nature portfolio

Peer Review File

Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes

Corresponding Author: Professor Huiming Bao

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Version 0:

Decision Letter:

Dear Professor Bao,

Your manuscript titled "Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes" has now been seen by 3 reviewers, whose comments are appended below. In the light of their advice we regret to inform you that we cannot publish your manuscript in Communications Earth & Environment.

You will see that the reviewers raise substantive concerns, particularly Reviewer 2, regarding the validity of your methodology, as well as the novelty and robustness of your work in light of the recent literature. Taking these points together with our editorial considerations, we are unable to conclude that your manuscript represents a sufficiently novel and compelling advance over the body of related work in the literature. Unfortunately, these reservations are sufficiently important to preclude publication of this study in Communications Earth & Environment.

Although we cannot offer to publish your manuscript, we believe the Editorial Board at Scientific Reports will find it interesting and recommend you transfer there. To transfer your manuscript there, please use our manuscript transfer portal. You will not have to re-supply manuscript metadata and files, unless you wish to make modifications. For more information, please see our manuscript transfer FAQ page.

We are sorry that we cannot be more positive on this occasion and thank you for the opportunity to consider your work.

Best regards,

Mengru Wang
External Editor
Communications Earth & Environment

Nicola Colombo, PhD
Associate Editor, Communications Earth & Environment
Consulting Editor, Communications Sustainability

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors study P recycling in stratified water columns of two lakes using a novel ESI-Orbitrap-MS technique. The method seems interesting. However, it would need more explanation. Perhaps, another journal format would enable to bring Methods section to the beginning, which could clarify the story. How many samples were analyzed and why were these two lakes selected and compared? Also, the problem itself is not clear enough.
Lines 15-18 Abstract. Please revise the sentence. “Low-P” lakes does not really make sense to me. In terms of TP, the lakes are not low-P lakes. Phosphate concentrations can be below the detection limit due to assimilation.
Lines 24-25: need be explained

Lines 27-28: Can it be specified, by which observation is this supported.
Lines 35-36: As above
Line 41: If water column is not mixed, then blooms are generated most likely by external nutrient loading. Why you do not consider this as a factor? Can you separate this with the method you use?
Lines 66-74: I cannot follow the logics, could you please explain what are your assumptions based on?
Lines 174-176: Please explain
Line 193: Start the sentence with the capital letter.
Lines 198-199: revise the sentence
Line 245: is "stratification" meant here (not "eutrophication")?

Reviewer #2 (Remarks to the Author):

This study investigates internal phosphorus cycling in stratified, low-phosphorus lakes using phosphate oxygen isotopes (δ^18O_P) combined with a one-dimensional diffusion–reaction model. While the work is carefully executed in several aspects, in my view the manuscript does not, in its present form, meet the scientific novelty and methodological rigor expected for publication in CEE. My detailed comments are as follows.

1. Assessment of Scientific Novelty

The manuscript focuses on internal phosphorus cycling in thermally stratified, low-P lakes, with particular emphasis on sediment P release and internal recycling processes. However, the release of phosphorus from lake sediments and the role of internal loading in sustaining eutrophication have already been extensively studied, and are widely recognized as key drivers of lake eutrophication. Focusing on such processes in relatively oligotrophic or low-P systems, although interesting, does not by itself constitute a clearly novel scientific question or conceptual advance.

Methodologically, the authors employ phosphate oxygen isotopes (δ^18O_P) as a tracer of P sources and cycling. This approach has become increasingly common in recent years and has been widely applied in rivers, lakes, reservoirs and marine environments to trace P sources and biogeochemical transformations. Thus, both the study object (internal P cycling in stratified lakes) and the analytical tool (δ^18O_P) fall within well-established research frameworks.

The manuscript does refine some existing understanding by providing higher-resolution vertical profiles and applying a reaction–transport model, but it does not clearly articulate a new conceptual model, a fundamentally new mechanism, or a substantial extension of current theory. Overall, the level of originality appears limited relative to what is typically expected for CEE.

2. Data Interpretation and Modelling Approach

The combination of δ^18O_P depth profiles with a one-dimensional diffusion–reaction model is potentially powerful, but I have several concerns regarding the robustness of the interpretations and the underlying assumptions.

Attribution of δ^18O_P signals
The manuscript essentially attributes the observed δ^18O_P variations entirely to biological processes (algal uptake at the surface and organic P remineralization at the thermocline), while other potential influences are not fully discussed. The high δ^18O_P values in surface waters are interpreted as enrichment due to rapid algal uptake, and the low values in the thermocline are attributed to remineralization of organic P. However, these conclusions are drawn from single-period (summer) profiles and model simulations, without support from multi-season or multi-year observations or independent biological rate measurements. As a result, the interpretations remain somewhat speculative.

Steady-state assumption and parameter identifiability
The diffusion–reaction model is formulated under a steady-state assumption and is calibrated by fitting vertical profiles to obtain key parameters such as algal uptake rates, mineralization rates, and apparent kinetic isotope effects (AKIEs). In natural stratified lakes, strict steady state is rarely achieved, especially during periods of strong stratification and bloom dynamics. This raises questions about the validity of inferring time-integrated process rates from a single snapshot. Additionally, the inverse problem of fitting multiple parameters to limited one-dimensional data can lead to non-unique solutions; the manuscript does not examine parameter sensitivity or identifiability in sufficient detail.

Magnitude and interpretation of AKIE values
The fitted AKIE for phosphate uptake in surface waters is on the order of −7‰, which is substantially larger than values typically reported from laboratory culture experiments (around −3‰). The authors ascribe this difference to strong fractionation under extremely low ambient phosphate concentrations and up-regulation of high-affinity uptake systems. While this is a plausible hypothesis, it is not substantiated by direct physiological or molecular evidence (e.g., measurements of transporter expression, independent uptake experiments, or rate constraints). Similarly, the AKIE values for organic P remineralization fall within the broad range reported in the literature, but the link between these values and specific enzymatic pathways in the studied lakes is not rigorously demonstrated.

3. Overall Evaluation and Recommendation

In summary, the manuscript provides an interesting case study of P cycling in stratified low-P lakes, but it is limited in both scientific novelty and methodological robustness:

The work largely refines and illustrates existing concepts of internal P loading and recycling rather than offering a clearly new conceptual framework or process understanding.

Key interpretations drawn from δ^18O_P data and the diffusion–reaction model are sensitive to assumptions (steady state, parameterization of uptake and remineralization, uniqueness of AKIE estimates) and are not extensively validated by independent lines of evidence.

The focus is predominantly on fundamental limnological and biogeochemical processes, with only indirect implications for engineering design, management, or broader environmental technology.

