Response to Reviewer 3 (https://doi.org/10.5194/bg-2023-169-RC3)

General comments:

This manuscript examines the ecological and environmental impact of the recently investigated cable bacteria on the geobiology of sediments in brackish environments, and their effect on the preservation of tests of calcareous foraminifera inhabiting these environments. Overall, the methods, results, and discussion demonstrate a scientific rigor that should engage the Biogeosciences readership. However, there's a need for further exploration from the foraminifera perspective, which this study initiates.

The primary concerns of this reviewer include the absence of qPCR results at Station 3, and a perceived lack of adequate consideration regarding the carbonate saturation state in discussing carbonate test dissolution, which is deemed a significant question. As explain to Reviewer 1, we encountered some administrative limitations during our collaboration with the Microbiology Institute of Biology in Aarhus University (Denmark). In this regard, we would like to make it clear in the Method section by adding:: "For administrative reasons, it was only possible to carry out these DNA analyses for stations 1 and 2."

About the carbonate saturation state: a second parameter of the carbonate system is needed in addition to pH to calculate it (such as DIC or alkalinity). We agree that this information is relevant to our subject but we have no such data, which is a shame. However, the stage of dissolution of the calcareous shells seems sufficiently edifying here to support the discussion about dissolution process.

Furthermore, detailing the ecological aspects of each station, such as the visual characteristics of the sediments, grain size distributions, limnological settings etc., could enhance the discussion on the study's results, yet such information is notably absent, for instance, in Table 1. It is recommended that these issues be addressed either through revision or in subsequent publications within Biogeosciences. Majority of those ecological parameters have been studied and have been discussed in Fouet (PhD report, 2022). She discusses the granulo-hydodyn-marine influence link and its correlation with the diversity and relative abundance of some foraminiferal taxa in the Auray estuary that are more closely associated with the marine environment. A cross-reference to this work can be added to Table 1.

The comments are noted in below:

P1L7 Change bold text to normal. This is the affiliation required by the laboratory and the university with which we are associated.

P3L72 In this context, Charrieau et al. (2018c) and Charrieau et al., 2022 https://doi.org/10.1038/s41598-022-10375-w to mention may also contribute to the depth of the discussion. We agree that Charrieau et al (2018c) contributes to the specific point about test dissolution below a certain pH value for estuarine foraminiferal species (*Ammonia* sp. and *Elphidium* sp.). Charrieau et al (2022) focuses on a large symbiont-bearing benthic species in warm coral reef environments (*Peneroplis* spp.), which is very different from our case study. This is the reason why we did not include it.

P5L113 The authors stated that the samples were washed with tap water, but it is unclear whether the weaken/broken tests were affected by the flow of water. Can authors confirm if any evaluation was made on this matter? Also, is it possible to observe the same tests in their natural setting by sorting raw samples and immersing them in seawater? This question arises from the fact that seawater usually has a carbonate saturation level greater than 1, which does not promote further dissolution. We agree that washing with seawater would probably be more appropriate, but we do not have such a system at Angers due to our distance from the coast. However, the Loire river providing tap water is relatively hard as it crosses the calcareous Parisian basin. The washing method used during this study was the same as for other projects on other marine sites carried out in the laboratory at the same time. No signs of dissolution of the foraminiferal tests were specifically reported that could incriminate this washing method. It will be pointed out in the Methods section that, to avoid further damaging the already fragile shells or organic linings, the wash was quick and gentle.

P6L131 Do authors measure carbonate saturation state or other related factors (e.g., calcium concentration or alkalinity) to understand calcium carbonate dissolution? As previously stated, we unfortunately do not have any chemical data other than pH and O_2 for further study of the saturation state of carbonates.

P6L147 I fully support the decision made by the authors. In my opinion, it is necessary to address the issue at the genus level and there is no need to narrow it down to the species level. Nonetheless, if there are any references that demonstrate species compositions that are unique to studied water, those should be included. We agree with the reviewer. We are not aware of any such studies to date.

