

Dear Editors and Reviewers,

We sincerely appreciate your valuable suggestions and the insightful comments regarding our paper submitted to *Biogeosciences*, entitled “*Enhanced Carbon Sequestration in Alkaline Lakes through Recycled CO₂ Utilization by Diatoms*”. Having carefully considered all the comments and suggestions, we have made substantial revisions and are now preparing to submit a revised version to the Journal. The major revisions are summarized as follows:

- (1) The manuscript title has been revised to “*Enhanced Carbon Retention in Alkaline Lakes through Recycled CO₂ Utilization by Diatoms*”, and the manuscript has been revised accordingly to distinguish carbon retention from long-term carbon sequestration.
- (2) The interpretations of recycled DIC utilization and sedimentary organic matter sources have been substantially revised to clarify the links among organic matter remineralization, respiration-derived DIC, and diatom carbon assimilation.
- (3) We have expanded the discussion of the limitations and uncertainties associated with using C₂₅ HBI carbon isotopes to trace recycled DIC utilization, including potential effects of DIC sources, isotope fractionation, and producer ecology.

Our point-by-point responses to the comments and suggestions are included at the end of this letter for your reference. The **major revisions** made to the manuscript are highlighted in **RED COLOR** in the manuscript (Revised version), as well as in the **comment responses**. The page number mentioned below refers to the revised manuscript (clean version) unless otherwise stated. We believe that these improvements will strengthen the quality and impact of our research, aligning it more closely with the high standards of *Biogeosciences*.

Once again, we sincerely appreciate the time and expertise that the Editors and Reviewers have devoted to evaluating our work.

Thank you for your consideration.

Yours sincerely,

Manuscript ID: egusphere-2026-2998

Title: Enhanced Carbon Retention in Alkaline Lakes through Recycled CO₂ Utilization by Diatoms

A handwritten signature in black ink, appearing to read 'Yongge Sun'.

Yongge Sun

The corresponding author, on behalf of all co-authors

Reply to Referee' (Reviewer #1) Comments:

Reviewer #1:

This study uses diatom-specific C₂₅ HBI biomarkers and compound-specific carbon isotopes to demonstrate that diatoms in Lake Haixihai reassimilate microbially respired DIC. The method is innovative and the core finding advances understanding of inland water carbon cycling. The writing is clear, figures and tables are all well documented. However, the key assumptions and interpretations require further validation, minor revision of this manuscript is recommended.

Authors' reply:

Thank you for your positive evaluation and constructive comments.

Here are specific comments:

Clarify whether C_{25:2} HBI derives mainly from planktonic or benthic diatoms?

Authors' reply:

Thank you for raising this important point. Yes, the ecological habitat of HBI-producing diatoms is highly relevant to our interpretation because benthic and planktonic diatoms may utilize distinct DIC sources.

To our best known, C_{25:2} HBIs cannot be assigned exclusively to either benthic or planktonic diatoms. Several identified producers are benthic diatoms, including *Haslea ostrearia*, *Pleurosigma strigosum*, and *Berkeleya rutilans* (Johns et al., 1999; Kaiser et al., 2016). However, the marine planktonic diatom *Pseudosolenia calcar-avis* has also been shown to biosynthesize C_{25:2} HBIs, with the same compound detected in water-column particulate matter and associated sediments (Kaiser et al., 2016). C_{25:2} HBIs are also produced by sea-ice diatoms, including *Berkeleya adeliensis*, which produces a sea-ice index: IPSO₂₅ (Belt et al., 2016). More broadly, Belt et al. (2001) proposed that known HBI-producing diatoms occupy benthic, planktonic, marine, and freshwater habitats. These observations suggest that the dominant ecological source of C_{25:2} HBI may differ across environments.

We have therefore revised the manuscript to clarify that diatoms producing C_{25:2} HBIs can occupy diverse ecological habitats, rather than regarding these compounds as exclusively benthic or planktonic biomarkers (Lines 64-71 and 479-481 in the revised manuscript).

Revised text:

*Lines 64-71 in Introduction section: The C₂₅ highly branched isoprenoids (C₂₅ HBIs) are produced by specific diatom groups occupying diverse ecological habitats (Rowland & Robson, 1990; Summons et al., 1993; Belt et al., 2000). Identified producers of C_{25:2} HBIs include several benthic diatoms, such as *Haslea ostrearia*, *Pleurosigma strigosum*, and *Berkeleya rutilans* (Johns et al., 1999; Kaiser et al., 2016), as well as planktonic diatoms such as *Pseudosolenia calcar-avis* (Kaiser et al., 2016) and sea-ice diatoms such as *Berkeleya adeliensis* (Belt et al., 2016). These C_{25:2} HBIs have been successfully applied to source*

determination in marine environments (e.g., Rowland & Robson, 1990; Summons et al., 1993; Belt et al., 2008; Belt et al., 2016; Kaiser et al., 2016) and are now being extended to lake systems (e.g., Aichner et al., 2010; Zhang et al., 2018; Diefendorf et al., 2025).

Lines 479-481 in Discussion section: The C_{25:2} HBI itself also introduces biomarker specific uncertainties. Its producers include both benthic and planktonic diatoms. Because these ecological groups may have access to distinct DIC sources, changes in their relative contributions could influence $\delta^{13}\text{C}_{\text{HBI}}$ independently of changes in recycled DIC availability.

References

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- Kaiser, J., Belt, S.T., Tomczak, M., Brown, T.A., Wasmund, N., and Arz, H.W. 2016: C₂₅ highly branched isoprenoid alkenes in the Baltic Sea produced by the marine planktonic diatom *Pseudosolenia calcaravis*. *Org. Geochem.* 93: 51-58. <https://doi.org/10.1016/j.orggeochem.2016.01.002>.
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C₂₅ HBI biomarkers are the only indices for tracking the CO₂ sequestration? Any errors can be created for this biomarker?

Authors' reply:

Thank you for this important comment. We agree that C₂₅ HBIs are not the only indicator of carbon sequestration, nor should they serve as a direct proxy for total carbon sequestration. In this study, we used it only to demonstrate and quantify a process affecting carbon retention: refixation of respiration-derived DIC by diatoms. Our interpretation is therefore based on multiple independent lines of evidence, including $\delta^{13}\text{C}_{\text{Org}}$, $\delta^{13}\text{C}_{\text{carb}}$ signatures, hopanoid indicators of microbial reworking, lignin degradation indices, and organic matter source biomarkers. These proxies constrain organic carbon sources, microbial remineralization, recycled DIC production, and its subsequent assimilation by diatoms.

We also acknowledge that the application of C_{25:2} HBI carbon isotopes is subject to several potential uncertainties, and we have now expanded the discussion accordingly (Lines 469-

486 in the revised manuscript). First, $\delta^{13}\text{C}_{\text{HBI}}$ values are modulated not only by the isotopic signature of assimilated DIC, but also by carbon isotope fractionation during carbon uptake by diatom. In particular, variations in CO₂ availability, bicarbonate utilization, and carbon concentrating mechanisms can substantially alter photosynthetic isotope fractionation, thereby affect the estimated contribution of recycled DIC. Second, C_{25:2} HBIs can be synthesized by both benthic and planktonic diatoms. Since these ecological groups utilize distinct DIC sources, shifts in their relative contributions can influence $\delta^{13}\text{C}_{\text{HBI}}$ values independently of variations in recycled DIC availability. Finally, biosynthetic conversion of assimilated carbon to HBI lipids can introduce an additional isotope offset. In this study, we used a fixed $\epsilon_{\text{b-l}}$ of 3.7‰ in the model, although this offset may vary among HBI-producing taxa and physiological conditions. We have therefore clarified in the revised manuscript that $\delta^{13}\text{C}_{\text{HBI}}$ values are best regarded as a tracer of carbon sources utilized by HBI-producing diatoms, rather than a direct quantitative measure for whole-lake carbon sequestration. Further refinement of quantitative estimates for recycled carbon utilization requires improved constraints on producer community compositions, respiratory endmembers, photosynthetic fractionation, and HBI biosynthetic offsets.

