Responses to Reviewer Comments

Manuscript “Stable iron isotope signals indicate a “pseudo-abiotic" process driving deep iron release in methanic sediments“ by Henkel et al., https://doi.org/10.5194/egusphere-2024-1942

RC1

General comments:

In this paper, the authors carried out a thorough analysis of porewater and solid phase chemistry throughout a core of marine sediments, with a focus on patterns of Fe isotope composition. The goal was to use these patterns to explain patterns of Fe biogeochemistry throughout the depth of the sediments. The thoroughness of the differential extractions was really impressive. They were operationally defined (e.g. roughly corresponding to adsorbed Fe(II), poorly crystalline Fe(III), crystalline Fe(III)) but those operational definitions are still meaningful from the standpoint of Fe biogeochemistry. The authors found that patterns of Fe isotope compositions of Fe(II) and Fe(III) phases were inconsistent with those observed to result from dissimilatory/respiratory Fe(III) reduction in lab experiments. The authors indicate that adsorption/atom exchange does not contribute to the observed isotope patterns even though they also indicate that the rates of Fe(III) reduction are likely quite low.

I am struggling with the fermenter conclusion a little bit, because it seems like the authors are saying “patterns of Fe isotopes in all of these different pools are inconsistent with dissimilatory/respiratory Fe(III) reduction, so it must be from the fermenters dumping electrons.” Additionally, conduction of electrons from fermenters to methanogens would not result in a net reduction of the Fe (the fermenter would reduce it, but the methanogen would oxidize it). Granted, it is likely that an initial reduction would have to occur, because most hypotheses for interspecies electron transfer via Fe involve magnetite or pyrite, but after that initial reduction, no net redox change would occur with the Fe.

We thank the reviewer for this generally positive feedback. We are aware that the conclusions of this manuscript still remain a bit speculative. The link to the fermenters is actually indicated by the previous microbial study by Aromokeye et al. (2021).

The reviewer wrote “the fermenters would reduce it” (Fe), but this is in fact not easy to prove. Fermenters may use crystalline Fe oxides to conduct electrons towards methanogens. We discuss this as an option that some of the electrons are “redirected” and are used (by fermenters *or* other microbes) for Fe reduction. Kato et al. (2012) and Cruz Viggi et al. (2014). demonstrated the use of Fe oxides as conductors are Meanwhile, some doubt is building up that those electrons are really conducted in an electronic fashion without reduction and reoxidation occurring. This is summarized in the review article by Xu et al. (2019). So, this supports our interpretation and we will revise the text accordingly to point out more clearly that there are many studies that see an enhancement of syntrophic activity in the presence of conductive magnetite or semiconductive iron minerals. But the details on how this mechanistically works on the molecular level and whether it involves the reduction of Fe(III) or not have not been elucidated yet.

The fact that we don't see a significant Fe isotope fractionation at the depth of the deep Fe^{2+} release at our study site indicates that the underlying reduction process is different to what dominates in shallow marine sediments. This absolutely makes sense, because in shallow sediments the electron donor for microbial iron reduction is acetate, which is less abundant in methanic sediments. The latter, in contrast, contain more CO_2, and CO_2-dependent methane formation is prevalent.

The reviewer states that "after that initial reduction, no net redox change would occur with the Fe" because "the fermenter would reduce it, but the methanogen would oxidize it". As far as we can judge, methanogens have not been conclusively shown to perform iron oxidation. There is a statement by Dinh et al. (2004) that implies this: "Similarly, a newly isolated *Methanobacterium*-like archaeon produced methane with iron (Fe^0) faster than do known hydrogen-using methanogens, again suggesting a more direct access to electrons from iron than via hydrogen consumption". But this paper was published 20 years ago and was targeting the oxidation of metallic iron (Fe^0); by now we know that DIET (Direct Interspecies Electron Transfer) between fermenting bacteria and methanogens plays an important role. TDinh et al. 2004 is about corrosion of metallic iron. The authors proposed the direct oxidation of metallic iron to Fe^{2+} and electrons taken up by SRB. For methanogens, the picture is less clear based on that study, but it seems to follow the same idea. A more recent study to the same topic is Holmes et al. (2022). However, metallic iron is regarded as a scarce substrate in the natural environment, which is why our study focuses on the reduction of Fe(III) or the use of conductive minerals such as magnetite and hematite.

I am also struggling to see how the authors incorporated advection or diffusion of dissolved Fe(II) into their models and interpretation. Depending on the rates of Fe(III) reduction, those would be a major controller of the extents of atom exchange (i.e., is Fe(II) exported quickly enough that no atom/electron exchange can occur?).

