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# Widespread release of dissolved organic carbon from anoxic boreal lake sediments

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# Abstract

Sediments in boreal lakes are important components of the carbon cycle because they receive and store large amounts of organic carbon (OC), and at the same time are a source of greenhouse gases. Under anoxic condition, sediment OC can be lost through dissolution from the solid phase and subsequent diffusion to the water column. Even though this process may have implications for sediment OC budgets, it has yet not been studied systematically. We combined laboratory sediment incubation experiments from four boreal lakes in central Sweden that differed widely in their biogeochemical conditions with data from the Swedish monitoring program covering >100 lakes, to analyze the frequency of occurrence of sediment DOC loss in boreal lakes, and to identify lake characteristics and the conditions that are related to high DOC fluxes from anoxic sediments. We found DOC diffusion from anoxic sediments in all the anoxic sediment incubations, however at different mean rates (0.7 – 3.7 mmol $m^{-2}$ $d^{-1}$). Similarly, 16 out of 17 of the monitoring lakes that developed anoxic bottom water exhibited an increase in bottom water DOC concentration, corresponding to a mean DOC diffusion flux from anoxic sediment of 11.1 ± 35.4 mmol $m^{-2}$ $d^{-1}$. The observed variability between lakes was related to particularly large DOC fluxes in humic-rich lakes, which we attribute to their low pH and high share of terrestrial OC favoring the formation of OC-Fe aggregates. Accordingly, increasing pH might facilitate the dissolution of sediment OC since high DOC fluxes from anoxic sediments were accompanied by high microbial iron and sulfate reduction resulting in concomitant pH increase.

# Introduction

Boreal lakes receive, transport, transform, and store large amount of terrestrial-derived organic carbon (OC) (Cole et al. 2007). The sediments of boreal lakes are of great importance because they usually receive large amounts of terrestrial OC, which is either buried and stored in the sediment, or degraded to the greenhouse gases $CO_2$ and $CH_4$, which eventually can be released to the atmosphere (Sobek et al. 2009). Accordingly, boreal lake sediments have both been described as significant and stable long-term carbon sinks (Chmiel 2015; Kortelainen et al. 2004), and as important sources of $CO_2$ and $CH_4$ to the atmosphere (Bastviken et al. 2011; Kortelainen et al. 2006). In addition to these relatively well-described sediment C fluxes, OC can be lost from the sediment under anoxic conditions through dissolution from the solid phase and subsequent diffusion of dissolved organic carbon (DOC) across the sediment water interface, as has been shown for marine sediments (Skoog and Arias-Esquivel 2009). A recent study of a boreal lake demonstrated that diffusive DOC loss from anoxic sediment can affect the sediment C budget (Peter et al. 2016). Sediments of boreal lakes are typically rich in OC (e.g., Chmiel 2015), and the hypolimnia of small boreal lakes are frequently oxygen-deficient thanks to the high water color promoting stable stratification (Fee et al. 1996). Hence, it seems plausible that diffusive DOC loss from anoxic sediments may be a common feature in boreal lakes. However, currently it is unclear how widespread this process is in boreal lakes, and which factors regulate it. Furthermore, boreal lakes are very numerous (Verpoorter et al. 2014) and play an important role in global C cycling (Raymond et al. 2013); hence studies on anoxic DOC diffusion from boreal lake sediments are warranted.

DOC diffusion from anoxic sediments is related to reductive iron (Fe) dissolution in sediments (Peter et al. 2016; Skoog and Arias-Esquivel 2009). Under oxic condition, DOC sorbs to Fe(III) oxide-hydroxides which promotes aggregation and sedimentation of the complex (Lalonde et al. 2012; Tipping and Woof 1983). The sorption of OC to Fe(III) strongly controls the stability of OC in soils and sediments, as it protects the OC from microbial degradation (Boudot 1989; Jones and Edwards 1998; Keil et al. 1994; Lalonde et al. 2012). Under anoxic conditions, Fe(III) is reduced and dissolves as Fe(II) into pore water. OC that was previously sorbed to Fe(III) is concomitantly released (O'loughlin and Chin 2004) and can diffuse across the sediment water interface into anoxic bottom water (Peter et al. 2016; Skoog and Arias-Esquivel 2009). Similarly, Fe reduction in anoxic lake sediments has been repeatedly shown to be important for the release and cycling of other compounds like phosphate ($PO_4^{3-}$) (Mortimer 1941) and arsenic (Smedley and Kinniburgh 2013).

Besides anoxia, other environmental factors may potentially affect OC-Fe interaction and sediment DOC diffusion, in analogy to sedimentary phosphorus (P) exchange which is a complex process depending on sediment characteristics and biogeochemical conditions (Hupfer and Lewandowski 2008). Sediment DOC diffusion upon reductive Fe dissolution has previously been observed in contrasting aquatic systems: a boreal lake (Peter et al. 2016), a drinking water reservoir (Dadi et al. 2015a) and in a marine sediment (Skoog and Arias-Esquivel 2009), suggesting that this process may occur under different environmental conditions. For example, the formation conditions of the OC-Fe complex, e.g., pH and solute concentrations, can be important for its stability (Kleber et al. 2015). Increasing molar metal-to-carbon ratios increase the probability that the metal will react with OC while pH controls the solubility and speciation of the reaction partners, leading to stronger sorption and co-precipitation at low pH. Dadi et al. (2015a) found that DOC fluxes were not only hindered by oxygen, but also by nitrate ($NO_3^-$) availability and that DOC release was strongly coupled to Fe and sulfate ($SO_4^{2-}$) reduction activity. In addition, they identified temperature as an additional controlling factor by mediating microbial reduction activity. Accordingly, also the source of sediment OC (e.g., autochthonous vs. allochthonous OC) and nutrient availability could have an influence on OC release through reductive Fe dissolution by affecting microbial activity (Sobek et al. 2009). Land-derived, humic OC has been shown to sorb to Fe(III) preferentially (Kaiser and Guggenberger 2000; Riedel et al. 2013) and accordingly the flocculation of terrestrial DOC, in part together with Fe(III), has been shown to be a major precursor of sediment material in boreal lakes (von Wachenfeldt et al. 2008; von Wachenfeldt and Tranvik 2008). Consequently, the quality of the OC might play a role in controlling OC release through reductive Fe dissolution. However, sediment OC might additionally be released from the solid phase through processes other than reductive Fe reduction like changes in pH and microbial enzymatic hydrolysis (Grybos et al. 2009; Li et al. 2012). Although Fe-DOC interactions are probably a prominent feature of boreal aquatic systems, as they typically contain large amounts of terrestrially derived humic OC (Sobek et al. 2007; Thurman 1985) and are characterized by tightly coupled OC and Fe cycles (Köhler et al. 2013; Kritzberg and Ekström 2012; Weyhenmeyer et al. 2014) it is likely that anoxic DOC diffusion will vary in magnitude between different lake systems, and it is important to get an understanding of the underlying biogeochemical mechanisms in order to gauge the generality of this process.

In this study, we investigated DOC diffusion from anoxic sediments in boreal lakes with the expectation that DOC diffusion is a common phenomenon in anoxic boreal lake sediments. We hypothesize that: (1) there are differences in DOC fluxes between the studied lakes, (2) these differences are driven by biogeochemical characteristics of the lakes, namely OC quantity and quality, Fe concentration, productivity and pH and (3) DOC flux from anoxic sediments is driven by increasingly reducing conditions and increasing pH. We studied in detail four typical boreal lakes in central Sweden that differed widely in their biogeochemical conditions (i.e., pH, nutrients and DOC concentration) using laboratory incubation experiments. Additionally, we analyzed data from the Swedish lake monitoring program for bottom water DOC concentration during periods with anoxic hypolimnion, to relate anoxic DOC fluxes to different prevailing biogeochemical conditions and lake characteristics. The combination of incubation data and monitoring data help to generate a more general picture of the occurrence, regulation and importance of sediment DOC diffusion during anoxia in boreal lakes.

