Todorova, S. G. & Costello, A. M. Design of *Shewanella*-specific 16S rRNA primers and application to analysis of Shewanella in a minerotrophic wetland. *Environ. Microbiol.* **8,** 426–432 (2006).
19. Ziemke, F., Brettar, I. & Hofle, M. G. Stability and diversity of the genetic structure of a *Shewanella putrefaciens* population in the water column of the central Baltic. *Aquat. Microbial. Ecol.* **13,** 63–74 (1997).
20. Dichristina, T. J. & Delong, E. F. Design and application of rRNA-targeted oligonucleotide probes for the dissimilatory iron- and manganese-reducing bacterium *Shewanella putrefaciens*. *Appl. Environ. Microbiol.* **59,** 4152–4160 (1993).
21. Lovley, D. R., Phillips, E. J. P. & Lonergan, D. J. Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. *Appl. Environ. Microbiol.* **55,** 700–706 (1989).
22. Jiang, J. & Kappler, A. Kinetics of microbial and chemical reduction of humic substances: Implications for electron shuttling. *Environ. Sci. Tech.* **42,** 3563–3569 (2008).
23. Watson, V. J. & Logan, B. E. Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures. *Biotechnol. Bioeng.* **105,** 489–498 (2010).
24. Marsili, E. *et al. Shewanella* secretes flavins that mediate extracellular electron transfer. *Proc. Natl Acad. Sci. USA* **105,** 3968–3973 (2008).
25. Kotloski, N. J. & Gralnick, J. A. Flavin electron shuttles dominate extracellular electron transfer by *Shewanella oneidensis*. *Mbio* **4,** 1–4 (2013).
26. Blodau, C. Carbon cycling in peatlands - A review of processes and controls. *Environ. Rev.* **10,** 111–134 (2002).
27. Madigan, M. T., Martinko, J. M., Stahl, D. A. & Clark, D. P. *Brock Biology of Microorganisms* (Pearson Education, 2012).
28. Straub, K. L., Benz, M. & Schink, B. Iron metabolism in anoxic environments at near neutral pH. *Fems Microbiol. Ecol.* **34,** 181–186 (2001).
29. Hegler, F., Posth, N. R., Jiang, J. & Kappler, A. Physiology of phototrophic iron(II)-oxidizing bacteria: implications for modern and ancient environments. *Fems Microbiol. Ecol.* **66,** 250–260 (2008).
30. Myers, C. R. & Myers, J. M. Ferric iron reduction-linked growth yields of *Shewanella putrefaciencs* MR-1. *J. Appl. Bacteriol.* **76,** 253–258 (1994).

Acknowledgements

This research was financially supported by grants from the Swiss National Science Foundation to M.S. and L.K. (Project 200021–135515) and from the German Research Foundation to A.K. and A.P. (KA 1736/3-2, research group eTrap). We thank M. Aeschbacher, S. E. Page, J. Keller, K-H. Knorr, C. A. Gorski, K. Wammer and K. McNeill for discussions.

Author contributions

The concept of redox cycling of HAs was developed by A.P., A.K., L.K. and M.S. M.S. and A.K. coordinated the overall project. L.K. and M.S. conducted redox cycling and electrochemical analyses of HA and model compounds, analysed the data, and wrote the paper. A.P. and A.K. prepared the HA and model compound solutions and the *S. oneidensis* MR-1 inocula, and contributed to writing the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.K. and M.S.

Competing financial interests

The authors declare no competing financial interests.