

Reviewer 1

We thank the reviewer for his/her careful review of our manuscript, which highly helped us improving the new version of the manuscript to be submitted. We have addressed all of the Reviewer's concerns with new additional data, namely:

- Pore water SO_4^{2-} , Ca^{2+} , Mg^{2+} and Mn^{2+} concentrations were added to Figure 2a and are discussed in the revised manuscript
- Based on geochemical modeling of pore water major ions, we calculated saturation indices for specific minerals. The revised manuscript contains a new table listing saturation indices for diagenetic minerals relevant of the present system (e.g. vivianite, siderite, calcite).
- The description of the methods and results (SO_4^{2-} , PO_4^{3-} , Fe^{2+} , Mn^{2+} , Ca^{2+} , Mg^{2+}) has been carefully reviewed and corrected.

We did our best to fulfill all the remarks and suggestions brought by the Reviewer. Each comment has been addressed in separate answers, and all the corresponding changes are highlighted in red in the text. Please, find here after our point by point answers to Reviewer 1.

Yours sincerely,

Aurèle Vuillemin

Specific comments

Section 2.4

- **line 27:** Phosphate was measured by ion chromatography. This method has the disadvantage of having a rather poor detection limit. The authors announce a limit of quantification of 14.3 μM , which is insufficient here. Commonly used colorimetric methods give at least 20x better detection. The main problem here is that all the concentrations presented in pore water are between 0 and 1 μM , well below the limit of quantification. Figure 2 shows a PO_4 profile with variations between 0 and 1 μM . There is therefore a problem with these PO_4 data, either in the description of the method or in the results; this must be seriously corrected.

Answer 1: Thank you for detecting this serious mistake from our side. By inadvertence, we reported the method used for major ion quantification. We indeed measured phosphate concentrations using spectrophotometry. We corrected the corresponding method part as follows:

“Concentrations of PO_4^{3-} in pore water were measured by spectrophotometry. We aliquoted 0.5 mL pore water to 1.5 mL disposable cuvettes (Brand Gmbh, Germany) and added 80 μL color reagent consisting of ammonium molybdate containing ascorbic acid and antimony (Murphy and Riley, 1962). Absorbance was measured at 882 nm with a DR 3900 spectrophotometer (Hach, Düsseldorf, Germany). Detection limit of the method is 0.05 μM .”

Additional reference:

- Murphy, J., and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters
Anal. Chim. Acta, 27, 31-36, 1962.

- **line 29:** pH is measured in the supernatant after homogenization of 2 mL sediment in 2 mL deionized water. I have never seen that the pH of pore water can be measured in this way. Is there a reference? The pH is measured here in pore water diluted with deionized water. This cannot give the in-situ pH value. In addition, the authors solve the carbonate system with this questionable pH measurement. Why did the authors not measure the pH in the water used to measure alkalinity?

Answer 2: What we presently applied is a method commonly used to measure pH in soil samples. We followed the published method no. 9045B from Black (1973) and calibrated our results based on the Standard Reference Material Catalog (Seward, 1986). This information has been added to the text.

“We homogenized 2 mL of sediment in 2 mL of deionized water and measured the supernatant after 2 min, which is the method commonly used to measure pH in organic-rich soil samples. We followed the published method no. 9045B from Black (1973) and calibrated our results based on the Standard Reference Material Catalog (Seward, 1986).”

Additional references:

- Black, C. A. (Ed.): Methods of Soil Analysis: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, 9045B Soil and Waste pH, American Society of Agronomy, Madison, USA, 1973.
- Seward, R. W. (Ed.): NBS Standard Reference Material Catalog NBS, Special Publication 260, National Bureau of Standards, Gaithersburg, USA, 1986-1987.

Section 3.4

- **line 25:** “Given that vivianite is a Fe^{2+} -bearing mineral phase, the isotopically light $\delta^{56}\text{Fe}$ values we measured in vivianites from Lake Towuti are consistent with the direction of fractionation occurring during Fe^{3+} reduction.”

This interpretation is based on the principle that Fe(II) is the result of the reduction of Fe(III) . But there is no evidence of that. The interpretation given here is indeed the one that would be given for "classical" sediments. But here, the context is very particular and it cannot be excluded that the Fe^{2+} that produced the vivianite does not come from the dissolution of an initial Fe(II) phase. This point should be discussed.

