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Peer Review File

Coastal ecosystem degradation driven by decades of unregulated terrestrial mining

Corresponding Author: Dr Mathisse Meyneng

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Version 0:

Decision Letter:

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Dear Dr Meyneng,

Please accept our apologies for the delay for sending a decision on your manuscript. Your manuscript titled "Coastal ecosystem degradation driven by decades of unregulated terrestrial mining" has now been seen by 3 reviewers, and we include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. Please highlight all changes in the manuscript text file.

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We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

Please do not hesitate to contact us if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Yang Yang, PhD
Editorial Board Member
Communications Earth & Environment
orcid.org/0000-0002-9513-4911

Alice Drinkwater, PhD
Associate Editor
Communications Earth & Environment
Consulting Editor
Communications Sustainability

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Furthermore, please align your manuscript with our format requirements, which are summarized on the following checklist:
Communications Earth & Environment formatting checklist

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In particular, the Data availability statement should include:
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- Accession codes where appropriate
- If applicable, a statement regarding data available with restrictions
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If a community resource is unavailable, data can be submitted to generalist repositories such as figshare or Dryad Digital Repository. Please provide a unique identifier for the data (for example a DOI or a permanent URL) in the data availability statement, if possible. If the repository does not provide identifiers, we encourage authors to supply the search terms that will return the data. For data that have been obtained from publically available sources, please provide a URL and the specific data product name in the data availability statement. Data with a DOI should be further cited in the methods reference section.

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This study reports a sophisticated multi-proxy analysis documenting recent changes in microeukaryote communities archived in a sediment core spanning the last couple millennia. The study provides compelling evidence for major community, geochemical, and sedimentary regime changes correlative with temporal changes in mining activities. The analytical methods are excellent (although the details could be explained better) and effectively and thoroughly supplemented with high-quality figures and tables. The manuscript is logically structured, and the quality of technical writing is superb. Interpretations are well supported by the data and analyses. This is an important study. The methodology is transferable to other study systems, and the outcomes are intellectually significant and should be of broad interest. I support publication of this study. A few general comments and some minor suggestions are provided below in hope that they can further improve this excellent contribution.

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The paper is well written and detailed with an abundance of supplementary information and data. I enjoyed reading this paper and I make only relatively minor comments that would improve the text which the authors can hopefully address

easily.
Specific section comments below
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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 Dr Meyneng,

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We therefore invite you to revise your paper one last time to 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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Best regards,

Alice Drinkwater, PhD
Associate Editor
Communications Earth & Environment
Consulting Editor
Communications Sustainability

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

I evaluated the previous version of this manuscript as an excellent contribution, but provided some suggestions, especially regarding the clarity of analytical methods, explanation of figures, and the issue of variable sediment accumulation rates that can affect estimates of diversity and turnover rates. The authors diligently addressed all my comments. Th authors also made substantial efforts to address comments of other reviewers, although I will defer to those reviewers as to the adequacy of those revisions. Overall, the revised submission is greatly improved and I have no further comments on the manuscript. I find it highly suitable for publication in Communications Earth and Environment.

Reviewer #3 (Remarks to the Author):

I'm glad to see all the comments have been well addressed by the authors. The manuscript looks good to me for publication.

Version 0

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Yang Yang, PhD

Editorial Board Member
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3. Supplementary Figure 6 is important to show the compositional changes among sediment units and their controlling factors. You may consider moving it to the main text if space allows.
4. The foraminifera analysis with genus level occurrence data is a bit coarse. Is it possible to show the relative abundance changes of different genera to better capture the biological trend? Also, varying abundance and thus sampling effort could severely affect the diversity patterns observed. Sampling standardization is recommended. Or at least you should plot the foram abundance (i.e., the number of individuals you picked and identified) changes along the core and discuss this.

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Version 1

Decision letter:

Dear Dr Meyneng,

Your manuscript titled "Coastal ecosystem degradation driven by decades of unregulated terrestrial mining" 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 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".

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

Best regards,

Alice Drinkwater, PhD
Associate Editor
Communications Earth & Environment
Consulting Editor
Communications Sustainability

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

I evaluated the previous version of this manuscript as an excellent contribution, but provided some suggestions, especially regarding the clarity of analytical methods, explanation of figures, and the issue of variable sediment accumulation rates that can affect estimates of diversity and turnover rates. The authors diligently addressed all my comments. Th authors also made substantial efforts to address comments of other reviewers, although I will defer to those reviewers as to the adequacy of those revisions. Overall, the revised submission is greatly improved and I have no further comments on the manuscript. I find it highly suitable for publication in Communications Earth and Environment.

Reviewer #3 (Remarks to the Author):

I'm glad to see all the comments have been well addressed by the authors. The manuscript looks good to me for publication.

Point-by-point response

On behalf of all co-authors, we sincerely thank the three reviewers for their careful reading of our manuscript and for providing constructive comments and suggestions. We believe that their feedback has significantly helped us improve the quality and clarity of our work.

Each comment has been addressed in detail; our responses are highlighted in blue, and page and line numbers refer to the revised version of the manuscript (without track changes).

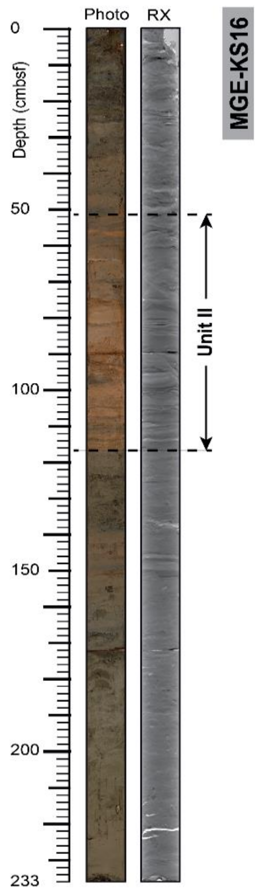
Reviewer #1:

This study reports a sophisticated multi-proxy analysis documenting recent changes in microeukaryote communities archived in a sediment core spanning the last couple millennia. The study provides compelling evidence for major community, geochemical, and sedimentary regime changes correlative with temporal changes in mining activities. The analytical methods are excellent (although the details could be explained better) and effectively and thoroughly supplemented with high-quality figures and tables. The manuscript is logically structured, and the quality of technical writing is superb. Interpretations are well supported by the data and analyses. This is an important study. The methodology is transferable to other study systems, and the outcomes are intellectually significant and should be of broad interest. I support publication of this study. A few general comments and some minor suggestions are provided below in hope that they can further improve this excellent contribution.

1. Scale and trends in sediment mixing – It is not clear from the text if the core is affected by vertical sediment mixing due to bioturbation (although this can be indirectly guesstimated from 210Pb profiles).

The core MGE-KS17 has been sliced into one-centimetre-wide layers for the analyses presented in this study. A twin sediment core MGE-KS16 has also been recovered during the MARGEST cruise at a distance of less than 20 m from the MGE-KS17. X-ray images were obtained from split sections using a Geotek-XCT system at the IFREMER laboratories. To avoid confusion between datasets, we did not include MGE-KS16 analyses into the main reconstruction, but we used its X-ray and photographic observations to assess sedimentary structures and potential mixing.

Visual assessment of X-ray images and optical photographs was used to qualitatively evaluate mixing extent and intensity. We did not observe typical mixing from bioturbation, except for sparse traces near the base of the core (around 180 and 220 cmbsf), which indicate the presence of potential sparsely distributed burrows. Gani et al. (2007) indicate that sporadically distributed traces of bioturbation are characteristic of narrow colonisation windows and persistent physico-chemical stresses under high sedimentation rates of several centimetres per year. Although bioturbation can be frequent in the fine-grained deltaic deposits, it has not been observed in the red-coloured Unit II deposits (lateral equivalent of Unit II in twin core between 50 and 120 cm). As proposed by Bhattacharya et al. (2020), the high sedimentation rates measured in the



Photography and X-Ray picture of the twin core (MGE-KS16) core associated with fluid-mud substrates that are difficult to colonise probably preclude extensive biogenic reworking.

