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Interactive comment on "Decoupled carbonate chemistry controls on the incorporation of boron into *Orbulina universa*." *by* E. L. Howes et al.

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First of all, we like to thank Michael for his extensive, critical and well justified comments.

1) "I have some concerns about the spread in the data and the calculation and propagation of uncertainties, and would suggest the authors at some point explicitly mention that the uncertainty poses limitations for how much can be interpreted from these data". In contrast to "wet chemistry" does Laser ablation record the inhomogeneous B distribution ("boron banding" see Branson et al., 2015 EPSL) and individual shell analysis captures intra-specimen differences (see reply to reviewer 2). As impressively demonstrated by Sadekov et al. (2016) is the variability in Both B/Ca and ïĄď11B recurring in each chamber and therefore represents real data of high quality. This is supported by

the fact that the value of average laser data are very close to wet chemical analyses were multiple specimens are dissolved and the intra- and inter-variability is "averaged" before the analysis. Therefore, we disagee that the "uncertainty poses limitations for how much can be interpreted from these data". We have added the following text in the method section: "Laser ablation, in contrast to "wet chemical" analysis, records the inhomogeneous boron distribution ("boron banding" see Branson et al., 2015) within a specimen and individual shell analysis captures inter-specimen differences. As impressively demonstrated by Sadekov et al. (2016) is the variability in both, B/Ca and ïĄď11B recurring in each chamber and therefore represents real data of high quality. This is supported by the fact that the values of the averaged laser data are very close to wet chemical analyses were multiple specimens are dissolved and the intra- and inter-variability is "averaged" before the analysis. The intra-specimen ïĄď11B variability in Cibicidoides wuellerstorfi is up to ca. 10 permil (Sadekov et al. (2016), while the inter-specimen δ11B variability of Amphistegina lessonii from the same treatment is ca. 6 permil (Kaczmarek et al., 2015b). Histograms of single-foram δ11B measurements from each of our pH treatments (supplementary Fig. 1) show that the laser ablation data is normally distributed (p-values from Shapiro-Wilk-tests are all higher than 0.05). This is confirmed by the box plots were the average and median values are very close to each other. Therefore, the relatively large standard errors of laser ablation analyses do not present a limitation for how much can be interpreted from the data." References: Sadekov, A., Kerr, J., Langer, G., de la Fuente, M., Skinner, L. and Elderfield, H. 2016. Understanding the mechanisms behind boron elemental and isotopic fractionation in the benthic foraminifera Cibicidoides wuellerstorfi. Poster, ICP12, Utrecht. Branson, O., Kaczmarek, K., Redfern, S.A.T., Misra, S., Langer, G., Tyliszczak, T., Bijma, J. and Elderfield, H. (2015) The coordination and distribution of B in foraminiferal calcite. Earth and Planetary Science Letters 416, 67-72.

2) "Including some measurements of widely analysed calcium carbonate standard material (such as JCP or JCT) would be really helpful in clearly demonstrating the efficacy of the technique". JCP and JCT are biological samples and probably have variable

B/Ca and ïĄď11B even at the micro-scale (they are powders). The laser spot will be too large to analyze individual grains and we have no means to analyze powders with laser ablation (yet). We agree that it would be very valuable to demonstrate how (the average of) LA data relate to wet chemistry analyses of both standards. This is something that we plan to do in the near future and has our first priority as soon as we have our own laser set-up properly. For the BG msc we will refer to papers demonstrating matrix independency (see our response to reviewer 2) and that we can use glass standards to relate to carbonate samples and standards. We have added some text to the method section: “It should be noted that the fs laser ablation process is fundamentally different from ns laser ablation. When the pulse length is shorter than 10 ps (Hergenröder and Hommes, 2006) the laser energy can be deposited into the material before it can thermally equilibrate. Femtosecond ablation also provides smaller aerosol particle sizes. Due to the short pulse length, fs laser ablation is matrix independent (e.g. Chmeleff et al., 2008; Horn et al., 2006; Oeser et al., 2014; Schuessler and von Blanckenburg, 2014; Kaczmarek et al., 2015; Lazarov et al., 2015; Lazarov and Horn, 2015), i.e. it does not require a matrix matched standard and therefore allows us to use NIST SRM 610 (a glass) as a reference for carbonates. As the boron concentrations are different between sample and standard and different matrices require more or less energy for ablation, the repetition rate was chosen such that the signal of sample and standard at the ion counters was comparable. This is important for normalization of the sample to the known ïĄď11B of the standard. Most previous publications on boron isotopes have used “wet chemistry” for which NIST SRM 951 is a perfect standard. We have also used this standard for the analysis of the culture waters. The foraminiferal shells, however, were measured using laser ablation, for which we used a different standard (referenced against NIST SRM 610). As shown by several studies (Kasemann et al., 2001; le Roux et al., 2004; Fietzke et al., 2010), both standards are, within analytical uncertainty, isotopically equal. Hence, for comparison between δ11B O. universa and δ11B of B(OH)4- the isotopic difference between the two standards can be neglected and it does not make a difference if values are reported versus one

or the other standard. ” 3) You suggest "inclusion of measurements of open ocean O. universa". You are absolutely right again that we could have done that. However, this paper focusses on the controls of boron incorporation only and we have chosen to leave the comparison of field and laboratory grown specimen as well as the impact of increased boron concentrations in culture water for a separate paper. In the past we have increased the boron concentration in culture water for obvious practical reasons. Hönisch et al. (2003) and Zeebe et al. (2003) demonstrate that it should not have an impact on ïĄď11B but we would like to investigate that a bit further. Hönisch, B., Bijma, J., Russell, A.D., Spero, H.J., Palmer, M.R., Zeebe, R.E. and Eisenhauer, A. (2003) The influence of symbiont photosynthesis on the boron isotopic composition of foraminifera shells. Marine Micropaleontology 49, 87-96. Zeebe, R.E., Wolf-Gladrow, D.A., Bijma, J. and Honisch, B. (2003) Vital effects in foraminifera do not compromise the use of delta B-11 as a paleo-pH indicator: Evidence from modeling - art. no. 1043. Paleoceanography 18, 1043-1043.