# Material and methods

## *Sampled lakes*

Laboratory incubation experiments were carried out on sediment from four lakes in central Sweden. The lakes are typical for the boreal region (i.e., small and shallow), and they were selected to achieve strong variation in biogeochemical conditions that are expected to have an influence on DOC diffusion from the sediments, such as sediment OC quality (C to nitrogen (N) ratio), DOC concentration, productivity and pH (Table 1). Lake Övre Skärsjön is the largest and deepest of the investigated lakes and located in a mineral-rich former Fe-mining area (Andersson 2004). It is oligotrophic and had the lowest pH and DOC concentration among the investigated lakes. Lake Skogsjön is the smallest and shallowest of the four lakes and completely surrounded by forest. It is a mesotrophic brown water lake with high DOC concentrations and low pH. Lakes Lötsjön and Tvigölingen are both meso- to eutrophic lakes of high pH. Lake Lötsjön has low DOC concentration while Lake Tvigölingen is high in colored DOC concentration.

## *Sediment sampling and incubation experiments*

From each of the four lakes, 12 intact sediment cores were retrieved during summer 2014 with a Uwitec gravity corer (using tubes of 57 mm inner diameter and 100 cm length) at the deepest point of the lake. Additional water to fill the sediment incubations in the laboratory was collected close to the sediment with a Ruttner sampler. Sediment incubations followed with minor adjustments the protocol by Peter et al. (2016). The top 7 cm of sediments were directly sliced as intact cores without mixing into oxygen impermeable UPVC incubation tubes (57 mm inner diameter and 6 mm wall thickness), using a custom made slicing device and filled with lake water to 25 cm above sediment. The cores were kept in a dark, temperature controlled water bath at 10°C, which was close to sampling *in situ* temperature. Inside the cores a floating stirring device (a buoyant 2 mL Eppendorf vial containing small magnets and construction foam), was agitated by an axle with 4 magnetic arms rotating in the water bath outside the cores at 24 rpm. This assured continuous mixing of the water column inside the incubation tubes, without resuspending the sediment. Before starting the experiment, the cores were incubated open for one week to adjust them to the experimental temperature and assure that the water and the upper sediment layers were oxic before starting the experiment. Six cores per lake were then made anoxic by flushing the water above sediment for 15-30 minutes with nitrogen gas, and immediately closed with an air-tight lid equipped with triple o-rings. Six other cores per lake were incubated without closing to allow the water and the upper sediment layer to remain oxic. Water samples were taken immediately after closing the cores and at day 7, 22 and 37, based on experience with previous sediment incubations from the Swedish lake Erssjön where DOC diffusion fluxes did not start before day 3 and continued until day 24 (Peter et al. 2016). Water overlying anoxic sediment was sampled by pushing the top stopper downward, forcing water to flow out through a Tygon tube connected to the stopper's outlet. From there water for DOC and Fe analyses was collected in oxygen free syringes, which were attached to the Tygon tube by a luer-lock valve. pH was instantly determined after sampling on a mobile pH meter (Metrohm 826, equipped with Aquatrode Plus electrode and calibrated with Merck Certipur buffers) (Table S1). Samples for DOC and Fe from the anoxic cores were immediately transferred to a glove box (Belle Technology MR3) filled with a $N_2$ atmosphere that was constantly monitored for $O_2$ concentration to avoid oxygen contamination of the samples ($O_2$ concentration inside the box was usually around 12 µmol $L^{-1}$) and filtered through GF/F glass-fibre filters. Fe fixation with ferrozine was started in the glove box immediately after filtration. Water from oxic incubations was retrieved from open cores with a Tygon tube connected to a 50 mL syringe. Oxygen concentration was measured with an oxygen optode sensor (PreSens OXY-10 mini) and was in anoxic incubations 0 µmol $L^{-1}$ from the first sampling occasion, and in oxic incubations on average 348 µmol $L^{-1}$, corresponding to ~100% saturation. Changing volume in the incubation tubes due to sample retrieval was previously confirmed to have no significant influence on the diffusive fluxes (Peter et al. 2016). One replicate of oxic incubations from Lake Skogsjön and three replicates from anoxic Lake Övre Skärsjön incubations had to be excluded from the results due to accidental sediment disturbance during sampling. Areal DOC and Fe fluxes (mol $m^{-2}$ $d^{-1}$) from sediment into the overlaying water were calculated by multiplying per day concentration difference (mol $m^{-3}$ $d^{-1}$, equivalent to mmol $L^{-1}$ $d^{-1}$) between samplings with the height of the water in the incubation tubes (m). Since the mixing speed of the overlying water and temperature were identical for all cores and throughout the experiment, physical conditions such as diffusive boundary layer thickness and thus diffusion path length stayed constant and did not contribute to the observed variability in DOC diffusion fluxes (Gudasz et al. 2010; Peter et al. 2016).

## *Chemical analyses*

DOC was determined by high-temperature catalytic oxidation on a Shimadzu TOC-L analyzer with an ASI-L auto sampler. For Fe(II) and Fe(III) measurements, samples were immediately analyzed for absorbance at 562 nm (Perkin Elmer, Waltham, USA) after adding a ferrozine solution following an adjusted protocol of the ferrozine method by Viollier et al. (2000), detailed in (Peter et al. 2016). Sediment OC, N and S content were determined on freeze dried sediments, combusted in an elemental analyzer (NA 1500, Carlo Erba instruments).

## *Swedish lake monitoring data set*

Data on concentrations of DOC and $O_2$ were obtained from the Swedish monitoring program of lakes, which is provided by the laboratory of the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences. Starting in 1983, 110 lakes, distributed across the country, were sampled 4–8 times a year at 1–3 depths and analyzed according to standard limnological methods. Method descriptions and all data can be freely downloaded at http://webstar.vatten.slu.se/db.html. For this study, we included only lakes that contained bottom water $O_2$ data points below 63 µmol $L^{-1}$ at two consecutive months (17 lakes). Since the deepest bottom water sample is typically taken at 1 m or more above the sediment, an $O_2$ concentration of <63 µmol $L^{-1}$ indicates a high probability of the sediment surface being anoxic. Most of these lakes (except Gipsjön and Älgsjön) were also part of the Swedish reference lake monitoring program, and were in addition to the long-term monitoring intensely sampled and described during 1989-1993 (Persson 1996). The Swedish monitoring program measures TOC instead of DOC, but since >90% of the TOC is in dissolved form in boreal surface waters (Mattsson et al. 2005), we use the term "DOC" in this paper. When we found two subsequent months with suboxic bottom water, DOC diffusion fluxes were calculated as the change in DOC concentration per day multiplied by the hypolimnion depth of the respective lake (see below). We assumed that no mixing of water masses has occurred between samplings, and that the observed changes in bottom DOC concentrations can be ascribed to diffusion from the sediment. Similar hypolimnetic accumulation approaches have been applied to derive P fluxes and were found to match laboratory flux estimates (e.g., Nürnberg 1987). However, assessing fluxes through a hypolimnion accumulation approach rather represent net fluxes of the compounds, because rates are assessed over relatively long time periods and compounds might undergo additional biogeochemical processes during that time, and are therefore not always directly comparable with laboratory fluxes (Nowlin et al. 2005). Average hypolimnion depth, the distance from the lake bottom to the thermocline, was calculated from hypolimnetic lake volume and hypolimnetic bottom area data that is available as multi-year average for the Swedish reference lakes (Persson 1996), in analogy to calculations in (Liboriussen et al. 2009). For Lakes Gipsjön and Älgsjön no lake morphometry data except lake depth were available and hence, hypolimnion depth was estimated based on the linear relationship between max lake depth and average hypolimnion depth observed in the other lakes: hypolimnion depth = 0.2779 * lake depth −0.4476, $R^2 = 0.787$, $p < 0.01$. In the same way we calculated, whenever data was available from the Swedish monitoring program, fluxes of redox sensitive compounds (Fe, $SO_4^{2-}$, $NO_3^-$, ammonium ($NH_4^+$), $PO_4^{3-}$ and manganese (Mn)) as well as changes of temperature and pH during the same periods as for DOC fluxes.

