

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>

RC2:

General Comments:

Overall, this is a very interesting study, presenting a comprehensive dataset and a scientific approach that combines field data with modeling. The illustrations are clear, the manuscript is well-written, and the interpretations, while complex and occasionally probably speculative, remain cautious. This is well-explained throughout the manuscript.

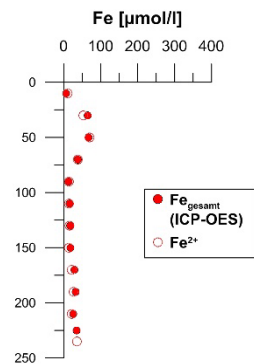
This work contributes to advancing our understanding of the iron cycle in methanic sediments. For all these reasons, I recommend the publication of this work.

I have two major remarks, in addition to several minor points listed in the PDF as comments:

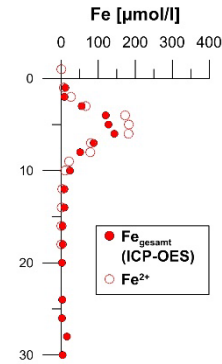
- The authors appear to assume, given the chemical conditions, that dissolved iron is always reduced iron (Fe-II). However, what is referred to as dissolved iron is, in fact, filtered iron (0.1 μm). Was the redox speciation of iron measured in all the pore waters? In the oceanic water column, 'dissolved' iron encompasses a variety of physicochemical species, including small particles (<0.1 μm containing Fe-III) or colloidal complexes (which can also contain Fe-III). The process of 'non-reductive dissolution' of iron – which does not require reductive conditions as it can involve desorption or ligand-promoted dissolution – seems to be an important process for the release of 'dissolved' - in fact filtered - iron at the sediment-seawater interface (Radic et al. 2011, Labatut et al. 2014, Homoky et al. 2021). Could this 'non-reductive dissolution' process play a role in these methanic sediments?**

This is in fact a very important point raised by the reviewer and we will clarify the issue of Fe speciation in the revised version of the manuscript. For the MUC at the investigated station HE443/10 and also for MUCs and GCs at other stations of the same expedition, we measured both, total dissolved Fe (by ICP-OES) and Fe^{2+} by the ferrozine method after Stookey (1970). The profiles match quite well as can be seen here:

HE443/074-1 SL



HE443/010-2 MUC



So, we are confident that the dFe pool at the sites investigated in the Helgoland Mud Area consists mainly of Fe^{2+} and don't see a reason to include NRD into the discussion. The reason why we don't have Fe^{2+} data for the gravity core samples is that before the addition of ferrozine we usually keep the Fe^{2+} stable by addition of an ascorbic acid solution. For the gravity core samples, however, the dissolved Fe concentrations were too high for the ascorbic acid mixture. Ascorbic acid was not present in excess. This was unfortunate and as consequence we changed the way we treat the samples directly after sampling (direct transfer of sample into cuvettes pre-filled with ferrozine).

Nonreductive iron dissolution (NRD) is a process that is usually brought up to explain heavy Fe isotope signals of dissolved Fe at the very surface or above the surface of the sediment. We are a bit sceptic concerning this, because a heavy dissolved Fe isotope signal can also be produced by the kinetic precipitation of Fe oxides (e.g., Staubwasser et al. 2013) that is without doubt happening at the oxic/anoxic boundary. We are not saying, NRD can't play a role in areas other than the Helgoland Mud Area. However, NRD is in our view for many of the sites for which this process has been proposed to take place, not *needed* in order to explain the pattern of stable iron isotopes in pore water or the deep water when considering that kinetic Fe oxide precipitation leads to a preferential incorporation of light Fe isotopes and only by aging and with equilibrium fractionation, Fe oxides become "more heavy".

- **The validation of the isotopic measurements appears too superficial. Potential artifacts (isotope fractionation, contamination) related to chemistry, preconcentration, purification, and partial dissolution seem not to have been thoroughly investigated. Yields and blanks from the various steps are not consistently reported. Repeatability seems not to have been quantified across the entire protocol (including the chemistry), and the error bars reported in the graphs seem too small. For instance, the repeatability of the instrument for pore waters appears to be 0.26 ‰, which in my view implies that no measurement of pore waters can have an error bar smaller than 0.26 ‰.**