Revised text:

However, further study is needed to more accurately constrain both the efficiency and the flux of recycled CO₂(aq) fixation by diatoms. One important uncertainty lies in the isotopic composition of the respiratory endmember. In this study, sedimentary $\delta^{13}\text{C}_{\text{org}}$ was used to approximate the organic substrates undergoing respiration because Lake Haixihai is oligotrophic and contains very low concentrations of dissolved and particulate organic matter. Nevertheless, microorganisms may preferentially degrade organic substrates whose isotope compositions differ from bulk sedimentary organic matter. This uncertainty may become greater in eutrophic lakes, where algal organic matter can contribute substantially to respiration and generate ¹³C-enriched DIC (Morales-Williams et al., 2017). Carbon isotope fractionation during diatom carbon acquisition introduces an additional uncertainty. Enhanced carbon concentrating mechanisms can reduce photosynthetic fractionation, particularly in alkaline waters with low CO₂(aq). Under very low pCO₂ conditions, the ϵ_{photo} can decrease to -3‰ to -1‰ or even to 0‰ (Sharkey & Berry, 1985; Erez et al., 1998). Applying these smaller fractionations would increase the inferred f_{resp} in our study. The C_{25:2} HBI itself also introduces biomarker specific uncertainties. Its producers include both benthic and planktonic diatoms. Because these ecological groups may have access to different DIC sources, changes in their relative contributions could influence $\delta^{13}\text{C}_{\text{HBI}}$ independently of changes in recycled DIC availability. In addition, conversion of assimilated carbon into HBI lipids introduces a biosynthetic isotope offset. We used a fixed $\epsilon_{\text{b-l}}$ of 3.7‰ in the model, but this offset may vary among HBI producing taxa and physiological conditions. Thus, $\delta^{13}\text{C}_{\text{HBI}}$ provides a useful indicator of the carbon sources assimilated by HBI-producing diatoms, but it does not directly quantify carbon sequestration at the ecosystem scale. Further refinement of quantitative estimates for recycled carbon utilization requires improved constraints on producer communities, respiratory endmembers, photosynthetic fractionation, and HBI biosynthetic offsets.

Were there both water sample or sediment samples both taken? What is the exact depth of water samples taken? Only the surface water or take the water samples based on line 105~106 “17 shallow water sites (< 10 m) and 18 deep water sites (> 10 m)”

Authors' reply:

Sorry for the confusion. No water samples were collected in this study. All 35 samples are surface sediments collected from the upper 0-3 cm of the sediment. The terms “shallow water” and “deep water” refer solely to the water depth at each sediment sampling site. Specifically, 17 sediment samples were collected from sites with water depths < 10 m, and 18 were collected from sites with water depths >10 m. Although C₂₅ HBIs can also occur in suspended particulate matter in the water column, this study focused exclusively on sedimentary C₂₅ HBIs. We have revised the sampling description to clarify this point (Lines 110-114 in the revised manuscript).

Revised text:

A total of 35 sediment sampling sites were established across Lake Haixihai (Fig. 1b), including 17 sites located in shallow water (< 10 m water depth) and 18 sites located in deep water (> 10 m water depth). Surface sediments representing the top 0 ~ 3 cm at each site were collected using a Renberg® gravity corer in March 2023.

Would season variations affect the results? It seems that sampling was only taken on March.

Authors' reply:

Yes, seasonal variability may influence the processes investigated in this study. Basically, seasonal shifts in precipitation, water level, temperature, thermal stratification, organic matter supply, and primary producer communities modulate the production and availability of recycled DIC. For instance, elevated temperature and much more organic matter supply during warmer and wetter season can stimulate microbial remineralization, whereas stronger thermal stratification may facilitate the accumulation of regenerated DIC in deeper waters while restrict its upward transport into the surface photic zone. Enhanced precipitation and runoff can also increase inputs of terrestrial organic matter and external DIC, thereby altering both $\delta^{13}\text{C}_{\text{org}}$ and the isotopic composition of the DIC pool available to diatoms. Furthermore, the abundance and community composition of HBI-producing diatoms may undergo seasonal variations in response to shifts in temperature, stratification, light, and nutrient availability. Collectively, these processes induced by seasonal shifts can affect C_{25:2} HBI abundance, $\delta^{13}\text{C}_{\text{HBI}}$, and the estimated contribution of recycled DIC.

However, our study focused on the surface sediments (0-3 cm), rather than a representative of discrete season. Instead, HBI signal in surface sediments records the integrated effect accumulated over a certain period depending on the sedimentation rate of Lake Haixihai. You are right. Although $\delta^{13}\text{C}_{\text{HBI}}$ in surface sediments of Lake Haixihai can reveal the utilization of recycled CO₂ by diatoms, it cannot resolve seasonal cyclic variations. This constitutes one

limitation of the present study, which needs to be addressed in future work through refined sampling design. Thank you for your insightful suggestions.

We therefore highlight this limitation in the revised manuscript: “Future studies combining seasonal measurements of water column DIC, suspended particulate HBIs, diatom communities, and sedimentary records will be required to quantify the seasonal dynamics of recycled carbon assimilation” (Lines 487-497 in the revised manuscript).

Revised text:

Seasonal variability represents a further limitation. The present study is based on a single basin wide survey conducted in dry periods, and therefore cannot explicitly resolve seasonal changes in recycled DIC production and utilization. The analyzed samples are surface sediments from the upper 0 ~ 3 cm and thus integrate deposited material over an unresolved period rather than recording only water column conditions at the time of sampling. Nevertheless, seasonal changes in water level, temperature, thermal stratification, runoff, organic matter supply, and diatom communities may alter the carbon sources available to HBI-producing diatoms. During warmer periods, enhanced microbial mineralization may increase recycled DIC in deeper waters, whereas stronger stratification may restrict its transport into the surface photic zone. Increased runoff may also modify terrestrial organic matter and external DIC inputs, thereby changing both $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{DIC}}$. Seasonal changes in the abundance and ecological composition of diatoms may further affect HBI production and carbon acquisition. Future studies combining seasonal measurements of water column DIC, suspended particulate HBIs, diatom communities, and sedimentary records will be required to quantify the seasonal dynamics of recycled carbon assimilation.

In Fig. 2 caption: ion chromatograph or mass spectra? Did authors mix all shallow or deep-water samples, or just use random one? This point should be specified.

Authors' reply:

Thank you. The main profiles presented in Fig. 2 (in the last version, now it's Fig. 3) are total ion chromatograms (TICs) acquired via full-scan GC-MS analysis, rather than mass spectra. All extracts from shallow and deep-water sediments were analyzed by GC-MS. The red and blue TICs in Fig. 2 (in the last version, now it's Fig. 3) are not the mixtures of all shallow or deep-water samples. They are derived from two individual representative samples. Sample S15 is the representative of the deep-water sediments, whereas sample S30 for the shallow-water sediments. The corresponding sample numbers have now been specified in the revised figure caption (Lines 239-242 in the revised manuscript).

Revised text:

Figure 3: Total ion chromatograms (TICs) of hydrocarbon fractions showing n-alkanes and C_{25:2} HBIs distributions in surface sediments of Lake Haixihai. The red and blue chromatograms represent sample S22 from a shallow-water site (< 10 m water depth) and sample S30 from a deep-water site (>10 m water depth), respectively. The inset in the upper-left corner shows the mass spectrum and molecular structure of C_{25:2} HBI.

The content of line 335-340 and 345-450 should be put into the section of materials and methods.

Authors' reply:

Thank you for this suggestion. We agree that the description of the isotope mixing model and the associated calculations should be presented in the Materials and Methods rather than in the Discussion. We thus follow your suggestion and reorganized this part of the manuscript.

- (1) As suggested, we have moved these sections from the Discussion to a new subsection in the Materials and Methods (**Section 2.5 *Quantification of recycled DIC utilization by diatoms***, Lines 181-202 in the revised manuscript). The Discussion has been revised accordingly and now focuses only on the model results and their biogeochemical interpretation.
- (2) The model results are now presented in a new Results subsection (**Section 3.4 *Contribution of recycled CO₂(aq) to diatom carbon uptake***, Lines 284-291 in the revised manuscript).
- (3) The previous Discussion subsection on the “Efficiency of diatoms in utilizing recycled carbon” has been removed as an independent section. Its interpretive content has been incorporated into the revised **Section 4.3 *Implications of recycled CO₂(aq) assimilation for aquatic carbon cycling***. The opening paragraph of this section has been substantially rewritten to avoid repeating model methods or numerical results (Lines 439-452 in the revised manuscript).