### *Data analysis*

With the incubation data, linear mixed effects (lme) models were performed: (1) for each lake to test the effect of anoxia on DOC and Fe concentrations in the water of the sediment incubations and (2) to test for significant differences between the lakes in DOC fluxes from the anoxic sediment incubations. The explanatory variables in lme models are included as either fixed effect (influencing the mean of the response variable) or random effect (influencing its variance) (Crawley 2007). The basic model included incubation $O_2$ conditions (anoxic or oxic) or the different lakes as fixed effects and the core replicates and time as random effects. Model extensions were tested for a variance function which allows different variances of the response variable per level of the fixed effect, and/or a first order temporal autoregressive process, which assumes that correlation between measurements decreases with increasing time distance. If this improved the relative goodness of the model fit, the model was included in the basic model described above. The models were checked using diagnostic residual plots and the significance of the fixed effect was assessed by analysis of variance (Crawley 2007). To confirm the robustness of the statistical test, and in cases when the lme models were too complex to converge, multivariate repeated-measures analysis of variance (rMANOVA) was used with $O_2$ conditions or lakes and time as interacting explanatory variables (Gueorguieva and Krystal 2004). The lme model is generally more conservative compared to rMANOVA. Simultaneous inference procedures were used to adjust for multiplicity and corrected *p* values are reported (Hothorn et al. 2008).

Data from the Swedish monitoring program were analyzed for: (1) significant DOC fluxes during anoxic periods, (2) significant differences in DOC fluxes between the lakes, (3) biogeochemical characteristics of the lakes favoring increased DOC fluxes and (4) effect of temperature, pH and redox sensitive compounds on DOC fluxes. To test the effect of anoxia on DOC fluxes (1), we performed Student's *t*-tests for every lake and assessed if DOC diffusion fluxes were significantly positive during anoxic periods. The occurrence of significant differences in sediment DOC fluxes (2) was tested by a Kruskal-Wallis test. To investigate how DOC fluxes are related to lake biogeochemical conditions (3) we used partial least square (PLS) regression (R package plsdepot). We used PLS regression for statistical analysis of the monitoring data because it is relatively tolerant against interdependence of variables on each other, deviations from normality and missing values. Also, PLS regression extracts the variability in the explanatory (X) variables that explains the variability in the response (Y) variable. Hence, it is capable of depicting how the different explanatory variables, by themselves and in concert with other variables, are related to DOC diffusion from anoxic sediment. Mean DOC diffusion fluxes were related to biogeochemical variables available from the Swedish lake monitoring program (including all lake water concentrations over all depths and seasons of DOC, Fe, total N (tot-N), $PO_4^{3-}$, Mn, $NH_4^+$, total P, $SO_4^{2-}$, magnesium (Mg) and calcium (Ca), water alkalinity and pH, content of sediment OC, Fe, aluminum (Al) and N and sediment ratios of C:N and Al:Fe). Variable importance in projection (VIP) was calculated as described in (Chong and Jun 2005). Additionally, with the strongest predictors identified by the PLS we performed single correlation analyses between DOC flux and lake characteristics (3). How pH and temperature changes and fluxes of other redox sensitive compounds during anoxic periods are related to DOC fluxes in the lakes (4) was tested by means of correlation analyses of DOC fluxes with changes in pH and temperature and fluxes of Fe, $SO_4^{2-}$, $NO_3^-$, $NH_4^+$, $PO_4^{3-}$ and Mn during anoxic periods. If necessary, variables were log or square root transformed to approach normal distribution. The statistical analyses were implemented using the software R (version 3.1.1). Generally, results are reported as mean values ± 1 standard deviation. Units were all reported in mol (e.g., transforming mg DOC to mmol DOC by dividing by the molar mass of C (~12 g/mol)).

# Results

## *DOC and Fe diffusion in sediment incubations*

In anoxic incubations, DOC concentration in water overlaying the sediment increased in 37 days within a relatively narrow concentration range for all the different lakes (0.15 ± 0.06 – 0.76 ± 0.47 mmol $L^{-1}$; Fig. 1), resulting in time-weighted mean DOC diffusive fluxes between 0.7 ± 0.8 and 3.7 ± 3.8 mmol $m^{-2}$ $d^{-1}$ (Fig. 2). Sediment DOC fluxes in the anoxic sediment incubations were not significantly different among the lakes (lme $p < 0.279$; rMANOVA $p = 0.056$), but there seemed to be tendency towards a higher mean DOC flux in Lake Övre Skärsjön and lower DOC flux in Lake Lötsjön incubations. Generally, it appeared that the lakes with low pH and high sediment C to N ratios (i.e. Skogsjön and Övre Skärsjön) had higher average DOC diffusive fluxes (Fig. 2A; Table 1). The individual lakes showed different temporal dynamics in DOC diffusive fluxes over the course of the anoxic incubations. While fluxes in Lake Lötsjön and Övre Skärsjön were highest at the end of the incubation (day 37), fluxes in Lake Tvigölingen and Skogsjön peaked at day 22 and decreased at the end of incubation (Fig. 2B).

In all lakes, DOC concentrations in overlying water of anoxic incubations were significantly different from DOC concentrations in overlying water of oxic incubations when tested with rMANOVA (Fig. 1). The lme models confirmed the significant differences between DOC concentration in oxic and anoxic incubations for Lake Lötsjön and Skogsjön, and almost for Lake Övre Skärsjön ($p = 0.055$); for Lake Tvigölingen, the lme model did not converge, i.e., was too complex to be computed. Oxic incubations revealed a decreasing trend in DOC concentrations over time, except in incubations with Lake Övre Skärsjön sediments (Fig. 1).

