

Referee no 1

Summary of the study:

The study aims to quantify the impact of heat waves on estuarine sedimentary fluxes, and particularly the subsequent CO₂ flux from the overlying water into the atmosphere. For this purpose, two experiments were carried out, one in winter, one in summer. For each experiment, three temperatures were chosen that reflect “ambient low”, “ambient high”, and “extreme” conditions. The key findings of the study are that higher temperatures speed up chemical reactions and turnover rates. These in turn increase CO₂ release first from sediments and subsequently from the water to the atmosphere.

Major comments:

The abstract is somewhat fuzzy. There is no introductory sentence and some of the statements are factually wrong (e.g. μatm as a unit for a rate).

Reply: We thank the reviewer for pointing this out. The abstract has been revised to include an introductory sentence that provides appropriate context for the study. We have also corrected the issue related to the reported units.

Abstract: *Estuaries are highly sensitive ecosystems in which increasingly frequent and intense heatwaves, driven by global warming, can profoundly alter biogeochemical processes and benthic-pelagic fluxes. Here, we studied how short-term heatwave extremes affect biogeochemical cycling and fluxes in a temperate estuary in a semi-dry climate using temperature-controlled benthic incubations. The results demonstrated a strong thermal effect, notably under extreme warming events, on shifts in exchanges across the sediment-water interface. Extreme heatwave conditions (+5 °C of the seasonal mean) intensified acidification (CO₂ production), oxygen depletion, and ammonification due to accelerated remineralization rates, resulting in strong effluxes of NH₄, Si(OH)₄, and PO₄ to the overlying water. These excessive nutrient inputs may increase the likelihood of eutrophication, especially in nearby oligotrophic coastal environments. Heatwave-induced CO₂ production reached ~4000, 2.3-fold higher than the ambient conditions, with a maximal flux of ~27.0 mmol m⁻² d⁻¹. Our experiments demonstrate that marine heatwaves enhance CO₂ emissions and alkalinity in temperate estuaries, indicating intensified organic matter remineralization and carbonate system turnover.*

The methodology is scarcely described which makes it difficult to comprehend the results. The experimental setup is ambiguous: Why do you expose sediments collected in winter to heatwave conditions and vice versa sediments collected in summer to “ambient low” temperatures?

Reply: The rationale for exposing sediments collected in both summer and winter to the full temperature range is to simulate the sharp temperature fluctuations that characterize this region. It was added in lines **78-85**:

“Temperature variability in the study area is largely driven by episodic “Sharav” (heatwave) events, during which hot, dry southerly air masses affect the region, especially during spring and autumn (Hochman et al., 2023). Therefore, under recurring heatwave conditions, sediments collected in both summer and winter may experience the region’s full temperature range. Moreover, exposing sediments to the full temperature range was necessary because temperature not only controls the magnitude of sediment nutrient fluxes but can also alter their direction, with sediments acting as either sources or sinks of nutrients under different thermal conditions. (Zhou et al., 2016; Rios-Yunes et al., 2023).”

R1: In addition, and more profoundly: The sediments were possibly mixed. That alters the entire chemistry in sediments as well as the microbial community. In that case the entire study might be just a record of artefacts.

Reply: We appreciate this comment and agree that sediment mixing can influence sediment fluxes and microbial activity. Therefore, we have revised the manuscript to better classify and describe the experimental setup. These changes are now reflected in Lines **93-101**:

“Because the natural sediment structure was disrupted and the overlying water was not continuously stirred, this experimental setup is best classified as a slurry incubation, which provides a valuable approach for assessing potential nutrient regeneration rates. However, conventional slurry incubations often yield flux estimates that exceed those under in situ conditions because sediment mixing disrupts the sediment matrix and enhances solute exchange. In our setup, such overestimation is unlikely because no mixing was applied after the initial preparation, allowing a sediment-water interface to re-establish during the 12 h equilibration period. Thus, our incubations were designed to assess temperature effects on flux dynamics yet do not represent in situ fluxes.”

The language of the manuscript is very often extremely generic. The authors talk about “other related modifications” and similar things instead of naming them. That reduces the clarity of the study.

Reply: We rephrased that sentence in Lines **39-42** and throughout the MS:

“These experiments, however, did not account for the likely adaptation of microbial communities to the warmer conditions prevailing in tropical and subtropical regions, nor for modifications to the underlying biogeochemical processes governing these systems, such as warming-induced suppression of infauna and, consequently, bioturbation (Lubinevsky et al., 2026).”

Overall, the study fails to point out what new questions it wants to answer. Eventually the discussion fails to answer any real question anyway. The result and discussion part is very much a result part. The discussion occasionally drifts into a review but does not manage to point out what the new findings of this study (are they new?) imply. There is some explanation of the observed data, but no attempt to put the data into context. For example: What is the total magnitude of flux change we look at? How many estuarine systems is the chosen estuary representative for?

Reply: We thank the reviewer for this important comment. As stated in the revised manuscript, the aim of the study is to assess the temperature control on benthic biogeochemical processes in temperate estuaries of warm regions, such as the eastern Mediterranean. We now clarify that the current experimental setup quantifies the temperature dependence of potential nutrient regeneration rates and related sediment processes, rather than actual in situ benthic fluxes. This distinction has been explicitly addressed in the revised manuscript, as described point by point below.

The fact that the conclusion is extremely short underscores that the study does not provide many new findings in its current form.

