

Reviewer 2

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

- Pore water SO_4^{2-} , Mn^{2+} , Ca^{2+} and Mg^{2+} concentrations were added to Figure 2a and are discussed in the revised manuscript.
- Downcore profiles for both highly reactive and total iron in bulk sediment are provided in Figure 2a.
- Based on geochemical modeling of pore water major ions, we calculated saturation indices for specific minerals (e.g. vivianite, siderite). These indices are listed in a new table (Table 1) now included in the revised manuscript.

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 2.

Yours sincerely,

Aurèle Vuillemin

Major comments

- **Comment 1:** The authors should clarify and emphasize their findings. For example, the authors should present an important finding regarding vivianite formation already in the title. The same applies to the abstract and later on, and what is novel regarding the formation of vivianite should be emphasized here in comparison to previous publications (e.g. Slomp, Paytan, Marz, Kasten).

Answer 1: We changed the title to “Microbially mediated formation of vivianite during early diagenesis in ferruginous sediments, Lake Towuti, Indonesia.”

We implemented the abstract with the following sentences to emphasize, as expressly demanded, the novelty and implications of our findings:

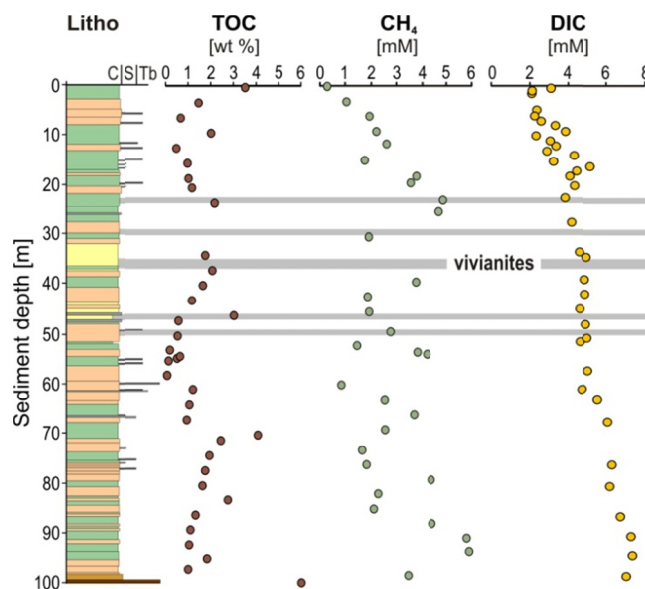
“...Together with pore water profiles, these suggest that the precipitation of millerite, siderite, and vivianite in soft ferruginous sediments stems from the gradual microbial reduction of pore water electron acceptors. Based on solute concentrations and modeled mineral saturation indices, we inferred vivianite formation to initiate around 20 m depth in the sediment as the likely result of the decrease in microbial activity and associated P recycling in pore water. Negative $\delta^{56}\text{Fe}$ values of vivianite indicated incorporation of kinetically fractionated light Fe^{2+} into the crystals, likely derived from active reduction and dissolution of ferric oxides and transient ferrous phases during early diagenesis. The size and growth history of the nodules indicate that, after formation, continued growth of vivianite crystals constitutes a sink for P during burial, resulting in long-term P sequestration in ferruginous sediment.”

- **Comment 2:** It is hard to judge the vivianite formation in response to paleoconditions, because: there is no quantitative investigation of the amounts of vivianite; there is a lack of context on pore water redox conditions in the vivianite-bearing layers (e.g. SMTZ redox sensitive elements other than iron). Where are the other pore water profiles (at least Mn^{2+} , SO_4^{2-} , methane)?

Answer 2: We provide additional pore water data, namely downcore profiles for SO_4^{2-} , Ca^{2+} , Mg^{2+} , and Mn^{2+} concentrations. The downcore profile for Mn^{2+} concentrations (revised Figure 2a) covaries with PO_4^{3-} and correlates the increased Mn content measured via EDX on early stage vivianite crystals. This, in addition to the saturation indices modeled for siderite and vivianite (Table 1), allows us to infer the depth at which pore waters reach initial saturation with respect to vivianite (see answer no. 3).