4) “. . .. . ..inclusion of measurements of open ocean O. universa.. . .. . .would test the hypothesis put forward for the apparently muted vital effects.” . . .. . . “ It would also address the issue of bicarbonate control- since there are a number of studies that show that just because these patterns can be seen in culture, it doesn’t mean they will hold up outside of the lab.” Analysis of open ocean O. universa can only partly address the point you make. In the lab we can perfectly control the environment the forams "see" and we can decouple parameters and extend each of them individually beyond the natural range while keeping the rest constant. Although, there is no a priori reason to assume that they would respond differently in the lab than in the field, this is difficult to prove. In the field there are many variables that are not constant, parameters cannot be decoupled and usually the ranges are limited. There may be ontogenetic migration, etc. I think that your recent paper in EPSL is a nice example and we will refer to it as Henehan et al., (in review) if it is not out yet. Your suggestion that the impact of photosynthesis on ïĄď11B of O. universa (or shell geochemistry in general) as observed in the lab may be muted in the field is a valid point and in line with my observations on their population

dynamics (Hemleben et al., 1994): “Orbulina universa d’Orbigny shows a subsurface maximum” and our data in the Red Sea indicate a depth habitat between 20-60 meters (Fig. 5). We have added some text to clarify this issue: “Interestingly, Henehan et al. (2016) propose a field calibration for O. universa that is very close to ïĄď11B of borate, suggesting that their “vital effects” are muted in the real ocean, especially the symbiont impact of raising the calibration curve above ïĄď11B of borate. This is supported by the observation of Hemleben et al., (1994) that O .universa occupies a subsurface maximum (in the Red Sea) between 20-60 meters (Hemleben et al., 1994; Fig. 5) and could explain why B/Ca in this species is not (completely) masked by symbiont photosynthesis (Salmon et al., 2016).” Hemleben, C. and Bijma, J. (1994) Foraminiferal population dynamics and stable carbon isotopes., in: Zahn, R., Pedersen, T.F., Kaminski, M., Labeyrie, L. (Eds.), Carbon Cycling in the Glacial Ocean: Constraints on the Ocean′s Role in Global Change. Elsevier, Fellhorst, pp. 145-166. 5) you suggest that "In the face of the data from Babila et al., Henehan et al., Salmon et al. (2016, EPSL) and the excellent review by Allen and Hönisch (2012), the authors should remove suggestions of using B/Ca as a proxy for the second carbonate system parameter, unless they can show evidence to support this relationship standing up out of the culture lab." You are right that it will probably be impossible for G. ruber (the most "autotrophic” of all symbiont bearing foraminifera) to use B/Ca downcore, but for non-symbiotic planktonic forams or benthics, it may still be a viable option. Below we will explain why we think that the relationship between PO4 and B/Ca that you describe for G. ruber in the G3, 2015 paper may be a red herring (but we cannot prove it because it would require new culture experiments using micro-electrodes). First of all, we fully agree that we were not clear at all regarding our comment related to a possible correlation between PO4 and B/Ca as suggested by Henehan et al. (2015). We wrote: "....we believe that this relationship results from a co-variation between ocean carbonate chemistry and nutrients because respiration of organic matter will release both carbon and nutrients." We were not referring to bulk ocean conditions but to their ambient environment. Forams, and especially symbiont bearing planktonic forams never “see” the bulk ocean carbon-

ate chemistry (see e.g micro-electrode studies by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only see their ambient carbonate chemistry as modulated by their own life processes as well as by symbiont photosynthesis and respiration (therefore our calibrations are empirical and not mechanistic). Surface dwelling deep sea benthics probably come closest to recording real bulk carbonate chemistry conditions, although the nutrient and carbonate chemistry gradients in the fluffy layer (which is the time when they grow and reproduce, I assume) may be very strong. In section 2.3 of your G3 paper you describe how you produce the environmental data (nutrients and carbonate chemistry). This is indeed the best you can do, but you will agree that the average (annual) estimates you get from extrapolation from grid point, etc... may not reflect real conditions during growth of the forams. In general, we would expect a positive linear relationship between nutrients and DIC and a negative one between nutrients and pH. When nutrients (PO4) are high, DIC is usually high and pH is low (normal deep water conditions or classical upwelling but also temperate ocean after winter mixing). High nutrients lead to higher primary productivity, consuming nutrients and DIC and increasing pH (normal sfc ocean conditions during spring bloom). The bottom line, in our view, is that the absence of the above mentioned relationships question the consistency between analysed B/Ca and the estimated environmental data. If we then turn to the data used for the cultures and the plankton tows in the Gulf of Eilat, those are of a very different quality as everything has been analysed/determined when the forams where actually adding carbonate. For the following it is important to realise that, of all symbiont bearing planktonic forams, G. ruber is the most "autotropic". Your fig. 4 demonstrates the close relationship between carbonate chemistry and B/Ca (when PO4 constant!). Fig 7. shows a clear correlation between bulk PO4 and the loss of a correlation with bulk carbonate chemistry (pH). However, we are convinced that if you would measure ambient pH (or [CO32-]) at elevated PO4, both (pH and [CO32-]) would be significantly higher! Hence, even if the correlation with bulk pH is lost, the correlation with ambient pH (or [CO32-], [HCO3-]) will probably still be there. We cannot prove it to you but micro-electrode measurements could!

