Replay on RC1 EGUSPHERE-2025-3780

We would like to thank the reviewer for the positive comments and constructive feedback. We have carefully revised the manuscript and re-examined the data to address all comments and suggestions. Below, we provide a point-by-point response to each comment. The updated figures, and table are included in the supplementary material.

Review of Manuscript: egusphere-2025-3780,

Title: Marine eukaryote community responses to the climate and oceanographic changes in Storfjordrenna (southern Svalbard) over the past ~14.0 kyr BP: Insights from sedimentary ancient DNA analysis

by Nethupul et al. 2025

The manuscript provides a reconstruction based on eDNA of the 'eukaryotic' community of Storfjordrenna over approximately 14K years before present. The samples were retrieved from a previously analysed sediment core (gravity core JM09-020-GC) and aims to use ancient sedimentary DNA to identify a more complete community of eukaryotes over time, adding to the microfossil work that has been previously reported by Lacka and colleagues. The goal of the study was to fill an information gap.

The introduction provides a brief synopsis of the region and core site, which can be influenced by either Arctic or Atlantic currents.

A concern is whether the site, with a constantly moving fronts of Atlantic and Arctic is useful as a proxy for detecting warmer periods. How integrative is the data within the strata sampled?

We appreciate the reviewer's concern. The study site was carefully selected in proximity to the present-day Arctic Front because this location is particularly suitable for detecting subtle changes in oceanographic conditions. According to Łącka et al. (2015), the Arctic Front experienced large-scale migrations during the Early Holocene, however palaeoceanographic evidence indicates that since that time, our study site has remained under the dominant influence of Atlantic Waters. This makes it a robust reference location for reconstructing past and present changes associated with the Atlantification process.

The biological and geochemical signals preserved in the sediments represent an integrated response to both gradual and abrupt climatic and oceanographic changes. Therefore, we argue that, rather than being a limitation, the site's location near the Arctic Front provides an

advantage for detecting subtle ecosystem responses to long-term warming and changing oceanographic regimes. Suitable information has been added to the Study area section.

A second concern is whether the information from the bioinformatically classified amplicons is sufficient for the detailed interpretation of ecology for different epochs. The amplicons were assigned at the order, family, and sometimes genus level, and then used to infer functional roles and provenance of the community members. While this approach could be sufficient for some groups, for others, accurate species identification is critical. Although statistically possible, the assignment to providence is questionable, and the interpolation of trophic status is doubtful given the lack of confirmatory experimental data.

Thank you for raising this issue The functional role is not assigned to ASV but to the higher taxonomic group, to which the ASVs have been assigned. We are confident that the assignment at such high level is correct because the reference database of ribosomal genes is sufficiently large. We know that in some taxa the trophic function can change even within the life cycle, but we were extremely cautious to assigned such complicated groups. Even if we cannot exclude some erroneous functional assignments, we hope that this is the minor part of our datasets.

As example; taxa such as dinoflagellates, ciliates, the species or genus level might be necessary to determine whether the ASVs should be assigned to a given functional group. We have made considerable efforts to assign ASVs as best as possible, especially in the ecological complex groups such as dinoflagellates, and ciliophora etc. However, we agree that the lack of precise species identification, mainly due to the incompleteness of the reference database, could introduce bias to our interpretation and might be a limitation. There are no experimental data for most of marine eukaryotes, so their functional role and ecology should be treated with caution. The relevant information has been added to the “Conclusions” section.

A major question is why the V1V2 region was targeted given most microbial eukaryotic studies primarily target V4, with V9 the second most used. It seems V1V2 sequences would greatly limit the taxonomic resolution of the study.

