Boron incorporation in the foraminifer *Amphistegina lessonii* under a decoupled carbonate chemistry

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ABSTRACT

A number of studies have shown that the boron isotopic composition ($\delta^{11}B$) and the B/Ca ratio of biogenic carbonates (mostly foraminifers) can serve as proxies for two parameters of the ocean's carbonate chemistry, rendering it possible to calculate the entire carbonate system. However, the B incorporation mechanism into marine carbonates is still not fully understood and analyses of field samples show species specific and hydrographic effects on the B proxies complicating their application. Identifying the carbonate system parameter influencing boron incorporation is difficult due to the co-variation of pH, CO_3^{2-}, and $B(OH)_4^-$. To shed light on the question which parameter of the carbonate system is related to the boron incorporation, we performed culture experiments with the benthic symbiont-bearing foraminifer *Amphistegina lessonii* using a decoupled pH – CO_3^{2-} chemistry. The determination of the $\delta^{11}B$ and B/Ca ratios was performed simultaneously by means of a new *in situ* technique combining optical emission spectroscopy and laser ablation MC-ICP-MS. The boron isotopic composition in the tests gets heavier with increasing pH and B/Ca increases with increasing $B(OH)_4^-/HCO_3^-$ of the culture media. The latter indicates that boron uptake of *A. lessonii* features a competition between $B(OH)_4^-$ and HCO_3^-. Furthermore, the simultaneous determination of B/Ca and $\delta^{11}B$ on single specimens allows for assessing the relative variability of these parameters. Among different treatments the B/Ca shows an increasing variability with increasing boron concentration in the test whereas the variability in the isotope distribution is constant.

Keywords: benthic foraminifer, B isotopes, B/Ca, carbonate chemistry, laser ablation

1. INTRODUCTION

The oceans carbonate system comprises six co-varying parameters ($[CO_2]$, $[HCO_3^-]$, $[CO_3^{2-}]$, pH, total alkalinity (TA), and dissolved inorganic carbon (DIC)). Changes of the carbonate system caused by past changes in the atmospheric pCO_2 can be reconstructed if at least two of these parameters are known. A number of studies have shown that the boron isotopic composition ($\delta^{11}B$) and the B/Ca ratio of biogenic carbonates (mostly foraminifers) may serve as proxies that can provide these two parameters.

In seawater boron (B) mainly exists as boric acid ($B(OH)_3$) and borate ($B(OH)_4^-$). The isotopic composition and concentration of both species are pH dependent (Fig. 1). Since the B isotopic composition of biogenic carbonates precipitated at a certain pH value is similar to that of $B(OH)_4^-$, Hemming & Hanson (1992) concluded that only $B(OH)_4^-$ is incorporated into biogenic carbonates. Therewith, the B isotopic composition can be used as a proxy to infer the pH that prevailed during the formation of the biogenic carbonate. However, several studies show a deviation between the B isotopic composition of the biogenic carbonates and $B(OH)_4^-$ of the sea water (Sanyal et al., 1996; Sanyal et al., 2001; Foster, 2008; Rae et al., 2011). This deviation is often explained by physiological processes like photosynthesis and respiration of symbionts (e.g. dinoflagellates) which modify the pH in the micro-environment around the foraminifera (Zeebe et al., 2003) leading to shifts in the B equilibria. Yet another explanation for the observed deviation is that not only $B(OH)_4^-$ is incorporated during the formation of calcium carbonate but to some extend also the isotopically heavier $B(OH)_3$ (Klochko et al., 2009). To account for physiological effects, species specific calibration experiments have been carried out to be able to apply this proxy and reliably reconstruct seawater pH (Sanyal et al., 2001; Hönisch et al., 2003; Henehan et al., 2013).

While the B isotope composition of biogenic carbonates is used to reconstruct past seawater pH, the B/Ca of foraminiferal calcite is often used to infer past seawater CO_3^{2-} concentrations e.g. (Yu et al., 2007; Brown et al., 2011). Inherent to all field studies and most experimental studies is that pH

and CO_3^{2-} concentration of natural seawater are correlated. It is therefore impossible to determine which parameter of the carbonate system is in control of B/Ca. Not surprisingly, correlations between B/Ca and pH were described in addition to B/Ca and CO_3^{2-} concentration (Yu et al., 2007; Tripati et al., 2011). The latter studies are based on field samples, but experimental studies suffer from the same ambiguity if the experimental setup uses a classical carbonate system manipulation, i.e. either DIC or TA manipulation. To identify the parameter of the carbonate system responsible for foraminiferal B/Ca, it is necessary to decouple pH and CO_3^{2-} concentration. Such an experimental setup will allow for excluding up to five out of the six parameters of the carbonate system. In an experimental study on the relationship between B/Ca and the seawater carbonate system Allen et al. (2012) showed "a competition between aqueous boron and carbonate species for inclusion into the calcite lattice" for *Orbulina universa, Globiberinoides ruber,* and *Globigerinoides sacculifer*. In this study we cultured *A. lessonii* under conditions in which pH and CO_3^{2-} concentration were decoupled in order to assess the controlling carbonate system parameter for B incorporation. The simultaneous determination of $\delta^{11}B$ and B/Ca on single specimens by means of a newly developed technique (based on a femto second laser ablation MC-ICP-MS connected to a fiber optic spectrometer) allows for the first time the determination of the elemental and isotope B variability among single specimens.

2. MATERIAL AND METHODS

2.1 Culturing and experimental setup

Live specimens of the benthic symbiont-bearing foraminifer *A. lessonii* were obtained from a coral reef aquarium at the Burgers Zoo (Arnheim, The Netherlands). SCUBA divers collected approximately 1 kg of sediment containing different species of foraminifers (Ernst et al., 2011). The sediment was transported to the Alfred Wegener Institute (Bremerhaven, Germany) immediately and transferred into a small aquarium (5L) filled with filtered (0.2 µm pore-size) North Sea seawater (NSW). The aquarium was equipped with a circulation pump to supply air and a time switched light source providing a light/dark cycle (12 h/12 h). About 100 specimens of *A. lessonii* were transferred to

well plates containing NSW and placed in a temperature controlled room at 25°C (again exposed to a 12 h/12 h light/dark cycle). After two weeks ~20% of the specimens had asexually reproduced, yielding 10-30 juveniles per specimen. Subsequently, juvenile foraminifers were transferred into petri dishes containing NSW with a dedicated carbonate system (see 2.2. Preparation of culture media). Each petri dish was placed into one of six boxes each receiving a concentration of pCO_2 that was in equilibrium with the corresponding carbonate chemistry of the prepared NSW media. The supply of pCO_2 was realized by a gas-mixing system producing a constant gas flow of 40 L per hour for each box. Concentration of CO_2 was logged using CO_2 sensors (type FY0D00CO2B10 Ahlborn) and did not deviate by more than 25 µatm from the target-value. In order to avoid evaporation of culture media in the petri dishes, the gas was saturated with water by bubbling it through a fritted wash bottle filled with de-ionized water. The complete experimental setup was placed in a temperature-controlled (25°C) room. Because of heat produced by the lamps the temperature within the boxes containing the petri dishes increased by up to 2°C during the light cycle. Since this holds for all treatments, it did not impair the interpretation of results. Light intensity was 100-150 µmol photons $m^{-2}s^{-1}$. Every third day the culture media was replaced by a freshly opened aliquot from the corresponding batch of culture media, which was stored without headspace at ~3°C. Approximately 24 hours before the culture media was replaced it was filled in a petri dish and placed in the corresponding gas box to equilibrate. Each time when the culture media was replaced, foraminifers were fed with concentrated and sterilized algae *Dunaliella salina* (20000 cells/ml). Before feeding algae were centrifuged to minimize dilution of the culture media, and exposed to 90°C for 20 minutes after centrifugation in order to reduce bacterial activity in the culture media. Foraminifers grew for three months. Afterwards specimens were harvested, bleached in NaOCl (active chlorine: 4.6%) for six hours, rinsed four times using de-ionized water, and dried for 12 hours at 50°C. For laser ablation analysis specimens were mounted on a glass slide using double sided adhesive tape.

