Nancy, July the 5th, 2026

Dear referees, dear editor

Please find as a separate file the new version of our submitted article, with all the additions and modifications already detailed in our replies to the referees.

First, we would like to thank again the referees for the constructive and challenging reviews. We provide new figures, modified interpretation and strengthened discussions in this version, following our detailed replies.

We would like to point however that a few things were not added to the manuscript.

Because SEM views of *Rosalina* sp. are already available in Thaler et al. (2023), we did not perform new ones but refer the readers to our previous work.

We did not expand Table 2 (now table 3) because tests and cytoplasms of the other foraminfera are very heterogeneous. Instead of an average value, we provide a few profiles across the test of F5(2)B1, which seemed more relevant to us.

Overall, the NanoSIMS data are provided as a separate document (see Data availability).

We hope you will find this new version clearer and improved.

Best regards,

Guillaume Paris and co-authors.

Referee 1

The manuscript, "Tracking sulfate, magnesium, phosphorus and amorphous phases in Rosalina-like benthic foraminifera", by Paris et al., presents results of correlative TEM and NanoSIMS imaging to track the distribution of sulfur, phosphorus, calcium and magnesium in cultured foraminiferal cells. The foraminifera were cultured within ^{33}S-labeled seawater to track the incorporation of inorganic sulfate into the test walls.

The paper is generally well-written and the results are novel and exciting. Nevertheless, there are some serious issues with the interpretation of the results. While the interpretation of the sulfur distribution is generally fine, I have problems to agree with the interpretations about the phosphorus distribution. Many studies showed that inorganic phosphorus is completely discriminated in foraminiferal calcite, which is different to corals (see Science paper by Boyle, 2006). Most of the P in foraminiferal calcite is associated with the organic matrix and efficiently removed by bleaching of the tests and in my opinion the results of the study by Paris et al. show exactly this. In addition, there are certain other major points of revision that are in described in detail below. In my opinion, the paper is definitely suited for Biogeosciences, but it needs substantial major revisions before publication.

We thank referee 1 for their thorough and stimulating review. We provide here answers on their main points and criticisms. In red, the answer for which we have already modified the text (mostly the typos). In green, our answers with new elements included in the new version of the draft.

The review led us to be more cautious about the description of P distribution within the cytoplasm and to be even more explicit about the limitations of the method we used, and thus of the observations we performed, which we hope strengthened some of our conclusions and provide a more nuanced discussion about the interpretations we provide within the cytoplasm.

Combined with the comments of referee 2, the points raised by referee 1 led us to reevaluate the connection between P, S and phases distribution within the tests. We provide new figures where we group the trends not by profile, but by trends, in order to calculate the correlation coefficients of each group to better explore the nature of the phase(s) possibly carrying the elements we analyzed.

Revisiting the data allows us to establish that trend B corresponds to the Primary Organic Sheet, and thus that the P, Mg and S enrichments correspond to the granules that we observe within the POS.

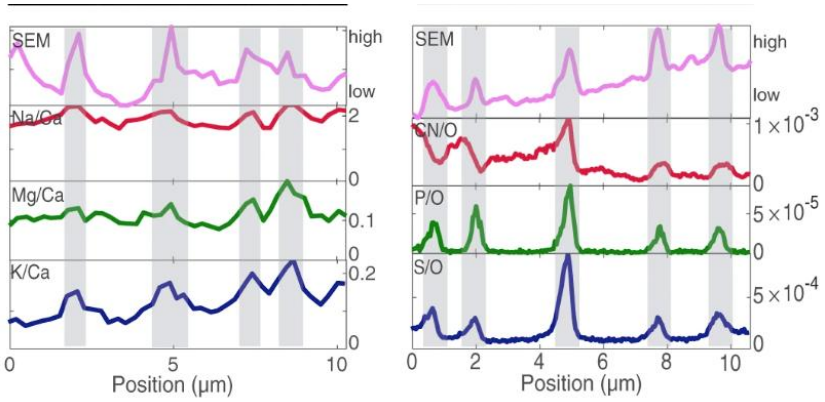
Below you can find my major points of revision:

1.: As mentioned above, the interpretation of the P distributions in the test walls are problematic and not really supported by the results of this study. Previous studies showed that P associated with foraminiferal calcite either is located in inorganic coatings (Boyle, 2006) or sits in the organic matrix of the test walls (Geerken et al., 2019; Glock et al., 2019). Nevertheless, the authors interpret their results as evidence for P that is bound in the calcite lattice. This is questionable, especially by looking at the plots in figure 4. Figure 4b shows the plot of P vs. CN. CN tracks the distribution of nitrogen in the test and is basically a proxy for organic matter in the test. The plot shows that there are two or

three slightly different phosphorus bearing phases in the test but in all these phases P is strongly correlated with organic matter. While this is similar to the correlation between S and CN (Fig. 4a), the intercept with the CN axis for all three different phases is zero or close to zero for the correlation between P and CN (Fig. 4b). This is not the case for the correlation between S and CN where the intercept indicates always significant S concentrations even when nitrogen is absent. In addition, the plot in Fig. 4g indicates, that S and P certainly are sitting in different phases, when normalized to CN. To really show that there is inorganic P within the calcite, I would have recommended to bleach the shells before the analyses to remove the organic matter in the walls. As it is now, I really think the authors have to do some major revisions to their interpretation here. Also, I have a bit of doubt about the interpretation of amorphous calcium phosphate (ACP) phases in the test walls.

The referee raises a very important point and we thank them for their comments.

First, we did not intend to say to all P sits in the mineral lattice. We said however that, in lack of raman data for instance, our data can be interpreted in different ways, and that some P in the mineral lattice and or as inorganic ion/ACP is possible.



In this figure from Geerken et al (2019), if S and P are strongly correlated, P is not so well correlated to CN. Thus, if CN is OM, and if S has a hybrid distribution between OM and calcite, so could P, and Mg.

Furthermore, when compared to the actual positions of the OOLs, thanks to the SEM and STEM views, we note that no substantial N or P is seen, but instead that the element distribution is more complicated.

In any case, we went back to our data and produced new figures. We also now chose to plot figures grouped by trends, instead of profiles. This has the advantage of allowing us to calculate correlations for each trend, and thus to be more rigorous in discussing them.

The new figures confirm the existence of the trends across all profiles. Trend A correspond to both OCLn-1 and OCLn and is defined by endmembers 1 and 2. Trend B corresponds to the POS and is defined by endmembers 3 and 5. Trend C corresponds to the edges of the tests and is defined by endmembers 4 and 6.