Given the above, I do not recommend this manuscript for publication in CEE in its current form.

That said, the work may still be of interest to journals that focus more specifically on aquatic biogeochemistry, nutrient cycling, and limnology. After substantial revision and further strengthening of the process interpretation (e.g., via additional data, sensitivity analyses, and clearer discussion of uncertainties), the manuscript might be more suitable for outlets such as Biogeochemistry, Limnology and Oceanography, or Journal of Hydrology: Regional Studies, which place greater emphasis on fundamental understanding of aquatic nutrient cycles.

Reviewer #3 (Remarks to the Author):

This study employs an innovative analytical approach to elucidate the underlying mechanisms of persistent eutrophication in low-phosphorus lakes, effectively addressing a long-standing paradox in lake eutrophication research. Overall, the study is clearly designed, the data are robust, and the conclusions hold some scientific significance. However, before possible publication, the manuscript would benefit from improvements in language clarity and logical flow to further enhance its academic presentation. The abstract should be re-organized, and I think more discussion is beneficial instead of the raw data. The current manuscript feels like a technical report. In addition, the authors should provide more discussion on mechanisms in the paper, and clarify the significance to conduct studies in these lakes.
General Comments:
1. It is recommended to state the main research objectives and the key scientific questions to be addressed more explicitly and directly in the introduction.
2. It is recommended to supplement the discussion with deeper research implications: for example, the potential of this method for predicting lake phosphorus dynamics under scenarios of climate warming/changes in stratification intensity, and its potential contribution to lake water quality management and predicting the evolution of trophic status.
3. Strengthen logical connections and transitions between paragraphs. Appropriately add transitional sentences between the results and discussion sections, to clarify how each part supports the overall conclusions.
4. Ensure the reproducibility of results and statistical inferences. Where claims such as “significantly increased” or “significant change” are made, the corresponding statistical test methods and p-values (or confidence intervals) should be provided. If statistical tests were not performed, remove terms like “significant” or replace them with non-statistical wording such as “marked/apparent trend”
5. Include details for methodological reproducibility. Supplement the methods section with the measurement precision/uncertainty for instruments like ESI-Orbitrap-MS and TC/EA-IRMS to enhance the reliability description of the methods.
6. Carefully check the writing conventions and grammar throughout the text.
Lines 45–46: “they are an insufficient to separate from alternative release pathways” contains a grammatical error.
Lines 91–92: “Lake Ahabetween” should be corrected to “Lake Aha between”.
Line 193: The first letter of the sentence needs to be capitalized.

Version 1:

Decision Letter:

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Professor Bao,

Your manuscript titled "Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment.

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table".

*****Please take care to match our formatting and policy requirements. We will check revised manuscript and return manuscripts that do not comply. Such requests will lead to delays. *****

Please outline your response to each request in the right hand column. Please upload the completed table with your manuscript files as a Related Manuscript file.

If you have any questions or concerns about any of our requests, please do not hesitate to contact me.

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Mengru Wang
External Editor
Communications Earth & Environment

Nicola Colombo, PhD
Associate Editor, Communications Earth & Environment
Consulting Editor, Communications Sustainability

REVIEWERS' COMMENTS:

Reviewer #4 (Remarks to the Author):

Review of: Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes

The authors present an interesting paper which uses stable isotope and other proxy evidence to present modes of P cycling within stratified lake systems. The paper combines several emerging methodologies within lake and isotope sciences (ESI-Orbitrap-MS and δ18O-PO4) to provide more evidenced conclusions on the role of algae and lake stratification on lake P dynamics and availability. Whilst I do not find the mechanisms presented here paradigm shifting (as the mechanisms and associated isotopic shifts in relation to algal P uptake are known), I do think this is one of the better evidenced studies I've seen. Importantly the authors use 18O-PO4 to elucidate the role of the surface-thermocline P cycle and disentangle this from traditional thoughts regarding direct sediment P release as the main driver for P based lake eutrophication. The stable isotope data presented here tell far more of a convincing story than concentration data alone and the findings are in an accordance with data and ideas being produced within my own group (unpublished).

I think the major concern with regards to publishing in CEE is impact as highlighted by other authors. There have been attempts to improve this but lake eutrophication is a global issue with huge environmental, economic and health implications. This study may go some way to help understand our best mitigation routes which is very important, however this large scale impact is lost as currently written.

Reviewer thoughts on edited manuscript

I was not asked to specifically re-review the manuscript but instead to concentrate on the response to previous review comments by the authors. Brief comments I have from my reading of the re-submitted draft are however listed below.
• In the abstract you state that 18O-PO4 values of +19 are extraordinarily high. This is not the case. These are above your expected equilibrium but well within the range found in many other lake systems. I would re-phrase this.
• Lines 22-27 I see these were added to give clarification to Rev1 however they appear as methods or results to this work not an introduction as such. Maybe either move these or state that "one-dimension diffusion models can be used to…" and cite sources
• I think the inserted lines 57-63 need some clarification. You also talk about equilibrium values, without characterising why this equilibrium is established and through what process. Are you talking here about a biogenically controlled equilibrium or just the simple re suspension of sedimentary PO4 in an inorganic process, with no isotope fractionation? Maybe not super relevant for the wider context of this study but it is worth noting that the sediments are not always in equilibrium the lake bottom water.
• Maybe it is cited somewhere but please clarify in the results text the equation used to calculate the theoretical equilibrium values for Fig 2.
• I think you need to be clear on if/ how you prepared the international isotope standards and if these were run, in-run via ESI-Orbitrap-MS. What were the 1stdv values for these standards in the run? Are the cited errors in the methods from your run or from previous papers?

Reviewer thoughts on rebuttal comments to previous reviews

Comments to Rev 1 seem sensible and well expanded upon.
Comment re the novelty of the study as raised by Rev2. I would actually agree with the authors here and add that the 18O-PO4 model and conclusions drawn here are conceptually novel. Whilst Rev2 highlights that it is know that sediments feedback into the lake P system (which is true) the paper clearly demonstrates that PO4 from sediments is not the major driver for lake eutrophication in this case. I have not seen to date a paper that offers such strong evidence for and mechanistic explanation of the surface-thermocline recycling of P as the driver for eutrophication. I think this, more than the methods, is the novelty here and is important for the community.

Rev2 has concerns over the testing and robustness of the diffusion model. I'm not a modelling expert and cannot speak to the extent of standard rigor that would be expected in testing the parameters of such a model.

