

Referee no 2

This manuscript presents an experimental investigation of the impact of temperature changes related to heatwaves on sedimentary metabolism. While the overall conclusion is not very surprising – higher temperatures increase microbial metabolism and thus increase oxygen consumption, nutrient release, and DIC production – the study could be a valuable addition, as it shows experimentally what rates can be expected for an estuary that is exposed to higher temperatures.

In its current form, however, this manuscript is not ready for publication. The experimental design and methods are vague and do not make it clear why the specific approach was chosen. As I read it, this seems to be a combination of two separate approaches: classical flux incubations – which are typically done with cores and mixing of the overlying water, and slurry incubations – which are typically done with continuous mixing of the slurry to get rates, and really only allow comparison between treatments. The authors now seem to set up slurry incubations, but they discuss the results using flux units ($\text{mmol m}^{-2} \text{d}^{-1}$), which they then compare to other fluxes in the literature. The problem with this becomes quite clear when they discuss oxygen fluxes, which are way lower than what you would expect from these types of sediments.

To make this manuscript publication-worthy, the authors would need to

(i) rewrite the methods so the experiment can be reproduced (which should always be the case for any publication), and also clarify the reason why a certain design was chosen (e.g. you use annual mean and mean winter, and then call the treatments ambient winter and ambient summer -> why not take the annual summer temperature?)

Reply: We are grateful for these comments and agree that a better classification of the experiment and the rationale behind this classification should be presented more clearly. We have therefore substantially revised the Methods section, rewriting it to provide a more detailed description of the experimental setup, justify its classification as a slurry incubation experiment, and clarify the implications of sediment disturbance and mixing for the interpretation of the measured fluxes. We believe these revisions improve both the transparency of the methodology and the reader's understanding of the experimental approach.

We added a section describing the chosen experimental temperature range.

Lines 77-84: *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).

We then added a sentence about the Na'aman Estuary being extremely shallow and therefore highly sensitive to temperature shifts

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.”*

The issues related to the sediment matrix disturbance are then introduced

Lines 92-100: *“Because the natural sediment structure was disrupted, and since the stirring of the overlying water was not employed, this experimental setup is best classified as a “slurry” incubation. Slurry incubations provide a valuable approach for evaluating potential rates of nutrient regeneration; however, sediment matrix disturbance can lead to flux estimates that exceed those under in situ conditions. We considered such an overestimation unlikely, as oxygen uptake rates were 2-3 times lower than those previously measured in marine sediments from the same region (Sisma-Ventura et al., 2021, 2022). Since the incubations do not represent in situ fluxes, our analysis focuses on the temperature-driven changes in nutrient flux rates and directions rather than on their absolute magnitude.”*

Subsampling is now described in detail:

Lines 115-125: *“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.”*

Flux calculation

Lines 167-174: *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.

(ii) present all results in rates (e.g., $\mu\text{mol cm}^{-3} \text{ d}^{-1}$) – not fluxes. Your experimental setup does not allow you to derive fluxes that are comparable to in-situ situations

Reply: Thank you for noting this issue. Fluxes in our setup were calculated under the assumption that an intact sediment-water interface had been re-established following the 12 h equilibration period, as the sediment was not mixed during the subsequent incubation (Lines **172-180**).

We note, however, that the incubations do not represent in situ conditions. Accordingly, our analysis emphasizes temperature-driven changes in nutrient flux rates and directions rather than their absolute values (Lines **90-100**).

(iii) adjust the discussion accordingly, the experimental design makes it almost impossible to compare to other studies, not does it allow you to make large inferences about any system-scale processes (even of your own site), the mixing you did before starting the experiment almost certainly introduced artefacts, which implies that most of the oxygen consumption is likely related to reoxidation processes, which might not have the same temperature dependence as carbon oxidation.

Reply: As kindly observed by the reviewer, the oxygen fluxes are 2-3 times lower compared to fluxes measured in coastal water of this region. To the best of our knowledge, several factors could affect oxygen fluxes, 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. We also agree that fluxes absolute values in our setup can't be compared with benthic fluxes representing in situ conditions. We do argue, however, that the temperature-dependent rates and flux direction can be compared with other heatwave control experiments.

We now acknowledge the low oxygen fluxes in lines **187-192**:

“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.”