Thank you for your comment. We agree that V4 and V9 are more commonly used in eukaryotic metabarcoding studies. Yet, the majority of these studies focused on planktonic diversity (e.g. de Vargas et al. 2015). In our study, we have targeted both planktonic and benthic organisms, including metazoans. The V1V2 region was already successfully used for characterizing benthic metazoan fauna, in particular meiofauna (Fonseca et al., 2010; Lindeque et al., 2013; Sinniger et al., 2016) and therefore, we selected this region rather than V4 or V9. We argue that the taxonomic resolution of V1V2 is comparable to other variable regions (or even better than V9) and that the reference databases, such as SILVA and PR2, contain a rich dataset of V1V2 sequences, which improves taxonomic assignment.

A second question on the use V1V2 is that, since they are at an end of the gene, it seems that the region would be more prone to degradation over time.

We thank the reviewer for raising this interesting question regarding DNA degradation over time. We agree that the position of an amplified fragment within a gene can influence its recovery in ancient DNA studies, along with several other factors, as shown by other authors (e.g. Llamas et al. 2016). Although, we cannot exclude that this process is also affecting ancient DNA in our study, we are not aware of this type of degradation being reported for ribosomal genes in marine sediments. by using metabarcoding method we somehow removed the problem of degradation sequences. Moreover, if this is true, similar degradation would potentially affect the V9 region, which is situated at the 3' end of the 18S gene. Nevertheless, none of the sedaDNA studies using the V9 region has raised this issue as far as we know. Additionally, we also have not observed any substantial differences between taxonomic composition inferred from V1V2, V4 and V9, when all these markers were analyzed on the same material**.**

The 'missing 3.5 kyr is also problematic, does this fit with other mid Holocene anomalies with warmer river inputs (more terrestrial?).

Thank you very much for your comment. These samples were excluded from further analyses due to their low total read count (fewer than 1000 reads). This removal was necessary to ensure statistically robust downstream analyses. This issue was primarily caused by the low eukaryote DNA concentrations in the samples, which are consistent with the findings of previous studies that reported reduced nutrient flux during the mid-Holocene period (e.g., Łącka et al., 2019; Łącka et al., 2015). Also, most of these samples dominantly represented by gymnamoebae. We also check their taxonomy to see the species were either found in specific condition or recorded in specific time intervals. Their abundance patterns and stratigraphic consistency were evaluated alongside other taxa, and showed signatures consistent with contamination risk.

Would the region be affected by Siberian rivers (e.g. see Dong et al. Nature communication 2022. Doi: 10.1038/s41467-022-33106-1)?

It is an interesting suggestion and it is theoretically possible. However, there are no clear signs of freshwater in other proxy records (see Łącka et al., 2019 and Łącka et al., 2015)**.**

How does this fit with the low mass accumulation rates given in Lacka et al. 2015? Despite the low accumulation those authors still reported forams in those sediments. So, was the lack of signal due to higher DNA degradation rates?

As noted above, samples from around the missing 3.45 kyr interval may show low DNA recovery due to degradation and are predominantly composed of fungi, and gymnamoebae DNA. The dominance of bacterial DNA may reflect elevated microbial activity, which in turn could have accelerated DNA degradation. added to discussion regarding mid Holocene

Most of the discussion seems to rely on supplementary figures and data, and the key points should be put in an integrating figure at the end. Overall, I am not sure how the analysis and conclusions have improved our understanding of the future trajectory of the Arctic biota, given that this region has always been on the front between Atlantic and Arctic waters

Thank you for your comment. We have created a new indicator figure based on the most significant indicators for both AW and ArW (now Fig. 5). Additionally, the supplementary figure "Heatmap of co-occurrence based on Spearman rank correlation between eukaryote families represented in the study" has been moved to the main text as Fig. 6. Together with Figs. 3 and 4, these figures relate to the most important parts of the discussion.

As mentioned above, the site's proximity to the Arctic Front offers an advantage in detecting subtle ecosystem responses to long-term warming. Since the Early Holocene, our study site has remained under the influence of Atlantic water, making it an ideal reference location for studying past changes and predicting those associated with ongoing atlantification.

Specific comments.

Abstract:

Line 25: define acronyms such as ArW on first mention. Please check elsewhere as well.

