

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 1:

Anonymous Referee #1 Received and published: 23 March 2020

The manuscript is a modification of previous work published by Clayer et al 2018 in *Geochimica et Cosmochimica Acta*. It builds on the hypothesis that the degradation of organic material under anaerobic conditions has two ultimate sinks – CO₂ or CH₄, the most reduced and oxidized states of carbon. The relative abundance of these two should therefore provide information on the average oxidation state of the degraded organic material accounting for transport and other sources of CO₂. This principal approach has been presented in Clayer et al (2018) *GCA*. The current paper is very similar to this published work and contains many data that are shared. It was not obvious to me where this work is a significant novel contribution beyond what has also ready been published. The authors use a simple steady state reaction transport model for diffusive and advective transport to determine microbial process rates based on sediment porewater concentration gradients. Furthermore, the isotope composition of DIC is used to improve the mass balance calculations.

Below I question the validity of this approach to obtain a meaningful mass balance using the Berg et al model at steady state.

Thank you for your rigorous and comprehensive comments, they have been very useful to improve the quality of the manuscript. We appreciate the time invested by the reviewer.

#1 - This manuscript is difficult to understand manuscript and very technical in its description. What makes this manuscript so hard to read and understand is the multitude of R subscripts that are used in the text and the extensive treatment of the methodology in the appendix. The fractional equations are nowhere introduced. Deserves an explanation. In practice, one has to have a table on the side to look up what reaction a particular R subscript refers to and, in addition, know all the notations from Clayer et al (2018) to follow this work.

We agree that the manuscript is very technical, which makes it harder to follow. In order to rigorously distinguish between net reaction rates, provided by PROFILE, and effective (or gross) reaction rates, we need these numerous R notations. We have now better introduced the effective reaction rates R_i . They were only introduced in Table 1 captions, in the original manuscript. We also better describe the term R_{net}^{Ox} . Note also that we have clarified the description of reaction r1 (see response to comment #9)

Note that all notations are described in the manuscript, there is no need to have notations from Clayer et al., 2018.

L. 138 now reads

"The main reactions retained in this study to describe carbon cycling in the sediments of the two lake basins are shown in Table 1. R_i and α_i denote, respectively, the effective (or gross) reaction rate and the carbon isotopic fractionation factor associated with each reaction r_i (Table 1)."

Note also that each net reaction rates are explicitly defined.

L.121 " $R_{\text{net}}^{\text{solute}}$ (in mol cm⁻³ of wet sediment s⁻¹) is the solute net production rate (or consumption rate if $R_{\text{net}}^{\text{solute}}$ is negative)"

L. "the net rate of CH₄ production, $R_{\text{net}}^{\text{CH}_4}$ "

L. "The net rate of DIC production, $R_{\text{net}}^{\text{DIC}}$,"

L. 153 " $R_{\text{net}}^{\text{Ox}}$ is the net reaction rate of all relevant oxidants consumption, i.e., O₂, Fe(III) and SO₄²⁻ only because NO₃⁻ and Mn(IV) are negligible (see above)." And there after (see also response to comment #15 below).

#2 - Still, to follow the conclusions becomes increasingly confusing as one reads along, until one is either lost or exhausted. In the current form, the manuscript cannot be digested. I recommend that the authors outline the hypothesis, mathematically, in the materials and methods section, of how their methodology allows them to get at the oxidation state of oxidized organic matter. In the current form, the reader has to wade through too much text to get to this most interesting point of the manuscript. This paper requires a much better didactic approach to get methods and goals across and the authors get sidetracked in many details that make it hard to follow their ultimate goal. It is, in its current form, not streamlined enough and requires very significant rewriting and restructuring to make the approach more understandable and possible to evaluate critically. At present, I cannot evaluate the quality of the manuscript, but am left in doubt about its novelty given the similarity to the 2018 GCA paper. While the fundamental goal, to arrive at the oxidation state of metabolizable organic material, is of some significance, the presentation of the approach is not well developed and can be improved considerably. Data and basic approach (although I did get lost in the complicated d13C treatment) are, in principle feasible, but overall I am concerned that the instrumental and modelling analytical uncertainty is too great to pin down the COS sufficiently (although an error is given).

We have reorganized the method and discussion sections to better describe the approach and outline the hypothesis mathematically (see our response to comment #3). We have also modified the abstract, introduction and conclusions for consistency and to highlight to novelty compared to the 2018 GCA paper.