The main sediment features highlighted by X-ray are parallel to low-angle laminations, in particular in the Unit II fine-grained sediments. These laminations are characteristic of high-frequency river flood and storm deposits, leaving reduced colonisation windows. As flood and storm domination increases, shallow marine deposits typically show a decrease in bioturbation and an increase in preservation of physical sedimentary structures (Bhattacharya et al., 2020).

Finally, very few deformations due to the coring operation could also be observed but they are limited to the contact with the PVC liner of the MGE-KS16. These deformations were expected and this is why we subsampled sediment in the inner part of the core (L434 and L545).

References:

Gani, M.R., Bhattacharya, J.P., MacEachern, J.A. Using ichnology to determine relative influence of waves, storms, tides, and rivers in deltaic deposits: Examples from cretaceous Western Interior Seaway, U.S.A. In: MacEachern, J.A., Bann, K.L., Gingras, M.K., Pemberton, S.G. (Eds.), Applied Ichnology. SEPM Short Course Notes, vol. 52. pp. 209–225 (2007).

Bhattacharya, Janok & Howell, Charles & MacEachern, J. & Walsh, J.P. Bioturbation, sedimentation rates, and preservation of flood events in deltas. Palaeogeography, Palaeoclimatology, Palaeoecology. 560. 110049 (2020). 10.1016/j.palaeo.2020.110049.

It is also emphasized repeatedly in the text and evident in figures that sedimentation rates accelerated and became more continuous toward the present. Trends in vertical mixing, sediment accumulation rates, and sediment sources not only can influence the communities but also result in trends in temporal resolution (age mixing or time averaging). This is particularly relevant here because species diversity can be inflated by increased time averaging and turnover rates through time can be suppressed by time averaging (see Olszewski and Kidwell, Paleobiology; Tomasovych and Kidwell, Paleobiology, and especially Tomasovych et al. 2024, Ecology Letters). The decrease in diversity and turnover rates can be thus potentially due to the upward decrease in time averaging driven by increase in sediment accumulation rates.

We thank the reviewer for this important comment. We fully agree that changes in sediment accumulation rates can influence both apparent diversity and turnover through time-averaging effects, and we appreciate the suggestion to address this more explicitly.

Following this comment, we have revised Figure 2 and now present alpha diversity corrected for sedimentation rate, following the recent work of Tomasovych et al. (2024, Ecology Letters). Because diversity in sedimentary records can increase mechanically with the duration over which assemblages accumulate, we modelled the relationship between diversity and sedimentation rate and retained the residuals of this model as a measure of sedimentation-corrected diversity. Prior to modelling, both diversity and sedimentation rate were log-transformed to account for their non-linear relationship and to reduce the influence of extreme values. This correction allows us to remove the inflation of diversity caused by time averaging and to focus on temporal changes not explained by sediment accumulation. Importantly, the sedimentation-corrected diversity still reveals a marked deficit in ASV richness during the most impacted interval, indicating a genuine decline in microeukaryote diversity that cannot be explained by time-averaging effects. In contrast, foraminifera richness does not show a clear directional decrease once corrected, but rather exhibits pronounced instability and increased variability, suggesting strong ecological perturbations affecting community structure. Taken together, these results indicate that both biological proxies record real biodiversity changes, and that the observed patterns are not artefacts driven by changes in sedimentation rate or temporal resolution.

Regarding turnover (or replacement), we note that our estimates are not based on the whole temporal dynamic but on a moving-window approach, which inherently smooths temporal variability and reduces the sensitivity of turnover estimates to local changes in sedimentation rate.

We agree that this methodological point was not sufficiently clear in the original manuscript. We have therefore clarified this in both the Methods section and the figure caption to explicitly state how turnover was calculated and why it is less sensitive to variable temporal resolution.

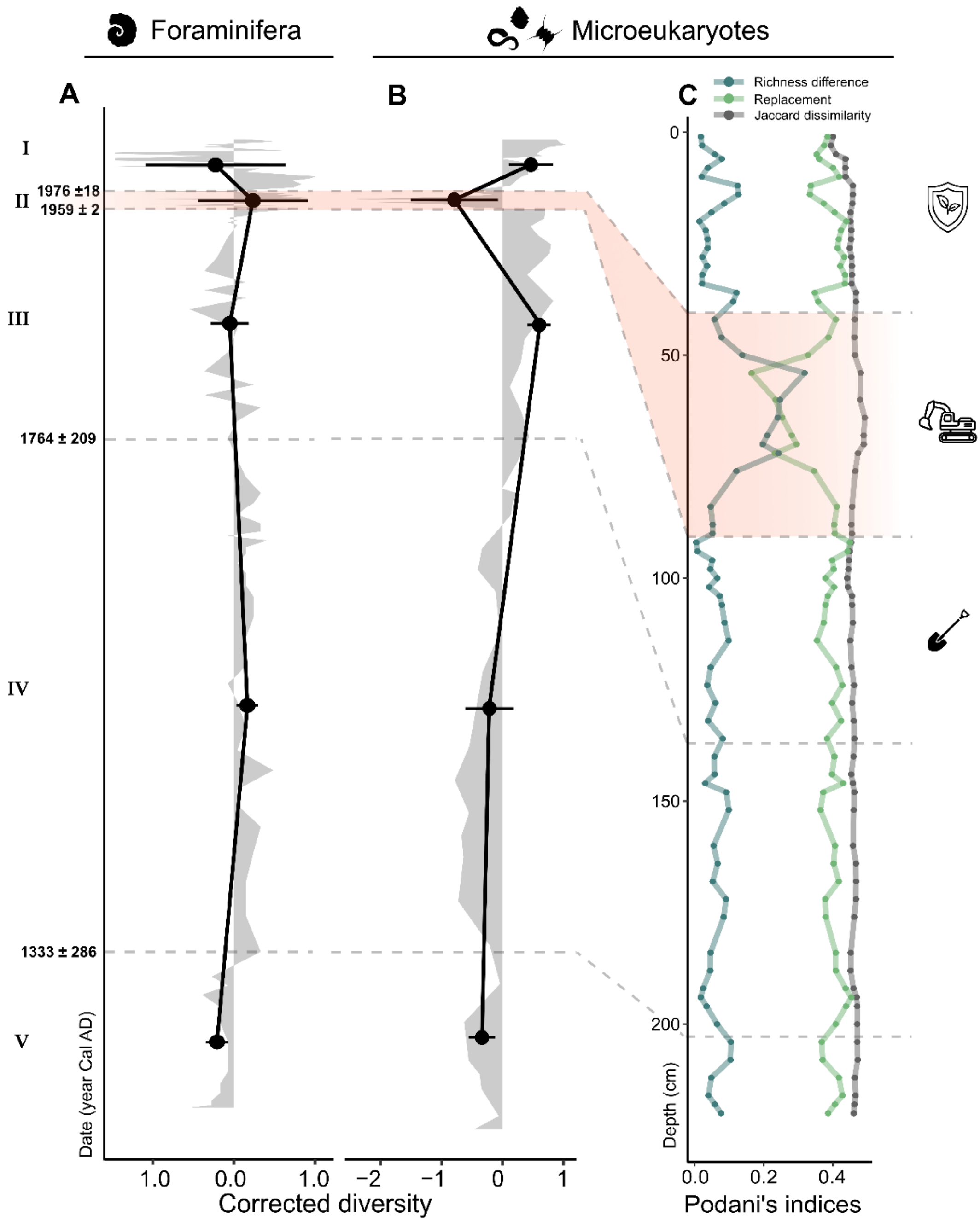
These additions strengthen the robustness of our interpretations by explicitly accounting for time-averaging effects and clarifying the methodological framework used to quantify biodiversity changes.

