



# Partitioning and Chemical Environments of Minor Elements in Individual Large Benthic Foraminifera Cultured in Temperature-Controlled Tanks

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Because reef-dwelling large benthic foraminifera (LBF) tests, which is composed of high-magnesium calcite, are the common components of modern reef sediments, and ancient reef carbonate rocks, they can provide a continuous record of the paleoenvironment of reef sediments. Evaluation of the responses of the minor element concentrations (Mg, Sr, Na, P, and S) of individual tests to ambient temperature and LBF growth modes was conducted with two cultured species, *Calcarina gaudichaudii* and *Amphisorus kudakajimensis*. The elements were assessed as tracers of temperature, salinity, nutrient concentration, and calcification rate. The Mg content of *C. gaudichaudii* and the Sr content of both species were controlled by temperature. It can be confirmed that the Mg of individual tests of *C. gaudichaudii* is a useful paleothermometer. There was a large biological modulation of Na, P, and S incorporation. *In situ* XANES analysis revealed that both inorganic and organic species contributed to the P and S content of LBF tests. We observed five sulfur components with variable oxidation states.

**Keywords:** large benthic foraminifera, proxy, element partitioning, culture experiment, chemical speciation, P and S K-edge XANES

## INTRODUCTION

The reconstruction of paleoenvironmental conditions is fundamental to understanding the mechanisms of climate change. In tropical and sub-tropical oceans, long-lived clacifiers such as massive corals and giant clams are geologically important producers of biominerals that provide high-resolution records of environmental conditions for up to hundreds of years (e.g., Gagan et al., 2000). Reef-dwelling large benthic foraminifera (LBF, >1 mm in diameter) account for 10% of the inorganic carbon production on seaward reef flats and in protected lagoonal communities (Fujita and Fujimura, 2008). Because LBF tests are common components of modern reef sediments and ancient reef carbonate rocks, they can provide a continuous record of paleoenvironmental changes in reef sediments, such as the Quaternary coral-reef sequences recovered by the Integrated Ocean Drilling Program Expedition 310 and 325 (Fujita et al., 2010; Yokoyama et al., 2011).

Environmental proxies of high-Mg calcite LBF tests also have the potential to provide continuous paleoceanographic records during climatically important periods such as the Paleogene (Evans et al., 2013).

The Mg/Ca ratio of foraminifera has been used extensively as a reliable seawater temperature proxy for reconstruction of paleoenvironments (Nürnberg et al., 1996; Toyofuku et al., 2000). Temperature-dependent Mg incorporation is clearly apparent in cultured LBFs (Evans et al., 2013; Maeda et al., 2017), but not in LBFs collected from actual reef beds (Raja et al., 2007).

The Sr/Ca ratio has been a tool for investigating Sr and Ca inventories in paleoceanographic studies (Elderfield et al., 2000). Little is presently known about Sr incorporation into LBFs, and previous studies reported that the Sr/Ca ratios of LBFs are not related to water temperature (Raja et al., 2005, 2007; Maeda et al., 2017). Because salinity is a major component in climate dynamics, reconstruction of past salinity variations in the surface waters have been investigated in the use of Mg/Ca or Sr/Ca paleothermometry with oxygen isotope measurements (Elderfield and Ganssen, 2000; Felis et al., 2009). Recently, it was found that skeletal Na/Ca ratio in low-Mg calcite tests of planktonic and benthic foraminifera is a function of ambient water salinity (Wit et al., 2013; Mezger et al., 2016; Hauzer et al., 2018). On a molar basis, Na is among the most abundant of minor elements in biogenic CaCO<sub>3</sub> (Yoshimura et al., 2017). The incorporation of Na into biogenic carbonates is complicated by differences in biogenic osmoregulation and salinity sensitivity (Delaney et al., 1985; Rosenthal and Katz, 1989), thus potential influences of temperature and growth changes and calcification processes on Na incorporation in LBF tests need to be verified.