Answer 3: We modified this sentence in part 3.4 with the following statement:

“...are consistent with the direction of fractionation occurring during Fe^{3+} reduction. However, dissolution of precursory ferrous phases could also be the source of the Fe^{2+} incorporated in vivianite crystals.”

We rewrote most of section 4.3 in the discussion in order to address Towuti's Fe mineralogy in terms of source to sink processes. We discuss the dissolution of initial and transient ferric/ferrous phases, the neoformation of minerals in the sediment and the related redistribution of Fe isotopes during reductive

diagenesis. We use new pore water data (i.e. Mn^{2+} , Ca^{2+} , Mg^{2+} and SO_4^{2-} concentrations) and modeled saturation indices for vivianite and siderite to support our interpretations (see answer no. 13).

Section 4.1

- **p.11, first paragraph:** “The ferrous Fe and P released from these reactions may produce vivianite, and/or be consumed through reactions with other dissolved elements, such as S.”

This first paragraph is not precise enough and is based on too many insinuations. Be more specific.

Answer 5: We replaced this sentence by the following one:

“In aquatic systems and surface sediments, Fe chemistry influences the distribution of dissolved sulfide, the solubility of trace metals, and bioavailability of phosphorus and thereby controls their rates of burial (Severmann et al., 2006)...”

Additional reference:

- Severmann, S., Johnson, C. M., Beard, B. L., and McManus, J.: The effect of early diagenesis on the Fe isotope compositions of porewaters and authigenic minerals in continental margin sediments, *Geochim. Cosmochim. Ac.*, 70, 2006-2022, <https://doi.org/10.1016/j.gca.2006.01.007>, 2006.

- **line 8 and 13:** HS^- and H_2S .

Answer 6: H_2S modified to HS^- consistently throughout the text.

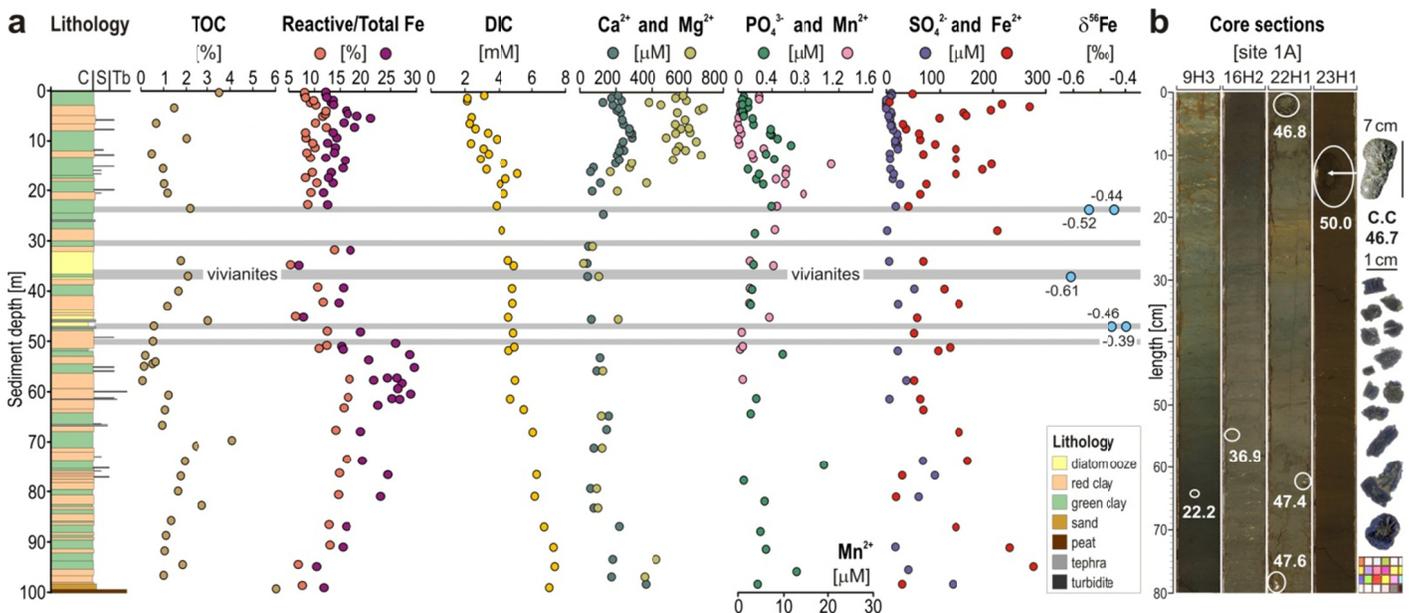
- **line 16** “Because rates of sulfide production are so low compared to the Fe delivery flux” and “in sediments such as Lake Towuti’s, siderite is an expected mineral phase” What are the quantitative elements to assert this?