Reply: We agree and added a full conclusion section

“Temperature appears to be an important driver of remineralization processes in estuarine sediments, and these effects may become more pronounced under future warming and marine heatwave conditions. In this study, simulated heatwaves were associated with enhanced benthic remineralization and generally higher sediment-water exchanges in a semi-arid, warm-adapted estuary. Extreme heatwave conditions were accompanied by higher rates of nutrient regeneration and CO₂ production, resulting in increased effluxes of NH₄⁺, PO₄³⁻, Si(OH)₄, and dissolved inorganic carbon. The observed covariance among oxygen depletion, acidification, and nutrient release suggests a close linkage between organic matter remineralization and carbonate system dynamics.

Notably, under extreme warming (35 °C), nutrient and carbon fluxes continued to respond to increasing temperature, whereas oxygen consumption did not differ significantly from the ambient-high treatment. This pattern may indicate that nutrient regeneration and carbon cycling respond differently to elevated temperatures than respiratory oxygen demand, although the underlying mechanisms remain uncertain. Overall, our results suggest that marine heatwaves could increase the potential for Mediterranean estuarine sediments to act as sources of regenerated nutrients and CO₂, with possible implications for coastal carbon cycling and eutrophication dynamics.

The study was based on sediment incubations designed to isolate potential sediment responses under controlled conditions, and therefore does not fully capture the complexity of natural estuarine environments. Consequently, the measured fluxes should be regarded as potential rates rather than direct estimates of in situ benthic

exchange. Future in situ observations and longer-term experiments are needed to determine the magnitude, persistence, and ecological significance of sediment biogeochemical responses to natural marine heatwaves.”

Precise criticism can be found in the line-by-line comments.

Summarizing it is actually very difficult to evaluate this study. The results might be worth being published (if the method section is revised), but then the entire structure of the study needs revision: I suggest a classic format with methods, results and discussion, and a completely revised discussion that clearly tackles the novelty of the results and their implications for the field.

Reply: Many studies adopt a combined Results and Discussion format, and we find that the current structure presents the results and their novelty in a concise and clear manner. This format facilitates the interpretation of the findings while avoiding unnecessary repetition between sections.

Line 6: The first sentence of the abstract is missing. Please insert a short sentence that guides the reader towards the research question and then start with what you did.

Reply: As kindly suggested, we have added a brief introductory sentence in the revised abstract to provide background and context for the subject.

Line 11 this sentence is somewhat weird – runoff ok, but tidal action? Is the idea to say that the enhanced nutrient fluxes lead to eutrophication in the ocean adjacent to the river? If so, just say that. The transport mechanism is somewhat self-explanatory.

Reply: As kindly suggested, the sentence has been rephrased to clarify the subject.

“These excessive nutrient inputs may increase the likelihood of eutrophication, especially in nearby oligotrophic coastal environments.”

Line 13: First: μatm is not a unit for a rate. Second: Where’s the difference between extreme hypoxia and anoxia? Third: Isn’t acidification the consequence of high CO_2 production?

Reply: Change in pCO_2 over time can be expressed as a rate:

$$\text{Rate of } \text{pCO}_2 \text{ change} = \frac{\Delta \text{pCO}_2}{\Delta t}$$

Nevertheless, as we report only the total amount of CO_2 produced rather than a temporal change, the term "**rate**" was removed from the sentence.

We agree with the reviewer. Hypoxia is generally defined as oxygen concentrations below approximately **2 mg L^{-1}** , whereas acidification refers to the decrease in pH resulting from

elevated CO₂ production. To avoid confusion and improve accuracy, we have rephrased the sentence in **Lines 12-14**:

“Extreme heatwave conditions (+5 °C of the seasonal mean) intensified acidification (CO₂ production), oxygen depletion, and ammonification due to accelerated remineralization rates, resulting in strong effluxes of NH₄, Si(OH)₄, and PO₄ to the overlying water.”

Line 14: Since alkalinity was not mentioned to this point the reduced buffering capacity appears very abruptly.

Reply: We thank the reviewer for raising this important point regarding buffering capacity. We agree that discussing buffering capacity requires consideration of total alkalinity, which is a key component of the carbonate system. To address this issue, we have rephrased the sentence in **Lines 17-19**:

“Our experiments demonstrate that marine heatwaves enhance CO₂ emissions and alkalinity in temperate estuaries, indicating intensified organic matter remineralization and carbonate system turnover.”

Line 14/15 This last sentence is quite detached from the rest of the abstract

Reply: We agree and therefore removed the sentence

Line 18: Please explain briefly in what way they buffer carbon and nutrients.

Reply: We thank the reviewer for this suggestion. To clarify the role of estuaries in biogeochemical cycling, we have added the following sentence briefly explaining how estuaries buffer carbon and nutrients, in **Lines 22-24**:

“Estuaries act as natural biogeochemical buffers by regulating carbon and nutrient cycling through alkalinity generation, carbon burial, biological uptake, and sediment nutrient retention and transformation (Borges and Abril, 2011).”

Line 20: Comma after Yet.

Reply: comma was added

Line 20: Is that really their depth? Or rather the fact that they are mainly influenced by a riverine system that depending on length and runoff is reflecting air temperatures and incoming heat radiation?

Reply: Indeed, the Na'aman Estuary is extremely shallow at its marine outlet. We have added a sentence to better describe the study site and clarify this characteristic in **Lines 87-90**:

“The Na'aman Estuary is extremely shallow (less than 1 m depth in its marine outlet) and therefore highly sensitive to fluctuations in air temperature, which can rapidly alter water temperature and associated biogeochemical processes. “

Line 23: What do you mean by “from these estuaries”? If you really mean from the estuaries to the adjacent sea then the rest of the sentence is weird as it is completely obvious that the adjacent sea might then be influenced by the fluxes. Or do you mean sedimentary fluxes in the estuary that can then influence the adjacent sea? Please clarify this sentence.