Similar to DOC concentrations, also Fe concentrations in overlying water increased during anoxic incubations, and stayed constant during oxic incubations, except for Lake Övre Skärsjön incubations where oxic Fe concentrations increased by 74.6 ± 31.1 µmol $L^{-1}$ (Fig.1). Fe concentrations increased within a range of 26.7 ± 9.7 to 175.3 ± 9.9 µmol $L^{-1}$, and highest time-weighted mean anoxic diffusive Fe fluxes were found in Lake Övre Skärsjön (701.1 ± 665.2 µmol $m^{-2}$ $d^{-1}$), followed by Lake Lötsjön (515.4 ± 477.7 µmol $m^{-2}$ $d^{-1}$), whereas Lakes Tvigölingen and Skogsjön exhibited lower fluxes (182.6 ± 191.0 and 108.7 ± 233.0 µmol $m^{-2}$ $d^{-1}$, respectively; Fig. 2D). Anoxic Fe fluxes were significantly different among lakes when analyzed with the lme model ($p < 0.05$) but not with rMANOVA ($p = 0.659$). Generally, the lakes with high Fe fluxes (Övre Skärsjön and Lötsjön) showed lowest sediment OC and S contents (Table 1). The increase of Fe concentration in anoxic sediment incubations was significantly different than in oxic incubations when tested with rMANOVA (Fig. 1). The lme model, which is the more conservative statistical analysis, did not converge except for Lake Skogsjön, where it indicated no significant difference between anoxic and oxic Fe concentrations. Unlike anoxic DOC concentration, Fe concentrations increased steadily during the incubations in all lakes. The contribution of Fe(II) to Fe(tot) increased during anoxic sediment incubations for all lakes (Table S2). Highest contribution of Fe(II) to total Fe after 37 days, the end of incubations, was found for Lake Lötsjön (92.1 ± 1.2%) while Lake Övre Skärsjön exhibited the lowest (65.0 ± 5.4%) and Lake Skogsjön and Tvigölingen had similar contributions (88.2 ± 4.8% and 86.2 ± 7.8%, respectively).

There was a significant and positive relationship between DOC and Fe concentration increase in anoxic incubations in all lakes, but the strength and slopes of these relationships were different among lakes (Fig. 3). While three of the lakes exposed strong and highly significant relationships between DOC and Fe concentration increase (linear regression, $R^2$= 0.60-0.84, $p < 0.001$), the relationship was weak in Övre Skärsjön ($R^2 = 0.34$, $p = 0.043$; Fig.

3). Further, the lakes exposed different ratios of the amount of DOC release to the amount Fe release during the incubations (ΔDOC:ΔFe). The ratio of ΔDOC:ΔFe at incubation end followed the same pattern as the slopes of the linear regressions (Fig. 3), with lowest ratios for Lakes Lötsjön (1.4 ± 0.4) and Övre Skärsjön (4.75 ± 3.21) and higher ratios for Lakes Tvigölingen and Skogsjön (6.7 ± 3.4 and 17.1 ± 7.0, respectively).

### *DOC fluxes from sediments in anoxic bottom water of monitored Swedish lakes*

Among the 110 examined lakes that have been frequently monitored, we found 17 lakes with bottom water DOC concentration data during suboxic (<63 μmol $O_2$ $L^{-1}$) conditions at two consecutive sampling occasions (e.g., during summer stratification). Since every lake was monitored over several years, and since some lakes had more than two consecutive suboxic sampling occasions, we were able to calculate in total 349 DOC fluxes in the suboxic bottom water (Fig. 4), of which 235 DOC fluxes were positive (67%). The mean diffusive DOC flux from suboxic sediment across all 17 lakes was 11.1 ± 35.4 mmol DOC $m^{-2}$ $d^{-1}$ (Fig. 4) which was significantly higher than zero (*t*-test, $p < 0.001$). When considering different months, mean DOC fluxes for all lakes were positive for all sampled months, except June where the mean DOC flux of 10 rates was negative (Fig. S1). Since 332 of 349 rates of DOC concentration change at suboxic bottom water can be attributed to summer stratification, an analysis of seasonality in sediment DOC diffusion was not possible. For individual lakes, the mean DOC fluxes were all positive, except for Lake Allgjuttern (Fig. 4), but significantly different from zero only for 5 of 17 lakes (*t*-test *p* for Brunnsjön = 0.002, Älgsjön = 0.011, Rotehogstjärnen = 0.001, Stora Envättern = 0.009 and St Skärsjön = 0.015; Table S3).

Comparing DOC fluxes between the lakes revealed a significant differences between them (Kruskal-Wallis rank sum test: $p = 0.029$). The first and second principal component in the PLS regression model with environmental concentration data (Table S3) explained 67% and 12%, respectively, of the variability in DOC fluxes between the monitored lakes (Fig. 5). Important predictors of DOC fluxes (VIP > 1 over the first two components) were lake water concentrations of DOC, Fe, $PO_4^{3-}$, total nitrogen (tot-N), and Mn, as well as sediment OC concentration and C to N ratio (sedOC, sedC:N), all of which were positively related to DOC flux. A negative relationship with sediment DOC flux was found for lake water pH. Hence, characteristics of humic-rich boreal lakes (high DOC, high Fe, high C to N ratio, low pH) were associated with high DOC flux from anoxic sediment. In accordance, correlation analyses with mean DOC fluxes were positive for sediment C to N ratio ($r = 0.53$, $p < 0.001$) and OC content ($r = 0.17$, $p < 0.001$), Fe ($r = 0.35$, $p < 0.001$), TOC ($r = 0.23$, $p < 0.001$), totN ($r = 0.10$, $p < 0.001$) and $PO_4^{3-}$ ($r = 0.17$, $p < 0.001$) concentrations. Negative relationships with DOC fluxes were found for pH ($r = -0.37$, $p < 0.001$) and Mn ($r = -0.04$, $p < 0.001$).

During anoxic periods, mean fluxes of Fe, $NH_4^+$, Mn and $PO_4^{3-}$ and increase in temperature were positive (Table 2). For these compounds, positive correlations with DOC fluxes were found for Fe ($r = 0.29$, $p < 0.05$), $NH_4^+$ ($r = 0.29$, $p < 0.0015$), and $PO_4^{3-}$ ($r = 0.14$, $p < 0.01$) and a negative correlation for temperature changes ($r = -0.11$, $p < 0.05$). During the same period, fluxes for $SO_4^{2-}$ and $NO_3^-$ and pH change were negative and correlation with DOC flux negative for $SO_4^{2-}$ ($r = -0.23$, $p < 0.01$) and pH change ($r = 0.18$, $p < 0.001$).

Fe fluxes in anoxic bottom water could only be calculated for 7 lakes due to lack of data during anoxic periods (Fig. 4). For most of the individual lakes, DOC concentration correlated significant positively with Fe concentration, however with different slopes of the regression (Stora Envättern slope = 10.16, $R^2 = 0.28$, $p < 0.01$, Älgsjön slope = 6.41, $R^2 = 0.64$, $p < 0.05$, Rotehogstjärnen slope = 4.53, $R^2 = 0.27$, $p < 0.01$, Brunnsjön slope = 3.83, $R^2$

= 0.28, $p < 0.05$ and St Skärsjön slope = 0.92, $R^2 = 0.27$, $p < 0.01$; Fig. S2). The ratio of mean DOC concentration changes to mean Fe concentration changes (ΔDOC:ΔFe) were positive for Brunnsjön (2.1), St Skärsjön (2.0), Älgsjön (1.6), Rotehogstjärnen (0.5) and Stora Envättern (0.3) and negative for Fräcksjön (−0.5).

# Discussion

## *DOC diffusion from anoxic sediments is widespread in boreal lakes*

This study shows that DOC concentration increase in anoxic bottom water of lakes is a widespread phenomenon that can occur across a variety of different boreal lakes. Incubation experiments using sediment from four boreal lakes that differed with respect to organic carbon content and origin, pH and nutrients, showed consistently that the DOC concentration increased in anoxic, but not oxic water overlying the sediment. Similarly, the DOC concentration increased in suboxic bottom water in 16 of 17 lakes from the Swedish lake monitoring program that matched the selection criteria of this study (see methods), although this change was statistically significant only for 5 of the lakes. Of 349 calculated DOC concentration changes in suboxic bottom water, 235 were positive.