Answer 7: We implemented pore water SO_4^{2-} concentrations to Figure 2a (here under). With the exception of four data points (i.e. 85, 81, 107, 121 μM), SO_4^{2-} concentrations are systematically below 50 μM and are on average 27 μM over the whole profile, which confirms quantitatively that production of HS^- in pore water is limited.

We also modeled mineral saturation indices based on the complete pore water dataset using the PHREEQC v.3 software, inclusive of all major cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} , Si^{4+} , Fe^{2+} , DIC), anions (Cl^- , SO_4^{2-} , NO_3^- , PO_4^{3-}), alkalinity, pH and borehole temperatures. These results show that pore water is oversaturated with respect to siderite (>0) in the entire sediment column, whereas vivianite remains close to, but slightly below saturation (-0.04). We report these modeled indices in a new table (Table 1, here under) and implemented the manuscript accordingly.

Additional reference:

- Parkhurst, D. L., and Appelo, C. A. J. (Eds.): Description of input and examples for PHREEQC version 3: a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations, book 6, chapter A43: U.S. Geological Survey Techniques and Methods, Denver, USA, 2013.



Revised Figure 2

| 5 m depth | Saturation | 10 m depth | Saturation |
|------------------|--------------|------------------|--------------|
| talc | 1.43 | siderite | 1.00 |
| siderite | 1.29 | quartz | 0.71 |
| quartz | 0.71 | vivianite | -0.04 |
| vivianite | -0.45 | talc | -0.31 |
| calcite | -0.68 | calcite | -0.83 |
| dolomite | -0.77 | aragonite | -0.97 |
| aragonite | -0.82 | dolomite | -1.27 |

Table 1

- **line 27:** “Because of the high concentrations of Fe oxides in Lake Towuti’s sediment (20 wt %), it is very unlikely that much P could escape to the bottom water.”

This is another unjustified statement. If the ferric phases have their PO₄ adsorption sites saturated, PO₄ can migrate.

Answer 8: We rephrased the second part of this sentence as follows:

“Concentrations of Fe oxides in Lake Towuti’s sediment are high (~ 20 wt %), and iron oxides such as goethite persist in the modern sediment even under full anoxia at the water-sediment interface and below (Sheppard et al., 2019). If PO₄³⁻ could diffuse out of the sediment,…”

- **line 30:** “In the deep sediments, pore water PO_4^{3-} concentrations are constantly low in the interval where vivianite crystals are observed (Fig. 2a), suggesting that vivianites acted as a main P sink during diagenesis”.

PO_4 concentrations are not quantified, given the method used. Are there not the elements to calculate the theoretical concentration of PO_4 that would be at thermodynamic equilibrium with vivianite? Such a calculation could strengthen the assertions.

Answer 9: As mentioned in answer no. 1, we corrected the description of the method. Since PO_4^{3-} concentrations were measured via spectrophotometry, our values can be considered reliable and quantitatively sound. As explained in answer no. 7, we also modeled mineral saturation indices based on the complete pore water dataset, which provide theoretical values on pore water saturation with respect to both siderite and vivianite. This new data is available as Table 1.

- **p. 12, line 18:** “they are...rather subject to variety of processes that are variable down core.”

The conclusion of this paragraph reflects its vague and speculative nature. The processes mentioned should be better described. Methane production suddenly appears without any other explanation. It would be better to specify the presentation.

