<u>Response to Referee Comment 2</u>

Dear Dr. Cibic,

We deeply thank you for your helpful comments, improving the quality and accuracy of the manuscript. Please find below the detailed explanations about how we have considered and answered each comment.

Concerning the modifications made on the revised version of the manuscript explained in the present document, please note that:
- additional sentences or words were highlighted in blue
- deleted sentences or words were written ~~in purple and crossed out~~

Please note that modifications made in response to the comments of the other referee (Referee 1, A. Regaudie-de-Gioux) are also indicated on the document, with the added parts highlighted in yellow and deleted parts ~~crossed out in red~~.

1. T. Cibic: The work by Soulié et al. "Simulated terrestrial runoff shifts the metabolic balance of a coastal Mediterranean plankton community toward heterotrophy" investigated the consequences of terrestrial runoff on plankton communities and some biological processes in the Thau lagoon on the Mediterranean coast. The results come from an in situ mesocosm experiment in which terrestrial runoff was simulated by adding soil and various chemical and biological variables were analyzed for 18 days.

The paper is well written, focused and interesting and should be published in Biogeosciences after revision.

A major criticism of this work is the fact that no real phytoplankton data are presented. All results were derived from Chl-a sensor data, including phytoplankton growth and loss rates. I know this is becoming more common and accepted lately, but similarly to Chl-*a* fluorescence and oxygen sensors data, for which some calibrations were done, actual phytoplankton counts and identifications should have been done on at least some samples to check if there is a match between Chl-*a* and microphytoplankton. Since flow cytometry was used in the paper to estimate heterotrophic bacterial abundance, the same method could have been used to assess the smaller phototrophic picoplankton. It is a pity that there is no information on the actual composition of the phytoplankton, as this is the topic of the article. If the authors have these (already published or unpublished) results, I think it would be a great addition to this publication to at least mention them in the discussion.

The authors: We agree that information on the composition of the phytoplankton community is important to complement chlorophyll-*a* data. In our experiment, phytoplankton community was analyzed by microscopy (identification and counting) and by flow cytometry. All these data are presented and published in Courboulès et al. (2023), which is cited at multiple times in the manuscript. These data show a very good agreement with chlorophyll-*a*, especially microphytoplankton, diatoms, identified and counted by microscopy, and nanophytoplankton, counted by flow cytometry, and this good agreement was already reported in Courboulès et al. (2023). As these data are already published and well-discussed in Courboulès et al. (2023), we feel that it is not necessary to present them in details again in the present manuscript. However, we agree that it should be mentioned in the Discussion section. Hence, we added this information in the Discussion section (L363, L377).

L363: "The phytoplankton community investigated in the present study was typical of Thau Lagoon in spring (Trombetta *et al*. 2019), mainly composed of diatoms, cryptophytes, and small nano- and picophytoplankton (Courboulès *et al*. 2023). The ~~This~~ negative effect of light limitation induced by the runoff on phytoplankton biomass is consistent with a mesocosm experiment performed in the Baltic Sea…"

L377: "As mentioned earlier, Chl-*a* strongly increased during the second part of the experiment in the runoff treatment. This positive response was mainly due to an increase in the abundance of diatoms, mainly *Chaetoceros sp.* and *Cylindrotheca sp*., cyanobacteria, and autotrophic dinoflagellates (Courboulès *et al*. 2023). In addition, the pico- and nanophytoplankton abundances counted with flow cytometry also increased at this time of the experiment (Courboulès *et al*. 2023). Overall, a very good agreement was found between the response of the Chl-*a* concentration and phytoplankton abundances, measured by both microscopy and flow cytometry, during the entire experiment (Courboulès *et al*. 2023). ~~This~~ The accumulation of phytoplankton biomass during the second part of the experiment in the runoff treatment was related to the strong increase in phytoplankton growth rate from d10"

2. T. Cibic: Introduction

I think it would be easier for the reader if some scientific questions or hypotheses were added at the end of the introduction section to structure the discussion.

The authors: We agree with this comment. Therefore, a sentence was added presenting some hypotheses at the end of the Introduction section (L77).

