ROUND 2

RC1

The revised manuscript has been substantially improved. However, I still have some comments and suggestions that require further attention.

Concerns regarding the use of a254 to indicate DOM accumulation or consumption: The changes in a254 only reflect variations in the concentration of aromatic organic compounds, rather than the entire DOM pool. This limitation should be explicitly stated in the manuscript to avoid misinterpretation. I suggest the authors add the relevant clarifications in both the Introduction and Discussion sections.

Authors reply: We fully agree with your observation and to address this point, we have added a clarification in the introduction (P3, L79-80) and discussion (P18, L499-502) sections and expanded the methodology section with an explanation as follows:

“Absorbance at 254 nm represents 50–60% of the carbon content in marine DOM (Görs et al., 2007). It primarily embraces unsaturated compounds with aromatic rings, conjugated systems, or carbonyl groups, mostly of biological relevance such as aromatic amino acids, nucleic acids, photosynthetic pigments, and steroids (Martínez-Pérez et al., 2017). As a result, a254 -although not representing all DOC present- can be used as reliable proxy for the evolution of DOM concentration during incubation experiments.” (P7, L203-208).

Incorporation of responses into the main text: While the authors indicated in the Response document that they added corresponding text to the revised manuscript, I noticed that some responses (e.g., comments 2 and 3 from Reviewer 2) were not incorporated into the main text. Please thoroughly cross-check the revised manuscript to ensure all responses are accurately reflected.

Authors reply: We apologize for this omission. While we initially addressed the reviewer’s comment, it was inadvertently removed during the final editing stage, where we significantly reduced the word count. We have now properly reinstated all comments from the first review round.

Specific Suggestions

Lines 46-48: The connection between the two sentences is not sufficiently clear.

Authors reply: We agree with this remark, we rewrote the sentence as follows:
“This effect likely arises from reducing the number of active, DOM-consuming bacteria and by providing egestion DOM compounds. At the onset of the productive season, high bacterial growth rates stimulate protistan grazing, which serve as a link between

bacterial biomass and higher trophic levels. However, as grazing pressure increases, protists can also contribute to the accumulation of a fraction of DOM.” (P2, L44-48).

Lines 78-89: The section is overly redundant. Consider presenting the purpose first, followed by the main findings in a concise manner.

Author reply: Thank you for this suggestion, we rewrote the paragraph to make it more concise as follows:

“Our study aimed to evaluate the role of protists in transferring bacterial and phytoplankton biomass under different productivity conditions and to assess potential changes in the chromophoric fraction of DOM and FDOM in these scenarios. We found that protistan grazing selectively targeted bacteria under both productivity regimes, and that intense grazing pressure on bacteria may contribute to the short-term accumulation of DOM substances, irrespective of productivity levels. These findings enhance our understanding of the ecological mechanisms influencing carbon flow in marine ecosystems.” (P3, L78-84).

Line 101: Revise to “an SBE9plus CTD profiler.”

Authors reply: Done (P4, L96).

Lines 104-105: Revise the ion abbreviations to “NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, SiO_4^{2-}” for consistency and clarity.

Authors reply: Thank you for your remark. We replaced Si to SiO_4^{2-} in the new version of the manuscript.

Line 107: Specify the pore size of the Whatman GF/F filter and review the manuscript to ensure all filter pore sizes are stated consistently.

Authors reply: Done.

Line 125: Provide the exact pore size of the Whatman polycarbonate filter.

Authors reply: We used a Whatman polycarbonate filter with a pore size of 0.2 µm, as stated in the manuscript (P4, L122).

Line 129: Check the unit, as “g m^2” seems incorrect or incomplete.

Authors reply: The unit represents grams of fabric per square meter, a standard term for describing a fabric's translucency or light permeability. To enhance clarity, we have revised its definition in the manuscript as "g of fabric per m^2." (P5, L126-127).

Line 130: Delete “(N, P, and Si)”.

Authors reply: Done.

Lines 198-199: The explanation of HIX provided here is inaccurate. HIX is primarily used to assess the degree of humification in DOM, which reflects its aromaticity and the extent of diagenetic alteration.

Authors reply: We have changed the definition according to the reviewer’s suggestion (P6, L195-196).

Line 366: In Figure 8b, the value of R2 is 0.759...

Authors reply: Thank you for catching this error. The correct R^2 value is 0.837, and we have now corrected it in both the main text and the figure.

Line 429: Why is the growth rate of heterotrophic bacteria high at all stations?

Authors reply: While our experimental design did not allow us to detect DOC excess or limitation as DOC was only measured at the surface layer and not at the depth where experiments were done (i.e., chlorophyll-a maximum), we can speculate that heterotrophic bacteria were under replete DOC conditions, in addition of being supplemented with inorganic nutrients during experiments. The concentration of DOC at the surface ranged between 52.3 and 95.7 µM, which is among the moderate to higher DOC range in open waters (Hansell et al. 2009, Oceanography). We added some text to clarify this point.