I would agree with Rev2 and Rev3 regarding the wider scope of the manuscript. This is an excellent paper for those studying lake P dynamics, but the wider impact expected for the Nature journals is somewhat lost as the paper stands. Whilst I actually hate unjustified upscaling and highly value site specific studies, I think the authors could do a better job of introducing the issues of lake eutrophication on a global scale (these processes are seen in these two lakes but are globally applicable). Can the authors give an idea of large scale impacts and consequences of this work. How many lakes globally may these findings be applied to, what are the impacts of P drive eutrophication, economically, socially for drinking water health etc.

I think Rev3 comment 2 is linked to the above. I appreciate the text that has been added but I think the overall comment is not quite addressed. Again, I think the reviewer is talking about wider, global scale impact. How can these findings be upscaled? Will these processes become more relevant in future climate change scenarios? How many more lakes maybe effected by stratification related eutrophication with an XX degree change in global temperature etc. I know this is a bit speculative but it scales up the impact of your findings from 2 local sites to possibly 1000's of lake systems globally, some of which will be critical for water resources and tourism.

I think Rev 3 comment 4 could be further expanded to ask are some of the differences you highlight statistically significant when the analytical error is considered. This may strengthen your arguments with regard to your results being significantly different to expected isotope equilibrium etc.

Reviewer #5 (Remarks to the Author):

General remarks

This study applies phosphate stable isotopes along vertical gradients in stratified lakes to identify internal phosphate sources sustaining algal growth under low external inputs. The narrative is clear, well structured, and focused, and I particularly appreciated the coherent message that organic phosphorus mineralization at the thermocline is key to understanding the sources of algal phosphorus. I have only a few minor remarks aimed at improving clarity in the introduction. Overall, I strongly support publication, as the study delivers an important message using a novel and timely isotopic approach to phosphate dynamics.

Specific remarks

Lines 37 - 38: What does "before stratification time" mean? Do you mean before stratification occurs?

Lines 53 - 56: Given the general audience of CommsEnv this is a tricky sentence. Especially this: What dou you mean by ".. with 1000 (AKIE-1) values reaching -30 permille and 5 permille, respectively." Please provide rephrasing for a more general audience.

Lines 59 - 60: PO43- released from sediments is not used by organisms in depth? What about uptake by microbial heterotrophs, which can utilize PO43-? Is it not rather that there is comparably low rates of usage?

Authors' point-by-point responses and resulted edits to *"**Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes**"*.

Reviewer #1:

The authors study P recycling in stratified water columns of two lakes using a novel ESI-Orbitrap-MS technique. The method seems interesting. However, it would need more explanation. Perhaps, another journal format would enable to bring Methods section to the beginning, which could clarify the story. How many samples were analyzed and why were these two lakes selected and compared? Also, the problem itself is not clear enough.

Reply: Thanks for your suggestions and questions. Lake Hongfeng and Lake Aha are two well-studied low-P (P < 1 μM), eutrophic lakes that exhibit stratification in the summer. The scientific problem is the debated cause of eutrophication in low-P lakes. We identified two hypotheses that would predict different vertical $\delta^{18}O_P$ profiles. And we tested the predictions using water-column $\delta^{18}O_P$ data of high-vertical resolution, which are only possible to obtain using the ESI-Orbitrap-MS approach our group recently developed. There are two papers (Wang et al., 2024; Wei et al., 2025) on the approach and we have cited them in the manuscript.

We analyzed 33 water samples and 2 sediment samples which we did not mention in the last version, and thanks to your suggestion now we have now added the information at Lines 267-268

To make the scientific problem statement clearer, we have revised in the introduction section the sentence in lines 36-37 to read: *"However, there is currently a debate on the causes of eutrophication in low-P lakes.*"

Lines 15-18 Abstract. Please revise the sentence. "Low-P" lakes does not really make sense to me. In terms of TP, the lakes are not low-P lakes. Phosphate concentrations can be below the detection limit due to assimilation.

Reply: Thank you for your comment. Here, we follow the convention and define a low-phosphorus lake as one where the total dissolved phosphorus concentration is P < 1 μM or PO_4^{3-} is less than 0.1 μM. At this P concentration, the growth of algae should have been limited. The observed concentrations in the surface layer (0-2 m) of Lake Hongfeng and Lake Aha confirm this classification:

TP (Total phosphors) is less than 0.5 μM, and PO_4^{3-} is less than 0.1 μM in the surface of lake water (0-2 m). These values fall squarely within the oligotrophic to mesotrophic range, confirming that the lakes selected for this study are, indeed, low-P systems.

To clarify this point in the manuscript, we have revised the Abstract and Introduction.

Abstract: Lines 14-16: Now it reads "*Sustained eutrophication in low-phosphorus (P < 1 μM and phosphate (PO_4^{3-}) < 0.2 μM) lakes poses a challenge to understanding P cycling in stratified lakes, which could be caused by sediment P release or efficient P recycling in the mixed layer.*"

And in Lines 32-34: it now reads "*Despite these measures, many lakes continue to experience eutrophication, with persistent blooms occurring even when ambient phosphate (PO_4^{3-}) concentrations are below the typical P limit (P < 1 μM and PO_4^{3-} < 0.2 μM)(Cao et al., 2024; Cao et al., 2016; Müller et al., 2019)*".

Lines 24-25: need be explained

Reply: Thanks for pointing out the potential ambiguity. We now added an explanation in lines 22-26. It reads:

"*A concentration-$\delta^{18}O_P$ coupled one-dimensional diffusion-reaction model constrained an apparent kinetic isotope effect of 0.9932 and 0.9906 for surface uptake and thermocline remineralization, respectively. Higher $\delta^{18}O_P$ at the surface and lower $\delta^{18}O_P$ at the thermocline zone suggest that the eutrophication was sustained by a rapid PO_4^{3-} uptake by algae at the surface layer and remineralization of organic P at depth.*"

Lines 27-28: Can it be specified, by which observation is this supported.

Reply: See the revised paragraph above. Lines 22-26: As above

Reply: Please refer to our response to your earlier question (Lines 15-18 in the Abstract).

Line 41: If water column is not mixed, then blooms are generated most likely by external nutrient loading. Why you do not consider this as a factor? Can you separate this with the method you use?

Reply: Thank you for your question. Lake stratification is concluded because of the observed thermocline in the lakes. That does not mean there is no mixing in the upper water body above the thermocline.

External nutrient loading may be possible, e.g., atmospheric deposition and river runoff. But observed eutrophication events correlate with summer warm temperatures and sunny weather, not with atmospheric dust or surface river runoff events.

Lines 66-74: I cannot follow the logics, could you please explain what are your assumptions based on?