There has been less examination of anion than cation incorporation into biogenic CaCO<sub>3</sub>. Phosphorus plays a central role in ocean fertility. Coral-based paleoenvironmental reconstructions have been linked to past changes in reef nutrient cycling caused by changes in river discharge, upwelling activity, and ocean circulation patterns (McCulloch et al., 2003). Recently, a link has been proposed between ocean phosphorus cycling and P/Ca ratios in reef-building and cold-water coral genera (Montagna et al., 2006; LaVigne et al., 2010; Anagnostou et al., 2011). Sulfur is an important component of organic compounds, and S concentrations in biogenic carbonates have been proposed as indicators of skeletal organic materials (Cuif et al., 2008) and structurally substituted sulfate (SSS) in biogenic CaCO<sub>3</sub> (Yoshimura et al., 2013; Tamenori et al., 2014). To date, little is known about S and P speciation in foraminifera tests. Because both elements are biological constituents, one key to understanding modes of element incorporation is *in situ* chemical speciation or chemical mapping (Cuif et al., 2008; Tamenori et al., 2014). Recent advances in synchrotron radiation (SR) X-ray spectroscopy, including improvements in signal stability and sensitivity, have facilitated exploring the chemical environments of minor elements in various materials. This study is the first investigation of the incorporation of phosphorus and sulfur into LBFs.

Culture experiments of foraminifera under controlled laboratory conditions provide a fundamental understanding

of element partitioning, isotope fractionation, and proxy development. For this study, we investigated the incorporation of the elements Mg, Sr, Na, S, and P in cultures of typical coral reef LBFs under four temperature conditions. We used high-sensitivity SR-XRF and XANES measurements to assess the influences of temperature and temperature-related growth changes on the incorporation of minor elements into individual tests. By revealing the dominant S- and P-bearing phases in biogenic CaCO<sub>3</sub>, XANES analyses can refine our understanding of the chemical environments of anions and thereby improve our ability to reconstruct paleoenvironmental changes at Earth's surface.

## MATERIALS AND METHODS

### Culture Experiments

We selected two species of algal symbiont-bearing LBFs, *Amphisorus kudakajimensis* and *Calcarina gaudichaudii*, which are common in northwestern Pacific coral reefs. *A. kudakajimensis* has porcelaneous imperforate test walls, is host to dinoflagellate endosymbionts, and attached to macroalgae in shallow reef areas. *C. gaudichaudii* precipitates hyaline perforate tests with diatom symbionts. Living adult individuals were collected from the near-shore reef flat in Bise, Okinawa Island on May 3, 2015 and cultured in a University of Ryukyus laboratory until asexual reproduction began. Soon afterward, asexually reproduced individuals were separated and maintained in glass vials with filtered seawater at ~21°C before culture experiments. We conducted culture experiments with *A. kudakajimensis* and *C. gaudichaudii* at the temperatures at 21–30°C (Table 1), and this temperature range is approximately in accordance with that observed around Okinawa Island, which is 21°C–29°C on average from 1993 to 2015 (referenced from the 11th regional Japan coast guard headquarters). The LBFs were maintained in glass vials in water baths at each culture settings within ±0.5°C of the target temperature. The mean daytime photon flux density was ~22 μmol m<sup>-2</sup> s<sup>-1</sup> on a 12 h:12 h light:dark cycle. Seawater in the glass vials was changed every 3 weeks. The details of the culture experiment are shown in Maeda et al. (2017).

The weights and lengths of *C. gaudichaudii* and *A. kudakajimensis* were measured for each individual test. The dry test weight of all specimens was measured 56 days after culturing began with a Cahn-33 microbalance which has the readability and reproducibility to 1 μg. We measured the major axis of the elliptical tests of *A. kudakajimensis*. Because the fragile spines of *C. gaudichaudii* were prone to breakage, we measured the radius of the median plane of *C. gaudichaudii*. It is difficult to estimate the precipitation rate of foraminifera due to those complicated forms, we used test length and weight for precipitation rate.

### Micro-X-Ray Fluorescence and X-Ray Absorption Near-Edge Structure

Whole tests were embedded in epoxy resin under vacuum and ground to expose a cross section across the test wall. The sections parallel to the long axis of the test were