L. 13-24 now read:

"To test the validity of this assumption, we modeled using reaction-transport equations vertical profiles of the concentration and isotopic composition ($\delta^{13}\text{C}$) of CH₄ and DIC in the top 25 cm of the sediment column from two lake basins, one whose hypolimnion is perennially oxygenated and one with seasonal anoxia. Furthermore, we modeled solute porewater profiles reported in the literature for four other seasonally anoxic lake basins. A total of seventeen independent porewater datasets are analysed. CH₄ and DIC production rates associated with methanogenesis at the five seasonally anoxic sites collectively show that the fermenting OM has a mean (\pm SD) carbon oxidation state (COS) value of -1.4 ± 0.3 . This value is much lower than the value of zero expected from carbohydrates fermentation. We conclude that carbohydrates do not adequately represent the fermenting OM in hypolimnetic sediments and propose to include the COS in the formulation of OM fermentation in models applied to lake sediments to better quantify sediment CH₄ outflux. This study highlights the potential of mass balancing the products of OM mineralization to characterize labile substrates undergoing fermentation in sediments."

And L. 68-74:

“In this study, the approach described in Clayer et al. (2018), combining concentration and $\delta^{13}\text{C}$ inverse modeling, is applied to the two newly acquired datasets. These datasets include centimeter-scale vertical porewater profiles of the concentrations and of the stable carbon isotope ratios ($\delta^{13}\text{C}$) of CH_4 and dissolved inorganic carbon (DIC), as well as those of the concentrations of EAs from hypolimnetic sediments of two boreal lake basins showing contrasted O_2 dynamics: one whose hypolimnion remains perennially oxygenated and the other whose hypolimnion becomes anoxic for several months annually. This procedure enables us to constrain the effective rates of OM mineralization reactions and calculate, using a mass balance equation, the COS of the substrates fermenting in the sediments in these two lake basins. In addition, we modelled solute porewater profiles gathered from the scientific literature or from our data repository for four other seasonally anoxic lake basins to estimate, using the mass balance equation, the COS of the substrates fermenting in these sediments. A total of seventeen independent datasets are analysed to provide additional insight into the COS of the fermenting OM in boreal lakes and the associated mineralization pathways.”

L. 448–453 now read:

“Reactive-transport modelling of twelve datasets of porewater profiles from three boreal lakes, i.e., Bédard, Tantaré (Basin B) and Jacks, as well as of the sub-alpine Lake Lugano (Melide and Figino sites) consistently showed that the main substrates for sediment methanogenesis at deep seasonally anoxic hypolimnetic sites have a mean COS value of -1.4 ± 0.3 . The OM in the sediment of the three boreal lakes, as well as their O_2 seasonal dynamics, is typical of boreal forest lakes. While Lake Bédard experiences prolonged episodes of extended hypolimnetic anoxia, Lake Tantaré Basin B and Jacks Lake show more moderate seasonal anoxia, where some years the hypolimnion of Lake Tantaré Basin B is only hypoxic (Clayer et al., 2016; Carignan et al., 1991). Hence, the selective mineralization of OM described by Clayer et al. (2018), involving that the most labile compounds are mineralized during OM downward migration in the water column and at the sediment surface leaving mainly reduced organic compounds to fuel methanogenesis in the sediments, likely applies to a large portion of boreal lakes.”

L. 460-461 now read:

“Introducing the average COS values reported in this study (-1.4 ± 0.3) into Eq. 15, the coefficients a and b would take values of 2.7 ± 0.15 and 0.65 ± 0.125 , respectively, and the CH_4 and CO_2 stoichiometric coefficients would be 0.68 ± 0.04 and 0.32 ± 0.04 , respectively. Note that the same stoichiometric formulation would be obtained for acetoclastic methanogenesis. Under these conditions, fermentation (r1) coupled to methanogenesis (r4) yields 2.2 ± 0.4 times more CH_4 than DIC for the studied lake sediments. Ignoring the implications of the present study regarding the COS of the fermenting OM could lead to the underestimation of CH_4 sediment outflux or of the rate of oxidant consumption required to mitigate this efflux by a factor of up to 2.6.”

