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Electron Transfer from Humic Substances to Biogenic and Abiogenic Fe(III) Oxyhydroxide Minerals

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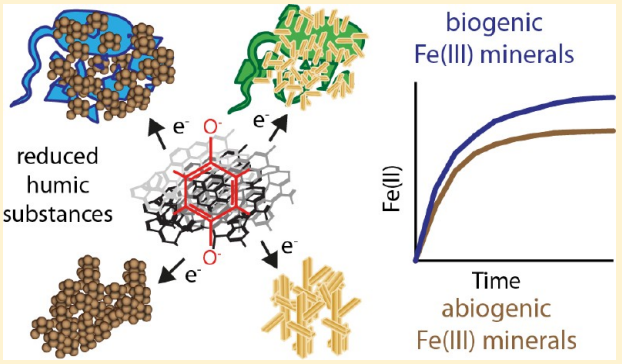
ABSTRACT: Microbial humic substance (HS) reduction and subsequent abiotic electron transfer from reduced HS to poorly soluble Fe(III) (oxyhydr)oxides, a process named electron shuttling, significantly increases microbial Fe(III) mineral reduction rates. However, the importance of electron shuttling in nature and notably the electron transfer from HS to biogenic Fe(III) (oxyhydr)oxides have thus far not been determined. In this study, we have quantified the rate and extent of electron transfer from reduced and nonreduced Pahokee Peat humic acids (PPHA) and fresh soil organic matter (SOM) extracts to both synthetic and environmentally relevant biogenic Fe(III) (oxyhydr)oxides. We found that biogenic Fe(III) minerals were reduced faster and to an equal or higher degree than their abiogenic counterparts. Differences were attributed to differences in crystallinity and the association of bacterial biomass with biogenic minerals. Compared to purified PPHA, SOM extract transferred fewer electrons per milligram of carbon and electron transfer was observed only to poorly crystalline ferrihydrite but not to more crystalline goethite. This indicates a difference in redox potential distribution of the redox-active functional groups in extracted SOM relative to the purified PPHA. Our results suggest that HS electron shuttling can also contribute to iron redox processes in environments where biogenic Fe(III) minerals are present.



INTRODUCTION

Humic substances (HS) are heterogeneous, polyfunctional organic molecules originating from the degradation of different types of organic matter.[1] They are present in almost all aquatic and terrestrial environments[1,2] and are redox-active due to a variety of redox-active functional units in the HS structure including quinoid functional groups.[3,4] These functional groups vary slightly in redox potential so that HS can accept and donate electrons over a range of redox potentials.[4] The redox potential of HS mostly lies in the range of +0.1 to −0.3 V;[4] however the distribution and frequency of the individual redox potentials varies between HS samples of different origin and composition.[4]

HS can be reduced by a variety of different bacteria including iron-reducing,[5] sulfate-reducing,[6] and even methanogenic[6] and fermenting[7] bacteria. Once reduced, HS can undergo different redox reactions acting as electron donors toward Fe(III) minerals,[5,8] oxygen,[8−10] organic pollutants such as chlorinated compounds,[11] and nitrobenzenes[12,13] or even bacteria.[14] Electron transfer from microbially reduced HS to Fe(III) minerals leads to the reduction of the Fe(III) minerals and to the reoxidation of the reduced HS, restoring them for further microbial reduction.[5] This so-called electron shuttling between HS-reducing microorganisms and Fe(III) minerals can significantly increase the reduction rates of poorly soluble Fe(III) minerals,[5,15] enable the microbial reduction of otherwise inaccessible Fe(III) phases,[16] and even facilitate the indirect Fe(III) reduction by bacteria that are not able to reduce Fe(III) directly.

In the environment, different iron minerals are formed, transformed and dissolved due to iron redox cycling, which is to a large part performed by microbial Fe(III) reduction and Fe(II) oxidation.[17−19] Fe(II) is oxidized by microaerophilic Fe(II)-oxidizers for example in groundwater seepage areas[20−22] or by nitrate-reducing and photoautotrophic Fe(II)-oxidizers under anoxic conditions as can be found for instance in freshwater sediments and aquifers.[22] Because of the low solubility of Fe(III) at neutral pH, microbial Fe(II) oxidation leads to the precipitation of Fe(III) minerals such as poorly crystalline ferrihydrite (approximate formula $Fe(OH)_3$),[21,22] but also more crystalline minerals such as lepidocrocite (γ-FeOOH) and goethite (α-FeOOH).[22,23] These so-called biogenic minerals, are often poorly ordered and nanocrystalline.[20,22,24,25] Compared to synthetic abiogenic Fe(III) minerals, biogenic Fe(III) minerals are characterized by a high degree of impurities and vacancies and especially by the association with and inclusion of large amounts of cell-derived organic matter.[22,26] Previous studies have indicated that natural and biogenic Fe(III) minerals are more reducible in microbial

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Fe(III) reduction experiments than synthetic Fe(III) minerals.[27−29]

Even though biogenic Fe(III) minerals are found in many different environments,[22] the electron transfer from HS to environmentally relevant biogenic Fe(III) minerals is only poorly understood until now, since previous studies mostly focused on synthetic Fe(III) minerals and highly purified HS samples. Bauer and Kappler[8] found that the extent of electron transfer from reduced HS to different Fe(III) minerals depends on the mineral identity and thus probably on the redox potential of the Fe(III) mineral that functions as electron acceptor. Nanocrystalline and organic matter-rich biogenic Fe(III) minerals are expected to have a different redox potential and consequently to show a different reaction behavior toward HS than abiogenic minerals. The goal of this study was to quantify the rate and extent of electron transfer from reduced and nonreduced humic acids and from fresh soil organic matter extracts to biogenic versus abiogenic Fe(III) minerals, in order to gain more information about the potential of HS electron shuttling in environmental systems that contain high amounts of biogenic Fe(III) minerals.