It has been corrected.

Introduction:

Line 48: It is more usual to list references in chronological order.

I followed the journal reference style which requires to arrange references alphabetically.

Line 54: change 'it' to "biodiversity". I was not sure; the sentence was quite long.

It has been corrected.

Line 64: what is meant by "significant' was there a statistical test?

Yes, there was a statistical test. The Grant et al., (2024) paper revealed a significant difference in the sedaDNA community in each sea ice condition, as determined by diversity analysis

Line 74: was the core taken from the trough south of Storfjorden? Please clarify.

Yes, the core was collected in Storfjordrenna, which is a through located south of Storfjorden. This information is already included in the description of study area.

Line 80-82: The study is correlative, and the detected changes could be a response to any number of environmental conditions. The results suggest trajectories.

It has been corrected.

Methods

Line 123: please justify the use of the V1V2 region, the vast majority of other amplicon 18S rRNA gene surveys target V4. See above comments

We agree that V4 and V9 are more commonly used in eukaryotic metabarcoding studies. Yet, the majority of these studies focused on planktonic diversity (e.g. de Vargas et al. 2015). In our study, we have targeted both planktonic and benthic organisms, including metazoans. The V1V2 region was already successfully used for characterizing benthic metazoan fauna (Fonseca et al., 2010; Lindeque et al., 2013; Sinniger et al., 2016) and therefore, we selected this region rather than V4 or V9.

Line 138; Why 50 cycles? 30 is more usual and more cycles can lead to artifacts, e.g. "point mutations" and primer dimers. Fewer cycles lead to higher taxa richness estimates (e.g. Wu et al. BMC Microbiology https://doi.org/10.1186/1471-2180-10-255).

In this study, we analyzed sediment samples with low DNA concentrations. Since 30 PCR cycles did not yield sufficient DNA for sequencing, we increased the number of cycles to 50. To ensure methodological consistency, we applied the same number of cycles to all samples.

Line 152: Ibarbalz et al. is not in the reference list.

It has been added to the reference list.

Line 205: Is there an explanation for the low number of reads between 4.0 and 7.5 kyr BP? See comment above.

As mentioned above, these samples had to be filtered out due to their low total read counts. This removal was necessary to ensure statistically robust downstream analyses. This issue was primarily caused by the low eukaryote DNA concentrations in the samples, which are consistent with the findings of previous studies that reported reduced nutrient flux during the mid-Holocene period.

Line 236. Figure 2, no time patterns are evident. What is the utility of this analysis?

This figure supports our findings that the major climatic intervals in the region did not significantly impact eukaryotic diversity, suggesting that overall eukaryotic biodiversity remained stable.

Line 243, what is meant by "stable" in the context of the missing 3.5 kyr?

We still have three samples belonging to the mid-Holocene period. Figure 3b shows the proportion of ASVs present in each climatic interval. As we can see, approximately 25% of the planktonic groups (phytoplankton, mixoplankton, and zooplankton) are present across all major climatic intervals except the Bølling-Allerød.

Line 277: The term microzooplankton is ambiguous, it can also mean very small metazoans, distinguished from e.g. large calnanus species. Referring back to the previous paragraph; how realistic is it to separate mixotrophic dinoflagellates and heterotrophic dinoflagellates at the taxonomic level compiled in figure 3. Figure 3b is confusing as it seems ecological groups and taxonomic gropes are given with the list of other heterotrophs. Perhaps this should be 2 separate sub figures

Thank you for your comment. We have replaced the term microzooplankton with planktonic heterotrophic protists to more accurately describe this group. It includes radiolarians (Polycystina and Acantharia), pelagic ciliates (Spirotrichea, Litostomatea, and Prostomatea), heterotrophic silicoflagellates (Pedinellales), and heterotrophic dinoflagellates (Peridiniales and Gymnodiniales). both main text, and supplementary doc. mentioned details information of the species included in the group.

