

### Author's response to reviewer's comments

Interactive comment on “Mineralization of organic matter in boreal lake sediments: Rates, pathways and nature of the fermenting substrates” by François Clayer et al.

#### Reviewer 3:

Anonymous Referee #3 Received and published: 22 April 2020

In this paper, Clayer et al. found the average carbon oxidation state (COS) is negative COS values by modelling solute pore-water profiles. They concluded that carbohydrates do not adequately represent the fermenting OM and that the COS should be included in the formulation of OM fermentation in models. It is an interesting work and the results can guide new biogeochemical model for OM degradation. However, the manuscript needs substantial improvement of the presentation before it can be recommended for publication.

We are thankful to the reviewer for constructive and rigorous comments. We believe that it helped improve the manuscript.

**The main issues is the lack of the OM and mobile labile information. There are no data for the deposition/sedimentation rate of OM, the chemical composition of OM (C,H,O,N,S,P,..), d13C distribution of OM et al. The results of COS from modelling solute pore-water profiles have not been validated. Even in the solute model there are too many fitting parameters and the conclusion is not convincing.**

Agreed, background information on OM was lacking.

As we understand it, the reviewer would also have appreciated to see chemical composition data on single organic compounds that corroborate our COS estimations. However, we do not dispose of such analytical methods nor of any additional samples to perform these analyses. We agree that it could have been an interesting complement. However, we believe that the strength of our demonstration resides is the consistency among the COS estimations reported for the seasonally anoxic basins. See also our response to your comment #5 below.

Regarding the solute model, we now have re-organized the methods description to better describe our approach in a convincing way. While the robustness of the net reaction rates obtained with PROFILE is clearly highlighted L. 226-232. Extracts of the method sections now reads

“Considering the net reaction rates obtained by inverse modelling, a realistic range of values can be given for each of the effective reaction rates  $R_i$  in each depth interval, as determined by PROFILE, using the general equations described below (Eqs. 3, 4 and 5). The detailed calculations for each  $R_i$  at both study sites are described in section S2.

(...)

Once the range of values have been determined for each of the effective rates  $R_i$  (see Table S2), they can be used in another reaction-transport equation to model the  $\delta^{13}\text{C}$  profiles of  $\text{CH}_4$  and DIC. Only sets of  $R_i$  values that yield acceptable modeled  $\delta^{13}\text{C}$  profiles, i.e., which fall within one standard deviation of the measured  $\delta^{13}\text{C}$  profiles (grey area fills in Fig. 4), were kept for COS calculation below (section 2.8). The  $\delta^{13}\text{C}$  modeling procedure is summarized below and described in detail in Section S.2. This procedure takes into account the effect of diffusion, bioirrigation (in Lake Tantaré Basin A) and the isotopic fractionation effect of each reaction  $r_i$ .

(...)

## 2.8 COS calculation

Considering the complete fermentation of metabolizable OM of general formula  $C_xH_yO_z$ , and making two assumptions, described below for clarity, the COS of the fermenting molecule is given by (combining Eq. S8 and S15; see Section S2 for details):

$$\text{COS} = -4 \left( \frac{R_{\text{net}}^{\text{CH}_4} - R_{\text{net}}^{\text{DIC}} - R_{\text{net}}^{\text{Ox}} + R_2}{R_{\text{net}}^{\text{CH}_4} + R_{\text{net}}^{\text{DIC}} + (1 - \chi_M)R_{\text{net}}^{\text{Ox}} - R_2} \right) \quad (9)$$

where  $\chi_M$  is the fraction of oxidants consumed by methanotrophy. Equation (9) is only valid if i) r1 is the only source of substrates for hydrogenotrophy and acetoclasty (this assumption is discussed in Section 4.2 below); and that ii) siderite precipitation (r7) is negligible (Saturation Index for siderite are negative except below 10 cm depth in the sediment of Lake Bédard, this case is considered in Section S2.1.2.2). With values of  $R_{\text{net}}^{\text{CH}_4}$  and  $R_{\text{net}}^{\text{Ox}}$  obtained from PROFILE (section 2.4), values of  $R_1$ ,  $\chi_H$  and  $\chi_M$  constrained by  $\delta^{13}\text{C}$  modeling (section 2.7), Eq. (9) can be used to calculate the COS of the fermenting molecule.”