Reply: To make more clear, we rephrased the sentence in **Lines 36-38:**

“These warming trends and thermal anomalies significantly affect carbon and nutrient fluxes in estuaries, with potential consequences for the functioning of adjacent coastal marine ecosystems that remain poorly understood.”

Line 27: What effect does temperature have? Please be specific.

Reply: Temperature's possible effects on estuaries are described in **lines 32-38.**

“The fundamental hypothesis is that with increasing temperatures, microbial metabolic rates increase (Price and Sowers, 2004), thereby accelerating the remineralization of organic matter (Fig. 1). Moreover, for nutrient-regulating reactions, such as denitrification or ammonia oxidation, temperature exerts a significant effect (Canion et al., 2014). Recent experimental observations, carried out in the mid-to-high latitudes estuaries, have demonstrated the expected metabolic effect as well as the potential impact of warming during heatwaves on carbon fluxes (Douglas et al., 2025; Palmas et al., 2025) and on nutrient fluxes (Fouet et al., 2025; Göbeler et al., 2025).”

Lines 20 – 30: Same Problem: What effect?

Reply: See the above answer.

Line 31: Please reduce the amount of “yet”s in the manuscript.

Reply: Agreed, some of the “yet” s were removed or replaced

Line 31: Likely should stand before microbial and ideally be replaced with “possible”.

Reply: The sentence was rephrased in **Lines 39-42:**

“These experiments, however, did not account for the likely adaptation of microbial communities to the warmer conditions prevailing in tropical and subtropical regions, nor for modifications to the underlying biogeochemical processes governing these systems, such as warming-induced suppression of infauna and, consequently, bioturbation (Lubinevsky et al., 2026).”

Line 32 What do you mean by “other related modifications to the fundamentals of these systems”? That formulation can mean anything. Please be precise in your language.

Reply: Please see the corrected sentence above

Line 33: Higher temperatures compared to what?

Reply: We replaced the term “higher” with “warm” temperatures

Lines 35 – 38: So far, the authors have not clarified which ecosystems might be affected in what way to justify that SEMS eco-systems can serve as a role model. Hence: Please rewrite the introduction here in a way that clarifies what ecosystems are likely to experience what kind of changes and in what way a SEMS ecosystem is showcasing these features already.

Reply: This is explained in detail in lines **42-49**.

The Mediterranean Sea, notably the Southeastern Mediterranean Sea (SEMS), already hosts such communities, adapted to warm temperatures and large thermal variabilities (Christaki et al., 2011; Givati, 2020; Marasco et al., 2023), and is experiencing an increase in marine heatwaves (Ibrahim et al., 2021). Additionally, microbes in the SEMS are also adapted to utilize low-abundance, highly refractory organic matter, which characterizes this region (Alkalay et al., 2020; Tselepides et al., 2000). Therefore, this region’s extreme environmental conditions establish a framework that could be considered a good model of future anthropogenically modified scenarios to study the impact of heatwaves on coastal ecosystems”.

Line 40: The hypothesis that higher temperatures increase metabolic rates has not really been established above. Please make sure that you explain this hypothesis “above”.

Reply: We agree with the reviewer that the hypothesis that higher temperatures increase metabolic rates was not sufficiently established in the original manuscript. To provide the necessary background and rationale for our study, we have added the following paragraph in **Lines 51-59**:

“Warmer conditions can stimulate microbial decomposition of organic matter, increasing oxygen consumption and nutrient regeneration from sediments. Because estuaries are shallow, dynamic ecosystems dominated by ectothermic organisms whose body temperatures closely track environmental conditions, even relatively small temperature shifts can trigger substantial ecosystem-wide responses. The ongoing warming of estuaries worldwide is therefore expected to alter metabolic processes, water quality, and biogeochemical cycling (Prum, Harris, & Gardner, 2024). Furthermore, heterotrophic respiration often increases more rapidly than primary production under warming, potentially shifting estuaries toward greater net CO₂ emissions and reducing their capacity to act as carbon sinks (Prum, Harris, & Gardner, 2024).”

Line 42: This would be a very nice spot in the manuscript to introduce DIC as an abbreviation for dissolved inorganic carbon.

Reply: The sentence was rephrased to introduce dissolved inorganic carbon (DIC) and improve clarity in **Lines 60-65**:

“To test the hypothesis that elevated temperatures enhance metabolic activity and nutrient regeneration, incubation experiments were performed with estuarine sediments collected during summer and winter. These experiments aimed to assess the response of sediment nutrient and dissolved inorganic carbon (DIC) fluxes across the in situ temperature range and under extreme warming conditions, including potential shifts in both the intensity and direction of nutrient exchange between sediments and the overlying water.”

Figure1: Please explain in the main text why you assume a lower degree of bioturbation and add to the caption that the bright brown features aim to reflect worm burrows and qualitatively depict the amount of expected bioturbation.

Reply: We agree that the role of bioturbation warrants consideration. However, this is a topic that we have intentionally treated only briefly because it overlaps with ongoing work by other groups, some of which has not yet been fully published. The decrease in bioturbation depicted in our conceptual framework is based on field observations from the region showing reduced infaunal abundance and activity under warmer conditions. While these observations have thus far been reported mainly through long-term monitoring efforts (e.g., Rahav et al., 2023, *The National Monitoring Program of Israel's Mediterranean Waters – Scientific Report on Biodiversity for 2022*), more detailed evidence from the southern Israeli Mediterranean shelf has recently been published (Lubinevsky & Tom, 2026), documenting substantial changes in benthic macrofaunal communities.