Reply: Two sources can contribute PO_4^{3-} to the surface layer and promote an algal bloom there. One is PO_4^{3-} released from the sediment, which may diffuse through the thermocline from below. The other is remineralized organic-P near the thermocline, which mixes into the surface layer. The two sources would both predict a higher PO_4^{3-} concentration near the thermocline, and thus cannot be distinguished by measuring the vertical PO_4^{3-} concentration profiles. However, the $\delta^{18}O$ profile of dissolved PO_4^{3-} in water columns can. The sediment PO_4^{3-} model would predict an unchanged $\delta^{18}O_p$ value at and below the thermocline while the orhanic P remineralization model would predict a much lower $\delta^{18}O_p$ value at or near the thermocline because remineralization process releases PO_4^{3-} that is much lower in $\delta^{18}O$ value, with expected 1000 (*AKIE*-1) values reaching -30‰ and -5‰, respectively (Liang and Blake, 2009).

To avoid misunderstanding, we have modified the paragraph in lines 57-63. It now reads:

"*In a lake ecosystem, the $\delta^{18}O_P$ of PO_4^{3-} released from the sediment is typically higher or close to the equilibrium value, due to equilibrium with porewater oxygen and limited oxygen isotope exchange with the overlying lake-water that may have a different $\delta^{18}O_P$ value(Jin et al., 2023; Joshi et al., 2015; Yuan et al., 2023). PO_4^{3-} released from sediment is not used by organisms in depth, and therefore its $\delta^{18}O_P$ value remains constant at and below the thermocline. For thermocline depth, the two PO_4^{3-} source models, including released from sediment at the bottom and remineralization of organic P near the thermocline, predict distinguishable vertical $\delta^{18}O_P$ profiles that can be tested.*"

Lines 174-176: Please explain

Reply: We are not sure what aspect of the sentence is unclear. So, we have expanded the discussion in lines 167-174:

"Our study revealed that a larger AKIE can be manifested in a low-P environment, likely due to a combination of specialized PO_4^{3-} transporters and a reservoir effect. In other P-starved environments (such as the polar tundra or the deep ocean), plants or microorganisms typically maximize their P acquisition capacity by actively regulating relevant gene expression or altering root architecture (Crombez et al., 2019; Yang et al., 2025). The correlation between this highly efficient P utilization strategy and isotope fractionation suggests that AKIE could potentially serve as an indicator of the degree of P limitation in ecosystems, offering a new perspective for understanding the dynamics of P cycling."

"

Line 193: Start the sentence with a capital letter.
Reply: Thanks. Corrected. It now reads "*The rate of PO_4^{3-} release through microbial remineralization of sinking biomass has exceeded the rate of PO_4^{3-} uptake.*" at lines 191-192.

Lines 198-199: revise the sentence
Reply: The revised sentences in lines 200-202 now read: "*The $\delta^{18}O_P$ values below the thermocline in Lake Hongfeng are higher, while in Lake Aha are lower than the predicted equilibrium values (Fig. 2b and Fig. 2d). This difference may be caused by the different $\delta^{18}O_P$ values of PO_4^{3-} released from sediments.* "

Line 245: is "stratification" meant here (not "eutrophication")?
Reply: Thank you for pointing out the ambiguity here. To clarify, we have revised the sentences in lines 258-259 to read "*All samples were collected in July during the stratification period when eutrophication occurred.*"

Reviewer #2:

This study investigates internal phosphorus cycling in stratified, low-phosphorus lakes using phosphate oxygen isotopes (δ^18O_P) combined with a one-dimensional diffusion–reaction model. While the work is carefully executed in several aspects, in my view the manuscript does not, in its present form, meet the scientific novelty and methodological rigor expected for publication in CEE. My detailed comments are as follows.

1. Assessment of Scientific Novelty

The manuscript focuses on internal phosphorus cycling in thermally stratified, low-P lakes, with particular emphasis on sediment P release and internal recycling processes. However, the release of phosphorus from lake sediments and the role of internal loading in sustaining eutrophication have already been extensively studied, and are widely recognized as key drivers of lake eutrophication. Focusing on such processes in relatively oligotrophic or low-P systems, although interesting, does not by itself constitute a clearly novel scientific question or conceptual advance.

Methodologically, the authors employ phosphate oxygen isotopes (δ^18O_P) as a tracer of P sources and cycling. This approach has become increasingly common in recent years and has been widely applied in rivers, lakes, reservoirs and marine environments to trace P sources and biogeochemical transformations. Thus, both the study object (internal P cycling in stratified lakes) and the analytical tool (δ^18O_P) fall within well-established research frameworks.

The manuscript does refine some existing understanding by providing higher-resolution vertical profiles and applying a reaction–transport model, but it does not clearly articulate a new conceptual model, a fundamentally new mechanism, or a substantial extension of current theory. Overall, the level of originality appears limited relative to what is typically expected for CEE.

Reply: We appreciate your comment. However, we respectively disagree with your characterization of the originality of our study.

Our story line goes like this: 1) There is a debate on the mechanism of low-P lake eutrophication and lake PO_4^{3-} concentration data alone cannot resolve the debate; 2) The $\delta^{18}O_P$ can, but conventional sampling resolution is too coarse to resolve competing models; 3) ESI-Orbitrap-MS method that our group just developed can do it because it uses 3 orders of magnitude smaller water volume than what the conventional method needs; 4) our high-spatial resolution $\delta^{18}O_P$ data combining with 1-D diffusion-reaction model support one of the models, i.e., the rapid organic-P remineralization model.

The conceptual models or the tested new mechanisms in our study may not meet the reviewer's expectation of "new" or "fundamental", but they are the state-of-art of the current field. Importantly, several prominent stable isotope research groups (Caltech, UFZ, …) in the world are racing to develop Orbitrap-MS-based methods for natural-abundance stable isotope measurement on oxyanions. At present, sulfate, nitrate and some small organic compounds have been worked out. Our group (International Center for Isotope Effects Research-ICIER) has succeeded in measuring phosphate and perchlorate, the two more difficult ones to be measured using the Orbitrap-MS approach. Our developed Orbitrap-MS methods for the $\delta^{18}O_P$ (Wang et al., 2024) is disruptive to the conventional method. What people are all looking forward to is an application of these Orbitrap-MS methods to science problems using natural samples, especially the $\delta^{18}O_P$ parameter. This manuscript of ours, if published, will be the first case of natural sample application of the Orbitrap-MS method.

2. Data Interpretation and Modelling Approach

The combination of δ^18O_P depth profiles with a one-dimensional diffusion–reaction model is potentially powerful, but I have several concerns regarding the robustness of the interpretations and the underlying assumptions.

Attribution of δ^18O_P signals

The manuscript essentially attributes the observed δ^18O_P variations entirely to biological processes (algal uptake at the surface and organic P remineralization at the thermocline), while other potential influences are not fully discussed. The high δ^18O_P values in surface waters are interpreted as enrichment due to rapid algal uptake, and the low values in the thermocline are attributed to remineralization of organic P. However, these conclusions are drawn from single-period (summer) profiles and model simulations, without support from multi-season or multi-year observations or independent biological rate measurements. As a result, the interpretations remain somewhat speculative.