We thus modified the main Figure 2A-B (see below), the associated text in the results, discussion and method sections:

Page 7 line 140: «*To account for variations in sediment accumulation through time, diversity patterns were corrected for sedimentation rate, which was negatively correlated with foraminiferal diversity ($R^2 = 0.32$, $p < 0.001$) and positively correlated with microeukaryotic ASV richness ($R^2 = 0.29$, $p < 0.001$). After accounting for this effect, clear temporal patterns emerged among sedimentary units. The strongest signal of mining impact occurred in Unit II, corresponding to the period of most intense disturbance. During this period, foraminiferal richness showed marked instability, with relatively high mean values but large variability among samples (mean residual = 0.23 ± 0.68; Figure 2A). In contrast, microeukaryotic communities exhibited a pronounced decline in corrected ASV richness (−0.79 ± 0.72, Figure 2B), indicating a substantial loss of diversity during this period.* »

Page 7 line 154: «*In the most recent sediments of Unit I, foraminiferal richness remained highly variable and showed an overall decrease in mean corrected diversity (−0.23 ± 0.87). Conversely, microeukaryotic richness increased markedly during this period (0.46 ± 0.37).*»

Page 27 line 646: «*The richness of the community was described using the number of ASVs and foraminiferal genera in each sample. Because richness measured in sedimentary archives can be artificially inflated by time-averaging effects when sedimentation rates are low, observed alpha diversity was corrected by sediment accumulation rate. The relationship between diversity and sedimentation rate was modelled using log-transformed values of both variables to account for their non-linear relationship and to reduce the influence of extreme values. As fossil diversity is expected to increase mechanically with the duration over which assemblages accumulate, independently of ecological processes, the residuals of this model were retained as a measure of sedimentation-corrected diversity. This approach removes the component of diversity explained solely by sediment accumulation and allows us to focus on temporal ecological changes not driven by variations in sedimentation rate (ref 94: Tomašových et al., 2024). Several Podani indices were used to describe more precisely the variations of ASV richness along the core, including richness difference and ASV replacement 95,96. These indices, together with Jaccard dissimilarity, were computed using a moving-window approach rather than fixed temporal bins. For each sample, Podani indices and Jaccard dissimilarity were calculated by comparing that sample to the two immediately preceding samples in the core (e.g. the sample at 10 cm was compared to those at 12 cm and 14 cm). This approach ensures comparisons among samples that are close in depth and therefore more comparable in terms of DNA preservation and sedimentation rate, thereby limiting artificial richness differences driven by DNA degradation. Using a moving-window framework also smooths short-term variability and reduces sensitivity to uneven temporal resolution caused by variations in sedimentation rate and time* *averaging. As a result, the estimated richness difference, replacement and dissimilarity metrics are less affected by vertical mixing and uneven sample spacing than estimates based on discrete and irregular temporal bins.»*



<u>*Revised Figure 2 with diversity corrected by the sedimentation rate*</u>

Caption: *« Temporal variations of the richness of (A) foraminiferal genera and (B) microeukaryotic ASVs, representing within-sample diversity. Richness values were corrected for sedimentation rate to account for mechanical dilution effects and better isolate ecological change. Grey shading indicates observed richness for each sample, while black dots represent the mean richness within each sedimentary unit. Error bars show the standard deviation of the corrected mean richness per unit. Different diversity components are described using (C) Podani's indices, illustrating changes in* *community structure in correspondence with sedimentary units and their estimated dating. Three components are shown: richness difference (blue; net change in ASV number), replacement (green; ASV turnover), and Jaccard dissimilarity (grey; overall compositional change). Each sample is compared with the two preceding samples to highlight short-term temporal shifts and periods of elevated compositional variability. Mining history is indicated by icons (see Figure 1 for the legend). »*

The issue of physical mixing is acknowledged for one unit of the core (Line 230), but the overall role of the upward trend in accumulation rates and the associated decrease in time averaging is not satisfactorily explored. Age mixing can be substantial in foraminiferal assemblages, as documented in previous studies (e.g., Lougheed et al. 2018 Climates of the Past – see also references cited there and other subsequent papers citing this work).

The physical mixing attested in the text supports the idea that the majority of the sediments deposited in the unit II have been transported by high hydrodynamic river conditions and therefore accumulated at sea as a product of mixed continental sediments (reference: Bertrand and Liébault, 2019). The physical mixing proposed in the text (initially L230) is not related to any marine processes of mixing (bioturbation, wave reworking, …).

The text has been clarified in this sense, page 11 line 254: "*These massive terrigenous inputs transported by strong river flood have led to a large accumulation of continental reworked sediment* [22] *which explains the difficulties in dating this section of the core.*"

The interpolated age-model and its uncertainties were obtained using Bayesian statistical modelling (Blaauw and Christen, 2011) of combined 210Pb and radiocarbon dates obtained from the core. All these chronological constraints have been set out of the unit II, too diluted in 210Pbxs activities and too poor in micro-shells content to obtain enough material for reliable direct dating (see method). The calculated time averaging of this part of the core (between 40 and 93 cmbsf) should thus not be affected by direct radiocarbon bias linked to possible sediment mixing.

Even if the visual description of the twin core MGE-KS16 (see above) does not show clear bioturbated mixed layers, we are fully aware that discrete bioturbation could possibly affect the age model and the uncertainties. Sedimentary bioturbated mixed layer can provide important offsets in the measured radiocarbon age between different species and between the fragments and whole shells of individual species (Ausín et al., 2019). Out of the unit II, only one level (out of every nine dated) has been measured by radiocarbon using multi-specimen assemblages of benthic foraminifera, which minimises the impact of such a possible averaged radiocarbon value and measurement error at this level on the complete statistically modelled age-model, and depending on the unknown heterogeneity of the sediment sample.

Age offsets are generally larger when sediment accumulation rates are low (Lougheed et al., 2018; Barker et al., 2007). Continuous deposition and a rapid sedimentation reduce the effects of bioturbation (Peng and Broecker, 1984; Manighetti et al., 1995). And the main depositional event recorded by unit II presents the highest sedimentation rate (1.0 cm yr-1 on average) of the core. The sediment accumulation rates even rose fivefold (from 0.2 to 1.0 cm yr-1) in few centimetres, from unit III to unit II, due to a drastic increase in ultramafic material. An intense diffused bioturbation should have levelled the sharp changes in sedimentation. Strong diffuse bioturbation would be expected to smooth such sharp transitions and damp high-frequency variability (Dolman et al., 2021), but these abrupt shifts are preserved across multiple independent proxies.

References:

Blaauw, M. & Christen, J. A. Flexible paleoclimate age-depth models using an autoregressive gamma process. Bayesian Analysis 6, 457–474 (2011).

Ausin, B., Magill, C., Haghipour, N., Fernandez, B., Alvaro, W., Hodell, D., Baumann, K. H. & Eglinton, T. (In)coherent multiproxy signals in marine sediments: Implications for high-resolution paleoclimate reconstruction. Earth and Planetary Sciences Letters. 515. 38-46 (2019). 10.1016/j.epsl.2019.03.003.

Lougheed, B. C., Metcalfe, B., Ninnemann, U. S., & Wacker, L.. Moving beyond the age-depth model paradigm in deep sea palaeoclimate archives: Dual radiocarbon and stable isotope analysis on single foraminifera. Climate of the Past, 14, 1–16 (2018). https://doi.org/10.5194/cp-2017-119

Barker, S., Broecker, W., Clark, E. & Hajdas, I. Radiocarbon age offsets of foraminifera resulting from differential dissolution and fragmentation within the sedimentary bioturbated zone, Paleoceanography, 22, PA2205 (2007). doi:10.1029/2006PA001354.

Peng, T. H. & Broecker W. S. The impacts of bioturbation on the age difference between benthic and planktonic foraminifera in deep-sea sediments, Nucl. Instrum. Methods Phys. Res., 233, 346–352 (1984).

Manighetti, B., McCave, I. N., Maslin, M. & Shackleton N. J. Chronology for climate change: Developing age models for the Biogeochemical Ocean Flux Study cores, Paleoceanography, 10, 513– 525 (1995).

Dolman, A. M., Groeneveld, J., Mollenhauer, G., Ho, S. L., & Laepple, T. Estimating Bioturbation From Replicated Small-Sample Radiocarbon Ages, Paleoceanography and Paleoclimatology, 36, e2020PA004142 (2021). https://doi.org/10.1029/2020PA004142, 2021b.

2. Analytical methods – It was difficult to fully discern analytical methods. For example, rarefaction was mentioned for ASV (but not foraminifera counts?). Similarly, multiple Wilcoxon tests were applied but it is not clear if any correction (e.g., Bonferroni correction) was applied to correct the resulting significance values for multiple independent tests. Whereas PCO and constrained analyses are explained in some detail, it is unclear if the data were standardized and/or transformed (relative abundance is mentioned) and how those standardizations may have differed between forams and ASV. It would improve reproducibility of this study if all those issues were spelled out clearly. Admittedly, I may have missed or misunderstood the methodological details, but this alone suggests that they need to be clarified and presented in a more accessible style.