We compare the temperature dependence, and not the absolute flux magnitude, to other heatwaves in lines **197-209**:

“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. Incubations of intertidal flat sediments (Arcachon Bay, Atlantic coast, S-W France), where in summer experiments, oxygen consumption measured in the “extreme heatwave” condition (25-29 °C) was significantly higher than the “ambient” condition (17-22 °C), yet not significantly different from their “strong heatwave” conditions (22-26 °C; Fouet et al., 2025). Likewise, an experimental heatwave setup, using in situ chambers over the sandy bay seafloor of the Finnish Archipelago, showed higher yet variable oxygen consumption in most chambers under heatwave conditions (Göbeler et al., 2025). A moderate heatwave boosted oxygen consumption in an experimental heatwave setup on muddy sediments in the Northern Baltic Sea estuary. In this setup, however, a strong summer heatwave suppressed oxygen consumption rates (Kauppi and Villnäs, 2022). Likewise, oxygen consumption rates in our experimental setup were significantly higher in extreme conditions than in the ambient-low conditions ($p > 0.05$ in both summer and winter experiments), but not significantly different from “ambient-high” conditions (Table S2). 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).”

Initial phase transient nutrient pulse. We added a paragraph explaining this issue in the revised version, lines **236-242**:

“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 setup preparation. 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.”

The limitation of the experimental setup, Lines **334-342**:

“Indeed, our experimental results suggest a substantial temperature influence on sediment biogeochemical processes under ambient-high and extreme warming, resulting in altered exchanges across the sediment-water interface. However, the experimental design was specifically intended to examine sediment responses under controlled conditions and therefore does not fully represent the complexity of natural estuarine environments. Previous studies have shown that marine heatwaves can also affect biogeochemical processes in the overlying water column, although the magnitude and direction of these responses appear to vary among systems (Douglas et al., 2025; Göbeler et al., 2025; Palmas et al., 2025). Therefore, this study measured fluxes should be regarded as potential sediment responses rather than direct estimates of ecosystem-scale fluxes.”

Instead, you could put more effort into extracting the coupled processes from your incubations – with the different production rates, you could try to make a mass budget or mechanistic model of your experiment to derive the processes (e.g, denitrification, aerobic respiration) and see if any processes respond non-linearly (although that is hard to derive from 3 different treatment points).

Reply: We are thankful for this comment; we have now fully addressed these issues:

Lines 250-258: *“Significant differences were observed between the NH_4^+ effluxes of extreme and ambient-high from ambient-low conditions in summer (slope comparison test; $p < 0.01$ and $p = 0.034$, respectively) but were not significantly different in winter (Table S2). We observed a strong negative covariance between NH_4^+ release and oxygen consumption during summer incubations (polynomial fit, $R^2 > 0.90$; Fig. 5), whereas we detected no comparable relationship in winter. NH_4^+ release was also negatively correlated with the acidification trend ($R^2 = 0.88$). These findings suggest that respiration-driven remineralization was more tightly linked to nutrient regeneration and carbonate system changes during summer than during winter (Devol and Abraham, 1993). “*

Lines 262:280: *Unlike NH_4^+ effluxes, NO_x fluxes were highly variable in both magnitude and direction. During the summer experiment, sediments acted as a net sink for NO_x under ambient-low ($-37.1 \pm 20.3 \mu\text{mol m}^{-2} \text{d}^{-1}$) and extreme ($-77.7 \pm 58.3 \mu\text{mol m}^{-2} \text{d}^{-1}$) conditions, whereas a slight efflux was observed under ambient-high warming ($5.4 \pm 15.3 \mu\text{mol m}^{-2} \text{d}^{-1}$; Fig. 4). In winter, ambient-low and ambient-high treatments resulted in net NO_x release to the overlying water (19.0 ± 19.8 and $46.6 \pm 33.8 \mu\text{mol m}^{-2} \text{d}^{-1}$, respectively), while the extreme treatment shifted to net NO_x uptake ($-10.1 \pm 16.7 \mu\text{mol m}^{-2} \text{d}^{-1}$). The tendency for enhanced NO_x uptake under extreme warming may be consistent with increased nitrate consumption through denitrification and other anaerobic nitrogen transformation pathways (Devol and Abraham, 1993). The substantially higher NH_4^+ fluxes relative to NO_x fluxes (up to ~26-fold) suggest that denitrification alone cannot account for the observed NH_4^+ production. Instead, the excess NH_4^+ likely originated from the remineralization of organic nitrogen compounds, such as amino acids and other nitrogen-rich organic matter. Under extreme warming conditions, enhanced microbial degradation of organic matter may have increased NH_4^+ production, while part of the regenerated nitrogen was subsequently transformed through nitrification-denitrification pathways (Devol and Abraham, 1993). The temporal patterns observed throughout both the summer and winter incubations (Figs. 2e, 3e-f) reveal alternating consumption and release of both NO_x and NH_4^+ , highlighting the dynamic coupling between nitrogen regeneration and removal processes.*