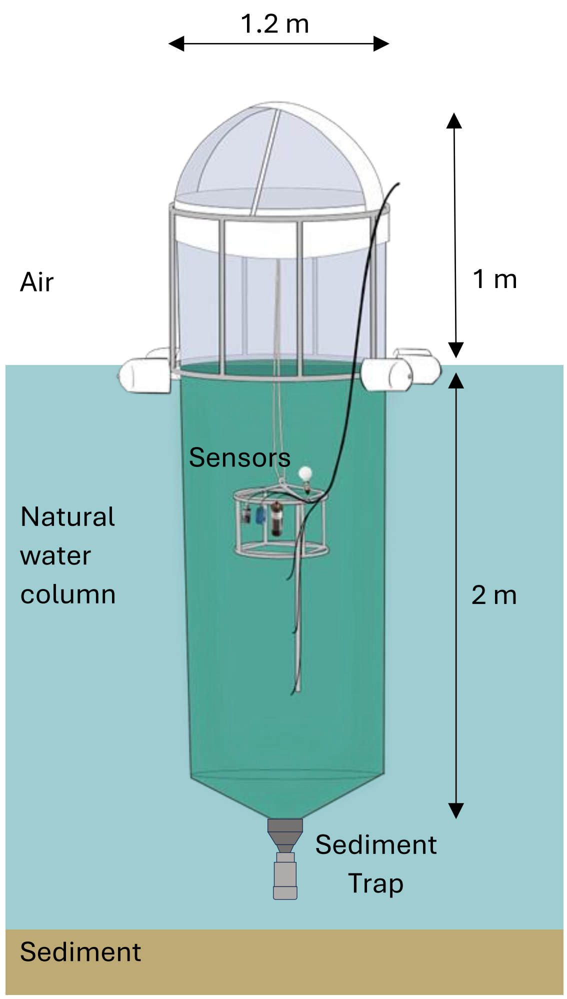
L77: "In the present study, high-frequency data from automated sensors immersed in the mesocosms were used to estimate GPP, R, μ and L in every mesocosm, and assess how both the metabolic and trophic indices of the community responded to the simulated runoff. Manual sampling was performed to assess dissolved and particulate materials as well as photosynthetic efficiency and carbonate system parameters. We hypothesized that (1) the metabolic index would be shifted by the runoff toward heterotrophy through light reduction and inputs of organic matter, and that (2) the terrestrial runoff would affect the trophic index by creating imbalance between phytoplankton and its factors of loss."

3. T. Cibic: M&Ms

Experimental design

It is not stated what the final volume in each mesocosm is. It is also not clear if the mesocosms are sealed at the top and what the bottom is like. Are the mesocosms open or sealed at the bottom? Are they floating? Please add this information.

The authors: We agree that this information was not clear, so we added some parts in the M&Ms section (L89, L100). We explained that, initially, each mesocosm was filled with 2200 L of natural lagoon water. Then, a total of 510 L for each mesocosm was sampling during the experiment, representing 23% of the initial volume. Mesocosms were sealed at the bottom by a sediment trap, floating, and attached on a floating pontoon, such as represented in the figure below that we added in Supplementary Information.



L89: "Six mesocosms were established in the lagoon. Each mesocosm consisted of a bag, sealed at the bottom, made of nylon-reinforced 200 µm thick vinyl acetate polyethylene film which was 280 cm high and 120 cm wide (Insinööritoimisto Haikonen Ky, Sipoo, Finland). Each mesocosm was equipped with a sediment trap at the bottom. A schematic representation of the mesocosm set-up can be found in Soulié *et al.* (2021) and in **Supplementary Information**."

L100:"Throughout the experiment, a total of 510 L was sampled for each mesocosm, representing 23% of the initial volume of the mesocosms."

4. T. Cibic: L76: The experiments were conducted for 18 days. In my opinion, this is a very long time to test the effects of a flash flood on the coastal area. There is a strong mixing effect by the seawater, and even in a confined area like a lagoon, the terrestrial runoff will eventually be diluted in a few days. The choice of the duration of the experiment should be discussed.

The authors: We agree with the fact that flash floods do not last as long as 18 days. However, the goal of the experiment was not only to study the short-term impact of flash floods and terrestrial runoffs, but also the longer-lasting consequences of such events, even when terrestrial matter has already sedimented and/or dissolved. As explained to the Referee 1, the duration of the experiment was set to 18 days to respond to multiple objectives. As explained above, the experiment needed to last long enough so that the responses of plankton at middle-term could be monitored. Indeed, we have already performed multiple mesocosm experiments in spring in Thau lagoon in the past (see Courboulès et al. 2022, Soulié et al. 2022a, 2022b) and reported that interesting dynamics as the occurrence of a phytoplankton bloom can occur up to almost 3 weeks after the beginning of the experiment, even in control mesocosms. Some sentences were added in the Material and Method section regarding the choice of the duration of the experiment (L84).

L84: "The duration of the experiment was set as 18 days to be able to monitor the responses of plankton at medium-term (multiple days to weeks), as interesting dynamics were already reported in control treatments during other experiments in Thau Lagoon up to almost 3 weeks after the start of the experiment (Courboulès *et al*. 2021, Soulié *et al*. 2022a), while coping with COVID-19 pandemics restrictions."

Courboulès, J., Mostajir, B., Trombetta, T., Mas, S. & Vidussi, F. Warming disadvantages phytoplankton and benefits bacteria during a spring bloom in the Mediterranean Thau Lagoon. *Front. Mar. Sci.* **9**, 878938. https://doi.org/10.3389/fmars.2022.878938, 2022.

Soulié, T., F. Vidussi, J. Courboulès, S. Mas, and B. Mostajir. Metabolic responses of plankton to warming during different productive seasons in coastal Mediterranean waters revealed by in situ mesocosm experiments. *Sci. Rep*. **12**:9001. Doi: 10.1038/s41598-022-12744-x, 2022a.