#3 -The authors provide statistical data to support their assertion, but it was not possible for me, based on the complicated description, to relate the outcome of these tests to the goal of the manuscript, i.e., the original oxidation of the degrading organic material. The authors must make sure, in a succinct and understandable and not too wordy fashion, how their methodology allows them to pin this value down sufficiently. Remove as much as possible reiterations of what has already been said and discussed in detail in Clayer et al 2018 GCA and restrict this paper to the novel information.

Thank you for a constructive comment. In consequence, we have (i) clarified the novel aspects (see response to comment above), and (ii) better described the modelling and COS estimation approaches, (iii) edited the conclusions (see also our response to comment #4) and (iv) simplified the discussion (See also our response to comment #4 and #5).

As a consequence of including Eq. 9 below, Eq. 11 and 12 were removed which simplified section 4.2. COS values displayed in Table 4 are directly calculated with Eq. 9.

The description of the approach 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 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 $\text{C}_x\text{H}_y\text{O}_z$, 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 χ_M is the fraction of oxidants consumed by methanotrophy. Equation (9) is only valid if i) r_1 is the only source of substrates for hydrogenotrophy and acetoclasty (this assumption is discussed in Section 4.2 below); and that ii) siderite precipitation (r_7) 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 , χ_H and χ_M constrained by $\delta^{13}\text{C}$ modeling (section 2.7), Eq. (9) can be used to calculate the COS of the fermenting molecule.”

L. 384-391 now read:

“The COS values displayed in Table 4 for all lake basins and dates were calculated by substituting the appropriate $R_{\text{net}}^{\text{CH}_4}$, $R_{\text{net}}^{\text{DIC}}$, $R_{\text{net}}^{\text{Ox}}$ and R_2 values in Eq. 9 and varying χ_M between 0 and 1, except for Lake Tantaré Basin A for which $\chi_M = 0.75$ (Table 3). When the value for R_2 was not available, we assumed that $R_2 = 0$. Equation 9 indicates that $R_2 > 0$, would yield lower COS values than those reported in Table 4.”

L. 406-442 now read:

“The COS values determined for the perennially oxygenated Basin A of Lake Tantaré (mean of -0.6 ± 1.1 ; range of -3.2 to 2.1 ; Table 4) are much more variable than for the five other seasonally anoxic lake basins including unrealistic values for October 2015 in the Z_1 (-3.2), September 2016 (0.4 – 0.6) and October 2005 (1.8 – 2.1). Indeed, the very negative value of -3.2 does not correspond to any degradable compound under anoxic conditions, whereas the positive values of 0.4 – 0.6 and 1.8 – 2.1 would involve either amino acids and nucleotides which are very labile (Larowe and Van Cappellen 2011) and tend to be degraded in the water column (Burdige 2007), or oxidized compounds, such as ketones, aldehydes and esters, known to be quickly reduced to alcohols. Possible sources of uncertainty in the COS estimation include mis-quantification of bioirrigation and DIC production through HMW OM fermentation (reaction r_2 ; Corbett et al. 2013). Clayer et al. (2016) provided

evidences that sediment irrigation by benthic animals is effective in Lake Tantaré Basin A and that reaction rates are sensitive to the bioirrigation coefficient. Nevertheless, 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). Bioirrigation might also be mis-represented. Indeed, the term used in Eq. 2 to calculate this contribution, i.e., $\phi\alpha_{\text{irrigation}}([\text{solute}]_{\text{tube}} - [\text{solute}])$, is indeed an approximation of intricate 3-D processes variable in space and time (Meile et al., 2005; Boudreau and Marinelli, 1994; Forster and Graf, 1995; Gallon et al., 2008; Riisgård and Larsen, 2005). On the other hand, DIC production through HMW OM fermentation (reaction r2; Corbett et al. 2013) was constrained by default in Lake Tantaré Basin A (Table 4). Indeed, fitting with Eq. 7 the experimental $\delta^{13}\text{C}$ data does not allow partitioning the production of DIC between r1 and r2 given that both processes share the same fractionation factor ($\alpha_1 = \alpha_2 = 1.000$). Equation 9 indicates that to obtain negative COS values for Lake Tantaré Basin A in September 2006 and October 2005, R_2 should be $>11 \text{ fmol cm}^{-2} \text{ s}^{-1}$ and $>110 \text{ fmol cm}^{-2} \text{ s}^{-1}$, respectively. These R_2 values correspond to transferring $>9\%$ and $>44\%$ of the rate of DIC production from R_1 to R_2 for September 2006 and October 2005, respectively. Hence, owing to the imperfection in the COS estimations for Lake Tantaré Basin A, COS values estimated for this site should be treated with caution. Note that the sediment surface was also oxic at the sites Melide and Figino of Lake Lugano in March 1989 (Table 4) as revealed by detectable bottom water $[\text{O}_2]$ (Table 4), and by low $[\text{Fe}]$, undetectable $\Sigma\text{S}(-\text{II})$ and $[\text{CH}_4]$ and relatively high $[\text{SO}_4^{2-}]$ in overlying water (Lazzaretti et al., 1992; Lazzaretti-Ulmer and Hanselmann, 1999). Despite this, the COS values determined for the two sites of Lake Lugano appear realistic and consistent with those calculated for Lakes Tantaré Basin B, Bédard and Jacks. This disparity between Lake Tantaré Basin A and Lake Lugano could be explained by the presence of benthic organisms in the former (Hare et al., 1994) but their absence in the latter, as shown by the presence of varves (Lazzaretti et al., 1992) and the absence of benthos remains in the recent sediments of Lake Lugano (Niessen et al., 1992)."