Results from a previous study using a similar experimental incubation procedure indicated a good match between DOC diffusion fluxes from sediment derived from laboratory experiments and *in situ* field measurements (Peter et al. 2016). Mean DOC fluxes derived from incubation experiments of the present study were in the same range (0.7 and 3.7 mmol $m^{-2}$ $d^{-1}$) as previously reported DOC fluxes from boreal Lake Erssjön (3.3 mmol $m^{-2}$ $d^{-1}$; Peter et al. 2016) and from a drinking water reservoir (2.31 ± 0.66 mmol $m^{-2}$ $d^{-1}$; Dadi et al. 2015b). However, the comparison of mean fluxes is difficult since fluxes are not constant over time, i.e., they approached zero towards the end of incubation when the concentration gradient between sediment pore water and water above the sediment diminishes. Fluxes from the 17 monitored Swedish lakes (mean, 11.1 ± 35.4 mmol DOC $m^{-2}$ $d^{-1}$) were generally higher than fluxes in sediment incubations. In the case of Lake Övre Skärsjön, which was part of both, experiments and the lake monitoring program, we were able to compare results from laboratory and *in situ* measurements and found that fluxes calculated from the monitoring data considerably exceeded fluxes derived from laboratory incubations (61 ± 50 mmol $m^{-2}$ $d^{-1}$ vs. 3.7 ± 3.8 mmol $m^{-2}$ $d^{-1}$). One reason for this might be that the applied hypolimnion depth, which was derived as a multi-year average based on monitoring data, was too high (e.g., the thermocline was assumed to be too shallow). This could have artificially increased the magnitude of the flux. Also, it has been shown that temperature can positively affect sediment DOC diffusion by stimulating microbial reduction activity (Dadi et al. 2015a), however, the temperature during experimental incubations (10°C) was higher than in most hypolimnia of the monitoring lakes (7.3 ± 3.0°C). Another reason could be the scarce temporal resolution of *in situ* measurements and that fluxes based on hypolimnetic accumulation rates represent net fluxes, i.e., that additional processes might increase or decrease hypolimnetic concentrations *in situ* (Nowlin et al. 2005). We cannot exclude mixing of water masses or groundwater intrusion between the monthly sampling occasions, which could have led to oxidation of bottom water and either, introduce additional DOC, lead to flocculation of DOC, or dilute hypolimnion DOC concentration. While the selection of suboxic instead of completely anoxic data points could have introduced additional uncertainty, no bias arising from the selection of data points <63 µmol $L^{-1}$ vs. <31 µmol $O_2$ $L^{-1}$ was evident (data not shown). More likely, other processes like groundwater inflow, heavy precipitation events, phytoplankton blooms and sinking organic material might mask the effect of DOC diffusion from sediments and complicate its assessment from monthly-spaced *in-situ* measurements. In addition, the national Swedish lake monitoring program measures TOC, not DOC concentration, and hence organic particles, which may be present, e.g., due to resuspension events, may to an unknown extent be included in the results. These are likely some of the reasons behind the observed considerable variability in sediment DOC fluxes and overall weak correlations calculated from the monitoring data. However, despite the inherent uncertainties in the DOC sediment flux data derived from the national monitoring program, the data demonstrate that in almost all the lakes, there was on average increase in bottom water DOC and Fe concentrations during anoxic periods, indicating that the accumulation of DOC and Fe is widespread in the anoxic hypolimnia of boreal lakes.

### *Lake characteristics favoring enhanced DOC release*

Sediment DOC fluxes at anoxia were different between the analyzed Swedish monitoring lakes. Particularly lakes rich in humic matter, which are characterized by a high DOC and Fe concentration, low pH and high sediment OC content and C to N ratio as a result of high terrestrial OC input, tend to release comparatively larger amounts of DOC from anoxic sediments. And this is consistent with results from the sediment incubation experiments. High pH weakens the binding between Fe and DOC (Kleber et al. 2015), thus in high pH lakes, DOC release from sediment through reductive Fe dissolution might be less relevant because less OC was originally bound to Fe in those sediments. Previous work has also shown that terrestrial OC preferably sorbs to Fe, especially high molecular weight and aromatic compounds (Riedel et al. 2013). To sustain high DOC diffusion from anoxic sediments, the proportion of terrestrial OC in sediments seems to be more important than lability of the OC which could fuel microbial Fe reduction, probably because of the higher susceptibility of allochthonous OC for interactions with Fe. Accordingly, the sediments of Lake Lötsjön, which have the lowest C to N ratio and thus the highest share of relatively easily degradable autochthonous OC (Gudasz et al. 2015; Sobek et al. 2009), released quite a lot of Fe but comparatively little DOC. Likewise, von Wachenfeldt and Tranvik (2008) showed that terrestrial DOC is an important precursor for sediment in boreal lakes and that Fe, next to light and temperature, is involved in the process of DOC flocculation and sedimentation. Possibly, sediment OC that was formed through flocculation of terrestrial DOC with Fe(III) is relatively easily dissolved to DOC as the Fe is reduced to Fe(II). In this respect, it could make sense that the sediment Fe content in itself was not among the most important predictors of sediment DOC flux.

## *Conditions influencing DOC release*

Redox conditions, e.g., oxic vs anoxic conditions, have a pronounced influence on DOC flux from the sediments. Given the high synchrony in DOC and Fe concentration increase in all the anoxic incubations and the Swedish monitoring lakes, it is likely that the release of DOC from sediment is triggered by microbial Fe reduction under anoxic conditions. This suggests that during reductive dissolution of Fe(III) to Fe(II) in anoxic sediments, organic carbon is released from the solid phase into the sediment pore water, from where it can diffuse into the anoxic bottom water. Similar conclusions were drawn in studies of a boreal lake (Peter et al. 2016), a drinking water reservoir (Dadi et al. 2015a), and of marine sediments (Skoog and Arias-Esquivel 2009). $PO_4^{3-}$ fluxes, which are known to be strongly controlled by Fe cycling (Hupfer and Lewandowski 2008; Li et al. 1999), were also correlated with DOC fluxes in the Swedish monitoring lakes, suggesting similar regulatory processes. The negative synchrony of DOC fluxes in the Swedish monitoring lakes with $SO_4^{2-}$ and positive correlation with $NH_4^+$ suggests that anaerobic microbial organic matter degradation and reducing conditions, i.e. low redox potential, are conditions that promote high DOC fluxes, and consequently that microbial activity is an important factor controlling organic matter degradation and reductive activity, as has also been described by (Dadi et al. 2015a). Hence, it seems plausible that the observed DOC increase in many different boreal lakes is related to anoxic DOC diffusion upon Fe(III) reduction in sediments. However, we did not observe a strong effect of temperature on DOC fluxes, as has previously been described for DOC fluxes from anoxic reservoir sediment (Dadi et al. 2015a), which was possibly related to the quite small temperature changes (0.3 ± 1.2°C) in our study. Also, a decrease of DOC release with increasing $NO_3^-$ concentrations can be expected due to the inhibition of Fe reduction by $NO_3^-$ availability (Dadi et al. 2015a). However, only a slight negative effect of $NO_3^-$ fluxes on DOC fluxes were observed. Furthermore, our sediment incubation data indicate that the coupling between reductive Fe dissolution and DOC diffusion to a certain extent is variable, based on different temporal responses of DOC and Fe diffusion and different stoichiometries of DOC and Fe released. It appears that the association of Fe and OC differs between the lakes and might therefore play different roles in anoxic DOC diffusion. Therefore, it is likely that next to microbial reductive Fe dissolution, other mechanisms play a role for DOC release from anoxic sediments. Fe reduction consumes protons, which can introduce an increase in pH leading to a general weakening of OC sorption and hence increased DOC release from solid phase (Grybos et al. 2009). We observed in the Swedish monitoring data that a pH increase during anoxic periods was positively correlated with DOC flux, and hence, it is plausible that pH changes are indeed important for release of sediment OC. Hence, pH not only influences the formation of OC-Fe complexes on the first hand, e.g., low pH lakes are more prone for OC-Fe associations, but also favors the release of OC from sediment material at increasing pH. In the anoxic incubation studies, pH dropped due to buildup of dissolved inorganic carbon in the closed system (Peter et al. 2016) and hence, the effect of increasing pH on DOC fluxes could not be studied in the incubations. Microbial uptake of DOC from sediment pore water can additionally act as a mechanism regulating DOC diffusion under anoxic conditions, since degradation of recalcitrant DOC is reduced under anoxic conditions (Bastviken et al. 2004). In a study with water from sediment incubations of another boreal lake, 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 DOC accumulation in anoxic water. On the other hand, Dadi et al. (2015a) attributed increased anoxic DOC flux rather to the release during Fe reductions than to decreased microbial DOC mineralization. In addition, other nonredox sensitive compounds such as aluminum-hydroxides can sorb OC and efficiently regulate the overall solubility of OC (Boudot 1989); however the analysis of Swedish monitoring data did not provide support for an influencing effect of Al content of the sediment. Without considering the exact mechanisms involved in anoxic DOC releases, the observed consistently significant correlation between OC and Fe and $SO_4^{2-}$ diffusion as well as increasing pH, suggests that in the study lakes, DOC fluxes will be highest when reducing conditions prevail (Fe and $SO_4^{2-}$ reduction) and pH is increasing.

