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41 method, we aimed to understand whether methodological errors or
42 (mis)interpretation of these extraction results may contribute to the
43 apparent limitation on OC-Fe_R values. Here, we synthesised OC-Fe_R
44 composites with a known Fe_R phase and known OM moieties, varying in
45 carboxyl content, at neutral pH. These were spiked into OC-free marine
46 sediment, and subject to a CBD extraction to investigate i) the efficiency of
47 CBD for OC extraction; ii) the efficiency of CBD for Fe_R extraction; ii) how
48 the OC moiety affects the physical parameters of associated Fe_R minerals;
49 and iii) the impact of OM moiety on OC and Fe release. We show that the
50 CBD method results in only partial dissolution of the most susceptible Fe_R
51 phase (ferrihydrite) and therefore incomplete removal of bound OC. While
52 as little as ~20% of Fe is released from OC-free ferrihydrite, structural
53 disorder of the mineral phase increases with the inclusion of more OC,
54 resulting in greater losses of up to 62% Fe for carboxyl rich OC-Fe_R
55 complexes. In addition, our results show that the NaCl control step
56 performed in the CBD method is capable of removing weakly bound OC
57 from Fe_R, such that inclusion of this OC in the total OC-Fe_R fraction may
58 increase marine sediments OC-Fe_R estimates by ~33%. Finally, we
59 suggest that the structure of OC involved in OC-Fe_R binding can affect
60 quantification of the OC-Fe_R pool. Our results have important implications
61 for assessing the Fe_R bound OC fraction in marine sediments and the fate
62 of this OC in the global carbon cycle.

63

64 **1. Introduction**

65 Marine sediments represent the largest organic carbon (OC) sink on Earth
66 (Hedges and Keil, 1995) and therefore play a crucial role in the regulation of
67 atmospheric oxygen (O₂) and carbon dioxide (CO₂) over geological time
68 (Berner, 1989). Despite the importance of OC burial however (Burdige, 2007;
69 Canfield, 1994), the factors that control OC burial and preservation in marine
70 sediments are unclear (Arndt et al., 2013). One important mechanism for OM
71 preservation appears to involve the association of OC with the reactive iron
72 fraction in soils and sediments (Fe_R, operationally defined as the fraction of
73 total iron that is extractable with sodium dithionite), with up to 40% of total
74 soil OC (Wagai and Mayer, 2007) and 21.5±8.6% of total marine sediment

75 OC (Lalonde et al., 2012) bound to Fe_R. This Fe_R “rusty sink” (Lalonde et al.,
76 2012) is assumed to protect OM from microbial degradation (Jones and
77 Edwards, 1998), and help preserve OM in the Earth system (Lalonde et al.,
78 2012).

79
80 The mechanisms responsible for OC-Fe_R associations are poorly
81 understood, but might include chemical sorption, physical occlusion or a
82 combination of both (Kaiser and Guggenberger, 2000; Lalonde et al., 2012;
83 Mu et al., 2016; Wagai and Mayer, 2007). For chemical sorption to protect
84 OC from microbial remineralisation over extended timescales, the
85 mechanism of OC sorption with Fe_R must involve strong chemical bonds and
86 a very high sorption coefficient (Henrichs, 1995). Work has demonstrated
87 this using a ramped pyrolysis or oxidation method to observe the
88 temperature decay profile of OC as a proxy for bond strength (Hemingway
89 et al., 2017). Strong chemical bonds between OC and Fe_R are thought to be
90 mediated via OM carboxyl (COOH) and mineral hydroxyl (OH) groups
91 (Karlsson and Persson, 2010; Karlsson and Persson, 2012; Mikutta, 2011).
92 The importance of carboxyl group bonding has been demonstrated by
93 Fourier Transform Infrared Spectroscopy (FTIR) of ferrihydrite precipitated
94 with dissolved organic matter (DOM) (Yang et al., 2012). Carboxyl groups
95 are prevalent in marine DOM, as evidenced by carboxyl rich alicyclic
96 molecules (CRAM) comprising a major component of refractory marine DOM
97 (Hertkorn et al., 2006). Carboxyl groups are thought to facilitate binding of
98 OM to Fe_R mineral surfaces via ligand exchange, whereby OM carboxyl
99 groups replace hydroxyl groups on the mineral surface (Chen et al., 2014;
100 Gu et al., 1994; Gu et al., 1995; Henneberry et al., 2012; Wagai and Mayer,
101 2007).

102
103 The citrate-dithionite-bicarbonate (CBD) method for evaluating the amount
104 of OC-Fe_R in a sediment sample involves reduction of the present Fe_R
105 phases, after which the reduced Fe then becomes dissolved in solution
106 liberating bound OC. Despite widespread utilisation of the CBD method,
107 previous studies have indicated that its efficiency may be less than originally
108 described, whereby it was thought to dissolve all solid reactive iron phases.

109 For example, Adhikari and Yang (2015) performed the CBD protocol of
110 Lalonde et al. (2012) on synthetic hematite-humic acid complexes and
111 reported incomplete reduction ($\leq 50\%$) of Fe_R . Further, the CBD method has
112 been criticised in the context of Fe speciation for its inability to fully dissolve
113 crystalline Fe_R minerals in ancient rocks and potentially modern marine
114 sediments (Raiswell et al., 1994). Thompson et al. (2019) showed that a CBD
115 extraction performed at circumneutral pH fails to extract a large proportion of
116 Fe_R minerals in modern sediments. While these studies have indicated that
117 CBD treatment may be less efficient than currently understood, it is unknown
118 as to why some OC- Fe_R complexes appear to be unextractable. By
119 evaluating the efficiency of the CBD method for OC- Fe_R extraction we aim to
120 understand the true extent to which OC- Fe_R can be extracted, and whether
121 the structural composition of the associated organic compound influences
122 the ability of CBD to successfully extract a particular OC- Fe_R complex. This
123 may additionally further our understanding as to why only a minority of the
124 sedimentary OC pool is found to be associated with Fe_R despite the fact that
125 OC- Fe_R interactions are found to promote OC persistence. For example, the
126 maximal OC- Fe_R content obtained by Lalonde et al. (2012) across their 42
127 global sediment samples was 41.69% (Wash. Coast 215), with a mean of
128 $21.5 \pm 8.6\%$. Other studies employing the same CBD method also concur with
129 this range (e.g., Zhao et al. (2018) ($15.6 \pm 6.5\%$), Salvadó et al. (2015)
130 ($11 \pm 5.5\%$). This poses a question as to why there is limited variability in the
131 OC- Fe_R values obtained for sediments under differing environmental
132 conditions, particularly where OC and Fe fluxes to the seafloor vary. To fully
133 understand the fate of OM in marine sediments it is important to determine
134 whether this apparent limitation in OC- Fe_R presence is a matter of
135 methodological error/interpretation in the quantification of OC- Fe_R (by CBD),
136 or an inherent feature of the OC- Fe_R interaction that requires further
137 investigation.

138

139 To address the uncertainty in CBD extraction efficiency we coprecipitated
140 model carboxylic acid compounds, containing either 1, 2 or 3 carboxyl
141 groups, with ferrihydrite, which is ubiquitous in marine sediments and acts
142 as a precursor for more stable Fe oxide phases (e.g., hematite) (Michel et

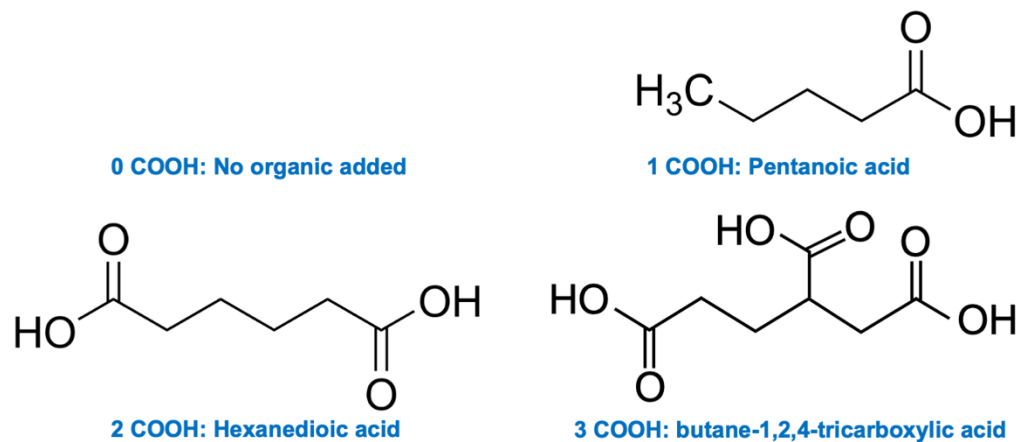
143 al., 2007). We then spiked OC-free natural sediment with our carboxylic acid-
144 ferrihydrite coprecipitates and subject them to a CBD extraction. We
145 investigate the efficiency of the CBD method for OC and Fe_R extraction and
146 discuss the implications for evaluating OC-Fe_R and OM preservation with
147 Fe_R in the marine system. We also comment on how the carboxyl character
148 of the OC might have a significant influence on OC preservation in marine
149 sediments.

