## Response to Reviewers (Round 2)

Reviewer comments in black, author response in blue.

We thank both new reviewers for their positive and helpful comments on our manuscript. Since Jeffery Cornwell had no further specific changes, the below responses relate to the comments raised in Bo Thamdrup's review.

The manuscript presents a methodological study of a widely used wet chemical extraction method for determining ”reactive iron” and associated organics (and phosphate) in soils and sediments – the dithionite-citrate-bicarbonate (CBD) extraction. It demonstrates how the selectivity of the method might deviate strongly from its intended target by leaving relatively large proportions of the reactive iron pool untouched and how this is affected by associated organics, and it provides guidelines for how to use the extraction to minimize these issues.

Wet chemical extractions for Fe, organics and P are a jungle with the exact protocols varying between labs, and calibrations with standard materials frequently being neglected. In this perspective, the present study has merit, although it also fails to some extent with respect to standards by only testing a homemade iron mineral. While this suffices to demonstrate the issues with the method, and OC-Fe materials might not be available, tests with some iron phases would have been useful.

The experiments have been carried out with care, the results are of good quality, and the manuscript is generally well written.

We thank the reviewer for their comments. As suggested, OC-Fe standard materials do not exist to the best of our knowledge. Synthetic ferrihydrite was used for this study because it is well established as a suitable sample material for both comparison of Fe extractions (e.g., Thompson et al., 2019, Poulton and Canfield, 2005) and OC-Fe interactions, as seen in the Eusterhues studies cited within this manuscript.

My greatest concern with the paper relates to a mixing up of Fe and organic carbon (OC) extractability. It starts with the title, which suggests that the subject is the extractability or Fe-bound OC, but really 90% of the results are about the extractability of Fe. It might be that the authors equate Fe and OC extractability, but then this needs to be discussed and justified.

The CBD extraction we use operates at circumnetural pH to prevent hydrolysis of OC. Well established, efficient methods already exist for the extraction of the target Fe phases, e.g., the pH 4.8 acetic acid buffered dithionite extraction of Poulton and Canfield (2005). Therefore, the extraction we employ is exclusively used for the extraction of iron bound organic carbon, hence the title. Reframing the title to suggest we are investigating dithionite based Fe extraction risks causing confusion since nobody uses this method solely for Fe extraction.

As the reviewer suggests, Fe and OC extractability are strongly linked, since the reductive dissolution of the Fe phases liberates associated OC. We have added a new sentence and reference to clarify the relative release of OC and Fe during the extraction. This extraction in particular results in a correlated release of OC and Fe because it targets the highly reactive Fe fraction to which OC associates. This is because overtime Fe phases become more crystalline and reactivity and surface sorption capacity decreases (Lalonde et al., 2012).

**On line 42-43** we now emphasise this by stating *"The CBD extraction for OC-Fe$_R$ operates on the principle that reductive dissolution of reactive Fe phases with sodium (Na) dithionite exclusively and quantitatively liberates Fe$_R$-bound OC from the sediment matrix. This extraction can be considered to target OC-Fe$_R$ since the vast majority of iron bound OC is associated with the highly reactive (Fe$_R$) fraction, dissolved by CBD, since more crystalline Fe phases have both reduced surface reactivity (Lalonde et al., 2012) and smaller specific surface area (Jelavić, Mitchell, Sand, 2020) for OC sorption. The reductive release of OC from an OC-Fe$_R$ complex has been shown to occur asynchronously and OC is mobilised to the dissolved phase at a greater rate than Fe (Adhikari et al., 2016)."*

Indeed, Fig. 1 demonstrates that these two parameters do not go hand in hand. In the introduction, l. 39-68, I recommend a clearer separation of studies focusing at Fe, OC, and P extraction with DCB, and I would like to see some discussion of the expected congruency between the extraction of these substances. Fe extraction would seem the natural starting point in this introduction, with OC-Fe and P-Fe extraction being later adaptations. Now it seems the other way around.

We have split the section into two paragraphs to create a greater distinction between historical use of CBD methods (Fe, OC and P) and the recent findings which drive our study. We have additionally changed the format of the start of the first paragraph to begin with Fe before moving on to OC and P as suggested. This paragraph now ends with a comparison between the Fe-P and OC-Fe methods, as we note it is difficult to compare the relative efficiency of these methods because of the variation in the reductive strength. **Line 51**: *"While Ruttenberg (1992) and Thompson et al. (2019) report 90-100% of synthetic ferrihydrite is extracted by the CBD method for Fe$_P$, the dithionite-to-sample ratio in their studies was more than double the ratio used in the OC-Fe$_R$ extraction by Lalonde et al. (2012)"*. We further compare the Fe-P study of Thompson et al., (2019) to an OC-Fe study of Adhikari and Yang (2015) and Fisher et al., (2020) in the new subsequent paragraph.