We thank the reviewer for this important comment and agree that the analytical workflow needed to be clarified and presented more explicitly to improve reproducibility.

A key point is that our study relies on two different types of biodiversity data**,** which required distinct preprocessing and analytical approaches. Microeukaryote data are based on ASV read counts (i.e. molecular sequence abundance), whereas foraminifera data consist of presence/absence at the genus level.

For the ASV dataset, we worked with both rarefied and non-rarefied count tables, depending on the objective of each analysis. Alpha diversity and Jaccard dissimilarity were calculated on the non-rarefied dataset, using approaches that account for mechanical bias (heterogeneous sedimentation rate and DNA degradation, through diversity correction and moving window comparisons). In contrast, all analyses based on read abundances were performed on rarefied data and expressed as relative abundances to ensure comparability among samples and to reduce sequencing-depth bias. We acknowledge that, as a methodological choice, we also provide the equivalent Figure 2 based on the rarefied dataset in the supplementary material to show that the qualitative pattern is similar **(Supplementary Figure 13)**.

For the foraminifera dataset, the data are inherently low-dimensional (genus-level presence/absence), which does not allow meaningful rarefaction or abundance-based transformation. Therefore, we analysed beta-diversity using Jaccard dissimilarity, which is appropriate for presence–absence data.

Additionally, to ensure comparability between the two biological datasets, we made several methodological choices. First, we did not attempt species-level resolution for the DNA dataset. Instead, analyses were conducted at a coarser taxonomic level comparable to the genus-level resolution used for foraminifera, and because metabarcoding with universal markers does not reliably support confident species-level assignments (e.g., Strube 2021). Second, for beta-diversity analyses, we applied the same dissimilarity metric to both proxies. Because the foraminifera dataset is based on presence–absence data, we used Jaccard dissimilarity for both datasets. This choice is also appropriate for ancient DNA metabarcoding data, as relative read abundances are known to be only semi-quantitative and do not directly reflect the original living community composition.

We clarified these choices in the Methods section and in the figure caption to avoid any misunderstanding.

Page 26 line 633: « *Because our study relies on two distinct types of biodiversity data, we applied different but harmonised preprocessing strategies. For the ASV dataset, diversity metrics based on richness and presence–absence (e.g. alpha diversity and Jaccard dissimilarity) were computed on the non-rarefied count table, using approaches designed to account for methodological biases (e.g. sedimentation rate correction). This choice was made to retain the oldest samples, which yielded fewer than 40,000 reads and would otherwise have been excluded by rarefaction. The equivalent figure based on the rarefied dataset is provided in the Supplementary Material and shows qualitatively consistent results* ***(Supplementary Figure 13)****. In contrast, all analyses relying on read abundances were performed on the rarefied dataset and expressed as relative abundances to ensure comparability among samples and minimise sequencing-depth bias. For the foraminifera dataset, the data are inherently low-dimensional and already limited in information content. Therefore, they were analysed as genus-level presence–absence data without further transformation.* »

Page 28 line 682: « *the genus level was chosen for homogenisation with the foraminifera dataset and because amplicon sequencing with universal markers does not reliably support confident species-level assignments (e.g., 98)*»

Page 29 line 690: « *Resulting p-values were adjusted for multiple testing using the Bonferroni correction.* »

Finally, to ensure full reproducibility, the analysis script was already openly available and has now been updated to include Bonferroni corrections, diversity metrics corrected for sedimentation rate, and a restructured file organisation to provide a clearer and more accessible workflow (see https://github.com/Matmey/Meyneng_NC).

References :

98: Strube, M.L., 2021. RibDif: can individual species be differentiated by 16S sequencing? Bioinformatics Advances 1, vbab020. https://doi.org/10.1093/bioadv/vbab020

3. Figure captions – This relates to the comment above. Captions (especially in supplementary figures)

are terse and do not explain figures in sufficient details. I suggest expanding captions considerably (even if repetitious with text): each figure should be fully understandable based on its caption. Explain all lines, symbols, and color-coding, especially if multiple color schemes are used on the same figure. For example, bars on Figure 2 are not explained in the caption and I could not find an explanation in the text.

We thank the reviewer for this comment. We agree that some figure captions lacked sufficient detail and that certain symbols were not fully explained. We have revised the figure legends to provide additional information for the main Figures 1, 2, 3, 4 (previously Fig. S6), 5, as well as Supplementary Figures S1, S2, S3, S4, S5, S6, S9, and S12, and Supplementary Tables S3 and S4.

Minor comments

Lines 41-42 “selective temporal dynamics” – It is not clear what the modifier ‘selective’ denotes. “distinct”?

We agree and made the modification.

Line 49 “a proposed geological epoch” – “an informal geological time interval (sensu e.g., Gibbard et al. 2022; J. Quat. Sci.)” may work better here. Anthropocene was indeed proposed as a formal “epoch” but was not approved as such.

The ongoing debate regarding the formal recognition of the “Anthropocene” as a geological epoch was indeed not mentioned in the previous version. We have revised the sentence accordingly to better reflect this nuance and have incorporated the reviewer’s suggested reference, which explicitly discusses the basis for this debate.

Paxe 3 line 48: « *Environmental changes have contributed to what many scientists now refer to as the Anthropocene, an informal geological time interval defined by significant human impact on Earth’s systems (ref 1,2)* »

Line 55: some citations would be useful here (e.g., Barnosky et al. 2017, Science; Dillon et al., 2020; Methods in Ecology and Evolution; Tomašových et al., 2020, Proceedings of the Royal Society B; Scarponi et al. 2022, Global Change Biology; Nawrot et al. 2024, Biogeosciences)

We agree that paleoecological studies not based on ancient DNA were underrepresented in the introduction, despite forming the historical foundation of the field. We have therefore expanded the reference list with the suggested papers to better reflect this broader paleoecological context.

Line 98-99: It would be useful to explain that this is referring to a matrix of pairwise Spearman correlation coefficients.

We modified this part of the main text as required to address the following comment from reviewer 3 (see below): we provided a clearer explanation of this choice, referred to the correlation matrix, and added a supplementary figure.

Line 315 (and elsewhere). I suggest avoiding compound nouns when possible. For example, “harmful species emergence” could be replaced with “the emergence of harmful species”.

We modified the main text in that sense (L121, L141, L255, L282, L430).

Line 451-452: “averaging 50–70 individuals per sample, although certain samples presented a complete absence of foraminifera”. – This statement implies that foraminifera samples varied in sample size. This means that raw species richness is not comparable across samples. Please clarify how this was addressed.

The exact same quantity of sediment was washed and sieved per considered sample, and all the foraminifers present within that exact same quantity of sediment were picked using a paintbrush.

This previous statement does not imply that foraminifera samples varied in sample size, but that within the different samples, most of them presented a total average of foraminifers of about 50 to 70 specimens, even though some samples presented a complete absence of foraminifera. To accommodate this comment, this statement was rephrased within the manuscript.

Page 22 line 531: « *126 samples containing the exact same amount of sediment* »

Figure 2 – explain what do the black bars (confidence intervals?) denote in the figure caption.

We modified the legend of Figure 2, adding the missing information (see the legend caption above).

Supplementary figures – provide detailed informative captions

As mentioned above, we detailed the captions of several main and supplementary figures.

Reviewer #2:

Summary:
The paper “Coastal ecosystem degradation driven by decades of unregulated terrestrial mining” presents a study on using sediment core biogeochemical parameters to reconstruct a historic change in the New Caledonia coastal zone. The sample and statistical analysis appear to be appropriate and valid. I believe this paper is of interest to the community and the wider field.
The paper is well written and detailed with an abundance of supplementary information and data. I enjoyed reading this paper and I make only relatively minor comments that would improve the text which the authors can hopefully address easily.
Specific section comments below

Intro:
Would be good to get a bit more detail on what changes are expected as a result of the mining (contaminants, sedimentation, organic matter) are expected in the core and more information on the timeline of mining activity and intensity (e.g., you mention a period of intense mechanised mining corresponding to Unit II. That way the reader can compare and confirm with what is presented in the results immediately following without referring to details in the methods.