We added a figure and some information on the sediment OM as follows:

“The sediment accumulation rates are 4.0–7.3 and 2.4–46.8  $\text{mg cm}^{-2} \text{yr}^{-1}$  at the deepest sites of Lake Tantaré Basin A and Lake Bédard, respectively (Couture et al., 2010). The relatively constant organic C ( $C_{\text{org}}$ ) content ( $20 \pm 2\%$ ; Fig. 2b), the elevated  $\{C_{\text{org}}\}:\{N\}$  molar ratio ( $17 \pm 2$ ; Fig. 2b), the  $\delta^{13}\text{C}$  ( $-29\%$ ; Joshani, 2015) and  $\delta^{15}\text{N}$  ( $+0.5\%$  to  $-2.5\%$ ; Joshani, 2015) values reported for the sediment OM over the top 30 cm in Lake Tantaré Basin A are typical of terrestrial humic substances (Botrel et al., 2014; Francioso et al., 2005). The  $C_{\text{org}}$  content ( $21 \pm 2.7\%$ ; Fig. 2a) and  $\{C_{\text{org}}\}:\{N\}$  molar ratio ( $14 \pm 1.9$ ; Fig. 2a) reported over the top 30 cm of Lake Bédard sediments show slightly more variation with depth, but are also typical of terrestrial OM. In addition, the  $\{C_{\text{org}}\}:\{S\}$  ratios of both lake basin sediments (50–200) are typical of those reported for soil OM ( $\sim 125$ ; Buffle, 1988).

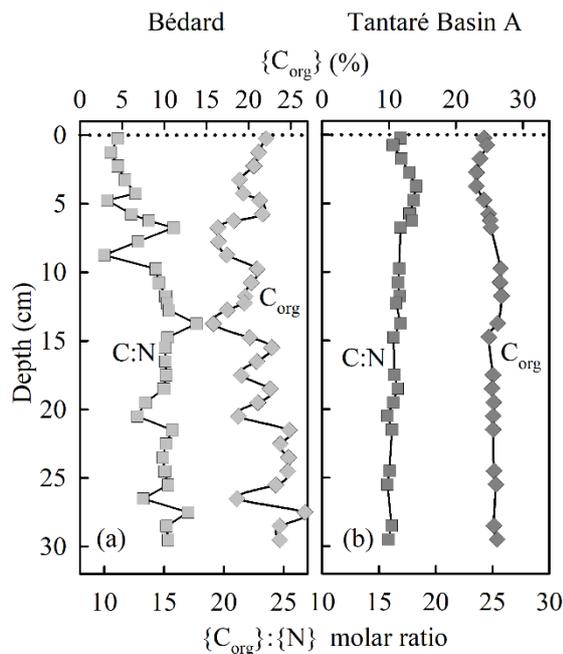


Figure 2: Depth profiles of the organic C concentrations and of the C : N molar ratio in sediment cores collected at the deepest sites of Lake Bédard (a) and Lake Tantaré Basin A (b).”

Here are some details:

**1. Reactions: Since the reactions the precipitation of siderite (r7) and sulfide oxidation by iron oxides (r8) were taken into account, the pyrite formation by Fe<sup>2+</sup> and H<sub>2</sub>S should be considered, too. The hydrogen H<sub>2</sub> in eq.(r8) is usually consumed easily by sulphate reducer bacteria rather than CO<sub>2</sub> reduction. The authors used general oxidant instead of O<sub>2</sub>, Fe(III) and SO<sub>4</sub>, which could have different oxidation rates, especially for CH<sub>4</sub> oxidation (r5).**

We agree that the description of the reactions was lacking some rigor in section 2.3. Regarding reducing Fe and S cycling, it now reads:

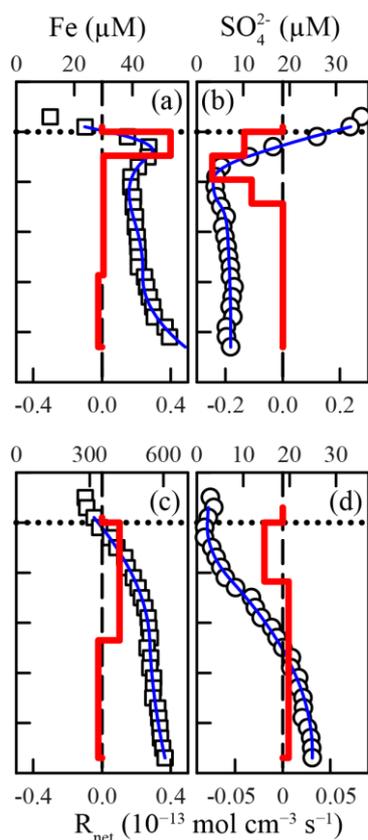
“Lastly, sulfide oxidation by iron oxides (r8), which can be a source of SO<sub>4</sub><sup>2-</sup> and H<sub>2</sub> (Clayer et al., 2018; Holmkvist et al., 2011), is also considered. Note that iron sulfide enrichments formed during past decades of elevated atmospheric SO<sub>4</sub> deposition are presently dissolving in Lake Tantaré Basin A (Couture et al., 2016). This process also occurs in the seasonally anoxic Basin B of Lake Tantaré (Couture et al., 2016) and is likely to also occur in Lake Bédard. Hence, other reactions involving reduced S and Fe species, such as pyrite precipitation, are believed to be insignificant for C cycling in the present study and are thus ignored.”

Concerning the consumption of H<sub>2</sub> by sulphate reducer, it possibly occurs in the top 5 cm in Lake Tantaré Basin A, but considering the low SO<sub>4</sub> concentrations, this process is likely negligible. Note that we have added a figure in the supplementary information showing modelled SO<sub>4</sub> profiles and reaction rates. This figure shows net SO<sub>4</sub> production below 5-7 cm depth at a very low rate.

Note that the various oxidation state of the oxidants are taken into account as now stressed L. 153

“R<sub>net</sub><sup>Ox</sup> is the net reaction rate of all relevant oxidants consumption, i.e., O<sub>2</sub>, Fe(III) and SO<sub>4</sub><sup>2-</sup> only because NO<sub>3</sub><sup>-</sup> and Mn(IV) are negligible (see above). For simplicity, R<sub>net</sub><sup>Ox</sup> is expressed in equivalent moles of O<sub>2</sub> consumption rate, taking into account that SO<sub>4</sub><sup>2-</sup> and Fe(III) have twice and one quarter the oxidizing capacity of O<sub>2</sub>, respectively. In practice, the value of R<sub>net</sub><sup>Ox</sup> was calculated by adding those of R<sub>net</sub><sup>O<sub>2</sub></sup>,  $\frac{1}{4}$ R<sub>net</sub><sup>Fe(III)}</sup> and 2R<sub>net</sub><sup>SO<sub>4</sub><sup>2-</sup></sup> where R<sub>net</sub><sup>O<sub>2</sub></sup>, R<sub>net</sub><sup>Fe(III)}</sup> and R<sub>net</sub><sup>SO<sub>4</sub><sup>2-</sup></sup> were estimated with PROFILE. In this calculation, we assumed that all dissolved Fe is in the form of Fe(II), and that the rate of Fe(II) consumption through reactions r7 is negligible compared to those associated with reactions r5 and r6. Under these conditions, R<sub>net</sub><sup>Fe(III)}</sup> = -R<sub>net</sub><sup>Fe</sup>. It should be noted that using R<sub>net</sub><sup>O<sub>2</sub></sup>, -R<sub>net</sub><sup>Fe</sup> and R<sub>net</sub><sup>SO<sub>4</sub><sup>2-</sup></sup> to calculate R<sub>net</sub><sup>Ox</sup>, we indirectly take into account the re-oxidation of reduced S and Fe(II), respectively, to SO<sub>4</sub><sup>2-</sup> and Fe(III) by O<sub>2</sub>. Indeed, with this procedure, we underestimate the terms  $\frac{1}{4}$ R<sub>net</sub><sup>Fe(III)}</sup> and 2R<sub>net</sub><sup>SO<sub>4</sub><sup>2-</sup></sup> because re-oxidation reactions are ignored, but we overestimate by the same amount the term R<sub>net</sub><sup>O<sub>2</sub></sup>. In other words, omission of these re-oxidation reactions affects only the relative consumption rates of individual oxidants and not the value of R<sub>net</sub><sup>Ox</sup>, which is of interest here.”