We emphasize in the manuscript the depth inferred for the SMTZ, which, due very low SO_4^{2-} concentrations, is located within the upper 50 cm of sediments. This was already addressed in a previous publication based on gravity cores (Vuillemin et al., 2018, Environ. Microbiol. 20).

Concerning methane concentrations, because a manuscript addressing methane production is presently under review, we refrain from providing this data. Since there is no preprint for this manuscript, we only cite an abstract related to the manuscript (Friese et al., 2018; EGU abstract). To answer the reviewer's demand, we provide at his/her discretion an insight into methane concentrations here under, which confirms that the SMTZ is found in very shallow sediments and that methane concentrations constantly increase with sediment depth. The trend for methane is similar to the one of DIC.



Because vivianites are only observed from 20 to 50 m depth intercalated with diatom oozes, we further addressed paleoproductivity and changes in the sedimentary regime. Still, we consider that we provide clear and sufficient evidence for the microbially mediated formation of vivianite during early diagenesis (see answer no. 9). We would also like to bring to the attention of the Reviewer that analyses for paleoenvironmental reconstructions are ongoing work and that a first manuscript detailing the lithostratigraphy and sedimentological processes is close to being submitted. The age model, which is the

most critical aspect in reconstructing past climate, is still under preparation. In the meanwhile, one has to accept some inferences and avoid speculations.

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.

- **Comment 3:** The paleointerpretation is confusing (page 12). When was the vivianite precipitated in the 20-50 m interval: Thousands years ago at the bottom of the lake or now due to current diagenetic processes? More data and discussion (e.g. diffusion modeling) are needed to support the first or second option. Otherwise, it is hard to suggest environmental interpretations without putting the current diagenetic processes into context or quantifying inferred past processes.

Answer 3: We now provide saturation indices modeled for specific minerals (e.g. vivianite, siderite) in a new table (Table 1, here under). These results show that siderite is the main mineral to reach saturation in the pore water at relatively shallow sediment depths (5 m), and that the saturation index modeled for vivianite increases with depth, but remains slightly below saturation. This, in addition to pore water concentrations (i.e. Mn^{2+} , Ca^{2+} , Mg^{2+}), allows to infer the depth at which pore waters are initially saturated with respect to vivianite, which is about 15 m depth. We consider that the downcore profile for pore water Mn^{2+} and PO_4^{3-} in parallel confirms this and correlate the increased Mn content measured on vivianite crystals via EDX and its incorporation at an early stage of growth.

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

Specific comments

Abstract

The first sentence is not relevant as it refers to ferric iron and phosphate adsorption and not to vivianite. Rewrite a general sentence that states that ferruginous lakes are important to the phosphorous cycle because of X, Y, etc.

Answer 4: We rephrased the first sentence as follows:

“Ferruginous lacustrine systems, such as Lake Towuti, Indonesia, represent specific cases of phosphorus cycling in which hydrous ferric iron (oxyhydr)oxides trap and precipitate phosphorus to the sediment, which reduces its bioavailability in the water column and thereby restricts primary production.”

- **line 34** is trivial. Also add “active” reduction on line 35 to make it also non-trivial. It is clear that the redox state is very low in this system to precipitate vivianite, the iron isotopes may suggest its active reduction in this zone. Be careful also with stating it is microbial reduction, as the isotope composition can be light also with abiotic reduction.

Answer 5: This sentence and rest of the abstract has been rewritten as follows:

“...Mineral inclusions like millerite and siderite reflect diagenetic mineral formation antecedent to the one of vivianite that is related to microbial reduction of iron and sulfate. Together with pore water profiles, these suggest that the precipitation of millerite, siderite, and vivianite in soft ferruginous sediments stems from the gradual microbial reduction of pore water electron acceptors. Based on solute concentrations and modeled mineral saturation indices, we inferred vivianite formation to initiate around 20 m depth in the sediment as the likely result of the decrease in microbial activity and associated P recycling in pore water. Negative $\delta^{56}\text{Fe}$ values of vivianite indicated incorporation of kinetically fractionated light Fe^{2+} into the crystals, likely derived from active reduction and dissolution of ferric oxides and transient ferrous phases during early diagenesis. The size and growth history of the nodules indicate that, after formation, continued growth of vivianite crystals constitutes a sink for P during burial, resulting in long-term P sequestration in ferruginous sediment.”

