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

Manuscript # egusphere-2025-6068

RC1: 'Comment on egusphere-2025-6068', Anonymous Referee #1, 14 Jan 2026

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

Summary

This manuscript explores the composition and dynamics of labile particulate iron (pFe) in the subtropical North Pacific, focusing on the split between biogenic and nonliving fractions. To achieve this, the authors undertook iron (Fe) uptake experiments (using the Fe isotope double spike method) and carbon (C) uptake to estimate how much Fe and C are incorporated into cells. They then use Fe:C uptake ratios in combination with particulate organic carbon (POC), particulate organic phosphorus (PP) and ATPase to estimate biogenic pFe. To estimate labile iron, Bates and Hawco used the chemical leach method of Berger et al. (2008) to estimate easily mobilised Fe from biogenic and non-living material. Finally, they connect the two and determine that biogenic Fe accounts for approximately 60% of labile Fe in the mixed layer, with the rest being associated with nonliving matter.

Overall, the manuscript is generally well written. One bugbear is that the manuscript keeps directing the reader to other papers for DFe and associated data (see examples below). I realise that the data has been published, but it would be useful to include plots in the supporting materials; otherwise, the reader has to sift through articles to check the claims.

We appreciate the reviewer’s consideration of our manuscript. We will add the associated Fe data published in our other manuscripts to this work.

Specific comments

Line 96: Are you saying that at the end of the incubation, there was ~a 50:50 split of the FeDS between the dissolved and particulate phases? Or is this total - probably the total as you added ~ 50 pM.

This is the total mean concentration of FeDS that was recovered at the end of the incubation; we have clarified this in the text which now reads:

“The total mean FeDS recovered from the dissolved and particulate phases was 55 ± 21 pM, …”

Line 119-120: Where is the evidence to support this? Please reference a figure or table here - as a reader, I really don't want to have to search through other references for the data. It’s your data (Bates and Hawco, 2025), so this should be easy to generate. Perhaps you could add an extra couple of panels to Fig 1 showing the iron data or add a new figure. Or you could add a figure to the supplementary information showing the DFe data and reference it came from Bates and Hawco, 2025.
Line 122-123: Again, please don't make me read other papers to see the primary data you are referring to - show it here and then reference where it came from.

We thank the reviewer for identifying how to make our work more accessible for readers. We will add the seasonal variability for euphotic zone dFe and labile pFe to Figure 1. We will also add the seasonal variability of lithogenic pFe and particulate Fe export to a Supplemental Figure so the reader does not have to seek out the other references.

Line 129: What about Synechococcus? was that measured? It can also be an important player in tropical and subtropical waters. Certainly, it is often found at shallower depths than Prochlorococcus (Flombaum et al., 2013).

Yes, we were also interested in the potential role of *Synechococcus* in Fe uptake. However, it did not show a strong correlation with Fe uptake rates (line 133). This is likely because *Synechococcus* cell numbers are relatively low at Station ALOHA, comprising just 2-8% of picophytoplankton biomass in both the upper and lower euphotic zones and ~2% of total ^{14}C productivity integrated across the euphotic zone (Rii et al. 2016).

Rii, Y., Karl, D. M., and Church, M.: Temporal and vertical variability in picophytoplankton primary productivity in the North Pacific Subtropical Gyre, Mar. Ecol. Prog. Ser., 562, 1–18, https://doi.org/10.3354/meps11954, 2016.

Line 134: Table S1 only show correlation data; perhaps you could show the population data for Prochlorococcus, Synechococcus, and picoeukaryotes at 25 m. That will allow the reader to check the data and the points about abundance made in the text.

We will add a supplemental figure showing the temporal variability of *Prochlorococcus*, *Synechococcus,* picoeukaryotes, and heterotrophic bacteria populations over the course of this study.

Line 134: How about presenting the 14C data. It would be nice to see how it varied temporally. We only have the Fe:C ratio data.

We appreciate the reviewer noting this and will add the HOT ^{14}C data to Figure 1.