Single, juvenile specimens of a clone were distributed equally between the different treatments to verify whether specimen specific effects on $\delta^{11}B$ would occur which, however, were not observed after the B analysis. The size of all foraminifers ranged between 400 and 900 µm before specimens

were harvested. The morphology of the tests was indistinguishable from the one of specimens grown in the natural habitat.

2.2 Preparation of culture media

Six treatments of manipulated NSW were prepared: treatments 1 to 4 had a constant pH but different [CO_3^{2-}]. The labels are: pH_8.1^{160}, pH_8.1^{260}, pH_8.1^{540}, and pH_8.1^{640}. The exponent represents the concentration of CO_3^{2-} in µmol/kg respectively. We will refer to the sum of treatments 1 to 4 as pH_8.1*. Treatment 5 yields a pH of 8.56 and a CO_3^{2-} concentration of 638 µmol/kg. It is labelled as pH_8.6^{640}. Treatment 6 has a pH of 7.86 and a [CO_3^{2-}] of 268 µmol/kg. It is labelled as pH_7.9^{260}. Since our treatments are not in equilibrium with a pCO_2 of 380 µatm (except pH_8.1^{260}), we used a CO_2 gas-mixing system providing each treatment with the associated equilibrium pCO_2. The required manipulation of the culture media was calculated by means of the computer program octave and the file csys.m (created by Richard E. Zeebe and Dieter Wolf-Gladrow, downloadable at http://www.soest.hawaii.edu/oceanography/faculty/zeebe_files/CO2_System_in_Seawater/csys.html. The csys.m file was modified to allow calculations of borate concentrations different from the natural concentration of seawater. The equilibrium constants of Mehrbach (for K1 and K2) and the total scale for pH were chosen. Temperature was set to 27°C, salinity to 32. Calculating the whole carbonate system chemistry requires at least two of its parameters. The input parameters for the pH constant treatments (pH_8.1*) were pH and pCO_2, for the [CO_3^{2-}] constant treatments (pH_8.6^{640} + pH_8.1^{640} and pH_7.9^{260} + pH_8.1^{260}) [CO_3^{2-}] and pCO_2. The basis for the different culture media was sterile filtered (0.2 µm pore size) NSW enriched in B (using $B(OH)_3$, chemical purity: > 99.5%) to a final concentration of ~4 mmol/kg, which is ~10 times the B concentration of natural seawater. The enrichment with B was done to obtain a higher concentration within the test for better B analysis. For each treatment two litres of culture media were prepared and filled without headspace into 50 ml (for the replacement of culture media) and 200 ml (for chemical analysis) gastight, boron free, silicate flasks and stored at ~3°C.

2.3 Analysis of the culture media

Since the amount of culture media in the petri dishes containing the foraminifers (which was replaced all three days) was not sufficient for all chemical analysis, approximately 200 ml of each batch of culture media were filled in polypropylene beakers and placed into the corresponding CO_2 box to equilibrate. Even though determining the chemical parameters once would have been sufficient, we performed this procedure bi-weekly to verify that all conditions stayed constant during the experimental period. After ~24 hours salinity and pH of these solutions were measured at *in situ* conditions and samples were taken for Ca, B, DIC, and TA analysis. Salinity measurements were performed using a conductivity meter (WTW Multi 340i) interfaced with a TetraCon 325 sensor. Measurements of pH were carried out by means of a combined pH glass electrode (Ectotrode Plus,Metrohm) interfaced to a Radiometer pH-Meter (PHM240). Repeated measurements of buffers show a reproducibility of 0.05 pH units. After calibration (NBS buffer) the conversion to total scale was performed by measuring a Tris/Tris-HCl seawater buffer prepared in accordance with the recipe described in (Dickson et al., 2007). Calcium and B concentrations were determined by a Thermo Elemental (TJA) IRIS Intrepid ICP-OES Spectrometer using Merck 4 (multi element standard) as reference material. The average external error as estimated by multiple measurements of the reference material was ± 3.5%. Total alkalinity was calculated from linear Gran plots (Gran, 1952) after triplicate potentiometric titration (Bradshaw et al., 1981) using a TitroLine alpha plus auto sampler (Schott Instruments). Culture media samples were calibrated against an in-house standard (NSW) which is calibrated regularly against certified reference material batch No. 54 of Dickson (Scripps Institution of Oceanography). The average reproducibility is ±10 µmol/kg. Determination of DIC was performed photometrically in triplicates with a TRAACS CS800 QuaAAtro autoanalyzer with an average reproducibility of ±10 µmol/l based on calibrations of an in-house standard (NSW) calibrated against Certified Reference Material Batch No. 54 of Dickson (Scripps Institution of Oceanography). Boron isotopic composition of the culture media were analyzed by means of a Thermo® Element XR, a single collector, sector field, high-resolution inductively coupled plasma mass spectrometer, fitted with a high sensitivity interface pump (Jet pump) as described in Misra et al. (2014). Boron isotopic composition is reported as per mil (‰) deviation from NIST SRM 951a ($^{11}B/^{10}B = 4.04362 \pm 0.00137$) (Catanzaro et al., 1970) where:

$$\delta^{11}B_{sample}(‰) = \left[\frac{\left(^{11/10}B\right)_{sample}}{\left(^{11/10}B\right)_{NISTSRM\ 951a}} - 1\right] \times 1000 \qquad (1)$$

Boron isotope analyses were made following a Sample – Standard Bracketing (SSB) technique. NIST 951a was used as the standard and samples were concentration matched, typically at ± 5%, with the standard and were analyzed in quintuplicate. The accuracy and precision of the analytical method was assessed by comparing $\delta^{11}B$ measurements of seawater (from the Atlantic Ocean) and secondary boron standards (AE 120, 121, 122) with published (accepted) results. Our estimate of $\delta^{11}B_{SW}$ of 39.8 ± 0.4‰ (2σ, n = 30) are independent of sample size and are in agreement with published values of 39.6 ± 0.4‰ (Foster et al., 2010) and 39.7 ± 0.6‰ (Spivack & Edmond, 1987). Moreover, our $\delta^{11}B$ estimates of SRM AE-120 (-20.2‰ ± 0.5‰, 2s, n = 33), SRM AE-121 (19.8‰ ± 0.4‰, 2s, n = 16), SRM AE-122 (39.6‰ ± 0.5‰, 2s, n = 16) are identical, within analytical uncertainty, to accepted values (Vogl & Rosner, 2012). Information about sample preparation for analysis can be found in the supplement.