Other heterotrophs mainly comprise heterotrophic microbes that are either non-planktonic or cannot be confidently categorized as plankton due to limited ecological information. Most of these were identified to their taxonomic group; the exception is "benthic ciliate," which we have reclassified as benthic ciliophora to maintain taxonomic consistency.

In Figure 3b, major ecological groups are highlighted with distinct colors, while other heterotrophs cluster separately at the bottom of the figure. This layout reduces confusion and allows readers to clearly distinguish between the primary ecological groups and the remaining heterotrophic taxa.

Discussion

Line 360: 'patterns" would be a better word than 'dynamics"

It has been corrected.

Line 427: Micromonas polaris is pan arctic and occurs throughout the euphotic water column all year round. The Grant et al. paper is only about sediment and ignores the huge amount of data records for this species. M. polaris is not adapted to increased warming.

We have corrected the sentence in line with your comment.

Line 474; how is extreme defined here and throughout.

In our study, cercozoans were consistently abundant, indicating their high tolerance to significant environmental changes. Therefore, the dominance of these highly tolerant species during the mid-Holocene suggests that this period may have experienced severe environmental conditions that were unfavorable for many sensitive species observed in other climatic intervals. The term 'extreme' may not be the optimal choice of vocabulary, as it may be too strong. For this reason, we opted to substitute it with the more neutral term 'unfavorable'.

Line 487: I believe it would be nutrient "resupply" rather than "resuspension" Nutrients are incorporated into biomass, which needs to be broken down by bacteria

etc. They then re-enter the euphotic zone as inorganic nutrient, by physical oceanographic processes; such as upwelling, deep mixing or lateral advection.

We thank the reviewer for noting this point. However, in the manuscript we refer to phenomena described by Łącka et al. (2019), so we decided to follow the term used in their paper.

Line 544: *Heterocapsa arctica* in the Arctic should not be confused with Heterocapsa bohalensis the HAB species. This is an example of the danger of generalizing (see above comment on the need for species identification.)

In our study, we identified one *Heterocapsa* species to the species level, and revised in the main text. This was *Heterocapsa rotundata* (ASV204), which was identified as a mixotrophic species recorded in cold-water environments (Stoecker and Lavrentyev, 2018; Rintala et al., 2010). Given the environmental conditions in the study region, which are characterized by low light and limited nutrients, mixotrophy likely plays a key role in maintaining growth when photosynthesis alone is insufficient. We revised the text in the manuscript as "Similarly, *Heterocapsa rotundata* a mixotrophic dinoflagellate commonly associated with harmful algal blooms in Arctic and North Atlantic waters (Rintala et al., 2010; Wu et al., 2022), was identified as a potential ArW indicator (Fig. S12)".

As we mentioned above, we agree that the lack of identification of ASVs to the species level itself may introduce a bias. However, we analyze and interpret the occurrence of specific taxa/ASVs in the context of other proxy records. Therefore, even when an ASV cannot be identified to the species level, its type can be inferred based on the location and environmental conditions, as supported by the information of sequence details in NCBI database.

Lin 602: all of the results are correlative and a more cautious interpretation is needed.

Thank you for your comment. We moved the supplementary figure to the main text as Figure 6 to illustrate interactions within eukaryotic families in our study. To avoid potential misinterpretation, we listed the species under each family rather than labeling families directly, since most families in this region are represented by only a few species. We also used cautious wording rather than making definitive statements about interactions. The relevant information has been modified under the Discussion: 5.3: Interactions within eukaryotic community structure in Storfjordrenna

Replay on RC2 EGUSPHERE-2025-3780

Review of Manuscript: egusphere-2025-3780,

Title: Marine eukaryote community responses to the climate and oceanographic changes in Storfjordrenna (southern Svalbard) over the past ~14.0 kyr BP: Insights from sedimentary ancient DNA analysis

by Nethupul et al. 2025

The manuscript addresses a relevant topic within the scope of Biogeosciences: the long-term response of Arctic marine eukaryotic communities to past climate and oceanographic changes, reconstructed using sedimentary ancient DNA (sedaDNA). The focus on Storfjordrenna, a climatically sensitive region influenced by interactions between Arctic Water and Atlantic Water, is well justified. Applying sedaDNA metabarcoding to reconstruct community composition and potential functional roles over the last 13.3 kyr BP represents a potentially novel and valuable contribution to Arctic paleoecology.

