

Response to review by Tom Jilbert.

Original comments in black, responses are in blue.

Fisher et al. present results of experiments into the extractability of OC-FeR (that is to say, sedimentary organic carbon bound to reactive Fe) during treatment with citrate bicarbonate-dithionite solution. CBD extractions are a commonly applied method, either stand-alone or as part of sequential extractions, for investigating elements associated with reducible phases in marine sediments. As the authors state, there is much heterogeneity in the details of applied CBD extraction protocols, even within the narrower context of studies into OC-FeR. This has led to difficulty in comparing results and the possibility that the currently used protocols may be sub-optimal for their stated goals. Therefore there is clearly a need for studies like this one, to eventually improve/harmonize the approaches used in the community.

Overall I had the feeling that the study delivers some interesting results although some of the interpretations are left only lightly justified. This leads to the idea that a more developed set of experiments could have yielded a more useful step forward. For example, the conclusion that complexation by citrate may be limiting the recovery of Fe in the highest-OC-FeR experiment deserves to be tested through a concentration series similar to the dithionite series the authors report. This is especially the case considering the comments of the first reviewer (Henkel) questioning whether citrate limitation is a feasible explanation for the observations in Fig. 1. I do not demand that the authors produce such additional data before publication but it is clear that their conclusion would be more robust if it was available, and therefore the overall impact of the study would be greater. A similar criticism could be leveled at the interpretations of the experiment comparing freeze-dried and wet samples, although I would say this is a complex topic that warrants a separate study.

We thank Dr Jilbert for their review and for seeing the value in our study. In the first review of this manuscript, Dr Henkel did raise the issue of whether citrate limitation could explain the reduced loss of Fe at high wt% Fe contents and in turn directed us to Henkel et al., (2016) which suggested the use of anoxic extractions instead. We happily included this point in our revisions to the manuscript; however, both anoxic extraction and increased citrate address the same issue of Fe precipitation in different ways so there appears to be some consensus on the underlying issue here. It is difficult to think of any other reason why Fe losses would be minimised, as there is nowhere else for the Fe to go besides staying in solution or precipitating.

We did not conduct experiments with increased citrate as the method we test is used for OC extraction and we were therefore very cautious about adding C containing compounds to the reaction. Indeed, we did not have a solution to this problem until Patzner et al., (2020) very recently incorporated a correction for the background DOC levels in the reaction.

We agree there is more work to be done on freeze-dried vs wet samples. Particularly around the effects of freeze-thawing, we unfortunately were unable to conduct these types of analyses as the sediment samples we had access to had all been previously frozen (as is common). However, our study provides a new perspective on this issue; for example, we are not aware of any studies which have quantified the effect of variable water mass on introducing error in determining the dry weight equivalent of sediments, or any studies which have aimed to compare the same synthetic samples under different preparation methods.

We have modified our interpretation of the freeze-drying experiment on the natural samples following the comments raised by Peter Kraal.

Another important point is that it seems that some of the content here may be an overflow from the authors' recent Chem. Geol. paper (cited Fisher et al. 2020), which is not a criticism as such but in some cases I had the feeling the reader is being referred there to explain what is going on in the experiments presented here, which should be avoided as this ms. must also be a stand-alone study, even if it is a Technical Note. I am specifically referring to the interpretation of the results of the experiment in which the degree of carboxylation of OM in synthetic OC-FeR is varied (Fig. 2). The discussion of the mechanisms here (Paragraph from Line 358) is too thin and the reader cannot understand why the degree of carboxylation makes such a difference to Fe extractability without accessing the other paper.

We have expanded our manuscript to provide an explanation for the carboxyl relationship identified in the previous study. We also expanded the methods section in response to a similar comment by a previous reviewer.

"Trends between Fe extraction and carboxyl content have been previously identified, with an increase in the number of carboxyl groups in an iron bound organic compound resulting in an increased proportion of Fe liberated from the sample (Fisher et al., 2020). This was explained by the greater amorphicity of ferrihydrite when carboxyl rich OC is incorporated into the mineral lattice, i.e., the resultant phase is less crystalline than pure ferrihydrite and therefore easier to reductively dissolve."