We have therefore added a brief statement in the main text and clarified the figure caption but prefer not to expand the discussion further so as not to pre-empt or potentially interfere with our colleagues' ongoing work.

We also note that the relationship between temperature and bioturbation is not universally positive. Studies from colder environments have reported increased bioturbation intensity with warming (e.g., Ouellette et al., 2004; Godbold & Solan, 2013). However, available evidence suggests that this response may be non-linear, with bioturbation peaking at intermediate temperatures and declining beyond a thermal optimum (e.g., Block et al., 2013). Given that background temperatures in the southeastern Mediterranean already exceed those reported for many temperate systems, a reduction in bioturbation under further warming is consistent with regional observations, although the mechanisms and generality of this response require additional investigation.

Lines 48 – 54: This section is completely detached from the rest of the methods and the information it contains is (at this point) not needed. Delete it and put the information to sections where it is needed.

Reply: We believe this section is more appropriately placed within the Methods section. In addition, we have expanded the description of the study site.

Line 55: Summer and winter are not very precise. Please specify at least the month, ideally provide dates.

Reply: information was added to the text.

Line 56: What kind of device was used for sampling?

Reply: We used a plastic grab.

Line 55 – 60: Was the sediment undisturbed? Or was it mixed? If it was mixed: How do the authors justify that the observed fluxes are not simply an artefact of the mixing?

Sandy silt implies very low permeability. Most likely the natural sediments' porewaters had distinct gradients. Likewise, the microbial community was set up for these gradients. If the sediment is mixed it can take weeks to several months before a sedimentary system is back to a natural equilibrated balance. In short: If the Sediments were mixed this might be an absolute red flag for publication. I want to urge the authors to clarify this in the manuscript and if need be, argue why they believe their results are still valid.

Reply: The sediments were indeed disturbed and likely mixed during the setup of the incubation system. We therefore reclassified the experimental setup; **lines 93-101:**

“Because the natural sediment structure was disrupted and the overlying water was not continuously stirred, this experimental setup is best classified as a slurry incubation, which provides a valuable approach for assessing potential nutrient regeneration rates. However, conventional slurry incubations often yield flux estimates that exceed those under in situ conditions because sediment mixing disrupts the sediment matrix and enhances solute exchange. In our setup, such overestimation is unlikely because no mixing was applied after the initial preparation, allowing a sediment-water interface to re-establish during the 12-h equilibration period. Thus, our incubations were designed to assess temperature effects on flux dynamics yet do not represent in situ fluxes.”

Reply: The oxygen consumption rate in our experiments was 2- 3 times lower than rates measured by reactors with undisturbed sediments and stirring of the overlying water, neglecting bottle effects.

Also, please see **182 – 195:**

“Dissolved oxygen (DO)-~~over time consumption rates~~, tracing respiration, were higher in our summer incubations compared to the winter experiment, and higher in the “extreme”

and “ambient-high” conditions compared to the “ambient-low” conditions (Fig. 2a and 3a). Average ($\pm$ SD) oxygen ~~concentration change rate~~ *influxes* in the summer experiment for ambient-low, ambient-high, and extreme treatments were -2.99 ± 1.51 , -4.66 ± 0.77 , and -3.67 ± 0.81 mmol m⁻² d⁻¹, respectively. These were higher than in the winter experiment, where the oxygen ~~influx concentration change rate~~ averaged -1.36 ± 0.4 , -2.35 ± 0.82 , and -2.23 ± 0.39 mmol m⁻² d⁻¹, respectively (Fig. 4; Table S1). These observations are in line with the expectation that higher respiration rates in temperate estuaries would be thermally controlled under ambient conditions (Apple et al., 2006). However, these rates were 2 to 3 times lower than oxygen fluxes measured on the southeastern Mediterranean shelf (Sisma-Ventura et al., 2021, 2022). The lower fluxes observed in our experiments may reflect methodological limitations, including the lack of stirring of the sediment-overlying water, the use of wide-neck bottles that were not fully airtight, and possible alterations of microbial activity caused by sediment mixing.”

Furthermore, the fluxes exhibited a clear temperature dependence, indicating that the observed responses were not merely an artefact of sediment mixing. Importantly, temperature influenced not only the magnitude of nutrient fluxes but also their direction.

Reply: We also added a paragraph about the unexpected effect of extreme warming on oxygen consumption, in lines 206-213:

“The lack of a significant increase in oxygen consumption rates under extreme heatwave conditions observed in our study is consistent with findings from several other estuarine systems. This recurring observation is somewhat unexpected, as higher temperatures are generally predicted to stimulate metabolic activity and, consequently, oxygen demand. The consistency of this pattern across different estuaries suggests that factors such as substrate limitation, shifts in microbial community structure, oxygen availability, or physiological adaptation may constrain the temperature-driven increase in respiration (Kauppi and Villnäs, 2022).”