Answer 10: We deleted this part of the sentence. We rephrased the sentences discussing methanogenic conditions and the role of CO_2 reduction by methanogenic archaea in controlling pore water activity of dissolved CO_3^{2-} ions. We also implemented Figure 2a with pore water Ca^{2+} , Mg^{2+} and Mn^{2+} concentrations and discuss their role in controlling PO_4^{3-} solubility and reactivity toward Fe^{2+} . The text (p. 12-13, lines 30-9) was modified as follows:

“...In contrast, Fe^{3+} concentrations in pore water are already depleted along the upper meter of sediment, whereas concentrations of DIC, which is produced during OM degradation, gradually increase with depth (Fig. 2a), suggesting that OM remineralization in shallow sediment is mainly driven by fermentation and methanogenesis rather than microbial Fe reduction (Vuillemin et al., 2018). In the vivianite-bearing intervals, DIC concentrations remain rather constant (4 mM). An explanation for this is that, once pore water Fe^{3+} and SO_4^{2-} concentrations are depleted as a result of microbial reduction within the first meter, OM remineralization mostly occurs by CO_2 reduction and methanogenesis (Friese et al., 2018). The onset of autotrophic methanogenesis is expected to reduce DIC activity in pore water and to draw down PO_4^{3-} cycling by microbes. Moreover, Ca^{2+} and Mg^{2+} concentrations in pore water, which are predicted to control the solubility of PO_4^{3-} in ferruginous systems (Jones et al., 2015), drop around these depths as divalent cations can precipitate during siderite formation (Vuillemin et al., 2019a), suggesting that PO_4^{3-} can then outcompete CO_3^{2-} for available Fe^{2+} and thereby saturate pore water with respect to vivianite (Table 1). Vivianite formation is indeed reported to occur under methanogenic conditions, often initiating below the sulfate-methane transition zone (Reed et al., 2011; Dijkstra et al., 2016), presently located within the upper meter of sediment (Vuillemin et al., 2018). Inclusions of millerite and siderite within vivianite crystals (Fig. 4) provide additional lines of evidence for microbial processes of pore water Fe^{3+} and SO_4^{2-} reduction with DIC production prior to vivianite formation. The saturation indices modeled for vivianite (Table 1) and downcore profiles of Mn^{2+} and PO_4^{3-} concentrations allow to infer a depth in the sediment at which pore waters initially reached saturation with respect to vivianite (ca. 20 m depth). Such relationship between dissolved Mn^{2+} and

PO₄³⁻ is also consistent with EDX punctual analyses of vivianite crystals that show Mn²⁺ incorporation at an early stage (Fig. 3b, Supplementary Fig. S4)....”

Additional references:

- Friese, A., Kallmeyer, J., Glombitza, C., Vuillemin, A., Simister, R., Nomosatryo, S., Bauer, K., Heuer, V. B., Henny, C., Crowe, S. A., Ariztegui, D., Bijaksana, S., Vogel, H., Melles, M., Russell, J. M., and Wagner, D.: Methanogenesis predominates organic matter remineralization in a ferruginous, non-sulfidic sedimentary environment, EGU General Assembly Conference Abstracts , 20, 7446, 2018.
- Jones, C., Nomosatryo, S., Crowe, S. A., Bjerrum, C. J., and Canfield, D. E.: Iron oxides, divalent cations, silica, and the early earth phosphorus crisis, *Geology*, 43, 135-138, <http://doi.org/10.1130/G36044.1>, 2015.

- **line 29:** “The presence of diatomaceous oozes, with vivianites below and above these sediments, indicates that P concentrations in the water column were much higher during this time interval compared to present-day levels”

The authors suggest here that the fossilization of more diatoms suggests that primary production was higher at the time of these deposits and that the increase in primary production was due to an increase in phosphate inputs. If this is the case, it should be detailed as such. But I am not sure that this is necessarily the case, because the exact opposite can be interpreted. Indeed, one could also imagine that an increase in eutrophication due to PO₄ input would favor cyanobacteria rather than diatoms, as observed in many lakes. Thus, more diatoms could indicate less PO₄. This should be discussed. In addition, the preservation of diatoms may also come from conditions more favorable to their fossilization than other periods.

Answer 11: We modified this sentence and added another one to address primary productivity by diatoms and cyanobacteria under increased P concentrations and their relative preservation in the sediment. These two sentences are as follows:

“...The substantial fossilization of diatoms, with vivianites below and above these sediments, could reflect higher P concentrations in the water column during this time interval compared to present-day levels...”

“...As P concentrations tend to affect algal phytoplankton productivity as a whole (Zhang and Prepas, 1996; Van der Grinten et al., 2004), high Si concentrations in the lake represent an additional factor promoting the preservation of diatoms over cyanobacteria during sinking and burial....”