Unfortunately I read Susann's review only after making my own comments on the original ms., then later noticed that she has done a very comprehensive job and found several of the same issues that I wished to highlight. It is good to see that the authors have responded thoroughly to Susann's comments and this will undoubtedly improve the next version. Therefore my list of additional comments is comparatively short.

We are grateful for this response to the changes we have already made to the manuscript and agree it has been significantly improved by the comments we have received.

General (in addition to the above; all Line numbers refer to the original ms):

- The Introduction can be better worded and arranged: First I suggest to move the para. starting Line 59 to directly above the short para. starting Line 81. This way you first describe the problems with the existing methodologies, then set out how you intend to solve them. Next, check a few key sentences: e.g. Line 26 "Understanding in which environments organic carbon (OC) persists": please clarify that you are referring to preservation of OC in sediments; Line 54 "fully reduce all solid reactive Fe phases and associated carbon"... I could not find this phrase in Lalonde et al. 2012, although it is presented here as a quotation. Please check.

We have moved this paragraph to the suggested location. On line 26 we are discussing the importance of understanding the global carbon cycle, we did not mention sediments here as we acknowledge the fact that OC does not just occur in marine sediments and its preservation is facilitated by many mechanisms (not just reactive iron). In the last line of the abstract, we now also mention that OC-Fe interactions exist in soils. On line 28 we begin the discussion about marine sediments.

The quote from Lalonde et al. (2012) appears on the first page of the PDF of that manuscript. On checking this, the original paper doesn't include the word "fully", so this has

been removed from our quote but this does not change the meaning of the quote as the original article uses “all”.

Original (Lalonde): *(The CBD method) “dissolves from the sediment matrix all solid reactive iron phases and the organic carbon associated with these phases (OC-Fe)”*

Our amended Line 54: *These findings contrast the previous understanding of the CBD method performed in an experimental context which states that this extraction will reduce “all solid reactive iron phases and the organic carbon associated with these phases” (Lalonde et al., 2012)*

- Throughout: the terminology in this field is easily misunderstood. E.g. the first reviewer thought for the whole time that % OC-FeR refers to % of total sediment, when in fact it refers to % of total OC. I also had major difficulties to get this upon first reading. So I suggest to clarify terminology early on, and modify figures and captions to make this easier to follow. E.g. I note that Barber et al. 2017 use more descriptive terminology in tables and figures e.g. "OC bound to Fe (% of total OC)" in their Table 1 and "Fraction of total sediment Fe" in their Fig. 4. Also check that CDB/CBD is used consistently. Both current appear.

We agree that the terminology can be misunderstood in these type of studies. A key difference between our study and that of Barber et al., (2017) is in the total OC content of samples. In our study, the natural sediment matrix has been made OC free, and we have added this aspect to the methods to emphasise how this sediment was prepared in response to the first review. The only OC source in our spiked sediments comes from the coprecipitation of organic acids with ferrihydrite (OC-Fe_R), while natural sediments contain various forms of unbound OC. Any addition of OC-Fe_R would represent 100% of the total sample OC pool regardless of how much was added, so this is not a useful metric. When we refer to a sample containing e.g. 20% OC-Fe_R that is not to say 20% of the OC present is Fe bound, but that 20 wt% of that sediment is the OC-Fe_R coprecipitate complex. Following this review and the previous, we have decided to refer to quantities in wt% as much as possible.

We have modified the definition given to OC-Fe_R on line 40 to “OC bound to reactive iron”.

Additionally we have removed the use of %OC-Fe_R following the previous review which asked us to consider the use of content vs concentration. Therefore, the amount of OC-Fe_R added is now referred to as the OC-Fe_R content in terms of wt%. This has also been changed in the text, e.g. from “20% OC-Fe_R” to “*the sample containing 20 wt% OC-Fe_R*”. Where reference is made to the total OC pool (when referencing other studies) this is explicitly stated; e.g. line 263 has been changed to “*OC-Fe_R has been observed at contents exceeding 40% (of total OC) in terrestrial environments*”.

In table 2, which describes the content of spiked samples, we have added “wt%” to describe the OC-Fe content of the sample. Table 2 also includes the actual composition of the sediment in mg to make it as clear as possible.