MATERIALS AND METHODS

Humic Acid Solutions and Soil Organic Matter Extract. Pahokee Peat humic acid reference (PPHA) was purchased from the International Humic Substance Society (IHSS). PPHA (0.5 g/L) was dissolved in 50 mM phosphate buffer and the pH was adjusted to 7. It has to be noted that the phosphate concentrations chosen (50 mM) are higher than typically observed in nature and that this can lead to the formation of Fe(II) phosphate minerals (e.g., vivianite) in our experiments that would not be observed in environmental settings. However, phosphate buffer was chosen as solvent to be able to compare our results to previous results from our and other groups with abiogenic Fe(III) minerals.[8] Fresh soil organic matter (SOM) extract was prepared after a protocol modified from Kaiser et al.[30] and Sharma et al.[31] Top soil was collected from the O-horizon of a spruce and birch forested area in Schönbuch, Baden-Württemberg, Germany. For SOM extraction, 100 g of sieved (2 mm) soil and 400 mL of Milli-Q-water were incubated oxically in a glass bottle on an overhead shaker for 24 h (15 rpm). Afterward, the SOM was allowed to settle for 24 h, and the supernatant was centrifuged (4000 rpm, 45 min) in small glass vials to remove particles prior to filtration (0.45 μm, mixed cellulose ester, Whatman, Germany). The SOM extract was sterile filtered (0.22 μm, cellulose-acetate, Fisher Scientific, Germany), the pH was adjusted to 7, and the extract was stored at 4 °C in the dark for up to 3 weeks. Chemical reduction of the PPHA solution and the SOM extract was performed by H_2/Pd as described in Kappler et al.,[32] and solutions were filtered (0.45 μm, cellulose−acetate, Merck Millipore, Germany) in an anoxic glovebox (100% N_2) to remove the palladium catalyst. The reducing capacity of reduced and nonreduced PPHA and SOM solutions was determined by incubation of the sample with 5 mM Fe(III) citrate for 1 h as described in Lovley et al.[5] followed by quantification of formed Fe(II) by the ferrozine assay,[33,34] and the DOC of the solutions was measured.

Biogenic and Abiogenic Fe(III) Minerals. Biogenic goethite was obtained by oxidation of approximately 7 mM of $FeCl_2$ by the nitrate-reducing, Fe(II)-oxidizing *Acidovorax sp.* strain BoFeN1[35] in filtered fresh water medium as described in Kleinert et al.[36] Briefly, the fresh water medium (0.3 g/L NH_4Cl, 0.5 g/L $MgSO_4$, 0.1 g/L $CaCl_2$, 0.14 g/L KH_2PO_4, 0.2 g/L NaCl, 22 mM $NaHCO_3$) was amended with 10 mM $FeCl_2$ and, after 48 h, precipitates of $Fe_3(PO_4)_2$ and $Fe(CO_3)_2$, which had formed after $FeCl_2$ addition, were removed by filtration (0.22 μm, polyethersulfone, Merck Millipore, Germany) in an anoxic glovebox. The filtered medium containing approximately 7 mM of dissolved Fe(II) was amended with acetate (5 mM) and nitrate (10 mM) and inoculated with BoFeN1. Biogenic ferrihydrite was produced by oxidation of 10 mM $FeCl_2$ by the phototrophic, Fe(II)-oxidizing *Rhodobacter ferrooxidans* strain SW2 in unfiltered fresh water medium. The medium composition was the same as described for BoFeN1, but the medium was not filtered and the precipitates remained in the medium since SW2 was shown to also completely oxidize the precipitated Fe(II).[23] Biogenic Fe(III) precipitates including the Fe(II)-oxidizing cells and associated biomolecules were harvested by centrifugation when Fe(II) oxidation was complete, washed three times with anoxic Milli-Q-water, and dried in an anoxic glovebox.

Abiogenic goethite (α-FeOOH, Bayferrox 920Z) was purchased from LANXESS (Leverkusen, Germany) and washed three times with Milli-Q-water and dried at room temperature. This type of goethite was chosen in order to allow the comparison to previous studies by our group[8,37] and others.[38] Abiogenic ferrihydrite was synthesized by neutralization of 200 mM $Fe(NO_3)_3$ by KOH as described by Cornell and Schwertmann,[39] washed four times with Milli-Q-water, and dried at room temperature. Despite the storage as a dried powder, the reactivity of the abiogenic FH still decreased with time (data not shown). Therefore, it was used for no longer than two months after synthesis.

For the experiments, dried Fe(III) minerals were resuspended in Milli-Q-water to a concentration of 5 mM for biogenic goethite, biogenic ferrihydrite, and abiogenic ferrihydrite and to 50 mM for the abiogenic goethite, respectively. The suspensions were made anoxic by evacuating the headspace three times (−0.9 bar, 3 min) and flushing with N_2. The different Fe(III) mineral concentrations were chosen to correct for the different BET surface areas of the minerals (9−12 m^2/g for abiogenic goethite[40] compared to 158 m^2/g for biogenic goethite[35] and 286 and 359 m^2/g for biogenic and abiogenic ferrihydrite, respectively (this study)).