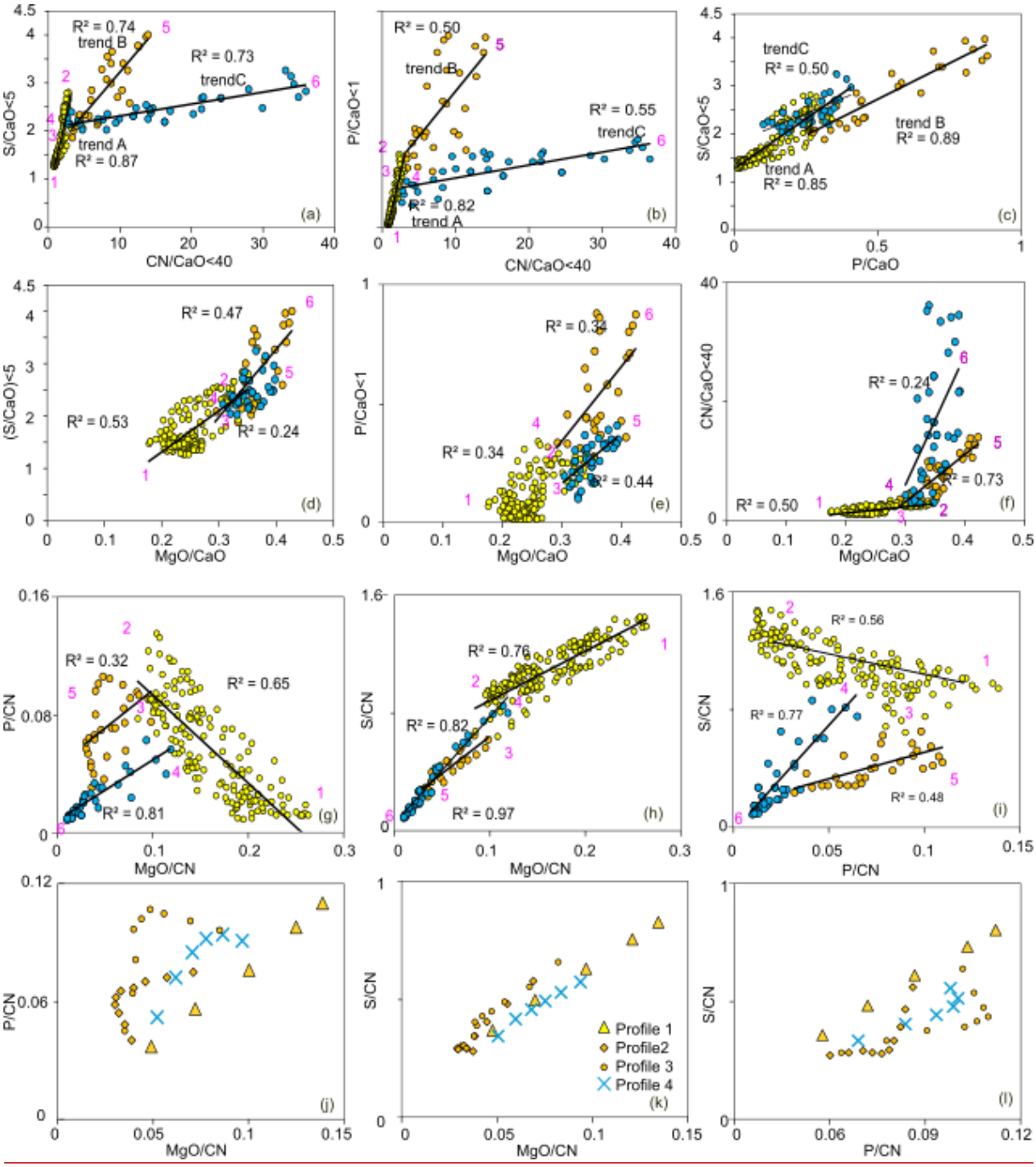


Figure 1 : New version of figure 4

We established a table of correlation coefficients (ratios normalized to Ca). It shows that the strongest covariation is between P and S, rather than between P and CN or S and CN, showing that these two elements share common patterns, that might not all be related to organic matter content. In green are the most significant correlation (>0.75), in orange are the moderately significant correlations (0.6<R^2<0.75) and in red are correlation factors R^2<0.5.

Ratios to Ca	P vs Mg	P vs CN	S vs Mg	S vs CN	CN vs. Mg	S vs P
trend A	0.34	0.82	0.53	0.87	0.50	0.85
trend B	0.34	0.50	0.47	0.74	0.73	0.89
trend B prof. 1	0.91	0.07	0.90	0.83	0.21	0.38

trend B prof. 2	0.71	0.74	0.80	0.90	0.9	0.93
trend B prof. 3	0.79	0.30	0.90	0.57	0.68	0.91
trend B prof. 4	0.90	0.55	0.60	0.86	0.75	0.95
trend C	0.44	0.56	0.23	0.73	0.24	0.50
trend A+C	0.58	0.44	0.67	0.43	0.50	0.85

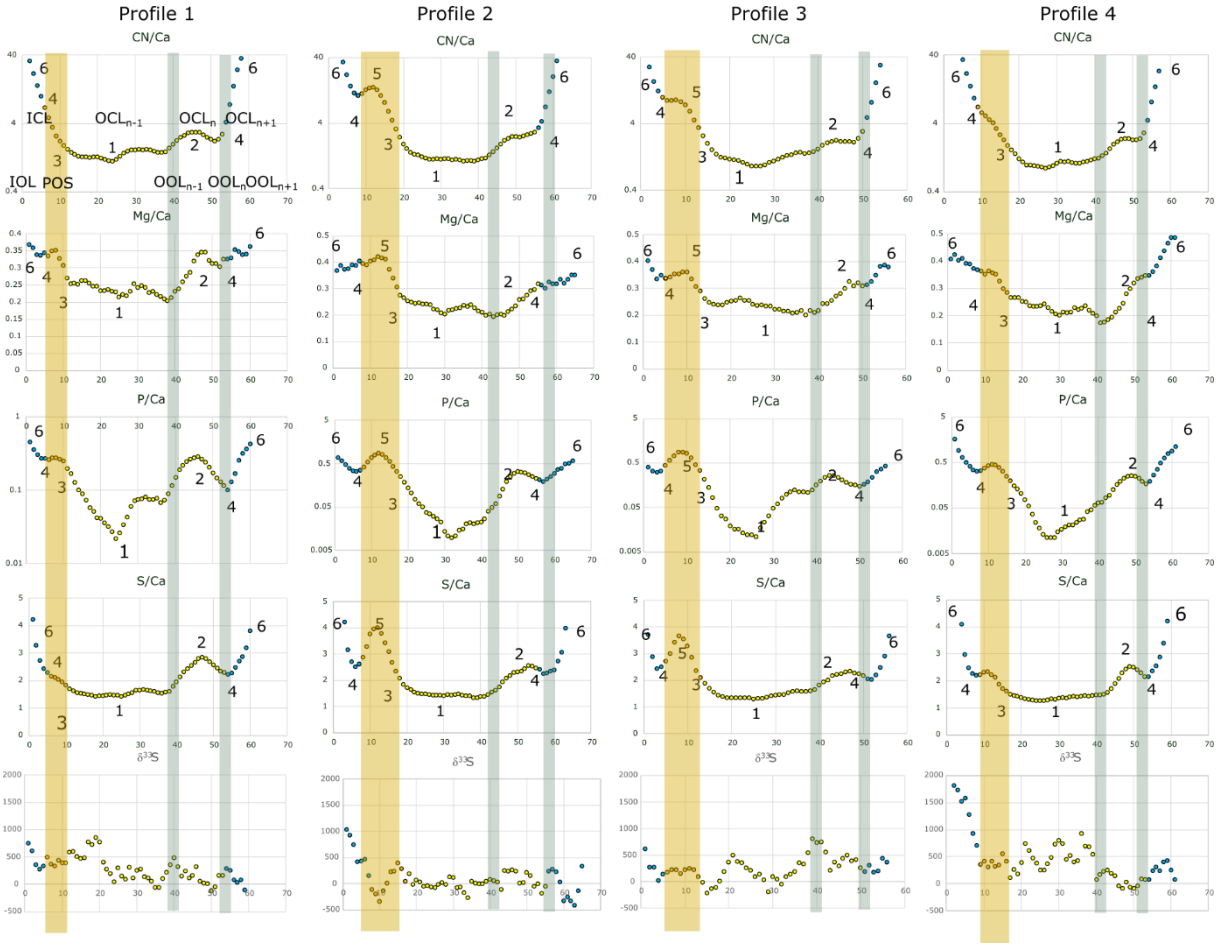


Figure 2 included in the new version of the supplementary materials. Points are color coded along the profiles according to each trend they belong to. In yellow : trend A, orange, trend B, blue, trend C. Numbers indicates the extremities of each trend and are the same as in the previous figure. The orange vertical bar for profile 1 corresponds to the POS, and the gray bars to the OOLs (see fig. 5). For the other profiles, the orange bars is placed based on trend B, and the gray bars represent the transition from endmember 1 to endmember 2 (most likely OOLn-1 by analogy to profile 1) and endmember 4 (most likely OOLn), while trend C represents the transition from OOLn to OOLn+1, including OCLn+1 and the transition from the POS to IOL, including the ICL.