Steady-state assumption and parameter identifiability
The diffusion–reaction model is formulated under a steady-state assumption and is calibrated by fitting vertical profiles to obtain key parameters such as algal uptake rates, mineralization rates, and apparent kinetic isotope effects (AKIEs). In natural stratified lakes, strict steady state is rarely achieved, especially during periods of strong stratification and bloom dynamics. This raises questions about the validity of inferring time-integrated process rates from a single snapshot. Additionally, the inverse problem of fitting multiple parameters to limited one-dimensional data can lead to non-unique solutions; the manuscript does not examine parameter sensitivity or identifiability in sufficient detail.

Magnitude and interpretation of AKIE values
The fitted AKIE for phosphate uptake in surface waters is on the order of −7‰, which is substantially larger than values typically reported from laboratory culture experiments (around −3‰). The authors ascribe this difference to strong fractionation under extremely low ambient phosphate concentrations and up-regulation of high-affinity uptake systems. While this is a plausible hypothesis, it is not substantiated by direct physiological or molecular evidence (e.g., measurements of transporter expression, independent uptake experiments, or rate constraints). Similarly, the AKIE values for organic P remineralization fall within the broad range reported in the literature, but the link between these values and specific enzymatic pathways in the studied lakes is not rigorously demonstrated.
Reply: Thank you for your comment. we will answer the 3 points you summarized.
1. We are testing two alternative models on the source of PO_4^{3-} that sustained eutrophication in low-phosphorus lakes. This is sufficiently done by employing high-resolution spatial measurement of PO_4^{3-} concentration and the $\delta^{18}O_P$ in summer days when the lakes were stratified. Multi-season or multi-year observations may help, but it is not necessary because other seasons do not have stratification or eutrophication.
2. We appreciate this comment as we had similar concerns in the beginning. While our model is based on a steady-state assumption, the following steps were taken to ensure robustness when doing the inverse model fitting:
a. Prior to parameter fitting, we reduced the non-uniqueness of the solution by constraining the parameter ranges using measured boundary conditions, including PO_4^{3-} concentration, organic P concentration, Chla, and the $\delta^{18}O_P$.
b. To address the multi-parameter coupling issue, we employed a stepwise fitting strategy: first fixing parameters supported by independent observation (concentration of PO_4^{3-} concentration, Organic P and Chla, $\delta^{18}O_P$), and then fitting the more sensitive, key parameters (*AKIE*s and rate of different P cycling step). This method effectively prevents excessive interference among parameters.
c. Furthermore, we conducted sensitivity tests for the core parameters (*AKIE*s), thereby validating the parameters fitted.
3. Justifying high *AKIE* values.
Yes, our observed *AKIE* values are higher than those reported in the literature, and this outcome aligns with predictions of recent research by Lis et al. (2019), which demonstrated that *AKIE* increases as PO_4^{3-} concentration decreases. We speculated in the manuscript a switch of enzymatic pathways occurs when PO_4^{3-} concentration drops to a certain low threshold. Exploring the underlying specific enzymatic pathways would be a research project worth pursuing (In fact, we have obtained a set of cultural experimental data, and the manuscript is in preparation), but is beyond the scope of this

manuscript; i.e., testing the hypotheses proposed in this manuscript does not require the details of enzymatic pathways.

3. Overall Evaluation and Recommendation

In summary, the manuscript provides an interesting case study of P cycling in stratified low-P lakes, but it is limited in both scientific novelty and methodological robustness:

The work largely refines and illustrates existing concepts of internal P loading and recycling rather than offering a clearly new conceptual framework or process understanding.

Key interpretations drawn from δ^18O_P data and the diffusion–reaction model are sensitive to assumptions (steady state, parameterization of uptake and remineralization, uniqueness of AKIE estimates) and are not extensively validated by independent lines of evidence.

The focus is predominantly on fundamental limnological and biogeochemical processes, with only indirect implications for engineering design, management, or broader environmental technology.

Given the above, I do not recommend this manuscript for publication in CEE in its current form.

That said, the work may still be of interest to journals that focus more specifically on aquatic biogeochemistry, nutrient cycling, and limnology. After substantial revision and further strengthening of the process interpretation (e.g., via additional data, sensitivity analyses, and clearer discussion of uncertainties), the manuscript might be more suitable for outlets such as Biogeochemistry, Limnology and Oceanography, or Journal of Hydrology: Regional Studies, which place greater emphasis on fundamental understanding of aquatic nutrient cycles.

Reply: Thank you for your criticism.

Our research quantifies the P cycling processes within low-P lakes, resolving the source of PO_4^{3-} that drives the observed eutrophication. Our model quantifies the fundamental isotopic fractionation factors for P uptake and P remineralization processes.

In summary, we believe our study has presented a clear scientific problem (debate), formulated two testable hypotheses, applied a truly novel (disruptive) analytical method, and employed a 1-D dynamic model to quantify the concentration and $\delta^{18}O_P$ profiles during low-P lake eutrophication. This study aligns well with CEE's scope.

Reviewer #3:

This study employs an innovative analytical approach to elucidate the underlying mechanisms of persistent eutrophication in low-phosphorus lakes, effectively addressing a long-standing paradox in lake eutrophication research. Overall, the study is clearly designed, the data are robust, and the conclusions hold some scientific significance. However, before possible publication, the manuscript would benefit from improvements in language clarity and logical flow to further enhance its academic presentation. The abstract should be re-organized, and I think more discussion is beneficial instead of the raw data. The current manuscript feels like a technical report. In addition, the authors should provide more discussion on mechanisms in the paper, and clarify the significance to conduct studies in these lakes.

Reply: We appreciate your recognition of the study's innovation, design, data robustness, and scientific significance, as well as your constructive criticisms for improvement.

In revision, we have revised the Abstract to add more to the discussion on the mechanism and the research significance.

General Comments:

1. It is recommended to state the main research objectives and the key scientific questions to be addressed more explicitly and directly in the introduction.

Reply: Thank you for your suggestion. As we responded to Reviewer#2 in the beginning, Our story line goes like this: 1) There is a debate on the mechanism of low-P lake eutrophication and lake PO4 concentration data alone cannot resolve the debate; 2) The $\delta^{18}O_P$ can, but conventional sampling resolution is too coarse to resolve competing models; 3) ESI-Orbitrap-MS method that our group just developed can do it because it uses 3 orders of magnitude smaller water volume than what the conventional method needs; 4) our high-spatial resolution $\delta^{18}O_P$ data combining with 1-D diffusion-reaction model support one of the models, i.e., the rapid organic-P remineralization model. We have adjusted the writing in the Introduction to better reflect this story flow.

