nature portfolio

Peer Review File

Microscale iron and sulphur isotopic compositions reveal pyritization pathways during early diagenesis

Corresponding Author: Dr Virgil Pasquier

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Version 0:

Decision Letter:

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Dr Pasquier,

Your manuscript titled "Microscale iron and sulphur isotope signatures reveal pyritization pathways and diagenetic overprints." has now been seen by 3 reviewers, whose comments are appended below. You will see that they find your work of some potential interest. However, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be interested in considering a revised version that fully addresses these serious concerns.

We hope you will find the reviewers' comments useful as you decide how to proceed. Should additional work allow you to address these criticisms, we would be happy to look at a substantially revised manuscript. If you choose to take up this option, please either highlight all changes in the manuscript text file, or provide a list of the changes to the manuscript with your responses to the reviewers.

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In particular, please ensure that the revised manuscript meets the following editorial thresholds:

** Provide firm and sufficient evidence to demonstrate that the isotopic compositions on pyrite can be used to constrain the pyritization pathway and microbial and physicochemical aspects of the depositional environment.

** Expand the justification for your methods and assumptions, and include the details of the methodology for quantifying AVS and CRS and measuring iron content in pyrite, as pointed out by reviewer #3.

**Discuss alternative possibilities for explaining how the light Fe+2 may be derived from other mechanisms different from DIR, and either acknowledge these in your study or justify their exclusion.

****

When resubmitting, please provide a point-by-point response to the reviewers’ comments. Please submit your responses as a separate file, distinct from your cover letter where you can add responses to the Editors’ comments that you do not want to be made available to the reviewers. Word files are preferred.

Important: The response to reviewers must not include any figures, tables or graphs. If you wish to respond to the reviewer reports with additional data in one of these formats, please add them to the main article or Supplementary Information, and refer to them in the rebuttal. Due to current technical limitations, any figures, tables, or graphs embedded in your rebuttal will not be included in the peer review file, if published.

Please bear in mind that we will be reluctant to approach the reviewers again in the absence of substantial revisions.

If the revision process takes significantly longer than three months, we will be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the reviewers' comments with a list of your changes to the manuscript text (which should be in a separate document to any cover letter), a tracked-changes version of the manuscript (as a PDF file) and any completed checklist:

Link Redacted

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Please do not hesitate to contact us if you have any questions or would like to discuss the required revisions further. Thank you for the opportunity to review your work.

Best regards,

Carolina Ortiz Guerrero, Ph.D.
Associate Editor
Communications Earth & Environment

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For your information, you can find some guidance regarding format requirements summarized on the following checklist: (https://www.nature.com/documents/commsj-phys-style-formatting-checklist-article.pdf) and formatting guide (https://www.nature.com/documents/commsj-phys-style-formatting-guide-accept.pdf).

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Comments on the manuscript entitled 'Microscale iron and sulphur isotope signatures reveal pyritization pathways and diagenetic overprints' submitted to Communications Earth and Environment.

In this contribution, the authors explore the microscale evolution of Fe and S isotopes within a range of sedimentary pyrite that formed within sediments under oxygenated water columns in the Black Sea. Their results show clear evidence for distinct isotope compositions for early formed pyrite to pyrite overgrowths and later pyrite formation. They therefore suggest that detailed examination of pyrite textures, together with microscale isotope compositions, help reconstruct pyrite formation pathways, with implications for deep time reconstructions.

I generally find the paper very well written, the methods are sound and I do believe the data of high quality. In fact, I only have comments on some of the author's interpretations, which appear quite old fashion in my opinion and not fully supported by the data, and some minor suggestions. I hope my comments will help improve the manuscript, which I recommend for publication.

Romain Guilbaud

At several occasions (e.g., L263-275; L454-465), the authors invoke the production of an isotopically light Fe2+ reservoir, derived from DIR, which then reacts with sulphide to form FeS at equilibrium, which itself reacts to form pyrite. This is, more or less, the way that was used to explain the very negative pyrite values in the Archean in 2005. Since then, there has been extensive work, cited by the authors, strongly nuancing this. First of all, in Guilbaud et al. (EPSL, 2010) and Guilbaud et al. (GCA, 2011), we found evidence that the core-particle size ratio in neoformed nanoparticles prevent equilibrium to be reached, even after 4 months of experiment, resulting in isotopically light FeS particles. Guilbaud et al. (GCA, 2011) and Wu et al. (2012) showed that at equilibrium, FeS becomes slightly isotopically heavy compared to Fe(II), but only at higher pH. This is one of the reasons why, as I suggest below, pH is so important and needs to be indicated in the main text. Then, there is the study by McAnena et al. (2023) which shows a range of isotopic compositions that are produced during reduction of Fe(III) (oxyhydr)oxides by sulphide at different pH values, and so without the intervention of any microbial activity. Finally, there is the pyrite-forming mechanism itself, which produces light early-stage pyrite (Guilbaud et al., Science 2011; Mansor and Fantle, GCA 2019; Heard et al, Science 2020). All in all, all these mechanisms produce isotopically light to very light Fe(II) and pyrite, and yet the authors prefer to invoke DIR. I would like to see more nuance in their interpretation. For instance, L464-465: ‘Expression of a KIE upon FeS transformation to pyrite (…) appears to be unnecessary’ is entirely unsupported. One could equally assert that ‘DIR appears to be unnecessary’, with the exact same logic! I believe that nowadays, with the large body of data we have on the matter, one really can (and should) do better when interpreting data…

L99: McAnena et al do suggest that the fractionation is sensitive to mineral dissolution rates, amongst other factors.

L104: similar values were obtained for the equilibrium fractionation between FeS and Fe2+ at both 25 and 2 degree C by Guilbaud et al. (2011), i.e. earlier than the Wu et al. (2012) paper.
L126-127 and the caption of Fig. 1. Sulphidic conditions are not defined by the lack of measurable Fe2+, as the authors suggest (e.g., Canfield and Thamdrup, 2009). Whilst it is correct to say that free Fe2+ quickly reacts with sulphide to precipitate as FeS, there is a range of Fe(II)-S aqueous species that are present (e.g., Rickard, 2006; Rickard and Luther, 2007). In fact this is precisely these species that give the porewater compositions of Fig. 1C. I think this is important to keep in mind that the Fe(II) speciation in porewaters of oxic, ferruginous and sulphidic sediments is fundamentally different, in order to interpret the range of measured isotopic compositions. Such dissolved Fe-S species should be discussed.