150

151 2. Materials and Methods

152 2.1 Ferrihydrite coprecipitate synthesis

153 Following a modified method of Schwertmann and Cornell (2000), 2-line
154 ferrihydrite was precipitated in the presence of either i) pentanoic acid
155 (C₅H₁₀O₂); ii) hexanedioic acid (C₆H₁₀O₄) or iii) butane-1,2,4-tricarboxylic
156 acid acid (C₇H₁₀O₆), which increase in their number of carboxyl groups
157 from 1 to 3, respectively. A pure ferrihydrite sample containing no organic
158 carbon was also precipitated as a control.



159 **Figure 1: Chemical structures of the organic acids coprecipitated**
160 **with ferrihydrite.** The chemical structures of each carboxylic acid used
161 in synthesising ferrihydrite coprecipitates are shown above their carboxyl
162 content abbreviation (1-COOH, 2-COOH etc.) and IUPAC name. 0 COOH
163 shows no structure as the 0 COOH coprecipitate contains no organic and
164 instead represents a 'pure' form of ferrihydrite.

165

166 Saturation concentrations for the three organic acids were determined by
167 increasing organic acid concentration in the mineral coprecipitation stage
168 and measuring end state wt %C (see **Table 1**). Saturation concentration
169 refers to the point at which additional increases in the amount of organic

170 acid added did not result in a further wt% C increase in the resultant
171 mineral coprecipitate. Coprecipitation of ferrihydrite with the named
172 organic acids was performed by dissolving organic acids in deionised
173 water (DI) with Fe (III) nitrate nonahydrate [Fe(NO₃)₃.9H₂O] (20 g/250 ml).
174 1 M potassium hydroxide (KOH) was then added by titration to achieve a
175 pH of 7.0 ± 0.3.

176

177 Following coprecipitation, all of the organomineral composites were rinsed
178 in 5L of DI water and left to gravitationally settle. This rinse step was
179 repeated 5 times. The pentanoic acid (1-COOH) coprecipitate was rinsed
180 three times due to slow settling of the lighter 1-COOH particles and
181 therefore a lower yield. The pH of the resultant slurry was raised to pH 7
182 through dropwise addition of 0.1 M NaOH, then centrifuged (2750 g, 20
183 minutes) with the precipitate being retained and the supernatant
184 discarded. Following this, the precipitate was frozen and freeze-dried. X-
185 ray diffraction (XRD) was used to confirm the Fe mineralogy of the
186 coprecipitate as 2-line ferrihydrite.

187 **Table 1: Organic acids used to form ferrihydrite coprecipitates.**

188

IUPAC Name	COOH Groups	Stoichiometry	Amount added ^a
Pentanoic acid	1	C ₅ H ₁₀ O ₂	5 ml
Hexanedioic acid	2	C ₆ H ₁₀ O ₄	3 g
butane-1,2,4-tricarboxylic acid	3	C ₇ H ₁₀ O ₆	3 g

189 ^a values of organic acid addition are relative to 20 g of Fe(NO₃)₃.9H₂O

190

191 **2.2 Sediment spiking**

192 Ferrihydrite-organic coprecipitates, representing iron bound organic
193 carbon (OC-Fe_R) were spiked into a OC-free natural sediment sample
194 from the Barents Sea (Water Depth 141 m; sediment core depth, 33.5 cm;
195 station B6, E40; Cruise JR16006) which acted as a 'carrier' for the OC-
196 Fe_R compound and replicated the physical properties of natural fine-
197 grained (silty clay) sediments. The sediment sample was freeze-dried and
198 homogenised by grinding using a pestle and mortar, followed by ashing
199 (650°C, 12 hours) to oxidatively remove all OC. While ashing affects
200 various natural sediment properties (e.g. dehydration/aggregation of

minerals), it is required as a preparatory step in our experiment to remove all natural OC from the sediment. However, the ashed sediment is used simply as a matrix for our OC-Fe_R additions, to better mimic the physical properties of a natural sediment than an artificial mixture of various marine sediment minerals. Inorganic carbon was removed by fumigation with HCl vapour, resulting in a sediment sample dominated by siliciclastic minerals. Spiked samples were prepared by mixing carbon free sediment with ferrihydrite coprecipitates at different ratios (see matrix in **Table 2**) in a centrifuge tube and agitating for 10 minutes at room temperature in light conditions.

Table 2: Concentration matrix for spiked sediments.

%OC-Fe: Sediment	0	10	20	30	40	50
OC-Fe (mg)	0	25	50	75	100	125
Sediment (mg)	250	225	200	175	150	125

Values shown are contextualised for a total experimental sample mass of 250 mg (Lalonde et al., 2012).

2.3 Extraction of iron bound carbon.

Extraction of dithionite reducible Fe_R and associated OC was conducted according to the citrate-dithionite-bicarbonate (CBD) method (Lalonde et al., 2012; Salvadó et al., 2015). Briefly, 0.25 g of spiked sediment was weighed into centrifuge tubes. Following this, 13 ml of 0.11 M sodium bicarbonate (NaHCO₃) and 0.27 M trisodium citrate (Na₃C₆H₅O₇) solution were added and the mixture pre-heated to 80°C in a water bath. Sodium dithionite (0.25 g) was then dissolved in 2 ml of 0.11 M NaHCO₃ and 0.27 M Na₃C₆H₅O₇ solution, added, and the mixture was agitated and heated again (80°C, 15 minutes). In parallel, a control extraction was conducted replacing sodium dithionite and trisodium citrate with sodium chloride at an equivalent ionic strength; 13 ml of 1.6 M NaCl and 0.11 M NaHCO₃, followed by 0.22 g NaCl dissolved in 2 ml of the NaCl and NaHCO₃ solution. All samples were centrifuged (10 minutes) and the supernatant was retained. This was followed by three rinses of the precipitate in artificial seawater with 15 ml of each of these three supernatants retained

232 and combined with one another. All supernatants were acidified to pH <2
233 with 12 N HCl to prevent Fe precipitation. Precipitates were oven dried at
234 60°C for 12 hours and decarbonated by acid fumigation to remove
235 inorganic carbon. Sample mass was recorded prior to elemental analysis
236 to correct for any mass loss.

237 **2.5 Elemental analysis - Iron**

238 Iron concentrations for samples following CBD extraction were
239 determined by atomic absorption spectroscopy (Thermo Fisher iCE3300
240 AAS). Calibration was performed using matrix matched standards and
241 quality control was confirmed following every 10 samples by repeat
242 sampling of calibration standards to check for drift. Total sedimentary Fe
243 content was determined by digesting ~2 mg of the spiked sediment mixes
244 in 1 ml 12N HCl at room temperature followed by an initial 10-fold dilution
245 with 1% HCl solution. Subsequent dilutions were made, dependent on Fe
246 content, using MilliQ water to produce a subsample within the detectable
247 window (1-10 ppm Fe). Control supernatants were diluted 20-fold to
248 prevent salt blockages and washed supernatants were undiluted except
249 for where the Fe concentration was >10 ppm, whereby these were diluted
250 10-fold. Extraction of Fe (ΔFe) was calculated as total Fe – CBD
251 extractable Fe, Fe liberated in the NaCl wash stage was subtracted from
252 the CBD extractable total however the NaCl values were negligibly small.
253 Error was determined by propagating the standard error of the mean
254 (SEM) calculated from duplicate results for the total Fe and CBD
255 extractable Fe pools.