Figure 1. Because you say that Prochlorococcus and picoeukaryotes dominate, is it possible to normalise the iron uptake to cell number to get an idea of uptake per cell? As you show in panels c-e, the strong coupling between uptake and Prochlorococcus and picoeukaryotes abundance indicates that it is driven by cell abundance, which is likely to vary seasonally. Based on the comments about the 14C data on lines 133 to 134, I assume that primary production (14C) and cell abundance are not coupled? It might be worth showing this as well.

We appreciate the reviewer's suggestion to calculate an uptake per cell. Due to co-occurrence of both groups with vastly different cell sizes, we do not feel that it is appropriate to cell number without creating new problems. However, we can use the slopes of the multiple linear regression model (Fig. 1e) to approximate an apparent Fe uptake per cell for *Prochlorococcus* and picoeukaryotes.

For *Prochlorococcus,* which dominate phytoplankton cell counts (but not necessarily biomass), our regression indicates a slope of 0.00045 pM Fe uptake per cell/mL per day. We can convert this to an apparent *Prochlorococcus* uptake rate of 45 x 10^{-19} mol Fe cell^{-1} d^{-1}, or 0.45 amol Fe cell^{-1} d^{-1}. This is in reasonable agreement with group-specific Fe uptake rates for *Prochlorococcus* from the South Pacific Ocean (Lory et al., 2022). A similar analysis for picoeukaryotes yields an apparent uptake rate of 39 amol Fe cell^{-1} d^{-1}. The ~100-fold difference in cell-specific Fe uptake rates is reasonable considering the ~100-fold difference in cell-specific C productivity for picoeukayotes (~30 fmol C cell^{-1} d^{-1} in the mixed layer; Rii et al., 2016) compared to *Prochlorococcus* (~0.3 fmol C cell^{-1} d^{-1} in the mixed layer; Rii et al. 2016). We have added the following text to paragraph two of section 3.1:

"From the slope of the multiple linear regression, we can calculate apparent cellular uptake rates of 0.45 amol Fe cell^{-1} d^{-1} for *Prochlorococcus* and 39 amol Fe cell^{-1} d^{-1} for picoeukaryotes. The *Prochlorococcus* rate agrees with previously reported *Prochlorococcus*-specific Fe uptake rates (Lory et al., 2022), while the ~100-fold difference between the two groups is consistent with the ~100-fold difference in group-specific ^{14}C productivity rates (Rii et al., 2016)."

^{14}C and cell abundances were not well correlated and we have amended line 133 to:

"^{14}C-based primary production was less variable than Fe uptake rates over these cruises (RSD = 0.13 for primary production compared to 0.40 for Fe uptake) and did not correlate well with cell abundances (R^2 < 0.1 for *Prochlorococcus*, *Synechococcus,* picoeukaryotes, and heterotrophic bacteria)."

Lory, C., Van Wambeke, F., Fourquez, M., Barani, A., Guieu, C., Tilliette, C., Marie, D., Nunige, S., Berman-Frank, I., and Bonnet, S.: Assessing the contribution of diazotrophs to microbial Fe uptake using a group specific approach in the Western Tropical South Pacific Ocean, ISME Communications, 2, 41, https://doi.org/10.1038/s43705-022-00122-7, 2022.

Rii, Y., Karl, D. M., and Church, M.: Temporal and vertical variability in picophytoplankton primary productivity in the North Pacific Subtropical Gyre, Mar. Ecol. Prog. Ser., 562, 1–18, https://doi.org/10.3354/meps11954, 2016.

Line 175: The assumption here is that the Berger method is getting all of the biogenic Fe - did you check the Fe/Al ratio for the labile and total pools to see if they jive with each other? Also, perhaps it should be mentioned that the Berger method was designed to look at labile iron from the Columbia River plume and coastal waters off the West Coast of the US. The values in that study were in the high nanomolar range for iron, whereas concentrations in the present work are subnanomolar. Since most of the iron in the present work is likely within organic molecules, dead and alive, it is possible that the Berger leach does not access this as molecules may need to be oxidised (noting the Berger leach is reducing) to break them down before iron can be accessed. Just a thought.

