Dear Dr Fujita,

Thanks for the thorough reading of our manuscript and your constructive comments

In general, the main concern of this referee was the focus of the manuscript, which should be more towards the potential of Zn/Ca and Ba/Ca as carbonate system proxies. By changing and restructuring the introduction and discussion session, we shifted the focus of the manuscript more towards the changes in Zn and Ba incorporation as a function of pCO_2. The conclusions and abstract are changed accordingly. Another one of the suggestions by both reviewers is to broaden the discussion by including other biomineralization and ion transport models to explain the species-specific trends in element incorporation.

The comments of the referee are posted below in italics. We address these issues point by point, in bold.

General comments:

1) Authors should reconsider what is the main purpose (hypothesis), what is the main results, and what are conclusions obtained from this study. In the Introduction, you may need more elegant story to explain why you conducted pCO2 controlled experiments as well as why you used symbiotic tropical large benthic foraminifera for this study, to understand trace element incorporation in foraminifera. I think the main important results in this study were increasing some trace element incorporation (Zn, Ba) with high pCO2 environments and a chemical model to explain this phenomenon.

Based on this comment we now somewhat changed the focus of the introduction more towards carbonate system proxies (e.g. Zn/Ca) and the necessity to study this across different taxa since they are known to 1) have different calcification mechanism and 2) a different D for elements studied so far. We used tropical foraminifera since they have a higher variability in Mg incorporation and both porcelaneous and hyaline species are readily available in this region.

This also led to a reorganization of the discussion. First, we now discuss the Zn- and Ba-incorporation as a function of pCO_2 (now 4.2) and then discuss overall differences between hyaline and porcelaneous foraminifera (4.1 in the old manuscript). Then, we discuss the chemical speciation of ions as a function of pCO_2 (4.3). Finally, we evaluate existing biomineralization models based on our results.

2) Authors are mainly concerned about a correlation, but not dealt with the quantity (amount) of elemental incorporation in foraminiferal calcite. Even if both Zn and Ba ion availability increases with high pCO2 conditions, the amount incorporated in foraminiferal calcite differs between the two elements (Zn is four times more incorporated than Ba). We know that ionic radius is related to trace element incorporation in calcite from many inorganic studies. How does your chemical model explain the incorporation of elements quantitatively?

We now added these observations to the discussion. Following the TMT mixing model, selectivity of Ca^{2+} channel differs for different elements, which probably also reflects in part ionic radius (see also, Nehrke et al., 2013).

3) This paper is not discussed anywhere (only briefly mentioned in the Introduction) about effects of temperature on element incorporation in foraminifera, which are well established by many papers. In Figure 3, you compared results of your study with those of previous similar studies. However, I wonder

these results are not simply comparable because of different species from different environments (water depths and latitudes, i.e. different temperature and salinity ranges) as shown in Supplementary Table S1. Temperature effects should be normalized to compare real DMg between different foraminiferal species. In addition, authors should discuss the relative sensitivity on E/Ca between temperature and carbonate chemistry when both parameters are variable.

We changed figure 3, to show the data of our study only. The previous figure 3 is now moved to the supplementary info, and we excluded data from foraminifera cultured/grown at low temperature, as they might partly have been grown under undersaturated conditions with repsect to calcite. Resulting species are all grown/cultured in a temperature range of 18-29 °C. We did not correct E/Ca for temperature, since the this effect on Na/Ca, Ba/Ca and Sr/Ca is not well constrained or unknown.

4) This study assumes only Ca-channel model as a possible ion transport mechanism. However, other possible mechanisms of ion transport have been proposed in foraminifera. I suggest that authors compare advantages and disadvantages of several transport models and justify the Ca channel model as the most appropriate to explain results in this study.

This issue was also raised by the second reviewer. The discussion is now put in a somewhat broader context, in which we also include other transport models, including seawater vacuolization (e.g. Ter Kuile and Erez, 1991; Erez, 2003, Elderfield et al., 1996; De Nooijer et al., 2014). This is added as a separated paragraphs in which we try to test/validate these models with our observations.

5) I wonder if laser abrasion (LA) method is appropriate for biocalcification study. As you know, hyaline foraminiferal shells are composed of the primary layer and the secondary layers (coating) with the organic matrix. The LA method cannot discriminate differences in element incorporation between these different layers. In addition, the spatial heterogeneity of E/Ca among calcite crystals in a chamber wall has been reported by many studies. How do authors overcome these problems by using the LA method?

Heterogeneity of elements in the chamber wall has only been observed in hyaline species, since they have (bi)laminar calcification. In this study we incubated adult foraminifera in culture media with calcein. LA-ICP-MS allows for targeting new chambers only, of which all layers of calcite were formed during the experiment. By using LA-ICP-MS we obtain an average signal of the chamber wall, averaging out any potential banding. Analysis of sufficient specimens/ chambers reduces uncertainty in average e.g. Mg/Ca values. Furthermore, we only ablated the final ~3 chambers, minimizing the potential effects of varying number of test carbonate layers. First paragraph of the discussion now lists this as one of the potential causes for element to calcium ratio (E/Ca) variability.