#4 - A lot of the discussion about the CH₄ isotopes are not really part of the goal of this paper. This should be separated.

Agreed, we simplified and moved the text L. 305-317 to section 3.4 to streamline the discussion. Note however that the discussion related to the importance of hydrogenotrophy in section 4.1 is kept and its implication is now emphasized in the Conclusions (see below).

L. 305-317 now, in section 3.4, read:

"The sharp upward depletion in $^{13}\text{C}\text{-CH}_4$ leading to a minimum $\delta^{13}\text{C}\text{-CH}_4$ value at 2.5 cm depth in Lake Tantaré Basin A sediments (Fig. 3a) was unanticipated since it occurs in the methanotrophic zone, i.e., where the remaining CH_4 is expected to be ^{13}C -enriched as a result of CH_4 oxidation. Marked $^{13}\text{C}\text{-CH}_4$ depletions at the base of the sulfate-methane transition zone, where CH_4 is consumed via SO_4^{2-} reduction, have often been observed in marine sediments (Burdige et al., 2017 and references therein). Such features are generally attributed to the production of CH_4 by hydrogenotrophy from the ^{13}C -depleted DIC resulting from the anaerobic CH_4 oxidation, a process referred to as intertwined methanotrophy and hydrogenotrophy (e.g., Borowski et al., 1997; Burdige et al., 2017; Pohlman et al., 2008). Here the modelled $\delta^{13}\text{C}\text{-CH}_4$ profile captured the minimum in $\delta^{13}\text{C}\text{-CH}_4$ in the Z_1 by simply assuming concomitant hydrogenotrophy and methanotrophy in this zone and an upward-increasing α_4 value from 1.085 in the Z_3 to 1.094 in the Z_1 (section S2.2.1 of the SI). A small variation with sediment depth in the fractionation factor α_4 is arguably possible since its value depends on the types of microorganisms producing CH_4 (Conrad, 2005)."

L. 444–447 now read:

"Our results show that fermentation and methanogenesis represent about 50% and 100% of OM mineralization in the top 25 cm of the sediments at the hypolimnetic sites in Lake Tantaré Basin A and Bédard, respectively, that methane is produced only by hydrogenotrophy and fermentation substrates have a negative COS at these two sites. The association of hydrogenotrophy with the fermentation of reduced OM (COS < -0.9 ; implying that labile compounds are depleted) in the studied

lake sediments is consistent with the fact that hydrogenotrophy becomes increasingly important when labile OM is depleted (Chasar et al., 2000; Hornibrook et al., 2000; Whiticar et al., 1986).”

#5 - There are a few assumptions whose impact I don't understand or that are difficult to assess, e.g., that there are no anaerobic reoxidation reactions for sulfide; elemental sulfur with FeOOH. The paper does not lost O₂ uptake rates for the oxic part of the year, which is an important constraint on the 'background CO₂ levels in the buried porewaters. The paper does not constrain oxygen penetration depths or the importance of bioturbation processes for DIC levels, and does not show O₂ microelectrode profiles, which would be necessary to constrain the inorganic oxidative processes. Therefore the constraints for the diagenetic system, e.g., by having total O₂ uptake rates are far and few. In principle, non-steady state reaction transport modelling with a much more advanced model are necessary to tackle this question, if it is possible at all.