2.4 Simultaneous determination of B isotopic composition and B concentration of single tests

For the simultaneous determination of the B isotopic composition and B concentration a Fiber Optics Spectrometer (Maya2000 Pro, Ocean Optics) was connected to the torch of a Thermo Finnigan Neptune multiple-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at the Leibniz University of Hannover. Laser ablation on reference material, NISTSRM 610, and samples was performed by an inhouse build UV-femtosecond laser ablation system based on a regenerative one box femtosecond laser (SolsticSpectra Physics).

The measured intensity for B in a standard is related to its known concentration. Based on this relationship the unknown B concentration of a sample can be calculated. However, in our case measurements of the reference material (NISTSRM 610) and samples have not been performed at the same laser repetition rate, hence their B ratio is not proportional. The correction for different laser repetition rates can be realized using an optical spectrometer by the collection of Ca on the two high intensity first order emission lines of Ca II at 393.48 and 396.86 nm in cps. The detection of Ca intensities of NISTSRM 610 and samples (whose Ca concentrations are known: [Ca] of NISTSRM

610 is 8.45%, [Ca] of $CaCO_3$ is 40%) allows to correct for different laser repetition rates as described in Longerich et al. (1996). A detailed description of this methodology can be found in Kaczmarek et al. (2015). A brief summary of the method is given in the supplement.

3. RESULTS AND DISCUSSION

3.1 Carbonate system

The determination of pH, TA and DIC of the culture media yielded three parameters of the carbonate system. In theory, any two of these parameters can be used to calculate the entire carbonate system. However, it has been shown that the results can differ depending on the choice of input parameters (Hoppe et al., 2012). To evaluate in how far the choice of input parameters (pH/DIC, DIC/TA, and pH/TA) would affect the calculated carbonate system within the same treatment, calculations have been performed with all three combinations of input parameters. As can be seen from Table S1 for this study the choice of input parameters does not result in significant differences. Therefore, further discussions and plots are based on the carbonate system calculated from the input parameters pH and DIC.

3.2 The B isotopic signature of *A. lessoniis'* tests

The measured boron isotopic composition of the foraminiferal tests is given in Table 1 (mean values calculated from single measurements of all foraminifers within one treatment) and Table S2 (single measurements of each foraminifer). For the treatments pH_8.1* the boron isotopic composition is identical (~ -32‰) while treatment pH_8.6^{640} shows an increase of the boron isotopic composition by 8.5‰. The boron isotopic composition determined for treatment pH_7.9^{260} shows a decrease of 3.4‰ compared to the values determined for the treatments pH_8.1*. The results show that the boron isotopic signature is clearly related to pH and independent of the CO_3^{2-} concentration (Fig. 2).

Under the general assumption that $B(OH)_4^-$ is the only species incorporated into the test of foraminifers, $\delta^{11}B$ of the test should equal the $\delta^{11}B$ of $B(OH)_4^-$. Therefore, theoretically the offset

between both ($\delta^{11}B_{foram}$ - $\delta^{11}B_{B(OH)4-}$) should be zero. Figure 3 shows the offset from the theoretical $\delta^{11}B$ of $B(OH)_4^-$ for each specimen and the inter-specimen variability in $\delta^{11}B_{foram}$. It can be seen that most foraminifers grown at a pH of 7.9 and 8.1 show an offset towards more negative $\delta^{11}B$ values. Foraminifers grown at a pH of 8.6 are shifted towards more positive $\delta^{11}B$ values. The inter-specimen variability in $\delta^{11}B$ spans a range of ~ 7‰ for foraminifers within the same treatment (the standard deviation for one foraminifera ranges from 1.20 to 1.97‰, see Table 1). In the following we address two questions: (1) what causes the offset of $\delta^{11}B$ of foraminifers from the theoretical $\delta^{11}B$ of $B(OH)_4^-$? (2) What are the potential reasons for the observed inter-specimen variability in $\delta^{11}B$?

3.2.1 The offset from the theoretical $\delta^{11}B$

Test size

It has been suggested that the $\delta^{11}B$ of foraminifers is related to its test size. Hönisch & Hemming (2004) report heavier $\delta^{11}B$ by 2.1 to 2.3‰ for individuals of *Globigerinoides sacculifer* in the sieve size class 515 - 865 µm than for shells in the 250 - 380 µm size class. This observation is explained by a reduced photosynthetic activity in smaller specimens at greater depth. A study by Walker (2004) showed a linear increase between size and symbionts in *A.lessonii*. If larger foraminifers accommodate more symbionts, smaller foraminifers experience less symbiotic activity, which might lead to lighter $\delta^{11}B$. However, in our study we do not observe either a correlation between the size of foraminifers and $\delta^{11}B$ or a correlation between growth rate and $\delta^{11}B$ (Fig. 4). In our experiment specimens grew for three months reaching a size between 400 and 900µm. Although we observed different growth rates within each treatment, we do not see a correlation between the test size and the boron isotopic composition. If such an effect really exists in *A.lessonii*, it is very small and not reflected in the boron isotopic composition.

Vital effects

For planktonic foraminifers symbiont activity strongly influences the pH in their micro-environment (Rink et al., 1998; Zeebe et al., 2003) affecting the $\delta^{11}B$ signature of the test. The photosynthetic activity of symbionts consumes CO_2 leading to a pH increase while symbionts'