The authors present interesting results and demonstrate the potential of eukaryotic communities as paleoenvironmental indicators, while also implicitly highlighting the need for further validation of their ecological meaning and the risks of over-interpreting single-proxy signals.

Overall, the manuscript presents promising data and is potentially very valuable in the context of future Arctic research. However, several revisions are required, mainly to clarify methodological choices, strengthen interpretation, improve the integration of key figures and tables into the main text, and correct minor technical issues.

Specific comments

Title: Consider changing "~14.0 kyr BP" to the more precise "13.3 kyr BP", as stated in the manuscript.

It has been corrected.

Abstract: Several technical terms and abbreviations (e.g. MAST, AW, ArW, ASV-based indicator analysis) are introduced without prior definition. These should be briefly explained in the abstract when they are first mentioned to improve accessibility for readers that are not familiar with the topic.

It has been corrected.

Introduction: The introduction would benefit from a clearer synthesis distinguishing what existing sedaDNA studies have already demonstrated from what remains unresolved, particularly with respect to functional ecological roles and ecosystem interpretation.

Most marine sedimentary ancient DNA studies remain largely taxonomically descriptive, focusing on patterns of species presence, richness, and turnover through time while providing limited insight into the functional ecological roles of detected taxa. Consequently, it remains unclear whether taxonomic shifts recorded in sedaDNA archives reflect fundamental ecosystem reorganization or merely the replacement of functionally equivalent species within otherwise stable ecological frameworks. Addressing this limitation requires moving beyond species diversity metrics toward functional, ecosystem-oriented approaches that integrate ecological roles and biotic interactions to better link past biodiversity changes with ecosystem functioning and environmental change, which were one of main focused in our study.

We included following text to the introduction section as "For example, recent studies have demonstrated that the sedaDNA approach can be used to reconstructing the interactions between sea -ice cover, ocean temperatures, and eukaryotic community composition (Armbrecht, 2020; Zimmermann et al., 2021; Zimmermann et al., 2023; Grant et al., 2024; Harðardóttir et al., 2024). However, there is still a significant lack of suitable-resolution marine eukaryotic sedaDNA records from the Arctic, especially those focusing on the, ecosystem-oriented approaches that integrate ecological roles and biotic interactions to better link past biodiversity changes with ecosystem functioning and environmental changes."

Study area: Figure 1 requires improvement for readability. The font size of Current abbreviations in panel A should be increased, and the coring location should also be indicated on panel A. While the definition of water masses (temperature and salinity) is clearly presented, it remains unclear whether these modern characteristics are assumed to be valid analogues for the entire 13.3 kyr record. A brief overview of known changes in these parameters in the region during the study period would strengthen the paleoenvironmental interpretation.

Figure 1 has been modified in accordance with the reviewer's suggestions. In the 'Study area' section, our aim was to present the area's modern hydrology. However, we were unable to precisely reconstruct the properties of the water masses as we had proxies for temperature but not salinity. However, previous multi-proxy records enabled us to trace the inflow of Atlantic and Arctic water masses, as well as the fluctuations of the Arctic Front. These changes are described and discussed in the context of our data in the 'Discussion' section. To avoid repetition, we have chosen not to include this information in the study area description.

Materials and Methods

L109 – How many radiocarbon dates were used to construct the age-depth model for the core? Since the data were recalibrated for this study, it may be appropriate to include the updated age-depth model within this manuscript.