We appreciate the reviewer's note of the complexity of interpreting results from the Berger leach. The mean mixed layer Fe/Al ratio of the refractory pool was 0.50 mol:mol, notably lower than the Fe/Al of the total pool (mean 0.69 mol:mol), but in agreement with the total Fe/Al ratio found in North Pacific aerosols (0.53 mol:mol, Buck et al. 2006). This suggests the Berger leach removed biogenic Fe but did not solubilize lithogenic Fe (which would have released Al). Rauschenberg & Twining (2015) also compared the Berger leach to direct measurement of phytoplankton cellular Fe and found good agreement in the subnanomolar range (including samples from the center of the North Atlantic subtropical gyre). We also note that the leach includes a heating step for the purpose of denaturing proteins (Berger et al., 2008), which we believe is effective for releasing Fe from colloidal biomolecules.

We have added the following sentence to the first paragraph of 3.2 Estimating pFe in biomass using three different approaches (line 160):

"The Berger et al. (2008) leach was originally developed to estimate labile pFe in a river plume and coastal waters, but subsequent applications in oligotrophic waters have shown that it can effectively solubilize biologically relevant pools of pFe (Rauschenberg & Twining, 2015)."

Berger, C. J. M., Lippiatt, S. M., Lawrence, M. G., and Bruland, K. W.: Application of a chemical leach technique for estimating labile particulate aluminum, iron, and manganese in the Columbia River plume and coastal waters off Oregon and Washington, Journal of Geophysical Research: Oceans, 113, https://doi.org/10.1029/2007JC004703, 2008.

Buck, C. S., Landing, W. M., Resing, J. A., and Lebon, G. T.: Aerosol iron and aluminum solubility in the northwest Pacific Ocean: Results from the 2002 IOC cruise: AEROSOL FE AND AL SOLUBILITY, Geochem. Geophys. Geosyst., 7, n/a-n/a, https://doi.org/10.1029/2005GC000977, 2006.

Rauschenberg, S. and Twining, B. S.: Evaluation of approaches to estimate biogenic particulate trace metals in the ocean, Marine Chemistry, 171, 67–77, https://doi.org/10.1016/j.marchem.2015.01.004, 2015.

Figure 3, panel b. The unit nM needs to be removed as this is a fraction calculation.

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

Figure 4 caption. "…authigenic (navy).." is mentioned, but the figure key is "Labile nonliving pFe".

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

Line 303 – I like this, it's always good to compare to other regions.

Thank you, we agree this is an important section for contextualizing our work.

References

Bates, E.S., Hawco, N.J., 2025. Dissolved Iron Seasonal Cycle and Residence Time in the North Pacific Subtropical Gyre. Geophysical Research Letters, 52(21): e2025GL118095.

Berger, C.J.M., Lippiatt, S.M., Lawrence, M.G., Bruland, K.W., 2008. Application of a chemical leach technique for estimating labile particulate aluminum, iron, and manganese in the Columbia River plume and coastal waters off Oregon and Washington. Journal of Geophysical Research-Oceans, 113.

Flombaum, P. et al., 2013. Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. Proceedings of the National Academy of Sciences, 110(24): 9824-9829.

Citation: https://doi.org/10.5194/egusphere-2025-6068-RC1

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?