L191: was the pH measured and what was it? It has strong implications for the speciation of both sulphide and iron mentioned in the previous comment, as well as on the pyrite growth mechanism.
Fig. 2C: could you indicate somehow the size range on the figure, instead of in the supplementary information?

L220-230: Butler et al. (2004) suggested that pyrite nucleation, which dominated by the so-called polysulphide pathway, inherits the S isotope composition of polysulphide, whereas later pyrite growth, dominated by the reaction between FeS and H2S, inherits the S composition of both FeS and H2S. How do your data compare to this suggestion?

Reviewer #2 (Remarks to the Author):

Review of “Microscale iron and sulphur isotope signatures reveal pyritization pathways and diagenetic overprints”
Author: Pasquier et al.
Manuscript #: 6554
Reviewer: M. S. Fantle

General comments

Overall Assessment

The dataset produced is quite interesting. The interpretation, on the other hand, operates based on a series of assumptions that are not well articulated. For example, there are a range of assumptions made regarding how particle distributions should look in particular settings/situations, but no real explanation of these assumptions. I think such an explanation is needed given the nature of the dataset; readers are familiar with bulk data, but less familiar with SIMS observations. Relatedly, there is no real explanation or quantification of expectations (e.g., Line 273).

There needs to be some kind of model that links observations and hypotheses. For example, (Line 311) how rapid is ‘rapidly’? Use a model to constrain this, and then ask if that value is reasonable. Use models to set expectations and to justify assumptions made regarding solute dynamics. There does appear to be some kind of calculation made to generate synthetic histograms, but I cannot for the life of me find a description of what is being done here. The calculations need to be explained (sorry if I missed that but I looked everywhere I could); without that, I cannot really evaluate the appropriateness of what is done here (e.g., how are ‘grain’ distributions generated?). These look like Rayleigh calculations but surely one has to use a reactive transport approach to capture dynamics properly. If not, one has to justify using a Rayleigh model in what is an open system. In addition, what assumptions are made in order to generate grain distributions? I would love to know more about this and I think it is important to present in the main text.

In addition, there is no real exploration of multiple hypotheses (at least I cannot find this). For the pyritization process, there is a single pathway presented (Lines 268-73) with no real statement regarding alternatives. The argument is not really built up effectively, but seems to be engineered in the opposite logical direction. Answers are given and the reader told that this is consistent with the data; it should be the other way around. Establish a theoretical interpretive framework and then compare the observations to this framework. The benefit of this is that the reader has a clear understanding of what the differences are between various pathways or whatever it is that affects particle distributions prior to bringing the data in.

Finally, the title mentions ‘diagenetic overprints’. I have read the manuscript a few times, and I still don’t see clear evidence for these overprints or even a discussion of them. The word ‘overprint’ doesn’t occur anywhere in the Discussion and there is no clear discussion of diagenesis either. I suppose this might come down to definitions…for instance, what is meant by diagenetic when you are talking about a mineral that primarily forms in the sedimentary column? Do the authors mean alteration that occurs after pyrite initially forms and stops growing? It might help to explain this…what do they consider to be the primary signal and at what point does an overgrowth become diagenetic? I think it would help me to read that, at least.

Writing and Organization

The writing is extremely dense in places (e.g., Lines 303-330), making it difficult for the reader. The style of the writing is more in the vein of ‘this is the answer’, rather than ‘these are possible answers, let’s evaluate them’. As a result, I am not completely clear what the authors are learning about the system under consideration and what they are simply assuming.

The epsilon jargon is unclear; both isotopic systems discussed have multiple stable nuclides. Thus, the alpha or epsilon values should contain the ratio being discussed (34/32 not 34) and the subscript should denote what is being compared to what (‘sulfide-sulfate’ not ‘mic’). Otherwise it is simply not clear what is meant. The reader shouldn’t have to search the text for an understanding of what a symbol means. It should be clear just from the symbol alone.

Although I like the Intro, I think there is material in there that reduces its effectiveness. For example, the dense text in Lines 90-113 can be shortened so that the main points are made without there being a detailed statement of isotopic effects. This material is relevant but can be introduced in the Discussion as needed. The same is true of Lines 115+. I don’t see this as Introduction material. This is discussion.

Title (and other instances): the word ‘signature’ is reserved for large homogenous Earth reservoirs (Sharp, 2017). Please amend the title; use ‘isotopic compositions’.

Specific comments

Line 22 and other instances: The ‘background’ terminology confuses me; why not call it ‘’shallow” or something a bit more descriptive? It really would help to be as descriptive as possible.

Line 40: “Over geologic timescales…” with an ‘s’

Line 41: word choice: replace ‘presents’ with ‘is’ the main sink.

Line 66: ‘lower’ not ‘lesser’

Line 71: sentence is wordy; detracts from effectiveness; strike “rather than…”. If it is important to state that the bulk analyses do not contain the same information, state that in the next sentences.

Lines 71-77: This passage seems critical, yet it is not written as effectively as it could be. If you are trying to sell the fact that microscale observations provide information that bulk observations do not, make that clear. I think the point that

Line 76: not sure why you use the word ‘passive’ here. Passive implies that the formation of the archive does not change the chemistry of the local system (e.g., trace Ca incorporated into barite). That cannot be true for pyrite, can it? It contains stoichiometric S (and Fe)…its formation must (or at least can) impact pore fluid chemistry, no?

Lines 86-88: There is some nuance that I think is missed in casting our work in this manner. Mansor and Fantle (2019) don’t dismiss diagenetic alteration…clearly: read the section in our paper on this point. We simply suggest that alteration is likely to be minor (as compared to something like calcite, which makes a lot of sense if you think about it). Our entire point is to understand exchange, and there is limited information information out there. Please don’t misrepresent our point.

Lines 92,109, etc: Please don’t use this terminology (56Fe-depleted, etc). It is unwieldy and confusing. Just state that the d56Fe values are low (or high)…that is much simpler. See Sharp (2017) for a justification of this point.

Line 116: There is no hyphen between ‘Fe’ and ‘isotopic’. Please correct everywhere this occurs.

Also, revise to: “…wide range of…observed in marine pore fluids (i.e., …..”

Line 118: not sure what is meant by ‘depth bands’; revise.

Lines 115+: This paragraph confuses me. I am not sure of its purpose here, in the Introduction. This reads more like interpretation than introduction to a problem (and its ostensible solution).

