Revised title: Living and nonliving particulate iron in the subtropical North Pacific Ocean

Manuscript # egusphere-2025-6068

RC2: 'Comment on egusphere-2025-6068', Anonymous Referee #2, 14 Feb 2026

Review of egusphere-2025-6068, “Biogenic and nonliving labile particulate iron in the subtropical North Pacific Ocean“ (Bates and Hawco)

(General Comments)

Estimation of biogenic and authigenic labile particulate Fe is important but challenging work. In previous studies, biogenic Fe fraction can be estimated based on Fe quotas and particulate organic carbon or phosphorus but the last two analytical fractions (C and N) contain organic detritus (e.g., dead cells, fecal pellets). According to the present authors’ idea on “biogenic” Fe (see below on terminology “biogenic”), the above approach can lead to overestimation of biogenic Fe. Instead, the authors introduce a new approach using particulate nucleotide adenosine-5’-triphosphate (ATP) to estimate “living organic carbon” to derive biogenic Fe. Labile particulate Fe minus this biogenic Fe was defined as “nonliving”, which was main labile pFe pool below the euphotic zone. These attempts are really fascinating. The authors also conducted Fe uptake experiments in the surface mixed layer on 12 cruises at Station ALOHA in the subtropical North Pacific for 3-year period coverage. The bulk Fe uptake rates increased with increasing Prochlorococcus and picoeukaryotes abundances. These data are important to understand the Fe availability for primary production in the subtropical North Pacific. Dataset is of good quality, mostly discussion is orderly, and the manuscript provides new findings and approach. Therefore, I highly evaluate these works and think the manuscript deserves to be published.

However, there are several points of concern including the terminology in this study. In this manuscript, the authors defined their “biogenic” Fe using particulate ATP as “living” because of the rapid hydrolysis of ATP in dead biomass. However, in previous studies (e.g. Sofen et al., 2023; Tagliabue et al., 2023), the “biogenic” particles reflect “biological uptake and accumulation in living biomass and differential remineralization from “dead biomass” (Sofen et al., 2023). I’m not so sure which definition is widely accepted, but I prefer to include dead biomass in the “biogenic fraction”. For example, I think Fe in freshly produced fecal pellets is “biogenic”. In the ocean, the term “biotic Fe” has sometime been used (e.g. Boyd et al., 2015), which might be better for this case (“living” biotic Fe). Thus, the authors should discuss the definition of “biogenic” and clarify the difference from the term in previous studies, like Sofen et al. (2023). Including the treatment of the term “biogenic”, several minor comments/questions to be addressed are given below:

We thank the reviewer for taking the time to review our manuscript. We appreciate the reviewer’s feedback on terminology, as we are eager to find the most appropriate terminology to use for these operationally defined terms. We will switch to the term “biotic” throughout and emphasize “biogenic” vs “biotic”, particularly in the context of comparison with Sofen et al. (2023).

We have modified the beginning of section 3.2 Estimating pFe in biomass using three different approaches:

“Quantifying the amount of Fe in biomass is challenging, due in part to issues in quantifying living biomass in the ocean generally. Here, we assess three different approaches to calculating particulate Fe in biomass (pFe_{Bio}). First, following the approach by Sofen et al. (2023) to determine biogenic pFe, we use particulate carbon (PC) and particulate phosphorus (PP), which likely include both living cellular and ‘dead’ organic detrital material, although to different extents. For the final approach, we estimate biotic pFe, or Fe in living biomass, using adenosine-5’-triphosphate (ATP) due to the rapid hydrolysis of ATP following cell death (Holm-Hansen & Booth, 1966). The pFe_{Bio} estimated by these approaches is compared to the suspended pFe_{Labile}, defined using the Berger et al. (2008) labile leach, which has been shown to capture the biogenic pool (Rauschenberg & Twining, 2015). Thus, labile pFe represents an approximate maximum for pFe_{Bio}, if there were no additional contributions from the remainder of the labile pFe pool (including authigenic pFe).”

1. Page 2, Line 36, “biogenic pFe pool”: Actually, the term “biotic” was used in Boyd et al. (2015). Please make sure of it.

We thank the reviewer for catching this, it has been fixed.

2. Page 3, Line 64, “the biogenic and nonliving labile pFe pools”: Previous studies (Sofen et al., 2023) divided labile pFe to biogenic and authigenic fractions to understand the marine Fe cycles. Considering the “authigenic” fraction, I understand that the biogenic fraction includes fresh organic detrital materials. Why do the authors newly define “the living and nonliving labile pFe pools” in this study? Please clarify the reason here.