We formulated more explicit hypotheses on the expected changes, based on previous studies and historical records of mining activities in Thio, addressing both the direct impacts of mining and the partial ecological recovery anticipated following the implementation of environmental regulations.

Page 4 line 88: « *We first hypothesised that intensified erosion associated with mining activities increased terrestrial inputs (including metal-rich ultramafic particles) to adjacent marine environments, thereby altering the structure of coastal communities. Second, we expected that the environmental* *regulations implemented after 1975 promoted a partial ecological recovery of Thio's coastal ecosystem*.»

Results:
Line 182: Needs more context around affiliation rate here.

The transition between paragraphs was indeed not clear; we thus modified the main text.

Page 9 line 207: « *To enhance the affiliation rate of these unassigned fungi and determine if it can be associated with terrestrial inputs, long-read amplicon sequencing was performed.* »

Line 206: "Thio coastal ecosystem"

Done

Discussion:
Line 230: You don't mention any potential for bioturbation by the infaunal community (is there a current bioturbating community?) and resultant mixing of contaminants across boundaries, although you mention strong hydrodynamic mixing has occurred.

See discussion above about bioturbation.

Methods:
Line 354: "the Thio regions mining history"

We modified the main text.

Page 17 line 406: « *A critical turning point in the mining history of the Thio regions occurred in 1975* […] »

Line 358: What is ORSTOM?

It is a French institute, we specified in the main text.

Page 16 line 388 : « *In response, SLN, supported by ORSTOM (Office of Overseas Scientific and Technical Research, now IRD, Research Institute for Development) scientists,* […] »

Line 370: Is MARGEST an acronym?

It is a project acronym meaning « Sedimentology, stratigraphy, and neotectonics of the eastern margin of New Caledonia ». It has been added to the main text L410.

Line 372: PVC?

PolyVinyl Chloride, we specified the acronym meaning L426.

Line373: Any immediate preservation for transport?

We clarified both points in the Methods section.

Page 19 line 438: «*The samples were placed in plastic 50 mL deoxygenated cryotubes and immediately frozen in liquid nitrogen for transport before being stored at -80 °C for sedaDNA extraction.* »

Line 393: Why was CIC method selected?

The ages based on 210Pb were calculated using a combination of the constant flux (CF), constant sediment (CS) and constant initial concentration (CIC) models. In the upper 40 cm section of the core (Unit 1), good agreement was found between the calculated ages for the seven measured sediment layers using the two models.

In Unit 2, however, 210Pb activity was negligible (probably due to dilution by high levels of mining-derived inputs), so these models could not be applied. Consequently, there is no 210Pb-derived age estimate for Unit 2.

In Unit 3, the few measured levels indicated that 210Pb activity was low just below Unit 2 and negligible at depths greater than 120 cm. Due to the absence of ages in Unit 2, the CF:CS model could only provide an estimate of a rough sedimentation rate in the upper section of Unit 3, not age. Therefore, the only way to calculate an age was to apply the CIC model to the 210Pb data in Unit 3.

An example of this combined use of the CF:CS and CIC models is illustrated in http://dx.doi.org/10.1016/j.margeo.2016.09.008.

Line 409: Can you expand on F14C?

Post-bomb dates are often reported as F14C or fraction modern carbon. This term has been introduced by Reimer *et al*. (2004) to report 14 C measurements of post-bomb samples.

Any radiocarbon-dated sample, when measured, provides an activity ratio or fraction modern carbon (F14C). The ratio corresponds to the measured radiocarbon activity in the sample, relative to the radiocarbon activity that is conventionally estimated at the year 0 BP (Stuiver & Polach, 1977).

F14C has been more detailed in the text:

Page 20 Line 463: "*The objective was to estimate the ages of sedimentary units, specifically unit II, where sediments produced a very diluted ^{210}Pbxs activity and accumulated too few micro-shells to obtain enough material to measure a robust radiocarbon activity in the sample and a reliable fraction modern carbon ($F^{14}C$) estimates because of strong terrigenous dilution*."

References:

Reimer, P. J., Brown, T. A. & Reimer, R. Discussion: Reporting and calibration of post-bomb14C data. Radiocarbon 46: 1299–1304 (2004).

Stuiver, M., & Polach, H. A. Reporting of 14C data. Radiocarbon, 19(3), 355–363 (1977). https://doi.org/10.1017/s0033822200003672

Lines 416-419: Grain size doesn't appear to be discussed within the results.

Grain size was not explicitly discussed in the Results. However, the sediment source modelling incorporates both metallic composition and grain size, so it is implicitly included in these results. We have clarified this in the legend of Figure 1 and added a description of the main changes in grain size in the Results section, particularly for Units II and III.

Page 5 Line 118: « *Unit III (136 to 90 cm depth, ending 1956 ± 2 yr Cal AD) marked the onset of mining impacts, with Ni concentration exceeding 2,243 mg kg^{-1} and an increase in Clay proportion associated with the first notable increase in ultramafic sediment proportion (+15% compared to Unit IV).* »

Page 6 line 123: « […] *finer sediment (39 ± 5.3 % of clay and 3.6 ± 1.9 % of sand),* […] »

Page 8 line 174: « *Among measured parameters, metal composition accounted for the largest share of explained variance (12.7%, 12.1%, 13.6%), followed by sediment source (5.5%, 5.6%, 9.5%), organic matter (5.4%, 4.6%, 7.6%), and grain size (5.4%, 3.5%, 9.8%).* »

Lines 430-435: Again doesn't' appear to be discussed in results section.

We briefly mentioned this finer sediment in Unit II associated with an ultramafic source (L253), as supported by previous work. But we have now included other mentions of these changes, especially that it's an important parameter shaping microbial communities.

Page 12 line 272: « *Both metallic composition and grain size are known to influence benthic communities 23–26, and changes in these parameters are therefore expected to affect community composition.* »

Page 12 line 276: « *The contribution of other factors differed between microeukaryotes and foraminifera, with, for example, grain size having the highest influence on foraminiferal communities, highlighting differences in responses among taxa.* »

Line 482: "one sample over two". I'm not understanding what this means as it seems to contradict the previous depth sampling description to me. Please clarify.

We agree and clarified the method :

Page 18 line 431: «*To optimise material usage, alternating layers were assigned to different analyses: one layer was used for foraminiferal examination, and the adjacent layer was used for sedaDNA analysis and environmental parameters.* »

Line 510: Were the blanks acceptable?

The blanks were indeed very acceptable. We included a high number of negative controls specifically to maximise contamination detection. Across 23 blanks, 85 ASVs (213,299 reads) were detected, and all were removed from the dataset before downstream analyses (see supplementary materials). We now state this more explicitly in the Methods.

Page 25 line 595: «*Across 23 negative controls (extraction and amplification blanks), 85 ASVs (213,299 reads in total, Appendix 1) were detected, and all of these ASVs were conservatively treated as contaminants and removed from the dataset prior to downstream analyses.*»

Figures:
I think it would be best to standardise whether you are plotting parameters vs depth (Figure 1) or time

(e.g., Figure 2). Time would be clearer and easier to compare between the over-core figures.

Indeed, all figures show changes with depth, except for Figure 2. This was a deliberate choice to better emphasise the abrupt impact and the large variability observed over a few decades, in contrast to the broader variability at the secular scale. When depth is used, the timescale of the impact is lost, and the changes appear more "continuous," which does not accurately reflect the true pace of change. Therefore, for most figures, the depth axis was retained mainly for visualisation purposes.

Here, we present the same data as in Figure 2A–B (see below; revised with diversity-corrected sedimentation rates), displayed on time and depth scales, respectively. The main message of this figure is the pronounced variability in richness through time, and the use of a time scale rather than a depth scale conveys this message more effectively. For this reason, we would prefer to retain the time-based representation.

