

Interactive comment on “Reduction of ferrihydrite with adsorbed and coprecipitated organic matter: microbial reduction by *Geobacter bremensis* vs. abiotic reduction by Na-dithionite” by K. Eusterhues et al.

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Referee #1 General comments: Iron oxide-organic matter interactions are important for the biogeochemical processes of iron and carbon cycling. Although existing studies suggest microorganisms use organic matter as electron shuttle to expedite iron reduction, it is likely that coating of organic matter on iron oxide surface or co-precipitation of organic matter with iron oxide can inhibit the iron reduction by preventing microbial access to mineral surface. This study was designed to examine the dissimilar effects of organic matter coating layer and co-precipitates on the abiotic/biotic reduction of iron.

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The results are interesting and potentially helpful in evaluating the iron geochemical processes in the presence of organic matter. This reviewer found following concerns for the manuscript, before it can be published.

The authors tried to compare the scenarios of surface coating of organic matter and co-precipitation of organic matter and iron. However, there are insufficient characterizations for complex produced through the two different ways. The physicochemical properties of two kinds of complex, including their XPS and surface areas, should be stated more clearly and used to interpret the iron reduction results.

Thank you very much for your in-depth review and the useful comments. In the revised version we now present FTIR and XPS spectra of the obtained ferrihydrite-organic matter complexes.

Specific comments: Line 65: What kind of certain conditions?

We changed the sentence into: Line 67: “At high Fh concentrations in solution (30mM), Amstaetter et al. (2012) even observed a decrease in Fe(III) reduction due to humic acid addition.”

Lines 108-112: More details about the synthesis process should be given.

We added more details to our description. For example the molar C/Fe ratio of the initial solution to allow for a better comparison the study of Shimizu et al. 2013 (Line 120).

Line 196: How was the second derivative of FTIR obtained? Basic description should be added to the method part.

We added: Line 133: “The spectra were baseline corrected by subtracting a straight line running between the two minima of each spectrum and normalized by dividing each data point by the spectrum's maximum. The second derivative was calculated using the Savitzky-Golay algorithm over 19-23 points.”

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Lines 184-201: Did authors analyze NMR and FTIR for iron oxide-sorbed and co-precipitated NOM? Under same concentration of organic matter, the chemical composition of NOM can be different for the sorbed and co-precipitated NOM.

NMR analysis on coprecipitates and adsorption complexes is not possible due to the paramagnetic Fe in ferrihydrite. Instead, we intended to collect NMR spectra from the supernatants, but the spectra turned out to be of such poor quality that they cannot be integrated. The poor quality can be explained by the likely presence of soluble Fe complexes in the supernatant.

FTIR spectra were collected from all samples. They show that coprecipitates and adsorption complexes are free of nitrate and that carboxyl groups of the forest floor extract are involved in bond formation between organic matter and ferrihydrite. Spectra of coprecipitates are very similar to spectra of adsorption complexes. However, the fact that we cannot see a compositional difference by FTIR does not necessarily mean that such a difference does not exist. In a previous experiment we found differences in the organic matter composition by NMR and sugar analysis, but not by FTIR. We therefore discuss possible effects of a different organic matter composition on reduction in the revised manuscripts (see below).

We did not add the surface area (BET) results, because in case of mixed mineral-organic phases they will not give the actual surface area of the ferrihydrite crystals, but show a strongly reduced surface area for all coprecipitates and adsorption complexes (masking effect of the organic matter during N₂ adsorption).

In the revised manuscript, we now show and discuss the FTIR spectra (See new Figure 1 and 2) and XPS spectra (See new Figures 4 and 5) of all incubated samples.

We added: Line 264: FTIR-spectra of adsorbed and coprecipitated organic matter differ from the original forest floor extract. The peak assigned to C=O in protonated carboxyl groups (1723 cm⁻¹) is reduced to merely a shoulder (seen only in the 2nd derivative of AFhD and CFhD at 1716 and 1712 cm⁻¹), while the signal related to deprotonated

