Biogeosciences
Discussions

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Interactive comment on "Coupled Ca and inorganic carbon uptake suggested by magnesium and sulfur incorporation in foraminiferal calcite" *by* Inge van Dijk et al.

Inge van Dijk et al.

inge.van.dijk@nioz.nl

Anonymous reviewer #1, Thanks for the thorough reading of our manuscript and your constructive comments. Below, our responses are listed directly below the comments. We try to answer all specific comments raised by your review.

Major comments - As far as possible, please report carbonate system details for both the culture experiment and the aquarium. What I found puzzling is that van Dijk et al. [2017] showed that S/Ca principally depends on the seawater [CO_3^{2-}], an exciting result, yet this is mostly not discussed in this manuscript. For example, in order to make sense of the data in Fig. 3B, we really need to know the [CO_3^{2-}] of the different

cultures. If they do not conform to a (temperature-driven?) S/Ca-[CO_3^{2-}] relationship, then why, and how does this impact the conclusions of van Dijk et al. [2017]?

For calculating the full carbonate system and thus CO_3^{2-} we need to determine 2 parameters. As the aim of the study was not relate S and CO_3^{2-} we (unfortunately) did not monitor e.g. alkalinity and DIC. We did occasionally measure pH of the culture media inside the culture bottle. However, beside the low number of measurements, pH is difficult to measure accurately. Still, we measured pCO_2 within the incubator, which was around 800 ppm. For the natural seawater we used, we assume a TA of $\sim$2300 umol/L (extrapolated from salinity). Using the CO2sys software, we estimate the [CO_3^{2-}] of the culture media to be between 115 and 145 umol/L over this temperature range. The experiment was specifically designed to study the effect of temperature on element incorporation. Hence, we focus the discussion on inter- and intra-specimen variation in S/Ca and Mg/Ca. We used CO2SYS to calculate the expected change in CO_3^{2-} due to differences in the used temperatures (assuming a constant alkalinity of 2300 umol/L and pCO_2 of 800ppm). The difference in temperatures between treatments (21 and 29 degrees) accounts for a change in CO_3^{2-} of $\sim$30 umol/L. This offset between treatments is relatively small compared to the sensitivity of foraminiferal S/Ca to [CO_3^{2-}]. and would result in a change in S/Ca of 6% or $\sim$0.08 mmol/mol (van Dijk et al., 2017, EPSL). Hence the S/Ca-[CO_3^{2-}] relationship is not sensitive enough to detect such variability between treatments (Fig. 3). Therefore, changes due to differences in [CO_3^{2-}] are most likely below the detection of this proxy. Even though our experiment was not designed to determine the impact of carbonate ion on S incorporation, and taking into account the limited control we have on the sea water carbonate chemistry of the experiments, values plot in the same order of magnitude as those of van Dijk et al., 2017 calibration, see the figure (R1: In red, average values for Amphistegina gibbosa (van Dijk et al., 2017, EPSL). In yellow, average values for Amphistegina lessonii from this study) for your reference.

Likewise, on page 12, line 24 onwards (Fig. 9), the shell SO_4^{2-}/CO_3^{2-} ratio is dis-

cussed, but what we are interested in is the shell SO_4^{2-}/CO_3^{2-} ratio as a function of the seawater SO_4^{2-}/CO_3^{2-} ratio. It would be more informative to replot the data in this way.

Although intriguing, we were not able to plot the data this way, since SO_4^{2-}/CO_3^{2-} is not known for all conditions. However, the reviewer provides a good point, we can use shell S/Ca to unravel SO_4^{2-}/CO_3^{2-} of the calcifying fluid at the site of calcification, which will be added to the last paragraph of the discussion. To be added to MS: "This implies that the S/Ca distribution in the foraminiferal chamber wall may reflect a change in SO_4^{2-}/CO_3^{2-} of the calcifying fluid in the site of calcification (SOC) during precipitation of the shell wall. Assuming a stable D during calcification (E.g. Dx1000= 0.013; Busenberg and Plummer, 1985), SO_4^{2-}/CO_3^{2-} at the SOC would be a factor of 3.6 higher during the thin, high-concentration band (with an S/Ca of 6.9 mmol/mol; Fig. 7) compared to the broader, low-concentration band (with an S/Ca of 1.9 mmol/mol). This decrease by a factor of 3.6 could be due to an increase in $[CO_3^{2-}]$ and/or a decrease in $[SO_4^{2-}]$ during precipitation. The latter could be the result of inclusion of small amounts of sulfate in the SOC at the beginning of chamber formation and ongoing incorporation of sulfate in the foraminiferal calcite. However, since the S/Ca is not decreasing towards the outer side of the shell in the low-concentration band, the former process, i.e. increasing CO_3^{2-}, might be more likely. An increase of CO_3^{2-} at the SOC from the first stage of chamber formation (high in S/Ca) to the broader second part (low in S/Ca) could be caused by an increase in internal pH due to proton pumping (Toyofuku et al., 2017). The band of high S/Ca would then be precipitated when proton pumping has not yet reached its maximum rate and the internal pH is still rising (Glas et al., 2013). However, to confirm this hypothesis, a more precise characterization of the calcification fluid's chemistry is necessary."