Lines 367-377: *Extreme heatwave conditions in our experimental setup were associated with enhanced sediment NO_x uptake, suggesting increased denitrification and/or other nitrogen loss pathways. This observation is noteworthy given that the adjacent Qishon Estuary has been reported to support heterotrophic nitrogen fixation (Geisler et al., 2020).*

At the same time, NH_4^+ production, likely driven primarily by organic matter remineralization, exceeded NO_x fluxes by approximately 26-fold, indicating limited recycling of regenerated nitrogen within the sediment. The strong covariance between oxygen consumption (and acidification) and the effluxes of NH_4^+ and Si(OH)_4 further supports enhanced organic matter degradation under extreme warming. However, because the present study did not directly quantify denitrification, nitrogen fixation, or organic matter composition, the mechanisms responsible for these patterns should be interpreted with caution.

I have listed some small remarks that appeared as I was reading the manuscript below, some of which are repetitions of what I have written here.

Kind regards

Sebastiaan van de Velde

L51: why annual average and not mean summer temperature?

Reply: Summer conditions in the region both represent an outsize portion of the year and have a very large temperature variability due to heatwaves. We have expanded on this in the text now.

L52: that statement should not be in methods

Reply: We considered it important to note here why we elected these temperatures, and thus this information is relevant.

L57: introducing mixing artefacts – are you sure you are measuring temperature effects?

Reply: As approximately the same amount of mixing was applied to all treatments, the difference should represent the effect of the treatments, even with the effect of mixing. We have now expanded in the text on the rationale of the experimental design and operation.

L71: did you collect several timepoints or just before/after?

Reply: For statistical robustness of the time series, six time steps were sampled in addition to initial conditions. This is now clearly stated in the text with expansion on the methodological approach.

L102: these fluxes are very low for intertidal sediments

Reply: We agree and actually consider this to be a good indication of limited reoxidation during the preparation of the experimental setup. We would note that overall, oxygen consumption in sediment in the region is not very high (see also Sisma-Ventura et al., 2022, Deep Sea Research Part I). We also speculate this has to do with relatively low concentrations of labile organic matter in the sediment, but this is outside the scope of this work.

Lines 178-191: *This study's experimental results show a significant thermal effect on the flux magnitude of most solutes, with differences observed across the tested seasons and thermal treatments. 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). Average ($\pm$ Standard Error) oxygen influxes in the summer experiment for ambient-low, ambient-high, and extreme treatments were -2.99 ± 1.51 , -4.66 ± 0.35 , and -3.67 ± 0.81 mmol m⁻² d⁻¹, respectively. These were higher than in the winter experiment, where the oxygen influx averaged -1.36 ± 0.4 , -2.35 ± 0.35 , 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.*

L117: not oxygen consumption but DIC production related to the metabolism is the reason for acidification

Reply: We consider these to be two sides of the same reactions, but changed the sentence to state change is due to “Production of dissolved inorganic carbon”.

Figure2: your oxygen profile suggests non-linear trends (I assume that red dot is overlying the other coloured dots?), so you would underestimate the actual oxygen consumption at timepoint 0 if you do not use a quadratic fit here. The same goes for other solutes (e.g. NH₄). The fact that the production is non-linear suggests that there are quite some transient effects occurring in the experiment – which might be related to the potential mixing artefact.

Reply: We now address this issue in lines **235-242**

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 setup preparation. 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.

L161: I would not be too sure that those NO_x fluxes are significant, so I would not put too much faith in those results

Reply: We did notice a tendency for enhanced NO_x uptake under extreme warming, which is consistent with increased nitrate consumption through denitrification and other anaerobic nitrogen transformation pathways (Devol and Abraham, 1993) in lines **268-270**. But we agree with the reviewer and therefore added a sentence about the limitation of this study setup, in lines **375-377**:

“However, because the present study did not directly quantify denitrification, nitrogen fixation, or organic matter composition, the mechanisms responsible for these patterns should be interpreted with caution.”

Figure5: why would you do cross-plots of the concentrations? It would make more sense to compare the fluxes

Reply: We wanted to present the trend rather than just the derivative; we think this presentation is more genuine.