Reply to Referee' (Reviewer #2) Comments:

Reviewer #2:

General comments

This manuscript presents molecular and isotopic signatures of 35 sediment samples collected across Lake Haixihai to investigate diatom recycling of microbially respired organic carbon, as a potential mechanism of carbon sequestration in lake sediments. Given the growing interest in freshwater "biological pumps", this study, employing a novel combination of advanced molecular and isotopic analyses, can appeal to the broad readership of Biogeosciences. Overall, the manuscript is well written and clearly focused on the central argument: the biological recycling of CO₂ as a key pathway for carbon sequestration in alkaline lakes. While this argument is based on intriguing findings (e.g., strong correlations in molecular and isotopic signatures between sediment organic matter and carbonates), these biogeochemical proxies provide primarily indirect evidence for the central argument. Below, I suggest several major revisions to help the authors sharpen the logic of the main hypothesis and better ground their conclusions in their empirical findings.

Authors' reply:

We sincerely thank the reviewer for the careful and constructive evaluation of our manuscript. We greatly appreciate these insightful comments, which have helped us improve both the scientific rigor and clarity of the manuscript. We have carefully considered all of the reviewer's suggestions and substantially revised the manuscript accordingly.

1. CO₂ recycling vs. C sequestration

In my view, the presented biogeochemical proxies are interesting per se, but provide primarily indirect evidence for the proposed mechanisms. First, the core hypothesis is conceptually weak: CO₂ recycling by diatoms, which can continue to fuel microbial decomposition without a bypass to sequestered DIC, does not inherently ensure long-term C sequestration (burial) in sediments. The authors need to clearly define these two key concepts (CO₂ recycling vs. carbon sequestration) and refine the theoretical link between them, supported by relevant literature.

Authors' reply:

We sincerely thank your important and insightful comment. We fully agree that our original manuscript did not adequately distinguish internal DIC recycling from long-term carbon sequestration, and that this distinction is essential for correctly interpreting the biogeochemical implications of our results. Your comment thus prompted us to thoroughly reconsider and refine the conceptual framework of the manuscript.

Yes, reassimilation of respiration-derived DIC by diatoms only represents an internal recycling process, whereby carbon released during organic matter mineralization is converted back into organic carbon rather than being immediately available for CO₂ evasion. We

therefore use the term **carbon retention** to describe the process directly supported by our observations: diatom reassimilation retains a fraction of the regenerated carbon within the lake carbon cycle and prolongs its residence in the aquatic system. Whereas long-term carbon sequestration requires subsequent export, preservation, and burial of this refixed carbon in sediments. These processes are governed by sediment accumulation, remineralization, and burial efficiency (Sobek et al., 2009; Sun et al., 2018) and were not investigated in the present study.

Nevertheless, a mechanistic link between carbon retention and potential long-term sequestration can still be established. Diatoms efficiently convert DIC into particulate organic carbon, and their siliceous frustules can facilitate downward particle export (Raven and Waite, 2004). Thus, reassimilation of respiration-derived DIC by diatoms provides an additional pathway for regenerated carbon to be incorporated into sinking particulate material and ultimately reach the sediments. However, only the fraction that subsequently escapes remineralization and is preserved upon burial can contribute to long-term carbon sequestration. We therefore revised our interpretation so that carbon retention represents the central process demonstrated in this study, whereas carbon sequestration is discussed only as a potential downstream consequence of this retention pathway.

Following this conceptual revision, we have made corresponding changes throughout the manuscript. First, the title has been changed from “Enhanced Carbon Sequestration in Alkaline Lakes through Recycled CO₂ Utilization by Diatoms” to “*Enhanced Carbon Retention in an Alkaline Lake through Reassimilation of Respiration-Derived DIC by Diatoms*” (Lines 1-2 in the revised manuscript). Second, the Abstract has been revised to state that biological refixation enhances carbon retention by returning regenerated inorganic carbon to primary production, while explicitly noting that this carbon contributes to sedimentary sequestration only if it is subsequently exported and preserved in sediments (Lines 22-25 in the revised manuscript; *These findings indicate that biological refixation of respiration-derived CO₂(aq) can enhance carbon retention within alkaline lakes by returning regenerated inorganic carbon to primary production rather than allowing its immediate loss through carbon evasion. This retained carbon may subsequently contribute to sedimentary carbon sequestration if it is exported and preserved in sediments*). Third, the final sentence of the Introduction has been revised to emphasize the implications of our results for internal carbon retention and carbon emission, while treating diatom-mediated sedimentary sequestration as a potential consequence rather than a directly quantified outcome (Lines 80-82 in the revised manuscript; *This study fills a gap in our understanding of internal carbon cycling in lake systems and provides new insights into the role of diatom-mediated DIC reassimilation in carbon retention and carbon emission, as well as its potential implications for longer-term sedimentary carbon sequestration*). Fourth, the Discussion has been revised throughout to distinguish internal carbon retention from long-term burial. In particular, we now state that assimilation by diatoms converts part of the recycled DIC back into organic carbon and retains it within the lake system, whereas its contribution to long-term sequestration depends on subsequent export and preservation (Lines 446-452 in the revised

manuscript; *Assimilation by diatoms converts part of this recycled DIC back into organic carbon and therefore represents an important component of internal carbon cycling. Diatom biomass can contribute to particulate organic carbon export, and their siliceous frustules can facilitate downward particle transport through the biological carbon pump (Raven and Waite, 2004). If a fraction of this refixed carbon subsequently escapes remineralization and is preserved in sediments, it may contribute to longer-term carbon burial.*). Finally, the Conclusions have been revised accordingly to avoid equating DIC reassimilation directly with sedimentary carbon sequestration (Lines 517-521 in the revised manuscript; *By linking degradation and fixation through a common DIC pool, the system maintains an efficient internal carbon turnover. This recycling pathway returns part of the respiration-derived DIC to primary production and thereby increases carbon retention within the lake. By converting regenerated DIC into particulate organic carbon, it may also provide an additional pathway toward sedimentary carbon sequestration if the refixed carbon is subsequently exported and preserved in sediments*).

References

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Second, the empirical findings, as detailed in my specific comments below, are presented inconsistently regarding the primary carbon source for sediment bicarbonates. The entire Results and Discussion sections require a thorough revision to resolve these internal inconsistencies and address the points outlined below.

- L 265: “Collectively, sedimentary organic matter in Lake Haixihai is mainly derived from aquatic macrophytes and terrigenous sources, with minor contributions from algae and bacterial input.” --> Then, how can you say that algal-derived C (including recycled C) can play a crucial role in C sequestration?

Authors' reply:

Many thanks for identifying this inconsistency. We acknowledge that we did not elaborate on this clearly in the earlier version of the manuscript.

Molecular and bulk geochemical evidence indicates that the sedimentary organic matter preserved in Lake Haixihai receives substantial contributions from aquatic macrophytes and terrigenous higher plants, with relatively smaller but non-negligible contributions from algal

and microbial sources. However, the relative proportions preserved in surface sediments do not necessarily represent the relative contributions of these sources to organic matter production and remineralization within the water column and sediment–water interface. Algal organic matter is generally more labile and can be preferentially remineralized during settling and early diagenesis, so its contribution to preserved sedimentary organic matter may be lower than its contribution to contemporary carbon cycling. The occurrence of biologically configured $\beta\beta$ -hopanes and hopenes, together with the prominent unresolved complex mixture (UCM), is also consistent with substantial microbial contribution to and reworking of the sedimentary organic matter pool. More importantly, recycled DIC utilized by diatoms does not need to originate from algal organic matter. Respiration-derived DIC can be generated through microbial mineralization of multiple organic matter pools, including terrestrial higher-plant material, aquatic macrophytes, algal biomass, and microbial organic matter. Thus, even where algae represent a relatively small fraction of the organic matter ultimately preserved in sediments, HBI-producing diatoms can still assimilate recycled DIC generated from the degradation of macrophyte- or terrestrial-derived organic matter. Aquatic macrophyte-derived organic matter may be particularly relevant in Lake Haixihai because it provides an abundant autochthonous substrate that can undergo microbial decomposition and mineralization within the lake.