- Throughout the manuscript, I was confused about what the term 'coupled' means. Is the implication that the Mg/Ca and SO_4^{2-}/CO_3^{2-} ratios covary in the EPMA maps because these ratios in the calcifying space are (coincidentally?) being coregulated?

If so, I recommend explaining more clearly what the inference is regarding how these ratios are modified through the process of chamber formation. In other places, the covariation ('coupling') of Mg and SO_4^{2-} when comparing different species is mentioned, and so it is sometimes ambiguous whether covariation within a chamber, or within foraminiferal calcite in general is being discussed. Is the argument that similar processes are operating on all scales (between species/within a chamber etc.)?

We tried to clear up the nomenclature used in the manuscript. We replaced coupled with coregulation and covariation.

- I appreciate that overall the authors have made an effort to consider how these data could fit into both the vacuolisation and TMT model. I would nonetheless urge more careful phrasing in places. For example, line 27 on page 1 states 'Mg incorporation is linked to the Ca-pump', but there is no strong evidence here for the TMT model here, and the previously published evidence has been disputed. Fig. 9 should also be reconsidered. Low-Mg foraminifera are not all 'Ca transport-dominated', indeed it is very difficult to see how the concentration of most trace elements could be reconciled with the TMT model. Perhaps Ammonia does differ in this respect from the planktonics, but a low shell Mg/Ca ratio does not necessarily imply Ca TMT. Also rephrase line 18, page 2, line 15, page 14 (Mg-transport is also possible/likely), line 18, page 14 (the phrase 'actively take up' is ambiguous but certainly not all foraminifera transport Ca which is what I think most would understand from this statement).

We rephrased these sentences, to also include differences in Mg transport out of the SOC as a process to change shell Mg/Ca. We removed only the word 'actively' from Page 14, line 18, since also with vacuolization, carbon and calcium are taken up together. In figure 9 we slightly modified the vector of SWV-dominated (see also comments reviewer 2).

Minor comments - Page 1, line 24. Rephrase or remove the word 'consistent'. If the behaviour of Mg and SO_4^{2-} incorporation is strictly consistent then surely some of the

inorganic processes mentioned earlier in the sentence could explain what you observe.

This sentence is now rephrased.

- Somewhere in the introduction it would be useful to state how big the SO_4^2 ion is compared to CO_3^{2-}. Would lattice distortion from Mg incorporation be expected to favour SO_4^{2-} incorporation?

This is now information added to the introduction based on the radii published in Jenkins&Thahur (1979). They state that SO_4^{2-} ions are larger than CO_3^{2-} ions, which would mean Mg distortion might increase the incorporation of SO_4^{2-}.

- Page 2, line 21. Consider reversing the sentence; the chemistry of the shell depends in part on the chemistry of the fluid at the calcification site, which in turn likely depends on the ambient seawater.

This sentence is now rephrased.

- Page 2, line 31. What does 'immobilization of these ions' mean?

Elements might not only be physically removed, but also unavailable in terms of e.g. speciation/complexation. We changed 'immobilization' for 'unavailability'.

- Page 3, line 1. Please clarify 'without a strong control on ions that inhibit calcification'. Do you mean that increasing DIC does not have a large effect on the speciation of most ions?

We rephrased: '..without needing removal of ions that inhibit calcification'

Page 4, line 6. I must be missing something obvious, but what does '(par)' mean?

PAR = Photosynthetically active radiation (μmol of photons m2 s-2), which equals to high light conditions. We stated this now more clearly in the revised manuscript.

- Page 4, line 31. Why was MACS-3 was used as the calibration standard? I understand the benefits of matrix-matching, but it has been argued that carbonate standard-

isation using NIST produces accurate results, and the issue with MACS-3 is that it is not as homogeneous as NIST610 [see e.g. Jochum et al., 2012], which is also borne out by the data in Tab. 1.

