

Response to Reviewer 1: Sebastiaan van de Velde

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General comments:

In this manuscript, Daviray et al. evaluate the impact of porewater acidification by the activity of cable bacteria on the preservation of benthic foraminifera in coastal marine sediments. The manuscript is well structured and the methods and aims were clear. My main comment would be that the introduction and discussion are not very in depth, and focus on one singled out factor (cable bacteria), while having not providing more context or considering other environmental factors. I have listed a few specific comments/questions below that could improve the impact of the manuscript. Overall, I believe this is a solid research paper, but some of the discussion should be a little more in-depth.

Specific comments:

The authors state that the aim of the study is to investigate the impact of cable bacteria activity on the preservation of forams. Throughout the MS they focus almost exclusively on this aim, and by doing so they provide very little broader context of why this work is important. I was left with a few questions that could be addressed by restructuring the introduction and discussion.

- Why is understanding the environmental parameters that control foraminifera important? As presented in the introduction (l. 58-62), to refine benthic foraminifera as bioindication tools or palaeoproxies, environmental parameters that influence species distribution, population dynamics, shell chemical composition and structuring... have to be better understood. This study is part of this effort to identify the causal factors of geochemical changes in microhabitats that have an impact on foraminifera.
- Are there any other processes that cause porewater acidification comparable to cable bacteria (e.g. reoxidation of reduced species in the oxic zone could lead to an acidic minimum), and why are they not considered? We agree to the reviewer and discuss about such processes in Discussion lines 336-338. As the sediment acidification continued well below the oxic zone, oxic processes did not appear to be involved here in such pH decrease. However, acidification due to the reoxidation of reduced iron by nitrate or MnO₂ in the suboxic zone could be more discussed (Soetaert et al., 2007; Middelburg et al., 2020). First, important remobilization of iron can be seen without test dissolution within the suboxic zone (Thibault de Chanvalon et al, 2015). Then, in Auray mudflats, nitrate is not a major component of bottom water and porewater chemistry in autumn as we have seen in other studies (< 4 μM, pers. comm.) so this hypothesis could be ruled out. Eventually, the involvement of MnO₂ can be discussed here, despite the lack of data. CBA involves iron reoxidation by MnO₂ within the suboxic zone (Sulu-Gambari et al., 2016). Then, this reaction seems to be a consequence of this bacterial activity, as is the sediment acidification that it may induce.
- Are there other locations aside from estuaries where these findings could be important? All marine environments where CB and carbonate-shelled benthic meiofauna may cohabit would be concerned (e.g. salt marshes, mudflats, lagoons, reefs, marine lakes; Burdof et al, 2017). Has this any impact on, e.g., climate records based on foraminifera isotopes? As things stand, there is no answer to this question. We can only assume that this bacterial activity could influence the isotopic composition of the foraminiferal test, if not a total loss of calcareous species in the sediment records, as Richirt and coauthors hypothesised (2022). This is why we open in the conclusion (l. 510-511) that

foraminifera could be used as paleoproxies of this bacterial dissolution process. Further investigations on this way should be performed.

Another factor that does not receive much consideration is the seasonality. So far, all laboratory studies of cable bacteria have shown a boom-and-bust cycle (rapid growth, followed by a collapse). Field studies on the other hand show a distinct seasonal pattern of alternations between cable bacteria, bioturbated macrofauna and *Beggiatoa* (e.g. Seitaj et al., 2015) or between cable bacteria and other sediment disturbance reworking events (e.g. van de Velde 2018). Indeed, the boom-and-bust cycle of CB in laboratory studies are observed, and the seasonal alternation of the sulphur-oxidising bacteria community on the field according to the hypoxia events inducing pH seasonal variability (Seitaj et al., 2015; Lipsewers et al., 2017; Malkin et al., 2022). However, such desoxygenation or reworking events have not been reported in this study area (Marie Fouet thesis; OFB and IFREMER data). Furthermore, the intertidal mudflats are reoxygenated at each low tide which could lead to the reactivation of cable bacteria activity in highly eutrophic environments. Unfortunately, there is little literature on cable bacteria activity under tidal cycle.

We agree that carrying out additional campaigns through time and laboratory experiments represent important issues for the future of the project. The seasonal alternation of bacterial communities is not the subject of this study and to enter these considerations risk weighing down the discussion. Discussing the potential temporal variability of CB and time integration by foraminifera as perspective seems sufficient to us for now.