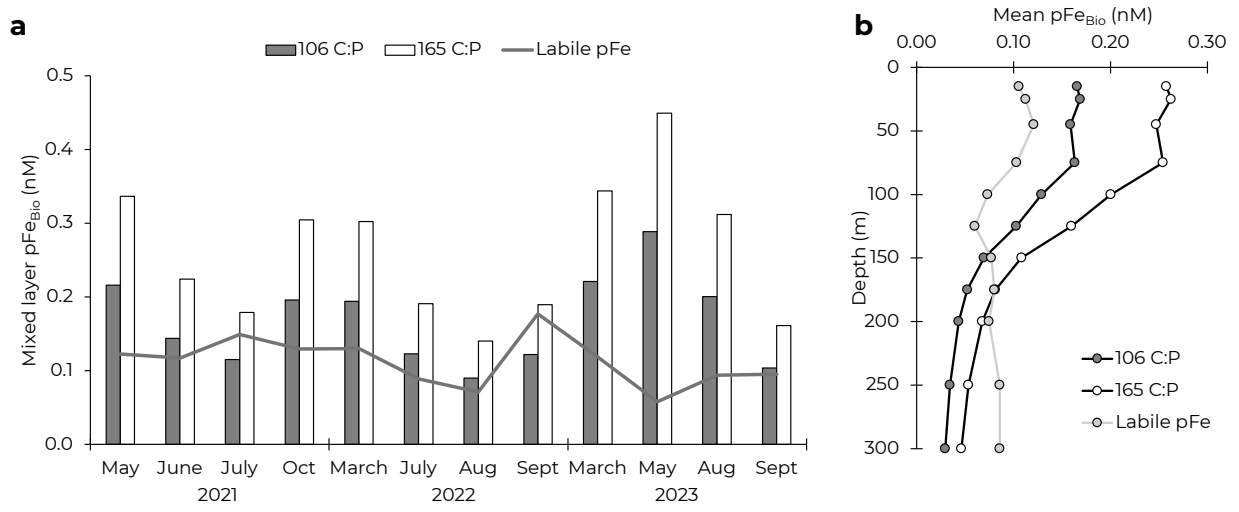
Concurrent increases in HPLC pigment fucoxanthin (HOT-DOGS, 2026) suggest particulate biogenic Si increases observed during this time series were indeed driven by diatoms. Note that particulate biogenic Si was elevated to a greater degree in summer 2022 (~7 mmol m^{-2} 0-150 m inventory in July 2022 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).”

Citation: https://doi.org/10.5194/egusphere-2025-6068-RC2

CC1: 'Comment on egusphere-2025-6068', Yang Xiang, 15 Jan 2026

Comments to the authors:

Bates and Hawco present a well-written manuscript demonstrating the partition between different particulate iron (pFe) pools, especially biogenic and nonliving pFe, during seasonal cruises at Station ALOHA. Such work is of great significance given the increasing attention on the role of authigenic mineral phases in the overall surface Fe cycling. The authors have done a good job presenting and interpreting the seasonal variations of particulate Fe data. The use of ATP to estimate biogenic is novel and quite interesting. The manuscript is clear, thorough, and nearly free of typos. However, I do have some substantive comments and editorial remarks as listed below. Overall, I recommend publication with major revisions.

We appreciate Dr. Xiang taking the time to provide feedback and have incorporated this feedback where possible.

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Major interpretation points

I have two major comments.

Firstly, the authors made many assumptions in their estimations of biogenic pFe, many of which were not mentioned in the text. For example, the Fe:C uptake ratio used in Equations 3-5 does not necessarily equal the cellular Fe:C quotas (Fe:C) used in Sofen et al. (2023). The authors need to acknowledge this and be clear about their assumptions.

This difference is discussed is lines 328-331, when comparing our data with that of Sofen et al. (2023). We have clarified this to:

"Note that the methods of estimating pFe_{Bio} differ between the two sites. Sofen et al. (2023) estimated pFe_{Bio} using Eq. 4 and cellular Fe quotas of eukaryotes determined by synchrotron X-ray fluorescence. 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)."

Additionally, Sofen et al. (2023) used PP_{Labile} in their calculations, while the authors used bulk PP in Equation 4. The authors should have the leachable PP data since they did the Berger leach. Al-Hashem et al. (2022; 10.1029/2022GB007453) found up to 60% of PP that cannot be accessed with the Berger leach, for example. Since the authors are

comparing their results with Sofen's, it's important to make sure the calculations have been conducted similarly. Otherwise, those results may not be directly comparable.

We will clarify in section 3.5 Comparison to the North Atlantic Subtropical Gyre that our approach does not allow for a perfect comparison to the Sofen et al. data, due to differences in methodological approach. We still see important value in comparing the data with the caveat of these methodological differences may have a small effect on the absolute values that we derive.

We disagree that the use of pPO4labile is the only valid measurement. The Al-Hashem et al. paper is based on a coastal transect in a very high dust region (directly off the coast of the Namib Desert), where detrital pPO4 is worth considering. In contrast, in open ocean conditions with low dust deposition like Station ALOHA, pPO4 overwhelmingly represents autochthonous 'biogenic' materials (living cells + organic detritus). Indeed, at the station closest to Station ALOHA on the GEOTRACES GP15 transect , >80% of small (0.2-51 μm) particulate P was labile (Lam et al., 2024; lability data not available for large particles, but large particulate P comprised ~10% or less of total particulate P).