Fe(III) Mineral Characterization. μ-XRD analyses of the dried biogenic and abiogenic Fe(III) minerals were performed using a Bruker D8 Discover X-ray diffraction instrument (Bruker AXS GmbH, Germany) with a Co Kα X-ray tube (λ = 0.179 nm), operating at 30 kV, 30 mA.[41] The EVA_ 10.0.1.0 software was used to merge the three measured frames of one sample and to identify the mineral phases present using the PDF-database licensed by ICDD (International Centre for Diffraction Data). The BET measurement of the biogenic and abiogenic ferrihydrite was done on an ASAP2000 (Micromeritics, Germany). For SEM imaging, dried Fe(III) minerals were ground and transferred onto aluminum stubs either dry or suspended in acetone. Samples were imaged as described previously.[42,43] For Mössbauer analysis of the reacted Fe(III) minerals, the entire content of two or three reaction vials was filtered (mixed cellulose ester, 0.45 μm Millipore) in an anoxic glovebox. The dried filters were covered with polyimide film (Kapton) to protect them from oxidation and transferred from the glovebox directly into the precooled cryostat (Janis cryogenics, USA). Mössbauer spectra were collected with a constant acceleration drive system in transmission mode

(WissEL, Germany) and with a ^{57}Co source in Rh matrix. Spectra were calibrated against a spectrum of alpha-Fe metal foil collected at room temperature. Spectra calibration and fitting was performed with Recoil software (University of Ottawa, Canada) using Voigt-based fitting methods. The samples were analyzed at room temperature, 140, 77, and 5 K.

Fe(III) Reduction Experiments. Experimental setup and all sampling were performed in an anoxic glovebox (100% N_2). The Fe(III) reduction experiments were started by mixing 10 mL of reduced or nonreduced PPHA or SOM solution with 5 mL of the respective Fe(III) minerals stock suspension (final concentrations: 16 mM of abiogenic goethite, 1.6 mM of all other Fe(III) minerals, 0.33 g/L PPHA (i.e., 0.19 gC/L PPHA), 0.077 gC/L SOM) in brown 20 mL headspace vials. The vials were closed airtight with butyl rubber stoppers and kept on an overhead shaker (5 rpm) at room temperature. Samples for quantification of dissolved Fe(II) were withdrawn with a syringe through the stopper and centrifuged, and Fe(II) in the supernatant was quantified with the ferrozine assay.[33,34] Fe(II) formation over time was quantified only in the dissolved fraction since it is not possible to completely remove the HS from the Fe(III) solids and dissolution of the Fe(III) minerals in the presence of the HS would lead to electron transfer during the extraction and thus to an overestimation of electron transfer. Therefore, Fe(II) in the solid phase due to sorption of Fe(II) to the Fe(III) minerals or the formation of secondary Fe(II)−Fe(III) minerals was determined only at the end of the experiments by Mössbauer spectroscopy. Additionally, the total amount of iron in each bottle was quantified in the beginning of the experiment by extraction with 6 M HCl followed by reduction of all Fe(III) to Fe(II) with hydroxylamine−hydrochloride and Fe(II) quantification with the ferrozine assay.[33,34] Controls consisted of Fe(III) mineral suspensions mixed with phosphate buffer (without HS) for quantification of Fe(II) present in and leaching from the minerals and of PPHA and SOM solutions mixed with Milli-Q-water (without Fe(III) minerals) in order to quantify Fe(II) present in the PPHA and SOM solutions. Reducing capacities of PPHA and SOM were calculated from the Fe(II) concentration at the end of the experiment after subtraction of the Fe(II) concentrations in the controls. Initial rates of Fe(II) formation were calculated by linear regression of the first sampling points which showed a linear increase in Fe(II) over time.

RESULTS AND DISCUSSION

Synthesis and Characterization of Biogenic Fe(III) Minerals. To quantify electron transfer from humic substances to biogenic Fe(III) oxyhydroxides, we synthesized two different biogenic Fe(III) minerals by two Fe(II)-oxidizing bacteria, the nitrate-reducing Fe(II)-oxidizer *Acidovorax sp.* BoFeN1 for biogenic goethite and the phototrophic Fe(II)-oxidizer *Rhodobacter ferrooxidans* SW2 for biogenic ferrihydrite.

The μ-X-ray diffractogram of the precipitates produced by BoFeN1 clearly corresponds to goethite (Figure 1) with no observable trace of any other crystalline mineral phases. The production of biogenic goethite during Fe(II) oxidation by BoFeN1 is consistent with previous studies from our laboratory.[35,36,44] Comparison to the diffractogram of abiogenic goethite (Figure 1) shows that the signals of the biogenic goethite are broader and less sharp than the signals of the abiogenic goethite. This indicates a smaller average crystallite size and/or lower crystallinity in the biogenic goethite,[45] which could be due to the inclusion of cell-derived organic matter or

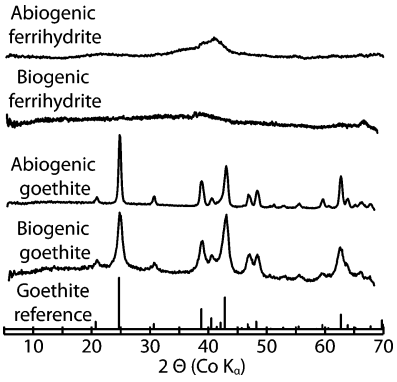

Figure 1. μ-XRD diffractograms of biogenic and abiogenic minerals before reaction with humic substances. Biogenic goethite is the mineral product of Fe(II) oxidation by *Acidovorax sp.* BoFeN1. Biogenic ferrihydrite is the mineral product of Fe(II) oxidation by *Rhodobacter ferrooxidans* SW2. μ-XRD data of biogenic and abiogenic goethite and of the biogenic ferrihydrite were recorded in this study, data of the abiogenic ferrihydrite is from Piepenbrock et al.[66] Reproduced with permission from ref 66. Copyright 2011 Elsevier.