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carboxyl groups (1622 cm⁻¹ in FFE) is increased and shifted to higher wavenumbers (1632, 1631 cm⁻¹). This pattern is explained by the formation of inner-sphere surface complexes between carboxylic acids and Fe oxides surfaces or dissolved metals (Kang et al., 2008; Persson and Axe, 2005). The peak at 1148 cm⁻¹ (C-O in carbohydrates) in the forest floor extract is not visible in the adsorbed or coprecipitated organic matter and the peak at 1089 cm⁻¹ is slightly shifted to lower wavenumbers (1082, 1079 cm⁻¹). Both changes point to a fractionation of carbohydrates during adsorption or coprecipitation. The absence of the sharp peaks at 1384 and 825 cm⁻¹ shows that coprecipitates and adsorption complexes are free of nitrate. We assume, the adsorption of organic matter has removed the surface bound nitrate, which could not be removed from ferrihydrite through washing (Fh in Figure 2), and the natural nitrate from the forest floor extract (FFE in Figure 2) did not react with the Fe oxides. FTIR spectra and their second derivatives of adsorbed and coprecipitated organic matter are remarkably similar. Small differences however exist for the main carbohydrate peak and its shoulders, but seem mainly related to the amount of mineral-bound organic matter: While carbohydrates are represented by peaks at ~1125 and ~1080 and ~1040 cm⁻¹ in samples with small C concentrations (AFhA; CFhA), samples with large C concentration show a strong peak at ~1080 cm⁻¹ and a shoulder at ~1040 cm⁻¹. (AFhD, AFhB, CFhD)

Line 146: After subtracting a Shirley-type background, P2p and N1s spectra were evaluated by fitting single pseudo-Voigt profiles (Lorentz portion = 0.2) to the measured data. Fe2p spectra were fitted by a pre-peak, a surface peak, and four multiplet peaks of decreasing intensity as proposed by McIntyre and Zetaruk (1977) and Grosvenor et al. (2004) for high spin Fe(III) compounds. Distances between multiplets were constrained to 1 eV, the FWHM was set to 1.4 eV and the Lorentz portion of the pseudo-Voigt curves was 0.2. The C1s peak was fitted using four pseudo-Voigt profiles with a fixed FWHM of 1.9 and a Lorentz portion of 0.2. The distances between the peaks were fixed to 1.6, 1.6, and 1.1 eV from lower to higher binding energies to distinguish the C1s binding states C-C, C-H, C-O, C-N, C=O, N-C=O and O-C=O.

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Line 286: High resolution XPS spectra of the C1s, N1s, Fe2p, and P2p lines are given in Figure 4. Weak S2p signals (data not shown) above the detection limit were found for the forest floor extract and for coprecipitates and adsorption complexes with low C concentrations (< 115 mg/g). The absence of S in complexes with higher organic matter contents may imply that adsorption of the forest floor organic material outcompetes adsorption of sulfate. The N1s and the P2p peaks show considerable noise (Figure 4), which leads to large scatter for C/N and C/P ratios (Figure 5). Nevertheless, the data show that the C/N ratio and the C/P ratio of coprecipitates and adsorption complexes are clearly higher than that of the original forest floor extract. While C/P-ratios for the coprecipitated organic matter are very similar to that of the adsorbed organic matter, a slightly, but significantly higher mean C/N-ratio (40) for the adsorbed organic matter is observed in comparison to a C/N of 35 for coprecipitated organic matter ($\alpha = 0.05$; T-test). The C1s peak can be deconvoluted into four peaks as shown exemplary for the forest floor extract (Figure 4) and assigned to 285.0 eV: C-C and C-H; 286.6 eV: C-O and C-N; 288.2 eV: C=O and N-C=O, and 289.3 eV: O-C=O (Arnarson and Keil, 2001). The adsorbed and coprecipitated organic matter was found enriched in aliphatic C (C-C, C-H) and carboxylic C (O-C=O), but compositional differences between adsorbed and coprecipitated cannot be seen (data not shown).

Lines 218-225 and Figure 2: XPS C/Fe ratio, is this atomic ratio or just signal ratio? It is better to convert X-axis to C/Fe ratio in bulk.

It is the C/Fe signal ratio. We cannot convert the X-axis to C/Fe, because we measured Fe concentrations only for the incubated samples.

We changed the Figure caption into: Figure 2: "Comparison of chemical surface composition expressed in XPS intensity ratios (C/Fe, C/N, and C/P) and bulk C content of Fh-OM associations.§"

Line 229 and Figure 3: Why did the reduction fraction decrease in control after 20 days? And in the control, the final reduction fraction is around 60-70%, with 30-40% of

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Fe resisting to microbial reduction. What is the mechanism for the reduction-resistance of Fe(III) in control samples?

In the original manuscript we had addressed the decrease in Fe(II) after 20 days only in the methods part:

Line 205:"The degree of dissolution was determined at day 17 for microbial experiments and after 75 min for abiotic experiments. Day 17 for microbial experiments was chosen, because the Fe(II)/Fe(total) of the ferrihydrite control at day 52 is much lower than at day 17 and therefore probably wrong. We assume that this is due to unintentional oxidation at the end of the experiment in this sample."

Now, we mention it also in the figure caption of Figure 3: "The Fe(II)/Fe(total) of the ferrihydrite control (red stars) at day 52 is much lower than at day 17 and therefore unexpectedly low, letting us assume that this is due to unintentional oxidation at the end of the experiment in this sample."