In addition, there is no obvious direct link between PO4 and B/Ca. Mechanistically, probably via increased photosynthesis leading to higher calcification rates. We show (Kaczmarek et al., 2015b) that boron partitioning increases with increasing growth rate in inorganic precipitation experiments. Early work by the pioneers of foram biology and calcification (Bé, Anderson, Hemleben, Spindler, Erez, Spero, Caron, etc.) has clearly demonstrated the huge impact of symbionts on foram shell growth, e.g.: Bé, A.W.H. (1965) The influence of depth on shell growth in globigerinoides sacculifer (brady). Micropaleontology 11, 81-97.) Bé, A.W.H., Spero, H.J. and Anderson, O.R. (1982) Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer globigerinoides sacculifer. Marine biol 70, 73-86. Caron, D.A., Bé, A.W.H. and Anderson, O.R. (1981) Effects of variations in light intensity on life processes of the planktonic foraminifer globigerinoides sacculifer in laboratory culture. J. Mar. Biol. Assoc. U.k 62, 435-452. Spero, H.J. and Parker, S.L. (1985) Photosynthesis in the symbiotic planktonic foraminifer Orbulina universa, and its potential contribution to oceanic primary productivity. Journal of Foraminiferal Research 15, 273-281. Jørgensen, B.B., Erez, J., Revsbech, N.P. and Cohen, Y. (1985) Symbiotic photosynthesis in a planktonic foraminiferan, Globigerinoides sacculifer (Brady), studied with microelectrodes. Limnology and Oceanography 30, 1253-1267. Hemleben, C., Spindler, M., Breitinger, I. and Ott, R. (1987) Morphological and physiological responses of Globigerinoides sacculifer (Brady) under varying laboratory conditions. Marine Micropaleontology 12, 305-324. I hope this explains our point a bit better. PO4 will increase symbiont photosynthesis, which raises ambient pH (and [CO32-]) and effectively decouples it from the bulk ocean carbonate chemistry. other references: Wolf-Gladrow, D.A., Bijma, J. and Zeebe, R.E. (1999) Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Marine Chemistry 64, 181-198. Babila et al. (2014) write: "The seasonal cycle of B/Ca in G.ruber white was more strongly correlated with light intensity than with temperature. Both observations suggest that the presence of symbionts in G.ruber and seasonal variability in their photosynthetic activity act to modify the internal pH during calcification, by up to âĹij0.2units relative to ambient sea-

water." This is again about G. ruber and supports the arguments we suggested above. Salmon et al. (2016) write: "We provide the first evidence for a strong positive relationship between area density (test thickness) and B/Ca, and reveal that this is consistent in all species studied, suggesting a likely role for calcification in controlling boron partitioning into foraminiferal calcite." This conclusion also supports our line of argumention above, that "Mechanistically, probably via increased photosynthesis leading to higher calcification rates." The subtropical gyre has negligible dissolved phosphate, hence they could not explicitly test your B/Ca relationship with [PO4], but we bet they would have found it. Besides G. ruber they also looked at orbulina and truncatulinoides. The sediment trap is at 1500m and calcification depths are calculated using ïĄď18O and Mg/Ca. This is the best they can do but also means that there is no real control on depth habitat. As the waters around the site are oligotrophic, you can expect that symbionts may overprint the primary relationship between B/Ca and carbonate chemistry parameters (as Babila et al. (2014) and you (Henehan et al., 2015) demonstrated for G.ruber). If available, it would be great to correlate B/Ca with monthly/seasonal light attenuation data for that site. Their fig 3 shows, first of all, that G. ruber, O. universa and and G. trunc are all over the place but that the non-symbiont species (G. bulloides and G. inflata) and even G. sacculifer seems to show a trend with the three carbonate parameters (our guess showing a primary control by carbonate chemistry parameters). We would even argue that there is still a positive trend for O. universa (but for borate/bicarbonate the range is too small and the system in the field it is not decoupled). Second, you will agree that T and carbonate chemistry parameters at this site are correlated. Eventhough, O. universa is also a symbiont "battlestar", it is noteworthy that Salmon et al. conclude that "Higher B/Ca values significantly correlate with larger tests but only in G. ruber" (as it is the most autotrophic of all symbiont bearing species and larger specimens harbor more symbionts). The bottom line is that field studies are not suitable for elucidating the mechanisms but are VERY helpfull in showing which species are not good for paleo reconstructions of the carbonate system, and we agree with you that G. ruber is one of them. We have substantially changed our discussion

of using B/Ca as a proxy for the second carbonate system parameter and do justice to the fact that the above mentioned studies show a decoupling of the primary relationship. We have added the following text to the last part of the discussion: “Recently, Henehan et al., (2015) showed that B/Ca in G. ruber collected with a plankton net was perfectly correlated to [PO43-] and not to any carbonate chemistry parameter, despite the fact that their culture study demonstrated a highly significant relationship between B/Ca and e.g. B(OH)4-]/[HCO3-]. Based on plankton tow, sediment trap and core-top data, they concluded that, apparently, B/Ca in G. ruber is controlled by [PO43-]. However, it should be noted that foraminifera, and especially symbiont bearing planktonic foraminifers never “see” the bulk ocean carbonate chemistry (e.g. e.g micro-electrode study by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only “see” their ambient carbonate chemistry as modulated by their own life processes and symbiont photosynthesis and respiration (so called “vital effects”). Existing calibrations and field relationships are therefore purely empirical and not mechanistic. Water masses usually show a covariation between nutrients and carbonate chemistry driven by community photosynthesis and respiration. When nutrients are high, DIC is usually high and pH is low and vice versa. However, the ambient carbonate chemistry of the foraminifer and the bulk seawater chemistry can be decoupled. We note that, of all symbiont bearing planktonic Foraminifera, G. ruber is probably the most "autotropic" (Bijma et al., 1992). Although we cannot prove it, we assume that symbiont photosynthetic rates are higher at elevated [PO43-] (limiting nutrient) and therefore that ambient pH would be higher. Hence, even if the correlation between B/Ca and seawater carbonate chemistry is lost, the correlation with ambient pH (or [CO32-], [HCO3-]) may still hold up. At this point, there is no obvious direct link between [PO43-] and B/Ca and we believe that, mechanistically, it can be explained by increased photosynthesis and/or higher calcification rates. Kaczmarek et al., 2015b) show that boron partitioning in inorganic precipitation experiments increases with increasing growth rate and early work by the pioneers of foraminiferal biology and calcification (e.g. Bé, 1965; Bé et al., 1982; Caron et al., 1981; Spero and Parker, 1985; Jørgensen et al., 1985; Hemleben et