Reply: We further discuss these issues in lines 349-366:

“Oxygen concentrations remained well above hypoxic levels throughout the incubations, indicating that aerobic remineralization was maintained across all treatments. This is particularly relevant given the elevated concentrations of dissolved organic matter typically observed in southeastern Mediterranean estuaries during summer, which support high rates of bacterial activity and organic matter degradation (Bar-Zeev and Rahav, 2015). Furthermore, oxygen conditions in regional estuaries span a broad range depending on water depth, hydrodynamic conditions, and organic matter loading (Bar-Zeev and Rahav, 2015; Suari et al., 2019). Therefore, the continued increase in nutrient and carbon fluxes under extreme warming may reflect enhanced remineralization and nutrient regeneration pathways that are not directly proportional to oxygen consumption alone. Notably, the extreme heatwave treatment (35 °C) did not result in significantly different oxygen consumption rates compared with the ambient-high treatment, whereas

nutrient and carbon fluxes continued to differ between these warming scenarios. Nevertheless, rates under both warming treatments remained elevated relative to the ambient-low treatment, indicating that substantial changes in sediment biogeochemical functioning had already occurred under seasonally high temperatures. Together, these results suggest that the response of sediment oxygen consumption to warming may become less pronounced at the highest temperatures examined, while nutrient regeneration and carbon cycling remain sensitive to further temperature increases. However, the mechanisms underlying these contrasting responses remain uncertain and warrant further investigation.”

Line 57: What material were the bottles made of?

Reply: The bottles were made of High-density polyethylene (HDPE). Information was added to the text.

Lines 60 ff: For clarification: Sediments collected in Winter and Summer were exposed to the same three different temperatures? If so: Why? What was the temperature in the field when the sediment was collected? Why not do one set at “in situ” temperatures and the other two at elevated temperatures that are expected under certain emission scenarios?

Reply: The rationale for exposing sediments collected in both summer and winter to the full temperature range is to simulate the sharp temperature fluctuations that characterize this region. Explained in lines 78-85:

“Temperature variability in the study area is largely driven by episodic “Sharav” (heatwave) events, during which hot, dry southerly air masses affect the region, especially during spring and autumn (Hochman et al., 2023). Therefore, under recurring heatwave conditions, sediments collected in both summer and winter may experience the region’s full temperature range. Moreover, exposing sediments to the full temperature range was necessary because temperature not only controls the magnitude of sediment nutrient fluxes but can also alter their direction, with sediments acting as either sources or sinks of nutrients under different thermal conditions. (Zhou et al., 2016; Rios-Yunes et al., 2023).”

Line 64: delete internal. No direct sunlight, but windows? What would indirect sunlight look like?

Reply: agreed and deleted

Line 65: Why the different times?

Reply: The time differences are very small, resulting from some time constraints during the incubations.

Line 66/67: This sentence does not make much sense: “What does “these” refer to? What do you mean by “conditions that would be dominated by heterotrophic activity”?

Reply: The sentence was rephrased

“Given the low light availability in estuarine waters, these conditions likely approximate a predominantly heterotrophic environment.”

Line 67 – 70: These sentences (reasoning for measured values) belong either to the introduction or the discussion.

Reply: It is essential for the reasoning for dark incubations.

Line 71: What do you mean by removed? Permanently? What subset? (number, which bottles, and most importantly: why?)

Reply: The entire paragraph was rephrased, **Lines 116-125:**

“Unlike benthic core incubations, where repeated subsampling is typically compensated by replacing the withdrawn water, slurry incubations rely on sacrificial sampling. Accordingly, bottles were destructively sampled at each time point rather than repeatedly subsampled throughout the experiment. Throughout the experiment, a subset of bottles was periodically removed from the temperature-controlled baths for sampling. In total, six time steps were sampled throughout the experiment in addition to initial conditions. Water samples were collected using a syringe, carefully withdrawing water from the overlying water column without disturbing the sediment. Bottles were sampled sequentially from the 24°C, 35°C, and 16°C treatments in a room maintained at 21°C, minimizing temperature deviations during handling and allowing measurements of dissolved oxygen and pH (at in-situ temperature) to be conducted as close as possible to the respective incubation conditions.”

Line 71: How much water? Was it replaced?

Reply: roughly 250 mL water samples, **line 126:**

“Water samples (~250 ml) were immediately filtered (0.45 µm, PES, Minisart, Sartorius) and subsampled for the different analyses.”

Reply: This concerns the measurements taken immediately upon sampling, namely dissolved oxygen and pH at in-situ temperature, in **Lines 134-139:**

“Bottles were sampled sequentially from the 24°C, 35°C, and 16°C treatments in a room maintained at 21°C, minimizing temperature deviations during handling and allowing measurements of dissolved oxygen and pH (at in-situ temperature) to be conducted as close as possible to the respective incubation conditions.”

Line 74: Filtered at what size? What filter (method) was used?

Reply: The information was added in **Line 126**:

“Water samples (~250 ml) were immediately filtered (0.45 µm, PES, Minisart, Sartorius) and subsampled for the different analyses.”

Line 75: How was the pH meter calibrated? On what scale are pH values reported?

Reply: The pH was measured on different scales. The first pH was measured on the NBS scale using a Radiometer, PHM240. The second was in the total hydrogen scale at a constant temperature of 25.000±0.006 °C with the CONTROS HydroFIA pH flow-through system, based on the ratios of light absorption at 434 and 578 nm before and after adding the factory-calibrated color indicator m-Cresol purple (Aßmann et al. 2011). The last was used for CO₂ air-sea gas exchange.

We added the following information, in **Lines 133-136**:

“In marine carbonate chemistry, the total pH scale (pH_t) includes free hydrogen ions and sulfate-associated hydrogen ions and is commonly used for seawater and estuarine studies. It is the most appropriate pH scale for describing the marine carbonate system, providing more accurate calculations of pCO₂.”

Line 78: This is confusing. What pH method was used now?

Reply: see the above explanation

Line 79: Why no alkalinity for the summer experiments?