O₂ microprofiles were not measured for this study, but Couture et al. (2016) reported O₂ micro-profile measured previously in the sediment of Lake Tantaré Basin A. These microprofiles were the basis of our estimation of $R_{net}^{O_2}$, see L. 129-132. For the majority of the other study sites, the bottom waters were anoxic (O₂ < 0.1 mg L⁻¹), thus O₂ uptake was negligible. Furthermore, we acknowledge in section 4.3 (L. 406-442), the fact that bioirrigation, O₂ uptake and misattribution of DIC production can involve uncertainty in the COS estimation for Lake Tantaré Basin A. However, these factors are much less prominent, if not absent, for the other seasonally anoxic lake basins. We believe that the strength of our demonstration resides is the consistency among the COS estimations reported for the seasonally anoxic basins.

We also agree that the concentration profiles presented here were collected in October and are thus the result of “**background CO₂ levels in the buried porewaters**”, and of changing conditions at the SWI. Although our approach does not enable to resolve all aspect of the complex OM degradation cycling, e.g., explaining the magnitude of all the fluxes involved at a given depth, it allows us to estimate process rates in a given sediment zone, independently of the background concentrations.

Note that the term $R_{net}^{O_2}$ takes into account anaerobic reoxidation reactions for sulfide; elemental sulfur with FeOOH. Admittedly, this point was not stated clearly enough. See our response to comment #6 below.

We also clarify the importance of bioturbation. L. 122-123 now reads:

“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).”

Regarding non-steady state reaction transport modelling, see also our response to comment #17.

#6 - Another curious observation is the omission of NO₃ dynamics as part of O₂ consumption by nitrification; also here there are no constraints on the system concerning NH₄⁺ to accompany C mineralization dynamics.

The model also ignores O₂ consumption due to Fe oxidation, but curiously the authors choose to include instead sulfide oxidation with O₂ and FeOOH.

Our approach, admittedly not stated clearly enough in the original manuscript, has considered all oxidants. Some of them (NO₃ and Mn(IV)) have been shown previously to be negligible because of their low content in the sediment and porewaters (Clayer et al., 2016). Secondary redox reaction as O₂ consumption due to Fe(II) oxidation are also taken into account.

To better appreciate these points, we modified section 2.3 (L. 153) as follows:

“ $R_{\text{net}}^{\text{Ox}}$ is the net reaction rate of all relevant oxidants consumption, i.e., O₂, Fe(III) and SO₄²⁻ only because NO₃⁻ and Mn(IV) are negligible (see above). For simplicity, $R_{\text{net}}^{\text{Ox}}$ is expressed in equivalent moles of O₂ consumption rate, taking into account that SO₄²⁻ and Fe(III) have twice and one quarter the oxidizing capacity of O₂, respectively. In practice, the value of $R_{\text{net}}^{\text{Ox}}$ was calculated by adding those of $R_{\text{net}}^{\text{O}_2}$, $\frac{1}{4}R_{\text{net}}^{\text{Fe(III)}}$ and $2R_{\text{net}}^{\text{SO}_4^{2-}}$ where $R_{\text{net}}^{\text{O}_2}$, $R_{\text{net}}^{\text{Fe(III)}}$ and $R_{\text{net}}^{\text{SO}_4^{2-}}$ 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, $R_{\text{net}}^{\text{Fe(III)}} = -R_{\text{net}}^{\text{Fe}}$. It should be noted that using $R_{\text{net}}^{\text{O}_2}$, $-R_{\text{net}}^{\text{Fe}}$ and $R_{\text{net}}^{\text{SO}_4^{2-}}$ to calculate $R_{\text{net}}^{\text{Ox}}$, we indirectly take into account the re-oxidation of reduced S and Fe(II), respectively, to SO₄²⁻ and Fe(III) by O₂. Indeed, with this procedure, we underestimate the terms $\frac{1}{4}R_{\text{net}}^{\text{Fe(III)}}$ and $2R_{\text{net}}^{\text{SO}_4^{2-}}$ because re-oxidation reactions are ignored, but we overestimate by the same amount the term $R_{\text{net}}^{\text{O}_2}$. In other words, omission of these re-oxidation reactions affects only the relative consumption rates of individual oxidants and not the value of $R_{\text{net}}^{\text{Ox}}$, which is of interest here.”