**TABLE 1** | Element concentrations in cultured LBFs.

| Sample ID   | Temp. °C | Weight $\mu\text{g}$ | Length $\mu\text{m}$ | n   | Mg ppm $\pm 2$ SE | Sr ppm $\pm 2$ SE | Na ppm $\pm 2$ SE | P ppm $\pm 2$ SE | S ppm $\pm 2$ SE |
|-------------|----------|----------------------|----------------------|-----|-------------------|-------------------|-------------------|------------------|------------------|
| CAL2-21A-1  | 21.2     | 32                   | 174                  | 114 | 30980 $\pm$ 920   | 1110 $\pm$ 100    | 3302 $\pm$ 105    | 1.80 $\pm$ 0.25  | 1170 $\pm$ 229   |
| CAL1-25B-7  | 25.2     | 41                   | 174                  | 131 | 31940 $\pm$ 960   | 960 $\pm$ 50      | 3400 $\pm$ 103    | 1.57 $\pm$ 0.26  | 1148 $\pm$ 194   |
| CAL2-29A-13 | 29.1     | 55                   | 198                  | 126 | 34670 $\pm$ 900   | 900 $\pm$ 30      | 3303 $\pm$ 104    | 1.55 $\pm$ 0.13  | 1282 $\pm$ 142   |
| CAL4-30B-10 | 30.3     | 42                   | 194                  | 140 | 35140 $\pm$ 920   | 890 $\pm$ 40      | 3387 $\pm$ 109    | 1.61 $\pm$ 0.29  | 1270 $\pm$ 144   |
| AMP21-4     | 21.2     | 100                  | 1365                 | 263 | 29110 $\pm$ 760   | 1210 $\pm$ 60     | 1738 $\pm$ 84     | 12.87 $\pm$ 3.15 | 2264 $\pm$ 79    |
| AMP25-12    | 25.2     | 96                   | 1228                 | 522 | 35670 $\pm$ 550   | 980 $\pm$ 100     | 1691 $\pm$ 38     | 7.02 $\pm$ 0.92  | 2132 $\pm$ 35    |
| AMP29-6     | 29.1     | 94                   | 1201                 | 480 | 33590 $\pm$ 600   | 760 $\pm$ 40      | 1598 $\pm$ 44     | 7.48 $\pm$ 0.85  | 2475 $\pm$ 85    |
| AMP30-3     | 30.3     | 94                   | 1246                 | 272 | 32290 $\pm$ 750   | 750 $\pm$ 40      | 1630 $\pm$ 62     | 10.74 $\pm$ 1.83 | 2180 $\pm$ 60    |

The sample ID of CAL represents *Calcarina gaudichaudii*, and AMP represents *Amphisorus kudakajimensis*. n, total number of  $\mu$ -XRF data points used for calculation.

then polished. The  $\mu$ -XRF and  $\mu$ -XANES measurements were performed at the b-branch of the soft X-ray photochemistry beamline (BL27SU) at the SPring-8 synchrotron radiation facility. A double crystal Si(111) monochromator ensured an energy resolution of 0.35 eV. A silicon drift detector was used to obtain  $\mu$ -XRF mapping data for each element (Tamenori et al., 2011). The samples were fixed on an aluminum sample holder and installed in a vacuum chamber and fixed on a motorized XYZ stage. We obtained full XRF data at each mapping point and extracted the elemental concentrations in the tests from the XRF datasets. The  $\mu$ -XRF measurements were taken at photon energies of 3000.0 eV and with acquisition times of 1 s at 12 and 24  $\mu\text{m}$  intervals for the horizontal and vertical axis, respectively. For both species Mg concentrations, which is most sensitive to temperature changes, are variable at the innermost part in the tests (**Supplementary Figures S1, S2**). There parts are probably corresponds to juvenile tests, thus these measurement points were excluded from calculation. Other parts did not show compositional discontinuity, suggesting most of the data are average of the culture periods.

