Answers to reviewers, second round of reviews :

Reviewer Julien Richirt :

The manuscript was greatly improved since the first submission, and I have read it again with pleasure. I appreciated the addition of useful figures and supplementary modelling approaches well used in the discussion section. I also appreciated that all my previous comments were considered carefully, and I was satisfied with the authors answers. However, because authors made important changes compared to the previous one, I have additional remarks and comments regarding this new version.

We'd like to thank Julien Richirt for his attention to our proofreading work, and we're pleased that he's satisfied with it.

Here are the 2 main problems I currently see with the manuscript that must be overcome before publication:

While I found the section 4.1 very interesting, I think some parts are unclear regarding what you mean exactly by tolerance, survival, cellular activity, pseudopodial activity, reproduction and calcification. From your data, forams seem to tolerate (survive) all concentrations, because you can find individuals alive (same amount than at the start) at the end of all your experimental conditions. However, reproduction (and calcification? I guess they cannot reproduce without being able to calcify?) is hampered or even prevented by the absence or high sulfate concentrations in your experiment. The pseudopodial activity assessment is also not clear: did you considered all individuals without any cytoplasm movement as dead even if they were attached to the substrate? In all cases, a foraminifera considered as living should have some cellular activity (even strongly reduced, such as during dormancy for instance).

We considered as dead all individuals that were detached from the substrate, but recognised that some rare foraminifera may still be attached after the death, as stated in line 143:

"We counted live individuals each week, for each medium. Since the studied species live attached to a substrate, individuals that no longer stick to the Petri dishes were considered dead, even though rare dead individuals (empty tests or no reticulopodial activity) may remain attached and few living adults can detach themselves from the substrate as well."

On the question why they would develop and maintain such a tolerance vis-à-vis relatively high sulfate concentrations (almost 2 times higher than during the whole Phanerozoic, meaning they were never exposed to such high concentrations), could micro-habitat variability explain this mechanism? For instance, you mention volcanic activity or hydrothermal vents as causes of high sulfate content in SW. Could it be that forams developed such a "good" tolerance because they sometimes (temporarily) have to deal with higher sulfate concentration in their micro-habitat than 28.2 mM? This is very speculative but might be interesting to mention, especially for the potential consequences on paleo-proxies.

We doubt that our study permits to establish that this ability to tolerate variations in sulfate concentration was developed to deal with higher sulfate concentration. This is an interesting point, yet far beyond the scope of the current study. However, now that we know that this tolerance exists, we think it's important to take this feature into account when using foraminifera for paleoenvironmental reconstructions.

The consequences for paleoproxies is mentioned in the text:

"This limitation means that foraminiferal CAS could be used to trace deep time secular changes in seawater [SO_4^{2-}], which varies from about 5 mM to 28 mM today (Algeo et al. 2015), but not to trace past seawater [SO_4^{2-}] enrichments above 28 mM, such as those that could be caused by large volcanic eruptions or sulfate-rich volcanic hydrothermal fluids on the seafloor.

In addition, to be cautious, we have written that this threshold could be specific to Rosalinidae, see in 4.4 section of discussion: "Future works are therefore important to confirm whether or not the seawater [SO42-] threshold of 28 mM for CAS incorporation can be applied to other benthic and planktonic foraminifera, or whether it is restricted to Rosalinidae".

2. The data presentation and interpretation regarding sulfate/calcite ratios as a function of SW sulfate concentration in sections 3.3 and 4.2.

This concerns the value at which the plateau begins to occur. You indicate several different values in the main text at different places.

For example: from 28 to 60mM in abstract and results for instance lines 26 and 269.

From 35 to 60mM in discussion line 345.

5-40 mM in discussion as referring as the part where there is a linear correlation between sulfate/calcite and sulfate concentration is SW on line 395.

We are going to correct this, now it's stated everywhere: from 5 to 28 mM

While these value changes are making it very unclear trough the manuscript for the reader, I think that your data indicate a relatively clear linear relationship between 5 and 35mM with a very good r^2 (in the following graph NSW is omitted). For this reason, I do not understand why you stick to 28.2 mM as the threshold value for the plateau starting point (which is the most encountered value in the current manuscript). Seeing the error bars on fig 7 I would recommend maybe considering the 5-35 mM range because there is no difference between 35 and 40mM (then the plateau starts at 35mM). This is rather important to be clear and consistent on this through the paper because it impacts the clarity for the reader and your further interpretation/discussion.

We do not believe that the CAS concentration of our cultures grown in seawater can be set apart from the overall data set. When this variability, from one experiment to the other in the same 28.2 mM concentration (in NSW and in ASW) is taken into account, the plateau clearly starts at 28.2.