241 respiration generates CO_2 leading to a pH decrease within the micro environment around the
242 foraminifer. In theory, acidification of the microenvironment due to respiration and calcification
243 would result in lighter $\delta^{11}B$ of the test whereas consumption of CO_2 by photosynthesis leads to heavier
244 $\delta^{11}B$. The net impact of these different processes depends on their respective rates (Zeebe et al., 2003).
245 The effect of photosynthesis on $\delta^{11}B$ in two planktonic species of foraminifers was studied by Hönisch
246 et al. (2003). Based on a comparison between the field grown, symbiont-bearing species *Orbulina*
247 *universa* and the symbiont-barren *Globigerina bulloides* Hönisch and co-workers (2003) observed a
248 lighter $\delta^{11}B$ for *G. bulloides* by 1.4‰. The authors suggest that if photosynthesis and respiration are
249 the major processes causing deviations in foraminiferal $\delta^{11}B$, foraminifers with high symbiont activity
250 (like *O. universa*) should record heavier $\delta^{11}B$ values whereas symbiont-barren foraminifers (like *G.*
251 *bulloides*) should record lighter $\delta^{11}B$ values. In the same study Hönisch et al. (2003) also investigated
252 the impact of symbionts on $\delta^{11}B$ within one species. From culture experiments with *O. universa* (using
253 culture media with a similar B concentration as used in this study) the authors report $\delta^{11}B$ values to be
254 1.5 ‰ heavier under high light than under low light conditions. The impact of photosynthesis on $\delta^{11}B$
255 was also studied by Zeebe et al. (2003) based on a model approach which also includes the data of
256 Hönisch et al. (2003). The diffusion-reaction model of Zeebe et al. (2003) describes changes in the
257 carbonate chemistry and B equilibrium caused by vital effects in the micro-environment of
258 *O.universa*. Based on this model changes in $\delta^{11}B$ due to different symbiont activities (as observed for
259 high light and low light in the culture study of Hönisch et al. (2003)) can be calculated. In general, the
260 calculated changes in $\delta^{11}B$ are in good agreement with the changes observed in the cultured
261 *O.universa*. Furthermore, the model showed that the $\delta^{11}B$ of *O. universa* cultured at high light is
262 heavier than the $\delta^{11}B$ of $B(OH)_4^-$ in the culture media, whereas at low light the opposite is reported.
263 *Amphistegina lessonii* is a symbiont-bearing species. The $\delta^{11}B$ values of the this species are lighter
264 than those of $B(OH)_4^-$, a fact which is seemingly at odds with the conclusions of Hönisch et al. (2003)
265 and Zeebe et al. (2003). In order to shed light on the question whether symbiont activity may explain
266 the lighter $\delta^{11}B$ values in our study (as opposed to *O. universa*) we compare photosynthesis rates
267 (nmol O_2 h^{-1} foraminifer^{-1}) of *O. universa* (Rink et al., 1998) and *A.lessonii* (Walker 2004). Rink et al.
268 (1998) reported a net photosynthesis of 8.72 nmol O_2 h^{-1} for *O.universa* with a shell diameter of 554

μm at 700 μmol photons m^{-2} s^{-1}. The photosynthesis data for *A.lessonii* in the study of Walker (2004) is normalized to the surface area and is ~ 3.5 nmol O_2 s^{-1} mm^2 at 700 μmol photons m^{-2} s^{-1} (Fig. 19 in the study of Walker (2004)). Based on the round shape (sphere) of *O.universa* we first calculated the surface area ($A = 4\pi r^2$) of the sphere using a shell diameter of 554 μm and then normalize the photosynthesis rate to second per mm^2 as performed by Walker (2004). The comparison between the photosynthesis of *O.universa* (32557 nmol O_2 $s^{-1}mm^2$) and *A.lessonni* (3.5 nmol O_2 s^{-1} mm^2) shows that symbiont O_2 production and therefore photosynthesis is lower for *A.lessonii.* Walker (2004) showed that in *A.lessonii* photosynthesis reaches its maximum at 170 μmol photon $m^{-2}s^{-1}$. We used 120 μmol photons $m^{-2}s^{-1}$ which might have led to weak light limitation, further decreasing O_2 production. Thus it is likely that O_2 production in our *A. lessonii* specimens was at least three orders of magnitude lower than in the *O. universa* specimens analysed by Hönisch et al. (2003) and Zeebe et al. (2003). We hypothesize that respiration and calcification (counteracting photosynthesis) are of relative greater importance in *A. lessonii* than in *O. universa*. The latter assumption explains why $\delta^{11}B$ values of *A. lessonii* are closer to symbiont-barren species than the ones of *O. universa*.

In benthic foraminifers without symbionts (*Neogloboquadrina dutertrei, Cibicidoides mundulus, Cibicidoides wuellerstorfi*) studied so far a lighter $\delta^{11}B$ is observed than for planktonic species (Foster, 2008; Rae et al., 2011) due to a lower pH of the growth habitant of benthic foraminifers in deeper waters. Respiration and calcification of benthic foraminifers are the dominant processes leading to an acidification in the micro environment. In support of this inference Glas et al. (2012) showed that the micro-environment pH of the symbiont-barren benthic species *Ammonia spec.* is, during chamber formation, by ca. 0.65 lower than bulk seawater.

The role of $B(OH)_3$

The incorporation of $B(OH)_3$ could modify foraminiferal $\delta^{11}B$ (Klochko et al., 2009). This B species always has a heavier isotopic composition than $B(OH)_4^-$. Therefore, additional incorporation of $B(OH)_3$ would result in heavier $\delta^{11}B$ of the foraminifers. Assuming that $B(OH)_3$ incorporation is positively correlated to $B(OH)_3$ concentration of seawater, the foraminifers from the pH 8.6 treatment

should display the lightest $\delta^{11}B$. Contrariwise, this treatment features the heaviest $\delta^{11}B$. Therefore, incorporation of $B(OH)_3$ appears to be unlikely.

3.2.2 The variability in $\delta^{11}B$

A significant variability in $\delta^{11}B$ between specimens from the same treatment was reported by Rollion-Bard & Erez (2010). These authors described $\Delta\delta^{11}B$ (the difference between the heaviest and lightest $\delta^{11}B$ values) to be pH dependent in *Amphistegina lobifera*. In their study the $\Delta\delta^{11}B$ increased from 4.7‰ for foraminifers cultured at a pH of 8.45 to 12.2‰ for foraminifers cultured at a pH of 7.9. This variability is explained in terms of a calcification mechanism based on sea water vacuolization. It should be noted that the spot size of the analytical method they used to measure the $\delta^{11}B$ of the test (secondary ion mass spectrometry (SIMS)) was ~30 µm. This would require that areas, of at least this size, exist within the test, which are formed from vacuoles of the same pH. The latter is unlikely since the authors suggest themselves that the vacuoles cover a pH range starting at the bulk pH and ending with pH 9. Since in their study only a small portion of the test was grown under experimental conditions, the question arises whether the determined $\Delta\delta^{11}B$ would be the same if the whole test had been grown under experimental conditions. Furthermore, the hypothesis that seawater vacuolization is the only source for calcification in foraminifers is controversially discussed (Nehrke et al., 2013). We calculated $\Delta\delta^{11}B$ from our data as done in the study of Rollion-Bard & Erez (2010). The $\Delta\delta^{11}B$ are 5.82 ‰ (pH_8.1^{160}), 5.26 ‰ (pH_8.1^{260}), 5.21 ‰ (pH_8.1^{540}), 6.17 ‰ (pH_8.1^{640}), 6.4 ‰ (pH_8.6^{640}), and 5.07 ‰ (pH_7.9^{260}). For a change of 0.5 pH unit Rollion-Bard & Erez (2010) report a change in $\delta^{11}B$ by 6.5 ‰ which is clearly not supported by our results. A change of 0.5 pH unit, as shown by the comparison of the 8.1_pH* (average $\Delta\delta^{11}B$) and pH_8.6^{640} treatments exhibits a shift of $\delta^{11}B$ by only 0.79 ‰ and is lower than the error of a single foraminiferal measurement (2RSE, formula S3, supplement). Based on the $\Delta\delta^{11}B$ in our treatments (see above) we do not observe a correlation between $\Delta\delta^{11}B$ and pH in *A. lessonii*.