al., 1987) has clearly demonstrated the huge impact of symbionts on foraminiferal shell growth. Interestingly, Babila et al. (2014) write: "The seasonal cycle of B/Ca in G.ruber white was more strongly correlated with light intensity than with temperature. Both observations suggest that the presence of symbionts in G.ruber and seasonal variability in their photosynthetic activity act to modify the internal pH during calcification, by up to âĹij0.2 units relative to ambient seawater." This supports our line of argumentation above. In another recent paper on B/Ca, Salmon et al. (2016) write: "We provide the first evidence for a strong positive relationship between area density (test thickness) and B/Ca, and reveal that this is consistent in all species studied, suggesting a likely role for calcification in controlling boron partitioning into foraminiferal calcite." Their conclusion also supports our reasoning, that, mechanistically, increased photosynthesis may lead to higher calcification rates. Remarkably, Salmon et al. (2016) show that B/Ca of the non-symbiont species (G. bulloides and G. inflata) and even the symbiont bearing species G. sacculifer are related to [CO32-] and [B(OH)4-/HCO3-], showing a primary control by carbonate chemistry parameters not masked by symbiont photosynthesis. One could even argue that there is a positive trend for O. universa but that the natural range for borate/bicarbonate is small in comparison to the decoupling we carried out in controlled culture experiments.

Our final conclusion is that field studies are not suitable for elucidating the mechanisms of proxy incorporation but are very helpful in showing which species are the golden standard for paleo reconstructions of the carbonate system, and we agree with Henehan et al. (2015) that G. ruber is not a good choice as its primary relationship to carbonate chemistry parameters is not very robust. However, other symbiont bearing species, non-symbiotic planktonic foraminifera and deep sea benthics, may still be a viable option to use B/Ca for carbonate chemistry reconstructions.” References: Rink, S., Kühl, M., Bijma, J. and Spero, H.J. (1998) Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer Orbulina universa. Marine Biology 131, 583-595. Wolf-Gladrow, D.A., Bijma, J. and Zeebe, R.E. (1999) Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Ma-

rine Chemistry 64, 181-198. Bijma, J., Hemleben, C., Oberhänsli, H. and Spindler, M. (1992) The effects of increased water fertility on tropical spinose planktonic foraminifers in laboratory cultures. Journal of foraminiferal research 22, 242-256. Bé, A.W.H. (1965) The influence of depth on shell growth in globigerinoides sacculifer (brady). Micropaleontology 11, 81-97. Bé, A.W.H., Spero, H.J. and Anderson, O.R. (1982) Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer globigerinoides sacculifer. Marine biol 70, 73-86. Caron, D.A., Bé, A.W.H. and Anderson, O.R. (1981) Effects of variations in light intensity on life processes of the planktonic foraminifer globigerinoides sacculifer in laboratory culture. J. Mar. Biol. Assoc. U.k 62, 435-452. Spero, H.J. and Parker, S.L. (1985) Photosynthesis in the symbiotic planktonic foraminifer Orbulina universa, and its potential contribution to oceanic primary productivity. Journal of Foraminiferal Research 15, 273-281. Jørgensen, B.B., Erez, J., Revsbech, N.P. and Cohen, Y. (1985) Symbiotic photosynthesis in a planktonic foraminiferan, Globigerinoides sacculifer (Brady), studied with microelectrodes. Limnology and Oceanography 30, 1253-1267. Hemleben, C., Spindler, M., Breitinger, I. and Ott, R. (1987) Morphological and physiological responses of Globigerinoides sacculifer (Brady) under varying laboratory conditions. Marine Micropaleontology 12, 305-324. Babila, T.L., Rosenthal, Y. and Conte, M.H. (2014) Evaluation of the biogeochemical controls on B/Ca of Globigerinoides ruber white from the Oceanic Flux Program, Bermuda. Earth and Planetary Science Letters 404, 67-76. Salmon, K.H., Anand, P., Sexton, P.F. and Conte, M. (2016) Calcification and growth processes in planktonic foraminifera complicate the use of B/Ca and U/Ca as carbonate chemistry proxies. Earth and Planetary Science Letters 449, 372-381. 6) In the next paragraph you say: "More generally, there are a few instances in the intro and discussion where preference is given to detailing the findings of older, and since superceded studies, rather than cutting straight to the new data coming out of the community and dealing with the questions they raise." We fully agree, this is our mistake and due to the fact that this msc has been around for too long and we didn't properly update. We have added and discussed the newest literature. "Specific points" Lines 25-27: the sentence

here does not make the necessary link between borate substituting into carbonate and why this would then make it a carbonate ion proxy. Need to point out in a sentence like this that borate is more abundant at higher pHs. Has been changed to read: "As B(OH)4- is substituted into the biogenic calcite lattice in place of CO32- and both borate and carbonate ion are more abundant at higher pHs it has been suggested early on that B/Ca ratios in biogenic calcite are a possible proxy for [CO32-]." Line 37-40 (and throughout): I have some serious concerns over the point being made here- that B/Ca is a useful second carbonate system parameter. Many experiments have shown (including ours- Henehan et al. 2015, G3) that you can produce a pH dependent shift in B/Ca in culture experiments where you change only the carbonate system. However, in the open ocean these relationships often fall down, because there are other controls on boron incorporation- see for example my paper, or excellent papers by Babila et al, or Allen and Hönisch (2012) or a really great paper just out by Kate Salmon et al. in EPSL. This abstract, and indeed the paper, is strongly advocating the use of B/Ca to derive the whole carbonate system, but this is based only on culture experiments and ignores the evidence in other papers that shows that really B/Ca is not at all reliable in open ocean foraminifera as a tracer of the carbonate system. These open ocean studies must be considered and the claims on behalf of B/Ca as a proxy needs to be removed. Please see our reply to 5) above. We argue that there is possibly still a primary relationship between the boron uptake and [B(OH)4-]/[HCO3-] as shown in our culture study. At this stage we cannot prove this but microelectrode studies could (e.g. Rink et al., 1998). We further agree with you, that in the field, the primary relationship between B/Ca and [B(OH)4-]/[HCO3-] can be completely masked by other parameters and that therefore B/Ca loses its potential as a robust proxy. We have discussed this now in the last part of the revised msc under "Proxy implications". Line 68: Line beginning 'At low'.. This sentence would be better off earlier where you mention pH-dependent speciation in Line 65. As it stands the thread of the paragraph is a little disjointed. Done as suggested Line 79: Suggest removing pteropods from here since people don't tend to use them for boron work. Done as suggested Lines 81-85: Pi-