New figure:



“

**Figure S3: Comparison of the modeled (blue lines) and average (n = 3) measured (symbols) concentration profiles of SO<sub>4</sub> (a and c) and Fe (b and d) in Lakes Tantaré Basin A (a–b) and Bédard (c–d). The horizontal dotted line indicates the sediment-water interface. The thick red lines represent the net solute reaction rate ( $R_{\text{net}}^{\text{solute}}$ ).”**

**2. Rate calculation: The reaction rate were calculated by computer code PROFILE. This rates obtained from PROFILE were very rough. It is better to use reactiontransport model to calculate the rate by considering OM deposition and degradation.**

We agree with the reviewer that a limitation of inverse modeling methods is that the predicted rate profiles may not be unique. However, we are confident that the depth distributions of the net reaction rates that we present in Fig. 3g, h, o and p for [CH<sub>4</sub>] and DIC are robust. Indeed, as stated L. 226-232 the statistical F-testing implemented in PROFILE allows to objectively select, among all the possible solutions, the one that gives the simplest rate profile while providing a satisfying explanation of the averaged solute concentration profile. Also, as can be seen in Fig. S1, using another inverse modeling code, i.e., Rate Estimation from Concentrations (REC, Lettmann et al., 2012), produces consistent results with those obtained with the code PROFILE. Moreover, the values of the net rates are of similar magnitude. Note that REC uses the Tikhonov regularization technique. This statistical method implies the adjustment of one discrete parameter (i.e., the smoothing parameter  $\lambda$ ) and, in contrast to PROFILE, does not suggest a given number of zones.

After having seriously considered the suggestion of the reviewer, we decided to keep our inverse modeling approach since we believe it is reliable as described above. To our knowledge, a non-steady state model is not necessarily better suited to interpret the concentration profiles because it

requires a high number of adjustable parameters (e.g., the flux of labile organic carbon and of dissolved oxygen and other oxidants, the rate constants for each reaction of OM degradation) which is not the case for the inverse model. In addition, using a forward model would imply making additional subjective choices regarding the rate expressions and boundary conditions, e.g., parametrizing the O<sub>2</sub> sediment flux.

**3. The bioirrigation term was shown in the equation (2) but the bioirrigation depth and coefficient were not clear. How does the bioirrigation affect COS estimation was also not clear. General once bioirrigation is strong, bioturbation should be considered, too. The solid phase (OM, iron oxides) in the bioturbation zone is well mixed, which strongly affect OM degradation.**

Agreed, there was some information lacking regarding biological processes. Bioturbation has been shown previously to be negligible compared to diffusion or bioirrigation (e.g. Clayer et al., 2016; Couture et al., 2008) This is now fixed as follows (in section 2.3):

L. 124 “The values of  $\alpha_{\text{Irrigation}}$  in Lake Tantaré Basin A were calculated as in Clayer et al. (2016), considering that it varies linearly from  $\alpha_{0,\text{Irrigation}}$  at the SWI (calculated according to Boudreau 1984 based on an inventory of benthic animals Hare et al., 1994) to 0 at 10 cm depth (the maximum depth at which chironomids are found in lake sediments; Matisoff and Wang 1998), and were assumed to be 0 in Lake Bédard since its bottom water was anoxic (Fig. 1).”

L. 122-123 “considering steady state and negligible solute transport by bioturbation and advection. The validity of these assumptions has been previously demonstrated for the study sites (Couture et al., 2008; Couture et al., 2010; Clayer et al., 2016).”

To describe the sensitivity of COS values to the bioirrigation term we added the following sentence L. 422:

“Even if reaction rates are sensitive the value of the bioirrigation coefficient (Clayer et al., 2016), additional simulations show that changing the bioirrigation coefficient by a factor of 2 (increased and decreased) did not result in significant changes in COS values (<0.2).”

**4. I don't understand why the acetoclastic methanogenesis was absent here. Generally acetoclastic methanogenesis dominates in lake sediment and hydrogenotrophic methanogenesis in sea sediment. The two pathways generate different d13C-CH<sub>4</sub> and d13-DIC pattern. Diffusion and bioirrigation will also change this pattern. The authors should prove it.**

Here, we clearly show that methanogenesis is 100% hydrogenotrophic at both study sites. As stated L. 284-287:

“hydrogenotrophy becomes an increasingly important CH<sub>4</sub> production pathway: i) when labile OM is depleted (Chasar et al., 2000; Hornibrook et al., 2000; Whitticar et al., 1986), ii) with increasing sediment/soil depth (Conrad et al., 2009; Hornibrook et al., 1997), or iii) with decreasing rates of primary production in aquatic environments (Galand et al., 2010; Wand et al., 2006)”

We also replaced l. 269-279:

“Modeled  $\delta^{13}\text{C}$  profiles were considered acceptable only when they fell within one standard deviation of the measured  $\delta^{13}\text{C}$  profiles (grey area fills in Fig. 4). Acceptable modeled  $\delta^{13}\text{C}$  profiles were

obtained only when methanogenesis was 100% hydrogenotrophic, i.e., when  $R_3 = 0$  (see section S2.2.2.1).”