It is commonly found in such experiments that microbial reduction of Fe oxides is incomplete. This is most often explained by surface passivation due to adsorbed Fe(II). We had mentioned this in Line 406: "Partial reduction of Fe oxides during microbial reduction is explained by surface passivation by adsorption of Fe(II) (Roden and Urrutia, 1999, Liu et al., 2001)."

Lines 230-231: The initial reduction rates did not differ significantly from the control for sample with 44 and 98 mg/g C in the adsorption scheme. And the difference in the reduction degree is completely because of the drop of final point in the control system, which seems not reliable. This reviewer suggested only discussing reduction data within 20 days.

This is what we have done. The reduction degree was calculated for day 17 anyway and the initial reduction rates are not affected by the last day. That is, discussion and Table 1 refer to the data of the first 17 days, whereas only Figure 3 shows the results

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of the whole experiment.

Line 240: Did this have any relationships with potential difference in chemical composition of organic matter sorbed by or co-precipitated with iron oxide?

We cannot exclude that differences in reduction between coprecipitates and adsorption complexes are also influenced by the possibly different organic matter composition. However, we cannot come up with a substantiated conclusion, because the necessary parameters (exact adsorption mechanism for complex natural organic matter; electron donating/accepting capacity of mixed Fe oxide/organic matter samples) cannot be determined to date. We therefore discuss this issue in the revised manuscript.

We added: Line 336: "A possibly different composition of the mineral-bound organic matter in coprecipitates compared to adsorption complexes is a further aspect, which has to be taken into account. Although FTIR spectra and XPS spectra were very similar, we cannot exclude differences between adsorbed and coprecipitated material. In a previous experiment with a distinct forest floor extract (Eusterhues et al., 2011) FTIR spectra had also been very similar, whereas ¹³C NMR analyses of the non-reacted fraction had shown that the adsorbed organic matter was enriched in O-alkyl C (carbohydrates), but depleted in carbonyl C and alkyl C relative to the coprecipitated material. (It was not possible to obtain NMR spectra of reasonable quality of the material used in this study. Formation of soluble Fe complexes in the supernatant might be an explanation.) However, this knowledge does not help us to judge the possibly different efficiency with which the possibly different fractions may inhibit ferrihydrite reduction. The ability of molecules to form bi- or multinuclear inner sphere-bonds was recognized to make strong inhibitors with respect to mineral dissolution (Stumm, 1997), while the presence of electron accepting and electron donating groups in the organic material controls its ability to act as an electron shuttle and promote reduction. Quinones and condensed aromatic groups have been shown to be redox active in humic acids and chars (Dunnivant et al., 1992; Scott et al., 1998; Klüpfel et al., 2014). While we do not expect any condensed aromatics, we cannot quantify quinones or multinuclear inner-

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sphere bonds in the mineral-bound organic matter."

Lines 262-265: This statement is somewhat contradictory to conclusion at lines 189-191.

Line 262: However, the enhancement of electron shuttling might have been especially strong for the experimental conditions chosen by Shimizu et al. (2013), because the content of aromatic groups and quinones is usually much larger in HA than in forest floor extracts as used in this study.

Line 189: In comparison to the material used for previous adsorption and coprecipitation studies [. . .] this material had a higher content in aromatic groups and carbonyl C (ester, carboxyl or amide groups), but a lower content of carbohydrates.

We do not see a contradiction. Although we found a relatively high content of aromatic groups (24%) (and expect this material to be more redox active than that of the previous study), humic acids have still higher contents of aryl C (~45%). Also, it is not exactly the concentration of the aromatic groups what matters, but the number of redox active groups. Quinones, hydroquinones and condensed aromatic groups are expected to be redox active, but we did not have the possibility to quantify them for our material. We found (and cited) a single paper reporting the number of redox active groups in a forest floor extract (Piepenbrock et al., 2014). This article shows that the forest floor extract was only half as active as a humic acid.

Lines 268-282: Perhaps partial of this discussion can be moved to the introduction part.

These lines describe the different electron transfer mechanisms of *Shewanella* and *Geobacter*. We considered transferring it to the introduction, but found it difficult to squeeze in without substantial changes. As the need to discuss electron transfer arises only because we are relating our data to another paper, we feel the paragraph may stay in the discussion part as well. We did not change the text.

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Line 388: Such conclusion will be strong, if the authors compare two bacteria in their experiment.

We agree that these sentences are speculative. To stress this we inserted “If this hypothesis holds true. . .”

Line 506: “If this hypothesis holds true, in natural environments, the likely presence of mineral-bound organic matter on Fe oxide surfaces may increase or decrease Fe reduction, depending on the dominating types of microorganisms.”

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