oneering as these studies were, the field has moved on quite a lot since then, and I am not sure I see the logic in dwelling so long on the specific findings of these studies when they have been superceded by better estimates of these pH values. Indeed, this whole paragraph isn't really necessary. The authors could make their point very quickly with one statement that 'The boron isotope-pH proxy is a widely used palaeoceanographic tool (a few example references)." We appreciate the advice and do not dwell on their work anymore but included them in the references as we should do justice to the pioneers as well. Line 111-113: Which studies use this approach? That sentence was wrong and is part of the approach used by Pearson and Palmer (2000). We have changed the text accordingly. "Another approach is based on the assumption that seawater [Ca2+] has remained proportional to AT over time so that AT can be adjusted in a way that the water column is exactly saturated with respect to calcite at the lysocline (∼500 m above the CCD; Pearson and Palmer, 2000). Surface AT can now be estimated by assuming that increases in AT with depth were the same as in the modern ocean. Line 113-114: Surely a changing CCD depth wouldn't invalidate the approach strictly speaking, it would just mean you can't use one estimate for a whole long term time series- you have to estimate for each data point? correct. We have added the fact that Pearson and Palmer (2000) note themselves that the CCD record for the Palaeogene Pacific Ocean is relatively poorly constrained. Line 95: proven, rather than proved. done Line 107: I may be missing something but I'm not sure I see the link with the hydrological cycle? We have rephrased this to be more precise: "However, salinity and alkalinity may be decoupled in space and time through weathering and changes in riverine alkalinity input." For example, continental weathering was probably more intense during periods of warm climate and high pCO2, which would deliver more Ca and alkalinity to the ocean. Lines 93-114: I'm not convinced that the authors are right to present alkalinity as such a paralysingly big problem as the tone of this passage suggests- it surely depends what the goal is. If the goal is to reconstruct CO2 changes, yes alkalinity introduces some uncertainty and it would be better if we knew it, but alkalinity has a relatively small influence on pCO2 values reconstructed

from pH, and many studies can just factor in the uncertainty on these estimates into their error propagation. I would suggest the authors reword this somewhat to just make the point that knowing the second parameter would be great to reduce uncertainty in CO2 estimates, rather than present it as such a very acute problem with the proxy. The other question is whether the propagated uncertainty in alkalinity reconstructed from a second proxy (taking into account measurement and calibration uncertainty) would be any lower than the margin of error that can be garnered from things like CCD depth. In reality, given the error bars in figure 2 for example, it would probably be just as large. We disagree with Michael Henehan that "alkalinity introduces some uncertainty". The change in surface water [CO2] is twice as much when the same atmospheric pCO2 is reached solely via a change in alkalinity as in the coral reef hypothesis (Lea et al., 1999). We do agree that the propagated uncertainty in the second parameter, reconstructed from an independent proxy (taking into account measurement and calibration uncertainty) might not be any lower than the margin of error that is inherent to assumptions around e.g. total alkalinity We have added the following text to the manuscript: "Although ïĄď11B has proven to be a reliable proxy for pH and one can argue that ocean pH is the main driver of the past atmospheric CO2, it is important to remember that changes in past glacial interglacial atmospheric pCO2 can be achieved via two end-member scenarios (e.g. Sanyal and Bijma, 1999; Lea et al., 1999). In the first scenario, changes in carbonate chemistry are brought about by changes in DIC only. This is equivalent to varying the response of the biological pump as a reaction to variations in the nutrient content of the surface ocean. In the second scenario, changes in carbonate chemistry are solely controlled by addition (due to dissolution in sediments) or removal (due to production) of calcium carbonate. The change in surface ocean carbonate chemistry is very different in these two scenarios because the ratio of carbonate ion increase to pCO2 decrease depends on surface ocean alkalinity (Lea et al., 1999). A smaller change is associated with the drawdown of DIC under conditions of unchanging alkalinity (e.g. strengthening the biological pump without calcite compensation). The change in surface water [CO2] is twice as much when the same pCO2

is reached solely via a change in alkalinity as in the coral reef hypothesis (Lea et al., 1999). This is nicely demonstrated in Fig. 1.1.3 of Zeebe and Wolf-Gladrow (2001) and Fig.1 of Foster and Rae (2016). The real oceans operate somewhere between these endmember scenarios and basically depends on the relative delivery rates of calcium carbonate and particulate organic carbon (the CaCO3:POC "rain ratio") and the sensitivity of calcium carbonate preservation in deep ocean sediments. Although it has been questioned if changes in the CaCO3:POC rain ratio of biogenic material produced in the surface ocean are directly communicated to the sediments (Ridgwell, 2003), we still believe that knowing a second, pH independent, parameter could reduce the uncertainty in CO2 estimates. On the other hand, the propagated uncertainty in the second parameter, reconstructed from an independent proxy (taking into account measurement and calibration uncertainty) might not be much lower than the margin of error that is garnered using assumptions around e.g. total alkalinity. Line 130: This is not correct. See for example Fig. 2 of Allen et al. (2012), where pH is kept constant but carbonate ion concentration is varied. Indeed, the authors state that pH was kept constant but carbonate ion increased in Line 128. It's not clear to me what the distinction is between the decoupled chemistry of Allen et al and that of Kaczmarek et al? Allen et al. raised [DIC] and tweaked pH via acid and base addition, so did these authors- what's the big difference? You are absolutely right. Line 130 as stated is confusing and not correct. What we basically meant to say is that Allen et al (2012) did not vary pH at constant [CO32-]. The text has been changed accordingly: "However, they did not decouple pH and [CO32-] both ways. In their experiments, they kept pH constant and varied [CO32-] but did not vary pH at constant [CO32-], leaving the question open whether the B/Ca ratio in planktonic foraminifera is only a function of the ratio between [B(OH)4-] and CT or [HCO3-] or perhaps also modulated by pH or [CO32-]. The manipulations used in our study and Kaczmarek et al. (2015) are exactly the same as Allen et al. (2012). Except that we added experiments at constant [CO32-] and varied pH. In addition, we used 10X boron and did not prepare media at constant, ambient, pH and lower [CO32-]. Line 136-138: The authors state here that they believe that this is