Our methods access different iron pools than Sofen et al. (2023), thus we felt the “authigenic” terminology was not appropriate for the remainder of our labile pFe pool – which includes dead biomass in addition to the authigenic & labile dust

pool. Based on this and the rest of the reviewer's feedback, we have changed these terms to "biotic" and "authigenic + detrital".

3. Page 5, Line 126 – 127, "uptake rates were similar in May for both 2021 and 2023": How about cell counts of Prochlorococcus and picoeukaryote?

Unfortunately, flow cytometry data is not available for the 2023 cruises so we were unable to make this comparison. However, uptake rates were also similar for July 2021 and July 2022 (32 ± 16 and 37 ± 19, respectively). *Prochlorococcus* counts were similar for these two cruises (1.76 x 10^5 and 1.65 x 10^5 / mL) while picoeukaryotes were more variable (0.007 x 10^5 and 0.004 x 10^5 / mL).

4. Page 5, Line 131 – 132, "Prochlorococcus and picoeukaryotes together make up the bulk of picophytoplankton biomass and primary production at Station ALOHA (Rii et al., 2016).": According to Bates et al. (2015), particulate biogenic Si inventories in the upper 150 m showed marked increase in spring 2021. Did this reflect the diatom bloom? If so, was any feature found in Fe uptake ratio?

Concurrent increases in HPLC pigment fucoxanthin (HOT-DOGS, 2026) suggest particulate biogenic Si increases observed during this time series were indeed driven by diatoms. Note that particulate biogenic Si was elevated to a greater degree in summer 2022 (~7 mmol m^{-2} 0-150 m inventory in July 2022 compared to ~3.5 mmol m^{-2} in spring 2021; Bates et al. 2025), so we would expect to see any feature driven by diatoms in both spring 2021 and summer 2022. While the Fe uptake rate and ratio are elevated in May 2021, they agree with those found in May 2023, when no diatom bloom was observed. Similarly, summer 2022 data agree well with uptake rates and ratios observed in summer 2021 and 2023. In our previous study (Hawco et al. 2022 L&O), we performed calculations highlighting that the ability of larger cells to dominate bulk Fe uptake rates is muted by their comparatively small surface area to volume ratio compared to small phytoplankton like *Prochlorococcus*.

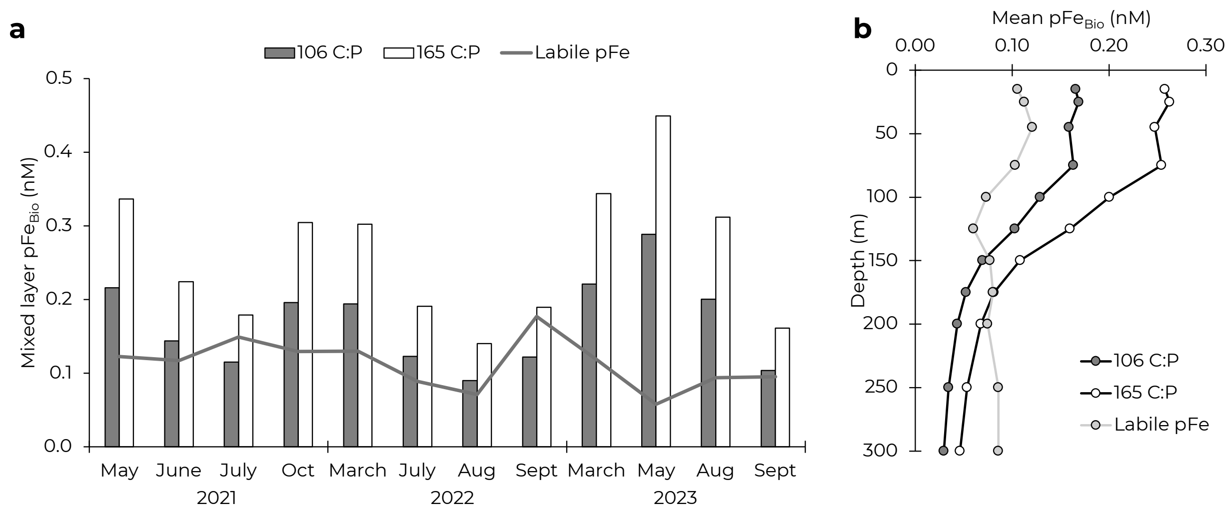
Hawco, N. J., Yang, S.-C., Pinedo-González, P., Black, E. E., Kenyon, J., Ferrón, S., Bian, X., and John, S. G.: Recycling of dissolved iron in the North Pacific Subtropical Gyre, Limnology and Oceanography, 67, 2448–2465, https://doi.org/10.1002/lno.12212, 2022.