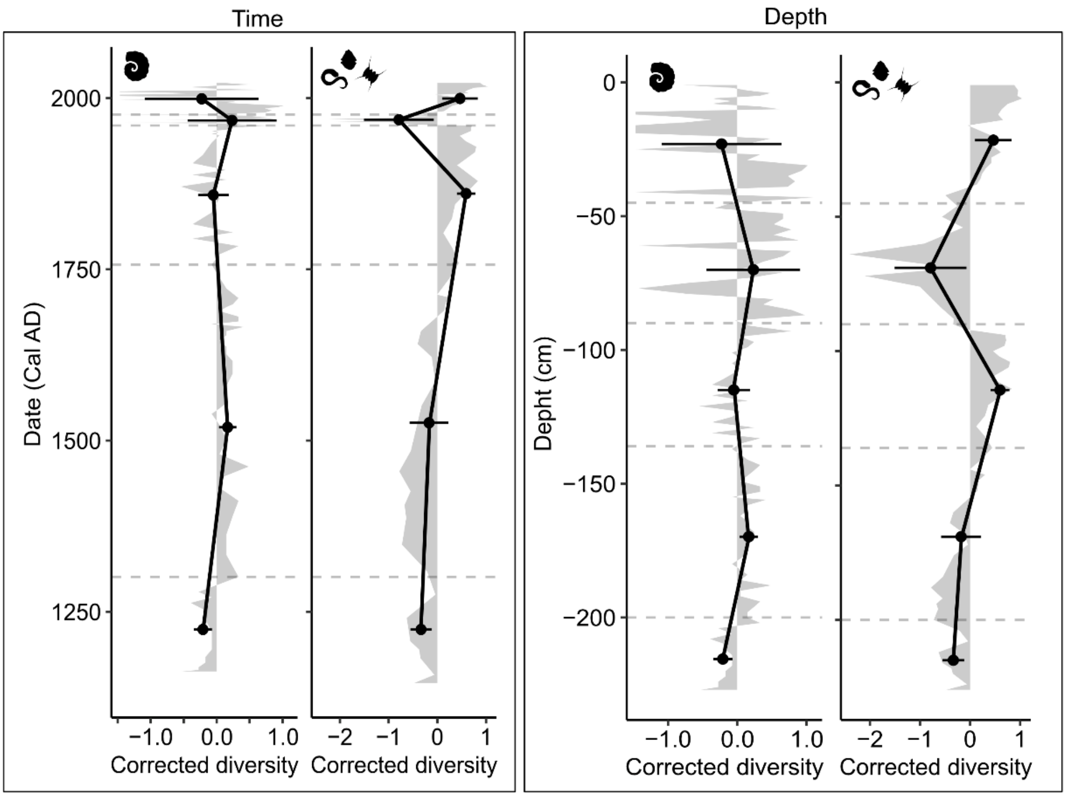


Figure to show the difference in visualisation between a time scale (left) and a depth scale (right).

Reviewer #3:

The manuscript is well written in general. It investigates the mining impacts on coastal marine ecosystems across hundreds of years and provides new knowledge about the loss and recovery of biodiversity in marine microorganisms. It is advantageous to adopt a multi-proxy approach with the integration of microfossil and ancient DNA data. The methodologies look appropriate for examining the biodiversity patterns and accomplishing the aims of the study.

However, the part about foraminifera analysis should be improved. I understand the use of genus level data perhaps to match the genus level identification of microeukaryotes by ASVs, but this should be discussed with a caveat paragraph because all information at species level is missing. The loss of species richness may be much more severe and indicate more explicitly the environmental impacts on marine benthic communities.

As noted by Reviewer 1, our treatment of the biological data (both microeukaryotes and foraminifera) was not sufficiently explained. We have clarified this in the revised manuscript. Specifically, we retained genus-level resolution for the genomic data to ensure consistency with the foraminiferal dataset and because metabarcoding did not allow robust species-level assignment. We have now explicitly discussed this limitation in the Methods section and added a caveat acknowledging that species-level assignment could provide additional ecological insight.

Page 28 line 679: « *We acknowledge that robust species-level assignment could enhance our understanding of the specific effects of mining, providing additional ecological information such as lifestyle, trophic strategy, or physiological tolerance, which may differ among species within the same genus.* »

Finally, we emphasise that biodiversity loss was observed at multiple taxonomic levels: genus-level for foraminifera (Figs. 2B and S13), selected microeukaryotic genera (Figs. 4 and 5, Table S2), and ASV-level for microeukaryotes (Fig. 2A). This demonstrates that both coarse- and fine-scale diversity metrics reveal the impact of mining on benthic communities, despite our lack of species-level assignations.

In addition, I think the current Discussion section does not present enough detailed information about the patterns and causes of biodiversity loss and ecological degradation. For example, in Unit I, why foraminifera diversity declined while microeukaryote diversity increased? Please try to add some explanation and discussion about this.

We have expanded the discussion of contrasting biodiversity patterns observed in Unit I. While some of this was previously included in the supplementary discussion, we have now moved a detailed explanation to the main text as it is indeed a contrasting result. The apparent increase in microeukaryote ASV richness in the upper layers of the core is partly due to methodological factors: relatively undegraded DNA in the first centimetre and the presence of living organisms both contribute to higher ASV counts. This pattern has been noted in previous studies (Holman et al., 2025) and limits the ability to distinguish true ecological change from this bias. We now explicitly acknowledge this limitation in the discussion:

Page 15 line 339: « *Reconstructing temporal dynamics in Unit I proved challenging due to the relatively undegraded DNA at shallow depths and the presence of living organisms, both of which increased ASV richness at the top of the core, a bias already reported in amplicon datasets (ref 13: Holman et al., 2025). These limitations highlight the importance of combining multiple proxies: in Unit I, amplicon sequencing suggested an increase in diversity, while foraminifera data showed a decline in genus richness, pointing instead to an overall reduction in biodiversity since the onset of mining. Whether the apparent increase in amplicon-based diversity reflects a true ecological signal or methodological bias remains unresolved, as community responses are known to vary among taxa (ref 57: Massana et al., 2011).* »

References :

Holman, L. E. et al. Navigating Past Oceans: Comparing Metabarcoding and Metagenomics of Marine Ancient Sediment Environmental DNA. Mol. Ecol. Resour. e14086, e14086 (2025)

Massana, R. Eukaryotic Picoplankton in Surface Oceans. Annu. Rev. Microbiol. 65, 91–110 (2011).

Another consideration is that you didn't directly attribute biodiversity loss to a specific environmental factor nor discuss the relative importance among factors. It could be because of increased sediment input, which affected turbidity, productivity, smothering the corals, or any other associated mechanisms, or because of the heavy metal pollution itself. Since the region was mined for nickel, any references and past evidence of biotic responses to this element would greatly strengthen your interpretation.

We thank the reviewer for this important comment. Because several environmental changes occurred simultaneously during the mining period, disentangling the relative contribution of each driver is indeed challenging. However, to better address this point, we have now strengthened our analysis and interpretation.

First, we incorporated the analysis previously presented in Supplementary Figure 6 into the main text (now Figure 4). This analysis uses hierarchical partitioning to quantify the relative contribution of several environmental factors (sedimentary unit, depth, metallic composition, organic matter, sediment sources and grain size) to the variability of the communities. This approach allows us to compare the relative influence of the different drivers and discuss this.

We agree that our study does not allow us to definitively attribute biodiversity loss to a single mechanism. Experimental approaches would be required to directly test metal sensitivity or the effects of turbidity and sedimentation. Therefore, in the revised manuscript, we expanded the Discussion to present more explicit hypotheses regarding the potential mechanisms involved, while clearly acknowledging the limitations of our observational approach in identifying a single dominant driver.

Page 12 line 272: « *Both metallic composition and grain size are known to influence benthic communities (28-30: Suzzi et al., 2023; Zhu et al., 2018; Pan et al., 2020), changes in these parameters are expected to induce different community composition.* »

Page 12 line 280: "*This dominant effect likely reflects additional environmental drivers not directly measured here, such as increased turbidity, pH variation, or sediment resuspension, which are known to strongly affect coastal marine communities (ref 33).*"

Page 13 line 296: « *These contrasting patterns among taxonomic groups suggest that certain taxa are subject to selective pressures imposed by high metal concentrations, as shown for Ni in some studies (ref 32,42,43: Gillmore et al., 2021; Garza Amaya et al., 2023; DeForest & Schlekat 2013), but experimental studies are needed to confirm their sensitivity and/or tolerance.*»

See also the response to specific comment 3 below.