Lam, P. J., Lee, J., Amaral, V. J., Laubach, A., Carracino, N., Rojas, S., Mateos, K. (2024). Size-fractionated major, minor, & trace particle composition and concentration from Leg 1 (Seattle, WA to Hilo, HI) of the US GEOTRACES Pacific Meridional Transect (PMT) cruise (GP15, RR1814) on R/V Roger Revelle from Sept to Oct 2018. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-01-30 doi:10.26008/1912/bco-dmo.918811.1

Secondly, some of the calculations are flawed. The use of PC in Equation 3 to estimate biogenic Fe is likely an overestimation.

With respect, this is the intention of the calculation. PC or POC is the primary particulate carbon measurement by HOT, BATS, and GEOTRACES, and thus is a natural starting point for estimation of biogenic Fe.

While phytoplankton production (uptake of dissolved inorganic carbon) is the dominant source of new organic matter in the open ocean, the standing stock of POC (here the authors use PC since those are the only data available) includes significant contributions from other sources. What about heterotrophic and allochthonous sources, for example?

We are not able to locate data arguing for allochthonous POC at Station ALOHA, which is generally representative of North Pacific subtropical gyre conditions and

has a turnover time with respect to photosynthesis on the order of a few days. Our recent work describing a coastal source of Fe from the Hawaiian Islands only requires a mixture of approximately 0.1-1% coastal seawater (Bates & Hawco, 2025) so we do not consider the islands to be a major source of carbon to Station ALOHA.

Bates, E. S. and Hawco, N. J.: Dissolved Iron Seasonal Cycle and Residence Time in the North Pacific Subtropical Gyre, Geophysical Research Letters, 52, https://doi.org/10.1029/2025GL118095, 2025.

Based on the authors' definition of biogenic pFe, the authors should possibly use phytoplankton carbon rather than total POC in Equation 3. Please refer to Graff et al. (2015; 10.1016/j.dsr.2015.04.006) for the nuances, with the former values much smaller.

While we appreciate this recommendation, measurement of phytoplankton carbon derived from fluorescence activated flow cytometry is nontrivial and come with its own sets of caveats and uncertainties. We are currently onboarding these techniques, but even when implemented successfully, they are low throughput, and therefore will inevitably require an extrapolation to compare with other data. Ultimately, our APT-based approach accomplishes the same goal.

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Interpretation points, by line #

Lines 14-16: Do the authors have data or literature to show how much of a fraction is labile pFe in dust deposition at Station ALOHA? I assume that it's a relatively small fraction, right?

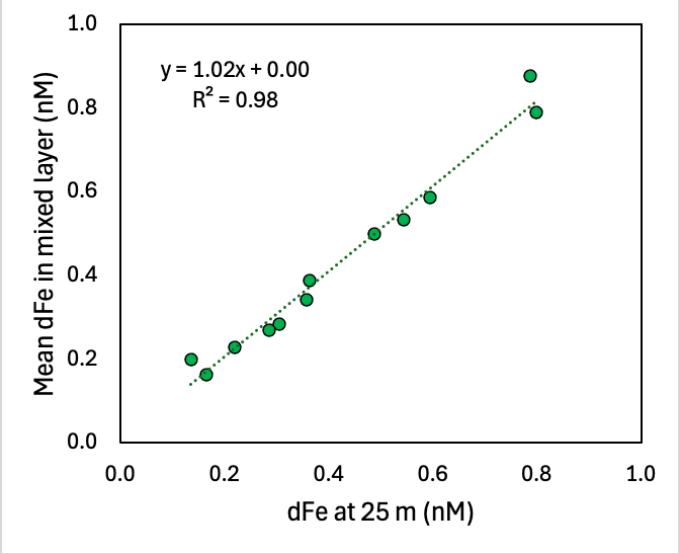
During dust deposition events at Station ALOHA, we observed labile pFe export from midwater sediment traps notably increased along with the increased lithogenic pFe export, resulting in an estimated 12-17% lability of the pFe export (Bates et al., 2025). Additionally, initial data from the Hawaii Aerosol Time-series suggests approximately 12-24% of aerosol Fe is labile (Kollman et al., 2024). Regardless, this would not change the conclusions from this study, as we are just trying to recognize that some portion of the "authigenic" pFe pool may be instead derived from dust, not formed *in situ* (lines 252-256).

