Postprint

1 **High variability in iron-bound organic carbon among five boreal lake sediments**

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1   **Abstract**

2   Being both stable carbon sinks and greenhouse gas sources, boreal lake sediments represent

3   significant players in carbon (C) cycling. The release of dissolved organic carbon (DOC) into

4   anoxic water is a widespread phenomenon in boreal lakes with impact on sediment C budgets.

5   The association of organic carbon (OC) with iron (Fe) is assumed to play an important role for

6   this anoxic OC release via the dissimilatory reduction of Fe, but also to influence the stabilization

7   of OC in sediments. To investigate the role of Fe-OC association for OC dynamics in different

8   boreal lake sediments, we compared the content of Fe-bound OC (Fe-OC, defined as citrate

9   bicarbonate dithionite (CBD) extractable OC) and the extent of reductive dissolution of solid-

10   phase Fe and OC at anoxia. We found high among-lake variability in Fe-OC content, and while

11   the amount of Fe-OC was high in three of the lakes (980 $-$1920 µmol g^{-1}), the overall

12   contribution of Fe-OC to the sediment OC pool in all study lakes was not higher than 11%. No

13   linkages between the amount of the Fe-OC pool and lake or sediment characteristics (e.g., pH,

14   DOC concentration, sediment OC content, C:N ratio) could be identified. The observed release of

15   OC from anoxic sediment may be derived from dissolution of Fe-OC in the lake sediments with

16   high Fe-OC, but in other lake sediments, OC release during anoxia exceeded the sediment Fe-OC

17   pool, indicating low contribution of reductive Fe dissolution to OC release from these lake

18   sediments. The range of the investigated boreal lakes reflects the high variability in the size of the

19   sediment Fe-OC pool (0 $-$ 1920 µmol g^{-1}) and CBD-extractable Fe (123 $-$ 4050 µmol g^{-1}), which

20   was not mirrored in the extent of reductive dissolution of Fe (18.9 $-$ 84.6 µmol g^{-1}) and OC

21   (1080 to 1700 µmol g^{-1}) during anoxia, suggesting that Fe-bound OC may play a minor role for

22   sediment OC release in boreal lakes. However, studies of redox-related OC cycling in boreal lake

23   sediments should consider that the amount of Fe-OC can be high in some lakes.

1 Introduction

1   **1 Introduction**

2   Boreal lakes receive, transport, transform, and store large amount of terrestrial-derived organic

3   carbon (OC; Cole et al. 2007) and boreal sediments have both been described as significant,

4   efficient and stable long-term carbon sinks (Chmiel 2015; Ferland et al. 2014; Kortelainen et al.

5   2004) and as important sources of greenhouse gases to the atmosphere (Bastviken et al. 2011;

6   Kortelainen et al. 2006). It is therefore important to understand factors affecting the

7   transformation of sediment OC in these systems.

8   In boreal surface waters, a generally tight coupling of Fe and OC has been observed

9   (Kritzberg and Ekström 2012; Köhler et al. 2013; Weyhenmeyer et al. 2014), and flocculation of

10   Fe-bound OC aggregates is seen as an important precursor of sediment formation in boreal lakes

11   (von Wachenfeldt and Tranvik 2008). Furthermore, in dissolved OC (DOC)-rich boreal lakes, the

12   diffusion of DOC from anoxic sediments to the water column seems to be a widespread

13   phenomenon, probably related to OC release via reductive Fe dissolution (Peter et al. 2017).

14   These observations indicate that Fe-OC interactions play an important role for OC dynamics in

15   sediments of boreal lakes. However, the fraction of Fe-bound OC in boreal sediments is currently

16   unknown, and therefore also the role of Fe in OC dynamics in boreal lake sediments.

17   In marine sediments, iron (Fe) has been proposed to play an important role in the

18   preservation of sediment OC and to create a "rusty sink" for OC, as ~20% of sediment OC was

19   found to be bound to Fe (Lalonde et al. 2012). Under oxic conditions, DOC sorbs to Fe(III)

20   (oxyhydr)oxides and Al , which promotes aggregation and sedimentation of the complex

21   (Lalonde et al. 2012; Tipping and Woof 1983) and protects the OC from microbial degradation

22   (Boudot 1989; Jones and Edwards 1998; Keil et al. 1994; Lalonde et al. 2012). However, this Fe-

23   bound OC pool can also be remobilized under anoxic conditions through reductive dissolution of

1   Fe and concomitant release of OC (Chin et al. 1998). Accordingly, it has been shown that DOC

2   diffusion during anoxia can constitute an important C loss from sediments, both in marine and

3   freshwater systems (Peter et al. 2016; Skoog and Arias-Esquivel 2009). Therefore, redox

4   conditions are an important controlling factor of DOC flux across the sediment-water interface

5   (Dadi et al. 2015).

6   DOC release from anoxic lake sediment may be related to reductive iron dissolution, but

7   may also be caused by increasing pH, induced e.g., through microbial iron reduction, which

8   weakens the sorption of OC to particles (Grybos et al. 2009). Also, OC mineralization can be

9   limited under reducing conditions (Bastviken et al. 2004) and hence, more OC can accumulate in

10   anoxic pore water compared to oxic sediment layers. OC solubility can additionally be regulated

11   through (i) competition with other anions (like phosphate) for the sorption to Fe and (ii) sorption

12   of OC to other non-redox sensitive compounds (like aluminum-oxides or clay minerals; Kleber et

13   al. 2015; Tipping et al. 2002). Environmental conditions in sediments can vary strongly between

14   lakes, and also DOC diffusion from anoxic lake sediment can be variable (Peter et al. 2017). It is

15   therefore likely that the relevance of Fe-OC coupling differs between lakes and a better

16   understanding of the role of Fe-OC interactions for sediment OC burial and mobilization is

17   warranted.