## RESULTS AND DISCUSSION

### Magnesium Thermometer

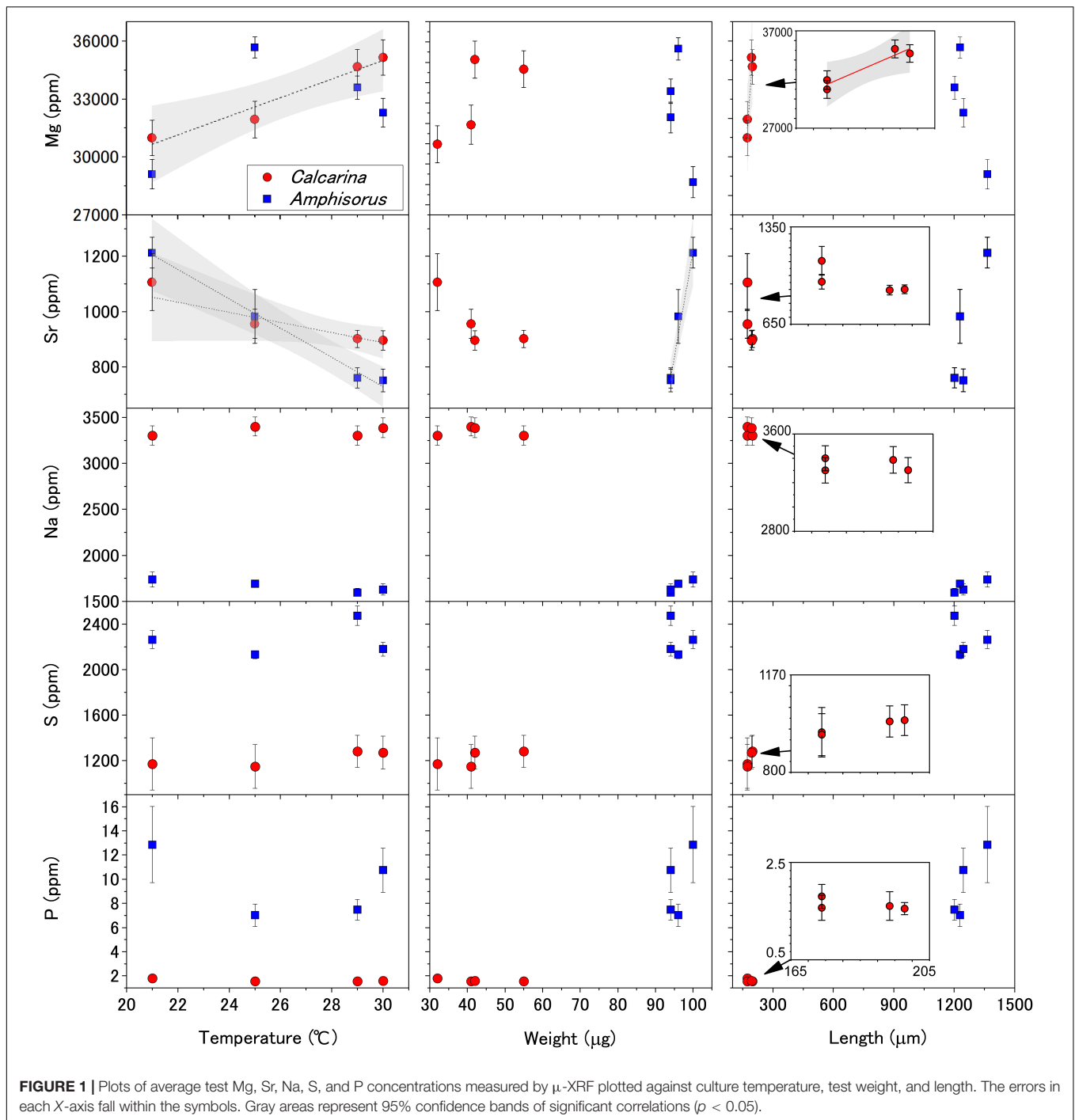
**Table 1** lists the LBF test element concentrations, and **Figure 1** plots them in relation to culture temperature, growth weight, and length. For *C. gaudichaudii*, the test Mg concentration clearly increased with culture temperature from 30,980  $\pm$  920 to 35,140  $\pm$  920 ppm (**Figure 1** and **Table 1**). The result supports the use of Mg/Ca ratios of individual tests of this species as a robust proxy for water temperature. Although Mg concentrations of both species fell within similar concentration ranges, those of *A. kudakajimensis* did not show a linear relationship with temperature; at 25°C, the Mg concentration of AMP-25-12 was an outlier. Mg concentration in *C. gaudichaudii* increased with length but not with test weight, and neither test weight nor length show significant difference with Mg concentrations in *A. kudakajimensis* (**Figure 1**). Growth factors do not affect higher Mg of AMP-25-12. The degree to which precipitation rate affects the partitioning of Mg is suggested to be related to solution chemistry, and abiogenic

calcite precipitated from solutions with high Mg/Ca ratios (>3 mol/mol) and high saturation states is insensitive to precipitation rate (Saenger and Wang, 2014). The response of LBF growth rates to temperature varied between species, but Mg/Ca ratios of all culture species have the linear relation with temperature between 21°C and 30°C (Maeda et al., 2017, 2018). The lack of a consistent pattern suggests that growth rates had only a minor effect on the Mg partitioning. The 1.9  $\pm$  0.3%/°C (95% confidence interval) increase in Mg/Ca of *C. gaudichaudii* (Maeda et al., 2017) is comparable to the previously reported values of the 1.9  $\pm$  0.3%/°C in *Operculina ammonoides* (Evans et al., 2015). However, there is a resolvable difference in the temperature sensitivity between LBF species (3.1  $\pm$  0.5%/°C for *A. kudakajimensis*, Raja et al., 2005). Therefore species-specific calibration should be needed in paleoceanographic studies.