## *Implications*

The combination of laboratory incubations and lake monitoring data demonstrated that DOC diffusion from anoxic sediments is widespread in boreal lakes. Particularly in humic and more acidic lakes and during anoxic periods accompanied by high Fe and $SO_4^{2-}$ reduction activity and increasing hypolimnetic pH, DOC fluxes can be expected to be largest. Interestingly, pH has two contrasting ways of acting on DOC fluxes: on one hand, increasing pH during anoxic conditions might contribute to DOC release through higher OC solubility at higher pH, but on the other hand, lakes that generally exhibit lower pH are more prone for high DOC fluxes, probably due to more OC being bound to Fe and consequently being available for dissolution in those lake sediments.

While it has been previously shown for one boreal lake that DOC dissolution from anoxic sediment may affect the overall sediment C budget (Peter et al. 2016), our study cannot draw any conclusion from the occurrence of sediment DOC release to the quantitative significance of this process. More studies are therefore needed that gauge the quantitative importance of sediment DOC release for lake or sediment carbon budgets. Nevertheless, this study shows that when establishing or interpreting the C balance of sediment, e.g., by calculating the OC burial efficiency, the potential effects of sediment DOC release should be considered, particularly in humic-rich boreal lakes.

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# Tables

**Table 1**. Different biogeochemical conditions of lake water and sediment surface layers used for incubations.

| Lake | Lötsjön (L) | Tvigölingen (T) | Skogsjön (S) | Övre Skärsjön (OS) |
|---|---|---|---|---|
| **Coordinates** | 59°51´45"N; 17°56´30"E | 60°4´39"N; 17°23´19E | 58°32´45"N; 14°20´3"E | 59°50´55"N; 15°32´47"E |
| **Depth (m)** | 10 | 3 | 2.5 | 28 |
| **pH** | 7.7±0.1[1] | 6.9±0.1[1] | 6.3±0.2[1] | 6.1±0.1[1] |
| **DOC (mmol $L^{-1}$)** | 0.95±0.03[1] | 3.4±0.1[1] | 2.7±0.1[1] | 0.9±0.03[1] |
| **Tot-N (µmol $L^{-1}$)** | 77.8±18.8[2] | 58.6–97.1[3] | 963[2] | 29.9±6.0[2] |
| **Tot-P (µmol $L^{-1}$)** | 0.97±0.35 (0.48–1.71)[4] | 0.42–1.10[3] / 0.24–0.48 [4] | 0.87[2] | 0.27±0.12[2] |
| **Fe (µmol $L^{-1}$)** | 8.6±18.7[1] | 24.7±0.4[1] | 60.0±0.8[1] | 34.0±7.6[1] |
| **Sediment OC (%)** | 13.9±0.2 | 29.2±0.2 | 38.2±0.7 | 17.6±0.4 |
| **Sediment C:N** | 10.1±0.1 | 17.2±0.2 | 19.3±2.9 | 20.4±1.1 |
| **Sediment S (%)** | 0.59±0.03 | 1.01±0.16 | 1.25±0.41 | 0.21±0.03 |

[1] starting conditions in sediment incubation experiments
[2] multi-annual mean (except for Lake Skogsjön only one data point was available) from Swedish lake monitoring program
[3] (Lindström 2000)
[4] (Brunberg and Blomqvist 1998; Langenheder et al. 2005).

**Table 2**. Changes in pH and temperature, and fluxes of redox sensitive compounds, calculated from two consecutive months of anoxic bottom water in lakes of the Swedish monitoring program.

| Flux/change | unit | mean | sd | *n* | $r^{§}$ |
|---|---|---|---|---|---|
| pH | | 0.10 | 0.19 | 352 | 0.18*** |
| temp | °C | 0.30 | 1.22 | 351 | −0.11* |
| Fe | mmol $m^{-2}$ $d^{-1}$ | 2.40 | 6.44 | 63 | 0.29* |
| Mn | mmol $m^{-2}$ $d^{-1}$ | 0.25 | 0.53 | 63 | ns |
| $NH_4^+$ | mmol $m^{-2}$ $d^{-1}$ | 0.42 | 1.32 | 352 | 0.29*** |
| $PO_4^{3-}$ | µmol $m^{-2}$ $d^{-1}$ | 0.37 | 18.3 | 352 | 0.14** |
| $NO_3^-$ | mmol $m^{-2}$ $d^{-1}$ | −0.20 | 0.50 | 146 | ns |
| $SO_4^{2-}$ | mmol $m^{-2}$ $d^{-1}$ | −0.88 | 1.71 | 175 | −0.23** |

§ Correlation coefficients and *p*-levels (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant) from Pearson's correlations with DOC fluxes.

# Figure captions

Figure 1. Mean DOC and Fe concentrations in anoxic (black circles) and oxic (open circles) water above sediments in incubation experiments (*n* = 6, except for Övre Skärsjön anoxic where *n* = 3 and Skogsjön oxic where *n* = 5). Grey shaded areas indicate standard deviations. *P*-values resulting from lme models and rMANOVA (rmaov) testing significant differences between concentrations in oxic and anoxic incubations are given (n.c. indicates that lme model did not converge). For the DOC lme model of Skogsjön one extreme outlier on day 22 was excluded.

Figure 2. DOC and Fe diffusion fluxes from anoxic sediment incubations for the different incubation periods (A and C) and as time weighted averages over the entire incubation period (B and D). (*n* = 6, except for Övre Skärsjön where *n* = 3). Error bars show plus one standard deviation.

Figure 3. Relationships between DOC and Fe concentration in the anoxic sediment incubations of the different lakes. *n* = 6, except for Övre Skärsjön where *n* = 3 and for Skogsjön one extreme outlier at day 22 was removed (result with outlier included: slope= 12.1, $R^2 = 0.188$, $p < 0.05$). Note that different scales are used for each lake.