P6L149 Authors use "living" to refer to fixed individuals stained with CTG, not live sorting. Absolutely, as mentioned in the Methods section.

P6L156 Charrieau et al. 2022, who conducted an experiment on *Peneroplis*, should be also introduced here. We agree and we will add it.

P10L176 I acknowledge that it is important to perform this calculation, but I need an explanation as to why the low ratio of calcareous foraminifera is regarded as a "loss" by the authors. Given that our assemblages are dominated by specimens with a calcareous test, and that previous studies on this site state the same (Redois et al, 1998; Fouet et al, 2022), we consider that a low ratio of calcareous specimens between the living and dead assemblages corresponds to a "loss" of calcareous foraminifera.

P10L199 It appears to be a reasonable statistical process. We thank the reviewer.

P11L217 Please specify why bacteria are not quantified in St. 3. Also, specify why quantification is done at St. 1 and 2 but not at St. 3. We will add a sentence about it: "For administrative reasons, it was only possible to carry out these DNA analyses for stations 1 and 2."

P11L229 Should pointing out or certifying if this distribution is a fingerprint be moved to the "Discussion" section? And, please provide citations or evidence that this distribution is “typical”. We agree the reviewer and will delete this sentence from the “Results” part because of the repetition lines 326-327 in the “Discussion” part.

P12L238 Is the CB density zero at St. 3? Or is it missing? As we have said previously, we will precise that we do not have this data for St 3.

P14L278 If the experimenters took care to use tap water and a gentle water flow while washing, it is important to include a thorough description of this in the methodology section. This information is crucial for others to be able to replicate the experiment accurately. We agree and we will precise it in “2.2 Sediment Sampling and Processing” part (l. 113).

P16L338 It is currently known that the presence of pore water in sediments is determined by sulfate, iron reduction, and CBA. However, it is still unclear to readers whether any deposits of calcium carbonate organisms, other than foraminifera, exist in the area. For example, there may be shells of bivalves such as clams, or sea snails, which shells are rich in calcium carbonate. These calcium carbonate contents can buffer the pH, but there is no information available on sediment composition, including alkalinity or calcium carbonate content. Hence, it is important to explain why CBA can be attributed to this. Is this a logical conclusion based on previous studies? We have observed few bivalve and snail shells within the sampled sediment. A study has been carried out on CBA in muddy bivalve reefs (Malkin et al, 2017). They reported no dissolution of living shellfish. They concluded that $CaCO_3$ and alkalinity accumulated on the reef were remobilised by the CBA from the sediments towards the bottom waters. The $CaCO_3$ dissolution process was therefore very active. It is likely that the dissolution process plays a different role depending on the scale considered. Indeed, the surface/volume ratio is very different depending on whether you're working on macro or meiofauna, and some macroorganisms can move and escape from these extreme conditions. There are also complex interactions between bioturbation and the cable bacteria activity which seem to buffer this bacterial activity (Malkin et al, 2014, 2022; Aller et al, 2019). Eventually, as presented to Reviewer 1, if calcareous foraminifera are decalcified so intensely, this means that despite the strong physical and biogeochemical dynamics of this kind of transitional environment in time and space, the corrosive conditions are sufficiently strong in intensity through time to generate dissolution in living organisms that can fight off these hostile conditions to a greater or lesser extent.