For instance, I am relatively sure (though I couldn't find my copy and check this) that Mehra and Jackson did NOT use CBD for OC-bound to reactive iron (as claimed in l. 39-40) but for the reactive iron itself.

Corrected:

**Line 40-42**: *"The method was originally applied to the extraction of iron oxides from soils (Deb, 1950; Mehra and Jackson, 1958) before being adapted for OC-Fe$_R$ quantification in marine sediments by Lalonde et al. (2012)"*

Specific comments

24: Please leave it to the reader to decide on the value of your work.

Removed "valuable"

61: Did they really say that CBD will REDUCE the Fe-associated organic C?

Changed to "dissolve".

64: Most of the tests concern Fe extraction, not OC-Fe

Changed to "$Fe_R$ associated with OC"

86: Same as above, most tests concern Fe extraction, not OC-Fe

**Line 90**: Expanded for clarity: "To address the question of how methodological variation affects OC-$Fe_R$ extraction, **due to variable dissolution of the associated $Fe_R$ phase"**

Methods, sect. 2.1 and 2.2: Are the procedures here not same as in Fisher et al. 2020? Or how do the procedures in l. 117-26 and 128-37 differ from the previous paper? If procedures for synthesis differ, how was the structure verified? If they don't, please make this clear and shorten the methods here.

The original version of this manuscript had a shorter methods section. In our first round of review we were asked by multiple reviewers to expand on the methods so readers did not need to consult Fisher et al., (2020) to understand what we did. In addition we were also asked to justify the use of 2-line ferrihydrite which added a new paragraph to section 2.1. The current methods section is as short as we can make it while retaining enough detail to reproduce the experiment. We have decided on balance it is better to retain this expanded methods section in line with the previous reviewers comments.

Results: Please use past tense for your results as customary.

Corrected.

Section 3.1: The first halves of each of the two paragraphs repeat results and should be strongly abbreviated.

This comment refers to repeating results, however we believe it likely refers to repeating methods (since the paragraph starts with an overview of what was done rather than results). We have shortened the overview in the first paragraph down to one sentence.

244: "incomplete Fe extractions" is difficult to understand, please rephrase.

Rephrased: "where the chosen method resulted in <100% extraction of the targeted phases."

248: 40% and 50% of total OC are confusing coming and not comparable to 7 wt% Fe in the previous sentence. These are not useful values here without information about the total OC content. If the systems were OC poor, the OC-Fe content could be low.

We have added two sentences to clarify the purpose of these values and put them into a better context within the paragraph. Line 248: *"Our high Fe content is driven by low C/Fe ratios since only short chain organic compounds are associated with the $Fe_R$ phases, and are therefore designed to be a mechanistic model rather than to simulate the types of compounds which occur naturally. Both the amount of reactive Fe and the amount of OC associated with Fe are highly variable, and the factors which control the OC-$Fe_R$ interaction remain poorly understood."*

**Line 260:** *"This large variability in environments containing OC-Fe$_R$, and the composition of such compounds, highlights the need to examine their extractability across a wide matrix."*

257-279: I suggest merging and condensing these two paragraphs. The discussion of 30-40% OC-Fe first and then 50% OC-Fe seems too repetitive and does not convey the big picture very well.

We have considered this suggestion but do not think it would improve the readability of the manuscript. The trends shown in 30-40% OC-Fe are very similar whereas 50% OC-Fe shows a different trajectory (Fig 1). It is important to consider these separately because there are specific factors which may be responsible for the limitation in OC-Fe and Fe extraction observed at 50%, as was raised in detail by Susann Henkel in the first round of review. We state this difference on **line 274** *"This differs from the previous compositions in reaching a maximum at ~60% Fe, as opposed to the ~90% achieved for 20-40 wt% OC-Fe$_R$."*. We appreciate the desire to present the big picture but feel this is best left to the conclusion while the more technical points of this technical note are presented in the discussion.

Fig. 1: The curves in the figure are not explained and look like parabolas with decreasing values at the highest dithionite additions. I recommend use of hyperbolas or other asymptotic functions instead to reflect the idea that the extraction saturates.

We have changed the curve fit for this figure to reflect a levelling off, reflecting saturation, as oppose to a decrease.

In addition we have changed all figures to remove error bars since these related to a systematic instrumental error yet gave the impression of independent repeats which could not be performed for reasons given in the manuscript. Figure captions have been adapted accordingly and instrument error is now provided in the open access data asset.