In terms of content, I think the point here is quite difficult to work out. One reason may be that pore fluid chemistry is a snapshot in time, while solids contain information integrated over time. Comparing them directly is likely not all that informative unless you have a quantitative construct that permits such a comparison.

Lines 135-8: So this seems to be the crux of the motivation for the current study (though this is belied by text later on in the Discussion), that the Fe isotopic composition of pyrite suggests a diagenetic origin. However, the authors are ignoring the hypothesis that Mansor and Fantle (2019) put forth to explain the range of bulk d56Fe values (not saying this is correct but it is one hypothesis out there). Without acknowledging that hypothesis, how can you state in Lines 141 that there are 'suspicions of a major diagenetic influence'? From where do these suspicions derive? I am missing the logical progression. Listen, I am definitely in favor of such explorations but the motivation needs to be much more clearly laid out.

Lines 210-8: For me, this is interpretation, not Results. Please move out of this section.

I also don't see the correlation mentioned in Line 215. There is no x-y plot between grain morphology and sulfide, for instance. If it is not a true correlation, don't call it that.

Line 253: Be clear on which value is which. I know it is obvious to you, but it will not be to every reader.

Lines 261 and 304: sorry, I am not sure what you mean by 'pooling' of the sulfide product (it is a poor choice of words). Can you be a little more precise? Are there zones of lower permeability and/or porosity that act to restrict solute diffusion? I think what you mean are zones within the column in which the production of a phase (like sulfide) exceeds its rate of either transport (and/or removal by oxidation and/or precipitation) out of that zone. But it is not 'pooling' in the sense of water sitting in a bucket. This is still a dynamic system and that matters.

Line 263: do you mean 'at all depths'?

Line 273: Sorry but on what is this expectation based? For me, this is what the discussion should present. Also, how unique is this? There really is only one hypothesis explored but very little to justify why it is preferred.

Line 282: The dependent clause here seems redundant; are you not saying the same thing? It reads that way to me, so please rephrase if you are trying to saying something else.

Line 339: I am sorry, but what do you mean by 'fractional reduction'? Some of the terminology used confuses me, and I am sorry for that. But I think it would benefit the paper to write a bit more clearly.

Lines 345-51: A confusing passage here; silicates might be reactive, you state. But no, says the literature, they are too unreactive. But then you have SEM images that suggest…what exactly? What do you SEE in the images that support the pyritization of silicates? You haven't made a statement of what you see! You also have Fe speciation data; pull on that thread a little more. What exactly is in those data that supports this hypothesis. And, finally, why did others say silicates are too unreactive and why would that be different here? These points all matter if you are to make a convincing argument.

Line 353-355: This is, unless I am mistaken, the first time I have seen this particular statement regarding the hypothesis that the authors hope to evaluate. If this is truly the objective, this should be stated at the end of the Introduction not in Line 353. It is out of place here.

Note that in the Introduction, the authors stated that they suspected a diagenetic influence but went no further than that in motivating the study. I think that a little bit more is needed, as I mention above, including a link between the dynamics in the sedimentary section and the grain-scale distributions that are the main product of this work.

Fig. 6: I have no idea how the second panel is generated. It says "Modelled" but I cannot find a single mention of how this was modeled. Maybe I missed it, but if not this needs to be explained. I see Fig. S8, and that looks what I am asking about. But I cannot tell what is actually being done to generate these histograms. Again, I am sorry if I missed it.

Line 442: I honestly don't see how this is an illustration of the impact of different pyritization pathways. To do this, you would need to know the pathway through time and then compare to the data. But the authors are supposing that there is a change in the pyritization pathways (Lines 454-5). I think the framing of this section needs to change so that this is clear.

Line 465: This is a good example of my issue with the writing; what makes these alternate hypotheses 'unnecessary'? You are communicating to the reader your hypothesis, which is only that, a hypothesis. So what you are saying is that if your hypothesis is supported, then one doesn't need to call upon a KIE? That, on its own, doesn't mean that a KIE isn't also occurring. The logic here is not clear to me, aside from the fact that you have model output that is not clearly explained that you claim matches the measured distributions.

Reviewer #3 (Remarks to the Author):

Pasquier et al. conducted systematic microscale iron and sulfur isotope analyses on pyrite from a gravity core collected in the Black Sea. Their study found that the microscale δ34S and δ56Fe values generally increase with depth, as anticipated. Interestingly, the Δ33S value initially increases and then decreases downcore. This unique isotopic pattern suggests variations in diagenetic processes and pyritization pathways as sulfide accumulates in the porewater. The authors extended their observations on δ56Fe to geological records, providing a novel contribution to our understanding of the role of diagenesis in the isotopic signatures of sedimentary pyrite. I recommend this study for publication with some minor suggestions.

Kind regards,
Jiarui Liu

Lines 175-188: It may be worthwhile to add a narrow column in Figure 2, showing the lithological units.

Lines 198-200: Unless I missed something, the method for quantifying AVS and CRS (termed solid total sulfide) is not provided in the method section.

Lines 253-254: One step is missing to link an offset of Δ33S value of 0.5142 to a λ-33 value of 0.5147. Put differently, what is the expected offset of Δ33S between sulfate and sulfide at such a λ-33 value?

Lines 259-261: This statement regarding sulfide pooling is not intuitive. The geochemical profiles do show a buildup and pooling of porewater sulfide.

Lines 308-310: The assertion that δ34S of free sulfide is relatively homogeneous at any given depth may not be accurate. In many marine settings, the δ34S of H2S increases with depth as it accumulates downcore. If the authors refer to sulfide pooling as the point where sulfide concentration reaches its maximum, they should clearly define what they mean by sulfide pooling.

Line 323: Realistically, as mentioned above, the δ34S of dissolved sulfide increases gradually with depth until it reaches +21 per mil. Therefore, I agree with the authors that sulfide pooling homogenizes the δ34S of the produced solid-phase sulfide at a given depth because the instantaneously produced sulfide with higher δ34S is “diluted” by the accumulated sulfide. Assuming steady-state deposition, pyrite would form throughout the sediment column. The pyrite collected at 100 cmbsf would represent a mixture of early- and late-formed pyrite, in which the early-formed pyrite would carry a much lower δ34S signal than its late-formed counterpart. Accordingly, a full range of SIMS δ34S values between -50 to 21 per mil is expected for pyrite at this depth. However, this is not the case, suggesting non-steady-state deposition. In other words, the pyrite at 100 cmbsf appears to lack low SIMS δ34S values as one would expect for steady-state deposition. The authors acknowledged that this pattern may reflect an artifact in SIMS analysis due to the large ion beam size for sulfur analysis. However, the role of non-steady-state deposition cannot be ruled out, as the authors mentioned later in the discussion.