Soulié, T., F. Vidussi, S. Mas, and B. Mostajir. Functional stability of a coastal Mediterranean plankton community during an experimental marine heatwave. *Front. Mar. Sci*. **9**:831496. Doi: 10.3389/fmars.2022.831496, 2022b.

5. T. Cibic: L104: Why was the sampled soil left to mature for two weeks? Why was this step necessary? In a flash flood there is no such step, the soil is washed away directly by the heavy rain and transported into a river and eventually into an estuary. Flash floods do not last 14 days, this is unclear and should be better explained.

The authors: The maturation process, during which the soil of the Puéchabon forest was mixed with water from the Vène river, aimed at recreating the natural transport of soil that can be transported for several weeks by rivers before reaching coastal waters. The goal of this was to mimic naturally occurring processes, as during natural terrestrial runoff, the most labile compounds of the soil can be degraded or transformed during their transportation by river water (Lobbes et al. 2000, Müller et al. 2018). We agree that the choice of this maturation step should be discussed. Therefore, we added some sentence in the Discussion section regarding this (L375).

L375: "Finally, Deininger *et al.* (2016) used a resin in their soil extraction procedure, yielding higher inorganic and organic nutrient concentrations in their extract compared to the protocol performed in the present study but being farther from natural terrestrial runoffs (Scharnweber *et al.* 2021). In the present experiment, a maturation step of the soil in river water of 14 days was performed, aiming at mimicking processes naturally occurring during the transportation of soil to coastal waters during terrestrial runoffs, such as the degradation of the most labile organic compounds (Müller *et al*. 2018). This duration of maturation can be considered as a long residence time in river water, regarding the fact that flash floods in the Mediterranean region are usually faster. Therefore, it can be supposed that the terrestrial matter added in the present study contained lower levels of labile organic compounds than what can be found during flash floods. This emphasises the need for extreme caution when comparing experimental studies investigating terrestrial runoff effects because protocols are often different from one study to another."

Lobbes, J. M., Fitznar, H. P., and Kattner, G.: Biogeochemical characteristics of dissolved and particulate organic matter in Russian rivers entering the Arctic Ocean. *Geochemica et Cosmochimica Acta* **64**(17):2973-2983. https://doi.org/10.1016/S0016-7037500°00409-9, 2000.

6. T. Cibic: Discussion

What I miss here is a discussion of the ecological implications of these results at the lagoon mesoscale. In particular, a detailed discussion of the trophic state of the lagoon and a comparison with other Mediterranean lagoons.

The authors: We agree with this comment. In accordance, we added some sentences discussing about the ecological implications of our results at the lagoon mesoscale in the Discussion section (L474). Please also note that upon this addition, several references were added to the Reference list accordingly (L534, L566, L621, L627).

L474: "The results of the present experiment suggest that the climate-change related intensification of terrestrial runoffs could temporarily alter metabolic and trophic indexes of the water column of the lagoon during productive seasons (Trombetta *et al*. 2019), potentially shifting it toward heterotrophy and disrupting its trophic balance. Coupled with terrestrial runoff-induced shifts of microbenthic net community production toward heterotrophy (Liess *et al*. 2015), these alterations could interact with ongoing shifts occurring in the lagoon, such as the changes in trophic functioning toward mixotrophy and heterotrophy related to oligotrophication (Derolez *et al*. 2020b). Such consequences may also be seen in other Mediterranean lagoons, as turbidity and extreme flood events were reported to control phytoplankton abundance and phenology in oligotrophic Mediterranean coastal lagoons in Southern France and Corsica (Bec *et al*. 2011, Ligorini *et al*. 2022)."

L534: Bec, B., Collos, Y., Souchu, P., Vaquer, A., Lautier, J., Fiandrino, A., Benau, L., Orsoni, V., and Laugier, T.: Distribution of picophytoplankton and nanophytoplankton along an anthropogenic eutrophication gradient in French Mediterranean coastal lagoons. *Aquat. Microb. Ecol*. **63**:29-45. https://doi.org/10.3354/ame01480, 2011.

L566: Derolez, V., Malet, N., Fiandrino, A., Lagarde, F., Richard, M., Ouisse, V., Bec, B., and Aliaume, C.: Fifty years of ecological changes: Regime shifts and drivers in a coastal Mediterranean lagoon during oligotrophication. *Sci. Tot. Env.* **732**:139292. https://doi.org/10.1016/j.scitotenv.2020.139292, 2020b.

L621: Liess, A, Faithfull, C., Reichstein, B., et al.: Terrestrial runoff may reduce microbenthic net community productivity by increasing turbidity: a Mediterranean coastal lagoon mesocosm experiment. *Hydrobiologia* **753**:205-218. https://doi.org/10.1007/s10750-015-2207-3, 2015.

L627: Ligorini, V., Malet, N., Garrido, M., Derolez, V., Amand, M., Bec, B., Cecchi, P., and Pasqualini, V.: Phytoplankton dynamics and bloom events in oligotrophic Mediterranean lagoons: seasonal patterns but hazardous trends. *Hydrobiologia* **849**:2353-2375. https://doi.org/10.1007/s10750-022-04874-0, 2022.