References:

Suzzi, A. L. et al. eDNA metabarcoding reveals shifts in sediment eukaryote communities in a metal contaminated estuary. Marine Pollution Bulletin 191, 114896 (2023).

Zhu, P., Wang, Y., Shi, T., Huang, G. & Gong, J. Genetic Diversity of Benthic Microbial Eukaryotes in Response to Spatial Heterogeneity of Sediment Geochemistry in a Mangrove Ecosystem. Estuaries and Coasts 41, 751–764 (2018).

Pan, Y., Yang, J., McManus, G. B., Lin, S. & Zhang, W. Insights into protist diversity and biogeography in intertidal sediments sampled across a range of spatial scales. Limnology and Oceanography 65, 1103–1115 (2020).

Chariton, A. A. et al. Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. Environmental Pollution 203, 165–174 (2015).

Garza Amaya, D. L. et al. Microbial impact to environmental toxicants Ni(II) and Co(II): Joint toxicity and cellular response in Paramecium. Chemosphere 345, 140434 (2023).

DeForest, D. K. & Schlekat, C. E. Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. Integrated Environmental Assessment and Management 9, 580–589 (2013).

In summary, I recommend this manuscript to be published with minor to moderate revision with an emphasis to deepen and refine the Discussion section.

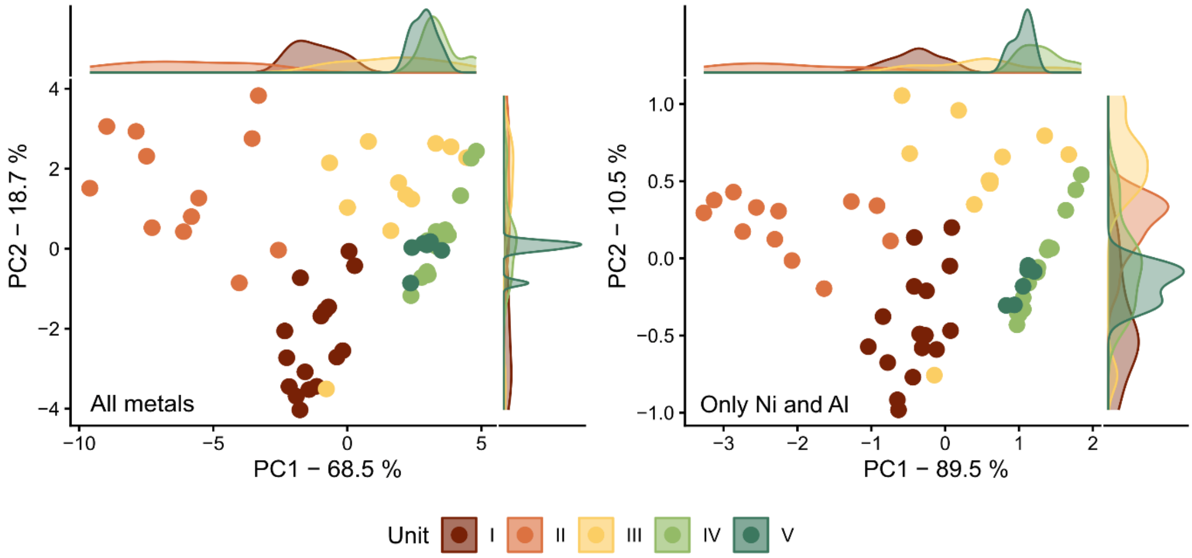
Specific points

1. Can you better justify the choice of aluminum to define periods of varying environmental conditions? I don't see a particularly strong correlation of Al with other trace metals in the supplementary figure 2. In addition, Ni and Al show opposite trends along the core. Any known mechanisms explaining this?

The strong correlation was not clearly shown in the manuscript, although it is very important for understanding our work. We provided more information on these correlations and their origin, explained by the type of soil in the watershed. Additionally, we add a figure in supplementary information, showing a principal component analysis on all measured metals and only the two selected, showing that they still well represent the samples' dissimilarity, acting as good representatives (Supplementary Figure 3, see below). We also added some references that support the link between these metals and their geologic origin. We had information lacking on the correlations and the groups of metals represented by Ni and Al.

The following text has been added :

Page 5 line 101: "*These chemical parameters were selected because they effectively represented two distinct groups among the 20 measured trace metals (Spearman correlation, p-value < 0.05, Supplementary Figure 2). In the first group, Ni was positively correlated with Co, Fe, Mn and Cr (Spearman coefficient = 0.99, 0.98, 0.97 and 0.85, respectively; Supplementary Figure 2), which are considered as representative elements of ultramafic rocks (ref 18–20: Dublet et al., 2012; Kierczak et al., 2021; Ulrich et al., 2019). In the second group, Al was positively correlated with Ba, Li, Pb, Sn and Ag (Spearman coefficient = 0.97, 0.97, 0.96, 0.95 and 0.95, respectively), which are classically considered to derive from volcano-sedimentary and/or metamorphic rocks (ref 21,22: Benson et al., 2025; Leach et al., 2005). The concentrations of Ni and Al also capture the main patterns of sample distribution observed in the full multi-metal dataset (Supplementary Figure 3), further supporting their use as representative indicators of the sediment core's overall metallic composition.*"



Supplementary Figure 3: Comparison of multivariate and bivariate PCA of sediment trace metals. *Each point represents an individual sample. Colours indicate the sedimentary unit (Unit I–V), and the shaded areas along the axes represent the distribution of samples along each principal component (marginal densities). The first two components (PC1 and PC2) explain the percentages of variance indicated on the axes. Pairwise dissimilarity between samples was quantified using the Euclidean distance. These analyses illustrate that the bivariate Al–Ni PCA retains the major patterns of sample clustering and dispersion observed in the full PCA, supporting the selection of Al and Ni as key indicators of metallic composition in the sediment core.*

References:

Dublet, G., Juillot, F., Morin, G., Fritsch, E., Fandeur, D., Ona-Nguema, G., Brown, G.E., 2012. Ni speciation in a New Caledonian lateritic regolith: A quantitative X-ray absorption spectroscopy investigation. Geochim. Cosmochim. Acta 95, 119–133. https://doi.org/10.1016/j.gca.2012.07.030

Kierczak, J., Pietranik, A. & Pędziwiatr, A. Ultramafic geoecosystems as a natural source of Ni, Cr, and Co to the environment: A review. Science of The Total Environment 755, 142620 (2021).

Ulrich, M., Cathelineau, M., Muñoz, M., Boiron, M.-C., Teitler, Y., Karpoff, A.M., 2019. The relative distribution of critical (Sc, REE) and transition metals (Ni, Co, Cr, Mn, V) in some Ni-laterite deposits of New Caledonia. J. Geochem. Explor. 197, 93–113. https://doi.org/10.1016/j.gexplo.2018.11.017

Benson, T.R., Jowitt, S.M., Simon, A.C., 2025. Special Issues on the Geology and Origin of Lithium Deposits—Introduction: Lithium Deposit Types, Sizes, and Global Distribution. Econ. Geol. 120, 503–511. https://doi.org/10.5382/econgeo.5149

Leach, D.L., Sangster, D.F., Kelley, K.D., Large, R.R., Garven, G., Allen, C.R., Gutzmer, J., Walters, S., 2005. Sediment-Hosted Lead-Zinc Deposits: A Global Perspective, in: Hedenquist, J.W., Thompson, J.F.H., Goldfarb, R.J., Richards, J.P. (Eds.), One Hundredth Anniversary Volume. Society of Economic Geologists, p. 0. https://doi.org/10.5382/AV100.18

2. In figure 2 A and B, you didn't specify the meaning of the grey shadowed area, nor the asterisks associated with richness values.

As requested by Reviewer 1, we have improved the descriptions of several figure captions, including Figure 2. The meaning of all graphical elements has been clarified.

3. Supplementary Figure 6 is important to show the compositional changes among sediment units and their controlling factors. You may consider moving it to the main text if space allows.