Line 339: FeHR/FePYR appears to be a typo.

Line 373: Additionally, the toxicity of sulfide to microbes in general should be considered.

Line 408: Sulfate reduction could still be the dominant anaerobic pathway for organic matter degradation because the decay of organic matter follows a power law. This means the rate of organic matter remineralization drops very sharply with depth.

Line 556: I assume the sediment used for SIMS analysis was preserved with NaOH as described in the section above. If so, this sampling protocol is less commonly used. Did the authors remove NaOH from the sediment before freeze-drying? Are there any effects on pyrite when sediment is preserved with NaOH?

Line 731: I don’t fully understand how the authors measured iron content in pyrite using the ferrozine assay. Did they use the supernatant after the chromium extraction? If so, the effect of chromium needs to be tested before applying the ferrozine method. A more common practice is to convert the sulfur content to iron, assuming a 1:2 stoichiometry in pyrite.

Line 740: I believe the two ratios are unitless.

Figure 2: Giunta et al. (2022, GCA) published sulfate and methane profiles for cores GC01 and GC02. These profiles look different from those shown in Figure 2. The gravity core used in this study was named GC06 in the methods section. It seems they were not collected from the same location, based on the water depth comparison. Clarification is needed regarding these profiles. Additionally, panels A to E in this figure are not labeled.

Figure 6: The modeling approach is not provided in the methods section.

General Note: Some of the labels and units are not properly displayed in most figures of the main text and the supplementary materials.

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Version 1:

Decision Letter:

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Dr Pasquier,

Your revised manuscript titled "Microscale iron and sulphur isotopic compositions reveal pyritization pathways during early diagenesis" has now been seen by our original reviewers #1 and #3, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment.

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table".

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Please outline your response to each request in the right hand column. Please upload the completed table with your manuscript files as a Related Manuscript file.

If you have any questions or concerns about any of our requests, please do not hesitate to contact me.

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Carolina Ortiz Guerrero, Ph.D.
Associate Editor
Communications Earth & Environment

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

I did review an earlier version of the manuscript some time ago. I read through the new version and the response made to the reviewers and I am pleased with the answer provided. Even if I may not agree with all interpretations, the authors have tackled each comment and provided sufficient arguments.

I do not have any further commments and support the publication of the manuscript.

Reviewer #3 (Remarks to the Author):

The authors have addressed the reviewers' comments, leading to an improved manuscript. I recommend it for publication.

Jiarui Liu

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Reviewer #1 (Remarks to the Author):

Comments on the manuscript entitled ‘Microscale iron and sulphur isotope signatures reveal pyritization pathways and diagenetic overprints’ submitted to Communications Earth and Environment.

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I generally find the paper very well written, the methods are sound and I do believe the data of high quality. In fact, I only have comments on some of the author’s interpretations, which appear quite old fashion in my opinion and not fully supported by the data, and some minor suggestions. I hope my comments will help improve the manuscript, which I recommend for publication.

Romain Guilbaud

We thank Dr. Guilbaud for the detailed and helpful review.

1. **Comment**: At several occasions (e.g., L263-275; L454-465), the authors invoke the production of an isotopically light Fe^{2+} reservoir, derived from DIR, which then reacts with sulphide to form FeS at equilibrium, which itself reacts to form pyrite. This is, more or less, the way that was used to explain the very negative pyrite values in the Archean in 2005. Since then, there has been extensive work, cited by the authors, strongly nuancing this. First of all, in Guilbaud et al. (EPSL, 2010) and Guilbaud et al. (GCA, 2011), we found evidence that the core-particle size ratio in neoformed nanoparticles prevent equilibrium to be reached, even after 4 months of experiment, resulting in isotopically light FeS particles. Guilbaud et al. (GCA, 2011) and Wu et al. (2012) showed that at equilibrium, FeS becomes slightly isotopically heavy compared to Fe(II), but only at higher pH. This is one of the reasons why, as I suggest below, pH is so important and needs to be indicated in the main text. Then, there is the study by McAnena et al. (2023) which shows a range of isotopic compositions that are produced during reduction of Fe(III) (oxyhydr)oxides by sulphide at different pH values, and so without the intervention of any microbial activity. Finally, there is the pyrite-forming mechanism itself, which produces light early-stage pyrite (Guilbaud et al., Science 2011; Mansor and Fantle, GCA 2019; Heard et al, Science 2020). All in all, all these mechanisms produce isotopically light to very light Fe(II) and pyrite, and yet the authors prefer to invoke DIR. I would like to see more nuance in their interpretation. For instance, L464-465: ‘Expression of a KIE upon FeS transformation to pyrite (…) appears to be unnecessary’ is entirely unsupported. One could equally assert that ‘DIR appears to be unnecessary’, with the exact same logic! I believe that nowadays, with the large body of data we have on the matter, one really can (and should) do better when interpreting data…

   Response: In principle, it is possible, by varying the rate of FeS transformation to FeS_2 (i.e., varying the fractionation associated with this transformation from -0.5‰ at slow transformation to -2.2‰ at rapid transformation) to explain the observed distribution of pyrite $\delta^{56}Fe$ values without invoking DIR. However, benthic chambers indicate Fe(II) fluxes into the water column in sulphide-free waters[1-6]. The sediments from which these benthic Fe(II) fluxes come are sulphide-free, making it unlikely that the Fe(II) comes from sulphide-driven dissolution of Fe(III) (oxyhydr)oxides. Furthermore, the $\delta^{56}Fe$ of the Fe(II) diffusing from the sediments to the water column is ~−3.28 ±1.3‰, offset by ~3‰ from riverine Fe(III) (oxyhydr)oxides[1-6]. The Fe isotope fractionation associated with sulphide-driven dissolution of Fe(III) (oxyhdr)oxides is ~0.85‰, and though this fractionation could vary under different conditions, it has never been recorded to approach large enough values to explain the isotopic composition of the benthic Fe(II) fluxes. Thus, we favor the DIR-related explanation of our measurements. Having said that, and in response also to Reviewer #2's comment #10, we now lay out several alternative hypotheses and discuss the evidence for/against those hypotheses before adopting a preferred explanation (lines 243-255, 266-276, 333-339, 341-413).

2. **Comment**: L99: McAnena et al do suggest that the fractionation is sensitive to mineral dissolution rates, amongst other factors.
   Response: We have corrected this statement (lines 110-113).

