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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#2 General comments Interactions of hydrous Fe oxides and organic matter have been recognised as important controls on numerous processes in soils and aquatic environments. One major research focus during the last two decades was on stabilization and accumulation of organic matter upon association with poorly crystalline mineral phases, such as ferrihydrites. In turn, work done during the 1980s und 1990s also showed that the surfaces of organic–mineral associations differ strongly from those of pure minerals, which causes different sorption and colloidal properties.

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Ferrihydrites tends to form in environments with organic-rich solutions. Some of these environments are also characterised by changes in redox conditions. Consequently, associated organic matter may not only be stabilised but could also become involved in the reductive dissolution and transformation into other minerals of ferrihydrite. Astonishingly, few studies addressed that topic so far. The presented, therefore, deserves attention and careful consideration. In general, the manuscript is well organised and the presentation of results and the discussion meet high scientific standards. Conclusions and implications are justified by the results. The overall experimental design is also well done; however, there could be a serious methodological flaw, which I hope the authors can address (see below). Considering the overall quality of the work and given that the authors can address the mentioned issue, I think the presented study would be an excellent contribution to the field.

Thank you for the excellent review and the time spent on our manuscript. We believe, we can allay your concerns regarding the nitrate contents and we are especially grateful for your comments regarding language and style.

Specific comments: Major concern: My major concern is the method used for preparation of ferrihydrite. Producing ferrihydrite from FeCl_3 requires careful control of pH to avoid undesired formation of akageneite as a side product. A standard method to produce pure ferrihydrite uses $\text{Fe}(\text{NO}_3)_3$, as done in the present study. Was that a good idea? I doubt. I am no specialist; I just have basic understanding of redox processes. However, a brief literature screening revealed *Geobacter bremesis* is Fe(II) oxidising and nitrate reducing species but can also oxidise organic compounds using either Fe(III) and nitrate as terminal electron acceptors. Could it be that some of the results relate to different contents of nitrate in the different organic–mineral associates? Could it be that the co-precipitated samples tended to larger contents of nitrate than those formed by sorption? Nitrate-richer systems would tend to less strong Fe(III) reduction, right? Also, could the presence of nitrate explain the re-oxidation of Fe beyond day 17 of the microbial incubation?

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It is true that the control ferrihydrite contains residual nitrate from $\text{Fe}(\text{NO}_3)_3$. The nitrate can be identified by IR and corresponds to a total N content of 1.3 %. Likewise the forest floor extract contains some natural nitrate (see below). However, the coprecipitates as well as the adsorption complexes are free of nitrate (FTIR, new Figure 2) and their total N relates to organic amide N. So, the adsorption of OM has removed the surface bound nitrate and the natural nitrate from the OM did not adsorb to the Fe oxide.

In previous ferrihydrite syntheses we could always fully remove the nitrate by the described washing procedure, therefore we did not expect problems during the ferrihydrite production for this study.

In more specific terms, this means that nitrate cannot explain differences in reduction between coprecipitates and adsorption complexes, nor the differences between ferrihydrite organic matter associations with different C concentrations.

However, the most important point is that *Geobacter bremensis* is not able to reduce nitrate (Straub et al., 1998 System. Appl. Microbiol. 21, 442-449; Straub et al., 2001) In Straub et al., 2004 *G. bremensis* was just co-cultured with nitrate-reducing Fe-oxidizing bacteria). Coby et al. (2011) write for example in their conclusion: “These results are analogous to those of a previous experiment with natural-sediment microflora in which Fe went through a single cycle of Fe reduction and oxidation (60) and to those of experiments with cocultures of *Geobacter bremensis* (53) or *Geobacter sulfurreducens* (7) (neither of which reduce nitrate) and the lithoautotrophic nitrate-reducing, Fe(II)-oxidizing enrichment culture of Straub et al. (52).”

This means that the presence of nitrate cannot have affected the Fe(II) production during reduction of the control ferrihydrite”.

We added: Line 243: “The control ferrihydrite as well as the ferrihydrites in coprecipitates and adsorption complexes displayed XRD patterns of a typical 2-line ferrihydrite (Cornell and Schwertmann, 2003). The specific surface area of the control ferrihydrite

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was 197 m² g⁻¹ as determined by N₂ gas adsorption and the concentrations of C and N were found to be 0.2% and 1.3%. The FTIR spectrum (Figure 2) showed that this high N concentration is due to nitrate, which has not been fully removed during ferrihydrite synthesis from Fe(NO₃)₃·9 H₂O. [Because *Geobacter bremensis* is not able to reduce nitrate (Straub et al., 1998; Straub et al., 2001), we assume that the nitrate contamination does not affect our microbial reduction experiments.]”

The IR spectra clearly indicate the presence of nitrate in the organic–mineral associates but did the authors attempt to determine the contents? Did they monitor the nitrate concentrations in the incubation solutions?