18   We compared the content of Fe-bound OC in sediments from five different boreal lakes

19   and evaluated the release of Fe and OC when these sediments were exposed to anoxic conditions.

20   We hypothesized that the lake sediments are generally high in Fe-bound OC, and that sediments

21   with high Fe-bound OC content will release significant amounts of OC under anoxic conditions.

22   We applied the citrate bicarbonate dithionite (CBD) method, which removes all reducible Fe

23   oxides, liberates the OC that was associated with this Fe fraction, and hence allows to measure

1   the Fe-bound OC fraction in the sediments (Lalonde et al. 2012; Mehra and Jackson 1960;

2   Poulton and Canfield 2005). Additionally, the total amount of Fe and OC that can be released

3   from sediments by microbially-driven reductive dissolution under anoxic conditions was

4   determined in sediment slurry incubation experiments.

5

1   **2 Methods**

2   **2.1 Study systems**

3   The investigated 5 lakes in central Sweden (Erssjön, Lötsjön, Tvigölingen, Skogsjön and Övre

4   Skärsjön, Table 1 and Fig. S1) were selected to span over wide gradients of organic carbon and

5   humic contents, e.g., they differ in DOC concentrations (0.9 – 3.4 mmol DOC L^{-1}), sediment OC

6   content (13.9 – 38.2 %) and molar C:N ratios (10.1 – 20.4, Table 1). The lakes also vary in pH

7   (6.1 – 7.7) and nutrient concentrations (29.9 – 963 μmol total nitrogen L^{-1} and 0.3 – 1.7 μmol

8   total phosphorus L^{-1}; Table 1). Lake Erssjön is a mesotrophic humic lake that develops stable

9   summer stratification with anoxic bottom water. Its catchment is mainly covered by forest with a

10   minor share of peat and agricultural land underlined by granite bedrock (Peter et al. 2016).

11   Oligotrophic Lake Övre Skärsjön is the largest and deepest of the investigated lakes and located

12   in a mineral-rich former Fe-mining area (Andersson 2004). Although the lake stratifies regularly,

13   deep water turns only occasionally anoxic. Lake Skogsjön, a mesotrophic humic lake, is the

14   smallest and shallowest of the lakes and completely surrounded by forest. It develops

15   stratification with anoxia, but due to the shallow depth it may mix also during the summer

16   stratification period. Lake Lötsjön is a eutrophic lake with little humic matter that stratifies with

17   anoxic bottom water in summer. Lake Tvigölingen is an oligotrophic humic forest lake and also

18   develops anoxic bottom water during summer stratification (Fig. S2).

19   From the deepest spot of each lake, four sediment cores were sampled by gravity coring.

20   The intact cores were previously used in another experiment for determination of carbon fluxes

21   under oxic conditions, as described in Peter et al. (2016; 2017). We chose to use sediment

22   material for further experiments only from cores that were previously used in oxic incubations, to

23   avoid potential depletion of OC and Fe by diffusion from sediment to overlying water, which

happens if cores are incubated under anoxic conditions, but not under oxic conditions (Peter et al. 2017). The cores were stored at 4° C prior to the previous oxic incubations (Peter et al. 2016; 2017) and we assume that during that time (~1 week), as well as during oxic incubation, changes in sediment OC and Fe inventory did not substantially deviate from changes that may occur in situ under oxic conditions. Three of the lakes (Tvigölingen, Lötsjön and Skogsjön) had anoxic bottom water at the time of core sampling (Fig.S2), which may imply that some of the reducible Fe-OC may not have been present in the sediment due to reductive dissolution and subsequent diffusion of Fe and OC to the bottom water (Peter et al. 2016) and that the estimates of the sediment Fe-OC pool presented here may be conservative. However, that the results of these three lakes did not differ systematically from the results of the other lakes, suggests limited influence of sampling conditions. Hence, while we cannot exclude an effect of the pre-experimental history on the sediment material, it seems likely that any such effect was comparatively small in terms of experiment outcome. We therefore regard the sediment material that was used in the experiments as representative for the conditions in the respective lake.

From the cores, the first 1 cm top sediment layers, representing sediment material of several years (sedimentation rate in boreal lakes is typically <0.2 cm year[1] (Chmiel et al. 2015)), were sliced, freeze dried and homogenized using pestle and mortar, and used for experiments as described in the following.