The caption to figure 3 has been updated to be more descriptive so they can be understood without reference to table 2.

Figure 3: “The sample used in this experiment was a spiked sediment comprised of 60 wt% carrier sediment and 40 wt% of a 2 COOH OC-Fe_R coprecipitate.”

Figures 1 and 2 already use “wt% of OC-Fe_R in sediment” to describe the varying OC-Fe_R contents.

The CBD/CDB interchangeability has been picked up on and corrected.

- Description of Fig. 1 results. The phrase "maximal extraction" is used repeatedly when describing the results, but it is only explained in the Discussion (Line 266). The best place for this description is actually Methods, because you can already state how you intend to use the data to estimate this value. That will make reading the paper a whole lot easier overall.

The definition for maximal extraction has been moved to the end of section 2.6 in the methods. We have rephrased line 266 to retain the example of maximal extraction and ensure this is not lost.

Specific - Line 67: maybe qualify with 'partial hydrolysis' or similar. For significant digestion of OC from sediments, either very low pH (and use of specific oxidizing acids) or very high pH are required.

Added "partial" to line 67 and subsequent occurrences.

-Line 89: misplaced comma after "suggesting"

Removed in review 1.

-Line 111: "and" should be "a"

Corrected

-Line 120: "varied" in preference to "differed"

Changed.

-Line 192-193: rephrase to "with those samples containing the least Fe showing the greatest proportional/relative extraction of Fe"

Rephrased from the first review to "*with the highest recovery of Fe in samples with low OC-Fe_R contents.*"

-Line 203-205: this looks more like part of a caption for Fig 1

Changed the phrasing of this section to refer to Fig.1.

-Line 244-246: Does this mean that the natural sediment samples in these experiments were freeze-thawed before the experiments? If so it will be important to state this in Section 2.2.

This refers to the sediment samples which we performed the extractions on rather than the carrier sediment that was spiked with the coprecipitate. Section 2.5 is the corresponding method section for this and includes the line "*half of which was freeze thawed and half was freeze dried*"

-Line 251: why give the formula? there are many Fe oxides that can be dissolved in dithionite so I suggest just to leave it out

Removed.

Line 259-263: Not clear how XAS can indicate clustering. If you are referring to locally enhanced concentrations ("hotspots"), yes this is a real phenomenon observed by high resolution mapping techniques. Still, I would be surprised if a homogenized sample of 0.25g would have a distinctly different OC-FeR content from the bulk sediment, so the logic of the statement is not clear and the paragraph does not really benefit from it.

Removed this reference, instead replaced with “*spatial and temporal variation in Fe fluxes to the seafloor can result in Fe rich sediments e.g. near hydrothermal vents (Poulton and Canfield, 2006) or in Fe-Mn nodules (Hein et al., 1997)*”

Line 293-294: This is a confusing opening sentence to the paragraph. Rephrase to make more concise.

Rephrased to: “OC-Fe_R was incompletely recovered for samples treated with a dithionite content equal to that which elicits maximal Fe extraction (Fig. 1).”

Line 304-305: It is not clear to me how an increase of DOC (citrate) during the extraction would impact on the quantification of OC after the extraction., if this is done on the solid phase. Can the sample not simply be rinsed before the drying and analysis?

A triplicate rinse stage is conducted as citrate is known to be retained in the solid matrix, however, in the Lalonde et al. (2012) study this was not a significant problem (<0.08% of dry weight was citrate). This problem is heightened in synthetic studies such as ours, as the OC/Fe ratio is much lower than for natural samples. Therefore, the ferrihydrite surface has much more space available for sorption of organics such as citrate. Further, citrate has 3 carboxyl groups and we previously showed that 3 COOH containing organics were very difficult to remove from ferrihydrite with the CBD method in Fisher et al., (2020). This would suggest that should citrate become bound, it is unlikely to be removed by the rinse.

To clarify this point we have added a sentence to the section highlighting this is of most concern for synthetic samples. *“This is particularly acute for the reduction of synthetically precipitated samples where low OC/Fe ratios leave more of the mineral surface available for sorption compared to Fe phases associated with natural organic matter.”*

Line 326-328: Clauses of the sentence are not well constructed

Restructured into two sentences.