6) What kinds of other trace elements except for those examined in this study are sensitive to pCO2 based on the chemical model? That is useful information to find new proxies for paleo-pCO2. In addition, I wonder what cause differences between sensitive and insensitive elements on pCO2.

In this model we only looked at elements which we also measured by LA-ICP-MS. We modelleed the elemenst which based on their know geochemical behavour are the most likely to show differences as a function of changes in carbonate chemistry. All elements have been modelled in some sort of other study previously as well (e.g. Keul et al., 2013). When considering the impact of [CO_3^{2-}] chemical speciation, the observed lack of sensitivity for Sr, Na and Mg might stem from their high concentration in seawater compared to e.g. Zn. Only a small amount of ions are hence complexed by [CO_3^{2-}]. Since there is a higher total amount of e.g. Mg ions in seawater, the amount of Mg-CO_3 complexation is relatively low. Due to their low concentrations and great affinity for

carbonate ions, elements like Cu, Co, Ni, Li, may be affected in the same way. We have added the possible behavior of these elements according to changes in speciation (4.3, chemical speciation).

7) Authors are confused about the terminology of Foraminifera. Throughout the text, the authors used "hyaline" and "miliolid" as comparable terms. But the term "hyaline" indicates the quality of shell appearance and the term "miliolid" is a taxon name belonging to the Order Miliolida. I suggest authors use comparable terms of "hyaline vs. porcelaneous" as shell appearance, "perforate vs. imperforate" as shell perforation, and "rotaliid vs. miliolid" as the two main taxonomic group.

To avoid any confusion, we changed the terminology in our paper to hyaline vs. porcelaneous and perforate vs. imperforate.

L1: Title is vague and general, should be changed to include keywords and reflect the main results of this paper; for example, Calcification model of some trace element (Zn, Ba) incorporation in foraminifera under high pCO2 environments.

We changed the title to: 'Trends in element incorporation in hyaline and porcelaneous foraminifera as a function of pCO_2' to better cover our main results.

Introduction:

L38-40: This sentence is strictly speaking incorrect. Diverse miliolid foraminifers belonging to larger benthic foraminifera (LBF) are found particularly in the Atlantic and the Caribbean. In addition, LBF do not cover a large Mg/Ca range, but only intermediate and high Mg/Ca ranges. The authors have to explain advantages to use LBFs for their study in more detail.

In our study we use intermediate to high $Mg/Ca_{CALCITE}$ (in our study ranging from 28.5 (*A. carinata*) to 141.3 (*H. antillarum*) mmol/mol), which is a large range in $Mg/Ca_{CALCITE}$.

New text: "A number of larger benthic foraminifera form hyaline shells, although the amount of Mg in their shells is often more than 10 times higher than that of planktonic and small benthic hyaline species, hence covering a larger range in $Mg/Ca_{CALCITE}$ values."

Methods:

L86: 90-600 µm fraction is too small for larger benthic foraminifers (almost juveniles).

We picked both directly from the macroalgae and from the 90-600 µm fraction, we now explain this in the methodology.

L86: As far as I know, Marginopora vertebralis (Quoy & Gaimard) is not distributed in the Caribbean and Atlantic (see Langer and Hottinger, 2000 Micropaleontology). Recheck if identification is correct.

We rechecked the identification of all of our species, and found that we misidentified *Sorites marginalis* as *Marginopora vertebralis.* This is now changed in the revised version of our manuscript.

L55: Is a paper in review OK to cite? If it is OK, it should be listed in the Reference.

L60: Not listed in the Reference.

L150, (Nardeli et al., 2016): not listed in the Reference.

L154, Barker et al. (2003): not listed in the Reference.

L94: Where is "Chapter 7 "? I also found other chapters in the text somewhere.

5 points above: We removed all references to chapters and manuscripts in review

L97-98: Add pCO2 unit. Explain what (A) means.

We added the *p*CO_2 unit (ppm) and explain the treatment names A-D

L108-109: Add the precision of temperature control.

The average temperature over the whole experiment was 25±0.2°C, which is now added to the revised manuscript.

L110-111: Note the light intensity level. In addition, I wonder if LEDs and yellow culture bottles (Fig. 1) affect wave length and hence the growth of symbiotic foraminifers?

The culture bottles themselves are not yellow, it's the calcein added to the culture media. Almost all incubated foraminifera grew new chambers. We now added a table with the growth parameters.

L113: Does food affect water quality and chemical composition?

The descripted *dunaliella* feeding solutions do not change the chemical composition, since these dunaliella were rinsed, centrifuged, freeze-dried and subsequently diluted in the culture media. Water was replaced every four days to keep organic waste buildup at a minimum

L155: Could the organic matrix in a shell be removed by this method? Does data not include any elemental incorporation in the organic matrix?