the incorporation of ions from the microbial growth medium. This is in agreement with previous studies which frequently observed a lower crystallinity for biogenic Fe(III) minerals.[22,46,47]

Electron microscopic imaging of the biogenic and abiogenic goethite (Figure 2) showed a needle-like structure that is typical for goethite for both the biogenic and the abiogenic goethite. However, the SEM images also showed that the particle size of the biogenic goethite is well below 1 μm in the range of 100−200 nm, while the particles of the abiogenic goethite are larger by more than 1 order of magnitude with a size of up to 3−4 μm (Figure 2). This correlates well to the BET surface area of the biogenic goethite produced by BoFeN1 of 158 m^2/g measured by Kappler et al.[35] compared to a surface area of 9−12 m^2/g for the abiogenic goethite used in our study.[40]

In contrast to the crystalline mineral product of Fe(II) oxidation by BoFeN1, precipitates formed during the oxidation of Fe(II) by SW2 did not show any defined signals in the μ-X-ray diffractogram and showed close similarities to abiogenic ferrihydrite (Figure 1). This is consistent with a previous study from our group[26] where a poorly crystalline Fe(III) mineral phase was identified at the end of Fe(II) oxidation by SW2 in unfiltered medium. Eickhoff et al.[48] identified the Fe(III) precipitates formed by SW2 as biogenic ferrihydrite based on a detailed Mössbauer analysis. The only difference to abiogenic ferrihydrite was the presence of 10% of a more magnetically ordered fraction in the biogenic ferrihydrite.[48] However this fraction was only visible at 77 K and not at room temperature[48] indicating that it is superparamagnetic, which makes it invisible for XRD. In SEM micrographs of the biogenic ferrihydrite, no crystalline structures were observed (Figure 2). Instead, aggregates were visible, which were slightly smaller and more loosely packed than those of the abiogenic ferrihydrite. In both cases, they probably consist of much smaller particles in the range of a few nanometers as described for ferrihydrite previously.[49] BET measurements yielded surface areas of 286 m^2/g for the biogenic ferrihydrite and 359 m^2/g for the abiogenic ferrihydrite confirming that the surface area and particle size of the two ferrihydrites are in the same range. On the basis of this characterization it seems that the biogenic and

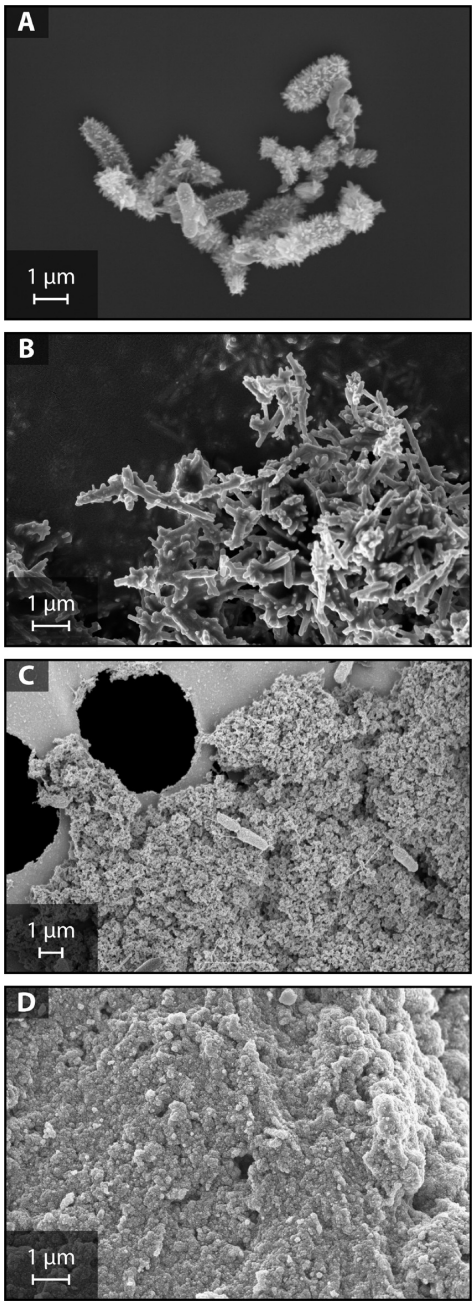

Figure 2. SEM micrographs of (A) biogenic goethite needles on cell surfaces, (B) abiogenic goethite, (C) biogenic ferrihydrite, and (D) abiogenic ferrihydrite. Biogenic goethite is the mineral product of oxidation of 7 mM dissolved Fe(II) by *Acidovorax sp.* BoFeN1. Biogenic ferrihydrite is the mineral product of the oxidation of 10 mM Fe(II) by *Rhodobacter ferrooxidans* SW2. Micrographs were taken at an acceleration voltage of 20, 5, 1, and 5 kV and a working distance of 4, 2, 4, and 3 mm for images A, B, C, and D, respectively.

the abiogenic ferrihydrite are very similar in crystallinity as well as particle size.