3. **Comment**: L104: similar values were obtained for the equilibrium fractionation between FeS and Fe2+ at both 25 and 2 degree C by Guilbaud et al. (2011), i.e. earlier than the Wu et al. (2012) paper.
   Response: We refer to the study of Guilbaud et al. (2011) in the revised manuscript (lines 105).

4. **Comment**: L126-127 and the caption of Fig. 1. Sulphidic conditions are not defined by the lack of measurable Fe2+, as the authors suggest (e.g., Canfield and Thamdrup, 2009). Whilst it is correct to say that free Fe2+ quickly reacts with sulphide to precipitate as FeS, there is a range of Fe(II)-S aqueous species that are present (e.g., Rickard, 2006; Rickard and Luther, 2007). In fact this is precisely these species that give the porewater compositions of Fig. 1C. I think this is important to keep in mind that the Fe(II) speciation in porewaters of oxic, ferruginous and sulphidic sediments is fundamentally different, in order to interpret the range of measured isotopic compositions. Such dissolved Fe-S species should be discussed.
   Response: We have specified that we refer to the accumulation of aqueous sulpide in the revised manuscript and figure caption. We also discuss the importance of Fe-S speciation.

5. **Comment**: L191: was the pH measured and what was it? It has strong implications for the speciation of both sulphide and iron mentioned in the previous comment, as well as on the pyrite growth mechanism.
   Response: Unfortunately the pH of the porewaters was not measured, although a global compilation of porewater pH in a variety of modern environments (including the Black-Sea margin) tends to show pH between 7 and 8. We have commented on this in the revised manuscript and discussed the implications for Fe-S speciation and the range of expected fractionations (lines 102-116, 393).

6. **Comment**: Fig. 2C: could you indicate somehow the size range on the figure, instead of in the supplementary information?
   Response: This figure is already dense, and adding the grain size range will decrease its readability. We prefer to keep the figure in its original design and provide the grain size range in the supplementary information.

7. **Comment**: L220-230: Butler et al. (2004) suggested that pyrite nucleation, which dominated by the so-called polysulphide pathway, inherits the S isotope composition of polysulphide, whereas later pyrite growth, dominated by the reaction between FeS and H2S, inherits the S composition of both FeS and H_2S. How do your data compare to this suggestion?
   Response: We agree with the Reviewer that under the more oxidizing conditions of the upper sediments, where electron acceptors are generally more abundant, the presence of polysulphides and their relatively rapid reaction with FeS is expected to dominate the formation of FeS_2. However, in most studied sediments, the $\delta^{34}S$ value of polysulphide is offset from that of sulphide approximately by the equilibrium polysulphide-sulphide S isotope equilibrium fractionation (~4-6‰) consistent with experimental evidence that polysulphides and sulphide rapidly reach S isotope equilibrium (Ref. 7). So, dominance of the polysulphide pathway in the upper sediments and the sulphide pathway in the deeper sediments could explain an offset between the $\delta^{34}S$ values of early and late pyrite of only ~5‰. We observe much larger variations, which are consistent with isotopic (Rayleigh-type) distillation of S isotopes during DSR, as observed in numerous past studies of the bulk $\delta^{34}S$ of pyrite[8-15] and in a few recent studies of in-situ pyrite $\delta^{34}S$ values[12,14-16]. We have clarified in the revised manuscript (lines 270-276).

Reviewer #2 (Remarks to the Author):

Review of "Microscale iron and sulphur isotope signatures reveal pyritization pathways and diagenetic overprints"
Author: Pasquier et al.
Manuscript #: 6554
Reviewer: M. S. Fantle

We thank Dr. Fantle for this detailed and constructive review.

General comments

Overall Assessment

8. **Comment**: The dataset produced is quite interesting. The interpretation, on the other hand, operates based on a series of assumptions that are not well articulated. For example, there are a range of assumptions made regarding how particle distributions should look in particular settings/situations, but no real explanation of these assumptions. I think such an explanation is needed given the nature of the dataset; readers are familiar with bulk data, but less familiar with SIMS observations. Relatedly, there is no real explanation or quantification of expectations (e.g., Line 273).
   Response: We have attempted to better articulate the expectations regarding the distribution of SIMS observations relative to bulk observations. We additionally attempted to justify our assumptions throughout the manuscript, in response to the comments provided by all three Reviewers.

9. **Comment**: There needs to be some kind of model that links observations and hypotheses. For example, (Line 311) how rapid is 'rapidly'? Use a model to constrain this, and then ask if that value is reasonable. Use models to set expectations and to justify assumptions made regarding solute dynamics. There does appear to be some kind of calculation made to generate synthetic histograms, but I cannot for the life of me find a description of what is being done here. The calculations need to be explained (sorry if I missed that but I looked everywhere I could); without that, I cannot really evaluate the appropriateness of what is done here (e.g., how are 'grain' distributions generated?). These look like Rayleigh calculations but surely one has to use a reactive transport approach to capture dynamics properly. If not, one has to justify using a Rayleigh model in what is an open system. In addition, what assumptions are made in order to generate grain distributions? I would love to know more about this and I think it is important to present in the main text.
   Response: We now provide an explanation of the model used to reproduce the Black Sea observations in our manuscript and discuss two main alternative explanations for the distributions (lines 243-255, 265-276, 333-339, 342-413). Please also see the response to Reviewer #1's comment #1 above.

10. In addition, there is no real exploration of multiple hypotheses (at least I cannot find this). For the pyritization process, there is a single pathway presented (Lines 268-73) with no real statement regarding alternatives. The argument is not really built up effectively, but seems to be engineered in the opposite logical direction. Answers are given and the reader told that this is consistent with the data; it should be the other way around. Establish a theoretical interpretive framework and then compare the observations to this framework. The benefit of this is that the reader has a clear understanding of what the differences are between various pathways or whatever it is that affects particle distributions prior to bringing the data in.
    Response: For readability, we did not go into the evidence in support of the preferred explanation in the main text and neither did we elaborate on the alternative hypotheses (which were explored). We have now restructured the discussion according to the Reviewer's suggestion and discuss alternative hypotheses (lines 243-255, 265-276, 333-339, 342-413)

11. **Comment**: Finally, the title mentions ‘diagenetic overprints’. I have read the manuscript a few times, and I still don’t see clear evidence for these overprints or even a discussion of them. The word ‘overprint’ doesn’t occur anywhere in the Discussion and there is no clear discussion of diagenesis either. I suppose this might come down to definitions…for instance, what is meant by diagenetic when you are talking about a mineral that primarily forms in the sedimentary column? Do the authors mean alteration that occurs after pyrite initially forms and stops growing? It might help to explain this…what do they consider to be the primary signal and at what point does an overgrowth become diagenetic? I think it would help me to read that, at least.
    Response: By “diagenetic overprint” we originally meant that late-stage pyrite formation in association with the migration of the SMTZ (by sulphidization of residual Fe(III) oxides) shifts the isotopic composition of both Fe and S in pyrite from that of the early pyrite formed in the upper sediments (by a combination of processes, including DIR and low S:Fe sulphidization of Fe(III) oxides). However, the Reviewer is correct that all of these processes are diagenetic. We have changed the title of the manuscript to better represent the processes that we believe to have taken place in the studied sediments. We have also made minor modifications to the text to this effect.