HOT-DOGS: the Hawaii Ocean Time-series Data Organization & Graphical System, 2026. https://hahana.soest.hawaii.edu/hot/hot-dogs/index.html

5. Page 8, Line 174 – 175, "The PC and PP approaches consistently overestimated pFe_{Bio}, which almost always exceeded measurements of pFe_{Labile}": What will

happen if the Redfield ratio was applied for this calculation? I'm not sure whether "biogenic Fe" should include organic detrital material or not. If the organic detrital material were included, the authors should consider wider ranges of the C:P ratio.

We appreciate the reviewer's suggestion and have repeated the PP analysis using the Redfield ratio, and plan to add this figure to the supplement. While it results in better agreement than the $C:P_{Phyto}$ of 165 mol:mol, it still on average overestimates pFe_{Bio} above pFe_{Labile}. Additionally, dissolved and particulate organic matter pools at Station ALOHA have been shown to exceed the Redfield ratio due to relative phosphate depletion (Björkman et al., 2000; Karl et al., 2001).



Björkman, K., Thomson-Bulldis, A. L., and Karl, D. M.: Phosphorus dynamics in the North Pacific subtropical gyre, Aquatic Microbial Ecology, 22, 185–198, https://doi.org/10.3354/ame022185, 2000.

Karl, D. M., Björkman, K. M., Dore, J. E., Fujieki, L., Hebel, D. V., Houlihan, T., Letelier, R. M., and Tupas, L. M.: Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA, Deep Sea Research Part II: Topical Studies in Oceanography, 48, 1529–1566, https://doi.org/10.1016/S0967-0645(00)00152-1, 2001.

6. Page 9, Line 198, can incorporating heterotrophic bacteria lead to increase or decrease C:P ratio or negligible contribution? Although relevant data is given as spreadsheet, please clarify this.

We appreciate the reviewer's note and have clarified this sentence:

"Additionally, the calculated $C:P_{Phyto}$ used in Eq. 4 omits a major contribution from heterotrophic bacteria to bulk biomass (Karl et al., 2022) and therefore may

overestimate the true C:P of living biomass (Fagerbakke et al., 1996; White et al., 2019)."

Fagerbakke, K., Heldal, M., and Norland, S.: Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria, Aquat. Microb. Ecol., 10, 15–27, https://doi.org/10.3354/ame010015, 1996.

White, A. E., Giovannoni, S. J., Zhao, Y., Vergin, K., and Carlson, C. A.: Elemental content and stoichiometry of SAR11 chemoheterotrophic marine bacteria, Limnol Oceanogr Letters, 4, 44–51, https://doi.org/10.1002/lol2.10103, 2019.

7. Pages 7–9, Since several previous studies also estimate biogenic Fe including detrital organic matter (e.g., Sofen et al., 2023) and the present authors' estimate is based on living organic carbon, so I think the use or definition of new terminology is necessary; e.g., "living biogenic Fe/ living biotic Fe" may be better than "biogenic Fe". I think the term "biogenic" can include not only living but also dead material and fecal pellets etc. In my opinion, "biogenic Fe" should not be confined to what is obtained by the use of ATP.

We appreciate this feedback and have changed the terminology to "biotic" Fe.

8. Page 9, Line 217 – 219, "Recent work based on shipboard experiments and Prochlorococcus measurements supports the use of a 250 C:ATP ratio at Station ALOHA (Karl et al., 2022), in agreement with a variety of other field and laboratory studies of marine organisms": In Howco et al. (2022), the contributions of both Prochlorococcus and heterotrophic bacteria were considered for Fe' uptake in seawater. However, the C:ATP ratio in heterotrophic bacteria was not discussed here. The authors should mention the uncertainty of C:ATP ratio in heterotrophic bacteria.