due to a covariation of nutrients and other carbonate system parameters. I would urge them to read the paper again- since the aim of this paper was to test the carbonate system control on B/Ca, we of course tested carbonate system parameters explicitly and B/Ca ratios show no correlations with carbonate system parameters. We also tested for covariation of phosphate with carbonate system parameters (some of these plots are given in the paper) and they show no relationships (p values greater than 0.05, and R2 values <0.1. On this point, the authors are mistaken, and this must be removed. What's more, the findings of the paper, which are directly contrary to the idea that B/Ca can be used in open ocean settings to derive a second carbonate system parameter, should be properly incorporated into the discussion (as with the findings of Babila et al. and Salmon et al.). I am happy to answer any questions the authors have to address any misconceptions about this study. Point well taken. Our comment related to a possible correlation between PO4 and B/Ca as suggested by Henehan et al. (2015) was way too short and didn't do justice to your paper were you tested carbonate system parameters explicitly and B/Ca ratios show no correlations with carbonate system parameters. We were not referring to bulk ocean conditions but rather to their ambient environment. As explained above (under 5), forams, and especially symbiont bearing planktonic forams never "see" the bulk ocean carbonate chemistry (see e.g micro-electrode studies by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only "see" their ambient carbonate chemistry as modulated by their own life processes as well as by symbiont photosynthesis and respiration (therefore all calibrations are purely empirical and not mechanistic). We have changed the wording to: "Recently, Henehan et al. (2015) demonstrated a very clear and close relationship between B/Ca and carbonate chemistry parameters (pH; [B(OH)4-]/[HCO3-] and [B(OH)4-]/DIC) in Globigerinoides ruber from culture experiments. However, this relationship is completely lost in the plankton tow samples and the sediments they analyzed. While they explicitly tested for a carbonate chemistry control on B/Ca, they found a strong relationship to [PO4-] and neither a correlations with carbonate system parameters nor a covariation of phosphate with carbonate system parameters. They concluded that apparently B/Ca

in Globigerinoides ruber is controlled by [PO4-]. We believe that the primary, mechanistic, relationship explaining B/Ca is probably still controlled via carbonate chemistry parameters in the ambient environment of the foraminifer but that it is masked in the field and decoupled from the bulk seawater carbonate chemistry. It should be noted that foraminifera, and especially symbiont bearing planktonic foraminifers never "see" the bulk ocean carbonate chemistry (e.g. e.g micro-electrode study by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only "see" their ambient carbonate chemistry as modulated by their own life processes and symbiont photosynthesis and respiration (so called "vital effects"). Existing calibrations and field relationships are therefore purely empirical and not mechanistic. Here we are specifically focusing on the primary controls of boron uptake and conducted experiments with a the planktonic foraminifer Orbulina universa and decoupled pH and [CO32-] in the same way as Kaczmarek et al. (2015b). We show that, in principle, combined measurements of δ11Bcalcite and B/Ca of the same species as conducted in our study might be used to fully constrain the carbonate chemistry in Earth history. However, based on recent publications (Allen and Hönisch (2012); Babila et al. (2014); Henehan et al. (2015) and Salmon et al. (2016)) it becomes increasingly clear that B/Ca may not be a very robust proxy in the field." Line 193: How were the seawater d11Bs measured? This is presumably a different way from the foraminiferal shells, and so there is the potential for the different analytical approaches to introduce absolute offsets here. These details are critical if we are to evaluate these numbers in an absolute sense, and need to be added. Measurements of natural seawater could also be added to give a feel for the typical reproducibility of the approaches for a standard everyone has. We have added a method section on SW boron analysis and added the individual SW analyses to the supplement Line 197: It's not clear to me why Martinez-Boti should be cited here for a salinity of 38. Not clear to us either, must have been some glitch. Has been taken out Line 261: Faraday should be capitalized Done Lines 306, 310: 'seawater scale' is a particular definition of pH, and not to do with boron. Better to say 'normalised to natural seawater'. Very good point. Has been corrected. Line 342