Writing and Organization

12. **Comment**: The writing is extremely dense in places (e.g., Lines 303-330), making it difficult for the reader. The style of the writing is more in the vein of ‘this is the answer’, rather than ‘these are possible answers, let’s evaluate them’. As a result, I am not completely clear what the authors are learning about the system under consideration and what they are simply assuming.
    Response: The original manuscript was organized to first present the preferred explanation, then more briefly discuss the alternatives. We realize that this may not be the order preferred by some readers, including the Reviewer. We have rewritten the revised manuscript to first present a set of hypotheses, then provide the arguments for and against these hypotheses. In addition, we have attempted to “lighten” the text.

13. **Comment**: The epsilon jargon is unclear; both isotopic systems discussed have multiple stable nuclides. Thus, the alpha or epsilon values should contain the ratio being discussed (34/32 not 34) and the subscript should denote what is being compared to what (‘sulfide-sulfate’ not ‘mic’). Otherwise it is simply not clear what is meant. The reader shouldn’t have to search the text for an understanding of what a symbol means. It should be clear just from the symbol alone.
    Response: Done.

14. **Comment**: Although I like the Intro, I think there is material in there that reduces its effectiveness. For example, the dense text in Lines 90-113 can be shortened so that the main points are made without there being a detailed statement of isotopic effects. This material is relevant but can be introduced in the Discussion as needed. The same is true of Lines 115+. I don’t see this as Introduction material. This is discussion.
    Response: We have shortened the introduction and introduced concepts in the discussion, as necessary.

15. **Comment**: Title (and other instances): the word ‘signature’ is reserved for large homogenous Earth reservoirs (Sharp, 2017). Please amend the title; use ‘isotopic compositions’.
    Response: We have modified the title according to the Reviewer’s suggestion.

Specific comments

16. **Comment**: Line 22 and other instances: The ‘background’ terminology confuses me; why not call it ‘’shallow” or something a bit more descriptive? It really would help to be as descriptive as possible.
    Response: We now term the pyrite formed in the shallow sediments “early pyrite”.

17. **Comment**: Line 40: “Over geologic timescales…” with an ‘s’
    Response: Corrected.

18. **Comment**: Line 41: word choice: replace ‘presents’ with ‘is’ the main sink.
    Response: Done.

19. **Comment**: Line 66: ‘lower’ not ‘lesser’
    Response: Done.

20. **Comment**: Line 71: sentence is wordy; detracts from effectiveness; strike “rather than…”. If it is important to state that the bulk analyses do not contain the same information, state that in the next sentences.
    Response: Done.

21. **Comment**: Lines 71-77: This passage seems critical, yet it is not written as effectively as it could be. If you are trying to sell the fact that microscale observations provide information that bulk observations do not, make that clear. I think the point that
    Response: We have attempted to clarify and increase the effectiveness of this passage.

22. **Comment**: Line 76: not sure why you use the word ‘passive’ here. Passive implies that the formation of the archive does not change the chemistry of the local system (e.g., trace Ca incorporated into barite). That cannot be true for pyrite, can it? It contains stoichiometric S (and Fe)…its formation must (or at least can) impact pore fluid chemistry, no?
    Response: Reworded.

23. **Comment**: Lines 86-88: There is some nuance that I think is missed in casting our work in this manner. Mansor and Fantle (2019) don’t dismiss diagenetic alteration…clearly: read the section in our paper on this point. We simply suggest that alteration is likely to be minor (as compared to something like calcite, which makes a lot of sense if you think about it). Our entire point is to understand exchange, and there is limited information information out there. Please don’t misrepresent our point.
    Response: We have rephrased these sentences in the revised manuscript to better represent the points made by Mansor and Fantle (2019).

24. **Comment**: Lines 92,109, etc: Please don’t use this terminology (56Fe-depleted, etc). It is unwieldy and confusing. Just state that the δ56Fe values are low (or high)…that is much simpler. See Sharp (2017) for a justification of this point.
    Response: Done.

25. **Comment**: Line 116: There is no hyphen between ‘Fe’ and ‘isotopic’. Please correct everywhere this occurs.
    Response: Corrected.

26. **Comment**: Also, revise to: “…wide range of…observed in marine pore fluids (i.e., …..”
    Response: Done.

27. **Comment**: Line 118: not sure what is meant by ‘depth bands’; revise.
    Response: We have rephrased.

28. **Comment**: Lines 115+: This paragraph confuses me. I am not sure of its purpose here, in the Introduction. This reads more like interpretation than introduction to a problem (and its ostensible solution).
    In terms of content, I think the point here is quite difficult to work out. One reason may be that pore fluid chemistry is a snapshot in time, while solids contain information integrated over time.

Comparing them directly is likely not all that informative unless you have a quantitative construct that permits such a comparison.
Response: Please also see the response to comment 14 above. We have reworded and removed some of this material from the introduction. We introduce it in the discussion, as necessary.

29. **Comment**: Lines 135-8: So this seems to be the crux of the motivation for the current study (though this is belied by text later on in the Discussion), that the Fe isotopic composition of pyrite suggests a diagenetic origin. However, the authors are ignoring the hypothesis that Mansor and Fantle (2019) put forth to explain the range of bulk δ56Fe values (not saying this is correct but it is one hypothesis out there). Without acknowledging that hypothesis, how can you state in Lines 141 that there are 'suspicions of a major diagenetic influence'? From where do these suspicions derive? I am missing the logical progression. Listen, I am definitely in favor of such explorations but the motivation needs to be much more clearly laid out.
Response: We have attempted to clarify these sentences, but now also lay out the various hypotheses proposed to explain the Fe isotopic composition of pyrite.