As pointed by the referee, S and Mg never reach the "zero" level. We note that P and CN don't reach zero either, even though we must point out that N dynamic contaminations are common and requires extra care to avoid. Except in trend A, P/Ca ratios show little to no correlation with CN/Ca, which suggest that P and CN are independently enriched. Furthermore, in trend A, the variations of CN/Ca and P/Ca are not very important, only a factor of 3 or 4.

In trend A, the best correlations are between S/Ca and CN/Ca or P/Ca and CN/Ca. Yet, when normalized to CN, they are also moderately correlated, but negatively, while Mg/CN and S/CN positively covary. In detail however, trend A corresponds to two areas : OCLn-1 and OCLn. OCLn has varying Mg/Ca with mostly uniform S and P and OCLn has a higher Mg/Ca, higher S/Ca and higher P/Ca. Thus endmember 1 corresponds to OCLn-1 and is an endmember with a non-unique Mg/Ca ratio. Within endmember 1, P/Ca and CN/Ca may be stable or vary up to factor of 3 or 4, with phases of stability. Both calcitic layers appear distinct, but the mechanisms of enrichments might be different, with more organic matter in the OCLn area, as supported by the STEM and TEM views. In such a scenario, the enrichment in S is indeed consistent with more S- and P- containing organic matter. Though we agree this is not a shared view, trend A could nonetheless also be consistent with a change of inorganic S, N and P content in the mineral lattice.

Trend B is more complicated, especially when normalized to CN. However, trends exist, especially when separated by profiles, though they are not simply explained by a correlation. When normalized to CN, the data clearly reveal that endmember 5 is significantly more enriched in P than Mg or S. As a result, we interpret trend B to possibly include three phases: calcite (endmember 3), (organic matter endmember 5) and another phase, either a specific P-enriched OM, or a P-enriched inorganic phase, possibly ACP. Because of this additional enrichment in P, endmembers 3 and 4 are identical except for the P/Ca ratio

In trend C, CN/Ca appear as more correlated with S than P/Ca variations. This could be related to a P-poor and S-rich organic matter. However, when trend A and C are grouped together, we note that Mg and S are moderately correlated through both trends, as well as P and Mg, more than they are to CN, which could be also indicative of a control that is not connected to organic matter only.

In conclusion, we agree with referee 1 that part of the S and P variations are due to changes in organic matter content and/or with type of organic matter, at least in trend C and possibly within endmember 2 of trend A. We also confirm that some of the variation is likely independent from organic matter, especially in trend B and could be due to a P, S and Mg enrichment in non-crystalline phases, with P being the most enriched, as, for example, ACP. However, the complicated shapes of the cross plots also contain the possibility that calcites from OCLn and OCLn-1 are not characterized by the same Mg, S and P/Ca ratios and also more Organic Matter and amorphous visible in OCLn-1 in TEM/STEM views

We have modified the text, images, abstract and introduction accordingly.

Then, the authors found some P-hotspots within the cells, which are partly associated with Ca and Mg. These have been interpreted as acidocalcisomes that have recently been found in foraminifera. In this context, those results could potentially be exciting. Nevertheless, there are some issues with the methodology, since classical drying for the preparation of thin sections for TEM, that the authors used, usually removes soluble content and dissolved ions from the cells (Ayache et al., 2010). This is also stated by the authors in line 174, although there is a reference missing there.

The reviewer is right, the sentence “This process removes all free ions and small molecules present in the cell.” needs a reference. We added the reference (Ayache et al., 2010) to the text.

That is also the reason why traditional fixation is not recommended, if acidocalcisomes are supposed to be visualized (Goodenough et al., 2019). Goodenough et al. (2019) recommend cryo-fixation to preserve acidocalcisomes and dissolved content. The only study that found evidence for acidocalcisomes in foraminifera so far also used cryo-fixation (Glock et al., 2025).

In addition, the authors used a phosphate buffer solution solution for the cell fixation (line 171), which potentially could create artificial P hotspots in the foraminifera. In line 372 it is written: “More phosphorus and sulfur are observed in the organic matter located close to the test compared to the center of the cell.” Could this maybe be an indication that the P originates from the buffer but did not intrude deeper into the cell? Nevertheless, there could be a slight possibility that the P hotspots in the cell are no artifacts, since they have a similar size and shape as the recently found P-bearing structures in foraminifera. This would have been incredibly lucky since acidocalcisomes in foraminifera have not been found yet by using classical TEM-fixation methods. Therefore, I would recommend to tone down the interpretation of the P hotspots in the cell and recommend to add a discussion of the possible artifacts, related to the preparation method of the thin sections.

We understand the reviewer’s point and agree that cryoscopy would have removed the ambiguities of our interpretations . We were not initially looking for acidocalcisomes specifically. Instead, we tried to interpret the P hotspots in agreement with the literature, hence the hypothesis that those hotspots are acidocalcisomes, based on their size and content. The cleaning should have removed the dissolved phosphate introduced during the protocol (Ayache et al., 2010), yet phosphate, either in the cytoplasm or the test, is not random.

Because none of our interpretations are new as such (acidocalcisomes and other P-bearing organelles) and are based on previous work, we modulate our interpretation so that the concerns raised by the referee appear clear to the readers of the article. The interpretation of P-rich grains as acidocalcisomes or any other type of structure is not critical for the current manuscript and we tune it down to reflect the uncertainties related to the method we used, be more explicit about possible contaminations from the method, correct the figures, abstract and conclusion accordingly.

The next point is not that severe but should maybe be considered by the authors: I was a bit puzzled by the use of the term “Rosalina-like foraminifera”. Either the species that the authors cultured is a Rosalina or not. I can imagine, where this is coming from: Most likely a foraminiferal species that has been optically identified as a Rosalina species has been cultured and later genetic analyses revealed that it is actually not a Rosalina. In this case, I would recommend to give this species (and genus) a name, rather then to use the term “Rosalina-like foraminifera”, which is objectively not correct and misleading.

We thank the reviewer for this important point, that we had discussed in Thaler et al. (2020) and should have further discussed here. The For1C1 strain (MNHN-CEU-2016-0075) has been designated '*Rosalina*-like' due to its morphological resemblance to the *Rosalina* genus.

The polyphyly of the ribosomal DNA sequences attributed to *Rosalina* and the uncertainties surrounding the morphological identification of the related specimens (Schweizer, 2008, 2009; Holzmann, 2017) make them unusable to confirm a taxonomic assignation.

Nevertheless, we agree with the referee 1 that 'Rosalina-like' may be removed, as the For1C1 strain can be assigned to the *Rosalina* genus based on its morphological characteristics according to the current knowledge. We propose to make this correction in the text, leaving the species-level identification open and using the designation *Rosalina* sp., as also suggested by the referee 2.