256 **2.6 Elemental analysis – Carbon**

257 Initial carbon content of synthetic samples was measured using a LECO-
258 SC144DR C&S analyser. Post extraction carbon was determined by
259 LECO analysis for the majority of sediment samples (OC-Fe
260 concentrations \leq 50%) or by a Vario PYRO cube (Elementar Analysis)
261 where post extraction sample mass was below the limits required for
262 LECO analysis (OC-Fe concentration >50%). Both instruments were
263 corrected for drift by running a known standard (LECO 502-694 and
264 Elementar sulfanilamide) following every 10-15 samples throughout the

265 analytical run. All carbon samples were analysed in a dried (oven or
266 freeze-dried) state following the removal of IC as described above.
267 Carbon loss was calculated according to supplementary equation 1,
268 adapted from Peter and Sobek (2018) and Salvadó et al. (2015).
269 Instrument error for C measurement is minimal ($\leq 1\%$ RSD) and in all
270 cases was less than the SEM calculated for independent repeat
271 measurements.

272 **2.7 X-Ray diffraction analysis**

273 Ferrihydrite-organic composites were structurally characterised for
274 crystallinity by powder x-ray diffraction (XRD). The pattern was collected
275 over a $2-90^\circ$ 2θ range in 0.01969° intervals with a step time of 930 ms
276 using a Bruker D8 diffractometer with Cu-K α radiation source ($\lambda \approx 0.154$
277 nm). Comparative analysis between the different coprecipitates was
278 achieved by normalising for relative intensity.

279 **2.8 Particle sizing**

280 Sizing of particle aggregates within the range of 0.1-10000 μm was
281 conducted via laser diffraction using a Malvern Mastersizer 2000. Wet
282 ferrihydrite coprecipitates were added dropwise to water in the suspension
283 mixer until within the detection limit of the machine. Detection of the
284 particle scattering pattern was conducted in triplicate by the detector array
285 and interpreted using the Malvern Mastersizer 2000 software (v6.01).

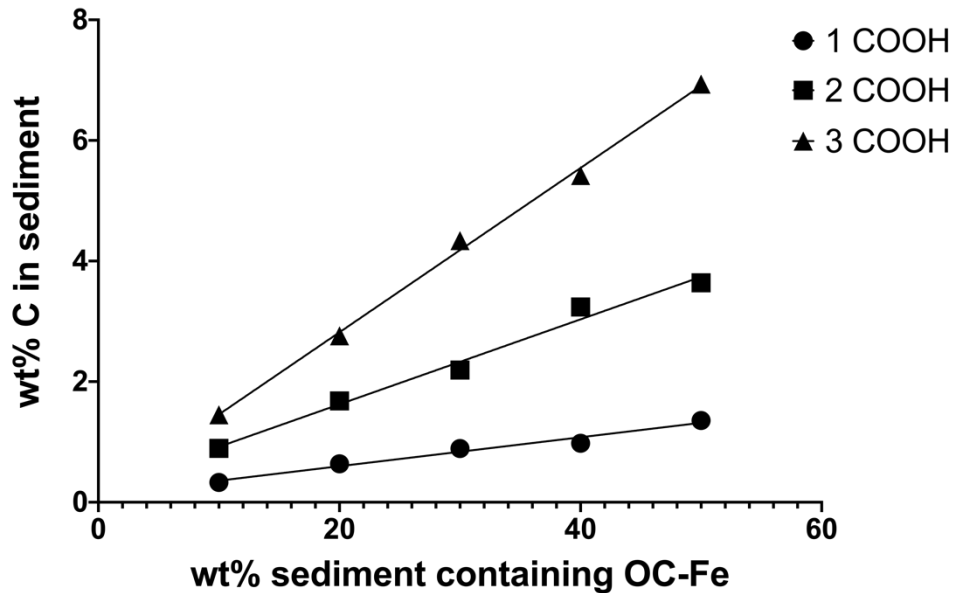
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287 **3. Results**

288 **3.1 OC content calibration of spiked sediments**

289 Coprecipitation of the three organic acids with ferrihydrite produced
290 coprecipitates with an increasing wt% C content, 1-COOH = 1.15%, 2-
291 COOH = 4.20%, 3-COOH = 8.51%. The molar C/Fe ratio similarly
292 increased (1-COOH = 0.2, 2-COOH = 0.5, 3-COOH = 1). These
293 coprecipitates were then spiked into the treated sediment across a
294 concentration gradient (as detailed in **Table 2**), producing a range of
295 composite samples variable in their C content (**Fig. 2**). The 15 unique
296 sample compositions represent a range of C concentrations from 0.33%
297 (10%, 1-COOH) to 6.94% (50%, 3-COOH). The C content of these

298 composites varied by both the number of C molecules in the three organic
299 acids and the amount of OC-Fe_R spiked into the sample relative to
300 sediment (see **Table 2**).

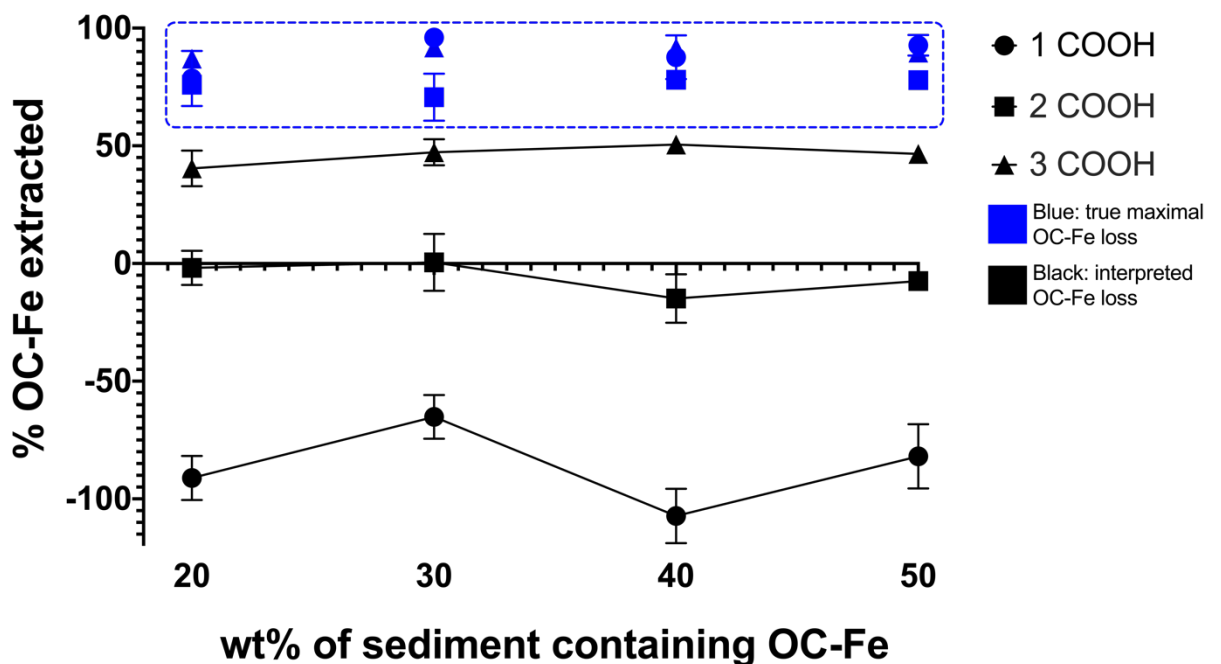


301 **Figure 2 - Calibration of sediment coprecipitate content and sediment wt% C.**
302 Coprecipitates show a tight linear regression trend across the series with R-squared
303 values of: 1-COOH = 0.97; 2-COOH = 0.98; 3-COOH = 0.99. 1/2/3 COOH refers to
304 the number of carboxyl groups per molecule in the three different organic acids
305 coprecipitated with ferrihydrite. %C refers to the concentration of carbon in the
306 overall sediment composite by wt%. Instrument error is too small ($\leq 1\%$ RSD) to be
307 visualised.
308