We have included a new figure showing the age–depth relationship for core JM09-020-GC, constructed based on the previous study and incorporating the additional age data. In addition, a table summarizing the AMS ^14C measurements and the corresponding calibrated ages has been added to the Supplementary Material.

L119 – What was the subsampling resolution? What sediment thickness does each sample represent, and how might variability in sedimentation rates affect the temporal resolution of the sedaDNA record?

Subsampling resolution was ~200–300 years for most of the record, with lower resolution during the mid-Holocene (ca. 4–6 kyr BP) due to removal of samples by eukaryote-specific quality control process, and also to the low sediment accumulation ratio during the mid Holocene compare to the other major time interval. Each sample represents 1 cm of sediment. High and relatively stable sediment accumulation rates minimize the impact of sedimentation variability on the temporal resolution of the sedaDNA record (Łącka et al., 2015; Łącka et al., 2019)

The sedaDNA workflow requires stronger grounding in the existing literature. The preparation protocol is supported by only a single reference; additional methodological references are needed unless this represents a newly developed method by the authors, in which case this should be clearly stated.

We followed the protocols established and positively tested by Pawłowska et al. (2014, 2019, 2020) and Lejzerowicz et al. (2013), with minor adjustments to the number of PCR cycles

based on sample-specific amplification performance. In addition, we included several published studies that used the V1V2 primer set for eukaryotic community analyses to strengthen the methodological grounding.

Please justify the choice of ~340 bp fragment length and the high number of PCR cycles (50). Does the high cycle number increase contamination risk? Why were shorter fragments not targeted? Please explain why this protocol is appropriate for the sediment type and age analyzed in this study.

In this study, we analyzed sediment samples with low DNA concentrations. Since 30 PCR cycles did not yield sufficient DNA for sequencing, we increased the number of cycles to 50. To ensure methodological consistency, we applied the same number of cycles to all samples. Such high number of cycles may increase the risk of PCR bias, but not contamination. Therefore, we perform a careful quality check to ensure the removal of all low quality or erroneous sequences.

We agree that V4 and V9 are more commonly used in eukaryotic metabarcoding studies. Yet, the majority of these studies focused on planktonic diversity (e.g. de Vargas et al. 2015). In our study, we have targeted both planktonic and benthic organisms, including metazoans. The V1V2 region was already successfully used for characterizing benthic metazoan fauna (e.g., (Sinniger et al., 2016; Fonseca et al., 2010; Lindeque et al., 2013)) and also V9 is shorter fragment which lack in taxonomy resolution impact to when assigning complex ecological functional taxa groups. Therefore, selecting V1V2rather than V4 or V9 much better option for our study.

Importantly, the higher taxonomic resolution afforded by the V1V2 region is essential for our downstream ecological interpretation. This study aims to categorize taxa according to ecological roles, an approach that requires reliable taxonomic assignment to minimize misclassification, particularly within complex and diverse groups such as dinoflagellates and ciliophorans.

L154 – Since fungi and gymnamoebae were removed due to contamination risk, were they evaluated as part of a quality control metric prior to removal?

Yes. Fungi and gymnamoebae were assessed during the quality control phase prior to removal and were not excluded a priori. We also check their taxonomy to see the species were either found in specific condition or recorded in specific time intervals. Their abundance patterns and stratigraphic consistency were evaluated alongside other taxa, and both groups showed signatures consistent with contamination risk.

L159 – Please justify the use of the CSS normalization technique. Critics note that CSS may over-represent low-abundance ASVs; alternative approaches such as CLR/ILR transformations or batch correction methods are often recommended.

We used CSS to normalize sequencing depth while retaining sensitivity to low-abundance ASVs, which is particularly important for sedimentary ancient DNA datasets characterized by low biomass, uneven sequencing depth, and a high proportion of rare but ecologically informative taxa.

Alternative compositional approaches such as CLR/ILR transformations were considered; however, their application requires imputation of zeros and assumes log-ratio relationships that can be unstable in datasets dominated by mostly low-abundance signals typical of ancient DNA.