The IR-spectrum shown in the first version of the manuscript belongs to the forest floor extract. The nitrate peak at 1384 cm⁻¹ corresponds here to 6.4% NO₃⁻, as determined by IC and is a natural part of the forest floor extract. However, as IR spectra of the organic matter-Fh complexes did not show this peak, the soil solution nitrate has not reacted with ferrihydrite.

Finally, did the authors monitor the redox potential during the incubation? That could give indications of the possible role of nitrate in the different systems.

No, we did not measure the redox-potential.

In short, can the authors rule out, estimate, or at least rate possible effects of residual nitrate? I feel that this could be crucial for the judging the study.

Because *Geobacter bremensis* cannot reduce nitrate (and because the nitrate is obviously not irreversibly bound to ferrihydrite, see FTIR), we are confident that the nitrate contamination in the control ferrihydrite does not significantly affect the Fe(II) production in our experiments.

Minor concerns: The study also addresses possible mineral transformation during incubation. The topic, however, has not been addressed in the introduction, and so, is poorly justified. I recommend introducing the topic in greater detail.

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We added: Line 70: The influence of mineral-bound organic matter on reduction and mineral transformation is less well investigated.

Line 81: In addition, the mineral-bound humic acid changed the mineral transformation during reduction. The formation of goethite was inhibited, the formation of magnetite decreased and the formation of a green rust-like phase stimulated (Shimizu et al., 2013). Such changes in the mineral assemblage will strongly affect the cycling of Fe.

The forest floor material used is a mixture of Of and Oh (consider using Oe and Oa instead; these terms are more common in English). Why not one single horizon? The most logic setup would have been using only the Oh (Oa) horizon, which is the horizon immediately overlying the mineral soil. Could it be that the composition differed from previous studies due to different portions of the two horizons?

We now use Oe and Oa instead of Of and Oh.

We collected both horizons because it would have been difficult to separate them and in order to gain more organic material for extraction. For a soil, we assume that leachates of both horizons will enter the mineral soil and that both horizons are meaningful sources for gaining water soluble organic matter. Although water extracts from Oi, Oe and Oa horizons are usually relatively similar, it seems possible that different portions of these horizons may also explain the differences between the previous extract and the one of this study.

Since the reasons for compositional differences between the extracts are not important with respect to our major questions we deleted the paragraph.

And why was sample dried but not extracted fresh? Air-dried samples tend to give water-soluble organic matter of a composition rather different from that in fresh samples. Could it be, an extract for a fresh Oh (Oa) horizon would have given more electron shuttling compounds?

We are aware of the fact that a water extract is not identical to the dissolved organic

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matter fraction of a horizon. The extraction of such a large amount of organic matter as needed for this study took us several months in the lab. Because it was not possible to keep the forest floor material under field conditions over this time, we decided to dry the material to prevent further uncontrolled degradation. We still believe that our extracts are relatively close to natural dissolved organic matter and we generally regard the drying of a forest floor as a process which occurs in the field, too.

Is possible to add a short explanation for using *Geobacter bremesis*?

Originally, we had planned to incubate our samples with *Geobacter* and *Shewanella*, because the electron transfer mechanisms of both organisms is well investigated but known to be distinct. For whatever reason *Shewanella* was not growing in our lab, so that we performed the experiments with *Geobacter* only. We added:

Line 91: *Geobacter bremensis* is common in soil and serves as a well investigated model organism for dissimilatory Fe(III) reduction.

Sorption and co-precipitation experiments were carried out a pH 5 but incubation experiments at pH 7. Why? The higher pH in the incubations may favour desorption, depending on the loading of organic matter. The released organic matter may have become an additional carbon source and may have been involved in complexation of Fe ions. I am a bit wondering; the authors cared for phosphate as a potential desorbing anion but not for pH. Also, the incubation media contained HCO_3^- , which interacts with ferrihydrite as well and may also favour desorption of organic matter.

The chosen pH was a compromise: In the presence of dissolved organic matter most coprecipitates form around pH 5, so that we were forced to do coprecipitation and adsorption syntheses at this pH. For optimum growth of *Geobacter*, however, a medium with pH 6.8 is recommended by both the DSMZ (Medium 579, pH 6.7 to 7.0) as well as the ATCC (Medium 1957, pH 6.8). This is in accordance to Straub et al. (1998) who isolated *Geobacter bremensis* on a pH 7 freshwater medium. Therefore we decided not to deviate from these settings.

We agree, that the shift in pH may have caused some desorption of organic matter, which may in turn have changed the organic matter loadings and led to a complexation of Fe ions. Because the general pattern in microbial and abiotic reduction experiments was very similar, we do not believe that the potentially desorbed organic matter has significantly stimulated microbial activity in samples with high organic matter loadings.