Bates, E. S., White, A. E., and Hawco, N. J.: Variability and Export Timescales of Upper Ocean Particulate Trace Metals in the North Pacific Subtropical Gyre, Global Biogeochemical Cycles, 39, https://doi.org/10.1029/2025GB008657, 2025.

Kollman, C., Marsay, C. M., Ohnemus, D., Stephens, M. P., Bates, E. S., and Buck, C. S.: Results from the Hawaii Aerosol Time-Series reveal seasonally contrasting aerosol solubility in the North Pacific Oligotrophic Gyre, driven by source diversity in spring and fall dust pulses, Abstract OS21H-0665, 2024 AGU Fall Meeting, 9-13 Dec, ADS Bibcode: 2024AGUFMOS21H0665K, 2024.

Lines 103-104: Are there any specific reasonings for using dFe at 25 m rather than the mean mixed layer concentrations? How different are dFe concentrations at 25 m and within the mixed layer?

The incubations were performed using seawater collected from 25 m; thus, the dFe concentration at 25 m is the most direct comparison for calculating an uptake rate. DFe concentrations at 25 m are well correlated with mean dFe concentrations in the mixed layer (R^2 = 0.98, m = 1.02) throughout our study period.



Line 117: Are sinking particulate Fe data from sediment traps at Station ALOHA? I assume that data were also extracted from the HOT-DOGS. If so, please add the data source to section 2.3.

We have clarified that these data are from sediment traps at Station ALOHA, which were generated as a result of our timeseries project. However, these data

are not available on the HOT-DOGS platform, but from Bates et al. (2025), as cited. This dataset is publicly available on BCO-DMO.

Lines 174-179: If carbon uptake rates (GPP) are underestimated by the ^{14}C incubation experiments (values between GPP and NPP), the Fe:C uptake ratios will be overestimated. This could help decrease the pFe_{Bio}.

The commenter points out a real issue with the use ^{14}C productivity measurements, but, with respect, we believe the issue is likely reversed. The best C-based denominator, in our opinion, would be net primary production, not gross primary production as implied in the comment above. This is because some fraction of the ^{14}C-determined productivity will be respired over the day while Fe would be only accumulate. If anything, the Fe:C stoichiometry would increase if we were able to precisely measure NPP, subsequently increasing the derived biogenic pFe.

Lines 189-191: Assuming the rest of POC are phytoplankton carbon for simplicity, the actual pFe_{Bio_PC} will decrease by 58-74%, which will make pFe_{Bio_PC} much more similar to pFe_{labile} and pFe_{Bio_ATP}.

We appreciate the commenter noting this, as this is why we included the ATP approach in addition to the PC approach. The 58-74% nonliving organic matter number comes from ATP measurements.

Lines 191-193: The way the Fe:C ratio is calculated is more like an average ratio with respect to the bulk pool of particles though. I don't think a lower Fe:C stoichiometry in detritus, a specific pool of particles, makes any difference to the overall calculation.

The Fe:C ratio is calculated as the uptake rate of Fe divided by the ^{14}C primary production, suggesting it represents the Fe:C ratio of bulk living primary producers. Using the PC approach, we are applying this bulk living Fe:C ratio to the entire PC pool. In these lines, we were explaining that this could be a cause of the overestimation if the PC pool contains particles that have a much lower Fe:C ratio than the live primary producers. Based on the mass balance constraints associated with pFelabile, a lower Fe:C in organic detrital material appears likely.

Lines 194-198: The authors could possibly quantify the effects from variations in phytoplankton community composition, since the C:P of living cells should range between 109 and 195 at Station ALOHA. With the minimum $C:P_{phyto}$ we have, the pFe_{bio_pp} is likely overestimated by up to 50%, which will result in values more similar to pFe_{labile}, but not for all the data.