2.2 Extraction of Fe-bound OC

The citrate bicarbonate dithionite (CBD) method to extract Fe-bound OC from sediments follows the protocol in Lalonde et al. (2012). Dried sediment (25 mg) was weighed in 10 mL glass

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1   centrifugation vials with teflon-lined caps and extracted with a 0.11 mol L^{-1} sodium bicarbonate

2   and 0.27 mol L^{-1} trisodium citrate (a metal complexing agent) solution. After heating the mixture

3   to 80°C in a temperature-controlled oven, a 0.1 mol L^{-1} sodium dithionite solution was added as

4   a reducing agent and the solution kept at this temperature for 15 minutes. To stop the extraction,

5   the vials were centrifuged at 1000 g for 10 min (higher speed caused glass vials to break) and the

6   supernatant collected for later analysis of dissolved Fe concentration. This procedure was

7   repeated twice to ensure complete dissolution of Fe. Subsequently, the sediments were washed 3

8   times with 5 mL artificial lake water (ALW) and supernatants combined. ALW was prepared

9   adjusting the protocol by Lehman (1980) by excluding the OC and Fe sources. Control samples

10 (to control how much OC is desorbed from sediments without Fe extraction) were run in parallel

11 using the same solid to solution ratios, temperature and time, but replacing metal complexing or

12 reducing reagents with NaCl solutions at the same ionic strength as applied in the extraction. The

13 supernatant was filtered (GF/F) and diluted in ALW before measuring total Fe concentration with

14 the Ferrozine method (Viollier et al. 2000) after 4 − 7 days of storage at 4°C. During storage,

15 dithionite in the samples was completely oxidized and did not interfere with Fe measurements

16 (Thamdrup et al. 1994). After termination of the experiment, sediments were freeze-dried and

17 analyzed for OC content. For each lake, extractions were carried out with sediment material from

18 four different cores.

19   The amount of OC that was removed together with Fe was determined as the difference

20 between OC content of control samples (OC$_{contol}$) and the CBD-extracted samples (OC$_{CBD}$) and

21 expressed as % of initial OC content (OC$_{initial}$) of the sediment (Lalonde et al. 2012)

22 % OC loss = (OC$_{contol}$ − OC$_{CBD}$) / OC$_{initial}$ ×100

Because the loss of Fe in some sediments was very high (>20% sediment weight), the specific weight of the sediment changed considerably. This weight change due to Fe loss was accounted for when calculating OC content in the extracted samples (OC_{CBD}). For example, the amount of Fe that was lost during CBD extraction was added to the sample weight measured in the OC analysis ($mass_{orrected} = mass_{measured}$ [mg] + $mass_{measured}$ [mg] * Fe_{loss} [%]) to calculate the OC content of the sediment ($OC_{measured}$ [mg] * $mass_{corrected}$ $[mg]^1$).

2.3 Sediment incubations – anoxic release of OC and Fe

This experiment aimed to determine the total amount of Fe and OC that could be released from sediments under anoxic conditions. Dried sediment (25 mg) from each lake was weighed in glass centrifugation vials, mixed with 10 mL ALW, and kept at both oxic (loosely covered with aluminum foil) and anoxic condition (in closed vials without headspace, stored in a N_2-filled glove box) at room temperature. For the anoxic incubations, the ALW was purged with N_2 prior to addition. The sediment slurries were regularly shaken (every second or third day) to mix sediment and ALW. At the end of an incubation, pH and O_2 were measured on a mobile pH meter (Metrohm) and a Needle-type Oxygen Sensor (PreSens), respectively. Subsequently, vials were centrifuged at 1000 g for 10 min and supernatant collected for DOC and Fe analyses. Incubations were repeated 5 times, for 29, 27 or 25 days each, with addition of fresh ALW. The whole experiment lasted 133 days, and at the last incubation, DOC and Fe release from sediments was close to zero (Fig. S3). Sediment material was incubated in four replicates, one from each of the four different cores for each lake, in order to cover the between-core variability that has been observed in previous experiments (Peter et al. 2016, 2017). One Skogsjön anoxic sediment replicate was lost due to vial breaking during centrifugation.

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2.4 Analytical methods

3 DOC concentration was measured through high-temperature oxidation on a Shimadzu TOC-L

4 analyzer. Sediment OC content was determined on freeze-dried sediments combusted in an

5 elemental analyzer (NA 1500, Carlo Erba instruments). For Fe measurements, an adjusted

6 protocol of the Ferrozine method by Viollier et al. (2000) was used (Peter et al. 2016). The

7 method is able to detect Fe(II) and, after a reduction step, Fe(III) in the same measurement.

8 However, in this study we report only total Fe concentrations for simplification (FeII contributed

9 $84 \pm 22\%$ to total Fe in anoxic incubation samples). Absorbance was recorded on a

10 spectrophotometer (Perkin Elmer, Waltham, USA) at 562 nm.

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3 Results and Discussion

3.1 Amount of Fe-OC can be high in boreal lake sediments

We found that in three of the study lakes, a considerable amount of OC was bound to Fe (Erssjön 1917 ± 300 µmol g^{-1}, Tvigölingen 1750 ± 458 µmol g^{-1}, and Övre Skärsjön 983 ± 642 µmol g^{-1}; mean ± standard deviation; Fig. 1, Table S1), which is substantially more than e.g., reported from marine sediments (300 ± 300 µmol g−1; Lalonde et al. 2012). For the other two study lakes, however, the amount of Fe-bound OC was low (Lötsjön 275 ± 475 µmol g^{-1}) or not distinguishable from zero (Skogssjön -817 ± 1250 µmol g^{-1}; Fig. 1). No linkages between the amount of the Fe-OC pool and lake or sediment characteristics (e.g. pH, DOC concentration, sediment OC content, C:N ratio; Table 1) could be identified. Our finding that the amount of OC bound to Fe can be high in the sediments of some boreal lakes is supported by studies reporting a coupling of OC and Fe concentrations in boreal surface waters, and the involvement of Fe in the precipitation of OC (Kritzberg and Ekström 2012; Köhler et al. 2013; Weyhenmeyer et al. 2014; von Wachenfeldt and Tranvik 2008). However, given the overall high OC content of the studied boreal sediments (14 − 38%; Table 1) the fraction of Fe-bound OC was relatively small. The highest share of Fe-OC was found for Lake Erssjön sediments (11.1 ± 1.5% of total sediment OC pool), followed by Lake Tvigölingen (7.2 ± 1.9%), Övre Skärsjön (6.7 ± 4.5%) and Lötsjön (2.3 ± 4.1%), and was not distinguishable from zero for Lake Skogsjön (-2.5 ± 3.9%). Only for Lakes Erssjön and Tvigölingen, the sediment OC loss due to Fe-bound OC extraction was significant (Student's *t*-test between sediment OC content of CBD-extracted and control sediments; $p <$ 0.001; Fig. 1, Table S1). In spite of the relatively low contribution of Fe-OC to the sediment OC pool and hence its minor role for the immobilization of OC in boreal lake sediments, the high amount of Fe-OC in some of the study lakes suggests that redox processes that affect the stability