Figure 4. Distribution of DOC (A and B) and Fe (C and D) diffusive fluxes during suboxic periods in all (A and C) and individual (B and D) lakes of the Swedish lake monitoring program. Numbers indicate number of data points. Boxes represent the upper and lower quartiles and central line the median of the data. Whiskers indicate the highest and lowest data points that are not outliers; outliers (open circles) are data points outside 1.5 times the respective quartile.

Figure 5. PLS loadings from analysis with lake chemistry data explaining variation in mean DOC fluxes for the evaluated lakes. Variables that are strongly correlated with DOC flux (VIP > 1 over both components) are DOC, Fe, sedOC, totN, pH, sedC:N, $PO_4^{3-}$, and Mn.

# Figures

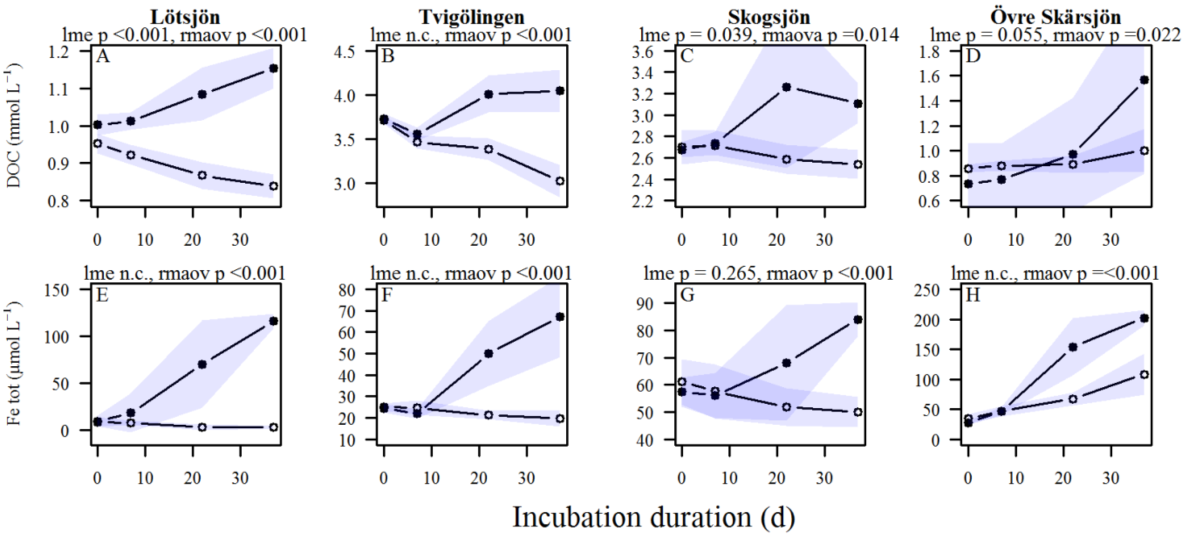
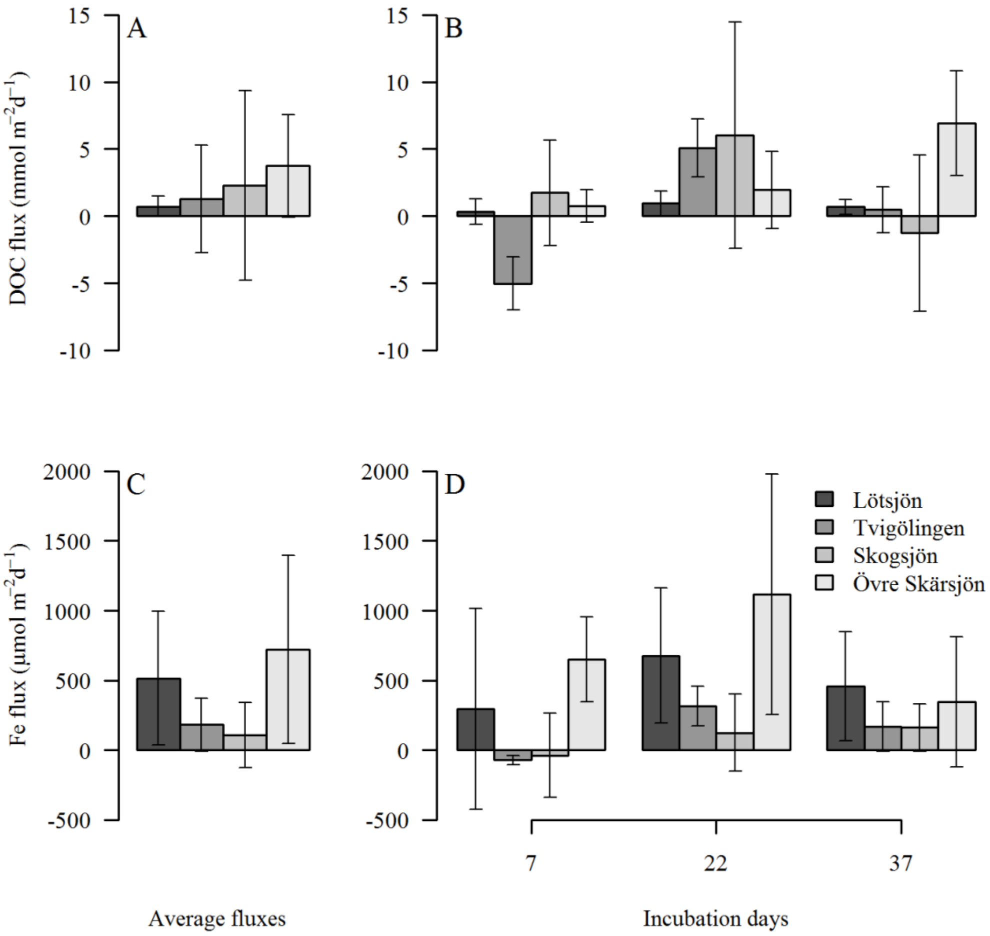



Figure 1




1

2 Figure 2

3

4

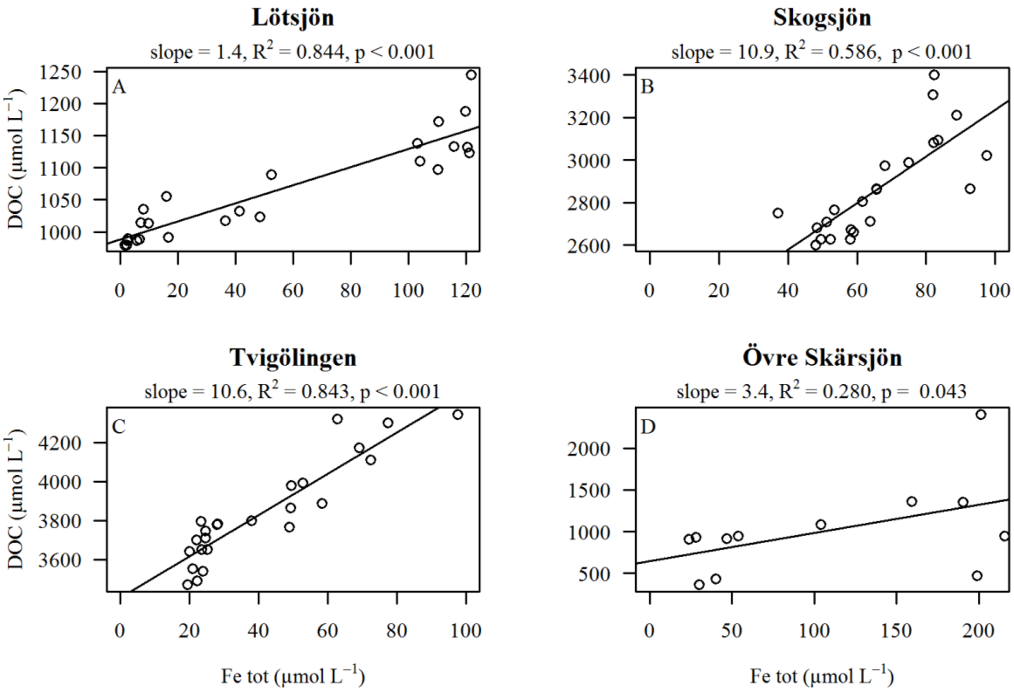

Lötsjön
slope = 1.4, R2 = 0.844, p < 0.001
A
Skogsjön
slope = 10.9, R2 = 0.586, p < 0.001
B
Tvigölingen
slope = 10.6, R2 = 0.843, p < 0.001
C
Övre Skärsjön
slope = 3.4, R2 = 0.280, p = 0.043
D
DOC (µmol L−1)
Fe tot (µmol L−1)