We agree MACS-3 is less homogeneous than NIST610, but the concentrations of various elements, including Ca, Mg, Na, are more close to the concentrations found in foraminiferal calcite. Furthermore, NIST standards are extremely rich in sodium, and therefore ablation leads to a memory effect, increasing the Na background for the next $\sim$30 measurements, changing the detection limit of this element considerably. Therefore, the multiple measurement of NIST at the start of the session decreases the quality of Na data. All in all, the MACS-3 element composition approaches the foraminiferal values, which leads to a more robust calibration (less extrapolation) of the sample values. Since the precision for MACS3 is still 5% or lower, we believe this standard is the better option/trade off and gives a more realistic indication of potential inaccuracies. We added this shortly in section 2.3.2. In manuscript: "We choose MACS-3 as a calibration standard, since element composition approaches the foraminiferal values closer than that of NIST 610 or 612 and therefore aids a more robust calibration, even though the MACS-3 is slightly less homogeneous (see precisions listed in Table 1)."

- Page 6, line 1. Please be specific instead of simply stating 'similar'.

We changed this to 'matching' to avoid confusion.

- Section 2.3.3. Please state accuracy and precision data for the SF S/Ca analysis, and how these were determined.

This are now added to the revised version of the manuscript (see also comments reviewer 2) in section 2.3.3.

- Page 6, line 8. Again, please be specific. Was the set-up similar but different to that of Barras et al. [2018]? If so, in what way?

We used an identical set-up, the only difference was the temperature (25°C) and

light cycle (12hr/12hr). The similarity and differences between experiments is now more clearly stated in the text (section 2.3.4) In manuscript: "Specimens of various foraminiferal species (Ammonia tepida, Bulimina marginata) from a recently published culture study (Barras et al., 2018), and Amphistegina lessonii cultured in the same culture set-up, were prepared for electron probe micro-analysis (EPMA) to investigate the intra-shell incorporation of sulfur and magnesium. These foraminifera were cultured under hypoxia (30% oxygen saturation) in controlled stable conditions and previously studied to investigate the Mn incorporation in foraminiferal calcite (for details and culture methodology, see Barras et al., 2018). Ammonia tepida and Bulimina marginata were cultured at 12°C, while specimens of Amphistegina lessonii were grown at 25°C. For the latter species, the set-up was equipped with a light system with 12 hr/12 hr light cycle."

- Page 7, lines 11-12. I understand that it's difficult to assess the most appropriate regression form from three data points, but it would be interesting to report the exponential slope here too given that it appears the slope may be exponential from the available data.

We included values for a linear regression as well as an exponential regression to the revised text to accommodate the reviewer.

Section 3.4, Tab. 2, and Fig. 6. Clarify exactly what these data represent. Is each EPMA map from a different specimen, or different chambers of the same specimen? Does each data point in Fig. 6 represent the average Mg/Ca and S/Ca ratios of all data within each transect map? Similarly, on page 9, lines 23-27, how repeatable are these values? Do the percentages represent the average of several maps from several specimens? This would be much easier to follow if there was a supplementary figure showing the location of all maps/transects used to calculate each data point in Fig. 6, or add a column to Tab. S1 stating how many specimens and which chambers the transects are from.

We agree with the reviewer that we can be more clear about the locations of the EPMA maps and transects. We therefore added three figures and expanded a table in the supplementary information, showing the SEM overview pictures with EPMA targets of all three species. We furthermore added more details in section 2.3.4. and 3.3 about the number of transects per chamber/specimens/species, and which transects are presented in Fig 6 and 7.

- Page 8, line 11. On line 7 the peak and base ratios are quoted as 56.5 and 10.2, which would equate to a ratio of 5.5, not 2.8.

An error occurred in the text, this value should be 20.2 and not 10.2, as also stated in Table 2 and Fig. 7. This is now corrected

- Page 8, lines 12-14. This is repetition of the first paragraph in this section.

We reorganized this paragraph. We first described the co-variation of Mg and S distribution profiles per transect (example shown in Fig. 1C). Afterwards we compare the average S/Ca and Mg/Ca values of all transects, as shown in Fig. 6.

- Page 8, line 20. Although the data are somewhat challenging to interpret, there are technically Mg/Ca-temperature data for Amphistegina reported by Raja et al. [2007] doi: 10.1029/2006GC001478.

We are aware of this field study, and indeed, these results are not straightforward. However, we now added it to the text for completion.

- Page 8, line 28. How is 0.9 derived? I calculate (35-20)*0.09 = 1.4 mmol/mol.