Based on the commenter's feedback, we estimated the variability expected in C:P based on 1) the variability in C:P for each group and 2) the variability in community composition. Using the interquartile range of observed C:P ratios for each group by Lomas et al. (2021), $C:P_{Phyto}$ varies between 155-179 mol:mol. Based on the variability in composition over an annual cycle reported by Rii et al. (2016), $C:P_{Phyto}$ would vary between 158-172 mol:mol. While there is variability in the C:P expected, it is still far from the 80 ± 36 mol:mol that would be needed to produced pFe_{Bio} values that would actually be in agreement with pFe_{Labile}.

Lomas, M. W., Baer, S. E., Mouginot, C., Terpis, K. X., Lomas, D. A., Altabet, M. A., and Martiny, A. C.: Varying influence of phytoplankton biodiversity and stoichiometric plasticity on bulk particulate stoichiometry across ocean basins, Commun Earth Environ, 2, 1–10, https://doi.org/10.1038/s43247-021-00212-9, 2021.

Rii, Y., Karl, D. M., and Church, M.: Temporal and vertical variability in picophytoplankton primary productivity in the North Pacific Subtropical Gyre, Mar. Ecol. Prog. Ser., 562, 1–18, https://doi.org/10.3354/meps11954, 2016.

Lines 198-199: The exact reason will make equation 3 an overestimation of pFe_{bio}.

Based on this feedback and comments from a reviewer, we have clarified that including heterotrophic bacteria may lead to an overestimation of C:P.

"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.

Line 242: If the authors name the remaining pool as nonliving, this implies what has been subtracted is "living". The pFe_{bio} is not necessarily pFe_{living}, right? Naming it as "non biogenic" is also not perfect, but it may be a better term. I agree that a more accurate term to name this pool of pFe is difficult…

Based on feedback from the reviewers, we have changed the terms to "biotic" and "authigenic + detrital".

Lines 247-258: The authors used a lot of text to explain how different their nonliving pFe is from the more common term used in the field, authigenic pFe. Their explanations rely on the fact that the operationally-defined Berger leach gets the pFe pool that is fully labile. What if the Berger leach also accesses some of the relatively reactive lithogenic pFe? Is that a possibility that could account for the differences and the similarity between $pFe_{nonliving}$ and pFe_{litho}? Please clarify.

Yes, the Berger leach appears to access some portion of dust-derived pFe, as evidenced by Berger leach-accessible Fe found in aerosol samples (e.g., Shelley et al., 2018). This is discussed in lines 252-254 as a possible contribution to what has been termed the "authigenic" pFe pool. While these minerals could be chemically similar to Fe oxyhydroxides that form readily in seawater, can they be considered truly authigenic if they are formed in the atmosphere instead of *in situ*?

Lines 250-251: If dFe adsorb onto or somehow get incorporated into nonliving organic particles, they likely exist as authigenic Fe minerals, right?

While this is true in some cases, there are other instances such as fecal pellets or biological detrital exudates where Fe may not be in mineral form (e.g. if chelated by organic moieties). We have clarified:

"First, this approach may include non-mineral Fe in or adsorbed to nonliving organic particles, such as in detrital organic matter, shown above to be a significant portion of the particulate C and P pools."

Lines 272-274: This conclusion is likely not to hold. What about adsorption, disaggregation, and redox?
Lines 291-293: This could also account for the similarity between pFe_{Bio} and pFe_{litho} at Lines 272-274.

We appreciate the commenter's feedback and have changed the lines 272-274 to:

"Refractory pFe is chemically inert and should only be affected by particle aggregation, disaggregation, and export processes. Based on the similarity between $pFe_{Auth+Det}$ and pFe_{Lith}, we suggest that the $pFe_{Auth+Det}$ profile is also primarily driven by these processes."

Lines 311-313: The summer-time dFe concentrations at BATS are much higher. I am not sure why such features were not discussed in the range of dFe.

The summertime dFe concentrations at BATS (mean in upper 100 m: 0.67 nM) are similar to those in winter at Station ALOHA (mean in upper 100 m: 0.64 nM)

highlighting differences in seasonal Fe cycling between the two sites. While this is discussed in lines 311-313, we chose not to plot winter dFe concentration at Station ALOHA in Figure 5 as comparable data from BATS in winter from the BAIT project are not available and because we were not able to perform Fe uptake experiments at ALOHA during winter.