1 of the Fe-OC aggregates in surficial sediments, such as reductive dissolution during anoxia, may

2 affect OC dynamics in boreal lakes (Peter et al. 2016).

3

4 **3.2 Among-lake differences in association between Fe and OC**

5 The association between sediment OC and Fe was remarkably different in the five investigated

6 lakes, as evidenced by the differences in both the content of Fe-bound OC, as well as by the

7 molar ratios of CBD-extracted OC:Fe (Fig. 1). The OC:Fe ratio was highest for Lakes

8 Tvigölingen (10.2) and Erssjön (8.7), the two lakes with also the highest amount of Fe-OC. These

9 ratios are within the range reported from lake sediments (10.1 ± 3.9; Peter et al. 2016) and marine

10 sediments (6.2 ± 7.7; Lalonde et al. 2012), indicating a strong association between OC and

11 available Fe in these two lakes. Moreover it suggests that most of the reducible Fe is aggregated

12 with OC in these sediments.

13 However, in the other three investigated lakes, the CBD-extracted OC:Fe ratio was very

14 low (Lakes Lötsjön: 0.8, Övre Skärsjön: 0.2 and Skogsjön <0), suggesting that there is almost no

15 aggregation between OC and reducible Fe in those sediments. For Lake Skogsjön, the high

16 sediment OC content (38%) complicated the detection of small differences in sediment OC, and

17 may have masked the comparatively small amount of Fe-OC. For example, even with an OC:Fe

18 ratio of 6 to 10, Fe-OC would only account for 2 to 4% of total sediment OC in Lake Skogsjön.

19 Even if this were the case, Fe-OC evidently makes up a negligible fraction of the sediment OC

20 pool, and association with Fe is therefore only playing a minor role for OC dynamics in Lake

21 Skogsjön. In contrast, Lake Övre Skärsjön had an extremely high content of reducible Fe (4050

22 μmol g^{-1}) but a low OC:Fe ratio, indicating the presence of reducible Fe that was not associated

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1   with OC. In addition, we observed that in Lake Övre Skärsjön the loss of Fe during CBD

2   extraction needs to be accounted for when calculating Fe-OC. When accounting for the high Fe

3   loss (>20% of sediment weight), the contribution of Fe-OC considerably increased (from -10.5 to

4   6.7% of sediment OC; Table S1). For the other lake sediments, where Fe was extracted in similar

5   amounts (123 − 347 µmol g^{-1}; Fig. 1) as in marine sediments (18 − 362 µmol g−1; Lalonde et al.

6   2012) correction for Fe loss only marginally changed the Fe-OC content (Table S1).

7   Lastly, also in Lake Lötsjön sediment, the ratio of OC:Fe was low (0.8), as a result of a

8   relatively high content of CBD-extractable Fe (350 µmol g^{-1}) and a low content of Fe-OC in

9   these sediments (2.3% of sediment OC, or 270 µmol g^{-1} sediment). The low OC:Fe ratio of Lake

10   Lötsjön may be explained by the high pH of the lake (highest among the investigated lakes; 7.7,

11   Table 1). Lower pH favors complexation and precipitation of Fe with OC (OC:Fe ratios >1)

12   while higher pH favors adsorptive (and weaker) association between Fe and OC (OC:Fe <1;

13   Tipping et al. 2002; Wagai and Mayer 2007). Since in general a high pH weakens the binding

14   between Fe and OC (Kleber et al. 2015), less sediment OC might be bound to Fe in high pH

15   lakes. Long-term monitoring data from the Swedish monitoring program from lake Övre Skärsjön

16   further corroborate the important role of pH for Fe-OC interactions, as the observed increase in

17   pH (from 5.0 to 5.6) over the past 30 years (1988 − 2012) coincided with increases in surface

18   water DOC (from 0.61 to 0.87 mmol L^{-1}) and Fe concentrations (from 10.0 to 17.2 in 1996 −

19   2002; http://webstar.vatten.slu.se/db.html). Accordingly, higher contribution of reductively

20   soluble OC was found in low pH soils (pH 4 − 5 for low vs. 5.5 − 6 for high pH soils; Wagai and

21   Mayer 2007) and increasing DOC concentrations in lake hypolimnia during anoxic periods were

22   found to be particularly pronounced in low pH lakes (5.2 for low vs. 6.6 for high pH lakes; Peter

13

et al. 2017). Hence, we propose that the high pH of Lake Lötsjön explains the low share of Fe-bound OC in its sediment.