Additional references:

- Van der Grinten, E., Janssen, M., Simis, S. G. H., Barranguet, C., and Admiraal, W.: Phosphate regime structures species composition in cultured phototrophic biofilms, *Freshwater Biol.*, 49, 369-381, <https://doi.org/10.1111/j.1365-2427.01189.x>, 2004.
- Zhang, Y., and Prepas, E. E.: Regulation of the dominance of planktonic diatoms and cyanobacteria in four eutrophic hardwater lakes by nutrients, water column stability, and temperature, *Can. J. Fish. Aquat. Sci.*, 53, 621-633, <https://doi.org/10.1139/f95-205>, 1996.

Section 4.3

- **line 12:** “Dissimilatory microbial reduction of iron releases Fe^{2+} in pore water that is up to 2 ‰ lighter than the original substrates ” the fractioning will depend on the rate of reduction.

Answer 12: Agreed. We modified the first part of this paragraph as follows:

“Previous Fe isotope studies of lakes identified either partial oxidation of Fe^{2+} in the water column or microbial iron reduction below the sediment-water interface as the main drivers for Fe pathways and isotope fractionation (Teutsch et al., 2009; Song et al., 2011; Liu et al., 2015). Depending on rates of Fe reduction and dissolution (Brantley et al., 2001), dissimilatory microbial reduction of iron releases Fe^{2+} that is up to 2 ‰ lighter than the original substrates (Crosby et al., 2007; Tangalos et al., 2010), therefore iron isotopes are commonly used to trace redox processes related to microbial activity in aquatic sediments (Percak-Dennett et al., 2013; Busigny et al., 2014). . . .”

- **line 21:** $\delta^{56}\text{Fe}$ values measured on vivianite are compared to “expected” values for iron oxides. From this point on, all the following in this paragraph is speculative and not supported by data.

Answer 13: As mentioned in answer no. 3, the second paragraph of section 4.3 was entirely rewritten to present Towuti’s Fe mineralogy in terms of source to sink processes. We address the dissolution of initial and transient ferric/ferrous phases, the neof ormation of minerals in the sediment and the related redistribution of Fe isotopes during reductive diagenesis. We further refer to previous publications on Towuti’s Fe mineralogy (Tamuntuan et al., 2015; Sheppard et al., 2019), and use new pore water data (i.e. Ca^{2+} , Mg^{2+} and SO_4^{2-} concentrations) and modeled saturation indices to support our interpretations. The second part of section 4.3 was modified as follows:

“Compared to the global bulk igneous rock reservoir ($\delta^{56}\text{Fe} = +0.1 \pm 0.1$ ‰) and ultramafic rocks (Dauphas et al. 2017) such as those present in Lake Towuti’s catchment, the $\delta^{56}\text{Fe}$ measured on whole vivianite crystals (-0.61 to -0.39 ‰) reveals incorporation of isotopically fractionated light Fe^{2+} (Fig. 2a), even though traces of detrital iron-bearing minerals and secondary oxides are present within vivianite crystals (Figs 4 and 5). Towuti’s Fe mineralogy from source to sink reflects complex cycling of Fe as iron minerals derived from catchment soils (e.g. goethite, hematite, magnetite) tend to transform into nanocrystalline Fe phases during reductive dissolution in the lake water column and sediment (Tamuntuan et al., 2015; Sheppard et al., 2019). Ferric/ferrous phases precipitating in equilibrium at the oxycline or during mixing events could be abiotically fractionated to 1-2 ‰ heavier/lighter isotope values than the remaining aqueous Fe^{2+} (Bullen et al., 2001; Skulan et al., 2002; Beard et al., 2010; Wu et al., 2011). After deposition, partitioning of the light Fe isotopes mainly transits through release to pore water (Henkel et al., 2016) implying a succession of mineral transformation and dissolution with internal diagenetic Fe redistribution during burial (Severmann et al., 2006; Scholz et al., 2014). For instance, mixed-valence iron oxides (e.g. green rust), which are authigenic phases that form initially under ferruginous conditions (Zegeye et al., 2012; Vuillemin et al., 2019a) are highly sorbent for pore water HCO_3^- and HPO_4^{2-} and can thereby transform into either siderite or vivianite as the sediment ages (Hansen and Poulsen, 1999; Bocher et al., 2004; Refait et al., 2007; Halevy et al., 2017). Because vivianite formation initiates in the sediment, we infer that vivianite crystals acted as additional traps for the reduced Fe^{2+} released to pore water and that their light $\delta^{56}\text{Fe}$ values are consistent with kinetic

fractionation related to microbial Fe reduction during early diagenesis, or eventually inherited from post-depositional dissolution of transient ferrous phases. In the latter case, pre-depositional processes of abiotic Fe fractionation related to stratified conditions will require further investigations.”