We discussed above several mechanisms that could cause the offset of $\delta^{11}B$ of *A.lessonii* from the theoretical value expected under the assumption that only $B(OH)_4^-$ is taken up into the test. Even though a combination of these mechanisms could explain the observed offset, they would have to operate with different magnitudes in different specimens (even for specimens from exactly the same treatment) to be in accordance with the observed variability. The latter is very unlikely and therewith no explanation on the observed offset can be given at this point. However, it is interesting to notice that for all experimental conditions the same variability between specimens is observed. Variability between specimens is documented for the uptake of other elements like e.g. Mg. This points towards a mechanism inherent to the biomineralization process itself, which is responsible for the observed variability.

3.3. The B/Ca of *A. lessonii*

The B/Ca data of the foraminiferal tests plotted against pH and $[CO_3^{2-}]$ of the culture media is shown in Figure 5. No correlation between the plotted parameters is observed. In a culture study of Allen et al. (2011) it was shown that the pH of culture media and B/Ca of foraminiferal tests are positively correlated. An increase of pH is associated with changes in the carbonate system: The concentrations of CO_3^{2-} and $B(OH)_4^-$ increase with increasing pH while the concentration of HCO_3^- decreases. Because of these coupled processes it is, in the framework of a classical carbonate system perturbation study like the one of Allen and co-workers (2011), not possible to identify the causal agent. In a second study Allen and co-workers (2012) suggested based on data from a culture study on three different planktonic foraminiferal species using a decoupled carbonate chemistry a "competition between aqueous boron and carbon species for inclusion into the calcite lattice". To further elaborate on this hypothesis we plot our B/Ca data against several possible candidates ($B(OH)_4^-/CO_3^{2-}$, $B(OH)_4^-/HCO_3^-$, and $B(OH)_4^-$/DIC) .The best correlation is given when B/Ca is plotted against $B(OH)_4^-/HCO_3^-$ (Fig.6). This is in good agreement with the data shown in the publication of Allen and co-workers (2012) for cultured *G. sacculifer*, *G.ruber*, and *O. universa*. To summarise: if pH and subsequently $[B(OH)_4^-]$ increase in the culture media, $[HCO_3^-]$ decreases resulting in less competition for $B(OH)_4^-$

347 for uptake into the foraminifer's test. In a natural system the competition between $B(OH)_4^-$ and HCO_3^-
348 support the underlying concept of the B/Ca proxy: the observed linearity of foraminiferal B/Ca and
349 $[CO_3^{2-}]$ can be inferred from the inverse correlated relationship between $[B(OH)_4^-]$ and $[HCO_3^-]$ with
350 increasing pH.

351 *Further observations*

352 At this point we would like to draw the attention of the reader to two interesting observations
353 within our data which cannot be elaborated further within the framework of this study, but that
354 represent an interesting basis for further investigations. 1) Since both parameters ($\delta^{11}B$ and B/Ca) were
355 determined simultaneously, the question arises whether a correlation between both parameters can be
356 identified. As can be seen from Figure 7 no preference for the incorporation of the lighter or heavier B
357 isotope as a function of the B concentration in the tests is observed. 2) It could be observed that the
358 standard deviation for B/Ca does show a significant increase with increasing B incorporation (Fig. 6).

359

360 **4. CONCLUSION**

361 Culture experiments based on a decoupled pH and CO_3^{2-} chemistry indicate that the $\delta^{11}B$ of
362 the test of *A. lessonii* is related to pH whereas the B/Ca of the foraminiferal shells show a positive
363 correlation with $B(OH)_4^-/HCO_3^-$. The latter observation suggests a competition between $B(OH)_4^-$ and
364 HCO_3^- of the culture media for B uptake into the test. The $\delta^{11}B$ values determined on single tests of
365 foraminifers show an offset from the values expected if only $B(OH)_4^-$ is incorporated into the shell and
366 a strong inter-specimen variability is observed. We evaluated potential processes responsible for these
367 observations such as test size, vital effects, and incorporation of $B(OH)_3$. However, we found that none
368 of these processes, or a combination of them, can explain the observed variability in the offset between
369 specimens.

370 The distribution of B in the tests is not homogenous: the variability in B/Ca increases with
371 increasing B/Ca in the tests. Our data shows no correlation between B concentration and isotope
372 fractionation.

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546 **TABLES**

547 **Table 1**

548 Mean values of the B isotopic composition and B/Ca of *A. lessonii*. Errors are expressed as SD (SD =
549 $\sqrt{\frac{\sum(\bar{x}-x)^2}{N}}$ information about N is given in the supplement). Also listed are the calculated isotopic
550 composition of $B(OH)_4^-$ (using eq. 3 and based on a calculated carbonate system using pH and DIC as
551 input parameters) and the offset between the isotopic composition of foraminifers and $B(OH)_4^-$
552 ($\Delta\delta^{11}B$).

Treatments	$\delta^{11}B$ (‰)	± $\delta^{11}B$ (‰)	$\delta^{11}B$ $B(OH)_{4\ cal}^-$ (‰)	$\Delta\delta^{11}B$ (‰)	B/Ca (mmol/mol)	± B/Ca(mmol/mol)
pH_8.1[160]	-32.71	1.27	-29.01	3.70	5.23	1.06
pH_8.1[260]	-31.88	1.20	-28.81	3.07	2.95	0.53
pH_8.1[540]	-31.69	1.20	-28.36	3.32	1.75	0.11
pH_8.1[640]	-32.45	1.43	-28.59	3.86	1.58	0.12
pH_8.6[640]	-23.65	1.97	-22.75	0.90	6.36	1.30
pH_7.9[260]	-35.59	1.22	-31.34	4.25	1.20	0.08

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556 **FIGURE CAPTIONS**

557 **Figure 1**

558 **(a)** Concentration of $B(OH)_3$ and $B(OH)_4^-$ in seawater. (**b)** Isotopic composition of $B(OH)_3$,
559 $B(OH)_4^-$, and B in seawater. Graphs are plotted for T = 20°C, S = 35, P = 380 µatm, [B] = 4.16
560 mmol/kg, pK_B= 8.5, $\alpha_{(B(OH)3\text{-}B(OH)4\text{-})}$ =1.0272.

561 **Figure 2**

562 **(a)** Boron isotopic composition versus pH of the culture media for all treatments. $\delta^{11}B$ data
563 represent mean values obtained from single measurements within one treatment. Error bars for
564 $\delta^{11}B$ represent SD. **(b)** Calculated carbonate ion concentration versus pH.

565 **Figure 3**

566 Difference between measured $\delta^{11}B$ in foraminifers and calculated $\delta^{11}B$ of $B(OH)_4^-$ (y-axis)
567 plotted against measured foraminiferal $\delta^{11}B$. The solid black line represents the B isotopic
568 composition of $B(OH)_4^-$. Error bars of single $\delta^{11}B$ values represent 2RSE and were calculated
569 according to eq. S3. The $\delta^{11}B$ of $B(OH)_4^-$ was calculated by (Zeebe & Wolf – Gladrow, 2001):

$$\delta^{11}B_{B(OH)_4^-} = \frac{\delta^{11}B_{CM} \times [B_{CM}] - \varepsilon_B \times [B(OH)_3]}{[B(OH)_4^-] + \alpha_B \times [B(OH)_3]}$$

570 Where $\delta^{11}B_{CM}$ and $[B_{CM}]$ are the $\delta^{11}B$ and B concentration of the culture media, α_B is the B
571 isotope fractionation factor between $B(OH)_3$ and $B(OH)_4^-$ (α_B = 1,0272 (Klochko et al.,
572 2006)), and $\varepsilon = (\alpha - 1) \times 1000$. In order to calculate $\Delta\delta^{11}B$ the isotopic difference between
573 NIST 610 (reference material to determine $\delta^{11}B_{foram}$) and SRM 951 (reference material to
574 determine $\delta^{11}B_{CM}$) has to be taken into account. As shown by several studies (Kasemann et al.,
575 2001; le Roux et al., 2004; Fietzke et al., 2010) both standards are within analytical
576 uncertainty isotopically equal.