Rates of Electron Transfer from Humic Substances to Different Fe(III) Minerals. To quantify the electron transfer from humic substances to biogenic and abiogenic Fe(III) oxyhydroxides, synthetic and microbially precipitated goethite and ferrihydrite were incubated with reduced and nonreduced Pahokee Peat humic acid (PPHA) and fresh soil organic matter extract (SOM). The rates of electron transfer were determined based on the rates of aqueous Fe(II) formation (see Materials and Methods section).

We found that chemically reduced PPHA readily reduced all four Fe(III) minerals used in this study, that is, biogenic and abiogenic goethite and biogenic and abiogenic ferrihydrite (Figure 3). A difference of about 1 order of magnitude was

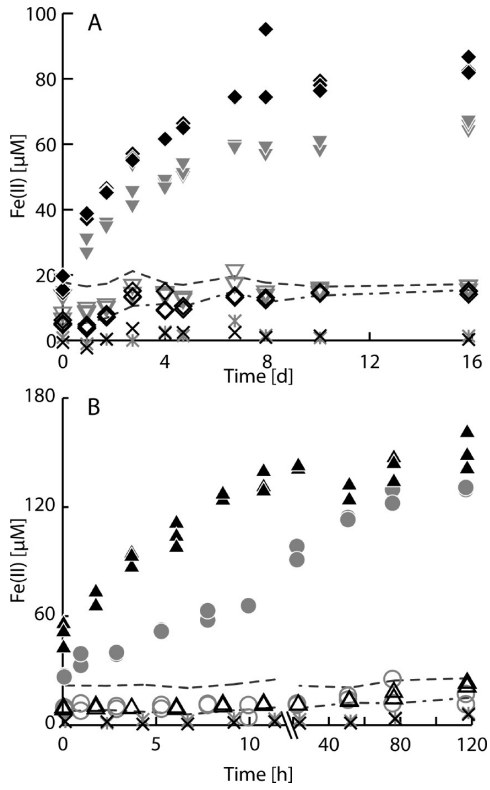

Figure 3. Electron transfer from reduced and nonreduced Pahokee Peat humic acid (PPHA) to biogenic and abiogenic (A) goethite and (B) ferrihydrite, depicted as formation of dissolved Fe(II) over time. Reduction of biogenic goethite (◆), abiogenic goethite (light gray ▼), biogenic ferrihydrite (▲), and abiogenic ferrihydrite (light gray ●) by reduced (closed symbols) and nonreduced (open symbols) PPHA. Controls of biogenic (×) and abiogenic (∗) minerals without PPHA and reduced (dashed lines) and nonreduced (dashed and dotted lines) PPHA without Fe(III) minerals. Note the different axes of the two graphs and the gap in the *x*-axis of (B).

observed between the initial rates of Fe(II) formation for goethite (82.1 ± 7.6 μmol/(d*g HA) and 40.1 ± 7.3 μmol/(d*g HA) for biogenic and abiogenic goethite, respectively) and ferrihydrite (954.7 ± 60.5 μmol/(d*g HA) and 347.2 ± 7.8 μmol/(d*g HA) for biogenic and abiogenic ferrihydrite, respectively). For both, goethite and ferrihydrite, aqueous Fe(II) formation was faster for biogenic minerals than for the abiogenic counterparts (Figure 3).

The initial Fe(II) formation rate in set-ups with the different Fe(III) minerals increased in the order abiogenic goethite < biogenic goethite < abiogenic ferrihydrite < biogenic ferrihydrite suggesting an increase in reduction rates with increasing mineral surface area. However, the variations in the Fe(II) formation rates were far greater than can be explained by the surface area alone. This is especially the case, because a ten times higher Fe(III) concentration of the abiogenic goethite was used in the reduction experiments. Even though leading potentially to a stronger aggregation of the mineral particles,

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the higher concentration of abiogenic goethite can be expected to partly correct for the very low surface area (9−12 m^2/g).[40] The factors controlling the reactivity and dissolution of Fe(III) minerals are discussed controversially in the literature.[50,51] Proposed controlling factors besides surface area[52] are solubility,[53] crystallinity and redox potential,[51] and aggregation of the mineral particles[52,54] and have been found to vary between microbial and chemical Fe(III) mineral reduction.[51,53,55]

Differences in mineral surface charge are not expected to be responsible for the differences in Fe(II) formation rates since both the biogenic and the abiogenic Fe(III) minerals are expected to be negatively charged under our experimental conditions.[56] This suggests that other factors than mineral surface properties influenced the Fe(II) formation rates. Postma[57] and Roden[52] found that the chemical reductive dissolution of synthetic Fe(III) minerals by ascorbate depended on the crystallinity of the Fe(III) mineral. In our experiments, there was a difference in crystallinity between the biogenic and the abiogenic goethite (Figure 1). This could indeed explain the observed lower initial Fe(II) formation rates for the abiogenic goethite compared to that of the biogenic goethite. However, no clear difference in crystallinity was visible between the biogenic and the abiogenic ferrihydrite (Figure 1), suggesting that other bulk mineral properties must play a role as well. The main difference of biogenic compared to abiogenic minerals is the incorporation of organic matter derived from the microbial cells.[26,58] These incorporated organic molecules could facilitate the reduction, as it was described also by other authors that biogenic Fe(III) minerals were generally reduced faster than abiogenic minerals.[28,55] Possible effects of incorporated organic matter on microbial Fe(III) mineral reduction were suggested to be due to electron shuttling between the cells and the Fe(III) mineral by incorporated redox-active organic molecules,[28] complexation of Fe(III) changing its redox potential and facilitating its reduction,[28] and complexation of Fe(II) which could increase the thermodynamic driving force for Fe(III) reduction[59] and secondly prevent its sorption to the Fe(III) mineral surface and thus the blocking of the Fe(III) mineral surface.[28] Similar processes could also have affected the electron transfer from HS to biogenic ferrihydrite in our experiments and even the electron transfer to biogenic goethite, although, in this case, it cannot be clearly separated from the effect of the lower crystallinity.

