July 17, 2025

Response for reviewers

We wish to thank the reviewer and Associate Editor for their attention to detail. Their comments are copied below, followed by our point-by-point responses in red.

First, we have modified our references in accordance with the standards of *Biogeosciences*. For papers with three authors, we have changed the in-text citations to the first author name followed by *et al.* The reference list has been reordered so that co-author papers appear before team papers led by the same first author. Finally, we have added 'a' and 'b' to the year of papers written by the same author in the same year to differentiate between different studies.

Reviewer 1 Comments

Thank you for your edits and clarification.

Lastly, I have one technical suggestion for your response regarding Fig. C in your supplementary files.
You mentioned the R-based commands you used (i.e., DEseq2) in your response letter.

However, the caption does not specify that you used Deseq2 and why you used these R plus bioconductor.

I now fully understand what DEseq2 does on your RNA-seq data after reading through "Why un-normalized counts?" at the Deseq2 home page:
https://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html#why-un-normalized-counts

As figure captions need to stand-alone, I suggest you reinforce the description of your strategy, possibly with citations (at least (Love, Huber and Anders, 2014)), why you adopted DEseq2?

Following the reviewer's suggestion, we have added the following statement to our Appendix C caption:

"Normalized *Fea1* counts were calculated using DESeq2's median of ratios method in R (Love, Huber and Anders, 2014)."

Further information regarding our RNA data analysis workflow is available in Methods Section 2.8:

"Raw reads were trimmed using Trim Galore 0.6.10 (Martin, 2011) and quality control was determined with FastQC (Andrews, 2010). A *de novo* metatranscriptome assembly was conducted using rnaSPAdes 3.15.5 (Bushmanova *et al.*, 2019) and CD-HIT-EST (Li and Godzik, 2006). Contigs were annotated using the Marine Functional Eukaryotic Reference Taxa (MarFERReT) database (Groussman *et al.*, 2023), which provides NCBI taxonomic annotations

(Federhen, 2012) and Pfam 34.0 functional annotations (Mistry *et al.*, 2021). Samples were mapped against the MarFERReT DIAMOND sequencing aligner and its compatible BLASTX command (e-value < 1e-06) (Buchfink, Xie and Huson, 2015). Trimmed samples were aligned using Salmon (Patro *et al.*, 2017). The package tximport (Soneson, Love and Robinson, 2016) was used to generate a comprehensive table of read count data for each sample and each contig. Only counts taxonomically mapping to Bacillariophyta (i.e., diatoms) were included. The normalized counts for all genes were then calculated using DESeq2's median of ratios method (Love et al., 2014)." (L365 – 376).

To avoid repetition, we do not reiterate our methods in the Appendix C figure caption.