309 **3.2 Extraction of iron bound organic carbon.**

310 The Fe_R bound OC fraction (%OC-Fe_R) lost from the composite material
311 during the extraction was determined for each of the carboxyl
312 coprecipitate containing samples at concentrations of 20-50% OC-Fe,
313 since C loss for coprecipitates at 10% OC-Fe was too small to be
314 accurately determined (**Fig. 3**). The wt% C values displayed in black
315 indicate those which have been processed through **Supplementary**
316 **equation 1**, a minor adaptation to the common equation used to remove
317 OC that is extractable with NaCl and believed to be unbound to Fe_R
318 (Barber et al., 2017; Lalonde et al., 2012; Peter and Sobek, 2018; Salvadó
319 et al., 2015). In blue, the maximal 'true' wt% C extracted are shown, these
320 refer to data which has only been transformed for mass loss, i.e. the
321 control and reduction extractions were not subtracted from one another.
322 For the 1- and 2-COOH samples the value shown in blue represents OC

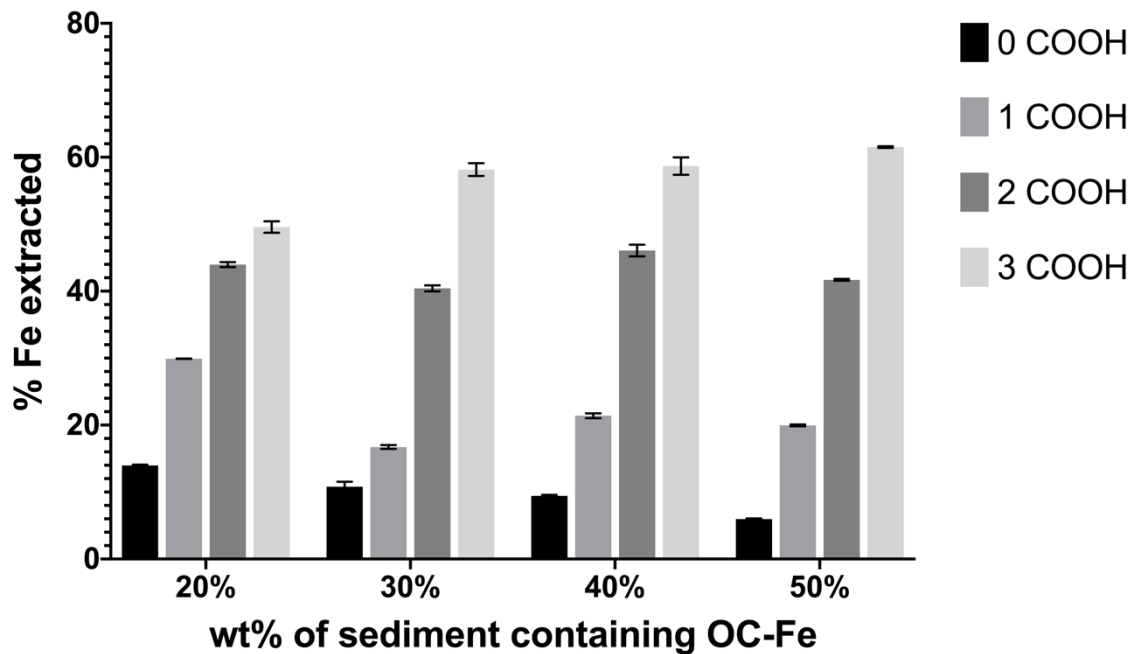
323 loss from just the NaCl extraction, as this value was greater than OC loss
 324 by dithionite reduction, while for the 3-COOH sample this shows OC loss
 325 from reduction as this was higher; all values can be found in
 326 **Supplementary table 3**. Consideration of why some samples
 327 experienced greater OC loss from treatment with NaCl than by dithionite
 328 reduction is explored in 4.1. Overall, these two sets of values can be
 329 grouped separately; the “true” extraction values are similar in nature
 330 (shown in the blue box), while the interpreted data (shown in black)
 331 become spread after inclusion of both the NaCl control and dithionite
 332 reduction values. For the interpreted values ~46% of the total OC-Fe was
 333 extracted from the 3-COOH coprecipitate, but ‘negative’ extractions of ~ -
 334 6% and ~ -90% were achieved for the 1- and 2-COOH coprecipitates,
 335 respectively. Negative extraction values can be explained as being an
 336 artificial feature of subtracting a greater OC loss in the control experiment
 337 from a lesser OC loss in the reduction. The mean difference between all
 338 three carboxyl coprecipitates was found to be significantly different for all
 339 pairwise combinations via a one-way ANOVA and Tukey’s test post hoc
 340 (99% significance level) (**Supplementary Table S1**). Precise values for
 341 all extractions including duplicate errors are detailed in **Supplementary**
 342 **Table S3**.



343 **Figure 3- Dithionite extractable fraction of OC from carboxyl coprecipitate**
344 **spiked sediments.** %OC-Fe extracted from artificial sediments spiked with 1,2 or 3
345 carboxyl organic acids. Error bars show standard error of the mean (SEM) of
346 duplicate values, where error bars are not shown this is because the error is too
347 small to be visualised on this scale. %OC-Fe extracted refers to wt% C removed by
348 reductive dissolution of the reactive iron phase for iron bound carbon. Key
349 descriptors, 1/2/3 COOH, represent the variation in the number of carboxyl groups
350 of the 3 different organic acids coprecipitated with ferrihydrite.
351

352 **3.3 Iron extraction**

353 Extractable Fe shows an increasing stepwise trend with the number of
354 carboxyl groups per coprecipitated acid molecule for all concentrations of
355 OC-Fe_R relative to sediment (**Fig. 4**). All extractions for easily reducible
356 Fe were incomplete (< 100% of added Fe) with a maximum yield of 61.6%
357 achieved for the 3-COOH coprecipitate at 50% OC-Fe, with a mean range
358 of 50.5-61.6% across all concentrations. In decreasing order, the 2-COOH
359 sediment yield was 40.0-45.7% Fe and 1-COOH sediment yield was 17.9-
360 29.9%. The OC free (0-COOH) sediment composite achieved the lowest
361 rate of Fe recoverability, between 6 and 14%. Fe removed by the NaCl
362 control treatment was subtracted from the Fe removed by CBD, however
363 the contribution of Fe removed by NaCl was minimal ($\leq 0.3\%$). Fe liberated
364 in wash stages for both CBD and NaCl treatment are included in the
365 overall %Fe extracted values. Relative to Fe liberated from the initial
366 chemical treatment, the wash removed a further 6.7-34.0% Fe for CBD
367 and 0.4 to 27.8% for NaCl. All sample means were calculated from the
368 average of independent duplicates. A significant difference was found
369 between the means of all coprecipitate pairwise combinations (>99%
370 significance) by one-way ANOVA analysis and Tukey's test post hoc. A
371 statistical summary is included in **Supplementary Table S2**



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Figure 4- Dithionite Figure 4- CBD extraction efficiency for reactive Fe in sediment samples differing in carboxyl richness. %Fe extracted from each carboxyl spiked sediment (0-3 COOH) is shown, values are control corrected. Error bars represent propagated error calculated from the duplicate values as detailed in 3.1.

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3.4 Coprecipitate grain size