577 **Figure 4**

578 Size and growth rate (defined as final size divided by the number of days in culture) versus B
579 isotopic compositions of foraminifers. If a specimen was measured several times the mean
580 $\delta^{11}B$ is presented here. Error bars of single $\delta^{11}B$ values represent 2RSE and were calculated
581 according to eq. S3.

582 **Figure 5**

583 **(a)** B/Ca plotted against pH of culture media and **(b)** B/Ca plotted against $[CO_3^{2-}]$ of culture
584 media. Both graphs show no correlation neither with pH nor with $[CO_3^{2-}]$. B/Ca data
585 represents mean values of all measurements of foraminifers. Error bars are expressed as SD.

586 **Figure 6**

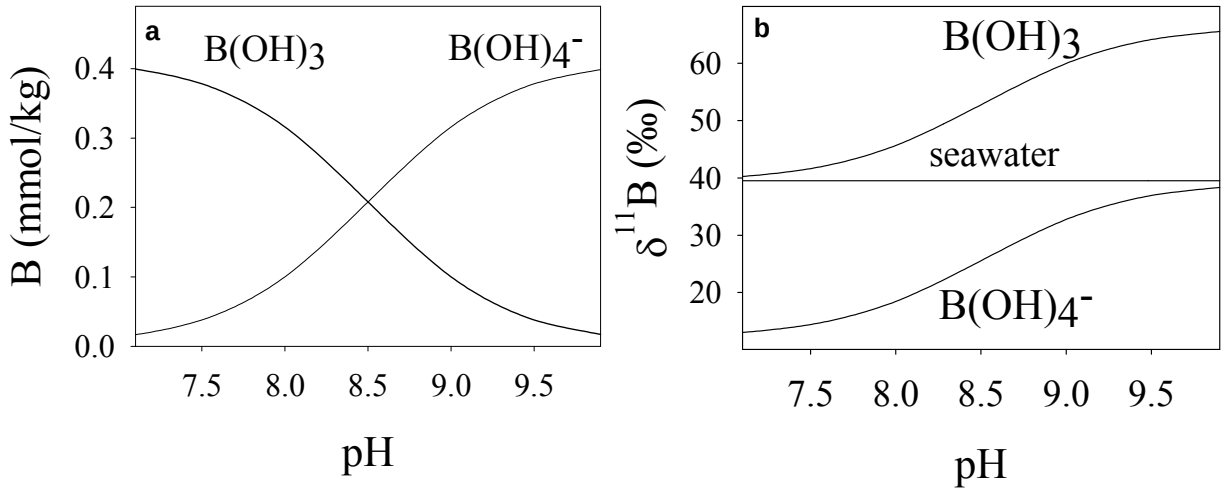
587 B/Ca plotted against $B(OH)_4^-/CO_3^{2-}$, $B(OH)_4^-/HCO_3^-$, and $B(OH)_4^-$/DIC. The best linear
588 regression is given when B/Ca is plotted against $B(OH)_4^-/HCO_3^-$. B/Ca data represents mean
589 values of all measurements. Error bars are expressed as SD.

590 **Figure 7**

Single B/Ca values plotted against single $\delta^{11}B$ values**.** No correlation exits between the plotted parameters.