Here below the proposition of the text, that we have included in the revised version of the manuscript, as new supplementary information a description of the main morphological characteristics that allow the For1C1 strain to be assigned to the *Rosalina* genus. We also explain why, at present, we prefer to leave the species-level identification open and use the designation *Rosalina* sp.

Text added to the supplementary material:

The test is plano-convex in shape, with a trochospiral pattern, and is rather compressed. The spiral side is in fact gently convex and the umbilical side is slightly concave. All the rapidly enlarging chambers are clearly visible on the convex spiral side, particularly under an optical microscope, the sutures are depressed and oblique, curving back at the periphery. On the umbilical side, the chambers appear subtriangular due to the presence of an acuminate extremity named 'flap' or 'folium', which extends over the umbilical region. The sutures are slightly curved. The interior of the chambers is simple and undivided. The periphery is subacute with no keel present. The aperture is a low interior marginal arch bordered by a lip and located near the periphery on the umbilical side. It is separated from a small secondary opening by the umbilical folium. The test is hyaline calcitic with a smooth, perforated surface.

However, there are certain differences compared to the type species of the genus, *Rosalina globularis,* d'Orbigny, 1826. These differences can be attributed to the morphological variability within this genus. These include a more evolute spiral coiling in the final whorl, which results in less overlap of the chambers on the umbilical side, and an ovoid rather than circular outline. Furthermore, significant morphological occurs within the For1C1 strain, with two distinct morphotypes (Thaler et al., 2023). Individuals of the first morphotype reproduce asexually every 12–15 days once their test has developed 11–12 chambers and morphologically resemble the Rosalina genus. Contrastingly, the others survive for several weeks and develop over 12 chambers, with those in the final whorl tending to become more elongated and arranged in a more irregular fashion. Both morphotypes of the For1C1 strain are attached to the substratum. High morphological variability is typical of attached forms, even more so in larger specimens (more than 12 chambers). Reproducing individuals of the For1C1 strain are likely to be diploid macrospheric schizonts that have entered an apogamic cycle of successive asexual reproduction (Thaler et al. 2023). During this cycle, the new generation of schizonts is produced by schizogony (i.e. the multiple fission of a multinucleate parental cytoplasm). Therefore, as already discussed in Thaler et al. 2023, it is difficult to identify them morphologically at the species level because the morphology of the diploid microspheric agamont and/or the haploid macrospheric gamont parent generation, on which species descriptions are often

based, is, to the best of our knowledge, unknown. Indeed, the morphology of the macrospheric diploid schizont, macrospheric haploid gamont, and microspheric diploid agamont generations can differ considerably. For this reason, we will use the species-level identification and use the designation *Rosalina* sp.

Moderate points of revision:

The introduction appears to be a bit long and detailed. It almost reads like a review. I have the feeling that the introduction can be shortened quite a bit and/or some parts could be moved to appropriate sections in the discussion. This is not completely obligatory, since Biogeosciences does not have a length limit but keep in mind that the length could deter some readers to read the text in detail.

Thank you for this suggestion. We have shorten the introduction for the sake of clarity and simplicity and removed superfluous details.

Minor points of revision:

Line 62: "CO_2" instead of "CO2". Done, thank you for pointing it out.

Line 70: Add "a" before sulfate reduction pathway. Corrected, thank you.

Line 113: See Boyle (2006) regarding phosphorus discrimination in foraminiferal calcite in contrast to incorporation of P in aragonite of corals.

We thank the reviewer for pointing out this article we had chosen not to include, because its targets fossil foraminifera and thus its conclusions about biomineralization are not direct. However, Boyle (2006) states that « Foraminifera engulf vacuoles of seawater into their interiors and then chemically modify these vacuoles as they are transported to the site of calcification (8). In contrast, coral anemone polyps transport Ca2+ and H+ ions and gaseous CO2 across their cell membrane into an extracellular reservoir at the base of the polyp, where the fluid is drawn from external seawater (9). As a result of this difference in the mode of calcification, the chemical compositions of corals are quite different from those of foraminifera, with coral elemental concentration ratios values close to seawater values for many elements such as Sr/Ca and U/Ca, whereas foraminifera—including an aragonitic species— strongly discriminate against these elements. ». We do not fully agree with this statement. It was shown in many studies that elemental ratios in corals are not "close to seawater", if this means that the elemental coral ratios should be close to elemental ratios in inorganic aragonite precipitated from seawater. For example, Sr/Ca ratios in corals are also strongly affected by vital effects (see for example Corrège, 2006, Case et al., 2010, Gagnon et al. 2007). Moreover, many more recent studies have shown that Centers of calcifications, or Rapid Accretions Fronts, have extremely variable ratios of those elements, depleted relative to expected from inorganic experiments, and thus that corals do actually significantly control the elemental composition of their aragonite and discriminate against some elements. However, we agree with Boyle – and with the referee's point – that foraminifera do also control the chemical composition of the precipitating fluid, from which they actively exclude Mg, as well as other elements, possibly including phosphate, sulfate, or nitrate. Yet, sulfate and Mg, despite being calcite inhibitors, are found in foraminiferal calcite, which in itself shows that the removal is not complete. As a result, traces of inorganic phosphate or nitrate could also be found in foraminifera, just as in corals, though to a lower level. Furthermore, as pointed by Boyle (2006), "LA-ICPMS does not determine the chemical form of the phosphorus, so we are not

certain whether the phosphorus is bound in the inorganic lattice or in organic phases or ferromanganese coatings. The authors argue against the importance of the latter phases, but others will demand direct proof eventually" and thus, does not exclude the possibility of inorganic phosphorus.

We agree that assuming all P to be inorganic would be too strong, but we stand by the interpretation that the presence of some inorganic P in the calcite lattice is possible and coherent with the non-zero level of phosphorus in the lowest concentration levels. In addition, the enrichment of P is not only connected to CN, opening the possibility of an additional phase carrying P in the test. We have however nuanced our discussion on this point throughout out the text.

Line 171: If the authors used phosphate buffer for cell fixation: Couldn´t P distribution in the cell be partly artificial? P in acidocalcisomes is usually lost during cell fixation, based on dehydration that removes free ions in the cell and cryo-fixation is the preferred method (see longer comment above).

The referee raises a very important point, which is critical for our interpretation. We agree that cryo-fixation would be a more relevant method and that the method we used removed some information. As a result, following the points made by the referee in their main comment, we tuned down our interpretation regarding P distribution in the cell, as explained in reply to the referee main point.

Line 220: This transparency regarding the problem of god standard materials is really appreciated. This is often a problem. I think it is indeed very unlikely that the strong fractionation of the S incorporated into the test is caused by artifacts from machine drifts. Thank you for your comment.

Line 244-245: "The laterally heterogeneous lightness or darkness of the grey in the outer calcitic layers is intriguing." In my opinion this sentence is a bit redundant and intriguing is a bit to pictorial for a scientific paper. We agree that the sentence is superfluous and we removed it. We would argue however that being intrigued is a good incentive for scientific research but this debate is probably beyond the scope of research on foraminifera.

Line 262: "chemically lighter" sounds a bit strange. Do the authors mean that the material is "composed out of lighter elements" or that it is "isotopically lighter"? To avoid ambiguity with the phrasing, we change it to "composed out of lighter elements" as suggested by the referee.

Line 264: "show" instead of "shows" (plural). Corrected. Thank you.