3.3 The role of reductive Fe dissolution for the release of DOC from anoxic sediment

We found concomitant DOC and Fe release from incubated sediments, which was for both, DOC and Fe, consistently and significantly higher in anoxic sediment incubations compared to oxic incubations (Student's t-tests $p < 0.05$, except DOC release for Lötsjön and Skogsjon: $p = 0.12$ and 0.1, respectively, Fig. 2). This is congruent with other findings of increasing DOC and Fe concentrations in anoxic bottom waters of lakes (Chadwick et al. 2006; Gonsior et al. 2013; Peter et al. 2017). The amount of DOC released from sediment during repeated anoxic sediment incubations (5 − 10% of sediment OC) was generally similar to the amount of CBD-extracted Fe-OC (0 − 11%; Fig. 4a). The total amount of OC released under anoxic conditions in the studied lake sediments ranged from 1080 to 1700 µmol g^{-1} (Fig. 3), with highest sediment OC release in Lake Lötsjön and Övre Skärsjön (10.2 ± 3.4% and 10.2 ± 1.4 of sediment OC, respectively), followed by Lake Tvigölingen (7.0 ± 0.9%), Erssjön (6.9 ± 1.5%), and Skogsjön (4.6 ± 0.4%; Fig. 2). Increasing DOC concentrations in anoxic lake hypolimnia were often attributed to diffusion from sediments upon reductive Fe dissolution (Brothers et al. 2014; Chadwick et al. 2006; Peter et al. 2017; Tipping and Woof 1983). Accordingly, the fraction of Fe-OC is not only thought to promote OC burial (Lalonde et al. 2012), but also to be a source for DOC release from sediments under anoxic conditions (Peter et al. 2016; Skoog and Arias-Esquivel 2009). For Lakes Erssjön and Tvigölingen, it is stoichiometrically possible that the pool of Fe-OC is responsible for the observed DOC release in anoxic sediment incubations (Fe-OC > anoxic DOC release; Fig. 4a). For the other lakes, however, the Fe-OC content in sediments was lower than the DOC

1 release under anoxic conditions (Fe-OC < anoxic DOC release). This implies that in these lakes,

2 it is unlikely that reductive Fe dissolution contributes substantially to DOC release from anoxic

3 sediments. In addition, we expected OC release via reductive Fe dissolution to result in a

4 proportional relationship between sediment Fe-OC content and anoxic DOC release. But since we

5 did not find such a relationship between sediment Fe-OC content and anoxic DOC release (Fig.

6 4a), we assume that OC-releasing processes other than Fe reduction are, at least additionally, at

7 play.

8   The pattern of DOC release among the lakes was not congruent with the lakes' Fe-OC

9 content (Fig. 4a) and DOC release during anoxic incubations of intact sediment cores (Peter et al.

10 2017). In contrast, Fe dissolution under anoxic conditions, as well as the amount of CBD-

11 extractable Fe, were remarkably corresponding to each other (Fig. 4b) and to the release of Fe

12 from intact sediment cores (Peter et al. 2017). Apparently, DOC release seems to be more

13 sensitive to different experimental conditions (slurries vs. intact cores) than Fe release, and the

14 association between Fe and OC seems to be far from being uniformly strong in different lake

15 sediments.

16

17 **3.4 Anoxic Fe dissolution is coupled to OC-bound Fe pool**

18 In contrast to OC, CBD-extractable Fe correlated well with released Fe in anoxic incubations,

19 although the CBD-extractable Fe was an order of magnitude higher than Fe released during

20 anoxic sediment incubations (Fig. 4b). Highest Fe dissolution under anoxic conditions was found

21 in Lake Lötsjön (84.6 ± 2.1 µmol g^{-1}), followed by Lake Övre Skärsjön (70.2 ± 24.3 µmol g^{-1})

22 and Erssjön (65.2 ± 15.4 µmol g^{-1}) and was lowest in Lakes Tvigölingen and Skogsjön ($39.9 \pm$

1 19.0 and 18.9 ± 0.4 µmol g^{-1}; Fig. 2). Hence, CBD-extractable Fe represents a pool of Fe that can

2 be mobilized during reductive dissolution, yet only partially; the majority of the CBD-extractable

3 Fe was not dissolved during anoxic incubations (Fig 4b). In Lake Övre Skärsjön, only a very

4 small fraction (2%) of CBD-extractable Fe was released during anoxic incubations owing to the

5 very large Fe release during CBD extraction (Fig. 1b), which may be related to the potential

6 presence of extractable Fe species derived from past mining activities in the immediate vicinity

7 of this lake (Andersson 2004). We propose that the fraction of Fe that was solubilized under

8 anoxic conditions is more important for sorption of OC than the bulk Fe that was only CBD-

9 extractable. Similarly, Wagai and Mayer (2007) suggested that in soils, the most easily reducible

10 Fe oxide phases are enriched in OC and therefore most important for OC cycling.

11

3.5 Processes involved in DOC release from anoxic sediments

13 During the anoxic incubations, the individual lake sediments released DOC and Fe at different

14 ratios. Molar ratios between mean DOC to Fe release were 18.1 for Erssjön, 17.9 for Lötsjön,

15 21.4 for Övre Skärsjön, 77.6 for Skogsjön and 43.0 for Tvigölingen. These ratios of DOC and Fe

16 release from anoxic sediments were considerably higher than CBD-extractable OC:Fe ratios in

17 these sediments (0 – 10, Fig. 1), or in marine sediments (6.2 ± 7.7; Lalonde et al. 2012),

18 supporting our conclusion that processes other than reductive Fe dissolution contributed to the

19 release of DOC from sediment.