**5. The chemical composition of individual molecules in OM pools can be detected from various state-of-the-art instrumentation including GC-MS, LC-MS/MS, HPLC-MS, NMR, Orbitrap MS, and Fourier transform ion cyclotron resonance (FTICR-MS). By combining a suite of previously developed thermodynamic theories ( Kleerebezem and Van Loosdrecht, 2010; LaRowe and Van Cappellen, 2011), one can calculate COS. If the results are consistent, the paper method is more convincing.**

Agreed, presenting data from state-of-the-art analytical methods on the composition of organic molecules could have been convincing, if we were able to isolate the compounds of interest. Indeed, the fraction of the organic C that is degraded in the sediment only represents <5% of the total  $C_{org}$  deposited. It could be challenging to isolate the compounds of interest that we believe are undergoing fermentation. This could well be the subject of a future study.

Nonetheless, fatty acids and alcohols, which are believe to be at the origin of methanogenesis here, are widespread compounds in lake sediments, and are major component of plant organic material (Cranwell, 1981; Matsumoto, 1989).

To better appreciate the point that only a insignificant fraction of  $C_{org}$  was degraded in the sediment we added the following text in section 4.1:

“Considering the sediment accumulation rate and sediment  $C_{org}$  content given in section 2.1, we calculate an average accumulation rate of  $C_{org}$  of  $4.7 \times 10^{-11}$  to  $1.0 \times 10^{-10}$  and  $2.9 \times 10^{-11}$  to  $7.6 \times 10^{-10}$  mol C  $cm^{-2} s^{-1}$  for lakes Tantaré Basin A and Bédard, respectively. Hence, the total sediment OM degradation rate ( $\Sigma R_1 + \Sigma R_2 + \Sigma R_6$ ) of  $1.3 \times 10^{-12}$  and  $1.4 \times 10^{-12}$  reported in this study for lakes Tantaré Basin A and Bédard, respectively, would involve only 1.2–2.8% and 0.2–4.8% of the total  $C_{org}$  deposited. Given that the remaining 95.2–99.8% of the deposited  $C_{org}$  is preserved in the sediment, it is not surprising that the sediment  $C_{org}$  concentration is constant with depth (Fig. 2).”

**6.  $\delta^{13}C$ -CH<sub>4</sub> in Lake Tantaré Basin A (Fig.3) is very negative (–107.0). Is there some explanation?**

A common explanation given in the literature is the intertwined hydrogenotrophy and methanotrophy. This process is also shown here to produce this local  $^{13}C$  depletion. See L. 305-317.

#### **REFs:**

Botrel, M., Gregory-Eaves, I. & Maranger, R. Defining drivers of nitrogen stable isotopes ( $\delta^{15}N$ ) of surface sediments in temperate lakes. *Journal of Paleolimnology* 52, 419-433 (2014).

Clayer, F., Gobeil, C. and Tessier, A.: Rates and pathways of sedimentary organic matter mineralization in two basins of a boreal lake: Emphasis on methanogenesis and methanotrophy: Methane cycling in boreal lake sediments, *Limnology and Oceanography*, 61(S1), S131–S149, doi:10.1002/lno.10323, 2016.

Conrad, R. (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*, 28(3), 193–202. <https://doi.org/10.1111/j.1574-6941.1999.tb00575.x>

Couture, R.-M., Gobeil, C. and Tessier, A.: Chronology of Atmospheric Deposition of Arsenic Inferred from Reconstructed Sedimentary Records, *Environ. Sci. Technol.*, 42(17), 6508–6513, doi:10.1021/es800818j, 2008.

Cranwell P. A. (1981) Diagenesis of free and bound lipids in terrestrial detritus deposited in a lacustrine sediment. *Org. Geochem.* 3, 79–89.

Francioso, O., Montecchio, D., Gioacchini, P., and Ciavatta, C. Thermal analysis (TG–DTA) and isotopic characterization ( $^{13}\text{C}$ – $^{15}\text{N}$ ) of humic acids from different origins (2005) *Applied Geochemistry* 20(3), 537-544

Matsumoto G. I. (1989) Biogeochemical study of organic substances in Antarctic lakes. *Hydrobiologia* 172, 265–289.