FIGURES

Figure 1



606 **Figure 2**

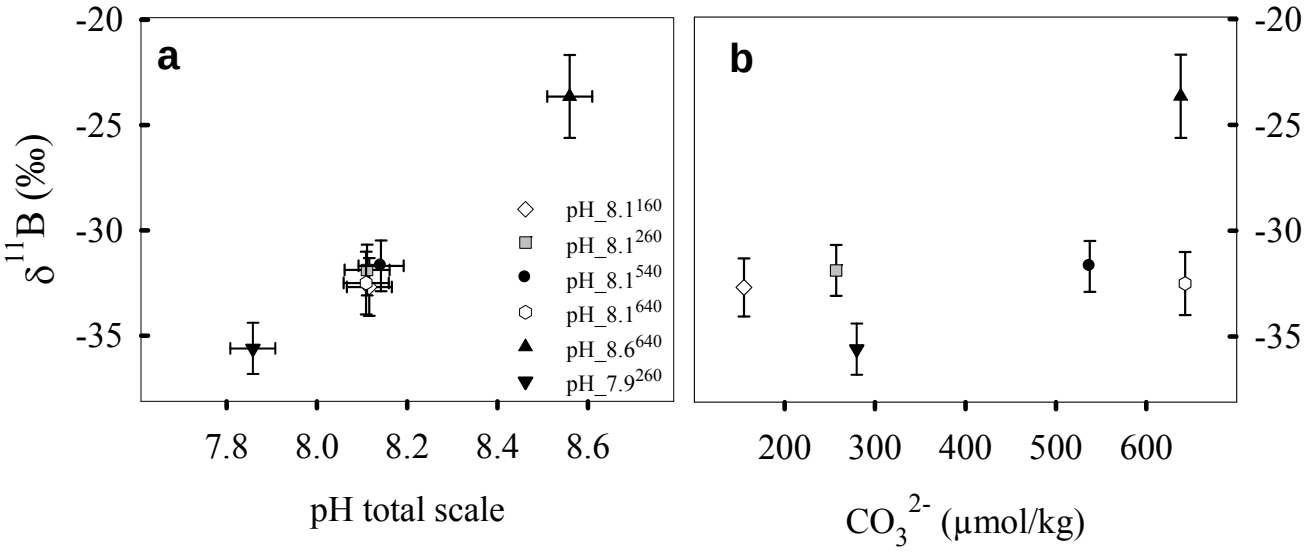


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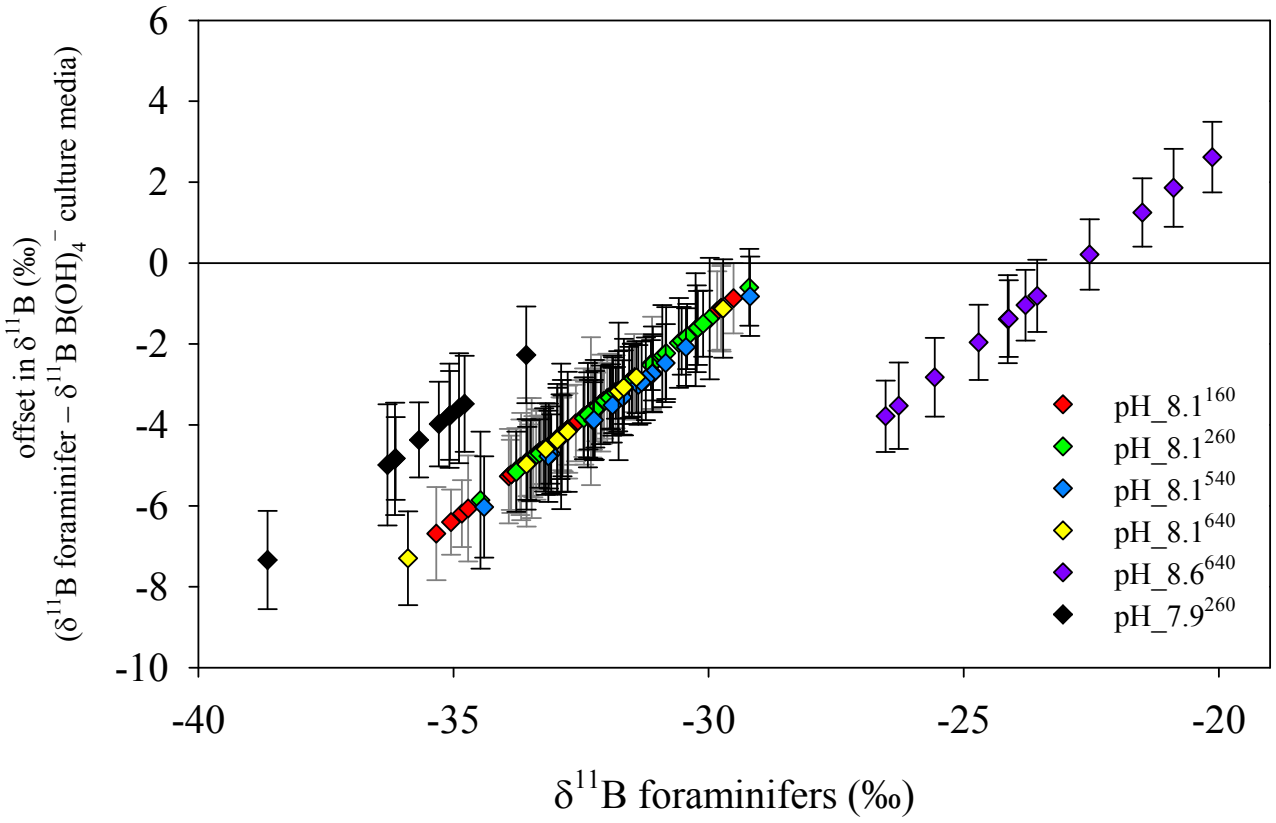
Figure 3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-2
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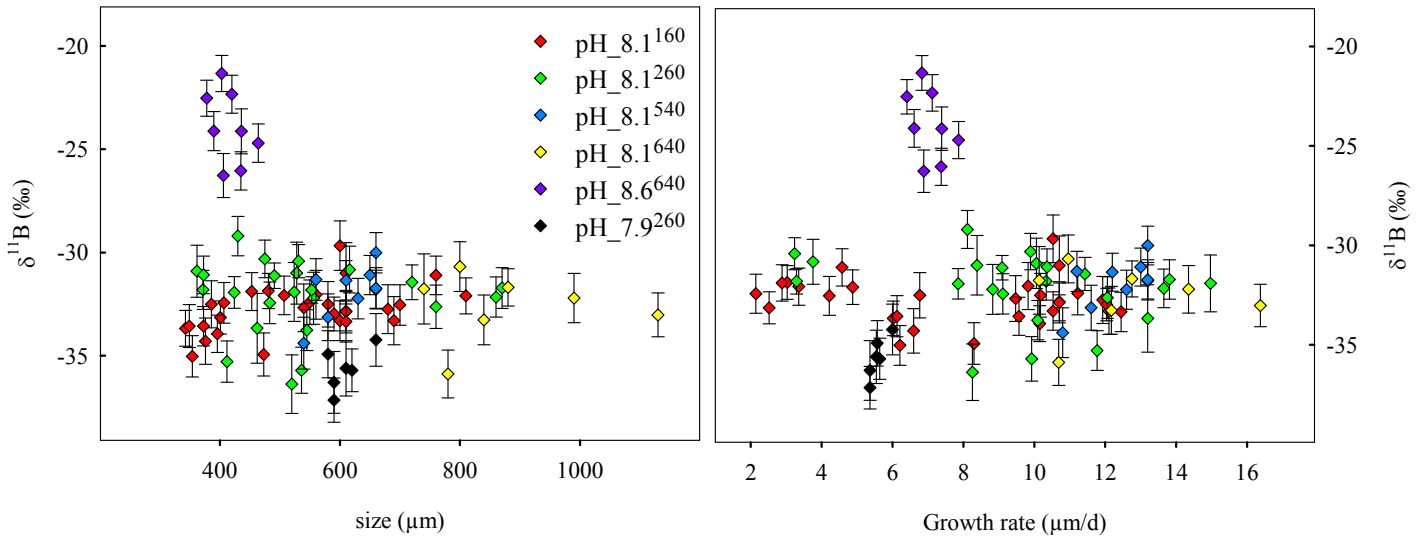
offset in δ11B (‰)
(δ11B foraminifer – δ11B B(OH)4− culture media)
δ11B foraminifers (‰)
pH_8.1160
pH_8.1260
pH_8.1540
pH_8.1640
pH_8.6640
pH_7.9260

Figure 4


pH_8.1160
pH_8.1260
pH_8.1540
pH_8.1640
pH_8.6640
pH_7.9260
δ11B (‰)
-20
-25
-30
-35
400
600
800
1000
size (µm)
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4
6
8
10
12
14
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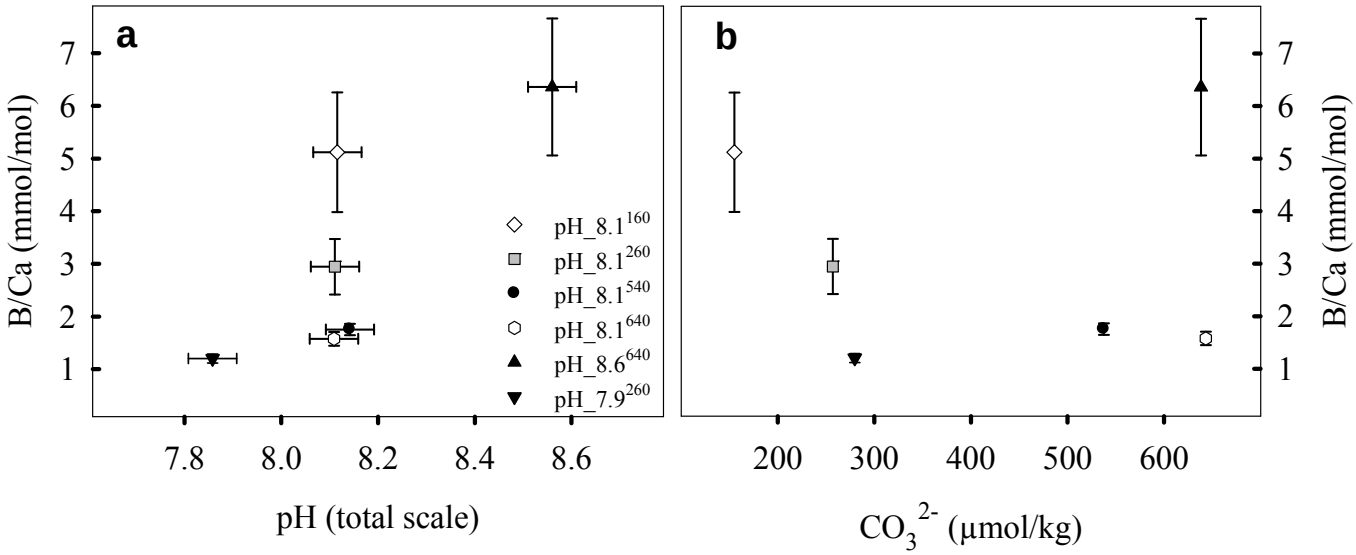
Growth rate (µm/d)