Line 271: Again "chemically lighter". See above. Just "lighter" would be also enough, I guess. We hesitated because lighter seemed more ambiguous. However, with the previous change suggested by the referee, "lighter" is probably clear enough here. Thank you for the suggestion.

Line 302-303: I would write "to the strongest P, S and Mg enrichments in relation to CN" instead of "to the strongest P, S and Mg enrichments compared to CN". We changed the sentence as suggested. Thank you.

Line 331: I don´t see that those distributions are very similar in fig. 5. Actually at the edges the distributions are very different. P gets really high at the edges and Mg not. S is in between. Also the minima and maxima in the inner parts are sometimes more or sometimes less pronounced in the

different elements. I would suggest to show a correlation plot and test if the correlation is significant. This could very well be the case but it is very hard to see in fig. 5.

We thank the reviewer for their comment, as this is an important point. Correlations of four profiles across the test shown in fig. 5 are provided in fig. 4. We realized that the relation to figure 4 was missing from figure 5 caption, so we added it. We also now refer to figure 4 in the sentence pointed out by the referee. Correlations are provided in the text following the table presented above.

Line 351 and 352: Replace “foraminifera” with “foraminifer” in both cases, since it is used for singular here and not for plural. We corrected it, thank you.

Line 396: The cited study doesn´t really show “Ca-phosphate” but most likely polyphosphate granules and gels that are associated with Ca in addition to dissolved pyrophosphate. Thank you for pointing this out. We have corrected the sentence to be more accurate.

Line 443: Maybe use “primarily” instead of “first”? We modified the sentence, thank you for the suggestion.

Line 444: Replace “foraminifera” with “foraminifer”, since it is used for singular here and not for plural. We corrected it, thank you.

Line 460: “but also to…”. To what? I have the feeling a word is missing in this sentence. The referee is absolutely correct, the word “control” was indeed missing. Thank you.

Section 4.1.1: There is a difference between precipitation of calcite and ion exchange between the lattice of already formed calcite in the surrounding medium. I have the feeling that both processes are a bit mixed up in this section. We reworded this section in order to avoid ambiguity.

Sections 4.1.1-4.1.3: I don´t really understand, why these three previously proposed biomineralization mechanisms have to be discussed and reviewed in such a detail in this section. They are partly not really related to the authors results. It felt necessary to us to explain the different ways ions could be brought to the tests, because we wanted to be conservative. We also believe that because we bring the possibility of active control of sulfate, we have to explain the main pathways in order to explore how this control could come into play in each of them. The reminder here is also necessary, because it shows that vacuolization, and passive transportation, both have the opportunity to deliver S, Mg, P and N as seawater ions to the site of calcification. However, we tried to reduce this section to the minimum.

Line 510: I think this sentence could be formulated a bit better. We tried to improve the sentence. Thank you for your comment.

Line 512 and following: Could the mixing of the labeled with the unlabeled P be related to mixture of unlabeled organic S with the labeled inorganic S that is incorporated into the test walls? Would the percentage fit to the plot in fig. 4a? For example in phase 1 it looks like up to 30-50% of the sulfur would sit in the organics. This would roughly fit to your 1/3 of non-labeled S.

There might have been a confusion here because we mention 1/3 of labeled S. Futhermore, this section is about the cytoplasm, we thus assume that all S is in organics there. We hope that the revisions of the text makes this section clearer to the readers.

Line 514: There is a word missing before “than”. More than? Less than? We wrote “than” instead of “that”. This is now corrected, thank you for spotting it.

Line 528-530: What would that mean? This section is a bit confusing. We modified and corrected the section. We hope it is more clear now.

Line 567: The use of “on the other hand” needs also “on the one hand”. We modified to sentence and used “Compared to trend A” instead.

Line 581: “Locally dark local”? Please reformulate. We changed the sentence to “locally dark areas”

Line 581-591: See my long comment above about the association of P to organic matter in foraminiferal tests and the discrimination of inorganic P in the calcite lattice. I really do not understand why the authors interpret their results as an evidence for inorganic phosphorus in the test. In my opinion all their results show the opposite and former literature is completely ignored. Especially the older works of Boyle (2006) and the studies that are cited within… The mentioned works of Boyle specifically address the question of cleaning on corals, though it also discusses the occurrence of P in foraminifera, though mostly to point the discrimination against P that is stronger in foraminifera than in corals. We certainly agree that foraminifera discriminate against many elements more than corals do. We hope that our longer response to the referee main comment, as well as the detailed correlations, make our interpretation stronger.

Line 606: Why co-distribution? P and S are not even clearly correlated when they are normalized to CN (the organics). They are only co-correlated, if they are not normalized to CN, because both elements are at least partly associated to organic matter (P more and S to a lesser content).

This sentence has been reworded following the discussion presented to the first point of referee 1. We stand by the observation that there are common trends,

Line 608: Phospho-aminoacids are not really polyphosphates. Polyphosphates are chained phosphate molecules, which could be completely inorganic or have an organic rest such in ATP.

Thank you for pointing this out. We must correct the text to be accurate.

Line 616: I cannot agree with this hypothesis and don´t see that the data are justifying this hypothesis. We don’t see any reason to exclude the idea there is at least traces of inorganic phosphate ions in calcite. If it can be included in inorganic calcite, and if there are phosphate ions in seawater, it is possible to find it in foraminiferal calcite, as we presented in our previous comments. We tuned this point down, as discussed previously.

Line 619: See above: The patterns are not similar, if normalized to CN. Thus, P and S are only co-correlated in the organic content.

As discussed previously to answer the main comments of the referee, our observations support a more complicated pattern. This text has been modified nonetheless.

Line 689: “plays” instead of “play” at the end of the line (singular).

Corrected, thank you

Line 698: How can you design an observation? The sentence has been corrected to “designed the experiments and established the sequence of observations to perform” for better understanding.

References from that review that are not cited in the paper:

Ayache, J., Beaunier, L., Boumendil, J., Ehret, G. & Laub, D. in Sample Preparation Handbook for Transmission Electron Microscopy: Methodology (eds Ayache, J. et al.) 125–170 (Springer, 2010).

Edward A. Boyle: A Direct Proxy for Oceanic Phosphorus? Science 312, 1758-1759 (2006). DOI:10.1126/science.1129723

Corrège, T. (2006). Sea surface temperature and salinity reconstruction from coral geochemical tracers. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *232*(2-4), 408-428.

Referee 2

With great interest I read the manuscript ‘Tracking sulfate, magnesium, phosphorus and amorphous phases in *Rosalina*-like benthic foraminifera’ by Paris and co-authors. They present interesting data of elemental distribution of foraminifera cultured in controlled conditions enriched in 33S. Although aware of the limitations of their analyses, the work is interesting to the global audience working on biomineralization and element distribution in foraminifera. I do however have some issues that the authors and some inconsistencies that have to be cleared up and fixed before publication, and below I post suggestions of revisions. I would also suggest the authors to present all the maps in the article / supplementary data / data repository, since certain maps are missing from the supplementary data.

We thank Dr van Dijk for her supporting words and for the following review. Her thorough, detailed and useful review helped us revisit our data, triggered new thoughts and led us to better explain our interpretations and fix the different points raised by the referee. We also made sure that all maps are present in the supplementary data or data repository, if they are referred to in the text.