Reply: we added the following information regarding the lack of alkalinity in the summer, in **Lines 139-140**:

“Due to limitations in water sample volume, alkalinity measurements were not conducted during the summer experiment.”

Line 81: Why at 25°C? Please rewrite the section on pH and alkalinity measurements in a clear and understandable way.

Reply: See the above explanation and the added information to the text in **Lines 133-136**.

Line 83: So far, I have not heard of undissolved organic nutrients.

Reply: Particulate organic nutrients are undissolved, yet it was not part of this study.

Line 95: Replace “through” with “throughout”

Reply: Corrected

Line 99: How were these fluxes calculated? How was oxygen measured?

Reply: we added the following section, **Lines 168-174**:

Unlike conventional slurry incubations, our flux calculations assumed an intact sediment-water interface after the 12 h equilibration period, since no mixing occurred during the incubation. For benthic fluxes, the standard calculation is based on the change in concentration over time in the overlying water:

$$F = \frac{\Delta C}{\Delta t} \times H$$

Where, F = benthic flux ($\text{mmol m}^{-2} \text{d}^{-1}$), $\Delta C / \Delta t$ = rate of concentration change in the water ($\text{mmol m}^{-3} \text{d}^{-1}$), and H = the overlying water height (m). Negative values indicate uptake by the sediment.

Line 101: These figures show concentrations, not consumption rates.

Reply: corrected in lines **180-183**:

*“Dissolved oxygen (DO) **change over time**, tracing respiration, was higher in our summer incubations compared to the winter experiment, and higher in the “extreme” and “ambient-high” conditions compared to the “ambient-low” conditions (Fig. **2a** and **3a**).”*

Line 99 – 105: Since it is not clear, how oxygen consumption was calculated, this section is difficult to follow. In addition, the described observations are of course very expected.

Reply: We now provide a full explanation of the flux calculation in Lines **168-174**.

As for the expectancy of the results, please see the added discussion in Lines **206-212**.

*“The lack of a significant increase in oxygen consumption rates under extreme heatwave conditions observed in our study is consistent with findings from several other estuarine systems. This recurring observation is somewhat **unexpected**, as higher temperatures are generally predicted to stimulate metabolic activity and, consequently, oxygen demand. The consistency of this pattern across different estuaries suggests that factors such as substrate limitation, shifts in microbial community structure, oxygen availability, or physiological adaptation may constrain the temperature-driven increase in respiration.”*

Further discussion in lines **349-366**:

“Oxygen concentrations remained well above hypoxic levels throughout the incubations, indicating that aerobic remineralization was maintained across all treatments. This is particularly relevant given the elevated concentrations of dissolved organic matter typically observed in southeastern Mediterranean estuaries during summer, which support high rates of bacterial activity and organic matter degradation (Bar-Zeev and Rahav, 2015). Furthermore, oxygen conditions in regional estuaries span a broad range depending on water depth, hydrodynamic conditions, and organic matter loading (Bar-Zeev and Rahav, 2015; Suari et al., 2019). Therefore, the continued increase in nutrient

and carbon fluxes under extreme warming may reflect enhanced remineralization and nutrient regeneration pathways that are not directly proportional to oxygen consumption alone. Notably, the extreme heatwave treatment (35 °C) did not result in significantly different oxygen consumption rates compared with the ambient-high treatment, whereas nutrient and carbon fluxes continued to differ between these warming scenarios. Nevertheless, rates under both warming treatments remained elevated relative to the ambient-low treatment, indicating that substantial changes in sediment biogeochemical functioning had already occurred under seasonally high temperatures. Together, these results suggest that the response of sediment oxygen consumption to warming may become less pronounced at the highest temperatures examined, while nutrient regeneration and carbon cycling remain sensitive to further temperature increases. However, the mechanisms underlying these contrasting responses remain uncertain and warrant further investigation.”

Lines 106/107: What experiments do you refer to here?

Reply: The different experiments and their results are described in lines **193-205**.

Line 118: What does “acidification rate” refer to? How is it defined/calculated?

Reply: Please see the following addition, lines **215-219**:

“Our acidification rates (decrease in pH over time) were the highest in the extreme heatwave conditions (varying between -3.1×10^{-3} and -5.5×10^{-3} pH units h^{-1} in winter and summer, respectively) and the lowest in the ambient-low conditions (varying between -0.7×10^{-3} and -1.4×10^{-3} pH units h^{-1} ; Fig. 2 and 3) (slope comparison test; $p < 0.01$ in both seasons; Table S2).”

Line 123: If temperature is the primary factor, which process is responsible for acidification? Is it only CO₂ partial pressure?

Reply: These issues were and still are addressed in Lines **311 -319**.

“As expected, pCO_2 concentrations showed a strong thermal regulation, with a production rate of 21.9 ± 1.69 , 9.47 ± 0.76 , and 1.97 ± 0.23 $\mu atm h^{-1}$ in the extreme, ambient-high, and ambient-low conditions, respectively ($p < 0.01$). In the extreme heatwave conditions, pCO_2 levels reached 4000 μatm . Thus, extreme heatwaves are expected to release massive amounts of CO₂ to the atmosphere. Comparable and even higher pCO_2 values were measured in different estuary systems (Borges and Abril, 2011). pCO_2 at 25°C was calculated to distinguish the thermal effect on partial pressure and the effect of anaerobic respiration under extreme heatwaves (Fig. 6). The thermal effect on partial pressure explains about 25.0% of the pCO_2 efflux; the rest is attributed to anaerobic respiration.”