We judged the effect of phosphate as being worse, because phosphate is known to form strong bonds with Fe oxides, to strongly compete with adsorbed organic matter and to inhibit the dissolution of the oxide (e.g. Stumm, 1997). HCO_3^- is much less reactive.

We added: Line 166: “The pH was chosen because recommended for optimum growth of Geobacter, by both the DSMZ (Medium 579, pH 6.7 to 7.0) as well as the ATCC (Medium 1957, pH 6.8). Adsorption and coprecipitation experiments were performed at pH 5, i.e. under pH conditions where most coprecipitates form in the presence of dissolved organic matter (Eusterhues et al., 2011). However, the higher pH during reduction experiments may have caused desorption of some of the mineral-bound organic matter.”

Determination of Fe^{2+} was done using the phenanthroline assay, which fails in detecting small Fe^{2+} concentrations and is not always truly reliable. Did the authors consider using the ferrozine assay?

We considered both methods. Both methods are very similar. We decided to use phenanthroline, because it was recommended for Fe(II) in such experiments by Braunschweig et al., (2012).

The text, especially the introduction, contains long and complicated sentences, which can be shorted without loss of information by omission of repetitive phrases and the use of a more active voice.

We tried to follow this advice as good as we could.

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Technical corrections Please avoid abbreviations; they are kind of jargon.

We removed all abbreviations (OM/organic matter; Fh/ferrhydrite; FFE/forest floor extract; HA/humic acid).

Please consider a shorter title.

We considered a shorter title. Although it would be more stylish and probably attract a larger number of readers, all of us agreed to stay with the long but precise title.

Please consider replacing expressions such as “reactivity towards reduction” by “reduction”.

We exchanged these two expressions throughout the manuscript.

p. 6041, l. 5–6: Consider changing the sentence into “Here, we investigated 2-line ferrihydrite, ferrihydrite with adsorbed organic matter, and ferrihydrite co-precipitated with organic matter for microbial and abiotic reduction of Fe(III).”

The sentence was changed accordingly.

p. 6041, l. 6–7: Consider dropping the sentence “As a surrogate ... forest floor”. It has little meaning.

We deleted the sentence

p. 6041, l. 18: Consider replacing “... when blocked by OM” by “... by attached organic matter”.

The sentence was changed accordingly.

p. 6041, l. 19: Consider dropping “... beside Fe(II) accumulation ...”.

The sentence was changed accordingly.

p. 6041, l. 19–20: Note, mineral-bound organic matter is not a mechanism but may be a factor. Consider replacing “... a further widespread mechanism to slow...” by “... a

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factor in slowing ...”.

The sentence was changed accordingly.

p. 6041, l. 24: Consider replacing “Because” by “Due to”.

The sentence was changed accordingly.

p. 6041, l. 25–26: Drop “it has been assumed that”.

The sentence was changed accordingly.

p. 6042, l. 1–4: Try to shorten; consider writing: Organic coverage may result in surfaces properties strongly different from those of the original oxides, with consequences for aggregation, mobility, and solubility.

The sentence was changed accordingly.

p. 6042, l. 5: Replace “frequently found” by “common”.

The sentence was changed accordingly.

p. 6042, l. 6: Replace “... which usually forms aggregates of only nanometer sized ...” by “... usually forming aggregates of nanometer-sized ...”.

The sentence was changed accordingly.

p. 6042, l. 13: Consider replacing “... so that coprecipitated Fhs are assumed to develop ...” by “... and so coprecipitated Fhs tend to ...”.

The sentence was changed accordingly.

p. 6042, l. 16: Consider replacing “widespread” by “common”.

The sentence was changed accordingly.

p. 6042, l. 17: Consider replacing “towards” by “to”.

The sentence was changed accordingly.

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p. 6043, l. 7–8. Replace “reactivity” by “reduction” and omit “toward reduction”.

The sentence was changed accordingly.

p. 6043, l. 9: Omit “Fh reactivity towards”.

The sentence was changed accordingly.

p. 6043, l. 19: Replace “compounds” by “chemicals”.

The sentence was changed accordingly.

p. 6045, l. 21: Replace “checked” by “monitored”.

The sentence was changed accordingly.

p. 6046, l. 22: Consider replacing “analogue” by “analogues”.

The sentence was changed accordingly.

p. 6047, l. 9: Replace “Fe-minerals” by “Fe minerals”.

The sentence was changed accordingly.

p. 6047, l. 11: Replace “modelling” by “fitting”.

The sentence was changed accordingly.

p. 6047, l. 25 – p. 6048, l. 1: Consider replacing “but a lower content” by “and less”.

The sentence was changed accordingly.

p. 6049, l. 8: Replace “is” by “for”.