Introduction

I do not agree that vivianite is not a studied mineral in sediments. Please correct.

Answer 6: We removed “...less studied...” from the sentence.

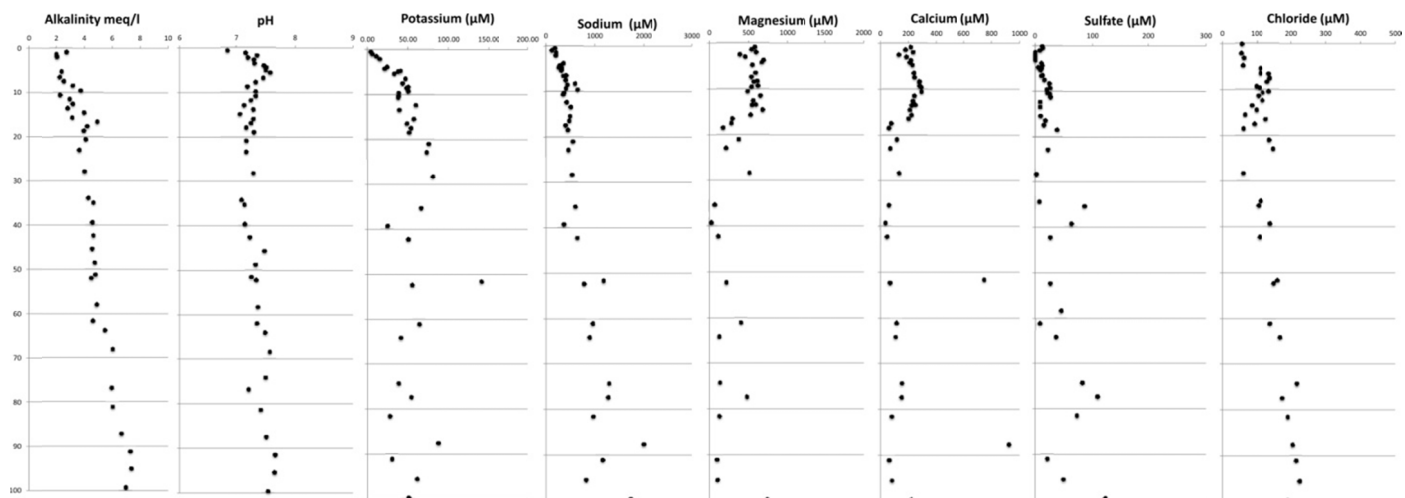
Methods

- **page 5:** Can the DIC be calculated indeed by this approach? How can the authors be sure the alkalinity is mostly carbonatic in this organic-rich sediment? Have they measured the carbonate alkalinity or the total alkalinity?

Answer 7: We measured total alkalinity along with the pH in pore water. In case, the relationship between total alkalinity and carbonate alkalinity according to Jenkins and Moore (1977) is the following:

Carbonate alkalinity (CaCO_3 mg/L) = $100,000 [K_2 (\text{HCO}_3^-)(10^{\text{pH}})]$.

We added the corresponding reference to the method part. We also measured pore water K^+ , Na^+ , Mg^{2+} , Ca^{2+} , SO_4^{2-} and Cl^- concentrations among others (here under, for information only) and checked the cation-anion mass balance. Thus, we are confident that the DIC calculated by this approach is consistent.



Additional reference:

- Jenkins, S. R., and Moore, R. C.: A proposed modification to the classical method of calculating alkalinity in natural waters, *J. Am. Water Works Ass.* 69: 56-60, <https://doi.org/10.1002/j.1551-8833.1977.tb02544.x>, 1977.

- **page 7:** I do not understand how the authors know that they isolate vivianite for the isotope measurement. Please clarify, also in consideration to the fact that diagenetic minerals are sometime more reactive to dissolution than detritus ones (refer to Henkel’s publications).