Additional references:

- Bocher, F., Géhin, A., Ruby, C., Ghanbaja, J., Abdelmoula, M., and Génin, J.-M. R.: Coprecipitation of Fe(II–III) hydroxycarbonate green rust stabilized by phosphate adsorption, *Solid State Science*, 6, 117–124, <https://doi.org/10.1016/j.solidstatesciences.2003.10.004>, 2004.
- Brantley, S. L., Liermann, L., and Bullen, T. D.: Fractionation of Fe isotopes by soil microbes and organic acids, *Geology*, 29, 535–538, [https://doi.org/10.1130/0091-7613\(2001\)029<0535:FOFIBS>2.0.CO;2](https://doi.org/10.1130/0091-7613(2001)029<0535:FOFIBS>2.0.CO;2), 2001.
- Halevy, I., Alesker, M., Schuster, E. M., Popovitz-Biro, R., and Feldman, Y.: A key role for green rust in the Precambrian oceans and the genesis of iron formations, *Nat. Geosci.*, 10, 135–139, <https://doi.org/10.1038/ngeo2878>, 2017.
- Hansen, H. C. B., and Poulsen, I. F.: Interaction of synthetic sulphate "green rust" with phosphate and the crystallization of vivianite, *Clay. Clay Miner.*, 47, 312–318, doi: 10.1346/CCMN.1999.0470307, 1999.
- Henkel, S., Kasten, S., Poulton, S. W., and Staubwasser, M.: Determination of the stable iron isotopic composition of sequentially leached iron phases in marine sediments. *Chem. Geol.* 421, 93–102, <https://doi.org/10.1016/j.chemgeo.2015.12.003>, 2015.
- Refait, P., Reffass, M., Landoulsi, J., Sabot, R., and Jeannin, M.: Role of phosphate species during the formation and transformation of the Fe(II–III) hydroxycarbonate green rust, *Colloid. Surface A.*, 299, 29–37, doi: 10.1016/j.colsurfa.2006.11.013, 2007.
- Scholz, F., Severmann, S., McManus, J., Noffke, A., Lomnitz, U., and Hensen, C.: On the isotope composition of reactive iron in marine sediments: Redox shuttle versus early diagenesis. *Chem. Geol.*, 389, 48–59, <https://doi.org/10.1016/j.chemgeo.2014.09.009>, 2014.
- Severmann, S., Johnson, C. M., Beard, B. L., and McManus, J.: The effect of early diagenesis on the Fe isotope compositions of porewaters and authigenic minerals in continental margin sediments, *Geochim. Cosmochim. Ac.*, 70, 2006–2022, <https://doi.org/10.1016/j.gca.2006.01.007>, 2006.

- **Last sentence of the conclusion:** “Although crystallization time was not constrained, supply of pore water PO_4^{3-} and Fe^{2+} during diagenesis maintained saturation with respect to vivianite and supported the continuous growth of crystals with depth.”

Here again, this statement is not validated by calculations. The degree of water saturation with respect to vivianite has not been calculated.

Answer 14: We deleted this sentence. As mentioned in answer no. 7, the revised manuscript now includes a new table of modeled saturation indices (Table 1) showing that pore water reaches conditions that are very close to saturation with respect to vivianite with depth. If pore water PO_4^{3-} was indeed incorporated in vivianite crystals during burial, this PO_4^{3-} cannot be accounted for in the pore water modeling. One can thus only infer a timeframe during which geochemical conditions in pore water were saturated with respect to vivianite. In this context, the downcore profiles for pore water Mn^{2+} and PO_4^{3-} concentrations allow to infer the depth at which pore waters reach initial saturation with respect to vivianite (ca. 20 m depth).

Technical corrections

- **Section 2.1, line 10:** Describe in more detail the chronology of the operations, i.e. date of drilling, extraction of pore water.... the minerals were extracted after more than 6 months of storage. Please specify it.