We thank the reviewer for spotting this error, and it is changed accordingly.

- Page 9, lines 1-2. How does this follow from the previous sentence? From Fig. 3 it appears that Mg and SO_4^{2-} are not necessarily coupled, why do these data imply that they are?

We rewrote this sentence: 'Since temperature-induced changes in Mg incorporation

do not increase foraminiferal S/Ca, Mg/Ca and S/Ca might therefore co-vary due to a different process, possibly by mechanisms involved in biomineralization.'

- Page 9, line 10. I don't think 'incorporated simultaneously' is the right terminology. Rather, the higher concentration bands are located in a similar place.

We changed this to: 'are spatial (and hence likely temporally) correlated'

- Page 10, lines 9-15. As it is written, it reads as if the mechanism for increased Mg/Ca resulting in increased alkali metal incorporation differs from that of the alkali earths. My understanding is that this is not necessarily the case, rather lattice distortion can result in increased incorporation into both lattice and interstitial sites (depending on ionic radius).

We do not disagree that incorporation of sulfate could be influenced by lattice distortion in theory, but we would expect to see this in Fig. 3, and in the base-peak values between species, as noted at the end of the paragraph. However, we concur to remove 'alkali' to not limit the discussion.

- Page 10, lines 23-27. I don't doubt that precipitation rate may be more sensitive to seawater Mg/Ca than temperature, but surely temperature will affect rate to some degree, if only because of the effect of temperature on carbon speciation through the temperature dependence of KW.

We rephrased the end of this paragraph

- Page 10, line 32. I suggest removing this sentence. There is no observational or theoretical evidence for inward-directed Mg transport, and it is difficult to see what the purpose of this would be. We changed this to 'passive transport or leakage of Mg'. Ca pump can accidently transport other ions than Ca. Due to (de)hydration of Mg, this ion would be less (or more) available for accidental transport, or leakage.

- Page 11, lines 1-3. The (de)hydration of any ion during attachment is a passive process depending on e.g. growth rate and the chemistry of the calcifying space. Why

is it only likely in one of the biomineralization models?

Because in the case of SWV, you would expect the opposite, a decrease of Mg/Ca due to less efficient export of Mg.

- Page 11, lines 12-15 and line 24. Given that MgCO3 is a small proportion of total Mg it seems unlikely that this is the explanation.

This paragraph is rewritten in the revised version of the manuscript and de-emphasized presence of MgCO3 in the foraminiferal shell.

- Page 11, lines 17-20. I don't follow the logic here. It reads as if the argument here is that the relationship between sulphate and temperature is counteracted either by the increased shell S/Ca being driven by the increased shell Mg/Ca, or that sulphur is actively transported to the calcification site to a greater degree at higher temperature. However, the first of these explanations is discounted elsewhere (e.g. page 14, line 12) and I do not see the mechanistic basis for the other based on the data presented here. To phrase it another way, surely the slope in Fig. 6 is being driven by the width or number of the co-located high-Mg, high-S bands in each transect, and unless the proportion of these changes as a function of temperature (does it?), why would the slope in Fig. 6 counteract a temperature-driven change in the activity of sulphate?

We restructured this paragraph to make our arguments more clear. However, since the data set as such does not allow to investigate a linkage between banding and temperature, we removed lines 17-20. This is not affecting the overall structure and discussion.

- Page 12, line 5. Evans et al. [2018] calculate that the Mg/Casw ratio at the calcification site of low-Mg foraminifera is <0.1 mol/mol, not 2 mol/mol as stated.

We thank the reviewer for spotting this error. The values is now changed to <0.1

- Page 12, line 27. Perhaps a bit picky, but it is better approximated to Ca/(CO32- + SO42-) = 1.

This is true, but then to be more precise, it also has to be Ca+Mg+Sr+Na etc. Since Ca and CO32- are the two main constituents, we leave the 'formula' as is for this exercise. We do change the 1 to ∼1

- Page 13, lines 16-18. I think this could be phrased more strongly, it is hard to see that the SO42-/CO32- ratio is less than one given âĹij30 mM [SO42-], unless the DIC concentration is very high in the calcifying space.

We phrased this more strongly now, and added a sentence to this paragraph.

- Page 13, line 22. It may well be species-specific, but note that the calcification site pH is not necessarily greater than 9 [âĹij8.75 according to Bentov et al., 2009].

We added the value of Bentov et al., 2009 to this line, to show variability between species (or methods).