Extent of Electron Transfer from Humic Substances to Different Fe(III) Minerals. In addition to the initial rates of electron transfer from the humic acids to the Fe(III) minerals, we determined the amount of electrons transferred, that is, the reducing capacities of the humic substances. We found that in contrast to the differences in Fe(II) formation rates, the reducing capacities of the reduced PPHA toward the different biogenic and abiogenic Fe(III) minerals varied only by a factor of up to two (Figure 4). More electrons were transferred to the biogenic than to the abiogenic goethite, while there was no significant difference in the reducing capacities obtained with the biogenic compared to the abiogenic ferrihydrite (Figure 4). Nonreduced PPHA and reduced as well as nonreduced SOM transferred electrons only to poorly crystalline ferrihydrite, but no electrons were transferred from the SOM extract to biogenic or abiogenic goethite.

A different extent of electron transfer from reduced PPHA to different Fe(III) minerals was described before by Bauer and Kappler[8] who attributed their observations to the differences in

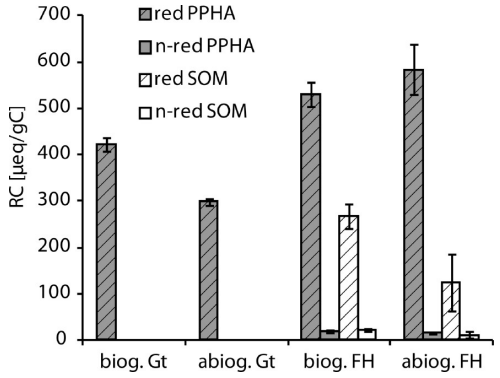

Figure 4. Reducing capacities (RC) of reduced (red) and nonreduced (n-red) Pahokee Peat humic acids (PPHA) and fresh soil extract (SOM) toward biogenic (biog.) and abiogenic (abiog.) goethite (Gt) and ferrihydrite (FH). Reducing capacities were calculated from the concentration of dissolved Fe(II) at the end of the experiment after subtraction of the Fe(II) concentration in the respective control bottles. Error bars are standard deviations of triplicate bottles.

redox potential of the Fe(III) minerals. The same is probably the case for the reducing capacities determined in our experiments for biogenic and abiogenic goethite since the redox potential of the biogenic goethite is expected to be different compared to the abiogenic goethite due to its lower crystallinity and the incorporation of organic matter. The higher extent of electron transfer suggests that the redox potential of the biogenic goethite is less negative than the redox potential of the abiogenic goethite of −274 mV.[8] The fact that the biogenic and abiogenic ferrihydrite were reduced to the same extent (Figure 4) suggests that the difference in redox potential between these two mineral phases was minor. This is supported by the similar crystallinity of the biogenic as that of the abiogenic ferrihydrite (Figure 1). The presence of cell-derived organic matter in the biogenic ferrihydrite, which apparently had a strong effect on the reduction rate (see above), seems to have no influence on the extent of reduction by reduced PPHA. The observed equal or higher extent of reduction of the biogenic minerals is consistent with the findings of Zachara et al.,[27] who described that direct and indirect (via the electron shuttle AQDS) microbial reduction of natural Fe(III) oxides proceeded to an equal or higher extent than reduction of synthetic Fe(III) oxides.

While reduced PPHA readily reduced all four Fe(III) minerals studied, nonreduced PPHA and reduced and nonreduced fresh soil extract (SOM) transferred electrons only to biogenic and abiogenic ferrihydrite (Figure 4). This is in good agreement with previous findings that nonreduced HS also had a low reducing capacity,[15,60,61] and transferred electrons only to poorly crystalline and dissolved Fe(III) phases.[8] The lack of electron transfer from reduced SOM to both abiogenic and biogenic goethite indicates that the redox properties, that is, the redox potential distribution and frequency, of this natural organic matter extract might differ from those of the highly purified PPHA. The electron accepting capacity (EAC) during chemical reduction (determined as the difference in reducing capacity (RC) toward Fe(III) citrate before and after reduction) of the SOM was 718 ± 158 μeq/gC (mean ± standard deviation, n = 5) compared to an EAC of 1519 ± 68 μeq/gC (n = 4) for the PPHA. Thus, the concentration of redox-active functional groups in the SOM is about half as high as in the PPHA. On the basis of the EAC, a lower RC of