“The moderate to high DOC concentration compared to other open waters (Hansell et al. 2009), appears to support sustained bacteria growth.” (P18, L496-497).

Line 467: Clearly state whether ciliates preferentially graze on smaller prey, such as bacteria?

Authors reply: Ciliates do not preferentially prey on bacteria; rather, they can thrive when the available prey are of uniform size. We revised the text to clarify this point.

“Ciliates primarily select prey based on size as their oral diameter limits ingestion. They can thrive on a diet composed exclusively of prey of uniform size, such as bacteria. In contrast, dinoflagellates employ diverse feeding strategies, allowing them access to a broader range of prey sizes.” (P17, L478-480).

Lines 491-508: These statements should be revised to improve logical flow and readability, ensuring that the connections between ideas are clearer and the overall message is more coherent.

Authors reply: We agree with this remark. We improved the readability of the paragraph as follows:

"The complexity of DOM, reflected by the prevalence of humic-like compounds ($FDOM_C$, $FDOM_A$, and $FDOM_M$), emerged as a key factor influencing the bacterial utilization of DOM. A net consumption of the DOM fraction, as estimated by a254, was observed when the initial DOM pool contained a significant proportion of low-reactivity compounds, influenced by the phenological status of the sampling site, as indicated by satellite chlorophyll-a trends. However, while stations 21 and 23 had high proportions of refractory compounds, they differed in labile compound content, leading to distinct experimental outcomes. Both stations had low pre-sampling chlorophyll-a, indicating limited recent autochthonous DOM production, yet showed contrasting bacterial activity. In station 23, high bacterial activity increased DOM complexity and net consumption under both treatments, suggesting dependence on an initial labile DOM pool (FDOMT). In contrast, station 21 had low bacterial activity, with the a254 DOM fraction shifting from consumption to accumulation without protists, indicating bacterial reliance on phytoplankton DOM exudates when the DOM pool was predominantly refractory." (P18, L508-519).

Lines 504-505: I didn't quite understand this statement. A low phytoplankton-to-bacteria biomass ratio indicates relatively high bacterial biomass. So, why would bacterial DOM consumption be masked by phytoplankton?

Authors reply: Thank you for identifying this error. The term was misspelled, and the correct ratio used in the manuscript is "bacteria-to-phytoplankton". We have corrected this in the text accordingly. (P18, L517).

Lines 538-541: Awkward sentence, please rephrase

Authors reply: We agree with this remark. We improved the readability of the paragraph as follows:

"Our experimental results indicate that the diversion of bacterial carbon from the DOM cycle by protistan grazing is only partial, as protistan grazing introduces additional DOM, while the reduction in bacterial abundance leaves a fraction of phytoplankton-derived DOM unutilized over short timescales." (P20, L563-566).

Figure 8b: I don't fully understand the positive relationship between the net a254 change and HB:Phyto. A higher HB:Phyto ratio indicates more HB biomass, which should correspond to a greater consumption of DOM. However, the figure shows the opposite trend.

Authors reply: This trend was observed only in the treatment without protists. We interpret this as a high dependence of bacteria on phytoplankton-derived DOM when bacterial biomass is low relative to phytoplankton (i.e., a low HB:Phyto ratio). Under these conditions, bacteria appear to rely more on phytoplankton-derived DOM than on other sources. If this interpretation is correct, the removal of protists—along with the absence of newly produced DOM—would lead to net a254 DOM fraction consumption in the incubation bottle.
We added some text in the discussion section to clarify this issue:

“Low bacteria-to-phytoplankton biomass ratios indicated that bacterial DOM consumption was masked by phytoplankton biomass but became evident without protists. That is, in stations were bacterial biomass is low relative to phytoplankton, bacteria appear to rely more on phytoplankton-derived DOM than on other sources. This effect become apparent upon removal of protists that implies the absence of newly produced DOM (Fig. 8b), in which a net a254 DOM fraction consumption was observed.” (P18, L522-526).

RC2

I think the manuscript by Lopez-Abbate has significantly improved since the first round of revision. I have some minor comments that I think prevent this manuscript from being accepted in it's current state that are discussed in the public revision section. Mainly, I do not agree with putting heterotrophic bacteria together with phytoplankton in reference to the visual display of the trophic pyramid. Heterotrophic bacteria are secondary consumers and should not be lumped in with phytoplankton as the bottom of a trophic pyramid. I skimmed through the McCauley et al 2018 mansucript they reference and here there are examples (e.g. Cho & Azam 1990, or even more pertinent Gasol et. al. 1997) where heterotrophic bacteria are clearly separated from phytoplankton in a trophic structure. Beyond this, the manuscript has some minor grammar in need of editing. Authors specifically reference Kang 2023 on food webs where phytoplankton and heterotrophic bacteria biomass are summed, specifically because they are eaten by protozooplankton - this manuscript summed them, and did not show food webs where phytoplankton and phytoplankton+heterotrophic bacteria both existed.