#7 - It seems very hard to see how the boundary conditions can be reasonably constrained to continue with the approach used by the authors.

As boundary conditions, we use for each solute their measured concentrations at top and bottom of the concentration profiles (as stated L. 128-129). The model assumes steady-state, and we now mention that this assumption is valid (see below). Under steady-state conditions, the concentrations of solutes at top and bottom of their profiles should not vary with time.

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).”

#8 - Line comments: Line 139: There are more products than acetate CO₂ and CH₄ and H₂: Formate, propionate, isopropionate, lactate, butyrate, isobutyrate, pyruvate, succinate, etc.; The sum of the latter can be as high as 30% of the total VFA. Ok, acetate is low, but why should it not, if it is consumed by terminal oxidizers? Line 191: No good explanation for the low acetate concentrations?

We agree, OM degradation occurs and produces other VFA. But eventually, to produce CH₄, it will be degraded to acetate and/or CO₂ and/or H₂ (see Conrad 1999). Reaction r1 is a representation of the fermentation reaction, considering all VFA is out of the scope of this study.

Note that when measuring ions with ion chromatography (L. 109-111), we also looked for VFA, but those were under detection limit (not mentioned in the manuscript).

Besides, we provide evidence that acetoclasty is negligible. Acetate is just not an important degradation product in these sediments. As stated L. 284-287:

“hydrogenotrophy becomes an increasingly important CH₄ production pathway: i) when labile OM is depleted (Chasar et al., 2000; Hornibrook et al., 2000; Whiticar 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).”

L.155 Profile underestimates the oxidation rate because it is a fit of a net rate, not a gross rate, e.g., cryptic cycling leads to CO₂ production by sulfate reduction in the absence of a curvature in the gradient.

Agreed, PROFILE provides net reaction rates. This is now clarified as:

“ $R_{\text{net}}^{\text{Ox}}$ is the net reaction rate of all relevant oxidants consumption, i.e., O₂, Fe(III) and SO₄²⁻ only because NO₃⁻ and Mn(IV) are negligible (see above). For simplicity, $R_{\text{net}}^{\text{Ox}}$ is expressed in equivalent moles of O₂ consumption rate, taking into account that SO₄²⁻ and Fe(III) have twice and one quarter the oxidizing capacity of O₂, respectively. In practice, the value of $R_{\text{net}}^{\text{Ox}}$ was calculated by adding those of $R_{\text{net}}^{\text{O}_2}$, $\frac{1}{4}R_{\text{net}}^{\text{Fe(III)}}$ and $2R_{\text{net}}^{\text{SO}_4^{2-}}$ where $R_{\text{net}}^{\text{O}_2}$, $R_{\text{net}}^{\text{Fe(III)}}$ and $R_{\text{net}}^{\text{SO}_4^{2-}}$ 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, $R_{\text{net}}^{\text{Fe(III)}} = -R_{\text{net}}^{\text{Fe}}$. It should be noted that using $R_{\text{net}}^{\text{O}_2}$, $-R_{\text{net}}^{\text{Fe}}$ and $R_{\text{net}}^{\text{SO}_4^{2-}}$ to calculate $R_{\text{net}}^{\text{Ox}}$, we indirectly take into account the re-oxidation of reduced S and Fe(II), respectively, to SO₄²⁻ and Fe(III) by O₂. Indeed, with this procedure, we underestimate the terms $\frac{1}{4}R_{\text{net}}^{\text{Fe(III)}}$ and $2R_{\text{net}}^{\text{SO}_4^{2-}}$ because re-oxidation reactions are ignored, but we overestimate by the same amount the term $R_{\text{net}}^{\text{O}_2}$. In other words, omission of these re-oxidation reactions affects only the relative consumption rates of individual oxidants and not the value of $R_{\text{net}}^{\text{Ox}}$, which is of interest here.”

#9 - Line 281 Equation 9 has not been introduced previously. I don't get those fractions.

As stated in the text, Equation 9 is a simplification of Reaction r1, where $x = \nu_1$. Reaction r1 is displayed in Table 1 and explained in section 2.3. For further clarification, the description of r1 now reads (L. 138):

“Once oxidants are depleted, fermentation of metabolizable OM of general formula C_xH_yO_z can yield acetate, CO₂ and H₂ (r1). The coefficient ν_1 in r1 constrains the relative contribution of acetoclasty and hydrogenotrophy.”