We appreciate the reviewer noting this oversight and have added discussion of the C:ATP ratio in heterotrophic bacteria:

"Additionally, there is some uncertainty in the influence of heterotrophic bacteria on the C:ATP ratio, which has been reported for heterotrophic bacteria at ~100 g g^{-1} (Hewes et al., 1990)."

Hewes, C. D., Sakshaug, E., Reid, F. M. H., and Holm-Hansen, O.: Microbial autotrophic and heterotrophic eucaryotes in Antarctic waters: relationships between biomass and chlorophyll, adenosine triphosphate and particulate organic carbon, Marine Ecology Progress Series, 63, 27–35, https://doi.org/10.3354/meps063027, 1990.

9. Page 10, Line 240, “Overall, the ATP approach provides the most reasonable pFe_{Bio} estimates of the three approaches assessed.”: I’m not sure whether some biogenic fraction including fresh organic detrital materials should be categorized to “nonliving labile pFe” or not. Please explain the merit to consider fresh organic detrital materials and authigenic fraction together as “nonliving labile pFe”.

Based on this feedback, we have changed the “nonliving labile” terminology to “authigenic + detrital”. We have chosen to consider these pools together as we do not have reliable methods to further separate this pool. While conceptually we may be able to use the PC or PP approach to estimate biotic + detrital particulate Fe, this results in an Fe pool approximately double the size of that accessed by the Berger leach (Figure 2b), perhaps due to uncertainties in the Fe:C ratio of detrital material.

10. Page 11, Line 262, “euphotic zone”: Please indicate the approximate depths of euphotic zone.

Corrected to “upper euphotic zone (0-75 m)”.

11. Page 11, Line 272, “majority nonliving below the DCM”: Isn’t the heterotrophic bacteria included in this fraction?

With the change in terminology, this has been changed to “majority authigenic + detrital below the DCM”. Additionally, heterotrophic bacteria should be included in the biotic pFe fraction as they contain ATP.

12. Page 15, Line 324, Given comparable values of particulate biogenic Fe ranging 0.03–0.10 nM for ALOHA and 0.03–0.08 nM for BATS, and overestimates by 2023Sofen’s method including detrital organic materials, this leads to higher biogenic Fe at ALOHA than BATS. Is it possible to give a likely explanation for the higher biogenic Fe at ALOHA since this is an intriguing feature?

We appreciate the reviewer’s suggestion and have added the following discussion:

“Given the much higher concentration of pFe_{Labile} at BATS, the possible overestimation of pFe_{Bio}, as shown here for Station ALOHA, is less impactful. Regardless, the inclusion of detrital organic material in the pFe_{Bio} estimates at BATS would suggest that biotic pFe in living cells would be comparatively lower than at Station ALOHA, which may be due to differences in phytoplankton community composition or biomass. While the two sites have overall similar magnitudes of phytoplankton biomass (Selph et al., 2022), BATS shows

significantly more variability in *Prochlorococcus* over the seasonal cycle, typically only reaching the levels observed at Station ALOHA during spring blooms (Malmstrom et al., 2010). "

Malmstrom, R. R., Coe, A., Kettler, G. C., Martiny, A. C., Frias-Lopez, J., Zinser, E. R., and Chisholm, S. W.: Temporal dynamics of *Prochlorococcus* ecotypes in the Atlantic and Pacific oceans, The ISME Journal, 4, 1252–1264, https://doi.org/10.1038/ismej.2010.60, 2010.

Selph, K. E., Swalethorp, R., R Stukel, M., B Kelly, T., N Knapp, A., Fleming, K., Hernandez, T., and Landry, M. R.: Phytoplankton community composition and biomass in the oligotrophic Gulf of Mexico, J Plankton Res, 44, 618–637, https://doi.org/10.1093/plankt/fbab006, 2022.

13. In Figure 5 and the caption, and LINE 325, "HOT" had better be replaced by "ALOHA" as I believe the former is the program/cruise name and the latter is the station name.

We thank the reviewer for catching this, it has been fixed.

14. Page 15, Line 330, the description seems to state "overestimation comes from synchrotron X-ray fluorescence". I believe it is ascribed to the use of organic P, not to X-ray fluorescence.

We appreciate the reviewer pointing this out and have change the wording to:

"This approach, using labile particulate phosphorus, includes detrital organic material as part of pFe_{Bio} and assumes Fe quotas from living, eukaryotic phytoplankton cells are representative of the entire microbial pool (Sofen et al., 2023)."

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