2. It is recommended to supplement the discussion with deeper research implications: for example, the potential of this method for predicting lake phosphorus dynamics under scenarios of climate warming/changes in stratification intensity, and its potential contribution to lake water quality management and predicting the evolution of trophic status.

Reply: We appreciate your recommendation. In this revised version, we have added at Lines 167-174:

"*Our study revealed that a larger AKIE can be manifested in a low-P environment, likely due to a combination of specialized PO_4^{3-} transporters and a reservoir effect. In other P-starved environments (such as the polar tundra or the deep ocean), plants or microorganisms typically maximize their P acquisition capacity by actively regulating relevant gene expression or altering root architecture (Crombez et al., 2019; Yang et al., 2025). The correlation between this highly efficient P utilization strategy and isotope fractionation suggests that AKIE could potentially serve as an indicator of the degree of P limitation in ecosystems, offering a new perspective for understanding the dynamics of P cycling.*"

And at lines 229-234: "*The $\delta^{18}O_P$ profile allows for isolating and quantitatively assessing the relative rates of key P cycling processes, including organic P remineralization and sediment release. This capability provides a data-driven foundation required to design and implement highly targeted remediation strategies (e.g., hypolimnetic aeration or sediment capping). Ultimately, by providing a mechanistic understanding of spatially resolved P recycling, this approach enhances the reliability of predictive models used to forecast future trophic status and effectively prevent eutrophic conditions.*"

3. Strengthen logical connections and transitions between paragraphs. Appropriately add transitional sentences between the results and discussion sections, to clarify how each part supports the overall conclusions.

Reply: Thank you for your comment. We have added several transitional sentences between the results and discussion sections to improve the flow. For example,

Lines 130-132: "*Collectively, the physicochemical data and the high-resolution $\delta^{18}O_P$ profiles presented in the Results section provide a foundation to distinguishing the source of PO_4^{3-} driving eutrophication in these low-P systems.*"

Line 211-212: "*In contrast to previous studies that relied solely on concentration gradients, our $\delta^{18}O_P$ profile provides isotopic insights into the P uptake by algae and remineralization of organic P pathways.*"

4. Ensure the reproducibility of results and statistical inferences. Where claims such as "significantly increased" or "significant change" are made, the corresponding statistical test methods and p-values (or confidence intervals) should be provided. If statistical tests were not performed, remove terms like "significant" or replace them with non-statistical wording such as "marked/apparent trend"

Reply: Thank you for your suggestion. We checked all sentences in the manuscript that contain "significantly increased" or "significant". We removed or replaced them when it was used as an adjective.

5. Include details for methodological reproducibility. Supplement the methods section with the measurement precision/uncertainty for instruments like ESI-Orbitrap-MS and TC/EA-IRMS to enhance the reliability description of the methods.

Reply: The measurement standard deviation (1 SD) of the $\delta^{18}O_P$ by TC/EA-IRMS and ESI-Orbitrap-MS are 0.4‰ and 0.8‰, respectively. We have added this information in the method section. The updated sentences read:

Lines 313-314: "*The standard deviation (1 SD) of the* $\delta^{18}O_P$ *by TC/EA-IRMS measurements was 0.4‰.*"

Line 350: "*The standard deviation (1 SD) of the* $\delta^{18}O_P$ *by ESI-Orbitrap-MS was 0.8‰*[3, 4]."

6. Carefully check the writing conventions and grammar throughout the text.
Lines 45–46: "they are an insufficient to separate from alternative release pathways" contains a grammatical error.
Lines 91–92: "Lake Ahabetween" should be corrected to "Lake Aha between".
Line 193: The first letter of the sentence needs to be capitalized.

Reply: Thanks. We have corrected the grammatical errors. Now they read:

Lines 80-81: "*water T remains stable from 0 to 6 m in Lake Hongfeng (26.4 - 26.7 °C) and 0 to 4 m in Lake Aha (26.8 – 27 °C),*"

Line 191-192: "*The rate of PO_4^{3-} release through microbial remineralization of sinking biomass has exceeded the rate of PO_4^{3-} uptake.*"

Reference:

Cao, J., Wu, Y., Li, Z.-k., Hou, Z.-y., Wu, T.-h., Chu, Z.-s., Zheng, B.-h., Yang, P.-p., Yang, Y.-y., Li, C.-s., Li, Q.-h., Guo, X., 2024. Dependence of evolution of Cyanobacteria superiority on temperature and nutrient use efficiency in a meso-eutrophic plateau lake. Science of The Total Environment 927, 172338.

Cao, X., Wang, Y., He, J., Luo, X., Zheng, Z., 2016. Phosphorus mobility among sediments, water and cyanobacteria enhanced by cyanobacteria blooms in eutrophic Lake Dianchi. Environmental Pollution 219, 580-587.

Crombez, H., Motte, H., Beeckman, T., 2019. Tackling Plant Phosphate Starvation by the Roots. Developmental Cell 48, 599-615.

Jin, Z., Wang, J., Zhang, R., Liao, P., Liu, Y., Yang, J., Chen, J., 2023. Identification of the sources of different phosphorus fractions in lake sediments by oxygen isotopic composition of phosphate. Applied Geochemistry 151, 105627.

Joshi, S.R., Kukkadapu, R.K., Burdige, D.J., Bowden, M.E., Sparks, D.L., Jaisi, D.P., 2015. Organic Matter Remineralization Predominates Phosphorus Cycling in the Mid-Bay Sediments in the Chesapeake Bay. Environmental Science & Technology 49, 5887-5896.

Liang, Y., Blake, R.E., 2009. Compound- and enzyme-specific phosphodiester hydrolysis mechanisms revealed by δ18O of dissolved inorganic phosphate: Implications for marine P cycling. Geochimica et Cosmochimica Acta 73, 3782-3794.

Lis, H., Weiner, T., Pitt, F.D., Keren, N., Angert, A., 2019. Phosphate Uptake by Cyanobacteria Is Associated with Kinetic Fractionation of Phosphate Oxygen Isotopes. Acs Earth and Space Chemistry 3, 233-239.

Müller, B., Steinsberger, T., Schwefel, R., Gächter, R., Sturm, M., Wüest, A., 2019. Oxygen consumption in seasonally stratified lakes decreases only below a marginal phosphorus threshold. Scientific Reports 9, 18054.

Wang, Z., Hattori, S., Peng, Y., Zhu, L., Wei, Z., Bao, H., 2024. Oxygen Isotope Analysis of Nanomole Phosphate Using PO(3)(-) Fragment in ESI-Orbitrap-MS. Anal Chem 96, 4369-4376.