- Page 24, lines 24-28. In both biomineralization models, pH is elevated in the calcifying space (or vacuole) in order to promote carbon concentration, and presumably the two processes occur simultaneously (what would be the benefit of separating them?). We don't know precisely to what extent this takes place or at what time, so I understand the reason for calculating it in this way, however my recommendation would be to rephrase this sentence as a constraint on the maximum calcification site SO42-/CO32- ratio, given that the assumption of seawater DIC is probably not correct.

We added some sentences to clearly state this would be a maximum value, which will in the end depend on DIC. However, it is unlikely that DIC will increase to a point where SO42:CO32 will be <1, and we added a short sentence in the revised version of our manuscript.

- Page 14, line 13. I don't think anyone has suggested that hyaline and porcelaneous foraminifera are characterised by the same biomineralization model.

We rephrased this sentence.

- Fig. 1. State which species/treatment this map is from.

Done.

- Fig. 3. Please clarify whether the grey symbols represent repeat measurement of the same solution or different groups of 10 foraminifera.

We changed the caption, it now states that these are not repeat measurements but different groups.

- Fig 9. Proton pumping is a feature of both biomineralization models, so why should the arrow for 'SWV-dominated' be in the opposite direction to 'H+ pump-dominated'?

See comments above. We acknowledge that SWV also includes a small increase in pH (8.75 according to Bentov et al., 2009). However, the pH increase in the vacuoles is lower then observed inside small benthic foraminifera. Therefore, we reduced the size of the arrow, but kept the direction as it was.

- Tab. 1 could be moved to the supplement.

We decided to keep table 1 in the main manuscript, since it is an important part of the LA ICP MS method.

Typos - Page 2, line 31. There is a full stop missing after the parenthesis. Done - Page 5, lines 28 and 33. Presumably it should read μl/min. Done - Page 6, line 13. An emulsion refers to two immiscible liquids, replace with suspension. Done - Page 7, line 23. Replace 'is' with 'are'. Done
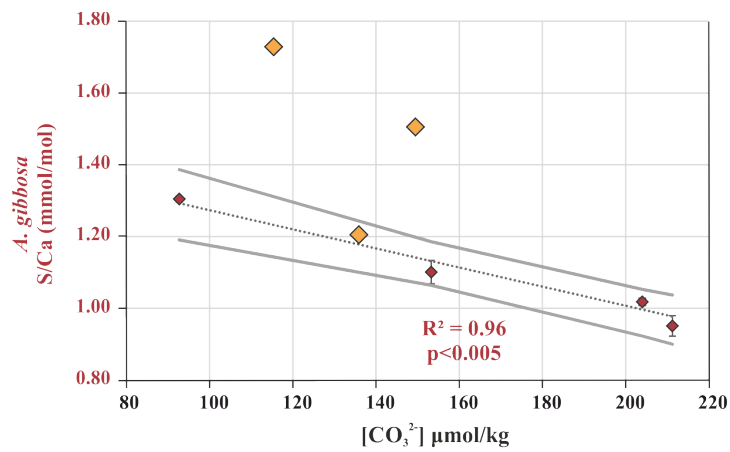
Fig. R1: In red, average values for Amphistegina gibbosa (van Dijk et al., 2017, EPSL). In yellow, average values for Amphistegina lessonii from this study.

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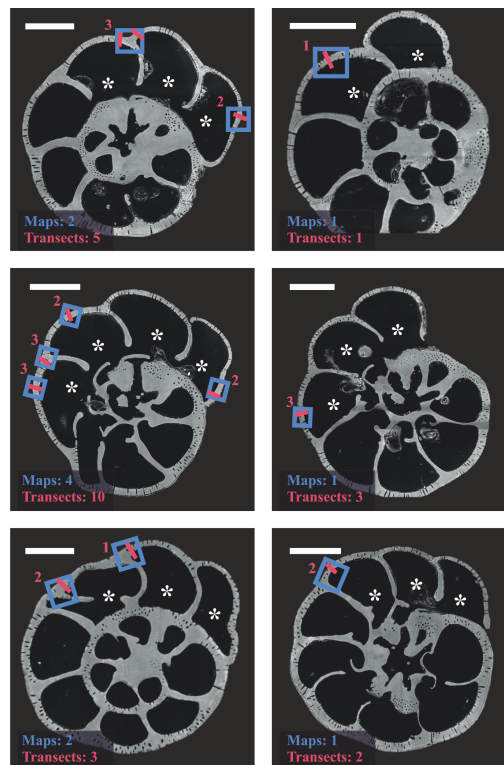
C13

Fig. 2. Example of on of the new supplementary figures (Fig. S1)

C14