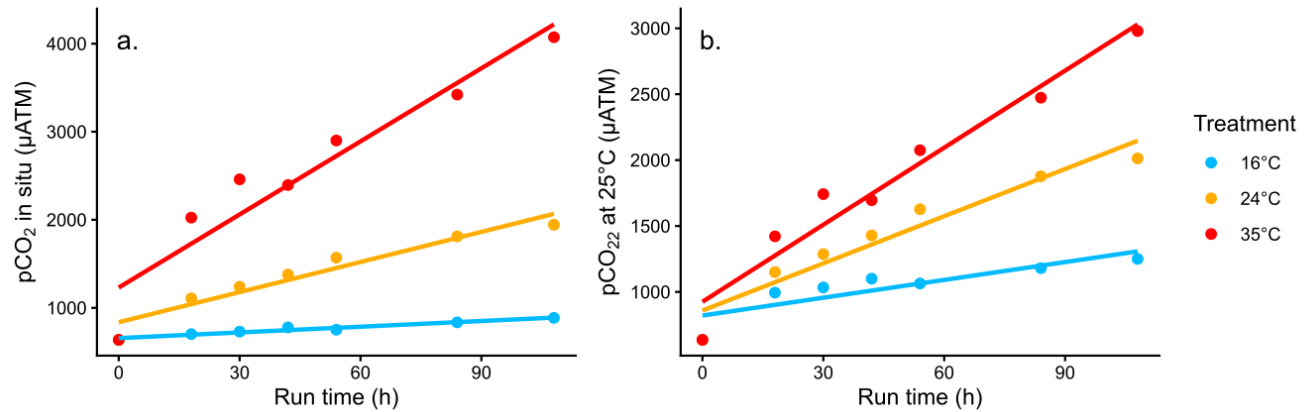


Figure 6: pCO₂ change with time for the winter experiment for (a.) in situ temperatures and (b.) corrected to 25°C.

Line 136: What do you mean by “setting up phase”?

Reply: See the full explanation in the comment below

Lines 138 – 141: “This initial pulse is not captured in detail in this work, as it represents a different mechanism related to the immediate response to the perturbation rather than the intermediate-term response investigated here, for which the post-initial-perturbation mode is the baseline.” Please rephrase this. What mechanism does the initial pulse represent, what do you mean by “the mediate response to the perturbation”, what does intermediate-term response even mean, and what exactly is a “post-initial-perturbation mode”?

Reply: We rewrite the section dealing with the initial transient nutrient pulse for better clarification in Lines **237-242**:

“The transient nutrient pulse observed during the first 12 h primarily reflects the initial phase of temperature adjustment. This is evident by the absence of a common intercept point for most of the nutrients, notably in the winter experiment (Fig. 3c-f). To a lesser extent, it can also reflect the disturbance associated with the setup preparation and sediment mixing. Consequently, the initial response is interpreted as an equilibration phase driven by experimental manipulation rather than the temperature-dependent biogeochemical response targeted in this study.”

Line 167: What model do you refer to here?

Reply: The sentence was rephrased for better clarity in lines **272-274**:

“Therefore, nitrogen cycling in our experimental setup appears to be dominated by organic matter remineralization, resulting in substantial NH₄⁺ production.”

Line 173: What does “these” refer to here? The mentioning of microbial community function comes very abrupt here. Is there any evidence for this? Please clarify your train of thought here.

Reply: Indeed, we lack evidence supporting such a shift in the microbial community; therefore, we removed this sentence.

Line 183: What you are saying here is: The fact that recycling of biogenic silica is also important in summer suggests strong recycling of biogenic silica. Need I say more? Please rephrase this.

Reply: Thank you for noting this point. The part “indicating that the recycling of biogenic silica is also important in summer” was removed from the sentence.

Line 195: What does “it” refer to here?

Reply: We replaced “it” with [This observation](#), in line 301.

Line 198: What do you mean by downstream?

Reply: Thank you for noting this point. This sentence was rephrased for clarity, in lines 303-305.

“Our results indicate that extreme heatwaves enhance the decomposition of both bloom-derived sedimentary deposits and downstream-transported organic matter, thereby enhancing nutrient fluxes to the overlying water.”

Line 201: “This stands in somewhat contrast to open marine systems, in which net CO₂ degassing decreases due to marine heatwaves”: This sentence stands without any reasonable context.

Reply: Thank you again for your attention to detail. We agree that the comparison between estuarine and open-ocean CO₂ exchange is not necessary in the context of this study. Accordingly, the sentence has been removed from the revised manuscript

Line 202: Rather use the plural “emissions”.

Reply: We replaced to emissions

Line 209: How did you calculate this percentage?

Reply: We added an explanation to the Figure 6 caption:

“The pCO₂ normalized to 25°C represents the biological contribution to CO₂ dynamics by removing the direct effect of temperature on CO₂ solubility and carbonate chemistry. In contrast, the in situ pCO₂ reflects the combined effects of biological processes and temperature. Therefore, the temperature effect can be quantified as the difference between the in situ pCO₂ and the pCO₂ normalized to 25°C.”

Line 216: What do you mean by footprint?

Reply: We rephrased the sentence for better clarity lines **327-329**:

“Although estuaries occupy only a small fraction of the Earth's surface (Laruelle et al., 2025), they constitute an important component of the global carbon sink (Borges and Abril, 2011; Deb and Mandal, 2021; Pusceddu et al., 2003).”

Line 217: Would you really describe a river mouth as a confined water body?