#10 - Line 322: This sentence is confusing, why should hydrogenotrophy produce DIC coupled to fermentation? Only fermentation r1 may produce CO2.

Agreed, DIC can only be produced by fermentation and not by hydrogenotrophy. We now clarify as follows:

“This high ratio indicates that DIC was not produced by fermentation (r1) alone in the Z₂ of this lake. Indeed, methanogenesis through the coupling of r1 and r4 yields a R₁/R₄ ratio of 2 if the fermenting substrate is carbohydrates (COS of 0) and lower than 2 if the fermenting substrate has a negative COS value.”

#11 - L.330: To avoid confusion, a carbon mineralization process leads to the formation of a mineral acid, e.g., carbonic acid. Neither fermentation nor methanogenesis can therefore be called mineralization processes. They are carbon degradation/decomposition processes.

We were not aware of this definition for the term “mineralization” since this term is widely used to refer to organic matter degradation/decomposition processes (i.e., transformation of organic matter to mineral molecules as DIC, Phosphate, Ammonium and CH₄) including oxidation and fermentation reactions, e.g., Burdige 1991; Larowe and Van Cappellen 2011; Arndt et al., 2013.

To avoid any confusion with other uses of the term “mineralization”, we precise in the introduction as follows (L. 50):

“Nonetheless, the performance of these models depends on the correct formulation of the complete OM mineralization reactions, e.g., OM decomposition to DIC, phosphate, ammonium and CH₄ through oxidation and fermentation reactions (Burdige 1991), particularly in terms of the metabolizable organic compounds involved.”

#12 - Line 332: Please change your terminology Methanogenesis by hydrogenotrophy cannot lead to CO2 formation

Agreed, we clarify as follows:

“Indeed, the sum of the rates of CH₄ production (ΣR_4), DIC production due to fermentation associated with CH₄ formation ($\Sigma R_1 - \Sigma R_4$) and HMW OM partial fermentation (ΣR_2)”

#13 - Line 337-340 This conclusion cannot truly be validated with the approach used here.

We agree that the text was lacking some clarity related to this conclusion. We now believe that our conclusion here is robust given our clarifications related to comment #XX regarding the use of the d13C data to constrain reaction rates. We also precise our point as follows:

“The inclusion of $\delta^{13}\text{C}$ data in the present modeling study thus allowed to better constrain the effective rates of CH₄ production (R₄). Indeed, a value of $R_4 = 119 \text{ fmol cm}^{-3}\text{s}^{-1}$ was required in Eq. (7) to produce an acceptable $\delta^{13}\text{C}$ -CH₄ profile (Table 3 and Fig. S3).”

#14 - Line 353-355 Again, the authors make the mistake of modelling net concentration profiles to extract information on gross rates. The H₂ production and CO₂ production rates by cryptic cycling are not reflected in curvatures of concentration gradients, these only represented the net effect.

We now clarify the text as follows:

“The progressive downward increases in dissolved Fe and SO₄²⁻ (Fig. 2e, f, m and n) below ~5 cm depth and decrease in ΣS(-II) (Fig. 2n) observed in the porewaters suggest a net production of H₂ from r8 in both lakes. However, in the Z₁ and Z₂ of Lake Tantaré Basin A, the rate of solid Fe(III) reduction (<3 fmol cm⁻³ s⁻¹; calculated from Liu et al. 2015) is much lower than that required from r8 (i.e., 1 to 2 times the additional H₂ production of 4R₄ - 2R₁; 70-424 fmol cm⁻³ s⁻¹) to produce sufficient amounts of H₂ to sustain the additional hydrogenotrophy. The net production rates of dissolved Fe (<10 fmol cm⁻³ s⁻¹) and SO₄²⁻ (<1 fmol cm⁻³ s⁻¹) and the net consumption rate of ΣS(-II) (<1 fmol cm⁻³ s⁻¹) are also consistent with this assertion (Fig. 2).”

#15 - A cryptic sulfur cycle is only used to argue for H₂ production. Why not CO₂ production by sulfate reduction?