Line 308: We replaced the sentence by “We therefore assume that the accessibility of the ferrihydrite surface for reducing agents or microbial cells is not systematically different in coprecipitates and in ferrihydrites with adsorbed organic matter.”

p. 6049, l. 11: Consider replacing “Fe reduction kinetics observed during incubation

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...” by “Incubation ...”.

The sentence was changed accordingly.

p. 6049, l. 16: Drop “toward reduction”.

The sentence was changed accordingly.

p. 6049, l. 17: Consider replacing “For example, in case of AFhA ...” by “In case of ...”.

The sentence was changed accordingly.

p. 6050, l. 1: Consider replacing “... was ruled out by XPS” by “... can be ruled out based on XPS results”.

The sentence was changed into: “A possibly larger accessible outer ferrihydrite surface in coprecipitates compared to ferrihydrite with the same amount of adsorbed organic matter can be ruled out based on XPS results”

p. 6050, l. 20–24: Consider differences could be due to the type of organic matter in solution.

Reviewer#1 raised the same point.

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 XPD 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

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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-sphere bonds in the mineral-bound organic matter.”

p. 6051, l. 8: Consider replacing “engage” by “use”.

The sentence was changed accordingly.

p. 6051, l. 23–24: The idea of weaker bonds becoming increasingly involved with increasing surface coverage is no standard concept. Either give reference or explain better.

We agree. In response, we deleted the sentence in brackets, because we feel a longer discussion would distract the reader from the main topic.

p. 6051, l. 27: “reduce extracellular OM” – could it be that that should read “oxidise extracellular OM”?

We think, “reduce extracellular OM” is correct. We are referring to the ability of Geobacter to reduce OM in a first step. The reduced organic matter may then donate electrons, for example to the ferrihydrite. The electron transfer will lead to the reduction of Fh and the re-oxidation of the OM. So, in the end the OM becomes oxidized, but this happens in consequence of the former reduction.

p. 6052, l. 1: Consider replacing “The often only partial ...” by “Partial ...”.

The sentence was changed accordingly.

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p. 6052/6053: Is section 3.3 really necessary? Consider omission.

We believe that this is an interesting side aspect of our work. On conferences it has already brought about considerable feedback. The fact that the other two recent studies about the influence of mineral-associated OM on reduction, (Henneberry et al., 2012; Shimizu et al., 2013) as well monitored and reported the mineral composition after reduction points in the same direction. Although the formation of secondary minerals in such experiments is always driven by the synthetic medium, our simple approach shows that growth of both goethite and Fe(II) minerals is inhibited by organic matter. This may explain why Fe(II) minerals such as siderite, magnetite and vivianite are rarely found in waterlogged soils.

p. 6053, l. 2: Replace “atoms” by “ions”.

The sentence was changed accordingly.

p. 6053, l. 3: How do the authors think that Fe ions might compete with OM? The two have opposing charges.

We do not fully understand this comment. Both Fe(II) as well as OM react with the Fe oxide surface. Catalysis by Fe(II) is usually used to explain goethite formation during reduction. The process of Fe(II) adsorption on Fe oxides can be analyzed with surface complexation models (Hiemstra & van Riemsdijk, GCA 2007) and the presence of adsorbed Fe(II) on hematite surfaces has been confirmed by Mössbauer spectroscopy (Larese-Casanova & Scherer, ES&T 2007).

However, the comment prompted us to add: Line 444: Furthermore, a preferential reaction of Fe(II) with the mineral-bound organic matter instead of the Fe oxide surface could be considered.

p. 6054, l. 8–9: Give a reference to the citation in quotation marks.

We now cite Roden (2004).

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p. 6054, l. 13: The standard dithionite–citrate–bicarbonate method involves heating and pH 8.3, which might reduce the inhibitory effect of attached organic matter.

We agree. Before creating confusion with respect to this common extraction method we should run more specific tests. We deleted the paragraph.

p. 6054, 15: Consider replacing “the reactivity of Fh towards both”.

We deleted “the reactivity of Fh towards both”.

p. 6054, l. 16: Consider replacing “Na-dithionite” by “Na dithionite of ferrihydrites”.

The sentence was changed accordingly.

p. 6054, l. 17: Consider omitting “which were”.

We deleted “which were”.

p. 6055, l. 10: Consider replacing “display” by “have”.

The sentence was changed accordingly.

Figure 2: The caption of Figure 2A is not easy understandable; it obviously a sorption isotherm, with the equilibrium C concentration given on the x-axis. Please, amend accordingly.

We changed the text into: “Ferrihydrite-associated C (normalized to the specific surface area of 197 m² g⁻¹ of the control Ferrihydrite) vs. C in the equilibrium solution. The line represents a BET-isotherm.”

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/11/C3797/2014/bgd-11-C3797-2014-supplement.pdf>

Interactive comment on Biogeosciences Discuss., 11, 6039, 2014.