(and Fig. 3): The choice of uncertainty calculation here seems wrong. The authors show that the repeat measurements of foram d11B within a test vary by far more than the calculated internal error of a single measurement described in Equation 2. It is therefore not reasonable to apply the single measurement uncertainty estimate to the 'whole test' d11B value, as it only accounts for the measurement uncertainty and not the variability between measurements. The best approximation of the uncertainty of the 'true' average bulk-test boron isotope ratio would in this case be 2 standard deviations of the variability within the test- these should be the error bars on Fig. 3 if they are to be truly representative of true variability. On top of this, when presenting the data in 'normal d11Bsw' space, the authors should also propagate the uncertainty stemming from the uncertainty in experimental seawater d11B- since these values are also critical and the uncertainty on these numbers is very large in some cases. Each data point respresents one single raster ablation of one single specimes (i.e. "whole tests"). The major difference between LA and "wet chemistry" data is that the latter method averages individual variability before analysis by analyzing multiple, dissolved, shells in one go, while LA captures individual variability (which is large and real as argued above) and averages afterwards. Regarding the error propagation of the "uncertainty" stemming from individual LA measurements and the analytical uncertainty from the seawater ïĄď11B analysis we have added table 4 in the supplement. The propagated error is of course large as it includes the individual ïĄď11B variability. We like to point out that this variability is data/information which is not related to the analytical uncertainty. We have added a calcite vs borate ïĄď11B crossplot (Fig. 4) to avoid the conversion into the seawater scale and making the error propagation obsolete. However, since not all studies (cited in Fig. 3) report the parameters required for the calculation of ïĄď11B of borate, we kept figure 3 but did not plot the propagated error. Line 355: Foster identify a relationship between carbonate ion and KD, not strictly B/Ca ratios. This is a really important distinction since Allen and Hönisch (2012) point out that the way KD is calculated can drive a correlation without any coherent pattern in raw B/Ca ratios. Good point. Has been changed accordingly. New text: "Foster (2008)

showed that the partition coefficient for the B/Ca ratio is influenced by [CO2−3] (and temperature). Although complicating the application as a proxy related to [B(OH)4-]/[HCO3-], he also demonstrated that B/Ca in combination with ïĄď11B can be used to fully constrain the carbonate system in downcore records. Nonetheless, he identified [CO32-] as having a major (secondary) control on B/Ca in samples of foraminifera from down core samples and core tops." Line 360-365: This is what Allen et al. (2012) did, and the conclusions reached are largely the same as this study. It seems therefore odd to mention two older studies in this paragraph first in Foster (2008) and Allen et al. (2011), but not mention the more relevant study right from the off. It has the effect of almost suggesting this study is the first to do this, but in fact it is largely replicating what Allen et al. (2012) did. This is not quite true. As argued before, even though the manipulations used in our study and Kaczmarek et al. (2015) are largely the same as Allen et al. (2012), we have added experiments at constant [CO32-] and varied pH, to decouple both the other way around. In addition, we also analysed ïĄď11B, next to B/Ca. Line 376-379: What is Fig. 5.1C? The discussion is a little odd here. Since the argument with competition is that B/Ca will correlate with the ratio of borate to carbon species, then these observations are to be expected: changing carbonate ion without changing pH changes the denominator, and changing pH without carbonate ion changes the numerator in altering boron speciation. So these are two sides of the same coin. Thanks for pointing this out. Fig. 5.1C should be Fig. 2E. You are right that the two opposing experiments could be seen as two sides of the B/Ca coin, where one manipulation affects the denominator and the other the numerator but in a chemical sense these experiments are very different. Biologically, the impact is very different as well. A priory we expect very different responses as the two opposing experiments will impact the ambient carbonate chemistry differently and therefore change the extent of the "vital effects". The ion channels taking up calcium and carbon during calcification may behave differently. [CO32-] changes at constant pH probably affect calcification rate, while constant [CO32-] at variable pH will not affect calcification rate, etc. Line 391-392: This sentence as it is currently phrased suggests that Yu et al. (2007) would

support bicarbonate being critical here rather than DIC or carbonate ion. However, this is not strictly true: Yu et al. (2007) never tried to regress against borate/DIC in that paper.. Just because they present borate/bicarbonate in this paper, it doesn't rule out the possibility that the correlation with borate/DIC or borate/carbonate might have been stronger. This passage therefore needs to be rewritten. We have rewritten this passage: "Although analysis of planktonic foraminifera from core tops revealed a good correlation between B/Ca and [B(OH)4-]/[HCO3-] it doesn't rule out a possible correlation with B(OH)4-/CO32-and/or B(OH)4-/CT.) (Yu et al., 2007). " Line 412-414: Is there any a priori reason for us to ever expect this? If so, it might be good to give it here. A priori no, but we did vary [CO32-] at constant pH and found, surprisingly, an effect of [CO32-] on ïĄď11B (see answer to reviewer 1)! This was previously not discussed in the manuscript but we have added text: "ïĄď11B increases with increasing [CO32-] at constant pHT from 17.2‰ at 238 μmol/kg CO32- to 19.9‰ at 534 μmol/kg CO32- (Table 3; Fig 3B). Applying ANOVA with a Bonferroni test, which is best suited for a limited number of pairs, the p-value of the overall ANOVA is 0.00203, demonstrating a significant difference between two or more population means. The difference between the mean ïĄď11B values of the [CO32-] treatments 239 and 286 μmol/kg were close to significance but only between 239 and 534 μmol/kg the difference was significant (Supplement Table 3). Because, this range in [CO32-] is beyond that of the real ocean and because pH and [CO32-] co-vary, we believe that this observation is only important for a better understanding of the ïĄď11B controls and does not significantly impact existing calibrations."

Line 423: Need to be clearer here – values for what? Borate ion. We have changed the sentence: "The δ11B values for O. universa found in this study match closely with the δ11B values of borate ion in artificial seawater given by Klochko et al. (2006).". Lines 437-441: Note again, this is fine in culture, but is ignoring plenty of open ocean data that suggests that the control of HCO3- on B/Ca is overwhelmed by competing controls. We have added a large paragraph in the section "proxy implications": "A wide range of [HCO3-] was necessary to facilitate de-coupling the carbonate system from

pHT. The high [HCO3-] in some of these treatments are unrealistic for natural seawater systems and more environmentally-relevant values should be used for future calibration experiments. The proxy should therefore be ground-truthed using water column and core top samples.