Wei, Z., Wang, B., Zhu, L., Hong, Y., Wang, Z., Yan, H., Peng, Y., Hattori, S., Bao, H., 2025. A sub-liter pretreatment method for Orbitrap–based freshwater phosphate oxygen isotope measurement. Applied Geochemistry 194, 106610.

Yang, G., Deng, M., Guo, L., Du, E., Zheng, Z., Peng, Y., Zhao, C., Liu, L., Yang, Y., 2025. Characteristics of leaf nutrient resorption efficiency in Tibetan alpine permafrost ecosystems. Nature Communications 16, 4044.

Yuan, H., Wang, H., Cai, Y., Yin, H., Zeng, Q., Liu, E., Li, Q., Wang, Y., 2023. Iron bound phosphorus predominates the contribution of phosphorus to lake system from terrigenous source: The evidence from the small watershed scale. Water Research 245, 120661.

Authors' point-by-point responses and resulted edits to *"**Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes**"*.

REVIEWERS' COMMENTS:

Reviewer #4 (Remarks to the Author):

Review of: Phosphorus Cycling Dynamics in Stratified Low-Phosphorus Lakes

The authors present an interesting paper which uses stable isotope and other proxy evidence to present modes of P cycling within stratified lake systems. The paper combines several emerging methodologies within lake and isotope sciences (ESI-Orbitrap-MS and δ18O-PO4) to provide more evidenced conclusions on the role of algae and lake stratification on lake P dynamics and availability. Whilst I do not find the mechanisms presented here paradigm shifting (as the mechanisms and associated isotopic shifts in relation to algal P uptake are known), I do think this is one of the better evidenced studies I've seen. Importantly the authors use 18O-PO4 to elucidate the role of the surface-thermocline P cycle and disentangle this from traditional thoughts regarding direct sediment P release as the main driver for P based lake eutrophication. The stable isotope data presented here tell far more of a convincing story than concentration data alone and the findings are in an accordance with data and ideas being produced within my own group (unpublished).

I think the major concern with regards to publishing in CEE is impact as highlighted by other authors. There have been attempts to improve this but lake eutrophication is a global issue with huge environmental, economic and health implications. This study may go some way to help understand our best mitigation routes which is very important, however this large scale impact is lost as currently written.

Reviewer thoughts on edited manuscript

I was not asked to specifically re-review the manuscript but instead to concentrate on the response to previous review comments by the authors. Brief comments I have from my reading of the re-submitted draft are however listed below.

1 In the abstract you state that 18O-PO4 values of +19 are extraordinarily high. This is not the case. These are above your expected equilibrium but well within the range found in many other lake systems. I would re-phrase this.

Reply: Thank you for your comment. We have revised the sentence to make it more specific. At lines 18-19, now it reads:

"The lakes showed high $\delta^{18}O_P$ values (19.3‰-19.8‰) at the surface (0–2 m), but low values (13.4‰-13.7‰) at thermocline."

2 Lines 22-27 I see these were added to give clarification to Rev1 however they appear as methods or results to this work not an introduction as such. Maybe either move these or state that

“one-dimension diffusion models can be used to…” and cite sources

Reply: This is part of the results. We have revised the sentence to read (at line 19-21): “A one-dimensional concentration-$\delta^{18}O_P$ coupled diffusion model constrains an apparent kinetic isotope effect of 0.9932 and 0.9906 for biological surface uptake and thermocline remineralization, respectively.”

3 I think the inserted lines 57-63 need some clarification. You also talk about equilibrium values, without characterising why this equilibrium is established and through what process. Are you talking here about a biogenically controlled equilibrium or just the simple resuspension of sedimentary PO4 in an inorganic process, with no isotope fractionation? Maybe not super relevant for the wider context of this study but it is worth noting that the sediments are not always in equilibrium the lake bottom water.

Reply: Thank you for your comment. The equilibrium we refer to is the biogenically mediated oxygen isotope exchange equilibrium between dissolved inorganic PO_4^{3-} and lake water. Mediated by microbial enzymes, the P-O bonds in PO_4^{3-} are broken and reformed, resulting in the oxygen exchange (Blake et al., 2005). That refers to PO_4^{3-} in the stratified water column. Yes, you are correct. PO_4^{3-} in sediments is not necessarily in equilibrium with the lake bottom water.

To clarify, we have updated the sentence in lines 55-57, now it reads “*In a lake ecosystem, the $\delta^{18}O$ of PO_4^{3-} released from the sediment is typically higher or close to the equilibrium value, due to oxygen isotope exchange with porewater oxygen and limited exchange with the overlying lake-water that may have a different $\delta^{18}O_P$ or $\delta^{18}O$ of water value.*”

4 Maybe it is cited somewhere but please clarify in the results text the equation used to calculate the theoretical equilibrium values for Fig 2.

Reply: We have the calculation of equilibrium $\delta^{18}O_P$ values in Method section. To display the calculation process, we have added the specific equation in the caption of Figure 2 at lines 621-623. Now it reads:

“***Fig. 2*** *The fitting results of a one-dimensional vertical diffusion model for PO_4^{3-} concentration and the $\delta^{18}O_P$. Open dots represent the measured $\delta^{18}O_P$ values, filled dots represent the calculated equilibrium $\delta^{18}O_P$ values according to equation (2), and the lines represent the fitted values.*”

5 I think you need to be clear on if/ how you prepared the international isotope standards and if these were run, in-run via ESI-Orbitrap-MS. What were the 1stdv values for these standards in the run? Are the cited errors in the methods from your run or from previous papers?

Reply: Thank you for your suggestion. International standards (Ag_3PO_4) were converted to NaH2PO4 solutions to match the chemical form of our samples. The specific steps were as follows: Approximately 8.4 mg of Ag3PO4 powder was dissolved in 100 mL of ammonia solution (for LC–MS, Aladin, China) to achieve a concentration of 200 mM. The solution was then passed through a cation exchange resin column (AG50W-X8, Bio-Rad) to convert NH4+ to H+, followed by pH adjustment to 7.0 using 10 M NaOH. These were in Methods section describing the ESI-Orbitrap-MS measuring process.