López-Abbate et al investigated how phytoplankton and bacterial mortality influence the pool of dissolved organic matter (DOM) on the Patagonian Shelf; a region of the ocean known as a hotspot of carbon sequestration. Dilution experiments revealed that phytoplankton mortality via grazing was greater than their growth rate, while bacterial growth and mortality were relatively balanced. By measuring colored dissolved organic matter (CDOM) and fluorescent dissolved organic matter (fDOM), authors observed a decrease in ‘fresh’ DOM and an increase in aromatic DOM. How carbon flows through multiple trophic levels is of importance for understanding marine ecosystems, especially in the context of carbon dioxide sequestration. I thank the authors for returning a heavily revised manuscript that demonstrates a marked improvement from the previous version, however I still have some comments that need to be addressed.

Authors responded to reviewer comments by stating they had edited the text to define what they consider to be a phytoplankton bloom. This seems to be missing from the current version of the manuscript and I believe this should be added to clearly define what they mean by a phytoplankton bloom.

Authors reply: We apologize for this omission. While we initially addressed the reviewer's comment, it was inadvertently removed during the final editing stage, where we significantly reduced the word count. We have now reinstated the definition of a phytoplankton bloom and carefully reviewed the manuscript to ensure that all comments from the first review round are properly incorporated.

I thank the authors for clarifying how they determined their trophic pyramids in figure 4A. I have now read through Kang et al 2023 and McCauley et al 2018. The Kang paper sums phytoplankton and bacteria in ecosystems where they are both prey items for protozooplankton. In these ecosystems, the trophic structure phytoplankton+heterotrophic bacteria, protozooplankton, and mesozooplankton - they do not create a pyramid that has phytoplankton, and phytoplankton+heterotrophic bacteria. The McCauley et al 2018 paper, on the other hand, treats heterotrophic bacteria as a higher trophic level than phytoplankton (see examples cited in text such as Gasol et al 1997 and Cho & Azam 1990). If the authors are citing Kang et al 2023 (which is not in the figure legend, at odds with author's response), they need to remove the phytoplankton from their trophic pyramid and only display Phytoplankton+Heterotrophic bacteria. Otherwise, heterotrophic bacteria are secondary consumers and at a higher trophic level than phytoplankton.

Authors reply: We modified the figure 4a according to the reviewer's suggestion and added the reference to Kang et al. 2023 into the figure's legend.

Minor comments:
Line 35 – 36: unclear phrasing, which microbes had a faster growth rate than the others?

Authors reply: Thank you for your remark, we rewrote the sentence as follows:

"Although phytoplankton biomass was higher than that of bacteria, protists selectively preyed on the faster-growing bacterial population, denoting trophic specificity of grazers." (P2, L35-36).

Lines 38 – 40: the terms top-heavy or bottom-heavy pyramid structure are not clear terms to me in this context, and I am not sure your data measures trophic efficiency, as respiration was not measured in this dataset.

Authors reply: We agree with this remark and have replaced 'trophic efficiency' with 'trophic coupling.' Additionally, we now refer to a top-heavy pyramid as an 'inverted trophic pyramid structure.' However, we have retained the term 'bottom-heavy pyramid' to clearly distinguish it from the less common inverted pyramid. The text now reads as follows:

“High trophic coupling was suggested by the biomass distribution of protistan consumers and their prey, which predominantly exhibited an inverted trophic pyramid structure. An exception to this pattern was observed at the highly productive shelf break front, where a traditional bottom-heavy pyramid emerged, indicating that most phytoplankton evaded protist predation despite evidence of herbivory.” (P2, L36-40).

Line 450 – 451: This sentence is speculative, and the citation (Taylor & Landry 2018) is not a study that demonstrates or even discusses this relationship in heterotrophic bacteria. I suggest this be removed.

Authors reply: We appreciate the reviewer's feedback and acknowledge the concern regarding the speculative nature of the sentence. However, we would like to clarify that the phrase 'the suggested mechanism underlying experimental observations' was already present in the original manuscript and remains unchanged in the revised version. Our reasoning aligns with Michel Landry’s Enhanced Microbial Loop Hypothesis and its extensions, which we believe provide a solid conceptual framework for our interpretation. Therefore, we prefer to retain the text as it stands unless the reviewer strongly suggests otherwise. Additionally, we agree that the reference to Taylor & Landry (2018) may not be the most appropriate and have accordingly replaced it with Landry et al. (2023) (P17, L452).