Following this comment and the reviewer's broader concerns regarding carbon sequestration, we have revised the manuscript to avoid implying that diatom assimilation of recycled DIC itself constitutes a quantitatively important long-term carbon sink. We now interpret this process primarily as **internal carbon recycling and retention**: reassimilation returns respiration-derived DIC to primary production and may prolong its residence within the lake, whereas its eventual contribution to long-term carbon sequestration depends on subsequent export, preservation, and burial.

- L 270-272 (Fig. 4) “In deep water sediments of Lake Haixihai, $\delta^{13}\text{C}_{\text{carb}}$ exhibits a significant positive correlation with $\delta^{13}\text{C}_{\text{org}}$ ($r = 0.748$, $p < 0.001$; Fig. 270 4a) and a strong negative correlation with TOC ($r = -0.852$, $p < 0.001$; Fig. 4b). This indicates that carbonate precipitation is strongly influenced by respiration-derived DIC (Sun et al., 2019).” --> Please explain the mechanisms, considering complex processes occurring in both water column and bottom sediments. For example, the observed correlation might be affected by separate processes such as CO₂ evasion or phytoplankton CO₂ uptake leaving heavy isotopes in water, which can occur irrespective of organic matter transformations in bottom sediments.

Authors' reply:

We thank the reviewer for this important comment. We agree that our original statements were lack of mechanism explanations.

The carbon isotope composition of authigenic carbonate largely reflects the $\delta^{13}\text{C}$ of the DIC pool from which it precipitates, although this DIC pool can be modified by several processes, including atmospheric exchange, CO₂ evasion, photosynthetic uptake, and organic matter remineralization (Leng and Marshall, 2004; Sun et al., 2019). These processes can produce different isotope effects. Organic matter mineralization introduces relatively ^{13}C -depleted DIC whose isotopic composition is related to that of the degraded organic substrate. In contrast, photosynthetic carbon uptake preferentially removes ^{12}C and therefore tends to enrich the residual DIC pool in ^{13}C . CO₂ evasion and air-water isotope exchange may likewise modify and generally enrich the remaining DIC in ^{13}C . In the deep-water sediments of Lake Haixihai, $\delta^{13}\text{C}_{\text{carb}}$ is positively correlated with $\delta^{13}\text{C}_{\text{org}}$ ($r=0.748$, $p<0.001$) and negatively correlated with TOC ($r=-0.852$, $p<0.001$). The negative relationship with TOC is particularly informative. If the observed isotope pattern were controlled primarily by enhanced primary production, greater photosynthetic carbon uptake would preferentially remove ^{12}C from the DIC pool and tend to leave the residual DIC and authigenic carbonate relatively enriched in ^{13}C . The process would therefore tend to produce carbonate enriched in ^{13}C , rather than in ^{12}C , with increasing organic carbon production. This expectation is opposite to the observed decrease in $\delta^{13}\text{C}_{\text{carb}}$ with increasing TOC. Moreover, simple photosynthetic drawdown does not readily explain the positive covariance between $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{org}}$. Taken together, these relationships suggest that photosynthetic fractionation alone is unlikely to be the dominant control on the observed carbonate isotope pattern and are instead consistent with an increasing contribution of ^{13}C -depleted DIC generated through organic matter remineralization to the DIC pool involved in carbonate precipitation (Sun et al., 2019). We have made relevant modifications in the revised manuscript (Lines 353-369).

Revised text:

The carbon isotope composition of authigenic carbonate largely reflects the $\delta^{13}\text{C}$ of the DIC pool from which it precipitates, although this DIC pool can be modified by several processes, including atmospheric exchange, CO₂ evasion, photosynthetic uptake, and organic matter remineralization (Leng and Marshall, 2004; Sun et al., 2019). These processes can produce contrasting isotope effects. Organic matter mineralization introduces relatively $\delta^{13}\text{C}$ -depleted DIC whose isotopic composition is related to that of the degraded organic substrate. In contrast, photosynthetic carbon uptake preferentially removes $\delta^{12}\text{C}$ and therefore tends to enrich the residual DIC pool in $\delta^{13}\text{C}$. CO₂ evasion and air-water isotope exchange may also modify the isotopic composition of the residual DIC, generally toward more $\delta^{13}\text{C}$. In the deep water sediments of Lake Haixihai, $\delta^{13}\text{C}_{\text{carb}}$ is positively correlated with $\delta^{13}\text{C}_{\text{org}}$ ($r = 0.748$, $p < 0.001$; Fig. 5a) and negatively correlated with TOC ($r = -0.852$, $p < 0.001$; Fig. 5b). The negative relationship with TOC is particularly informative. If the observed isotope pattern were controlled primarily by enhanced primary production, greater photosynthetic carbon uptake would preferentially remove ^{12}C from the DIC pool and tend to leave the residual DIC and authigenic carbonate relatively enriched in ^{13}C . The process would therefore tend to produce carbonate enriched in ^{13}C , rather than in ^{12}C , with increasing organic carbon production. This expectation is opposite to the observed decrease in $\delta^{13}\text{C}_{\text{carb}}$ with increasing TOC. Moreover, simple photosynthetic drawdown does not readily explain the positive covariance between $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{org}}$. Taken together, these relationships suggest that photosynthetic fractionation alone

is unlikely to be the dominant control on the observed carbonate isotope pattern and are instead consistent with an increasing contribution of $\delta^{13}\text{C}$ -depleted DIC generated through organic matter remineralization to the DIC pool involved in carbonate precipitation (Sun et al., 2019).

References

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- Sun, D., He, Y., Wu, J., Liu, W., and Sun, Y. 2019: Hydrological and ecological controls on autochthonous carbonate deposition in lake systems: A case study from lake wuliangsu and the global perspective. *Geophys. Res. Lett.* 46: 6583–6593. <https://doi.org/https://doi.org/10.1029/2019GL082224>.

- L 285-288 (& 5) “The remaining samples show a strong positive correlation between $\delta^{13}\text{CHBI}$ and $\delta^{13}\text{C}_{\text{org}}$ ($r = 0.946$, $p < 0.001$; Fig. 5a). Generally, this relationship can be readily interpreted as diatoms being the main contributor of organic matter. However, biomarker and geochemical evidence shows that organic matter in Lake Haixihai is mainly derived from aquatic macrophytes and terrestrial inputs, and minor from algae and bacteria inputs as discussed in Section 4.1. Thus, the synchronous isotopic variations reflect a close link between DIC utilized by diatoms and respiration-derived DIC within Lake Haixihai.” --> Contradictory descriptions against the preceding one and within the same paragraph.

Authors' reply:

We thank the reviewer for pointing out this again.

One possible explanation is a common-source effect. If diatoms contributed the majority of sedimentary organic matter, then $\delta^{13}\text{C}_{\text{HBI}}$ and bulk $\delta^{13}\text{C}_{\text{org}}$ could covary simply because both signals originated largely from the same algal biomass. However, our multiproxy source assessment does not support this interpretation as we discussed earlier. Therefore, bulk sedimentary $\delta^{13}\text{C}_{\text{org}}$ cannot be regarded simply as a diatom isotope signal. Under this source composition, the strong positive covariance between $\delta^{13}\text{C}_{\text{HBI}}$ and $\delta^{13}\text{C}_{\text{org}}$ is more consistent with isotopic coupling through internal carbon recycling. Microbial mineralization transfers part of the isotopic signal of degraded organic matter into respiration-derived DIC, and this regenerated DIC can subsequently be assimilated by HBI-producing diatoms. The isotopic composition of the organic substrate can therefore be indirectly transferred to diatom-derived HBIs through the DIC pool.

We have revised the paragraph accordingly (Lines 384-394 in the revised manuscript).