In material and methods, explain why the specimens are called *Rosalina*-like. Some sentences about the issues with determination would be welcome. Would it be possible to add some SEM photos of the species? I would explain issues with current taxonomy, and I would argue to use the name Rosalina sp. throughout the manuscript. I would remove the species name from the title, and change it for instance into ‘Tracking sulfate, magnesium, phosphorus and amorphous phases in cultured benthic foraminifera’

We thank Dr van Dijk for her comment. Similarly to the elements we developed in our reply to Referee 1, the For1C1 strain (MNHN-CEU-2016-0075) has been designated "*Rosalina*-like" due to its morphological resemblance to specimens of the *Rosalina* genus. However, the polyphyly of the ribosomal DNA sequences attributed to *Rosalina*, alongside the uncertainties surrounding the morphological identification of related specimens (Schweizer, 2008, 2009; Holzmann, 2017), mean that these sequences cannot be used to confirm a taxonomic assignment. Nevertheless, based on its morphology, the strain can be attributed to the *Rosalina* genus, according to current knowledge. In line with Referee 2's suggestions in the 'Materials and Methods' section (or in an annex), we added a description of the main morphological characteristics that allow the For1C1 strain to be assigned to the *Rosalina* genus. We also explain why we currently prefer to leave the species-level identification open and use the designation *Rosalina* sp. Following the suggestion of the Referee 2 we also changed the title to : "**Tracking sulfate, magnesium, phosphorus and amorphous phases in cultured benthic foraminifera".** SEM photos are available in Thaler et al., 2023. It thus did not seem necessary to add new ones here, but we mention it in the supplementary material, with the description of *Rosalina* sp.

Line 155: Can you explain the reason for the short duration + the different durations of incubations?

We wanted to explore whether unlabeled sulfate from seawater (from before the labeling experiment itself) could be stored within the cell (possibly in vacuoles) or if sulfate is incorporated quickly after introduction in the labeled media. Some foraminifera were sacrificed after 2 days and others after 5 days, depending on our observations. Beyond this period, the foraminifera reach a size close to the reproductive age, which would have disrupted the counting and recognition of cells. There are also foraminifera that stop growing after a certain size, and we would have compared individuals that spend a significantly longer time in the environment before precipitating a lodge.

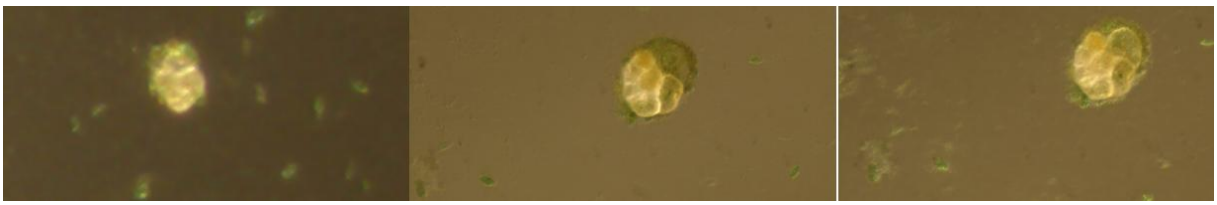
Line 156: this information should be added to table (fed with labelled food)

The referee is right. This has been done. Thank you.

Line 164: You did not observe it, but it could have been possible?

Thank you for your question, we realize more explanations are necessary here.

Chambers were counted before and after the experiments for juvenile foraminifer A1 and we have documented the growth of two chambers in two days in the labeled medium (we can add the photos documenting this in the manuscript).



Foraminifer F5(2)B and F2A2 were selected amongst different adult specimens after the experiment in the labeled environment. Combined with the absence of ^{33}S enrichment in the two last OCLs that would have been the ones to grow in the labeled medium, we strongly assume that no calcite grew during the labeling. We intend to disambiguate this aspect in the text.

The situation is more complicated Fa5B, adult as well, possibly more likely to have grown a new chamber during the experiment. However, the questions related to the labeled food are different and thus, it is less important for our observations to know whether or not a chamber grew during the food labeling experiment and we do not look at the last chambers. We are now more explicit in the text here as well.

Fig. 2 - Could the oblique lines indicating additional structures found in the OCL be the edges of cogwheel colums? See Nakajima et al., 2016, van Dijk et al 2020, Cisneros-Lazaro et al., 2022?

We thank the reviewer for this interesting suggestion. However, would cogwheel translate as oblique structures, and not perpendicular to the test ?

Fig. 3 - Panel f, the green lines represent the platinum deposit, but does the top green line not represent the cytoplasm, looking at the 12C14N map?

We thank the reviewer for this important point on which we actually spent time ourselves. During the cellular fixation process, cytoplasm contraction and cellular detachment likely occurred between the wall and cellular material in the chamber analyzed here (n-2), creating a gap. Then, during the cutting process to extract the FIB lamella, some of the cellular material was likely pulverised and redeposited in the gap between the wall and the cellular material, where it mixed with platinum.

This area therefore could represent the redeposition of cellular material, which could explain the layered appearance of it as well as the CN enrichment.

This redeposited layer is an artefact of the FIB lamella preparation process, this area has been excluded from any interpretation, except for d33S has any sulfur present in this part of the FIB would come from organic matter of the cytoplasm.

The presence of platinum could explain the high density observed in the STEM image. This area remained slightly thicker than the calcium carbonate wall, which could account for its black colour in the MET bright-field image and the presence of platinum could explain the high density observed in the STEM image.

The following sentence has been added to the result section to take this aspect of the process into account :

“The FIB section is surrounded by two thicks layers of platinum deposits, one of them constituting the dark (Fig. 2a) or white (Fig; 2b) layered area at the base of the FIB. The layers can be seen in the MEB view (Fig. 3f). The FIB was performed on chamber n-2, which could have retracted different the fixation process, creating a gap. The cutting process during FIB extraction likely pulverized some of the cellular material attached to the test, which was then redeposited in the gap between the wall and the cellular material, where it mixed with platinum. This layer is thus an artefact of the FIB lamella preparation process.”

I have some problems to interpretate figure 4, maybe I am missing something. The figure presents data from 4 profiles on the same chamber. The chamber wall is described in the text (line 294 and onwards) to be an alternation of bands parallel to the chamber wall that have each have a distinct chemical signature (e.g. high or low ratios). The profiles are taken perpendicular to the foram wall

and this banding, should there not in theory be points of each profile in each trend? For example, trend 2 is only observed for profiles 2 and 3. What could cause this heterogeneity?

Dr van Dijk is correct in the way she reads the figure, and there is undoubtedly a lateral heterogeneity that is visible in the different NanoSIMS maps, with areas that are more enriched in P or S or Mg within a given layer. Profiles 2 and 3, and to a lesser extent 4 as well, happen to capture two of these enrichments. This reflects the location of P, S, and Mg enriched grains, already documented in the text. In any case, we also hope that the new figures will help to show that all trends are present in each profile, though the extent of the enrichment is variable.

The foram shown in Figure 3, were not observed to have formed 2 new chamber in the experimental condition – it would be remarkably fast growth rates. Could you derive this from maybe photos taken before and after the incubation? Since there is some 33S enrichment (line 317), could you conclude based on the 33S signal that this n-1 chamber was formed during the experiment? Otherwise, is this enrichment coming from adsorption / isotope exchange? How does it compare to 33S measurements on the A1 specimen – that did calcify for certain.

We thank the referee for this comment,

The foraminifer shown in Figure 3, 4 and 5 (F5(2)B) did probably not grow any chamber in the labeled medium. Because foraminifera were sacrificed after living in the labeling medium, chamber n-1 most likely did not grow in the medium either.