L201 – Given the complexity of the statistical analyses, a supplementary figure or table summarizing key analytical steps, outputs, and decision thresholds would improve transparency and reproducibility.

The methodology is described in detail in the main text under the *sedaDNA workflow* and *Statistical analysis* sections, where all analytical steps are explicitly documented, including the R packages and software used at each stage. Furthermore, all raw DNA sequence data have been deposited in NCBI. Using the procedures outlined in the Methods, the analyses are fully reproducible, and independent reproduction of the results following these steps does not present any technical issues. Therefore, to avoid unnecessary expansion of the 'supplementary material' section, we decided not to add any additional figures or tables.

Results

The removal of 13 samples (nearly 25% of the dataset) due to low read counts is substantial and should be discussed more explicitly. Please report the read count threshold applied and clarify whether these samples share specific sedimentological or environmental characteristics. Although all are from the Mid Holocene, it would be useful to relate this to sediment properties reconstructed in previous studies of the same core.

Thank you for the comment. We have added details regarding sample removal, as it was previously misinterpreted that all 13 excluded samples were from the mid-Holocene. After initial quality filtering and denoising with DADA2 in SLIM, eight samples were excluded due to low DNA yield (<500 reads), primarily reflecting either insufficient/absent DNA or presence of DNA prohibition in the sediments. An additional five samples were removed during the downstream eukaryote-specific quality control process described in the methodology, which impact the resulting lack of samples spanned the period between 4.0 and 7.5 kyr BP. The relevant changes done under the 4. results:4.1: Metabarcoding data.

The exclusion of 13 samples (~25% of the dataset) was based on a stringent read-count threshold of 1,000 total reads per sample, as reported in the Methods. These counts reflect **eukaryotic sequences** retained after all quality filtering and decontamination, ensuring that only reliable data were used for downstream analyses. Inclusion of samples below this threshold would have compromised statistical robustness and potentially introduced noise.

Importantly, the low read counts in the excluded mid-Holocene samples, dominated by fungi and amoebozoan DNA, reflect genuine environmental and sedimentary conditions rather than methodological failure. Previous studies of the same core document reduced nutrient flux and lower marine productivity during this period (Łącka et al., 2015; Łącka et al., 2019), which likely led to reduced DNA input and preservation. The relevant information is included in the Discussion (chapter 5.1.4).

The statement that alpha diversity varied across time intervals contrasts with the non-significant Kruskal–Wallis result for Shannon diversity; this apparent inconsistency should be clarified.

Alpha diversity varied across time interval; however, Shannon diversity showed no significant differences among intervals (Kruskal–Wallis, $p = 0.48$; Fig. S2, Table S2). This apparent discrepancy reflects that species richness is sensitive to rare taxa, whereas Shannon diversity accounts for both richness and evenness, making it less responsive to occasional low-abundance ASVs.

Figure S1 should be moved to the main text and complemented with depth and age information for each sample.

We included depth and age information in Fig. 3a. Fig. S1 was not moved to the main text because it shows non-significant differences in Shannon diversity among time intervals.

L225 – Is it robust to draw conclusions about the Mid Holocene based on only three samples?

We agree that the limited number of Mid Holocene samples restricts the strength of inferences that can be drawn for this interval. Accordingly, results for the Mid Holocene are interpreted cautiously and are used primarily to indicate qualitative trends rather than to support strong statistical conclusions.

L229 – The FSO results are introduced rather abruptly. It is unclear how strongly each proxy influences community composition relative to the others. Providing effect sizes or comparative metrics would improve interpretation.

We included the MFSO correlation into results section, which explained environmental proxies together showed a moderate proportion of the variation in community composition.

L243 – Could differences in the number of analyzed samples per time period influence observed ASV richness?

Differences in sample number could affect observed ASV richness; however, our study maintains a consistent temporal resolution of ~200–300 yr, which mitigates this effect. Even for the Mid-Holocene, samples adequately cover the time period.