Revised text:

A possible explanation for this relationship is a common-source effect. If diatoms constituted the dominant source of sedimentary organic matter, $\delta^{13}\text{C}_{\text{HBI}}$ and bulk $\delta^{13}\text{C}_{\text{org}}$ could covary because both signals would largely originate from the same algal biomass. However, the multiproxy source assessment in Section 4.1 indicates that sedimentary organic matter in Lake Haixihai receives substantial contributions from aquatic

macrophytes and terrigenous higher plants, whereas algal and microbial sources are relatively smaller but non-negligible. Bulk $\delta^{13}\text{C}_{\text{org}}$ therefore does not primarily represent a diatom-derived isotope signal. Under this mixed-source setting, the positive covariance between $\delta^{13}\text{C}_{\text{HBI}}$ and $\delta^{13}\text{C}_{\text{org}}$ is more consistent with isotopic coupling mediated by internal carbon recycling. During microbial remineralization, respiration-derived CO₂(aq) can partly inherit the carbon isotopic composition of the degraded organic substrates. Assimilation of this regenerated CO₂(aq) by HBI-producing diatoms can subsequently transfer this isotopic signal into C₂₅ HBIs. Thus, the observed relationship suggests a close linkage between the organic matter undergoing remineralization and the DIC utilized by diatoms within Lake Haixihai.

- L 360-364 “This means that diatoms efficiently utilize respiration-derived DIC, returning it to the biological carbon pump. This process likely account for the high organic carbon sequestration flux observed in Lake Haixihai despite its oligotrophic state (Wang et al., 2019). Combined with CaCO₃ precipitation, these pathways capture respired-derived DIC that would otherwise escape to the atmosphere, thereby enhancing internal carbon cycling and stabilizing carbon storage.” --> Please explain how microbial-derived CO₂ ends up sequestered in sediments.

Authors' reply:

Thank you for this comment.

Respiration-derived DIC in Lake Haixihai can be generated through microbial degradation of multiple organic matter sources, including terrigenous higher-plant material, aquatic macrophytes, and algal and microbial organic matter. Once released, this regenerated inorganic carbon can follow at least two pathways toward the sedimentary carbon pool. First, a portion of the respiration-derived DIC can be incorporated into authigenic carbonate during carbonate precipitation and thereby enter the sediments as mineral carbon (Sun et al., 2017). Second, respiration-derived DIC can be reassimilated by primary producers, including diatoms, and converted back into particulate organic carbon. Diatoms are particularly relevant to this second pathway because their siliceous frustules and associated particle production can facilitate downward export of organic matter through the biological carbon pump (Raven and Waite, 2004). If part of this refixed organic carbon subsequently escapes remineralization and is preserved in the sediments, it can contribute to longer-term carbon burial.

Combining the previous comments, the former discussion of diatom “efficiency” and direct carbon sequestration has been removed and reorganized into the revised Section 4.3 (Lines 438-462 in the revised manuscript). The new text now distinguishes between internal **carbon retention** and **potential sedimentary burial**, and presents authigenic carbonate formation and biological refixation as two complementary pathways through which regenerated DIC may remain within the lake system.

Revised text:

Alkaline lake systems commonly sustain stable diatom communities (Karst-Riddoch et al., 2005; Oduor &

Schagerl, 2007; Duarte et al., 2008; Stenger-Kovács et al., 2014). Diatoms can utilize different forms of inorganic carbon and are sensitive to changes in DIC availability and composition (Tuite, 1981; Raven et al., 2011; Shi et al., 2017). Thus, the stable carbon isotopic composition of diatom-derived lipids can respond to changes in the DIC pool used for photosynthesis (Corcoran et al., 2025). In Lake Haixihai, this relationship is reflected by the $\delta^{13}\text{C}$ values of $\text{C}_{25:2}$ HBI and by the modeled contribution of respiration-derived DIC, which accounts for $31\% \pm 8\%$ of diatom carbon uptake. These results provide quantitative evidence that regenerated inorganic carbon can be substantially returned to primary production rather than being lost directly from the lake system. Assimilation by diatoms converts part of this recycled DIC back into organic carbon and therefore represents an important component of internal carbon cycling. Diatom biomass can contribute to particulate organic carbon export, and their siliceous frustules can facilitate downward particle transport through the biological carbon pump (Raven and Waite, 2004). If a fraction of this refixed carbon subsequently escapes remineralization and is preserved in sediments, it may contribute to longer-term carbon burial. In parallel, respiration-derived DIC can also be incorporated into authigenic carbonate and transferred to the sedimentary inorganic carbon pool. Thus, biological refixation and carbonate precipitation represent two pathways through which internally recycled carbon may be sequestered within alkaline lake systems.

References

- Sun, D., He, Y., Wu, J., Liu, W., and Sun, Y. 2019: Hydrological and ecological controls on autochthonous carbonate deposition in lake systems: A case study from lake wuliangsu and the global perspective. Geophys. Res. Lett. 46: 6583-6593. <https://doi.org/https://doi.org/10.1029/2019GL082224>.*
- Raven, J.A., and Waite, A.M. 2004: The evolution of silicification in diatoms: inescapable sinking and sinking as escape? New Phytol. 162: 45-61. <https://doi.org/https://doi.org/10.1111/j.1469-8137.2004.01022.x>.*

- L 410-412 “Lakes, particular alkaline lakes, should not be recognized easily as conduits for terrestrial carbon loss (Tranvik & Jansson, 410 2002). They are active systems in which biological re-fixation of respired carbon can substantially enhance long-term carbon sequestration and mitigate CO₂ emissions.” --> Is this a quantitatively significant sink?

Authors' reply:

We thank the reviewer for raising this important point. We acknowledge that the present study does not directly demonstrate that this pathway constitutes a quantitatively significant carbon sink at the ecosystem scale. Although a substantial fraction of regenerated inorganic carbon can be returned to primary production, however, converting this fraction into a net carbon sink would require additional constraints on the amount of refixed carbon exported from the water column, the fraction that escapes subsequent remineralization, sediment accumulation and burial efficiency, and the balance between carbon burial and CO₂ evasion.

We have therefore revised the manuscript to avoid describing diatom reassimilation as a quantitatively significant long-term carbon sink. The original statement that biological

refixation can “substantially enhance long-term carbon sequestration and mitigate CO₂ emissions” has been removed and replaced by a more cautious statement (Lines 511-514 in the revised manuscript).

Revised text:

Rather than functioning solely as conduits for carbon loss (Tranvik & Jansson, 2002), lakes, particularly alkaline lakes, can actively retain regenerated carbon by returning respiration-derived DIC to primary production. Such biological refixation prolongs the residence of recycled carbon within lake systems, reduces its immediate availability for carbon evasion, and creates additional opportunities for its transfer into sinking particulate matter and eventual sedimentary preservation.

2. Data presentation

Sections 3.1 & 3.2 (Tables 1 & 2) require more efficient data presentation, supported by rigorous statistical analyses. For example, in Section 3.1, if depth-dependent trends were observed in the measured variables, these would be far more effectively illustrated using statistical relationships or summary figures rather than relying solely on Table 1. Furthermore, any comparisons between shallow and deep sites should be backed by appropriate statistical tests (e.g., t-tests or non-parametric equivalents).

Authors' reply:

Thanks for this helpful suggestion. As suggested, we have added a new **Figure 2** that summarizes the principal bulk geochemical and molecular characteristics of the shallow and deep water sediments using boxplots, while retaining Tables 1 and 2 to provide the complete site-specific data. We further performed statistical comparisons between the shallow and deep water groups using Tukey's HSD test. Statistical significance is now directly indicated in Fig. 2 by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Lines 215-226 in the revised manuscript). The analyses demonstrate significant differences ($p < 0.05$) in several variables, including $\delta^{13}\text{C}_{\text{org}}$, TOC/TN, P_{aq} , TAR, $n\text{C}_{21-}/n\text{C}_{22+}$, C_{30} $\beta\beta$ -hopane + hopenes, $\Sigma 8$, and $P/(V+S)$, whereas TOC, CPI, and $\text{C}_{25:2}$ HBI concentrations do not show significant differences between the two groups. The corresponding Results sections have also been revised to emphasize these statistically supported differences.