30. **Comment**: Lines 210-8: For me, this is interpretation, not Results. Please move out of this section.
Response: These lines contain observations, not interpretations. The word "related" may be the source of confusion and it would have been better to use "correlated" instead. We have changed this and also clarified that we are describing observations, not providing interpretations.

31. **Comment**: I also don't see the correlation mentioned in Line 215. There is no x-y plot between grain morphology and sulfide, for instance. If it is not a true correlation, don't call it that.
Response: The correlation exists – please see the figure below. The correlation is observable by eyeballing the porewater sulphide concentration profile and the relative abundances of the various morphologies at comparable depths in the sediment. We now show a cross-plot of these quantities in the SI and refer to this figure from the main text.

32. **Comment**: Line 253: Be clear on which value is which. I know it is obvious to you, but it will not be to every reader.
Response: Reworded.

33. **Comment**: Lines 261 and 304: sorry, I am not sure what you mean by 'pooling' of the sulfide product (it is a poor choice of words). Can you be a little more precise? Are there zones of lower permeability and/or porosity that act to restrict solute diffusion? I think what you mean are zones within the column in which the production of a phase (like sulfide) exceeds its rate of either transport (and/or removal by oxidation and/or precipitation) out of that zone. But it is not 'pooling' in the sense of water sitting in a bucket. This is still a dynamic system and that matters.
Response: We have reworded to explain what we mean and now refer to this as sulphide accumulation.

34. **Comment**: Line 263: do you mean 'at all depths'?
Response: We have clarified in the revised manuscript.

35. **Comment**: Line 273: Sorry but on what is this expectation based? For me, this is what the discussion should present. Also, how unique is this? There really is only one hypothesis explored but very little to justify why it is preferred.
Response: Please see the responses to comments 9, 10, 12 and 29.

36. **Comment**: Line 282: The dependent clause here seems redundant; are you not saying the same thing? It reads that way to me, so please rephrase if you are trying to saying something else.
Response: Reworded.

37. **Comment**: Line 339: I am sorry, but what do you mean by ‘fractional reduction’? Some of the terminology used confuses me, and I am sorry for that. But I think it would benefit the paper to write a bit more clearly. **Response**: We mean reduction of only some of the Fe(III) oxides. We have reworded for clarity.

38. **Comment**: Lines 345-51: A confusing passage here; silicates might be reactive, you state. But no, says the literature, they are too unreactive. But then you have SEM images that suggest…what exactly? What do you SEE in the images that support the pyritization of silicates? You haven’t made a statement of what you see! You also have Fe speciation data; pull on that thread a little more. What exactly is in those data that supports this hypothesis. And, finally, why did others say silicates are too unreactive and why would that be different here? These points all matter if you are to make a convincing argument.
    Response: We have developed this point further and referred to Pasquier et al., 2022 (GCA), where we describe this phenonenon in more detail (lines 330-339).

39. **Comment**: Line 353-355: This is, unless I am mistaken, the first time I have seen this particular statement regarding the hypothesis that the authors hope to evaluate. If this is truly the objective, this should be stated at the end of the Introduction not in Line 353. It is out of place here.
    Response: Done.

40. **Comment**: Note that in the Introduction, the authors stated that they suspected a diagenetic influence but went no further than that in motivating the study. I think that a little bit more is needed, as I mention above, including a link between the dynamics in the sedimentary section and the grain-scale distributions that are the main product of this work.
    Response: Please see the response to comment 11. We consider all pyrite to be diagenetic in the studied sediments and thank the Reviewer for pointing out the misleading title. We have also reworded to introduction accordingly to state that we mean late diagenetic (as opposed to the early diagenetic pyrite).

41. **Comment**: Fig. 6: I have no idea how the second panel is generated. It says “Modelled” but I cannot find a single mention of how this was modeled. Maybe I missed it, but if not this needs to be explained. I see Fig. S8, and that looks what I am asking about. But I cannot tell what is actually being done to generate these histograms. Again, I am sorry if I missed it.
    Response: You have not missed it, we did not provide sufficient information on the modeling and thank the Reviewer for catching this. We have taken a different approach to the modeling, which we now better explain in the Methods.

42. **Comment**: Line 442: I honestly don’t see how this is an illustration of the impact of different pyritization pathways. To do this, you would need to know the pathway through time and then compare to the data. But the authors are supposing that there is a change in the pyritization pathways (Lines 454-5). I think the framing of this section needs to change so that this is clear.
    Response: We have changed this section completely, removing the text referred to by the Reviewer.

43. **Comment**: Line 465: This is a good example of my issue with the writing; what makes these alternate hypotheses ‘unnecessary’? You are communicating to the reader your hypothesis, which is only that, a hypothesis. So what you are saying is that if your hypothesis is supported, then one doesn’t need to call upon a KIE? That, on its own, doesn’t mean that a KIE isn’t also occurring. The logic here is not clear to me, aside from the fact that you have model output that is not clearly explained that you claim matches the measured distributions.
    Response: We have clarified the evidence for and against the various alternative hypotheses and now more clearly state why we do not think that differential expression of a KIE is occurring in the studied sediments. We also qualify that our findings do not mean that in other settings differential KIE expression does not occur.

Reviewer #3 (Remarks to the Author):

44. Pasquier et al. conducted systematic microscale iron and sulfur isotope analyses on pyrite from a gravity core collected in the Black Sea. Their study found that the microscale δ34S and δ56Fe values generally increase with depth, as anticipated. Interestingly, the Δ33S value initially increases and then decreases downcore. This unique isotopic pattern suggests variations in diagenetic processes and pyritization pathways as sulfide accumulates in the porewater. The authors extended their observations on δ56Fe to geological records, providing a novel contribution to our understanding of the role of diagenesis in the isotopic signatures of sedimentary pyrite. I recommend this study for publication with some minor suggestions.
Kind regards,
Jiarui Liu
Response: We thank Dr. Liu for this very helpful and positive review.

45. **Comment**: Lines 175-188: It may be worthwhile to add a narrow column in Figure 2, showing the lithological units.
Response: We have added the requested information to Fig.1.

46. **Comment**: Lines 198-200: Unless I missed something, the method for quantifying AVS and CRS (termed solid total sulfide) is not provided in the method section.
Response: The method used for their quantification was described in the original Fe speciation section.