Since the journal does not impose strict limits on figures and tables, it may be worth moving some supplementary materials into the main text. The frequent references to supplementary figures (e.g. Figures S8 and S9) make the Results section difficult to navigate.

We agree that more figures in the manuscript may facilitate navigating the text. Therefore, we decided to add two figures into main text: fig. 5 which present most significant ASV-based indicators to help navigating the indicator analysis section, and Fig. 6, which present co-occurrence based on spearman rank coefficient analysis between eukaryote families with their ecological roles.

Discussion

L366 – The title of Section 5.1 is very broad. In this section, the authors primarily discuss how oceanographic changes influenced eukaryotic communities, mediated through water-mass dynamics, sea ice, meltwater input, and stratification rather than direct climate variables. Rephrasing the section title would improve accuracy. In several places, sedaDNA read abundance is interpreted as a proxy for productivity. Since sedaDNA does not directly quantify biomass or productivity, the language should be softened to emphasize relative community signals, presence/absence patterns, or community restructuring instead.

Thank you for your comment. We changed “climate change” to “oceanographic changes” to make the title more precise.

L380 – The interpretation of freshwater or brackish taxa (e.g. Limnofila) as an ecological signal would benefit from considering alternative explanations, such as allochthonous input, riverine transport, or ice-rafted DNA. A discussion of potential transport pathways and clearer criteria for distinguishing local versus advected taxa would strengthen this section.

Thank you for your comment. The relevant information has been added to the Discussion: “The dominant cercozoan was *Limnofila* sp., a genus primarily found in fresh and brackish waters (Mylnikov et al., 2015; Nikolaev et al., 2003), the presence of *Limnofila* sp. may reflect either local ecological conditions or allochthonous input via riverine transport or ice-rafted debris (Łącka et al., 2015; Nguyen et al., 2026; Andruszkiewicz et al., 2019; Jo et al., 2025)”.

L463 – Although the authors appropriately caution interpretation of the Mid Holocene results, further discussion is needed to explain the low read counts observed during this period. Possible explanations could include sediment characteristics identified in previous studies, regional patterns observed at other sites, or methodological issues.

Samples from the Mid Holocene corresponding to the missing section of the record show low eukaryote DNA recovery, likely due to extensive degradation, and are dominated by fungi and amoebozoan DNA, particularly Acanthamoeba, which constitutes a large proportion of the

recovered sequences. The dominance of amoebozoan DNA may reflect elevated microbial activity, potentially accelerating post-depositional DNA degradation.

Moreover, as was already mentioned in the Discussion, previous studies of the same sediment core showed the reduction in productivity around the time period (Łącka et al., 2015, 2019), which was reflected in low abundance of Phyto- and zooplankton sequence reads.

L517 – While a very detailed table is provided in the supplementary materials, the manuscript would benefit from a shortened version in the main text containing only significant indicator taxa and associated paleoenvironmental proxies.

As mentioned above we add a new figure which included most significant ASV-based indicators from the study.

Although some of these points are addressed throughout the discussion, an additional dedicated subsection or a clearer concluding paragraph would help emphasize why studying eukaryotic communities is valuable, what new insights this approach provides beyond other proxies, the key advances of this study, and the limitations of sedaDNA-based reconstructions.

We included the mentioned points under the conclusion with additional details. The relevant information has been added to the Conclusion

Technical corrections: The texts have been corrected according to the Reviewer's suggestions

L13 – "sedaDNA" is inconsistently italicized; please standardize usage throughout the manuscript.

L41 – "IPCC" should be written in uppercase letters.

L89 – The unit of salinity is missing (‰ or PSU): We have added the PSU unit.

L183 – Missing separation between sentences ("methods/Seven").

L244 and L290 – Missing spacing.

L437 – Extra period in "development.".

L442 – Incorrect citation format; should read "According to Łącka et al. (2020)". The same issue occurs in L485.