Revised text:

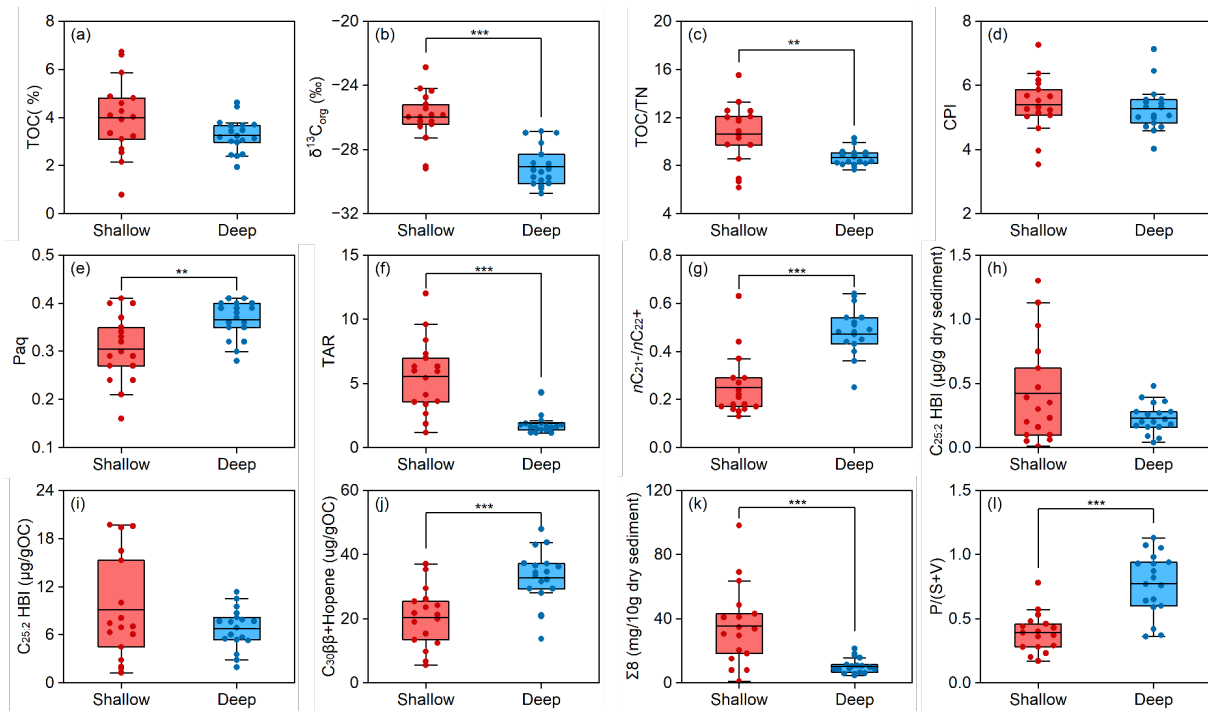


Figure 2: Comparison of bulk geochemical and molecular characteristics between shallow and deep water surface sediments of Lake Haixihai. Boxplots show (a) total organic carbon (TOC), (b) $\delta^{13}\text{C}_{\text{org}}$, (c) TOC/TN, (d) carbon preference index (CPI), (e) aquatic plant index Pa_q , (f) terrigenous-to-aquatic ratio (TAR), (g) $n\text{C}_{21+}/n\text{C}_{22+}$ ratio, (h) $\text{C}_{25:2}$ HBI concentration normalized to dry sediment mass, (i) $\text{C}_{25:2}$ HBI concentration normalized to organic carbon, (j) concentration of biologically configured C_{30} $\beta\beta$ -hopane + hopenes, (k) total abundance of the eight major lignin phenols $\Sigma 8$, and (l) the lignin degradation index $\text{P}/(\text{V}+\text{S})$. Red and blue represent shallow water (< 10 m) and deep water (> 10 m) sediments, respectively. Differences between shallow and deep water sediments were evaluated using Tukey's HSD test, with significance levels indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. In each boxplot, the center line represents the mean, the lower and upper limits of the box represent the first and third quartiles, respectively, the whiskers extend to 1.5 times the interquartile range, and points represent individual sediment samples.

3. Interpreting algal contributions to isotope signatures

In this study, source tracing relies on the simplified assumption that terrestrial plants and algae possess distinct isotopic signatures. However, given the overlap in $\delta^{13}\text{C}$ between plants and algae, as well as lower C/N ratios of soil organic matter (an important terrestrial source) relative to plant biomass that approach algal values, the simple dichotomy (algae-dominated deep water OM vs. plant-dominated shallow water OM) requires a more refined explanation as well as additional information for algal isotope signatures from the study site or other lakes.

- L 237-239: “In Lake Haixihai, $\delta^{13}\text{C}_{\text{org}}$ values ($-30.7\text{‰} \sim -22.9\text{‰}$) and TOC/TN ratios (6.16 $\sim$ 12.55) indicate that shallow water sediments contain a mixture of algal and terrestrial organic matter, whereas deep water sediments are mainly dominated by algal input (Fig. 3a).”

Authors' reply:

We thank the reviewer for this important comment. As suggested, we now first present the conventional source ranges used for interpreting bulk $\delta^{13}\text{C}_{\text{org}}$ and TOC/TN values and then apply this framework to the Lake Haixihai data. Shallow water sediments are characterized by relatively ^{13}C -enriched $\delta^{13}\text{C}_{\text{org}}$ values ($-26.0\text{‰} \pm 1.6\text{‰}$) and higher TOC/TN ratios (10.64 ± 2.51), whereas deep water sediments have more ^{13}C -depleted values ($-29.1\text{‰} \pm 1.3\text{‰}$) and lower TOC/TN ratios (8.58 ± 0.63). These patterns provide a rough indication of stronger terrestrial higher-plant influence in shallow water sediments and a relatively greater algal contribution toward the deeper basin. We now explicitly acknowledge, however, that this interpretation cannot be based on bulk proxies alone. Freshwater phytoplankton, including diatoms, can show $\delta^{13}\text{C}$ values that overlap those of terrestrial C3 plants (Vuorio et al., 2006), and soil organic matter can exhibit TOC/TN ratios similar to aquatic organic matter following microbial processing (Zhao et al., 2015). Instead, the revised discussion places greater emphasis on molecular biomarkers to refine the source interpretation (Lines 299-316 in the revised manuscript).

Revised text:

The availability and composition of organic substrates regulate microbial remineralization and therefore influence the production and isotopic composition of respiration-derived DIC (Bade et al., 2004; Karlsson et al., 2007; Sobek et al., 2009). Constraining sedimentary organic matter sources is therefore important for interpreting carbon recycling in Lake Haixihai. The crossplot of $\delta^{13}\text{C}_{\text{org}}$ values and TOC/TN ratios has been widely used for a rough assessment of organic matter sources in modern sediments (Meyers, 1997). Freshwater algal organic matter is generally characterized by relatively low TOC/TN ratios, commonly around 4 ~ 10, whereas vascular land plants typically have substantially higher C/N ratios because of their greater abundance of cellulose and lower protein contents (Meyers, 1994, 1997). Freshwater phytoplankton commonly shows $\delta^{13}\text{C}$ values within approximately -33‰ to -25‰, whereas terrestrial C3 plants generally fall within a broadly overlapping range of approximately -32‰ to -20‰ (Meyers, 1997; Lamb et al., 2006). In Lake Haixihai, shallow water sediments are characterized by relatively ^{13}C -enriched $\delta^{13}\text{C}_{\text{org}}$ values ($-26.0\text{‰} \pm 1.6\text{‰}$) and higher TOC/TN ratios (10.64 ± 2.51), whereas deep water sediments have more ^{13}C -depleted $\delta^{13}\text{C}_{\text{org}}$ values ($-29.1\text{‰} \pm 1.3\text{‰}$) and lower TOC/TN ratios (8.58 ± 0.63) ($p < 0.01$; Fig. b, c). These distributions suggest a stronger contribution of terrestrial higher plant organic matter in the shallow water sediments and a relatively greater contribution of algal organic matter toward the deeper basin (Fig. 5a). However, freshwater phytoplankton, including diatoms, can show $\delta^{13}\text{C}$ values that substantially overlap those of terrestrial C3 plants (Vuorio et al., 2006), while soil organic matter may exhibit TOC/TN ratios close to those of aquatic organic matter because of microbial processing (Zhao et al., 2015). Therefore, more specific constraints are required to distinguish the relative contributions of algae, aquatic macrophytes, terrestrial higher plants, and microbial sources, for which molecular biomarkers provide additional source information.