20 Fe reduction can lead to an increase in pH through proton consumption and a weakening

21 of the binding of OC to the particulate phase, thereby rendering it more soluble. In anoxic soil

22 incubation studies, Grybos et al. (2009) could show that pH was responsible for a larger fraction

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of DOC release from soil material at anoxic conditions than reductive Fe dissolution. Although pH dropped in all sediment incubations due to CO_2 production, pH was higher in anoxic (6.3 ± 0.3) than in oxic (6.0 ± 0.2) incubations and DOC and Fe release from sediments showed a significant positive correlation with pH in the first incubation cycle (Fig. S4), when also most of the DOC was released (Fig. S3). Hence, the high synchrony of Fe and DOC observed in anoxic incubations of the individual lakes (Fig. S3) may be partly due to changes in pH associated with iron reduction (Fig. S4). In addition, it was observed that high Fe-reducing conditions lead to high colloidal dispersal of OC in soils (Buettner et al. 2014), which may have contributed to the higher ratios of DOC to Fe under anoxic reducing conditions. It has also been shown that the degradation of recalcitrant DOC is reduced under anoxic conditions, while the degradation of labile DOC at reducing conditions was only slightly affected (Bastviken et al. 2004). In addition, highly reduced DOC was shown to be preferentially preserved in anaerobic environments because anaerobic electron acceptors (e.g. sulfate) provide too little energy to power the oxidation of highly reduced organic compounds in anaerobic microbial respiration (Boye et al. 2017). Similarly, in a previous study with Lake Erssjön water, loss of DOC through mineralization was found to be approximately 3 times higher under oxic than under anoxic conditions (Peter et al. 2016). Hence, decreased mineralization under anoxic conditions could potentially contribute to the higher DOC accumulation in anoxic compared to oxic sediment incubations. Likely, a combination of different processes, involving reductive Fe dissolution, decreased mineralization under anoxic conditions and increasing pH due to microbial Fe reduction activity led to the observed DOC release and accumulation during anoxic incubations and to the different patterns observed for the individual lake sediments.

4 Conclusions

Even though the contribution of Fe-OC to the sediment OC pool was relatively low in the studied lakes (<11%), the amount of Fe-OC can be comparatively high in boreal lake sediments, and the reductive dissolution of Fe and OC at anoxia might therefore be important for OC cycling in some boreal lakes. However, our results do not suggest that Fe-bound OC plays a large role for sediment OC dynamics in boreal lake sediments in general. The size of the Fe-OC pool, as well as the extent of OC and Fe release at anoxia, were notably different among the various boreal lakes, suggesting a complex interplay of multiple factors. Furthermore, DOC release during anoxic sediment incubations was not related to the chemically extractable OC-Fe pool, while anoxic sediment Fe release was, suggesting different controls over DOC and Fe release. The high between-lake variability in Fe-bound OC and DOC release illustrates that more studies on phase transitions (DOC-POC) in lake sediments are needed.

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1 **Tables**

2

3 Table 1. Characteristics of the studied lakes.

Lake	Lötsjön	Tvigölingen	Skogsjön	Övre Skärsjön	Erssjön
Coordinates	59°51´N 17°56´E	60°4´N 17°23´E	58°32´N 14°20´E	59°50´N 15°32´E	58°22´N 12°10´E
Depth (m)	10	3	2.5	28	5
pH[a]	7.1 - 8.1 (7.6±0.3)	6.4 - 7.0 (6.6±0.2)	5.0 - 5.7 (5.4±0.2)	5.4 - 6.1 (5.7±0.2)	5.3 - 6.6 (5.89±0.33)
DOC (mmol L^{-1})[a]	0.81 - 0.98 (0.89±0.05)	3.18 - 3.75 (3.47±0.22)	1.82 - 2.54 (2.1±0.24)	0.68 - 0.88 (0.78±0.07)	1.7 - 2.7 (2.05±0.37)
Tot-N (µmol L^{-1})	77.8±18.8[d]	58.6–97.1[e]	963[d]	29.9±6.0[d]	48 - 253 (98±46)[c]
Tot-P (µmol L^{-1})	0.97±0.35 (0.48–1.71)[f]	0.42–1.10[e]	0.87[d]	0.27±0.12[d]	0.7 - 2.8 (1.3±0.5)[c]
Fe (µmol L^{-1})[a]	0.4 - 98.5 (7.48±23.02)	9.9 - 42 (16.18±9.7)	8.1 - 68.2 (21.8±15.74)	5.6 - 19.8 (11.72±4.87)	5.6 - 86.6 (19.68±18.13)
Sediment OC (%)[b]	13.9±0.2	29.2±0.2	38.2±0.7	17.6±0.4	20.4±0.6
Sediment C:N[b]	10.1±0.1	17.2±0.2	19.3±2.9	20.4±1.1	16.1±0.5
Sediment S (%)[b]	0.59±0.03	1.01±0.16	1.25±0.41	0.21±0.03	0.43±0.03

4 a) min, max and mean ± 1 SD from 4 different depths during 3 sampling campaigns in summer 2014 for

5 lakes L, T, S and OS (Agstam 2015). For Lake Erssjön data from samples from 4-5 different depths

6 sampled in 2014 and published in Peter et al. 2016. Fe concentrations are dissolved concentrations.