However, going back to figure 5 thanks to Dr van Dijk's comment, we note that the OOLs and the IOL are enriched in ^{33}S, reaching values of 500 and 800 ‰, respectively. Another area, right at the transition from the POS to OCLn-1 is characterized also by non-zero values, up to ~800 ‰. This is significantly lower than the values for the chambers built in labeled seawater, with values up to 5500 ‰. This reveals however that seawater sulfate seems to circulate somehow across the organic layers of the test, which thus seems to remain active after formation of the chamber, especially the IOL. This seems also to be true for parts of the calcite, but not so much for the POS. The POS and the rest of the test are barely, but possibly slightly, enriched (values between 0 and 300 ‰). Overall, this observation supports the idea that at least some of the sulfur in the organic layers comes from seawater sulfate, and that the organic network remains active after the chamber is formed. This also suggests that the composition of calcite might evolve after it is formed, with new ions incorporated in previously formed chambers.

We also added individual profiles for profiles 2 3 and 4 in figure 2 of the reply to referee 1. The clearest pattern is the addition of labeled sulfate on the inner side of the test (ICL/IOL), virtually none in the POS and the OCL/OOLn+1, sometimes moderate enrichments in the OOLs or OCLn-1 close to the POS, but nothing systematic.

We included and discussed these details in the new version of the text.

Line 331-332: 'similar' = can you test this?

We did not try to test this. We rephrased the caption to use a less ambiguous wording.

Line 342+: Since the P/CaO and P/CN profiles show very similar patterns, is this not just proof that you P is coming from organics, especially since your forams did not undergo any cleaning? Could the oblique shape noted in the P/CaO map be caused by being close to a T section of two chambers?

Regarding the first question, we kindly refer to the first point of referee 1 where we discuss these trends in detail. Regarding the second question, the FIB lamella was not close to a T-section between two chambers. It has been cut perpendicularly to the surface within the chamber.

Line 346: when comparing to other studies, caution should be taken, since some of these studies use oxidatively cleaned or uncleaned samples (or both in the case of glock et al., 2019), which

Thank you for pointing this out. The text has been modified accordingly and the comparisons were nuanced to take this important aspect into account. Though the sentence appears to have been cut, we hope it doesn't alter the meaning of the referee's point.

Figure 6: Trend A and trend B are corresponding to profile of another foram? Why not take nanoSIMS profiles and see if the match the profiles of the other specimen used in Fig. 4?)

These trends corresponds to profiles of the same foraminfer as in Fig. 4 and 5. We hope that the new figures and corrections make this point clearer.

Line 375: there are white arrows missing in panel 7d and 7i.

Thank you for pointing it out. This has be fixed.

Line 378: Is the P coming from the chemical fixation of the cell due to the phosphate buffer? Would it no difusse in to the cytoplasm and bind to structures?

We understand the point of the referee, which is also pointed out by referee 1. We understand that our explanations were not sufficient, the text has thus been modified accordingly. However, because the method unfortunately removes non-fixed ions and molecules, it seems unlikely that it would be able to allow P to bind to structures. We thus think that the cleaning process should have removed the phosphate introduced during the cell fixation protocol, and that its distribution in the cytoplasm or the test is not random.

Line 371, 381-382: The enrichment of S at the outer edge of the cytoplasm, is it located in the cytoplasm, or is it in the calcite? What limit was used to mask the calcite (or cytoplasm) in all the figures? Is this area not heavily impacted by artifacts at the edges? The edges of the test pores and the edges of the test walls show the strongest topography differences on foraminiferal cross-sections after measurements with NanoSIMS (Geerken et al., 2019), which likely impact the ion yield during the NanoSIMS. Caution should be taken when interpretating measurements .

Thank you for your questions and remarks here. This is a very important point raised by the reviewer here again. The mask was performed from the Ca maps, the absence of calcium defines the limit of the mask. However, because of the spatial resolution of the NanoSIMS linked to the beam size and the difference of matrix between the test and cytoplasm, we probably should not interpret too deep the transition between the cytoplasm and the test and we removed the discussion about the enrichment when relevant.

Line 392-394: I see the vesicle near the IOL, which has points of increased Ca, but are the same pixels that are rich in Ca, also rich in 33S? Or is the rest of the filling of the vesicle (which is low in Ca) enriched? The d33 shows for me a very similar pattern to the vesicle below, were there is no Ca

The referee raises a very good point. We revisited the description and interpretations here and later in the text.

Table 2: The table is based on very little data, while you have more observations. Would it be worth to expand the calculation to the other specimens / maps? Also the d33 maps are missing in the supplementary materials.

All maps should be present in our data posted online (see Data Availability). We did not expand table because full sections of the cytoplasm are very heterogeneous and it didn't seem relevant, withtou mention the potential loss of free sulfate/sulfur during the fixation process that would affect the average ratios.

Line 419: reference to wrong figure? Should be 9? Why are only use these maps, and not the maps from previous images?

We apologize for this mistake. We corrected all wrong referencing of the images. We selected only some maps to allow a clear presentation.

Line 445: 7h referes to the panel of 31P. you mean 9h? If I look at the test of F2A2 in figure 7 (7j) there seems to be some enrichment?

Here again, we sincerely apologize for the mislabeling. We took extra care in correcting all references to figures. Thank you very much for taking the time.

Due to the low precision of ^{33}S measurements, we do not interpret any value between -200 and + 200 as different from zero.

Line 450-452: This species is calcifying following a laminar model? Why do we not see the more enriched 33S from the n layer of calcite in the n-1 chamber? Also therefore the measurements of n-1 are a mix of n and n -1 calcite ?

The analyzed strain calcifies in accordance with the laminar model (ICL-POS-OCL) model, which is typical of rotaliids. This is clearly evident in specimen F5(2)B (see Figure 2). However, specimen A1 is a very small juvenile with seven chambers (see Figure 1a). Thus, the analyzed chambers N and N-1 are very small, thin, highly curved and pore-less. This makes it difficult to obtain FIB lamella cuts perpendicular to the surface of the test. It is possible that slightly oblique lamella cuts were achieved instead, as the POS structure is not visible in the Ca maps (it should appear as a zone low in Ca within the wall; see Figures 9a and 9d). Furthermore, the wall in chambers N and N-1 is very thin at around 1–1.5 µm wide. Consequently, detailed ^{33}S according the ICL-POS-OCL and ICL-POS-OCL-OCL microstructures of the wall could not be created using the lamellae from chambers N and N-1, respectively (see Figures 9a, b, c, d, e and f). Alternatively, it cannot be excluded that the first chambers during the juvenile stage have almost a mono-layer structure, which requires confirmation through further studies. In any case, the interpretation that "the difference between the two chambers could suggest that more labelled sulfate is incorporated in the last chamber after the

individual spends more time in labelled seawater" (Lines 450-451) does not change, whether or not chamber N-1 is a mixture of calcite from chambers N and N-1. If the calcite in chamber N-1 is a mixture of calcite from chambers N and N-1, the difference in the incorporation of labelled ^{33}S, linked to the time spent in labelled ^{33}S media, is likely to be attenuated, but should still exist. However, because of the precision of the ^{33}S measurement, a 300% difference is not easy to detect precisely.

452-3: The food is unlabeled, why does the 33S homogeneity proof there is no sulfur from food present? The POS is not exclusively made up from organ matter from the food – or if so, add the reference. See Nagai et al., 2018. Also, does the size of your beams allow for the clear distinction of organic banding?

Our reasoning was that if all S is homogeneously labeled across the test, this may imply that all S is the same mixture of seawater sulfate included during the experiment and unlabeled sulfur. Thus, even across the POS, at least ¾ of the sulfur initially comes from seawater sulfate. We nuanced our statement and changed the sentence from "that no significant amount of sulfur from food is present." To "…that less than ¼ of the sulfur can be ultimately sourced from food."