We believe that further experiments will be needed to evaluate more precisely how CAS record sulfate concentration between 28 and 40Mm.

Minor and supplementary/more detailed comments are annotated in the following pdf file.

We would like to thank Julien Richirt for these thorough revisions, we addressed all of these corrections.

Reviewer David Evans

This is my second review of the manuscript Impacts of seawater sulfate concentration on sulfur concentration and isotopic composition in calcite of two cultured benthic foraminifera by Thaler et al., which presents S/Ca and δ34S measurements of two strains of rosalinid foraminifera cultured under different seawater [SO4 2-]. The authors have, in my view, adequately addressed all of the comments from the first round of review, resulting in an improved version of what was already a very interesting and comprehensive piece of work.

I have some further minor comments for the authors to consider but would suggest that they do so at their own discretion.

We would like to thank David Evans for his kind words, and for this second review, which helped us to improve the manuscript once again.

Main comment 1. Reproduction/growth in culture. If I understand what is written on lines 79 and sections 2.1.2/3.1, dead individuals (empty shells, or those not attached to the petri dishes) were removed, leaving only live foraminifera.

Is it therefore possible that foraminifera that did not die but remained dormant could be present in the final analyses? In most cases this is of course not important, as there was a large increase in the number of individuals during the experiment.

We believe that this is possible, but only in rare cases. In any case, they are outnumbered by the total number of foraminifera present in each analysis (more than 100).

A possible exception to this is the 60 mM experiment, in which the number increased from ~30 to ~100. Given that the interpretation of the [SO4 2-]swS/Cashell plateau hinges on this experiment, I suggest adding a note to explain whether or not this datapoint unambiguously does not contain pre-experiment shell material, or if it could represent a mixture of shell material grown under normal seawater and experimental conditions (which could then be an alternate explanation for the plateau if the foraminifera from reproduction in the experiment were smaller).

We do not believe that the interpretation of the plateau holds on the 60mM data point: At 28mM the CAS concentration ranges from 10800 to 14200 ppm and all following CAS concentration (at 35, 40 and 60mM) remain in that range. Additionnally, considering the hundreds of new foraminifera formed in media 35 and 40, we do not believe that the plateau can be explained by the presence of pre-experiment foraminiferal shells (less than the initial number of foraminifera, that is as low at 6 individuals in these experiments, still clearly lower than the final number of hundreds of shells collected).

On a similar note, Table 1 gives the numbers of foraminifera in each experiment through time, but how many were discarded during the experiment? Does this provide evidence for multiple generations? Even in the 60 mM [SO4 2-] experiment?

Considering 1 adult can give 20 to 40 juveniles, it is likely that only one generation of foraminifera was generated in the 60mM media (with enough first generation reproducing to go from 31 to 108 foraminifers) as illustrated with the stagnation in the population increase after 12 days.

In the 10mM experiment of set 2, starting with 6 foraminifers, even if all 6 had yield 40 juveniles, we would have only reach 240 specimens. So to explain a final amount of 1014 specimens we need at least 2 generations, which corroborates the fact the population is still increasing after 31 days.

Minor comments

1. Lines 26-29. An inhibitory kinetic effect would seem a more likely possibility to me, but I appreciate not every hypothesis can be listed in the abstract.

The inhibitory kinetic effect hypothesis is described in line 390 of discussion

2. Line 51. Sulphur and magnesium are not trace elements in seawater.

We changed the phrase to: "elements present in seawater get incorporated as traces in the biomineral structure"

3. Lines 58 and 451. I suggest using a different phrase to 'large volcanic events' as this possibly implies single eruptions, whereas Laakso et al. discuss large igneous provinces emplaced over thousands of years.

We replaced "large" by "important"

4. Lines 75-76. You could clarify that most studies that included material grown before culture attempt to account for this in some way, e.g. using size-mass relationships or labels.

We made the modification

5. Lines 117-118. Are the units mM or mmol/kg?

In mM

6. Lines 211-212. Were all samples run at the same concentration as the seawater standards? If not, does this approach potentially result in a reproducibility that is too low?

All samples were run on the instrument at a concentration similar to that of seawater. It has been demonstrated elsewhere that lower concentrations on the instrument do not affect the validity of the 34S value (Paris et al., 2013), but lower intensities do yield lower reproducibility, both as a result of counting statistics (and possibly Johnson noise) and a greater influence of the background subtraction.

7. Line 246. Please clarify which results you are referring to.

We modified the sentence: "take into account pH and DIC value nor foraminifera counts measured in that media after day 15. "

8. Lines 258-259. I think this explanation is unlikely (e.g. the DIC had increased by day 5 (Figure. 6) and there was no further increase.