References

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Vuorio, K., Meili, M. and Sarvala, J. 2006: *Taxon-specific variation in the stable isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of lake phytoplankton*. *Freshw. Biol.* 51: 807-822. <https://doi.org/10.1111/j.1365-2427.2006.01529.x>.

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Specific comments

- Line (L) 13: “investigate”

Authors’ reply:

Thank you. We have corrected the wording accordingly (Line 13 in the revised manuscript).

- L 16: “correlate”

Authors’ reply:

Thank you. We have corrected the wording accordingly (Line 16 in the revised manuscript).

- L 22-23 “lakes should not be recognized easily as conduits for terrestrial carbon loss.”: Lakes are actually well recognized as sources of terrestrial carbon loss. Please reformulate the sentence to clarify what you want to emphasize.

Authors’ reply:

Thank you for this helpful comment. In response to this comment and the related major comment above, we have revised this statement. The revised text now reads: “These findings indicate that biological refixation of respiration-derived CO₂(aq) can enhance carbon retention within alkaline lakes by returning regenerated inorganic carbon to primary production rather than allowing its immediate loss through carbon evasion. This retained carbon may subsequently contribute to sedimentary carbon sequestration if it is exported and preserved in sediments.” (Lines 22-25 in the revised manuscript)

- L 48 “autogenic carbonate”: Do you mean “authigenic carbonate”?

Authors’ reply:

Thank you for pointing this out. We have replaced “autogenic carbonate” with the term “authigenic carbonate” (Line 49 in the revised manuscript).

- L 94 (throughout the manuscript): not “u”g L-1 but “μ(micro)”

Authors’ reply:

Thank you for pointing this out. We have carefully checked the entire manuscript and replaced all occurrences of “ug” with the correct symbol “μg” throughout the text and tables (e.g., Lines 99 and 304, Table 2, and Figs. 7 and 8 in the revised manuscript).

- L 114: Vienna Pee Dee Belemnite (VPDB) as a reference material?

Authors’ reply:

Thank you. VPDB is the reference scale for reporting carbon isotope compositions rather than a reference material directly analyzed in our measurements. We have revised the Methods to clarify that all $\delta^{13}\text{C}$ values are reported relative to the Vienna Pee Dee Belemnite (VPDB) scale. The wording has been corrected accordingly (Lines 121-123 in the revised manuscript).

Revised text:

All samples were analyzed in duplicate, with analytical precision of $\pm 0.2\%$ for TOC and TN, and $\pm 0.2\%$ for carbon isotopes. All $\delta^{13}\text{C}$ values are reported in per mil (‰) relative to the Vienna Pee Dee Belemnite (VPDB) scale.

- L 167: characters or characteristics?

Authors’ reply:

Thank you for pointing this out. We have replaced “characters” with the more appropriate term “characteristics.” The subsection title has been revised to “***Bulk characteristics of organic matter in surface sediments from Lake Haixihai***” (Line 204 in the revised manuscript).

- L 170: “show”

Authors’ reply:

Thank you. We have changed “shows” to “show” (Line 207 in the revised manuscript).

- L 180 (&142) “carbon preference index”: You need to define this earlier (L 142) and use the acronym consistently. L 185 (& 242-244) “TAR”: The same goes for this term. How can we evaluate the values ranging from 1.14 to 12.01? Are any reference values available?

Authors' reply:

We thank the reviewer for pointing out these issues.

As suggested, we have therefore expanded Section 2.3 to provide the source significance of all three indices (Lines 150-158 in the revised manuscript): In this study, several *n*-alkane indices were calculated to infer organic matter sources. The carbon preference index (CPI) describes the odd-over-even predominance of long-chain *n*-alkanes (Silva et al., 2012). High CPI values, commonly observed in fresh plant-derived *n*-alkanes, indicate a pronounced odd-carbon-number predominance. The terrigenous-to-aquatic ratio (TAR) with higher values indicates a greater relative contribution of higher-plant-derived *n*-alkanes and with lower values indicates greater aquatic/algal contributions (Silva et al., 2012). The aquatic plant index (P_{aq}) < 1 is characteristic of dominant terrestrial plant input, values of 0.1 ~ 0.4 indicate increasing contributions from emergent macrophytes, and values of 0.4 ~ 1.0 are commonly associated with submerged or floating macrophytes (Ficken et al., 2000). These ranges are used as qualitative source constraints because overlap among terrestrial and aquatic plants has subsequently been documented (Mead et al., 2005).

The Results section has also been revised so that only the acronyms CPI, TAR, and P_{aq} are used after their definitions in the Methods, and interpretations based on these indices have been made more consistent with their established source significance (Lines 230-237 in the revised manuscript).

The CPI ranges from 3.53 to 7.26 (Table 2), indicating a pronounced odd-over-even predominance of long-chain *n*-alkanes in most samples. Most samples also display a distinct unresolved complex mixture (UCM) in the $nC_{23} \sim nC_{35}$ carbon range. However, deep water sediments contain relatively higher proportions of short- to mid-chain *n*-alkanes than shallow water sediments (Fig. 3), as indicated by higher nC_{21}/nC_{22+} ratios and P_{aq} values (Table 2). P_{aq} ranges from 0.16 to 0.41, with higher values in deep water sediments (0.37 ± 0.04) than in shallow water sediments (0.30 ± 0.07 , $p < 0.01$; Fig. 2e). TAR ranges from 1.14 to 12.01 and is substantially higher in shallow water sediments (5.56 ± 2.84) than in deep water sediments (1.93 ± 0.92 , $p < 0.001$; Fig. 2f), indicating a stronger relative contribution of higher-plant-derived *n*-alkanes in the shallow water area.

References

- Silva, T.R., Lopes, S.R.P., Spörl, G., Knoppers, B.A., and Azevedo, D.A. 2012: Source characterization using molecular distribution and stable carbon isotopic composition of *n*-alkanes in sediment cores from the tropical Mundaú–Manguaba estuarine–lagoon system, Brazil. *Org. Geochem.* 53: 25-33. <https://doi.org/https://doi.org/10.1016/j.orggeochem.2012.05.009>.
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[https://doi.org/10.1016/S0146-6380\(00\)00081-4](https://doi.org/10.1016/S0146-6380(00)00081-4)

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- L 248-256: Interpretations of the two indicators seem contradictory. “lignin parameters (S/V vs. C/V) show that most terrestrial organic matter is derived from non-woody angiosperms” vs. “The aquatic plant index (P_{aq}), an indicator reflecting the proportion of aquatic macrophytes based on the proportion of mid-chain to long-chain *n*-alkanes (Mead et al., 2005), is slightly higher in deep water sediments (0.37 ± 0.04) than in shallow water sediments” vs. “aquatic macrophytes and terrigenous inputs both contribute to sedimentary organic matter. Despite the small surface area of Lake 255 Haixihai (3 km²), nearshore sediments are strongly influenced by terrigenous input.”

Authors' reply:

We thank the reviewer for pointing out this inconsistency.

We have revised this section to clarify that the lignin S/V and C/V ratios primarily characterize the botanical type of lignin-bearing vascular plants rather than uniquely distinguishing terrestrial from aquatic sources. In Lake Haixihai, the non-woody angiosperm signal identified by the lignin parameters may originate not only from terrestrial herbaceous vegetation but also from aquatic macrophytes, because several abundant macrophyte taxa in the lake are themselves non-woody angiosperms. We have therefore removed the statement that the lignin data demonstrate that most “terrestrial organic matter” is derived from non-woody angiosperms.

We have also clarified the interpretation of P_{aq}. This index reflects the relative abundance of mid-chain *n*-alkanes commonly associated with aquatic macrophytes compared with long-chain homologues. The slightly higher P_{aq} values in deep water sediments (0.37 ± 0.04) than in shallow water sediments (0.30 ± 0.07) therefore indicate a greater **relative** aquatic macrophyte signal toward the deeper basin, but do not necessarily imply a greater absolute macrophyte contribution. This distinction is important because the decrease in terrigenous long-chain *n*-alkane input toward deeper waters can itself increase P_{aq}.