It thus seems that we agree with the referee. We do say that that we don't find sulfur from food, not that we don't see sulfur from organic matter. As we detail later, sulfur might be fixed by the organism and there it thus the possibility that sulfur in organic matter might come from seawater sulfate.

The size of the beam is a critical limitation that we must keep in mind during our interpretation and the referee raises a very important point. To answer clearly, no, the size of the beam does not always allow to clearly detect the organic bandings, because the organic lines themselves are much thinner (as seen in TEM / STEM views) than the beam size (please see fig. 5). However, an unlabeled S in the POS could still be detected, similarly to the P enrichments or other enrichments, and appear as a more-diffuse change

Line 455: There is no map for d33 in figure 5. You mean fig 9? Thank you for pointing this out. This is corrected in the text.

Line 456 ' new seawater sulfate….' I do not understand the first part of the sentence

What we meant to say is that, despite the test having been formed before the experiment, we find labeled sulfur here. This observation implies that "fresh" seawater sulfate, not the sulfate present in seawater at the time of growth, is found in the test. We changed the sentence to "Seawater sulfate is thus introduced during the duration of the experiment" .

Line 456-60 ' Rotaliid foraminifera', sentence incomplete? Thank you for seeing it. A word was missing. The sentence now reads "Rotaliid foraminifera are not only able to precipitate calcium carbonate but also to control its composition and mineralogy."

4.1.1-4.1.3 This feels very much as a review, and I think you are going a bit far in the interpretation of your limited data in terms of calcification pathways. I think it is interesting, but more to discuss in the light of how open / closed the system is, since you find enriched cytoplasms in forams that did not

calcify, and not focus on incorporation, since it is not cryo nanosims and you have no calcification fluids / vesicles left.

We thank the referee for her point. We need to reorganize and simplify this section, also in light of referee 1's comments and adding reference to the work of Nagai et al. (2018).

Line 468; I do not understand why you would expect labeled S on the outside of the calcite.

If there was an addition of sulfate through the seawater leak process, we would assume it to be incorporated from the outside and the inside, and thus to be found on both sides of the test. However, in the case of a process such as Nagai et al.'s, we would expect a different pattern.

Line 470: You mean you see uptake of 33S in pre-experimental chambers ?– this is in contrast what you say in line 456-457.

We believe that the two statements are identical and we hope that the new wording we propose, less ambiguous, reconciles them both for the referee. We see traces of labeled sulfur within tests that did not grow during the experiment (see fig. 5 for instance), in the OCLn-1, but not in the latest layers that were built just before the transfer.

Line 515: why are these specimens not added in table 2?

Table 2 (now table 3) is calculated only for the FIB of the test. Because the cytoplasm is so heterogeneous, it seemed irrelevant to calculate an average value, and the test is mostly free of 33S. We added however the profiles for d33S of F5(2)B1 in the supplementary material.

Line 564-566: For me, the plots in Fig. 4, in which P/CaO and P/CN show almost the exact pattern proofs the opposite...

This is coherent with the concerns raised by referee 1. We corrected this sentence, as well as other parts of the text, in line with our reply to referee 1. We kindly invite Dr van Dijk to also read our reply to referee 1.

Line 660: Already commented on this, but there is more evidence that the POS is formed by the cytoplasm/pseudopods, at least for Ammonia: Nagai et al., 2018 https://doi.org/10.5194/bg-. You would therefore not expect to be low in d33, since the maps show that the cytoplasm is enriched. The weaved POS would exhibit tiny gaps (holes within the framework) that may serve as pathways for passive ion exchange, which explains your high 33S in your cytoplasm

We thank Dr van Dijk for this comment. We carefully read this section again, but we believe that we are in agreement here. We only state here that sulfate in the POS comes from seawater sulfate, not that is not associated to organic matter. If it is associated with OM, it just means that this association was performed in the cell.

We carefully read again Nagai et al. 2018 about the possibility of passive ion exchange. First things first, we should have cited their work, highly relevant for our study. We agree that their work provides a detailed mechanism for direct precipitation from seawater.

"The organic layers (especially well-observed in the OOL and the POS) still contained numerous gaps < 1 µm in size, which we interpret as maintaining the exchangeability of seawater and elements

contained within, for the initial part of calcium carbonate precipitation. The site of calcification is therefore interpreted to be still open in the middle stage. In the late stage, however, the organic layers have been completely filled by membranous pseudopodia and no such gaps remain. At this stage, therefore, the site of calcification is closed from the surrounding seawater. Hence, we interpret that during the late stage the elements required for calcification must be selectively taken up by biological means such as exo-endocytosis or ion pumps through the OOL."

They do thus support that at least part of the calcification happens in controlled conditions, in agreement with our interpretations. We also don't observe a drastic change in Mg/Ca ratios across the test, which should be observed in the case of transition between the passive and active transport processes (Nagai et al., 2018, based on Nerkhe et al., 2014).

The last point of the comment concerns the possibility that the gaps would create a passive paths= that explains the presence of labelled sulfate in the cytoplasm. It seems unlikely to us that the cell would be entirely in direct contact with seawater through passive exchange to the extent that sulfate could circulate freely across the membrane and enter the cytoplasm.

We thank again Dr van Dijk for referring to this study and we included Nagai et al (2018) in our discussion, to outline our points of agreement and possible disagreement with how they interpret their observations.

Line 673: This 1/3 is referring to the cell, not the test right? This is absolutely correct. We changed the sentence to "Cells that lived in ^{33}S-labeled seawater revealed that at least 1/3 of the sulfate incorporated in the cytoplasm is directly taken from seawater" and modified the sentence associated to this point in the abstract.

Line 680: replace 'grew up' with 'formed'

We changed the sentence. Thank you.

Inconsistencies and typos

We have corrected all the typos and we thank Dr van Dijk for taking the time to point them out.

Line 145 wed = fed

Line 138: seawater

Line 118: ang = and

Line 210: consisted in suites = consisted of sets? We changes suites to series

Line 264: show

Line 285: point

Line 316: double in

Figures and tables: There is inconsistencies when referring to figures, which led to some confusion on my part to which figure was referred to by the authors. Double check the references. Thank you very much. We apologize for this.

Table 1: are there 2 specimens called A1? One that was in an experiment of 2 days, and one that was in an experiment of 5 days? This was a mistake on our end. It was the same experiment and it lasted 2 days. This is now fixed. Thank you for seeing it.

Fig. 2: (2)B1 = F5(2)B1? Absolutely thank you. This has been fixed.

Fig 4: Trend 1, 2, 3 = trend A, B etc in the text? Yes, this has been corrected

Fig 5: add panels, since you refer to them in text? (eg line 455) Thank you, this is a very good point and we added the panels.

Fig. 7: missing element/title for map 7g and 7j + missing magenta arrow in 7j (for the ca rich vesicle near the IOL Thank you, we changed the figure accordingly.

The authors talk about 'trend A' or 'trend B', this correspond to the numbers in figure 4? If so, rename the trends in the figure / text. This is an important point, thank you very much for pointing it out. This is corrected in the new version of the figure.

Name of the species: 'Pseudorosalina Banyuls' in the supplement, 'Rosalina-like' or 'Rosalina like' throughout the manuscript

We thank Dr. van Dijk for this comment. Following our reply to referee 1, we have corrected this to *Rosalina* sp. through the text.