We changed the sentence to: "DIC probably built up in the Petri dishes each week as the foraminifera respire".

9. Section 4.1 title. Bear in mind that there were large covariations in seawater [Na+] and [Cl-]. Worth mentioning in the discussion?

We made the following modification line 127

The amount of NaCl in those two media was adjusted to keep the total salinity constant (35.06 g/L). Na^+ concentrations for ASW[0] and ASW[180] were 479 mM and 402 mM, respectively, representing a maximum 24% change while the Cl^- concentrations were 612 mM and 175 mM, respectively, representing a maximum 71 % change, for a maximum 180% change in sulfate concentration.

10. Section 4.2. At some point (in the introduction?) it would be helpful to mention the Mg/Ca of these foraminifera if it is known. If they reduce the Mg/Ca of the biomineralisation site compared to seawater then the considerations regarding the effect of seawater [SO4 2-] on CaCO3 nucleation and precipitation will likely not apply/be more complicated than implied in some places in the manuscript (e.g. lines 338, 407).

We don't know the Mg/Ca of the tests. This experiment was focused as a first step on $[SO_4^{2-}]$ and we did not measure Mg/Ca ratio, in future work it might be indeed interesting to measure the two as suggested.

11. Line 338. On a similar note, I would suggest rephrasing this sentence. There may have been no precipitation in those experiments but it does not mean it is not possible, e.g. if higher degrees of oversaturation were to be achieved.

Although we agree that increasing the saturation of seawater to favor calcite precipitation might work, it would be a completely different system.

Line 338 we state "It is remarkable to note that foraminifera can reproduce and thus calcify at $[SO_4^{2-}]$ as high as 90 mM (Fig. 5), concentrations at which no inorganic calcite precipitation occurs (Bots et al., 2011; Barkan et al., 2020). However as discussed before, their reproduction is limited to the first week, which strongly suggests that they could only tolerate brief exposure to such a high level of sulfates in their environment. "

Our sentence refers to systems with defined concentrations described in papers that are referenced, hence the "concentration space" where our sentence applies is given. We thus decided to keep the sentence as is.

Likewise, I would not read anything into the 'fact that calcite precipitates' (line 369).

There is indeed calcite precipitating in our experiments, which constitute our fact. We then use that fact as a suggestion for a mechanism "The mere fact that calcite precipitates therefore suggests that sulfate is at least partially removed from the precipitating fluid"

We believe that stating that it is only " suggesting" is showing enough caution and we decided to keep the sentence.

12. Line 355. Please clarify, the same as what? Heterostegina?

We have now clarified that the presence of sulphated glycosaminoglycans has been documented in the organic matrix test of many foraminiferal taxa (see Langer 1992) and not only in *Heterostegina* (see Weiner and Erez, 1984). In line 355 we refer rather to the bilayer test construction in all rotaliids, not just *Heterostegina*.

We specified:

The organic matrix of the test of a wide variety of foraminiferal taxa contains over-sulfated glycosaminoglycans and proteins (Weiner and Erez,1984; Langer 1992). The benthic foraminifera Rosalinidae belong to the order Rotaliida and likely share the same mechanisms of biomineralisation and bilayer test construction as other rotaliid families.

13. Lines 416-418. Given that you include speciation modelling, can you say which ion pairs become relatively more abundant?

The relative abundance stay the same, NaSO4>MgSO4>>CaSO4. Here are the endmembers of our results for two different pHs (8.2 and 8.19) at 5mM of SO42- are (in mol/L):

CaSO4	$NaSO4^-$	MgSO4
2E-04	0.002	0.001
2E-04	0.002	0.001

While at 60mM at pH 8.2 and 8.15 we get :

CaSO4	$NaSO4^-$	MgSO4
0.002	0.019	0.015
0.002	0.0189	0.015

All these results are provided in the appendix,

14. Line 720. It also diffuses between the experiment and atmosphere.

While we agree that CO_2 diffuses between the experiment and atmosphere, we believe that no strong enough variation in the atmospheric level of CO_2 (that can vary in a lab depending on how many people are working in the same room) can explain our DIC concentration changes. Additionnally, to the best of our understanding, a strong CO_2 variation in the atmosphere could lead to a DIC increase, correlated to a pH change, which is not what we observe.

Typos

1.Line 65. Change 'interrogates' to 'suggests'. Done

2. Lines 137. Experiments. Done

3. Line 184. Aliquots, or delete 'of the'. Done

4. Lines 314-315, 390. Change four instances of 'Mm'. Done

5. Line 693. Forms. Done

6. Line 728. On a pool of a hundred to…'. Done

7. Line 816 and Fig. D1, it should be CaHCO3 + (also NaSO4 -). Done