Figure 3

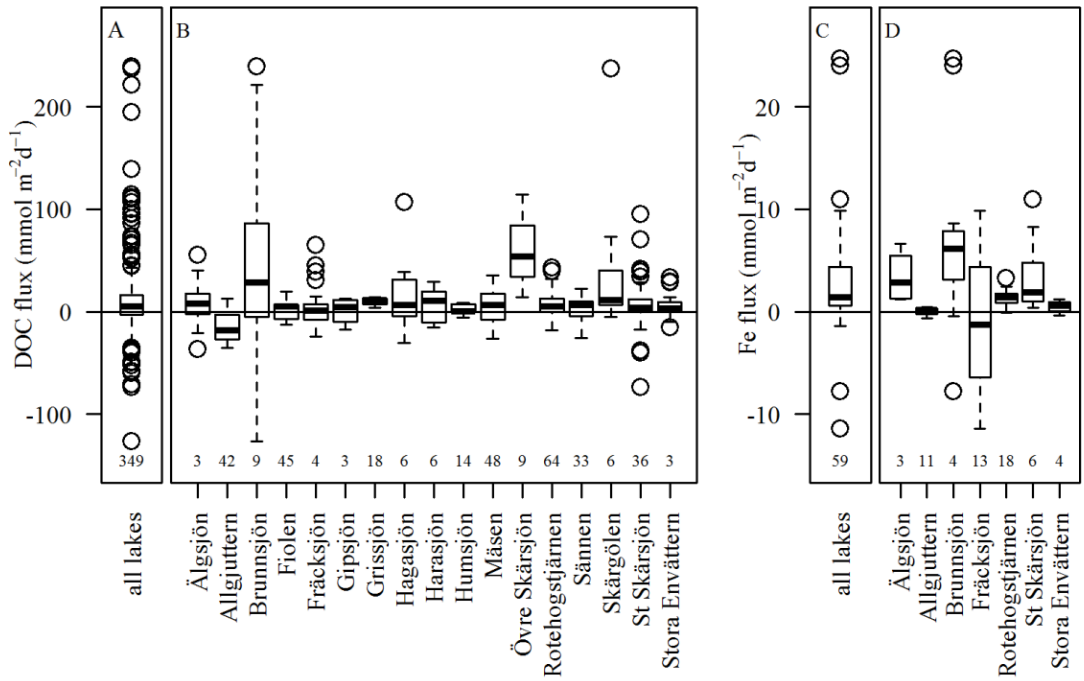
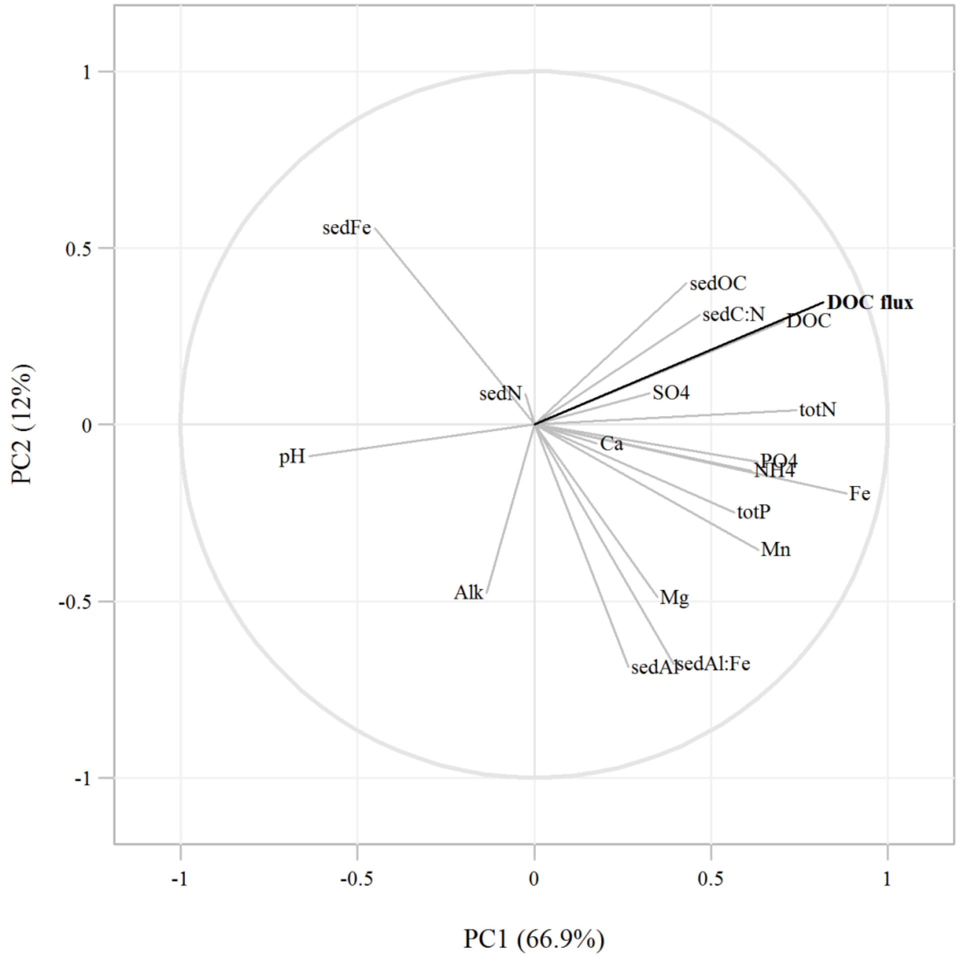

A
B
C
D
DOC flux (mmol m-2d-1)
Fe flux (mmol m-2d-1)
200
100
0
-100
20
10
0
-10
349
3 42 9 45 4 3 18 6 6 14 48 9 64 33 6 36 3
59
3 11 4 13 18 6 4
all lakes
Älgsjön
Allgjuttern
Brunnsjön
Fiolen
Fräcksjön
Gipsjön
Grissjön
Hagasjön
Harasjön
Humsjön
Mäsen
Övre Skärsjön
Rotehogstjärnen
Sännen
Skärgölen
St Skärsjön
Stora Envättern
all lakes
Älgsjön
Allgjuttern
Brunnsjön
Fräcksjön
Rotehogstjärnen
St Skärsjön
Stora Envättern

Figure 4



sedFe
sedOC
sedC:N
DOC flux
DOC
sedN
SO4
totN
pH
Ca
PO4
NH4
Fe
totP
Mn
Alk
Mg
sedAl
sedAl:Fe
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
PC2 (12%)
PC1 (66.9%)


Figure 5