Answer 8: Fe isotope analyses were carried out on entire vivianite crystals, not on pools obtained via Fe sequential extraction. We hand-picked vivianite crystals under the binocular from the dense mineral extracts. The separation and identification procedure is explained in part 2.4. These crystals were further used for XRD, SEM and TEM analyses, which resulted in the certain and definitive identification of the mineral vivianite. The same isolated vivianite crystals were processed for Fe isotope analyses. We clarified this in the text, for instance in:

- page 7, lines 9-10: “Minerals observed under a stereo microscope (Nikon SMZ800) included siderite, vivianite, millerite (i.e. NiS) and detrital pyroxene (i.e. $\text{Ca}(\text{Mg}, \text{Fe})\text{Si}_2\text{O}_6$). Pyrite (i.e. FeS_2) was not observed. Vivianite crystals, which were identified in the interval from 20 to 50 m sediment depth, were hand-picked under the stereo microscope for further analyses.”

-page 8, lines 17-19: “After density separation, vivianite crystals were hand-picked under the stereo microscope and the isolated crystals processed for Fe isotope analyses at the HELGES lab, GFZ Potsdam (von Blanckenburg et al., 2016); however, the presence of some minor inclusions of siderite, silicates and oxides within vivianite crystals could not be ruled out.”

Discussion

- **page 12:** see also above. More data is needed and calculations to support precipitation of vivianite at the last glacial.

Answer 9: We do not state anywhere that vivianite crystallization initiated during the Last Glacial. We only provide the paleolacustrine context corresponding to the sediments that surround vivianite nodules and how

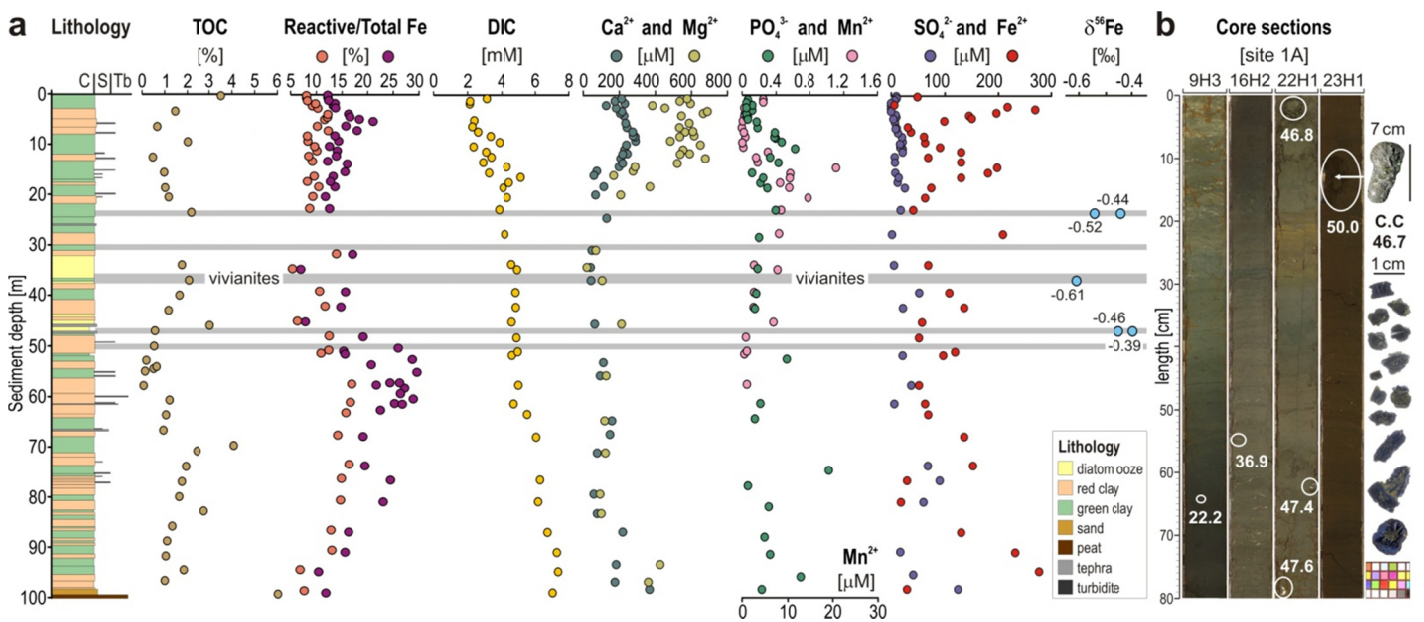
these sediments resulted in geochemical conditions that were favorable to the formation of vivianite during diagenesis, and thus later burial.