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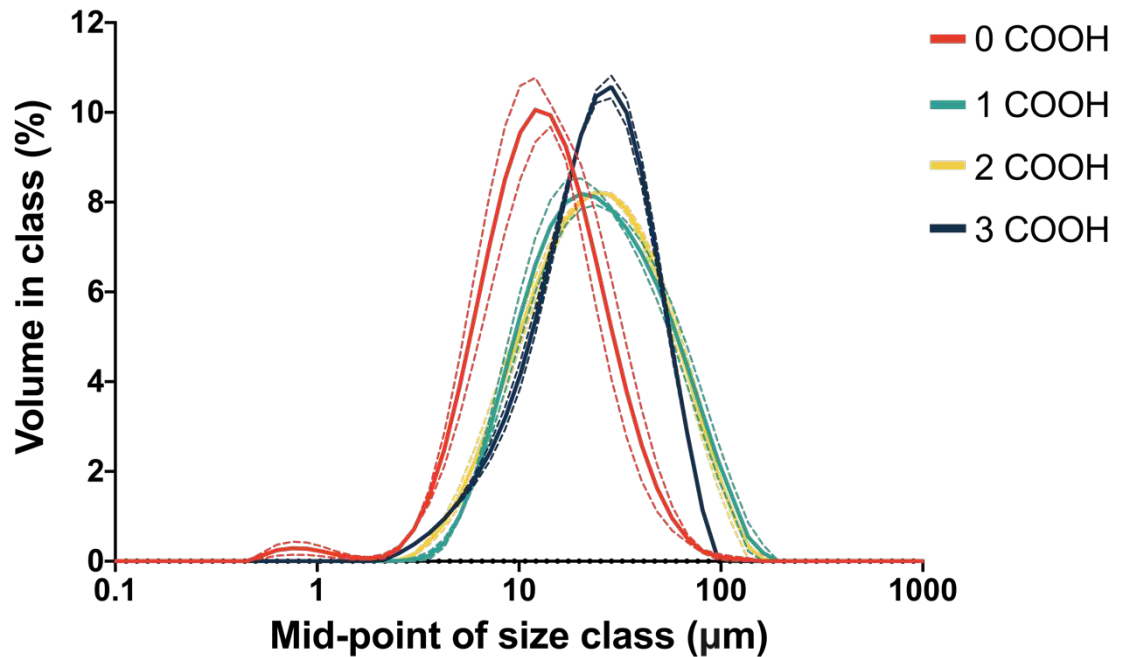
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Grain size of ferrihydrite composites was determined by laser diffraction analysis in the 0.01 to 10000 μm range. The 3-COOH coprecipitate showed a slight peak shift from 12.04 to 28.66 μm compared to the carboxyl free, pure ferrihydrite phase (0-COOH) (**Fig. 5**). One and two COOH coprecipitates showed no determinable difference in grain size distribution and sat between the 0- and 3-COOH sediments. The 0- and 3-COOH precipitates also had marginally taller, slimmer distributions indicating a narrower range of grain sizes and a greater proportion of the class existed at the mid-point compared to 1- and 2-COOH. These two samples have almost identical normal distribution curves and show no determinable difference in grain size or distribution. Due to the overlapping of all distributions, there is no statistically significant difference in the grain size of coprecipitates.



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Figure 5- Grain size distribution of carboxyl coprecipitates. Grain size distribution is shown as percent by volume in class. Values shown along the solid lines are the mean of triplicate measurements, dashed lines represent the SEM. A small number of data points in the range 0.01-0.1 μm and 1000-10000 μm are excluded from this graph to improve resolution, all these values were ~ 0 .

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3.5 X-ray diffraction analysis (XRD)

All samples show a characteristic two peak signature (**Supplementary Fig. S1**), indicating successful synthesis, and carboxyl free ferrihydrite resembles a characteristic two-line sample. As the number of carboxyl groups per coprecipitated acid molecule increases, a decrease in relative intensity and softening of the two peaks can be seen.

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4. Discussion

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Organic carbon bound to Fe_R represents an important mechanism by which OC is preserved in marine sediments with $\sim 22\%$ of total sediment OC bound to Fe_R (Lalonde et al., 2012). This “rusty sink” is important for regulating atmospheric O_2 and CO_2 (Torn et al., 1997) and understanding the fate of OM is crucial in accurately predicting the future of carbon cycling under global climate change (Adhikari and Yang, 2015). The CBD method, previously used to quantify OC- Fe_R in terrestrial environments, has been applied to marine sediments providing the first quantification of OC preserved in association with Fe_R on a global scale (Lalonde et al.,

417 2012). Here we applied the same CBD method to synthetic samples, in an
418 experimental investigation of OC-Fe_R, to investigate the performance of
419 the CBD method for OC and Fe extractability as a function of the
420 molecular characteristics of the associated OM. Previous studies have
421 typically produced organomineral coprecipitates using natural sources of
422 OM, e.g., forest floor extract (Chen et al., 2014; Eusterhues et al., 2014a;
423 Eusterhues et al., 2014b; Eusterhues et al., 2011). Whilst using naturally
424 sourced OM more closely replicates OC-Fe_R coprecipitates formed in
425 environmental settings, the speciation and structure of natural OM are
426 largely unknown and therefore the effect of OM molecular composition on
427 the efficiency of the CBD method cannot be easily evaluated. By using
428 short chain organic molecules, differing only in the number of carboxyl
429 groups per acid molecule, we explore how the CBD method is influenced
430 by the carboxylic character of the OM. We demonstrate important
431 inefficiencies in the CBD method through calibration against synthetic
432 standards which has the potential to transform our interpretation of CBD
433 extractions performed on natural sediments and thus our understanding
434 of marine sediment carbon preservation on a global scale (4.1, 4.2). We
435 also comment on how the carboxyl character of the OM might have a
436 significant influence on OM preservation (4.3).

437

438 **4.1 Current extraction methods may underestimate the extent of OC-** 439 **Fe_R interactions in soils and sediments**

440 Preservation of OM by Fe_R is thought to result from protection of OC from
441 microbial degradation, mediated through chemical bonding between OC
442 and Fe_R (Kaiser and Guggenberger, 2000). Protection is believed to
443 require high energy bonding between OC and Fe_R to maintain a
444 preservative effect (Henrichs, 1995). Weaker interactions (e.g., Van der
445 Waals forces) may exist but are assumed to be of insufficient strength to
446 facilitate OM preservation (Burdige, 2007). Treatment with CBD has been
447 described to dissolve “all solid reactive iron phases and the organic
448 carbon associated with these phases” in natural sediments (Lalonde et
449 al., 2012). Therefore, we would expect full liberation of Fe and C given
450 that the C content of our synthetic sample was equal to the amount of OC

451 associated with ferrihydrite, a solid reactive Fe phase. Across all
452 concentrations and C structures we observe incomplete recovery of Fe_R ,
453 not exceeding $50.51 \pm 1.32\%$ of the added Fe content (3-COOH, 40% OC-
454 Fe_R addition) (**Fig. 2**). As the amount of OC extracted is less than total
455 amount of OC added to the sediment samples this indicates either the
456 CBD method or the interpretation of CBD results contain a source of error.
457 However, the OC- Fe_R values obtained, in particular for the 3-COOH
458 containing sediment, are comparable to observations from many
459 environmental samples.

460

461 Where CBD has been applied to marine sediments the %OC- Fe_R (=
462 fraction of total OC bound to Fe_R) ranges between 0-42% on continental
463 shelves (e.g. Lalonde et al., 2012; Ma et al., 2018; Salvadó et al., 2015)
464 with typical averages for sediments under oxic bottom waters of $21.7 \pm$
465 7.8% (Lalonde et al., 2012). While the calculation for our 3-COOH
466 coprecipitate exceeds these ranges for %OC- Fe_R in marine sediments, it
467 is agreeable with terrestrial environments. For example, Zhao et al. (2016)
468 determined an %OC- Fe_R pool of $37.8 \pm 20\%$ for forest soils. The
469 appearance of a less than complete OC extraction might be explained by
470 the large loss of OC- Fe_R from NaCl treatment, designed to remove OC
471 not bound to Fe_R . Lalonde et al. (2012) note $7.2 \pm 5.4\%$ of OC is released
472 from treatment of natural sediments with NaCl, while we recorded 43.81
473 $\pm 3.06\%$ for our 3-COOH coprecipitate. The subtraction of this OC pool,
474 thought to be unbound to Fe_R , reduces the value for %OC- Fe_R from 90.00
475 $\pm 2.50\%$ removed by CBD treatment to $46.19 \pm 7.57\%$. Therefore, we
476 suggest that OC removed by CBD gives a more accurate assessment of
477 OC- Fe_R , and that the subtraction of the NaCl 'control' pool in previous
478 studies may have led to a severe underestimation of the actual size of the
479 %OC- Fe_R pool.

480

481 The effect of NaCl removal of OC is amplified for the 1- and 2-COOH
482 sediment composites compared to the 3-COOH composite. Here, the
483 substantial extraction of OC by NaCl results in final %OC- Fe_R calculations
484 of $\sim 90\%$ and $\sim 6\%$ respectively (**Fig. 2**), which is physically implausible.

485 This demonstrates that the NaCl control treatment is capable of removing
486 OC from a Fe_R mineral interface. While the effect of NaCl on the OC-Fe_R
487 association has not been previously investigated and is largely based on
488 assumptions, research in soils has indicated that NaCl has the ability to
489 interfere with Fe_R minerals, with Pereira et al. (2019) showing that 8-10%
490 of Na⁺ and Cl⁻ ions in a NaCl solution can adsorb to ferrihydrite.
491 Subsequently the presence of NaCl can affect the adsorption kinetics of
492 organic compounds to Fe_R with a positive relationship observed between
493 increasing NaCl concentration and adsorption of glyphosate (an organic
494 herbicide) to ferrihydrite (Pereira et al., 2019), while (Orcelli et al., 2018)
495 show an inverse trend for glyphosate adsorption to goethite.