Lines 316-320: Why not present the comparisons of labile pFe between BATS and Station ALOHA? It's more meaningful than the derived parameters, which are prone to large uncertainties.

We will add the labile pFe profile comparisons. However, we are choosing to keep the comparison of the derived parameters because the primary focus of the paper is exploring different approaches to understanding what's happening within the labile particulate Fe pool and how these parameters vary, not necessarily the labile pFe pool as a whole.

Lines 329-331: What if the authors conduct similar calculations as Sofen et al. (2023) by using their average Fe:C quota at Station ALOHA and labile PP, how will the values of biogenic and nonliving pFe change? Will this affect the conclusions? From what I can tell, Station ALOHA will possibly still have higher biogenic pFe within the mixed layer, but the values below 125 m will be potentially more comparable between these two sites.

This can be approximated following our PP approach, which uses the Fe:C quota and particulate P measurements from the HOT program. As discussed in section 3.2, this estimates the biogenic pFe pool as ~200% of the labile pFe pool, meaning nonliving pFe would be ~ zero for 0-150 m on average. While we would love to provide an exact comparison to Sofen et al.'s data, different choices were necessary based on the nature of the study sites and the data available to us.

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Editorial remarks (by line #):

Lines 138-142: Figure 1a- It seems like the data point from August 2022 is the only one without any error bars. Why is that?

The error bars are smaller than the data marker. We have amended the figure caption to include:

"Data points without visible error bars have uncertainties smaller than the marker size."

Line 260: I assume that the pFe_{Bio} hereafter is pFe_{Bio_ATP}. The authors should consider either pointing it out or just using pFe_{Bio_ATP} to avoid confusion.

We thank the commenter for pointing this out, it has been clarified.

Line 275: Figure 3a- How variable are the labile and lithogenic pFe? It may be helpful to plot the standard deviation at each depth with error bars.

We have added data showing the variability of labile pFe to Figure 1, and of lithogenic pFe to a supplemental figure. For further information on the variability of labile and lithogenic pFe and their drivers, we point the reader to Bates et al. (2025), where it is discussed in detail.

Lines 282-284: The authors should consider plotting this data point on Figure 4c. Additionally, to exclude a data point as an outlier, a statistical method will be preferred.

This data point is plotted on Figure 4c. We have changed the wording from "an outlier in October 2021" to "data from October 2021" to avoid confusion in our intention.

Line 285: Figure 4b- Is this data point at (0,0) real or of bad quality? I can only see one bar with nonliving pFe as 0 in Figure 4a. When is the other zero nonliving pFe observed?

We thank the commenter for pointing this out. The mean mixed layer concentration was plotted in Fig. 4a compared to the concentration at 25 m in Fig. 4b, which is why there was a difference. We have changed panel b to show the comparison between mean mixed layer concentrations of $pFe_{Auth+Det}$ and lithogenic pTi, which has a very similar correlation, to avoid confusion.

Line 285- Figure 4d- Is this correlation significant?

The correlation is not significant ($p > 0.05$), but given the role of picoeukaryotes in explaining Fe uptake rates (e.g. Fig. 1), we think this comparison is worthwhile. Indeed, if we were to exclude the anomalous October 2021 data point from the regression, the correlation would be significant ($p = 0.05$, $R^2 = 0.56$). We hope the change in lines 282-284 help to better clarify this figure. We do not wish to remove the Oct 2021 data point, merely point out that, aside from this cruise where *Prochlorococcus* numbers were significantly higher and appeared to drive a peak pFe_{Bio}, picoeukaryotes probably play a role in determining the concentration of pFe_{Bio}.

Lines 316: Figure 5g-i: The way biogenic pFe was calculated is different between this study (ATP method) and Sofen's method. The authors should consider labeling such differences clearly in the legend or captions.

We have changed our terminology to “biotic” and “authigenic + detrital” to make this more clear, and have clarified further in the caption.

Lines 355-357: For some reason, I cannot open any of these links. However, I can find the data page on BCO-DMO. It's weird, but the authors should make sure that all of these links work during revision.

We thank the commenter for noticing this, the dash mark between BCO and DMO was causing issues when copied into a web browser. This has been fixed.

Citation: https://doi.org/10.5194/egusphere-2025-6068-CC1