7 b) Sediment data from Peter et al. 2016 except Lake Erssjön from Peter et al. 2017

8 c) TP and TN lake Erssjön sampled during 2012-2013 at 0.5 and 4 m depth within the monitoring program

9 of the Skogaryd research catchment (http://www.fieldsites.se)

10 d) multiannual mean of different depths (except for Lake Skogsjön only one data point available) from

11 the Swedish lake monitoring program

12 e) Depth integrated samples (1-2 m intervals) from May, July, October 1996 and February 1997

13 (Lindström 2000)

14 f) interannual mean (1997-2003), Langenheder et al. 2005

15
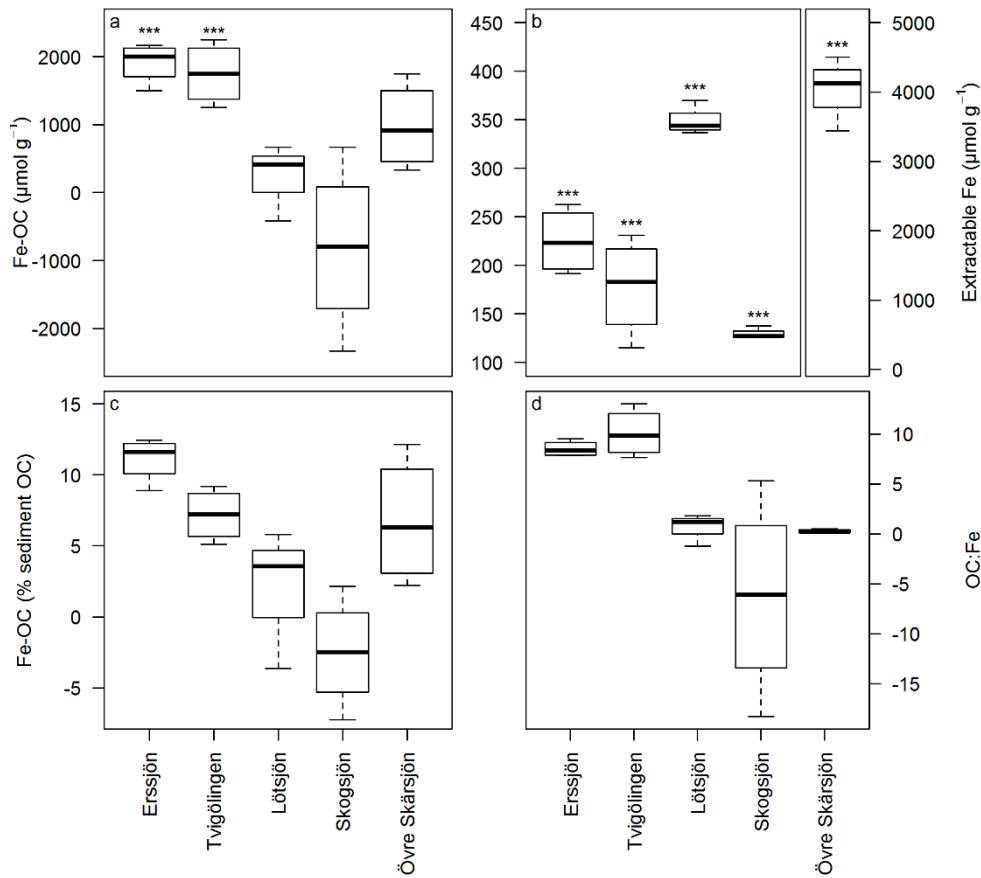
Figures

Figure 1. Fe-bound OC in different boreal lake sediments released during CBD-extraction,
expressed as amount Fe-bound OC per sediment mass (μmol g^1; panel a) and as proportion of the
total sediment OC pool (%; panel c). Amount of Fe released from sediment during CBD
extraction (μmol g^1; panel b) and molar ratios of OC:Fe released during CBD extraction (panel
d). Asterisks indicate significant differences ($p < 0.01$) from Student's t-tests between OC and Fe
content, respectively, of CBD-extracted and CBD-control samples. Boxes represent the upper and
lower quartiles of the data and whiskers the highest and lowest data points that are not outliers
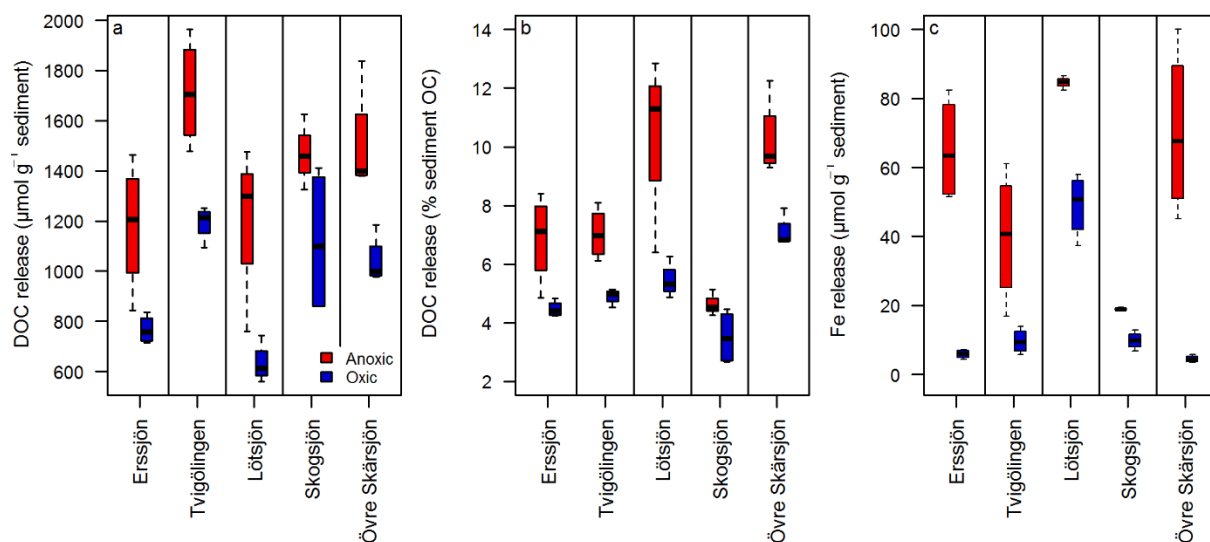(more than 1.5 times the respective quartile, indicated by points).