So far, there has been no study that showed a constant presence of cable bacteria throughout a year, so it is likely that your site also experiences reworking by fauna or other resuspension events. You partly allude to the importance of sediment mixing at L483, but this is not considered anywhere else in the MS. What is benthic fauna community at the field site? We agree with the critic. This prospect of sediment mixing was mentioned without giving a possible cause, as was the macrofauna. The benthic macrofauna (> 2 mm) of the mudflat is dominated by polychaetes (*Nephtys* spp.) known to burrow into the sediments (Michaud et al, 2021). There are also bivalves (*Cerastoderma edule*), as well as a few gastropods (*Peringia ulvae*) and arthropods (*Chaetogammarus marinus* and *Apohyale prevostii*). Total abundance is around 15 ind.50 cm⁻² (pers. comm. Oihana Latchere).

Are there many intense resuspension events (e.g. storm floods)? The field survey was carried out at the end of September, at the end of the low-water period. There were no floods or storms in the weeks preceding our sampling (archives Météo France). The Auray estuary and the Morbihan gulf are very enclosed systems mostly protected from marine storms. The most intense resuspension phenomenon here would be rising tide (Menier and Dubois, 2011; Menier et al, 2011) and bioturbation.

How would they influence your interpretation (e.g. seasonal sediment mixing homogenizes the top sedimentary layers and moves forams from within the acidic zone down to the deeper layers, or vice versa; see, e.g. Hülse et al., 2022)? We can assume that a homogenization phenomenon in the upper sediment layers, under biotic or abiotic influence, would reduce CB activity as observed on mudflats (Malkin et al, 2014, 2022; Aller et al, 2019). We would then expect an increase in pH in the suboxic zone, a weakening of the dissolution process, and a shorter time residence within the acidic zone. The calcareous shells of the foraminifera would then be less likely to be subject to decalcification and would probably be better preserved. Levels of decalcification would be lower (< stage 3), and calcareous shell specimens would probably remain in the majority of assemblages.

Eventually, 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 are able to fight off these hostile conditions to a greater or lesser extent.

Finally, I am not entirely convinced you can use forams to reconstructing the history of cable bacteria, there are so many parameters that cause dissolution that it will be impossible to relate this robustly to cable bacteria activity (let alone reworking of the sediment - see Hülse et al., 2022). How would you go about doing that? This would involve 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, (2) the study of foraminiferal species assemblages (C/T ratio), shell preservation and isotopic shell composition and (3) paleoenvironmental methods (like sedimentology) distinguish other factors responsible for the dissolution process and to infer it to bacterial activity.

Technical comments:

Title: 'a prelude' -> why not just say 'implications for ...'? It can be; it was just a personal fantasy...

L32: strongly -> omit. At several instances you use 'strongly', e.g. 'strongly consider', 'strongly explained'. In get this is for emphasizing the importance, but you can omit strongly on most of these occasions. The importance of your results is clear for scientists working in your field. This suggestion will be taken into account.

L115: why only station 1 and 2? The data from station 3 were added to the manuscript after the DNA analyses had been carried out on the samples from stations 1 and 2; the apparatus and the team of the Microbiology Institute of Biology in Aarhus University (Denmark) were subsequently no longer available to us to carry out measurements for the three stations together.

L123: Is this tip diameter or length of your tip? We have specified "tip diameter" in the manuscript.

L125: for microsensor profiling, you should not have a stepsize smaller than the tip size? We are aware of this methodological limitation and the theory about microsensors. However, Unisense is not able to make pH robust probes thinner than 500 μm for *in situ* investigations. We tried many times and most of them did not last until the end of the first profile...

L235: Geelhoed The correction will be done.

L337: pH minima are also generated by reoxidation of reduced iron species (and other reduced species) We agree. For us, this was the meaning of "iron reduction". As mentioned above, we're going to discuss these reactions in a little more detail.

L376: remove then This suggestion will be taken into account.

L507: But it is specifically the low pH generated by cables that is important? Very good point. We agree that the *in situ* variability of the dynamics of CB activity from one mudflat to another under the same hydrological system is a very interesting prospect that deserves to be studied further such as their temporal dynamics.

References:

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Hülse et al., 2022: 10.1016/j.earscirev.2022.104213

Soetaert et al., 2007 (10.1016/j.marchem.2007.06.008)

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Thibault de Chanvalon et al, 2015 (10.5194/bg-12-6219-2015)

Sulu-Gambari et al., 2016 (10.1016/j.gca.2016.07.028)

Burdof et al, 2017 (10.5194/bg-14-683-2017)

Richirt et al, 2022 (10.1016/j.palaeo.2022.111057)

Lipsewers et al., 2017 (10.1128/AEM.03517-16)

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

Marie Fouet's thesis

OFB (<https://professionnels.ofb.fr/fr/node/276>)

IFREMER ([https://wwz.ifremer.fr/envlit/DCE/La-DCE-par-613 bassin/Bassin-Loire-Bretagne](https://wwz.ifremer.fr/envlit/DCE/La-DCE-par-613-bassin/Bassin-Loire-Bretagne))

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Météo France

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