We are actually not saying that there is NO atom/electron exchange. In fact, we even say in line 510 that it's very likely that these processes occur. In the paragraph ~line 585 we elaborate on this: "If part of the adsorbed (heavy) iron is then exchanged with the reactive Fe oxide surface (Crosby et al. 2007) and might subsequently even migrate deeper into the iron oxide crystal (Larese-Casanova et al., 2023), it could cause an alteration of Fe oxide isotope signatures towards positive values without reducing the mineral. It might also be speculated that adsorption and the related electron and atom exchange are more prevalent at depths that have a high Fe oxide (Fe_{dith}) content, but this interpretation remains very speculative, in particular because our model does not indicate adsorption to be a dominant Fe sink." What the model indicates is only that adsorption (and atom/electron exchange) is not the main process leading to the present pore-water and $\delta^{56}Fe$-profile.

Despite these criticisms, I think the work is important because it contributes to what we know about patterns of Fe isotope compositions in different Fe pools – It's just hard to explain at this point. I think the authors have identified the major controller here: kinetics of Fe(III) reduction vs. kinetics of abiotic processes. I enjoyed reading this paper.

Specific comments:

Ln. 57. The authors do not include Fe(III) reduction by methanogens in their interpretation. The enzymology of that process is similarly understudied to that of fermenters.

The reviewer mentions that we should include whether Fe^{2+} release can also be linked to the reduction of Fe^{3+} by methanogens that switch between methane generation and Fe reduction (e.g., Sivan et al. 2016, Eliani-Russak et al. 2023, Gupta et al. 2024 and references therein).

In the revised version, we will consider this aspect a bit more. Respiratory methanogenic iron reduction might be a possible explanation for deep Fe release in methanic sediments. However, as the process is respiratory, we assume (can't prove) that is would lead to similar Fe isotope fractionation as Fe reduction in shallow sediments. Furthermore, in order to do respiratory Fe(III) reduction, methanogens would need to oxidize CH_4 or an organic substrate (e.g., acetate, methyl compounds). Methane oxidation seems unlikely to support growth coupled to iron(III) reduction (see Chadwick et al., 2024). Which other electron donor is there for methanic zone methanogens to abandon their primary metabolism of CO_2 reduction with H_2 to methane? Gupta et al. (2024) summarize this issue by stating: "… even though we and others have shown that methanogens like *M. acetivorans* are metabolically active and can conserve energy by iron respiration […], robust growth that spans multiple generations is yet to be demonstrated i.e., it is still not known whether methanogens can couple iron reduction to growth in addition to energy conservation. Regardless, redox transformation of iron species by methanogens has substantial biogeochemical ramifications in and of itself to merit further investigation."

We are curiously following this very controversial discussion, but we have the feeling that this goes a bit beyond the scope of our study, which – in the end – is about stable Fe isotope signatures and does not contain microbiological data.

ln. 345-351. avoid three sentence paragraphs. Also having trouble seeing how these observations are fitting into the broader story.

We will revise this paragraph and give a better context concerning the Mn data.

Ln. 620. this isn't completely true (benefitting both microbes). Unless the methanogen can use the reduced/conductive Fe phase as an electron donor, the Fe(III) just gets reduced and that's the end of it. This scenario is #1 on ln. 604.

This comment refers to the following text: "The fermenting bacteria that transfer electrons to crystalline Fe oxides do not directly profit from Fe(III) reduction beyond the removal of thermodynamic limitations brought about by accumulation of fermentation intermediates. In other words: The fermenters use the conductive Fe oxides to transfer electrons and to be able to continue with the fermentation of particularly aromatic OM. The transfer of electrons via conductive Fe oxides speeds up the degradation of aromatic compounds and is beneficial to both partner microbes (e.g., Jiang et al., 2013; Kato et al., 2012; Zhuang et al., 2015). The transfer of electrons via conductive Fe oxides speeds up the degradation of aromatic compounds and **is beneficial to both partner microbes** (e.g., Jiang et al., 2013; Kato et al., 2012; Zhuang et al., 2015)."

The reviewer is right and this is exactly what we wanted to express. It's beneficial for both if the methanogen receives (part of) the electrons that are shuttled through the Fe oxide. In the revised version we will reformulate this to avoid confusion: "is **metabolically and mechanistically beneficial to both partner microbes** …".

Ln. 622-624. I think Nathan Yee's group has done some work to address how fermenters reduce Fe(III).

Thank you for this information. We checked and indeed found one paper by a member of his group that we might include into the discussion.

Ln. 659-660. I agree with this, and think it's the strength of the paper.

Technical corrections:

Ln. 19. Please remove "unsurprisingly"

Ln. 386. Please change "conclusive" to "consistent"

Ln. 436. Please change "wit" to "with"

Technical corrections will be done as suggested.

Citation: https://doi.org/10.5194/egusphere-2024-1942-RC1

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