496

497 In our study we are able to show NaCl is capable of removing OC-Fe_R
498 because by synthesising coprecipitates, we have ensured that all OC in
499 the sample is associated with Fe_R: i) ferrihydrite is the only reactive
500 interface OC is exposed to; ii) residual OC not associated with ferrihydrite
501 is already removed by multiple rinses following precipitation, at which
502 point C/Fe ratio determination is recommended (Eusterhues et al., 2011;
503 Han et al., 2019). Presumably C/Fe determination would not be
504 recommended following rinses if it was plausible that non-Fe associated
505 OC remained in the coprecipitate, as this would skew these ratios by
506 including excess OC.

507

508 In addition to a high OC loss in the control, we may also be observing an
509 artificially low OC loss in the CBD treatment stage. This can explain
510 negative mean values for C% loss of -12.53% and -19.61% following CBD
511 extraction at 20 and 40% (1-COOH), respectively. Under reducing
512 conditions, the Fe_R phase is dissolved by sodium dithionite and the
513 coprecipitate experiences Fe loss, as shown in **Fig. 4**. However, this Fe
514 loss is incomplete and much of the phase (~77%) remains within the
515 sediment. Liberation of OC from the surface of ferrihydrite during reductive
516 dissolution will increase the number of available surface binding sites and
517 therefore could allow for either re-adsorption of liberated OC or sorption
518 of C containing reagents involved in the reduction stage (sodium

519 bicarbonate, trisodium citrate). While sorption of bicarbonate to Fe
520 (hydr)oxides at neutral pH is a thermodynamically favourable process via
521 monodentate inner-sphere complexation (Acelas et al., 2017),
522 bicarbonate contamination is not expected to be significant due to acid
523 fumigation of samples to remove carbonates. Lalonde et al. (2012) also
524 determined residual bicarbonate to be insignificant, contributing $\leq 0.08\%$
525 of dry sediment weight. Sorption of OC or citrate onto reduced Fe_R phases
526 could elevate the sediment carbon concentration determined and
527 therefore appear to artificially decrease %C loss in the reduction stage,
528 below what is observed in the non-dissolved control.

529

530 The CBD method assumes that treatment of sediment with NaCl removes
531 OC not associated with Fe_R . However, our results suggest that this NaCl
532 leached pool of OC is bound to Fe_R phases but less strongly than the CBD
533 extractable pool, with the susceptibility of an individual OC compound
534 towards removal with NaCl being linked to its carboxyl content. The extent
535 to which comparatively weak OC- Fe_R associations, removed from Fe_R by
536 NaCl, confer protection from microbial degradation upon OC is unknown,
537 however, a large co-localisation of OC with mineral phases (including Fe)
538 has been observed at depths far beyond quantification of OC- Fe_R (23.9
539 metres below seafloor) (Estes et al., 2019). This potentially suggests the
540 extent of OC association with Fe_R is larger than determined by CBD
541 extraction. We suggest that the OC pool extractable by NaCl is of
542 importance when calculating the overall OC- Fe_R budget. The scale of this
543 importance is demonstrated by the additional $7.2 \pm 5.4\%$ OC removed
544 with NaCl in treatment of surface marine sediment, previously exposed to
545 seawater (Lalonde et al., 2012). When accounting for the mass of C lost
546 in the extraction (as in this study and in Salvadó et al. (2015)) the removal
547 of OC by NaCl is even greater with $16.4\% \pm 7.1\%$ of OC- Fe_R being
548 discounted from an initial CBD extraction of $27.4\% \pm 5.4\%$ (Salvadó et al.,
549 2015). Therefore, the subtraction of OC removed by NaCl from the overall
550 OC-Fe pool has a large influence on the resulting value for OC- Fe_R
551 calculation. It is a question as to why exposure to NaCl should remove
552 such a large fraction of OC from marine sediments which have existed in

553 environments surrounded by seawater for many years. However, the
554 NaCl wash employed in the CBD method is of a much greater molar
555 strength than seawater (1.85 M vs 0.49 M). Our results show that NaCl
556 treatment does have the ability to remove Fe_R bound OC and therefore
557 subtraction of this value from CBD extractions has the potential to
558 underestimate the global OC-Fe_R budget. Given the ability of NaCl to
559 remove loosely bound OC, caution should be taken in interpreting OC
560 extracted in this way before removal from the dithionite extractable pool.

561

562 A recalculation on the presumption that all NaCl removable OC extracted
563 by Lalonde et al. (2012) represents weakly bound OC-Fe_R suggests that
564 the role of Fe_R in preserving OC could be underestimated by up to ~33%.
565 However, while in a synthetic experiment it appears that NaCl removable
566 OC is bound to Fe_R phases, this may not be fully translated to natural
567 sediments due to the loss of OC from other sources, such as those bound
568 to manganese oxides and other mineral surfaces (Allard et al., 2017;
569 Bernard, 1997; Tipping and Heaton, 1983) or DOC dissolved in the pore
570 waters (Fox et al., 2018; Rossel et al., 2016). A change in the extent to
571 which OC-Fe_R contributes to OM preservation has ramifications for both
572 the global OC-Fe_R budget and for biogeochemical models aiming to
573 include OM preservation mechanisms to reduce uncertainties related to
574 the fate of OM (Schmidt et al., 2011; Zhao et al., 2016). The bias towards
575 removal of organic compounds with a lower number of carboxyl groups by
576 NaCl suggests that these moieties are the most likely organic compounds
577 to be excluded from OC-Fe_R quantifications by the CBD method.
578 However, the underlying mechanisms controlling OC-Fe_R bonding and the
579 strength of these OC-Fe_R bonds requires further investigation.

580

581 **4.2 Current extraction methods may incompletely extract reactive** 582 **iron phases in sediments.**

583 Quantifying the efficiency of Fe_R extraction techniques has largely been
584 neglected within the literature due to the nature of chemical methods
585 being operationally defined, making it difficult to calibrate these against
586 alternative methods of measuring Fe_R phases such as Mössbauer

587 spectroscopy (Hepburn et al., 2020). In marine sediments, total Fe is
588 comprised of phases of differing stability, requiring extraction techniques
589 of increasing strength to remove them (Poulton and Canfield, 2005). Here
590 we isolated ferrihydrite, which is considered easily reducible by sodium
591 dithionite as part of the Fe_R pool (Lalonde et al., 2012). However, our
592 measure of Fe extractability shows a maximum extraction of only ~60 wt%
593 Fe when coprecipitated with a 3-COOH acid; comparable with the
594 incomplete (~31-50%) sodium dithionite reduction of hematite-humic acid
595 complexes observed by Adhikari and Yang (2015). We find that Fe
596 extractability appears to be even less efficient, for 1- and 2-COOH
597 coprecipitates (**Fig. 4**).

598

599 While reduction by Na dithionite is a well-established method, the
600 adoption of CBD treatment at circumneutral pH, to avoid hydrolysis of OC,
601 remains unique to the OC extraction application. Thompson et al. (2019)
602 found that a CBD extraction at pH 7.6 fails to extract a large proportion of
603 crystalline Fe oxide minerals in both modern and ancient sedimentary
604 rocks. We make a similar observation for OC-free ferrihydrite phases,
605 where only $9.91 \pm 0.95\%$ Fe is recovered post extraction. While it is
606 apparent that CBD treatment cannot fully extract easily reducible Fe
607 (hydr)oxide phases, and therefore the entire associated OC pool, there is
608 no obvious improvement that can be made to the current protocol due to
609 the constraints of maintaining a neutral pH.