To clarify the link between lacustrine paleoconditions and post-depositional processes leading to the diagenetic formation of vivianite in this specific sedimentary interval, we modified the title and introductory sentences of this subchapter as follows:

“Past lacustrine conditions promoting vivianite formation during burial

In sulfur-poor, ferruginous settings, vivianite, siderite and magnetite can be formed in the sediments (Postma, 1981) depending on the local pH, CO₂, PO₄³⁻, the amount and reactivity of ferric oxides and OM buried in the sediment (Fredrickson et al., 1998; Glasauer et al., 2003; O’Loughlin et al., 2013). By analogy to hydromorphic soils (Maher et al., 2003; Vodyanitskii and Shoba, 2015), redox conditions at the time of deposition and fluxes of OM and reactive ferric oxides to the sediment would select for siderite or vivianite as the main diagenetic ferrous end-members during burial.”

Otherwise, we provide substantial additional pore water data (i.e. Ca²⁺, Mg²⁺, Mn²⁺ and SO₄²⁻ concentrations; see revised Figure 2) along with modeled saturation indices for siderite and vivianite (Table 1) to allow us to infer the initial depth of formation of vivianite (see answer no 3). In regards of these additional data, we have carefully checked and clarified the discussion.



Revised Figure 2

Additional references:

- Maher, B. A., Alekseev, A., and Alekseeva, T.: Magnetic mineralogy of soils across the Russian Steppe: climatic dependence of pedogenic magnetite formation. *Palaeogeogr. Palaeoclimatol.* 201, 321-341, doi: 10.1016/S0031-0182(03)00618-7, 2003.
- Vodyanitskii, Y. N., and Shoba, S. A.: Ephemeral Fe(II)/Fe(III) layered double hydroxides in hydromorphic soils: A review, *Eurasian Soil Sci.*, 48, 240-249, doi: 10.1134/S10642293150, 2015.

- **page 13:** Again, also abiotic reduction can result in 2 ‰ fractionation.

Answer 10: We are aware of this fact. However, cases of abiotic reduction of Fe that reach 2 ‰ isotopic fractionation usually occur at oxic-anoxic interfaces (see Bullen et al., 2001; Wiesli et al., 2004; Wu et al., 2012). Pore waters are anoxic and it is unlikely that such abiotic fractionation could occur in the sediment. We provide multiple lines of evidence that vivianite crystals form in anoxic sediments, and not in the water column. Nevertheless, as answered to Reviewer 1, 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 neoformation of minerals in the sediment and the related redistribution of Fe isotopes during reductive diagenesis. This includes potential abiotic fractionation at the water column oxycline. The second paragraph of section 4.3 has been rewritten as follows:

“...Compared to the global bulk igneous rock reservoir ($\delta^{56}\text{Fe} = +0.1 \pm 0.1 \text{ ‰}$) 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.
- Bullen, T. D., White, A. F., Childs, C. W., Vivit, D. V., & Schulz M. S. Demonstration of significant abiotic iron isotope fractionation in nature. *Geology* 29, 699-702 (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.chem.geol.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.
- Welch, S. A., Beard, B. L., Johnson, C. M., & Braterman, P. S. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(II) and Fe(III). *Geochim. Cosmochim. Ac.* **67**, 4231-4250 (2003).
- Wiesli, R. A., Beard, B. L., & Johnson, C. M. Experimental determination of Fe isotope fractionation between aqueous Fe(II), siderite and "green rust" in abiotic systems. *Chem. Geol.* **211**, 343-362 (2004).
- Wu, L., Percak-Dennett, E. M., Beard, B. L., Roden, E. E., & Johnson, C. M. Stable iron isotope fractionation between aqueous Fe(II) and model Archean ocean Fe-Si coprecipitates and implications for iron isotope variations in the ancient rock record. *Geochim. Cosmochim. Ac.* **84**, 14-28 (2012).