Actually, all these issues (isotope fractionation, process blanks, preconcentration) have been thoroughly investigated, but they have been presented already in the papers Henkel et al. (2016) and Henkel et al. (2018) to which we refer. We will revise the manuscript to point out more clearly, where the respective information can be found. Furthermore, we will revise the figure caption to Fig. 2. In fact, the given uncertainty bars in the figure refer to the 20 cycles of one block of an analysis. So, all the 20 single analyses were done within ~2 minutes during one dip into the respective sample. The repeatability that is given with 0.26‰ in contrast, was determined by measuring the JM standard 15 times (so in fact 15 x 20) during the run of two separate sequences. We also included replicates of pore-water samples (at 1 and 8 cm depth) into the sequences. Those are displayed in Fig. 1 as well.

see the pdf for other comments

Copied in here from the pdf:

Line 14: “The low $\delta^{56}\text{Fe}$ values of dissolved iron liberated by microbial iron reduction are characteristic for shallow subsurface sediments and benthic Fe fluxes into the water column.”

Reviewer: I do not agree. In many places benthic Fe fluxes have been characterized by slightly heavy iron isotope signatures.

This refers again to the potential NRD that has been mentioned before. We will reformulate this sentence a bit and will put more emphasis to the fact that microbial iron reduction in shallow sediments preferentially releases light Fe. We will be more careful with the expression benthic flux *out of the sediment*, because indeed, the isotopic signature of the dissolved Fe pool may be altered in the top few millimeters due to Fe oxide precipitation and/or NRD.

Line 41: “Iron isotopes, expressed as $\delta^{56}\text{Fe}$ (‰), are thus considered as a tool for assessing the role of microbial iron reduction (MIR) for the mineralization of organic matter and for tracing benthic iron fluxes into the water column (e.g., Conway and John, 2014; Homoky et al., 2009; Severmann et al. 2006, 2010; Sieber et al., 2021).

Reviewer: Better refer to John, S. G., Mendez, J., Moffett, J., & Adkins, J. (2012). The flux of iron and iron isotopes from San Pedro Basin sediments. *Geochimica et Cosmochimica Acta*, 93, 14–29. <https://doi.org/10.1016/j.gca.2012.06.003>.”

We will add the paper by John et al. (2012).

Line 154: “Not sure an article can be accepted with incomplete references.”

This refers to the PANGAEA dataset for which I now included the full reference (incl. doi).

Line 176: “The measured value was $0.49 \pm 0.26\%$ (n=15, 2SD) and overlapped within uncertainty with previously published values ($0.42 \pm 0.05\%$, Schoenberg and von Blanckenburg, 2005; $0.46 \pm 0.20\%$, Walczyk and von Blanckenburg, 2005; $0.35 \pm 0.14\%$, Weyer and Schwieters, 2003).”

Reviewer: “This means the instrumental precision is 0.26 per mil... Repeatability of the whole procedure, including pre-concentration and purification should have been quantified. 2 duplicate samples are not enough to statistically evaluate repeatability. Trueness of the instrumental analysis has been quantified (no bias), but trueness of the entire procedure is not validated here. Trueness should have been quantified for the whole procedure from pre-concentration to Neptune analysis. Yields and blanks should have been presented.”

We will revise the respective paragraph and include details to the trueness of the entire procedure. The procedure is, however, not new. Details regarding the procedure are for example given in Henkel et al. (2016) and Henkel et al. (2018).

Lines 189-192:

“Sequential Fe extractions were performed after Poulton and Canfield (2005): ~50 mg of dry sediment were suspended in always 5 ml of a) MgCl_2 for adsorbed Fe, b) Na-acetate for Fe-carbonates and surface-reduced Fe(II), c) hydroxylamine-HCl for easily reducible Fe-oxides (ferrihydrite, lepidocrocite), d) Na-dithionite/citrate for reducible Fe-oxides (goethite and hematite) and e) ammonium oxalate/oxalic acid for magnetite.”

Reviewer: “All this partial extractions could be associated with isotope fractionations, leading to procedural artefacts. This should be discussed. On what basis can we conclude that this is not the case?”

A thorough analysis of these issues is presented in Henkel et al. (2016) to which we refer. But we will revise the text so that readers can easily find the details.

Line 198-205:

“The MC-ICP-MS was equipped with a SSI dual cyclonic spray chamber, a low flow 50 μl PFA nebulizer and a Ni skimmer cone (x-type). Samples were measured using the standard-sample bracketing with certified reference material IRMM-014. All ^{54}Fe data were Cr-corrected based on measurements of ^{52}Cr . In addition, all data were blank-corrected and samples were analysed in random order. The standard JM (see above) was analysed after each block of three samples. Samples bracketed by JMs that did not fall into the target range of $0.42 \pm 0.05\%$ were repeatedly measured. The repeatability precision resulting from up to 6 replicate sample measurements (not including replicate processing) was better than 0.34% (2SD) and on average 0.11% . The intermediate precision of JMs was $0.44 \pm 0.15\%$ (n=151, 2SD).”