649 **Figure 5**



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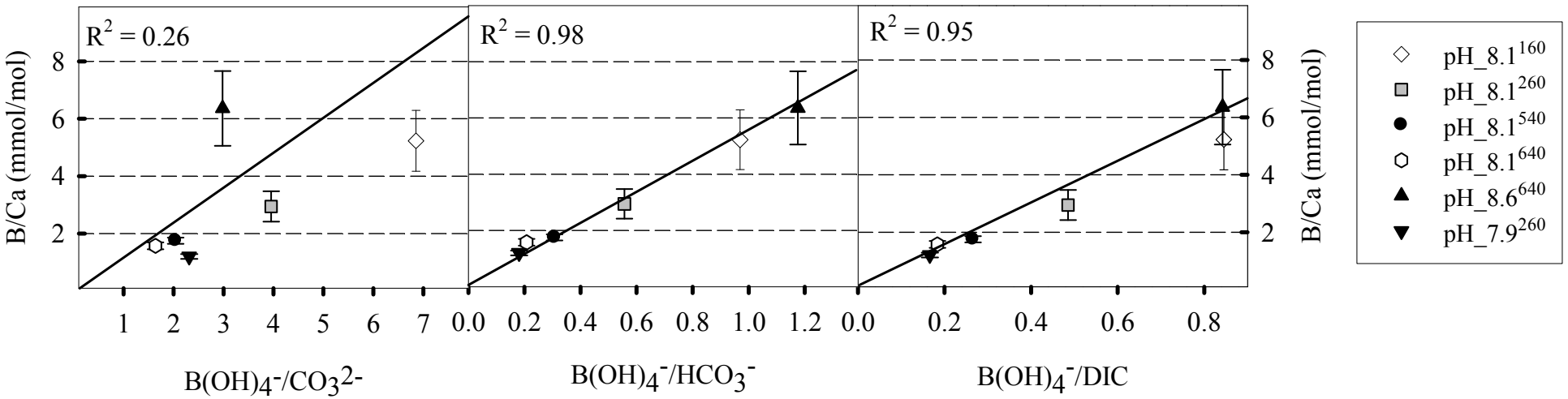
662 **Figure 6**



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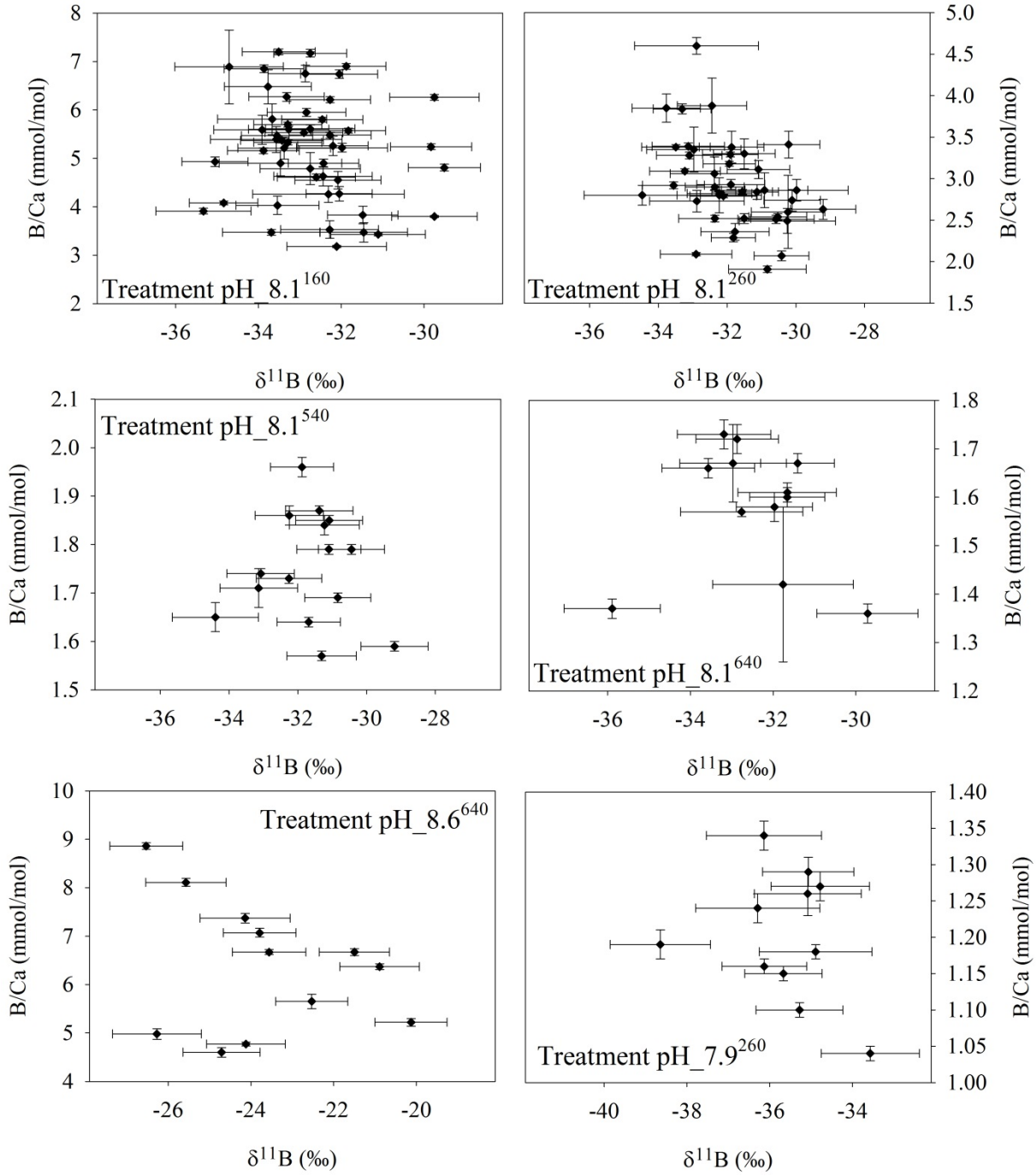
665 **Figure 7**



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