Recently, Henehan et al., (2015) showed that B/Ca in G. ruber collected with a plankton net was perfectly correlated to [PO43-] and not to any carbonate chemistry parameter, despite the fact that their culture study demonstrated a highly significant relationship between B/Ca and e.g. [B(OH)4-]/[HCO3-]. Based on plankton tow, sediment trap and core-top data, they concluded that, apparently, B/Ca in G. ruber is controlled by [PO43-]. However, it should be noted that foraminifera, and especially symbiont bearing planktonic foraminifers never “see” the bulk ocean carbonate chemistry (e.g micro-electrode study by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only “see” their ambient carbonate chemistry as modulated by their own life processes and symbiont photosynthesis and respiration (so called “vital effects”). Existing calibrations and field relationships are therefore purely empirical and not mechanistic and the ambient carbonate chemistry of the foraminifer and the bulk seawater chemistry can be decoupled. We note that, of all symbiont bearing planktonic Foraminifera, G. ruber is probably the most "autotropic" (Bijma et al., 1992). Although we cannot prove it, we assume that symbiont photosynthetic rates are higher at elevated [PO43-] (limiting nutrient) and therefore that ambient pH would be higher. Hence, even if the correlation between B/Ca and seawater carbonate chemistry is lost, the correlation with ambient pH (or [CO32-], [HCO3-]) may still hold up. At this point, there is no obvious direct link between [PO43-] and B/Ca and we believe that, mechanistically, it can be explained by increased photosynthesis and/or higher calcification rates. Kaczmarek et al., (2015b) show that boron partitioning in inorganic precipitation experiments increases with increasing growth rate and early work by the pioneers of foraminiferal biology and calcification (e.g. Bé, 1965; Bé et al., 1982; Caron et al., 1981; Spero and Parker, 1985; Jørgensen et al., 1985; Hemleben et al., 1987) has clearly demonstrated the huge impact of symbionts on foraminiferal shell growth. Interestingly, Babila

et al. (2014) write: "The seasonal cycle of B/Ca in G.ruber white was more strongly correlated with light intensity than with temperature. Both observations suggest that the presence of symbionts in G.ruber and seasonal variability in their photosynthetic activity act to modify the internal pH during calcification, by up to âĹij0.2 units relative to ambient seawater." This supports our line of argumentation above. In another recent paper on B/Ca, Salmon et al. (2016) write: "We provide the first evidence for a strong positive relationship between area density (test thickness) and B/Ca, and reveal that this is consistent in all species studied, suggesting a likely role for calcification in controlling boron partitioning into foraminiferal calcite." Their conclusion also supports our reasoning, that, mechanistically, increased photosynthesis may lead to higher calcification rates. Remarkably, Salmon et al. (2016) show that B/Ca of the non-symbiont species (G. bulloides and G. inflata) and even the symbiont bearing species G. sacculifer are related to [CO32-] and [B(OH)4-/HCO3-]. In our view, those results demonstrate the primary control by carbonate chemistry parameters not masked by symbiont photosynthesis. One could even argue that there is a positive trend for O. universa but that the natural range of [CO32-] variability (or borate/bicarbonate) is small (ca. 20 μmol kg-1 in the depth range 30 to 50m) in comparison to the decoupling we carried out in controlled culture experiments. Interestingly, Henehan et al. (2016) propose a field calibration for O. universa that is also very close to ïĄď11B of borate, suggesting that their “vital effects” are muted in the real ocean, especially the symbiont impact of raising the calibration curve above ïĄď11B of borate. This is supported by the observation of Hemleben et al., (1994) that O .universa occupies a subsurface maximum (in the Red Sea) between 20-60 meters (Hemleben et al., 1994; Fig. 5) and could explain why B/Ca in this species is not (completely) masked by symbiont photosynthesis (Salmon et al., 2016).

Our final conclusion is that field studies are not suitable for elucidating the mechanisms of proxy incorporation but are very helpful in showing which species best suited for paleo reconstructions of carbonate system parameters and if and how much vital effects determine species specific offsets from the target parameters. We agree with

Henehan et al. (2015) that G. ruber is not a good choice as its primary relationship to carbonate chemistry parameters is not very robust. However, other symbiont bearing species, non-symbiotic planktonic Foraminifera and deep sea benthics, may still be a viable option to use B/Ca for carbonate chemistry reconstructions."

Lines 462-464: Again, this is suggesting that these culture observations can be transferred to the open ocean when a number of more recent studies that the authors do not cite here (and should) show that these relationships don't hold up outside of the lab. see above Fig 3: In panel A, these data from other studies are all plotted on one d11B-pH plot. But critically, pK*B differs between each study. Therefore some of the variation in behavior of d11B with pH in each study can derive from a different pK*B in each case. This is why we have moved towards plotting things in d11Bcalcite-vs-d11Bborate space. To represent these data in an informative way, each datapoint needs to be normalised to a single pK*B, which is clumsy to try and do. I would advise that the authors plot these data in a calcite vs borate d11B crossplot instead. How also were the lines constructed between points? Also, as mentioned above, error bars should also account for the uncertainty in d11Bsw that is carried through into these normalised d11B values. Why are there no error bars on pH, also? There should be. Finally, I see no benefit in plotting the Kakihana et al borate ion curve in panel A at all- this value is defunct, and has been shown to be erroneous (Rustad et al. 2010), so why plot it? We have added a calcite vs borate ïĄď11B crossplot (Fig. 4) and added some text in the method section: "One could further argue that the uncertainty stemming from the analysis of culture water ïĄď11B should also be propagated when plotting in 'normal ïĄď11Bsw' space (supplementary table 4). The propagated error is of course large as it includes the individual ïĄď11B variability of the foraminifers. We like to point out that this variability represents true data which is largely unrelated to analytical uncertainty. We have added a calcite vs borate ïĄď11B crossplot (Fig. 4) to avoid the conversion into the seawater scale and making the error propagation obsolete. However, as not all studies report the parameters required for the calculation of ïĄď11B of borate we plotted for comparison in 'normal ïĄď11Bsw' space but did not

propagate the error related to the analysis of culture water ïĄď11B.". We have added the error bars on pH We leave the ïĄď11B borate curve with ïĄąïĂă= 20 permil (but do not mention Kakihana) in Fig. 3 for comparison of slope (which is less steep than the Klochko curve)

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