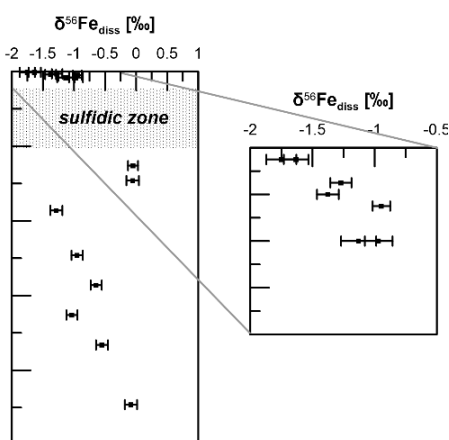
Reviewer: “Not clear why these details are given here but not in the previous section about pore waters.”

The reviewer is right. We will provide more details regarding the pore-water analyses.

Concerning the “on average 0.11‰”: **“I do not understand how this number is calculated”**

We will reformulate this. Basically, we wanted to express that we did repeated measurements for several samples that were measured each up to 6 times. In worst case, the repeatability was 0.34‰. For all other samples it was much lower, sometimes 2SD was 0.03‰ for 4 separate analyses of the same sample.

Fig. 2:



Reviewer: “Error bars are too small. If the JM Fe wire repeatability was 0.26 per mil (2SD), then, it seems to me that, no measurement can be considered more precise than that.”

This issue is addressed in the response to another comment.

Line 305-306 and Line 321: “Phosphorus concentrations measured by ICP-OES of acidified pore-water aliquots (not shown, but available under xxx)” and “... PANGAEA (xxx).”

Reviewer: “Cannot be accepted like that.”

The full reference to the PANGAEA dataset will be included into the revised version.

Figure caption to Fig. 6: “b) Keeling plot for $\delta^{56}\text{Fe}$ values of pore water with 95% confidence interval.”

Reviewer: “Why only 6 data points. This should be explained in the legend.”

This is true. It was only written in the main text (Line 493-495): “Here, we only used data from below those depths at which $\delta^{56}\text{Fe}_{\text{diss}}$ is mainly controlled by the reaction with H_2S , i.e., between 450 and 150 cm, where there is a rather linear $\delta^{56}\text{Fe}_{\text{diss}}$ trend...”. We will add “We only used data from between 450 and 150 cm, where there is a rather linear $\delta^{56}\text{Fe}_{\text{diss}}$ trend.” to the figure caption.

Lines 458 and 459: “This trend is related to 1) the progressive removal of ^{54}Fe from the reducible ferric Fe pool during burial and ongoing MIR as well as to 2) progressive preferential removal of ^{54}Fe during interactions with hydrogen sulfide at the sulfidization front (Severmann et al., 2006).”

Reviewer: “preferential removal of light Fe isotopes“

We will reformulate the sentence according to suggestion.

Lines 502-503: “The $\delta^{56}\text{Fe}_{\text{diss}}$ value at ~ 190 cm is $-1.28 \pm 0.10\%$ (2SD), so while diffusing upwards, the Fe_{diss} either (1) loses ^{56}Fe or (2) is affected by an additional process providing ^{54}Fe .”

Reviewer: “As noted above, Fe does not just have 2 isotopes. Therefore I believe that the authors should use 'light isotopes' instead of '54' and 'heavy isotopes' instead of '56'”

Yes, correct! We will also reformulate this sentence.

Very impressive and very nice work !

Thank you!

Citation: <https://doi.org/10.5194/egusphere-2024-1942-RC2>

Stookey (1970) Ferrozine-A New Spectrophotometric Reagent for Iron, *Anal. Chem.* 42, 7, 779-781, <https://doi.org/10.1021/ac60289a016>.

Henkel et al. (2016) Determination of the stable iron isotopic composition of sequentially leached iron phases in marine sediments. *Chem Geol.* 421, 93-102, <http://dx.doi.org/10.1016/j.chemgeo.2015.12.003>.

Henkel et al. (2018) Iron cycling and stable Fe isotope fractionation in Antarctic shelf sediments, King George Island, *Geochim. Cosmochim. Acta*, 237, 320-338, <https://doi.org/10.1016/j.gca.2018.06.042>.