We thank the reviewer for this suggestion. Considering it illustrates compositional changes among sedimentary units for all communities and their controlling factors, and since the journal guidelines allow one additional figure, we have moved Supplementary Figure 6 to the main text (now main Figure 4). We made some graphical modifications to enhance the homogeneity with the other main figures (see below). In the original supplementary figure, the individual influence of each factor was shown; here, we chose to highlight the total influence (individual + shared) of each factor. This choice has been clarified in the Methods section. We have also added further details about this figure in the Results and Discussion sections to highlight its relevance.

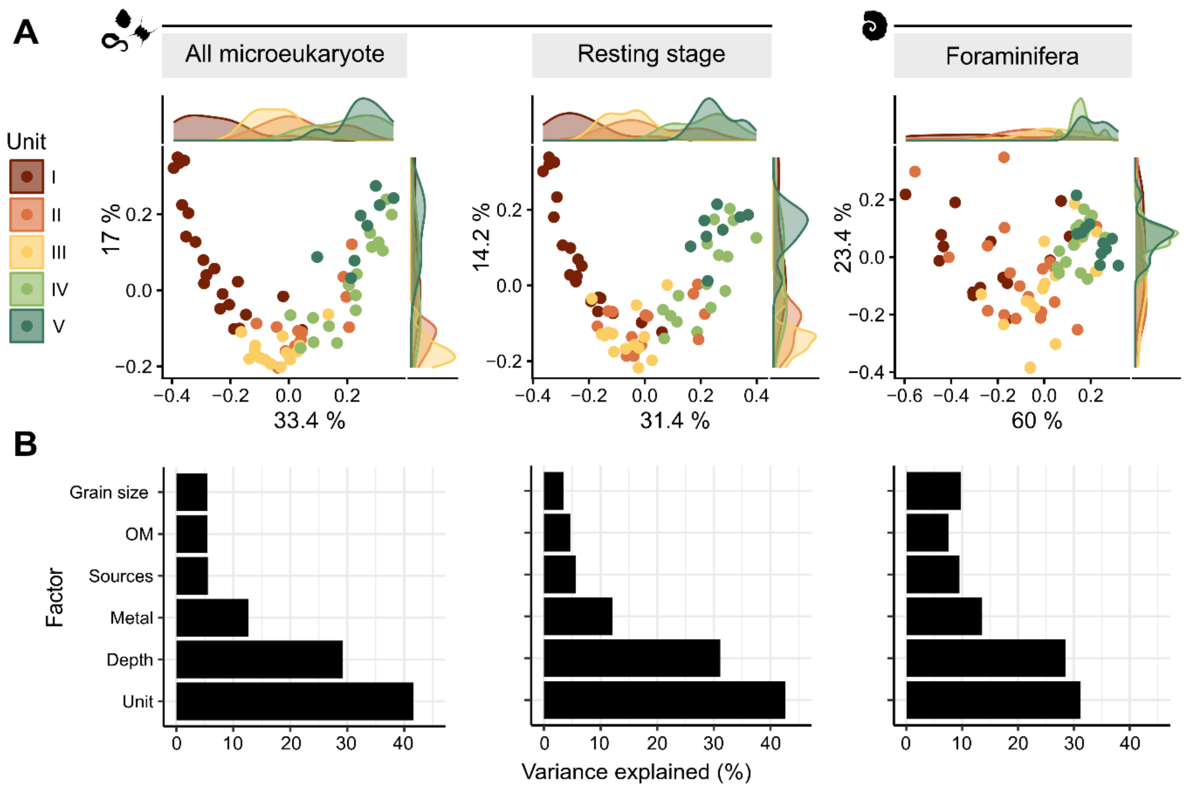


Figure 4: Changes in community structure among units.
(A) Principal coordinates analysis (PCoA at the genus level based on the Jaccard distance) of the entire microeukaryote community, the resting-stage subcommunity, and the foraminiferal community. Each dot represents a sample from the sediment core and is coloured according to sedimentary units. Shaded areas along the axes represent the distribution of samples along each principal component (marginal densities). (B) The percentage of variance explained was calculated using hierarchical partitioning to assess the influence of different groups of environmental variables: sedimentary unit, sample depth within the core (cm), metals (Ni and Al concentrations), grain size (proportions of sand, silt, and clay),

organic matter (POC and TN percentages), and the modelled sediment source (ultramafic and volcanosedimentary percentages).

Page 8 line 170: « *At the genus level, community structure varied across mining periods (Figure 4A-B), with sedimentary unit explaining 41.6%, 42.6%, and 31.2% of the total variance of microeukaryote, resting stage and foraminifera communities, respectively. Depth, representing temporal progression, was the second most influential factor (29.2%, 31.1%, 28.5%). Nevertheless, the environmental conditions of the sediment also contributed. Among measured parameters, metal composition accounted for the largest share of explained variance (12.7%, 12.1%, 13.6%), followed by sediment source (5.5%, 5.6%, 9.5%), organic matter (5.4%, 4.6%, 7.6%), and grain size (5.4%, 3.5%, 9.8%).*»

Page 12 line 266: « *This is further supported by the strong contribution of sedimentary units in explaining community structure* ***(Figure 4A–B)****.* »

Page 12 line 274: « *In our dataset, metallic composition showed the highest contribution among the measured environmental parameters in explaining community structure, consistent with previous studies in the area (ref 31,32: Meyneng et al., 2024; Gillmore et al., 2021). The contribution of other factors differed between microeukaryotes and foraminifera, with, for example, grain size having a stronger influence on foraminiferal communities, highlighting differences in responses. However, the highest overall influence was associated with sedimentary units corresponding to the different mining periods. This dominant effect likely reflects additional environmental drivers not directly measured here, such as increased turbidity, pH variation, or sediment resuspension, which are known to strongly affect coastal marine communities (ref 33: Chariton et al., 2015).*»

Page 28 line 675: « *The contribution of each factor to the explained variance was quantified as the total contribution, including both its individual effect and shared effects with other factors*.»

References:

Meyneng, M. et al. The Origin of the Matter Matters: The Influence of Terrestrial Inputs on Coastal Benthic Microeukaryote Communities Revealed by eDNA. Environmental DNA 6, e70041 (2024).

Gillmore, M. L. et al. Metabarcoding Reveals Changes in Benthic Eukaryote and Prokaryote Community Composition along a Tropical Marine Sediment Nickel Gradient. Environmental Toxicology and Chemistry 40, 1892–1905 (2021).

Chariton, A. A. et al. Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. Environmental Pollution 203, 165–174 (2015).

4. The foraminifera analysis with genus level occurrence data is a bit coarse. Is it possible to show the relative abundance changes of different genera to better capture the biological trend? Also, varying abundance and thus sampling effort could severely affect the diversity patterns observed. Sampling standardization is recommended. Or at least you should plot the foram abundance (i.e., the number of individuals you picked and identified) changes along the core and discuss this.

The foraminifera data were analysed at the genus level as a deliberate methodological choice, given that the primary aim of this study was to investigate broad-scale changes in foraminiferal biodiversity over time in response to environmental perturbations. Hence, the genus level was preferred to ensure determination consistency across the many samples of the core, with varying quality in sediment, leading to shells sometimes being damaged. Sampling was standardised, with identical amounts of sediment washed, dried, and picked prior to genus determination. We acknowledge that quantitative abundance data would have been valuable and could have provided additional insights. Nevertheless, even based on genus-level presence/absence alone, our data clearly reveal significant disturbances, with shifts in foraminiferal genus distribution closely reflecting the historical timeline of mining activities in Thio.

We add more details on the method to explain our choice of genus-level identification and on the standardisation of sampling.

Page 21 line 504: *« To isolate benthic foraminifera, 126 samples containing the exact same amount of sediment (sampled with a normalised size tube, pressed upside down over the whole thickness of the selected one-centimetre-wide sediment layers), were preserved in 100 mL of ethanol. Once back in the lab, they were washed separately using demineralised water and sieved through mesh sizes of 300 µm and 150 µm, to only retain adult specimens. All the foraminifera present in the sediment samples were hand-picked under a binocular microscope using a fine paintbrush and placed on black numbered grids with double-sided adhesive tape for identification and geochemical analysis*. »

Page 22 line 516: « *The genus level was chosen to ensure consistent taxonomic identification across the numerous core samples, which varied in sediment quality and often contained partially damaged shells that limited reliable species-level determination*.»