47. **Comment**: Lines 253-254: One step is missing to link an offset of Δ33S value of 0.5142 to a λ-33 value of 0.5147. Put differently, what is the expected offset of Δ33S between sulfate and sulfide at such a λ-33 value?
Response: We have reworded for clarity.

48. **Comment**: Lines 259-261: This statement regarding sulfide pooling is not intuitive. The geochemical profiles do show a buildup and pooling of porewater sulfide.
Response: The Reviewer is correct, we have rephrased this in the reviewed manuscript (lines 294-307).

49. **Comment**: Lines 308-310: The assertion that δ34S of free sulfide is relatively homogeneous at any given depth may not be accurate. In many marine settings, the δ34S of H2S increases with depth as it accumulates downcore. If the authors refer to sulfide pooling as the point where sulfide concentration reaches its maximum, they should clearly define what they mean by sulfide pooling.
Response: The Reviewer is correct, the S isotopic composition of pooled sulphide is expected to increase with depth (see lines 305-307 of the original manuscript) both because of the progressive isotopic distillation and ^{34}S enrichment of the residual sulphate from which the sulphide is produced and because the sulphide produced deeper within the sediment diffuses upward and mixes with sulphide produced in-situ. The isotopic composition of sulphide at any single depth then reflects the mixing of in-situ production and upward diffusion of more ^{34}S-enriched sulphide. Consequently, with increasing depth (or more precisely with increasing aqueous sulphide content), the S isotopic composition of free sulphide is expected to be increasingly homogeneous with depth. We have clarified in the revised manuscript (lines 292-311).

50. **Comment**: Line 323: Realistically, as mentioned above, the δ34S of dissolved sulfide increases gradually with depth until it reaches +21 per mil. Therefore, I agree with the authors that sulfide pooling homogenizes the δ34S of the produced solid-phase sulfide at a given depth because the instantaneously produced sulfide with higher δ34S is "diluted" by the accumulated sulfide. Assuming steady-state deposition, pyrite would form throughout the sediment column. The pyrite collected at 100 cmbsf would represent a mixture of early- and late-formed pyrite, in which the

early-formed pyrite would carry a much lower δ34S signal than its late-formed counterpart. Accordingly, a full range of SIMS δ34S values between -50 to 21 per mil is expected for pyrite at this depth. However, this is not the case, suggesting non-steady-state deposition. In other words, the pyrite at 100 cmbsf appears to lack low SIMS δ34S values as one would expect for steady-state deposition. The authors acknowledged that this pattern may reflect an artifact in SIMS analysis due to the large ion beam size for sulfur analysis. However, the role of non-steady-state deposition cannot be ruled out, as the authors mentioned later in the discussion.

Response: Our preferred explanation is exactly one of non-steady-state deposition, where an increase in the sedimentation rate causes a shoaling of the SMTZ and a burst of late-stage pyrite formation. The late-stage pyrite does not come at the expense of early diagenetic pyrite, and the Reviewer is correct that SIMS histograms at depths in which late-stage pyrite formed are expected to show both. However, if the late-stage pyrite is sufficiently more abundant than the early diagenetic pyrite, if it infills and coats the early pyrite, and/or if analytical issues exist (e.g., the beam size), then the early diagenetic pyrite may be underrepresented in the SIMS histograms. We think that this is the reason that the low-δ^{34}S pyrites are not present in our sulphidic-zone histograms. We have clarified this in the revised manuscript (lines 454-502).

51. **Comment**: Line 339: FeHR/FePYR appears to be a typo.
    Response: Corrected to Fe_{PYR}/Fe_{HR}, thanks for catching this.

52. **Comment**: Line 373: Additionally, the toxicity of sulfide to microbes in general should be considered.
    Response: Done.

53. **Comment**: Line 408: Sulfate reduction could still be the dominant anaerobic pathway for organic matter degradation because the decay of organic matter follows a power law. This means the rate of organic matter remineralization drops very sharply with depth.
    Response: The Reviewer is correct. A rapid increase in the sedimentation rate translates into two opposing effects: (1) a shallower onset of organoclastic sulphate reduction leading to more rapid sulphate drawdown with depth, and (2) a shallowing of the SMTZ, which provides a larger and 'fresher' organic matter pool for methanogens. One of the consequences is that a greater proportion of the sulphate reducers will now feed on CH_4, through AOM-SR, leading to sulphide accumulation at and above the SMTZ. We have clarified in the revised manuscript (lines 472-476).

54. **Comment**: Line 556: I assume the sediment used for SIMS analysis was preserved with NaOH as described in the section above. If so, this sampling protocol is less commonly used. Did the authors remove NaOH from the sediment before freeze-drying? Are there any effects on pyrite when sediment is preserved with NaOH?
    Response: The sediment were not conserved in NaOH prior to SIMS analyses. We only used NaOH to poison methanogen populations, as is commonly done. The sediment core was stored unopened at 4°C prior its opening on land.

55. **Comment**: Line 731: I don't fully understand how the authors measured iron content in pyrite using the ferrozine assay. Did they use the supernatant after the chromium extraction? If so, the effect of chromium needs to be tested before applying the ferrozine method. A more common practice is to convert the sulfur content to iron, assuming a 1:2 stoichiometry in pyrite.
    Response: The Reviewer is correct, we have clarified the methodology in the revised Methods (lines 747-749).

56. **Comment**: Line 740: I believe the two ratios are unitless.
    Response: Corrected.

57. Comment: Figure 2: Giunta et al. (2022, GCA) published sulfate and methane profiles for cores GC01 and GC02. These profiles look different from those shown in Figure 2. The gravity core used in this study was named GC06 in the methods section. It seems they were not collected from the same location, based on the water depth comparison. Clarification is needed regarding these profiles. Additionally, panels A to E in this figure are not labeled.
Response: The Reviewer is correct, these CH_4 profiles were not included in the study by Giunta et al. (2022). We have corrected the figure caption. The panel labels are in the upper left corner of each panel.

58. Comment: Figure 6: The modeling approach is not provided in the methods section.
Response: We provide a description of the modeling approach in the revised Methods (lines 765-846).

59. Comment: General Note: Some of the labels and units are not properly displayed in most figures of the main text and the supplementary materials.
Response: We thank the Reviewer for pointing this out and have corrected this.

Reference used in the response to the Reviewers:

1 Lenstra, W. K. *et al.* The shelf-to-basin iron shuttle in the Black Sea revisited. *Chemical Geology* **511**, 314-341, doi:10.1016/j.chemgeo.2018.10.024 (2019).

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