610

611 The apparent trend between the number of carboxyl groups per
612 coprecipitated acid molecule and Fe extracted (**Fig. 2**) may be explained
613 by considering how the association of OC with iron (hydr)oxides effects
614 the structural order of the resulting mineral phases. Previous work shows
615 that with increasing OC content, the mineral is more poorly ordered
616 (Cismasu et al., 2011; Mikutta, 2011; Mikutta et al., 2008). Thus, because
617 we see increased OC uptake with the number of carboxyl groups per acid
618 molecule, it is expected that our ferrihydrite becomes more poorly ordered
619 and thus less chemically stable compared to OC-free ferrihydrite. Our
620 XRD analysis supports this theory as a weakening of peaks identifying 2-

621 line ferrihydrite is observed when OC content in the coprecipitates is
622 increased (**Supplementary Fig. S1**). Decreasing crystallinity was
623 similarly observed for samples where total OC (polygalacturonic acid)
624 content was increased (C/Fe 0-2.5) which inhibits transformation of
625 ferrihydrite to more stable phases (ThomasArrigo et al., 2018). In addition,
626 upon extraction the presence of organic matter in OM-Fe_R complexes has
627 previously been attributed to the reductive release of Fe through electron
628 shuttling effects, due to the ability of organic compounds to act as electron
629 carriers between redox reactions (Adhikari and Yang, 2015).

630

631 **4.3 Carboxyl content of Fe associated OC effects OC extraction**

632 As the carboxyl content of our coprecipitates increases, the ability of
633 dithionite to remove OC increases, while the ability of NaCl to remove OC
634 diminishes. For the sample spiked with 50% OC-Fe, the 1-COOH sample
635 was extractable for $92.74 \pm 4.39\%$ of its OC-Fe content by treatment with
636 NaCl while dithionite removed only $10.85 \pm 9.32\%$ (**Supplementary Table**
637 **S3.**) The balance between OC liberated by dithionite vs NaCl
638 subsequently shifts in favour of dithionite with increasing carboxyl content,
639 for the 2-COOH sample the values are roughly equal (dithionite = $70.42 \pm$
640 1.23% , NaCl = $77.89 \pm 2.12\%$). Finally, for the 3-COOH sample dithionite
641 is twice as effective than NaCl, removing $89.60 \pm 0.49\%$ of OC compared
642 to $43.04 \pm 0.49\%$, the trend described for the 50% OC-Fe sample was
643 similarly observed across all coprecipitate concentrations (**Fig. 3**).

644

645 It is assumed that NaCl is only likely to remove weakly bound OC, since
646 OC-Fe bonds are known to be very strong (Barber et al., 2017; Henrichs,
647 1995) and NaCl was previously attributed to only removing OC not
648 associated with Fe_R (Lalonde et al., 2012). Further, the extraction of less
649 OC from sediments upon treatment with NaCl compared to dithionite (e.g.
650 Lalonde et al., 2012; Salvadó et al., 2015) demonstrates that the NaCl
651 treatment must be less aggressive than dithionite, which is to be expected
652 since NaCl has no reducing power. Therefore, the greater loss of OC by
653 NaCl treatment compared to dithionite for the 1- and 2-COOH containing
654 samples suggests a greater proportion of this OC is weakly bound

655 compared to the 3-COOH sample. In turn this suggests that polycarboxylic
656 acids and more carboxyl rich compounds are more strongly bound to Fe_R ,
657 possibly via the formation of multiple strong carboxyl ligand exchange
658 bonds, and hence that carboxyl rich OM might be preferentially preserved
659 in soils and sediments (Kaiser and Guggenberger, 2007).

660

661 For our coprecipitation experiments, the OC source was isolated to
662 carboxyl groups as far as possible, with unavoidable inclusion of one
663 additional short chain C molecule in the 1- and 2-COOH acids (4 non-
664 COOH C's) compared to the 3-COOH acid (3 non-COOH C's). As COOH
665 and OH groups are thought to be important for OM-Fe binding (Karlsson
666 and Persson, 2010; Karlsson and Persson, 2012; Mikutta, 2011; Yang et
667 al., 2012) individual moieties of carboxylic acid were used to reproduce
668 natural OM binding to Fe_R , but in a controlled system where the potential
669 for different sorption mechanisms due to different OM functional groups
670 was reduced. If polycarboxylic acids and more carboxyl rich OM
671 compounds are more strongly bound to Fe_R then environmental conditions
672 favouring high concentrations of Fe, such as continental margins and
673 hydrothermal vents (Tagliabue et al., 2017), as well as carboxyl rich OM
674 inputs (e.g., regions of high primary productivity and shallow waters) are
675 likely to be more efficient at preserving OC.

676

677 The translation of this implication into an environmental context requires
678 an understanding of the moieties present in marine DOM. Much of the
679 work aimed at probing the OC- Fe_R interaction has involved
680 coprecipitations of Fh with terrestrial DOM (e.g., Eusterhues et al., 2014a;
681 Eusterhues et al., 2014b; Eusterhues et al., 2011). Typical coprecipitation
682 experiments for Fe_R bound OC use DOM from forest floor extracts or
683 standards (e.g., Suwannee River). Molecular level work using scanning
684 transmission X-ray microscopy coupled with near-edge X-ray absorption
685 fine structure (STXM NEXAFS) spectroscopy suggests the importance of
686 OM carboxyl content for OC binding to Fe_R by using forest floor extract
687 enriched for carboxyl content (Chen et al. (2014). Coprecipitation with
688 terrestrial organic matter may not be representative of marine DOM

689 however, as marine DOM might be enriched in carboxyl content, for
690 example, a significant fraction of refractory marine DOM is composed of
691 carboxyl-rich alicyclic molecules 'CRAM' (Hertkorn et al., 2006).
692 Additionally, marine (algae) OM further differs from terrestrial sources by
693 exhibiting a lower C/N ratio (4-10) than terrestrial OM (>20) (Meyers,
694 1994). Higher plants, the largest contributor to terrestrial OM, contain less
695 than 20% protein, hence have lower nitrogen contents and higher C/N
696 ratios than protein rich (~80%) marine bacteria and benthic animals
697 (Müller, 1977). OM preserved in ancient sediments (since 11.6 Ma) shows
698 increased C/N ratios (>10) relative to marine surface (algal) OM (Twichell
699 et al., 2002). This change is attributed to partial OM degradation during
700 sinking and potentially post deposition. Verardo and McIntyre (1994),
701 however, suggest that alteration of C/N ratios from shallow to deep burial
702 in sediments occurs due to favourable decomposition of N-rich (C/N low)
703 OM by microbial communities. This would imply that N-rich (C/N low)
704 marine OM undergoes more extensive structural alteration during
705 decomposition than C/N high terrestrial OM. Future work should focus on
706 coprecipitation of carboxyl rich OM and marine OM with Fe_R to further
707 elucidate the controls on OC-Fe_R associations.

708

709 **5.0 Conclusion**

710 Determining of the amount of OM associated with Fe_R in sediments is an
711 important parameter in understanding the fate of OM in the marine
712 environment and the biogeochemical controls on the global C cycle more
713 widely. Currently, only the CBD method exists for quantifying the extent of
714 this interaction, however, by estimating OC-Fe_R using a chemical
715 extraction, this means any resulting value is operationally defined by its
716 susceptibility to chemical treatment and not by its persistence or
717 association with Fe_R. Despite this uncertainty, little work had been
718 performed to understand the limitations of the CBD method and therefore
719 results are currently considered to be representing all of the Fe_R and
720 associated OC in a sediment sample, with some studies using this value
721 to estimate regional or global OC-Fe_R budgets. Here, we performed CBD
722 extractions on synthetic ferrihydrite-organic acid coprecipitates varying in

723 their carboxyl context to understand how samples which should be
724 recoverable for their total Fe and C contents performed under the CBD
725 method. In contrast with assertions that CBD treatment was able to fully
726 reduce all solid Fe_R phases and associated OC, we found that CBD is
727 unable to fully reduce ferrihydrite. Further, we show Fe reduction to be
728 variable based on the chemical composition of OM, specifically carboxyl
729 group content. As an increasing number of carboxyl groups become
730 associated with a ferrihydrite-organic coprecipitate this exerts a strong
731 control on the crystallinity and therefore reactivity of Fe, with a significant
732 increase in the amount of Fe reduced for carboxyl rich coprecipitates.
733 Additionally, we found that the NaCl wash stage has the ability to remove
734 a large amount of Fe bound OM and that further consideration should be
735 given in interpreting OC loss from the NaCl stage before subtracting this,
736 often large value, from the dithionite extractable pool. These conclusions
737 have significant implications for the quantification of OC-Fe_R as NaCl
738 extractable OC was originally thought to only extract non-Fe bound OC
739 and therefore this sum is subtracted from the dithionite extractable pool of
740 OC-Fe_R. Our results therefore indicate that a combination of incomplete
741 Fe_R reduction by CBD and removal of Fe_R bound OC by NaCl have likely
742 contributed towards an underestimation of the extent to which OC-Fe_R
743 interactions persist in marine sediments.
744

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Supplementary Information

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Mass balance calculation

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To account for the mass loss during the extraction experiment we

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applied the mass balance calculation of Salvadó et al. (2015) combined

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with Peter and Sobek (2018). The %OC loss is calculated by applying

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bulk %C to the pre extraction mass and post extraction %C to the final

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mass. Comparison by change in raw %C is likely to overestimate the

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final %C if start and end carbon concentrations are calibrated to the

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initial mass, as when %C reduces so does the sample mass.