In contrast, higher TAR values and greater lignin abundances in shallow water sediments indicate stronger overall higher-plant and terrigenous influence in the nearshore area. We therefore now interpret the lignin and *n*-alkane data together as evidence for mixed inputs from terrigenous vegetation and aquatic macrophytes, with stronger terrigenous influence in shallow water sediments and a relatively greater aquatic macrophyte contribution toward deeper water sites.

The relevant text in Section 4.1 has been revised accordingly (Lines 325-338 in the revised manuscript).

Revised text:

Lignin phenol compositions provide additional information on the vascular-plant component of sedimentary organic matter. The S/V and C/V distributions indicate an important contribution from non-woody angiosperms (Fig. 4b; Tareq et al., 2004). However, this signal cannot be attributed exclusively to terrestrial herbaceous plants because several abundant aquatic macrophytes in Lake Haixihai, including Potamogeton perfoliatus, Vallisneria natans, Myriophyllum spicatum L, Najas minor, and Utricularia, are also non-woody angiosperms and may contribute lignin-derived phenols to the sediments. The P_{aq} values provide a complementary constraint on the contribution of aquatic macrophytes. Values range from 0.16 to 0.41 and are slightly higher in deep water sediments (0.37 ± 0.04) than in shallow water sediments (0.30 ± 0.07) (Table 2). Because P_{aq} expresses the relative abundance of mid-chain n-alkanes commonly associated with aquatic macrophytes compared with long-chain homologues (Mead et al., 2005), the higher values in deep-water sediments indicate a greater relative contribution of aquatic macrophyte-derived n-alkanes toward the deeper basin, rather than necessarily a greater absolute macrophyte input. In contrast, the higher TAR values and lignin abundances in shallow water sediments indicate stronger overall higher-plant and terrigenous influence in the nearshore area. Thus, the combined lignin and n-alkane evidence suggests mixed inputs from terrigenous vegetation and aquatic macrophytes, with stronger terrigenous influence in shallow water sediments and a relatively greater aquatic macrophyte signal toward deeper water sites.

- L 262: Can this indirect evidence be translated to “strong microbial reworking of organic matter”?

Authors' reply:

Thank you. Hopanoids are primarily derived from bacteriohopanepolyols occurring in the cell membranes of bacteria. Biologically synthesized hopanoids predominantly possess the 17 β (H), 21 β (H) configuration, whereas increasing diagenetic and thermal maturity progressively favors the more thermodynamically stable $\alpha\beta$ - and $\beta\alpha$ -hopanes hopane configurations. Consequently, the dominance of $\beta\beta$ -hopane and hopenes in young sediments is generally interpreted as evidence for recent bacterial input and thermally immature organic matter rather than mature petroleum-derived hydrocarbons (e.g., Naraoka et al., 2000; Sun, 2018). The unresolved complex mixture (UCM) also provides information on microbial alteration. During biodegradation, microorganisms preferentially consume readily degradable straight-chain hydrocarbons, whereas more resistant branched and cyclic compounds and microbial transformation products become relatively enriched. Because many of these compounds cannot be individually resolved by conventional gas chromatography, they appear as a broad UCM hump in total ion chromatograms. Experimental and sedimentary studies have shown that bacterial reworking can simultaneously reduce the original n-alkane odd-even predominance and generate a pronounced UCM, whereas prominent UCMs are also

widely observed in biodegraded hydrocarbon mixtures (e.g., Silva et al., 2012; López et al., 2015).

In Lake Haixihai, we found no evidence for substantial petroleum contamination, and we therefore interpret the combination of abundant biologically configured $\beta\beta$ -hopane and hopenes with a pronounced UCM as being consistent with substantial microbial contribution and reworking of sedimentary organic matter. We have accordingly revised the text and supplemented the references (Lines 342-348 in the revised manuscript).

Revised text:

The distributions of hopanoids in the hydrocarbon fractions of surface sediments are dominated by biological configured $\beta\beta$ -hopane and hopenes, with minor contributions from geological configured $\alpha\beta$ - and $\beta\alpha$ -hopanes (Fig. S2). The predominance of biological configurations is consistent with recent bacterial input and the low maturity of sedimentary organic matter (Naraoka et al., 2000; Sun, 2018). In addition, the prominent unresolved complex mixture (UCM) in the TICs, in the absence of evidence for substantial petroleum contamination, is consistent with extensive microbial alteration of sedimentary organic matter (Silva et al., 2012; López et al., 2015). These molecular characteristics collectively support the microbial contribution to the sedimentary organic matter pools and their reworking effects.

References

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- Sun, D., Tang, J., He, Y., Liao, W., and Sun, Y. 2018: Sources, distributions, and burial efficiency of terrigenous organic matter in surface sediments from the Yellow River mouth, northeast China. *Org. Geochem.* 118: 89-102. <https://doi.org/10.1016/j.orggeochem.2017.12.009>.
- Silva, T.R., Lopes, S.R.P., Spörl, G., Knoppers, B.A., and Azevedo, D.A. 2012: Source characterization using molecular distribution and stable carbon isotopic composition of n-alkanes in sediment cores from the tropical Mundaú–Manguaba estuarine–lagoon system, Brazil. *Org. Geochem.* 53: 25-33. <https://doi.org/10.1016/j.orggeochem.2012.05.009>.
- López, L., Lo Mónaco, S., and Volkman, J.K. 2015: Evidence for mixed and biodegraded crude oils in the Socororo field, Eastern Venezuela Basin. *Org. Geochem.* 82: 12-21. <https://doi.org/10.1016/j.orggeochem.2015.02.006>.

- L 373-374: This is a simplification of complex causal relationships. For example, how can you exclude the possibility that freshwater diatoms use ¹³C-depleted CO₂ from other sources (not from reworking)?

Authors' reply:

Thank you for this comment. As discussed in our responses to the preceding comments, we agree that the carbon source utilized by diatoms can potentially be influenced by several processes, including external DIC inputs, atmospheric exchange, and photosynthetic modification of the ambient DIC pool. However, these alternative processes do not readily explain the observed isotopic and molecular relationships in Lake Haixihai. Instead, $\delta^{13}\text{C}_{\text{HBI}}$ closely covaries with bulk $\delta^{13}\text{C}_{\text{org}}$, while its variations are also associated with molecular indicators of microbial reworking and organic matter degradation, including biologically configured hopanoids and lignin-derived P/(V+S). In addition, the covariance between $\delta^{13}\text{C}_{\text{carb}}$, $\delta^{13}\text{C}_{\text{org}}$ and TOC is consistent with a contribution of isotopically depleted DIC generated through organic matter remineralization. Taken together, these relationships support isotopic coupling between organic matter remineralization and the DIC assimilated by HBI-producing diatoms. In addition, following the reorganization of the Results and Discussion, the original text at Lines 373-374 has been removed. The corresponding discussion has been substantially rewritten in the revised Section 4.3 (Lines 439-462).

- Fig. 5 legend: Please provide more details about the indicators. Otherwise, the figure is not self-sufficient.

Authors' reply:

Thank you for this helpful comment. As suggested, we have therefore expanded the figure legend to define the variables shown in each panel and to explain their biogeochemical significance.

The revised legend now reads (Lines 415-422):

Figure 6: Relationships between the carbon isotopic composition of C_{25:2} HBI ($\delta^{13}\text{C}_{\text{HBI}}$) and bulk sedimentary organic carbon ($\delta^{13}\text{C}_{\text{org}}$) (a), the concentration of biologically configured C₃₀ββ-hopane + hopenes (b), and the lignin degradation index P/(V+S) (c). C₃₀ββ-hopane + hopenes represents the summed concentration of C₃₀ββ-hopane, hop-17(21)-ene, hop-13(18)-ene, and diploptene and is used here as an indicator of recent microbial contribution and organic matter reworking. P/(V+S) is calculated as the ratio of *p*-hydroxyl phenols to the sum of vanillyl and syringyl phenols and reflects the extent of lignin demethylation/demethoxylation degradation, with higher values indicating more extensive degradation. Solid lines represent linear regressions, and shaded areas indicate 95% confidence intervals.