In this cleaning step we remove the organic matrix from the foraminiferal shells. Although, in theory, it might be possible there are small amounts of matrix remaining in the carbonates, the amount of organic matter in foraminiferal shells is low, even before cleaning (e.g. 0.1-0.2 wt% of the total shell of *Heterostigina depressa*; wenier and Erez, 1984). However, for future work it might be interesting to analyze the organic matrix, to evaluate its potential contribution to the total E/Ca.

L167: What is the main difference between this paper and Van Dijk et al. (in review)?

We removed this reference, since this article is still in review. The differences with this study and Reichart et al. (2003) are summarized in this paragraph. For instance, we use different ICP-MS's, different cells, and used additional standards.

Results:

L217-218: Explain the rationale (hypothesis) why the authors compare Mg/Ca with other TE/Ca?

We included this figure in our manuscript because of the obseverd link between Mg and other TE published earlier (like e.g. Evans et al,., 2015).

L218-219: not only significant values, but also R2 values should be noted.

The R^2 values are presented in Table 3

Discussion:

L249-251: Compare advantages and disadvantages of several ion transport models and justify a Ca-channel model as the most appropriate to explain results in this study.

L260: I think miliolid foraminifera still need the major removal of Mg ions even if carbonate is directly precipitated from seawater.

We broaden the discussion to include also other transport models and how these might differ for porcelaneous and hyaline species, when comparing them to our observations.

L289: PHREEQC needs explanation

L290: llnl database?

PHREEQC and the llnl database are now explained in more detail in the new paragraph 4.3, which focusses on the modelling of chemical speciation.

Section 4.3: this section is mostly a review of previous studies. I suggest that authors explain an incorporation model shown in Fig. 6 in detail.

Due to a reorganization in our discussion, we now spent two paragraphs on different transport models.

Section 4.4: Does size matter? authors mentioned that they measured only small size (L86). Calcarinids (Neorotalia in #15 in Fig. 3) are similar in size to Amphistegina, but have high Mg contents similar to a bigger Heterostegina. You may need another interpretation to explain the difference between two taxa. In addition, I think larger benthic foraminifers (in particular some taxa dwelling at a lower euphotic depth) have a strategy to attain a high surface area to volume ratio by flatting to get light for algal C6 symbionts. Please show the surface area to volume ratio between comparing taxa to justify your interpretations. Less Ca channels in the membrane of LBFs are also unlikely, because LBFs are bigger thus have much more membranes and channels than smaller foraminifers if channel density are the same. I think the second process is more feasible than the first process.

L347-350: I think this explanation is more plausible. Hyaline foraminifers are highly diverse and may have similar but slightly different calcification strategy acquired during evolution. I guess the relative contributions of primary and secondary layers and organic matrix may depend on hyaline foraminiferal taxa, which may cause interspecific variability of E/Ca compositions.

We removed this part of our discussion, since we agree it is the less likely explanation of our observations for hyaline species. We end our discussion with a paragraph on the contribution of different mechanisms, which might explain our observations for both hyaline and porcelaneous species.

Section 4.5. L359: I think the major removal of Mg is necessary because your results show that DMg is much lower than 1.

With the seawater vacuolization model (Erez, 2003) it is indeed necessary to remove Mg ions from the calcification fluid. But the removal of Mg is not necessary when ions are transported by TMT,

since these channels mainly import Ca to the site of calcification. Only very few Mg ions would be transported (1 for every 10.000 Ca ions).

L366-369: Is this correct? lower? I think higher or similar based on slope inclinations in Fig. 4. I do not understand how to estimate the relative contribution of seawater endocytosis and transmembrane transport. I guess some trans-membrane ion exchanges (Mg removal) occur between seawater vesicles and intrashell cytoplasm. High pCO2 seawater contains relatively large amounts of Zn and Ba ions, which are incorporated into foraminiferal cytoplasm via seawater vacuolization. Calcite needles are then precipitated from seawater vesicles with modifications by trans-membrane ion exchanges between seawater vesicles and intrashell cytoplasm.

No, high pCO_2 contains the same amount of Zn and Ba ions, only speciation differs. Seawater vacuolized at different pCO_2 will have the same Zn and Ba concentration. The calcite needles which are precipitated from these vacuoles will have the same Zn/Ca and Ba/Ca, unrelated to ambient seawater carbonate chemistry. However, by combining TMT and seawater vacuolization, calcification fluid starts with ambient seawater, which is diluted with Ca^{2+} by TMT. During this processes, the amount of ions other than Ca^{2+} transported to the site of calcification depends on the chemical speciation (amount of free ions), the relative abundance compared to Ca^{2+} and the selectivity of and thus discrimination by the Ca^{2+} channels. This is now described in more detail in the discussion section.