DIC production by sulfate reduction is considered where the value of Rox_net is positive. Admittedly our phrasing was confused. We now clarify as follows:

“R_{net}^{Ox} is the net reaction rate of all relevant oxidants consumption, i.e., O₂, Fe(III) and SO₄²⁻ only because NO₃⁻ and Mn(IV) are negligible (see above). For simplicity, R_{net}^{Ox} is expressed in equivalent moles of O₂ consumption rate, taking into account that SO₄²⁻ and Fe(III) have twice and one quarter the oxidizing capacity of O₂, respectively. In practice, the value of R_{net}^{Ox} was calculated by adding those of R_{net}^{O₂}, $\frac{1}{4}$ R_{net}^{Fe(III)}} and 2R_{net}^{SO₄²⁻} where R_{net}^{O₂}, R_{net}^{Fe(III)}} and R_{net}^{SO₄²⁻} 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_{net}^{Fe(III)}} = -R_{net}^{Fe}. It should be noted that using R_{net}^{O₂}, -R_{net}^{Fe} and R_{net}^{SO₄²⁻} to calculate R_{net}^{Ox}, we indirectly take into account the re-oxidation of reduced S and Fe(II), respectively, to SO₄²⁻ and Fe(III) by O₂. Indeed, with this procedure, we underestimate the terms $\frac{1}{4}$ R_{net}^{Fe(III)}} and 2R_{net}^{SO₄²⁻} because re-oxidation reactions are ignored, but we overestimate by the same amount the term R_{net}^{O₂}. In other words, omission of these re-oxidation reactions affects only the relative consumption rates of individual oxidants and not the value of R_{net}^{Ox}, which is of interest here.”

#16 - The overall problem with the approach is that a balance based on CO₂ and CH₄ and the OM oxidation state is too poorly constrained. In reality, in addition to a mass balance an independent charge balance should be achieved to constrain the original oxidation state of the OM. The current approach balances the electrons between the mass of methane and total CO₂ accounting for diffusive transport. This could likewise be achieved by adjusting the alkalinity.

Agreed, an independent charge balance would have been very useful, but our current dataset does not enable to perform it. Note, however, that our mass balance is corroborated with the isotopic mass balance which add some robustness to our approach. In addition, the number of sites where our approach yields consistent results (i.e., a COS value < -1.0), especially for the seasonally anoxic sites where oxidation reactions, DIC production through partial fermentation (r2) and bioirrigation

do not prevent the accurate estimation of fermentation reaction rates (see also l. 423 – 442), provide a strong support for the robustness of our approach.

#17 - I think that the authors use the model the wrong way. It is perfectly fine for comparing rates in the different zones, but it is not possible to balance the inventories in the respective zones with this model. A more sophisticated reaction transport model that accounts for the cumulative amount of DIC formed during burial needs to be used to explain the amount and isotope composition of DIC. The model Profile only captures a snapshot of a concentration distribution, i.e., the steady state, and it does not allow for calculating cumulative effects during burial, which is important for a diagenetic model and for this case to account for the buried amount of DIC from oxic respiration. In addition, the steady state assumption is invalid for most natural cases except for very small distance, e.g., at the micrometer scale where diffusion is extremely fast. A time-dependent model that includes mass accumulation rates must be used here.

We agree that our dataset only provide a snapshot of the complex OM degradation cycling. The concentration profiles presented here are the result of “**the cumulative amount of DIC formed during burial**”, and of changing conditions at the SWI. However, the inverse modelling tools used here with the assumption of steady state, whose validity has been discussed previously several times for the study sites (e.g., Clayer et al., 2016; Couture et al., 2008; Feyte et al., 2012), enables us to obtain the net reaction rates for this snapshot for CH₄, DIC and Oxidants (relevant here are O₂, Fe(III), and SO₄) independently of the background concentrations.

We are confident that the depth distributions of the net reaction rates that we present in Fig. 3g, h, o and p for [CH₄] and DIC are robust. Indeed, 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 measured 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.

After having seriously considered the suggestion of the reviewer to use a non-steady state model, 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, of dissolved oxygen and other oxidants, the rate constants for each reaction of OM degradation) which is not the case for the inverse model.

We do not have the pretention to resolve all aspects of the complex OM degradation cycling, e.g., explaining the magnitude of all the fluxes involved at a given depth. We use the net reaction rates obtained by PROFILE in our isotopic model to constrain the gross rates and estimate process rates in a given sediment zone.

REFs:

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.