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Supplementary Equation 1 was devised to determine %OC-Fe loss.

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Supplementary Equation 1

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$$\Delta C(\%) = \frac{(M^{\text{Pre(R)}} \times \%C^{\text{Bulk}}) - (M^{\text{Post(R)}} \times \%C^{\text{Post(R)}})}{M^{\text{Pre(R)}} \times \%C^{\text{Bulk}}}$$

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$$\frac{(M^{\text{Pre(C)}} \times \%C^{\text{Bulk}}) - (M^{\text{Post(C)}} \times \%C^{\text{Post(C)}})}{M^{\text{Pre(C)}} \times \%C^{\text{Bulk}}} \times 100$$

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$M^{\text{Pre/Post(R/C)}}$ = Mass pre/post (R) reduction / (C) control.

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$\%C^{\text{Post(R/C)}}$ = %C Post reduction/control experiment.

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$\%C^{\text{Bulk}}$ = %C in the sediment pre extraction (same for control and

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reduction).

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X-Ray Diffraction Analysis

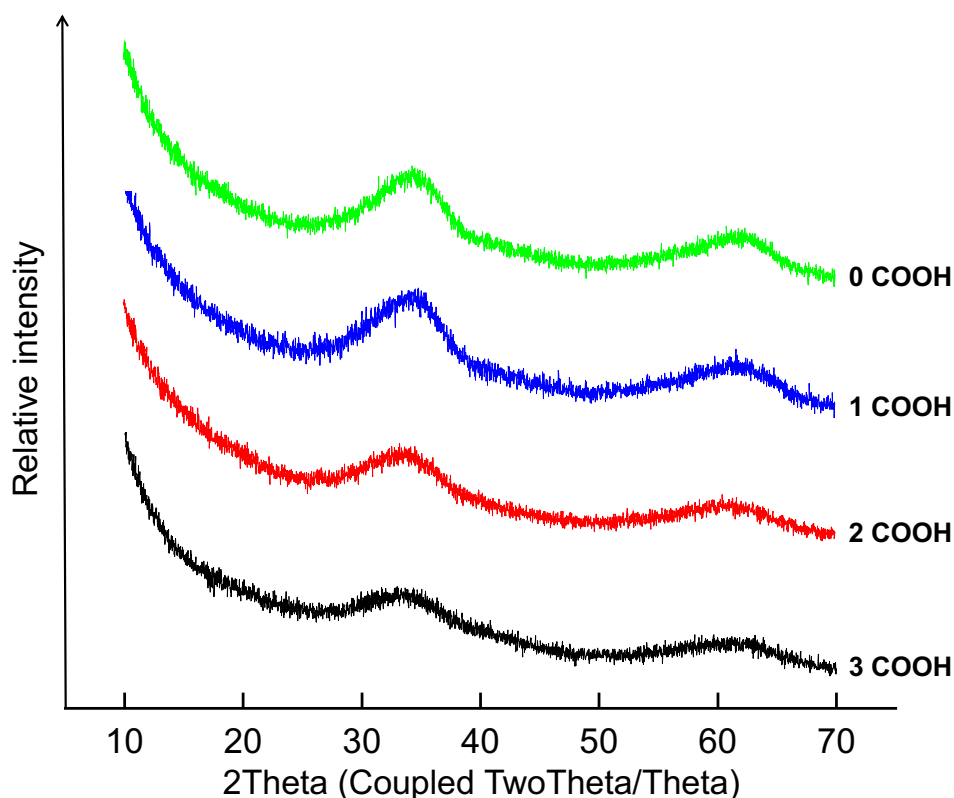


Figure S.1- Stacked XRD of coprecipitates with increasing carboxyl rich organic content.

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Statistical Analysis

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Ordinary one-way ANOVA

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970 A one-way ANOVA test was conducted for %OC-Fe extraction values
971 (Figure 2) and %Fe extraction values (shown in Figure 4) across the
972 carboxyl range. The one-way ANOVA was applied to these two data sets
973 as a comparative measure of sample means across the groups. The
974 one-way ANOVA is limited by only having the ability to report whether
975 there is a difference in means between two groups. Hence, a Tukey
976 honest significant difference (HSD) test was applied post-hoc to
977 determine the exact groups for which a statistically significant difference
978 in means occurred for at the 99% significance level (**Table S1 and S2**).
979 All analyses were conducted in GraphPad Prism version 8.30, GraphPad
980 Software, La Jolla California USA, www.graphpad.com.

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Table S1: Tukey's multiple comparisons test of extracted %OC-Fe for carboxyl coprecipitates.

Comparison	Adjusted P Value	Significant?*
1 COOH vs 2 COOH	<0.0001	Yes
1 COOH vs 3 COOH	<0.0001	Yes
2 COOH vs 3 COOH	0.0001	Yes

*Significance was determined at the 99% significance level.

Table S2- Tukey's multiple comparisons test of extracted %Fe for carboxyl coprecipitates.

Comparison	Adjusted P Value	Significant?*
0 COOH vs 1 COOH	0.0050	Yes
0 COOH vs 2 COOH	<0.0001	Yes
0 COOH vs 3 COOH	<0.0001	Yes
1 COOH vs 2 COOH	<0.0001	Yes
1 COOH vs 3 COOH	<0.0001	Yes
2 COOH vs 3 COOH	0.0019	Yes

*Significance was determined at the 99% significance level.

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Determination of %OC-Fe

Table S3 shows the raw data for the reduction and control stages of the CBD treatment which were used as inputs to Equation 1 in order to calculate %OC-Fe as shown in Figures 2 and 3

Table S3- Raw carbon data for determining %OC-Fe.

wt %OC-Fe in sample	Bulk %OC*	Reduction (Δ %OC)	Control (Δ %OC)	%OC-Fe extracted
1 COOH				
20	0.636	-12.527 (\pm 21.056)	78.606 (\pm 11.716)	-91.132 (\pm 9.340)
30	0.968	17.398 (\pm 7.741)	95.968 (\pm 1.162)	-65.189 (\pm 9.346)
40	0.978	-19.607 (\pm 2.243)	87.622 (\pm 9.317)	-107.229 (\pm 11.560)
50	1.357	10.854 (\pm 9.315)	92.742 (\pm 4.386)	-81.888 (\pm 13.702)
2 COOH				
20	1.681	74.066 (\pm 5.818)	75.944 (\pm 1.457)	-1.879 (\pm 7.275)
30	2.192	71.080 (\pm 2.150)	70.613 (\pm 9.976)	0.467 (\pm 12.126)
40	3.237	63.203 (\pm 8.030)	78.079 (\pm 2.245)	-14.876 (\pm 10.275)
50	3.639	70.423 (\pm 1.234)	77.892 (\pm 2.123)	-7.468 (\pm 3.357)
3 COOH				
20	2.761	87.043 (\pm 3.224)	46.620 (\pm 4.390)	40.423 (\pm 7.615)
30	4.339	91.764 (\pm 1.881)	44.503 (\pm 3.625)	47.260 (\pm 5.506)
40	5.420	91.628 (\pm 0.686)	41.114 (\pm 0.629)	50.514 (\pm 1.315)
50	6.935	89.602 (\pm 0.487)	43.036 (\pm 0.485)	46.566 (\pm 0.973)

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*Bulk %OC refers to the OC content prior to any treatment. Values shown are a mean of duplicates, \pm indicates the standard error of the mean (SEM).

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