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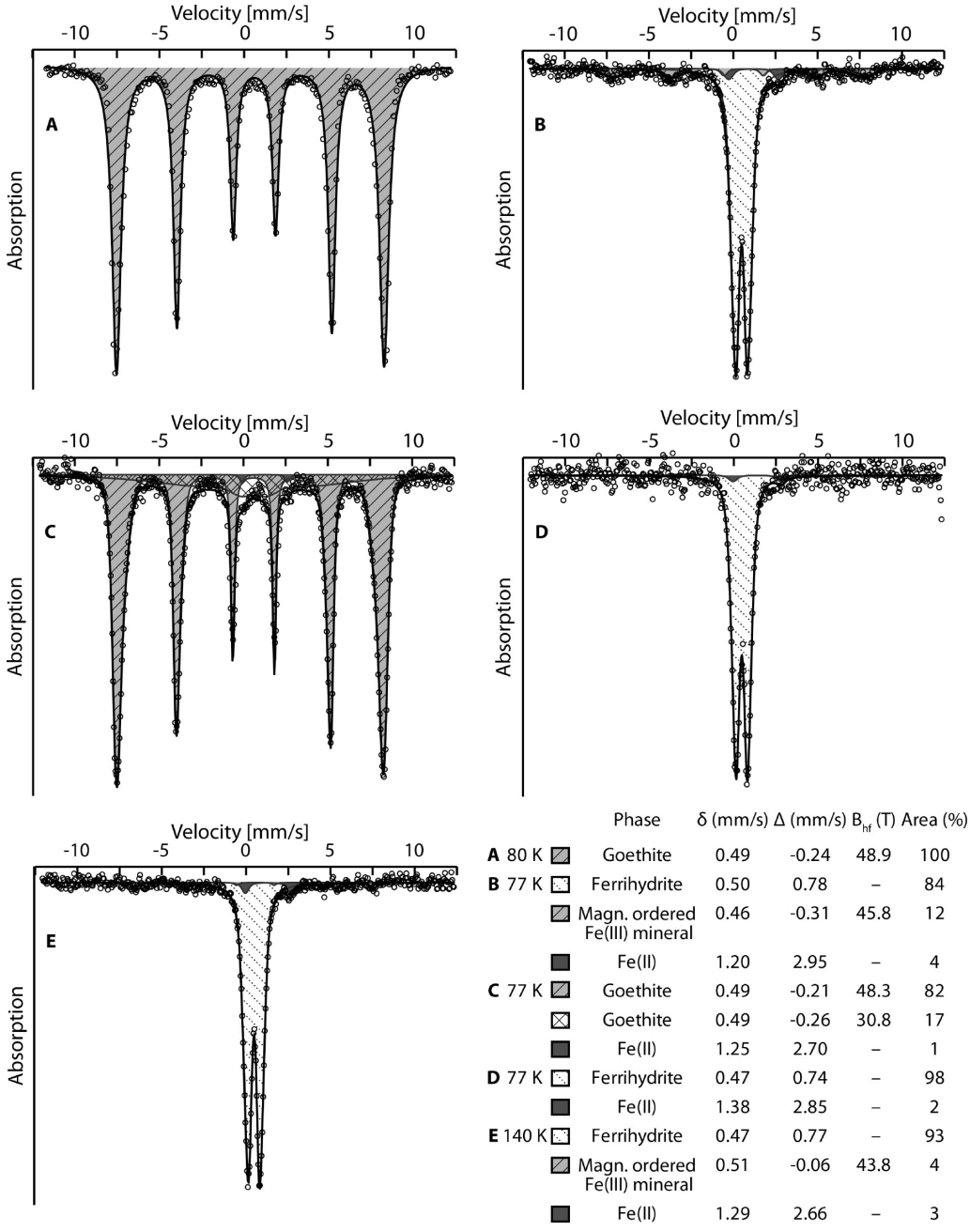

	Phase	δ (mm/s)	Δ (mm/s)	B_{hf} (T)	Area (%)
A 80 K	Goethite	0.49	-0.24	48.9	100
B 77 K	Ferrihydrite	0.50	0.78	–	84
	Magn. ordered Fe(III) mineral	0.46	-0.31	45.8	12
	Fe(II)	1.20	2.95	–	4
C 77 K	Goethite	0.49	-0.21	48.3	82
	Goethite	0.49	-0.26	30.8	17
	Fe(II)	1.25	2.70	–	1
D 77 K	Ferrihydrite	0.47	0.74	–	98
	Fe(II)	1.38	2.85	–	2
E 140 K	Ferrihydrite	0.47	0.77	–	93
	Magn. ordered Fe(III) mineral	0.51	-0.06	43.8	4
	Fe(II)	1.29	2.66	–	3

Figure 5. Mössbauer spectra of biogenic goethite (A) before reaction with reduced Pahokee Peat humic acid (PPHA), and biogenic ferrihydrite (B), biogenic goethite (C), and abiogenic ferrihydrite (D) after reaction with reduced PPHA. For the biogenic ferrihydrite after reaction with PPHA (B), a second spectrum recorded at 140 K (E) is shown to illustrate the superparamagnetic character of the magnetically ordered Fe(III) mineral phase. Temperatures of all Mössbauer analyses and fitting parameters are given in the table.

reduced SOM compared to that of reduced PPHA would be expected, but not the complete absence of electron transfer to goethite. Therefore, not the concentration but the quality of the redox-active functional groups could be the reason for the different RCs. Aeschbacher et al.[4] showed that humic acids were able to accept electrons over a range of redox potentials of 0.2 to 0.4 V and therefore described their redox potential as a continuum over this range. They also showed that the range of redox potential and the frequency of moieties of a certain redox potential vary between different HA samples. On the basis of this knowledge, we hypothesize that the redox potential distribution of the functional groups is in a less negative range in SOM as compared to PPHA and, thus, less favorable for the reduction of low-E_h electron acceptors like goethite which has an E_h of −274 mV.[8] Additionally, differences in the Fe complexation capacity or in the accessibility of the redox-active functional groups of the different humic materials could also have influenced the electron transfer to the different Fe(III) minerals. These differences between SOM and PPHA could be due to the different origin of the organic matter samples or they could be the result of changes in the PPHA during extraction and purification with alkaline and acidic solutions such as condensation or polymerization reactions.