Reply: Under some conditions, notably in arid and semi-arid regions can be confined, in lines **329-330**:

“As shallow, at times confined, water bodies, they are also highly sensitive systems to temperature anomalies (Ahmed et al., 2025; Des et al., 2025).”

Line 221/222: This sentence is rather a review of previous literature that would be suitable for the introduction, not the discussion section.

Reply: It is, nevertheless, useful here to set the scene for the discussion.

Line 225: Did you mean represents instead of presents?

Reply: Corrected.

Line 227: What effects? These generic formulations are used very often in the script making it very hard to read.

Reply: We have modified to expand on this.

Line 230/231: This sentence is a bit weird. Especially the part “but possibly also aerobic ones”. That is super generic and does eventually not provide any information.

Reply: The sentence has been modified for clarity.

Line 231: What do you mean by “this region”?

Reply: We have modified this to make it clearer that we refer to the region on the margins of the southeastern Mediterranean.

“Oxygen concentrations remained well above hypoxic levels throughout the incubations, indicating that aerobic remineralization was maintained across all treatments. This is particularly relevant given the elevated concentrations of dissolved organic matter typically observed in southeastern Mediterranean estuaries during summer, which support high rates of bacterial activity and organic matter degradation (Bar-Zeev and Rahav, 2015). Furthermore, oxygen conditions in regional estuaries span a broad range depending on water depth, hydrodynamic conditions, and organic matter loading (Bar-Zeev and Rahav, 2015; Suari et al., 2019).”

Line 234/235: The fungi story comes quite out of the blue and lacks explanation.

Reply: We considered it important to note this is another possible contributor. However if this raises confusion, we deleted the sentence.

Line 238: See comment on the abstract.

Reply: Corrected

Line 240: There is a “that” missing after fact.

Reply: Sentence corrected.

Line 252: You already mentioned the 4000 μatm .

Reply: We removed this repetition and rephrased the following sentences in lines 385-391. We define it as an increase in the potential CO₂ release

“Under experimental heatwave conditions, CO₂ production yielded a maximum estimated air-sea flux of 27.13 mmol m⁻² d⁻¹, assuming an average wind speed of ~4.0 m s⁻¹ (Sisma-Ventura et al., 2017) and an atmospheric CO₂ concentration of 420 ppm. By comparison, the maximum daily flux under ambient-high conditions was 11.71 mmol m⁻² d⁻¹, while the CO₂ flux of adjacent coastal water was 6.2 mmol m⁻² d⁻¹ during the warm season (Sisma-Ventura et al., 2017). These estimates suggest a substantial increase in the potential for CO₂ release during heatwave conditions.”

Line 253: How was this flux correlated to wind speed? Please provide formulas for the respective calculations.

Reply: We added the calculation to the Methods section, lines

Air-sea CO₂ fluxes were calculated using the bulk gas-exchange formulation:

$$F = k \times K_0 \times (pCO_{2,sw} - pCO_{2,air})$$

where F is the air-sea CO₂ flux (mol C m⁻² d⁻¹), k is the gas transfer velocity, K_0 is the solubility coefficient of CO₂ in seawater, $pCO_{2,sw}$ is the partial pressure of CO₂ in surface seawater, and $pCO_{2,air}$ is the atmospheric CO₂ partial pressure (contemporaneous atmospheric ~420 μatm). The gas transfer velocity was estimated using the quadratic wind-speed parameterization of Wanninkhof (2014):

$$k = 0.251 U_{10}^2 \left(\frac{Sc}{660} \right)^{-0.5}$$

where U_{10} is the wind speed at 10 m above the sea surface (m s⁻¹) (from Sisma-Ventura et al., 2017) and Sc is the Schmidt number of CO₂, calculated as a function of water temperature. CO₂ solubility (K_0) was calculated from in situ temperature and salinity according to Weiss (1974).

Line 254: How was this estuary flux calculated/measured? What estuary are you referring to?

Reply: See the above corrections

Lines 262 – 266: The fact that the conclusions are four sentences over four lines speaks for itself.

Reply: The conclusions were expanded, now including the main findings of this study.

“Temperature appears to be an important driver of remineralization processes in estuarine sediments, and these effects may become more pronounced under future warming and marine heatwave conditions. In this study, simulated heatwaves were associated with enhanced benthic remineralization and generally higher sediment-water exchanges in a semi-arid, warm-adapted estuary. Extreme heatwave conditions were accompanied by higher rates of nutrient regeneration and CO₂ production, resulting in increased effluxes of NH₄⁺, PO₄³⁻, Si(OH)₄, and dissolved inorganic carbon. The observed covariance among oxygen depletion, acidification, and nutrient release suggests a close linkage between organic matter remineralization and carbonate system dynamics.

Notably, under extreme warming (35 °C), nutrient and carbon fluxes continued to respond to increasing temperature, whereas oxygen consumption did not differ significantly from the ambient-high treatment. This pattern may indicate that nutrient regeneration and carbon cycling respond differently to elevated temperatures than respiratory oxygen demand, although the underlying mechanisms remain uncertain. Overall, our results suggest that marine heatwaves could increase the potential for Mediterranean estuarine sediments to act as sources of regenerated nutrients and CO₂, with possible implications for coastal carbon cycling and eutrophication dynamics.

The study was based on sediment slurry incubations designed to isolate potential sediment responses under controlled conditions, and therefore does not fully capture the complexity of natural estuarine environments. Consequently, the measured fluxes should be regarded as potential rates rather than direct estimates of in situ benthic exchange. Future in situ observations and longer-term experiments are needed to determine the magnitude, persistence, and ecological significance of sediment biogeochemical responses to natural marine heatwaves.