Figure 2. Amount (μmol g^{-1}; a) and percentage (%; b) of DOC release, and amount of Fe release (μmol g^{-1}; c) during oxic (blue) and anoxic (red) sediment incubations. Boxes represent the upper and lower quartiles of the data and whiskers the highest and lowest data points that are not outliers (more than 1.5 times the respective quartile, indicated by points).
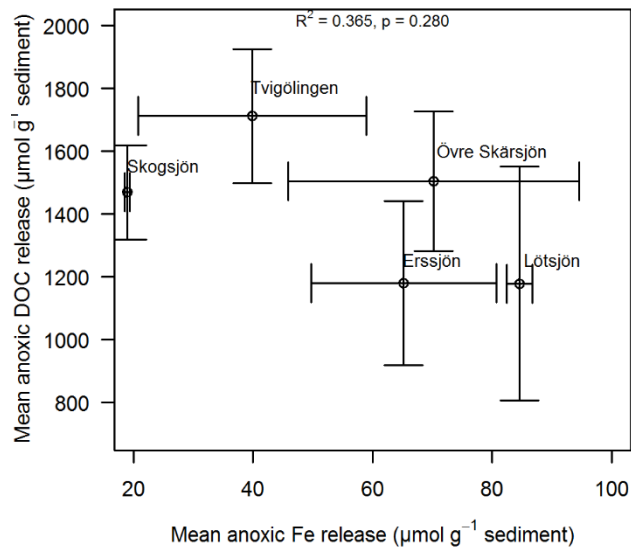
Figure 3. Mean total DOC vs. Fe release of all the lakes during the anoxic sediment slurry experiment. Error bars indicate standard deviations.
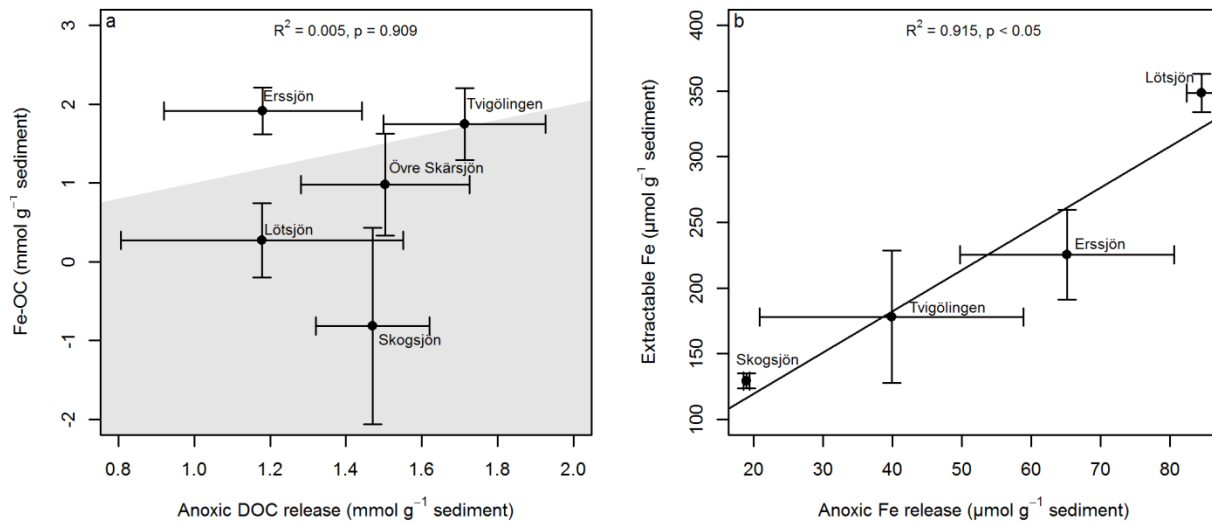
Figure 4. Comparison between DOC (panel a) and Fe (panel b) release during anoxic sediment slurry incubations and CBD extraction. The white-grey boundary represents the 1:1 line for Fe-OC to anoxic DOC release and hence, in lakes lying in the grey shaded area, anoxic DOC release exceeded the amount of CBD-extractable Fe-bound OC. In the regression in panel (b), Lake Övre Skärsjön was excluded since Fe release during CBD extraction was over one order of magnitude higher than in the other lake sediments; see Fig. 1b and text for details. Error bars indicate standard deviations.