Answer 15: For detailed information on field sampling and core processing, the readership can refer to (Russell et al., 2016; Friese et al., 2017). Following the reviewer's suggestion, we implemented part 2.1 as follows:

“The TDP coring operations were carried out from May to July 2015 using the International Continental Scientific Drilling Program (ICDP) Deep Lakes Drilling System (Russell et al., 2016). Hole TDP-TOW15-1A (156 m water depth; hereafter TDP-1A) was drilled in May 2015 with a fluid contamination tracer used to aid geomicrobiological sampling (Friese et al., 2017). Samples were collected from cores from TDP-1A immediately upon recovery and over 450 samples were subsequently processed in the field for analyses of pore-water chemistry, cell counting and microbial fingerprinting, and organic geochemistry. Core catchers from TD-1A were packed into gas-tight aluminum foil bags flushed with nitrogen gas and heat-sealed to keep them under anoxic conditions until mineral extraction. Pore water was extracted on site from 5-cm-long whole round cores (6.6 cm diameter) that were cut from the core sections, immediately capped and transferred to an anaerobic chamber flushed with nitrogen to avoid oxidation during sample handling (Friese et al., 2017). In January 2016, the unsampled remainders of the cores from TDP-1A were split and scanned at the Limnological Research Center, Lacustrine Core Facility (LacCore), University of Minnesota, described macroscopically and microscopically to determine their stratigraphy and composition (Russell et al., 2016) and then subsampled. Minerals from core catcher sediments were extracted after 3 month of storage, and macroscopically visible vivianite crystals were hand-picked from split TDP-1A cores after 8 months of storage. Except as otherwise noted, all our samples and measurements come primarily from hole TDP-1A.”

- **Section 2.2, line 24:** The carbonate removal technique to measure the total organic carbon goes through a washing and centrifugation phase. Doesn't this step cause the loss of organic matter less dense than water?

Answer 16: This is the standard procedure applied for TOC and bulk organic matter using HCl (5%) on freeze-dried samples. We tested this method on pure siderite to ensure that this carbonate phase dissolves in the same way as calcite. Such sample processing is routine analysis at the Forschungszentrum Jülich.

- **Section 2.4, line 17:** Dissolved Fe is measured using Ferrozine. Authors have used the method described by Viollier et al., 2000. This study recommends using ascorbic acid to transform all dissolved iron (FeII + FeIII) into FeII. Was ascorbic acid used here?

Answer 17: In Viollier et al., (2000), the authors mention the addition of hydroxylamine hydrochloride as the reducing agent used to measure pore water Fe^{3+} , and not ascorbic acid as stated by the reviewer (which is included in the measurement of PO_4^{3-} concentrations). Fe^{3+} concentrations can be calculated as the difference between total Fe and Fe^{2+} . We did process the samples using hydroxylamine hydrochloride and therefore we corrected the method description part accordingly. However, total Fe concentrations measured in pore water were identical to those of Fe^{2+} , and thus Fe^{3+} is absent in pore water. We also referenced the initial protocol from Stookey (1970). The method part was revised as follows:

“Dissolved ferrous and ferric iron concentrations were measured in the field via spectrophotometry (Stookey, 1970). Directly after pore water retrieval, we aliquoted 1 mL of pore water sample to 1.6 mL Rotilabo single-use cells (Carl Roth, Karlsruhe, Germany) and stabilized dissolved Fe^{2+} by adding 100 μL of Ferrozine Iron Reagent (Sigma-Aldrich Chemie Munich, Germany). Absorbance of the colored solution was measured at 562 nm with a DR 3900 spectrophotometer (Hach, Düsseldorf, Germany). To determine pore water total Fe concentrations, 150 μL of hydroxylamine hydrochloride were added to 800 μL of the previous mixture, left to react 10 min to reduce all dissolved Fe^{3+} , stabilized by adding 50 μL ammonium acetate and absorbance of the solution measured a second time (Viollier et al., 2000). Pore water total Fe concentrations were found to be the same as Fe^{2+} concentrations, and thus Fe^{3+} is absent in pore water. Detection limit of the method is 0.25 μM .”

Additional reference:

- Stookey, L. L.: Ferrozine - A new spectrophotometric reagent for iron, *Anal. Chem.*, 42, 779-781, <https://doi.org/10.1021/ac60289a016>, 1970.