P17L346 Based on the information given in the introduction, it appears that the discussion is addressing one of the objectives of the study. However, there seems to be a lack of material to support the argument. It is not possible to determine the reproducibility of the discussion about the extent of CBA, especially with the absence of CB abundance data at St. 3. For instance, during sediment sample processing, was the presence of CB confirmed visually or through other means? Or was it only left on photographs? If the presence of CB was confirmed, it would be possible to describe the amount of CB present in Table 1 qualitatively. If you have microscopic or visual observations in the form of a bacterial mat, it would be supportive. If such data is available, it would be a good idea to add it to the figure or supplement data. The presence of CB in the sediments was not confirmed visually in 2020. We recently attempted to make microscopic observations and take photographs of CB by incubating sediment from stations 2 and 3 using the method described by Thorup et al. (2021). We have been able to observe balls of filaments whose scale and structures seemed to indicate that they were indeed CB. These observations were in low abundance. This is not surprising given the calculated densities, which remain within the low range values reported in the literature (see “Discussion”, lines 330-341). However, the combination of pH and oxygen microprofiles provides a very high degree of confidence that they are active and therefore present at station 3.

P18L403 I am largely in agreement with the authors' perspectives. However, I recommend a more extensive engagement with the discussions on test dissolution from prior studies as outlined in Introduction lines 61-65, and subsequently, a further elaboration on the assertion that "the influence of CBA cannot be overlooked and may be predominant in certain locales.” Following these remarks and those of Reviewer 2, we plan to restructure the Discussion section. The dissolution process in Auray would be discussed in more detail based on the bibliography and the data in Marie Fouet's thesis. It would conclude with the hypothesis that the CBA seemed to be the main contributor in this case. The section titles and abstract will be modified accordingly.

We will also add a brief development of the hypothesis of dependent species response based on the work of McIntyre-Wressnig et al. (2014), Haynert et al. (2014), Charrieau et al. (2018c) and Mojtahid et al. (2023).

While the manuscript addresses the mineralization of organic carbon and the intensification of acidic environments, the discourse concerning carbonate saturation appears to be insufficient. As delineated by the authors in lines 63 and 69, test dissolution corresponds with a reduction in carbonate saturation. Carbonate saturation is functionally related to the concentrations of carbonate and/or calcium ions. Hence, it would be pertinent to include a discussion on calcium ions (even if there are no observed variations), as opposed to solely focusing on pH. The solitary mention of carbonate saturation at line 444 falls short of providing a comprehensive understanding. We share this criticism. In our view, it goes without saying that the acidification processes that coastal environments undergo, and which are discussed here, include the carbonate saturation. We can mention this more during the discussion. We are keen to remain general about the complexity of the chemical processes involved so as not to confuse the reader and to avoid making statements that would be highly speculative given the data available to us.

P20L449 I agree with the importance of the authors' perspective in incorporating the new perspective of CBA into the discussion of foraminifera distribution. We thank the reviewer for agreeing with our thesis.

P21L490 It is crucial to consider the authors' point of view. For instance, it would be beneficial to develop a proxy that can detect the existence and strength of dissolution by CBA in the future. Additionally, it might be necessary to acknowledge the potential of modifying the process of micropaleontological sediment treatment due to the assumption of shell dissolution. A multivariate approach coupling (1) the identification of lipid biomarkers in cable bacteria or eDNA and their investigation in ancient sediments to determine their presence and (2) the study of foraminiferal species assemblages (C/T ratio), shell preservation and isotopic shell composition, could be a good candidate to try out.

References

Aller et al, 2019 (10.1126/sciadv.aaw3651)

Charrieau et al. (2018c, 10.1016/j.marenvres.2018.03.015)

Charrieau et al. (2022, 10.1038/s41598-022-10375-w)

Redois (1998, PhD report, fr)

Fouet (2022, PhD report, fr/en, http://www.theses.fr/s227694)

Fouet et al. (2022, 10.3390/w14040645)

Haynert et al. (2014, 10.5194/bg-11-1581-2014)

Malkin et al, 2014 (10.1038/ismej.2014.41)

Malkin et al., 2022 (10.1002/lno.12087)

McIntyre-Wressnig et al. (2014, 10.2113/gsjfr.44.4.341)

Mojtahid et al. (2023, 10.1016/j.chemgeo.2023.121396)

Thorup et al. (2021, 10.1016/j.syapm.2021.126236)