Mineral Products of Electron Transfer from Humic Substances to Fe(III) Minerals. To determine if electron transfer from reduced PPHA to biogenic and abiogenic Fe(III)

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minerals led to the formation of any secondary minerals, Mössbauer spectroscopy was performed on the solid phase reaction products (Figure 5). The first goal of the Mössbauer spectroscopy analysis was to determine if any Fe(II) was present in the solid phase after reaction of the minerals with the humic substances. In Mössbauer spectra of the reduced Fe(III) minerals, indications of a small Fe(II) doublet could be found (Figure 5B–E). However, these doublets only accounted for up to 4% of the total iron, which is very close to the detection limit of Mössbauer spectroscopy, and could not in all cases be clearly separated from the background noise. Therefore, the exact quantification of the Fe(II) present in the solid phase was not possible. Nevertheless, the Mössbauer data indicated that in addition to dissolved Fe(II), there was also a small amount of Fe(II) present in the solid phase after reaction with PPHA.

To make sure that the Fe(II) detected in the Mössbauer spectra was formed during the reaction with the PPHA and was not already present in the biogenic minerals before the reaction as a result of incomplete microbial Fe(II) oxidation, we determined the amount of Fe(II) in the nonreacted biogenic goethite and ferrihydrite by complete dissolution of the minerals followed by the ferrozine assay. While we did not find any Fe(II) in the biogenic goethite, the biogenic ferrihydrite contained 1.5 ± 0.1% of Fe(II). We thus conclude that if any formation of solid-phase Fe(II) or sorption of dissolved Fe(II) to the Fe(III) minerals took place during reaction with PPHA, this amounted to a maximum of 2.5% of the total iron.

Besides the formation of Fe(II), no new mineral phases could be detected in the Mössbauer spectrum of the biogenic goethite after the reaction (Figure 5C) compared to the spectrum of the same mineral before the reaction with PPHA (Figure 5A). The biogenic ferrihydrite after the reaction with HS contained an admixture of a magnetically ordered component, which amounted to 12% at 77 K (Figure 5B) and 4% at 140 K (Figure 5E), but the fact that Eickhoff et al.[48] found a similar fraction of a magnetically ordered mineral phase in their biogenic ferrihydrite that was produced exactly as in our study suggests that this component was already present before the reaction with HS. Similarly, the spectrum of the abiogenic ferrihydrite after reaction with reduced PPHA (Figure 5D) showed no formation of further mineral phases beside ferrihydrite. Thus, no formation of secondary minerals due to the reduction of the different Fe(III) minerals by reduced PPHA was found.

The lack of secondary mineral formation in our experiments could be due to the low amount of Fe(II) formed in all set-ups (6–10% for biogenic goethite and biogenic and abiogenic ferrihydrite and <1% for abiogenic goethite). This finding is consistent with reports from Cutting et al.[51] describing that crystalline Fe(III) phases did not undergo any bulk mineralogical transformation during microbial reduction in the presence of AQDS as electron shuttle, even if small amounts of Fe(II) accumulated on the mineral surface. Ferrihydrite, on the other hand, is known to undergo mineral transformations, for example to goethite or magnetite, due to the reaction with adsorbed Fe(II).[62−64] However, this transformation can be inhibited by high phosphate concentrations[65] as was present in our study and by HS sorption to the ferrihydrite.[66] Therefore, the lack of secondary mineral formation in our experiments is not surprising. However, since such high concentrations of phosphate buffer are not expected to be present in environmental systems and, furthermore, oxidized humic substances are continuously rereduced by microorganisms providing the possibility of a higher extent of Fe(III) reduction, it is possible that electron transfer from reduced humic substances to Fe(III) minerals will lead to secondary mineral formation in the environment.

Environmental Implications. In the present study we could show that biogenic Fe(III) minerals, produced by the oxidation of Fe(II) by two different Fe(II)-oxidizing bacterial strains, readily accepted electrons from reduced PPHA. The rates of Fe(II) formation for biogenic goethite and ferrihydrite by reduced PPHA were always higher than the rates for the respective abiogenic Fe(III) minerals and also the extent of reduction was equal or higher for the biogenic minerals. These differences were attributed to a slightly higher redox potential of the biogenic Fe(III) minerals compared to that of their abiogenic counterparts caused by the lower crystallinity and due to the incorporation of organic matter into the biogenic minerals. These findings indicate that, in environmental systems with a high content of biogenic Fe(III) minerals, for example freshwater or marine sediments where iron-oxidizing bacteria have been described,[21,22] electron transfer from reduced HS and thus electron shuttling from bacteria to Fe(III) minerals could be even more important than previously estimated based on studies with abiogenic Fe(III) minerals.

Furthermore, we showed that a fresh soil organic matter (SOM), extracted by the incubation of an O-horizon of a forest soil with water, showed an electron accepting capacity similar to that of highly purified PPHA. However, the redox potential distribution of the SOM was less favorable for electron transfer to Fe(III) minerals than that of PPHA, and reduced SOM transferred electrons only to poorly crystalline ferrihydrite but not to goethite. Nevertheless, our results demonstrate that Fe(III) mineral reduction and electron shuttling is not restricted to highly purified HS samples, but is also possible with an untreated organic matter extract as it might spontaneously form upon rainwater filtration through a forest soil. Taken together, the findings presented in this study confirm the potential contribution of HS electron shuttling to electron fluxes and biogeochemical processes in the environment.

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