In response to the suggestion, we have updated the Methods section to provide a detailed description of the standard preparation at lines 324-327, it reads:

"*International standards (Ag_3PO_4) were converted to NaH_2PO_4 solutions through dissolution in ammonia, cation exchange (AG50W-X8 resin), and pH adjustment to 7.0 (Wei et al., 2025). The standard deviation (1 SD) of the $\delta^{18}O_P$ measured by ESI-Orbitrap-MS was 0.8‰, as was reported in our previous study (Wang et al., 2024; Wei et al., 2025).*"

Reviewer thoughts on rebuttal comments to previous reviews

Comments to Rev 1 seem sensible and well expanded upon.
Comment re the novelty of the study as raised by Rev2. I would actually agree with the authors here and add that the 18O-PO4 model and conclusions drawn here are conceptually novel. Whilst Rev2 highlights that it is know that sediments feedback into the lake P system (which is true) the paper clearly demonstrates that PO4 from sediments is not the major driver for lake eutrophication in this case. I have not seen to date a paper that offers such strong evidence for and mechanistic explanation of the surface-thermocline recycling of P as the driver for eutrophication. I think this, more than the methods, is the novelty here and is important for the community.

Rev2 has concerns over the testing and robustness of the diffusion model. I'm not a modelling expert and cannot speak to the extent of standard rigor that would be expected in testing the parameters of such a model.

6 I would agree with Rev2 and Rev3 regarding the wider scope of the manuscript. This is an excellent paper for those studying lake P dynamics, but the wider impact expected for the Nature journals is somewhat lost as the paper stands. Whilst I actually hate unjustified upscaling and highly value site specific studies, I think the authors could do a better job of introducing the issues of lake eutrophication on a global scale (these processes are seen in these two lakes but are globally applicable). Can the authors give an idea of large scale impacts and consequences of this work. How many lakes globally may these findings be applied to, what are the impacts of P drive eutrophication, economically, socially for drinking water health etc.
Reply: Thank you for this suggestion. According to your suggestion, we have added a cautious expansion of the global implication of our finding at line 220-222. It reads: "*As global warming intensifies thermal stratification, the rapid water-column P recycling identified here may become a dominant driver of eutrophication in thousands of low-P lakes globally.*"

7 I think Rev3 comment 2 is linked to the above. I appreciate the text that has been added but I think the overall comment is not quite addressed. Again, I think the reviewer is talking about wider, global scale impact. How can these findings be upscaled? Will these processes become more relevant in future climate change scenarios? How many more lakes maybe effected by stratification related eutrophication with an XX degree change in global temperature etc. I know this is a bit speculative but it scales up the impact of your findings from 2 local sites to possibly 1000's of lake systems globally, some of which will be critical for water resources and tourism.

Reply: Thank you for your suggestion. Please refer to our response to your comment #6 above.

8 I think Rev 3 comment 4 could be further expanded to ask are some of the differences you

highlight statistically significant when the analytical error is considered. This may strengthen your arguments with regard to your results being significantly different to expected isotope equilibrium etc.

Reply:

Reply: Thank you for your suggestion. We have added statistical tests which have confirmed that the differences between our measurements and equilibrium values are robust.

Now the sentences have been modified to read at lines 105-108: "*The highest $\delta^{18}O_P$ value was observed in Lake Hongfeng at 0 m (19.8‰) and in Lake Aha at 0.5 m (18.7‰), both of which deviate significantly from their calculated equilibrium values ($p < 0.01$), i.e., 14.3‰ for Lake Hongfeng and 14.4‰ for Lake Aha, considering the T and the $\delta^{18}O$ of lake water.*"

And in Statistical analysis in the Methods section, we have added at lines 395-397: "*Statistical comparisons between measured $\delta^{18}O_P$ and $\delta^{18}O_{Eq}$ were conducted using paired-sample t-tests. Independent-sample t-tests were used to distinguish surface (depth ≤ 1 cm) and subsurface layers. All analyses were performed in R.*"

Reviewer #5 (Remarks to the Author):

General remarks

This study applies phosphate stable isotopes along vertical gradients in stratified lakes to identify internal phosphate sources sustaining algal growth under low external inputs. The narrative is clear, well structured, and focused, and I particularly appreciated the coherent message that organic phosphorus mineralization at the thermocline is key to understanding the sources of algal phosphorus. I have only a few minor remarks aimed at improving clarity in the introduction. Overall, I strongly support publication, as the study delivers an important message using a novel and timely isotopic approach to phosphate dynamics.

Reply: Thanks for the encouragement. Below are our point-by-point responses to your specific remarks, along with the corresponding edits we have made to improve the clarity of the introduction.

Specific remarks

1 Lines 37 - 38: What does "before stratification time" mean? Do you mean before stratification occurs?

Reply: Thank you for pointing this out. Yes, we did mean before thermal stratification occurs. We have revised the sentence by simply deleting "*before the onset of stratification*" for clarity at lines 34-35.

It now reads: "*One prevailing hypothesis points to the internal loading of PO_4^{3-} from bottom sediments as the trigger for these blooms.*"

2 Lines 51 - 54: Given the general audience of CommsEnv this is a tricky sentence. Especially this: What dou you mean by ". with 1000 (AKIE-1) values reaching -30 permille and 5 permille, respectively." Please provide rephrasing for a more general audience.

Reply: Good catch. We have simplified the explanation to read at lines 51-54:

"*The apparent kinetic isotope effect (AKIE) for PO_4^{3-} oxygen during the remineralization of phosphomonoester and phosphodiester, the main form of organic P in lake water, can result in ^{18}O-depleted PO_4^{3-}, with the degree of isotopic fractionation (i.e., 1000 (AKIE-1)) reaching -30‰ and -5‰, respectively*"

3 Lines 59 - 60: PO43- released from sediments is not used by organisms in depth? What about uptake by microbial heterotrophs, which can utilize PO43-? Is it not rather that there is comparably low rates of usage?

Reply: Thank you for your correction. You are right; microbial heterotrophs in the deep water do utilize PO_4^{3-}, but the utilization rate is relatively low. We have revised the sentence to accurately reflect this point.

The original sentence in lines 57-59 has been modified to read:

"*The utilization rate of PO_4^{3-} by microbial heterotrophs at depth is relatively lower than microbials in surface layer, and therefore its $\delta^{18}O_P$ value may remain constant at and below the thermocline.*"

References:

Blake RE, O'Neil JR, Surkov AV. Biogeochemical cycling of phosphorus: Insights from oxygen isotope effects of phosphoenzymes. *American Journal of Science* 2005, **305**(6-8)**:** 596-620.

Wang Z, Hattori S, Peng Y, Zhu L, Wei Z, Bao H. Oxygen Isotope Analysis of Nanomole Phosphate Using PO(3)(-) Fragment in ESI-Orbitrap-MS. *Anal Chem* 2024, **96**(11)**:** 4369-4376.

Wei Z, Wang B, Zhu L, Hong Y, Wang Z, Yan H*, et al.* A sub-liter pretreatment method for Orbitrap–based freshwater phosphate oxygen isotope measurement. *Applied Geochemistry* 2025, **194:** 106610.