The Mg/Ca ratios of benthic foraminifers with high-Mg calcite tests, *Planoglabratella opercularis* and *Quinqueloculina yabei* (Toyofuku et al., 2000), *O. ammonoides* (Evans et al., 2015), and *Neorotalia calcar*, *Baculogypsina sphaerulata* (Maeda et al., 2018) were also linearly related to temperature. The Mg/Ca ratios of two LBFs grown in the same culture batch as the samples used in this study were positively correlated with temperature (Maeda et al., 2017). Aside from this general trend, individual differences occurred in *A. kudakajimensis*. For conventional ICP-AES or ICP-MS analysis, each data comes from analyses of multiple tests with intensive cleaning. Conversely, our  $\mu$ -XRF samples were embedded in epoxy resin following mild cleaning via brief ultrasonification in ultrapure water and a methanol rinse. Although the analytical protocols differed from previous studies, the present results suggest that the Mg/Ca ratios of the perforate species *C. gaudichaudii* are more robust proxies for water temperature than the Mg-thermometer of *A. kudakajimensis*.

### Proxies for Seawater Chemistry: Strontium and Sodium

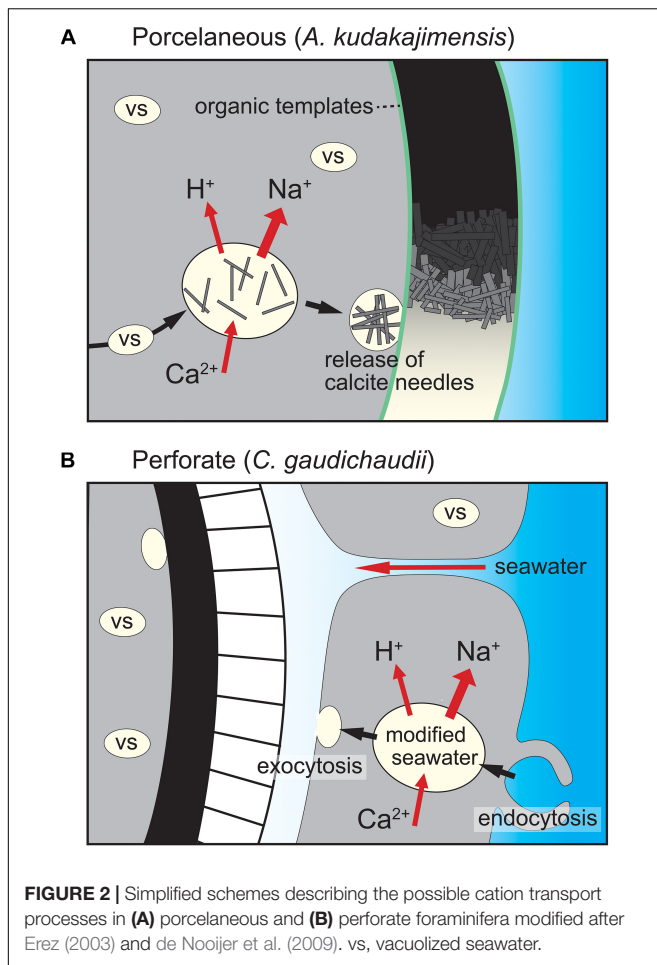
The Sr/Ca in LBFs ranged from 1.8 to 2.4 mmol/mol (Maeda et al., 2017), which is higher than that of planktonic foraminiferal low-Mg calcite ( $\sim$ 1.4 mmol/mol<sup>-1</sup>, Elderfield et al., 2000; Cl  roux et al., 2008). Foraminiferal calcite is a potential recorder of past seawater Sr/Ca ratios, but core-top and culture calibrations suggest that factors such as growth temperature and sensitivity to dissolution affect foraminiferal



Sr/Ca ratios to different degrees (Elderfield et al., 2000). Although the Sr/Ca ratios in *C. gaudichaudii*, *A. kudakajimensis*, and three other species living on coral reefs are poorly correlated with temperature (Raja et al., 2007), the Sr concentrations of both cultured LBF species was negatively correlated with temperature. Strontium distribution coefficient in abiogenic calcite precipitation experiments increases with increasing calcite precipitation rate (Lorenz, 1981). Neither growth weight nor length was correlated with Sr content of *C. gaudichaudii*,

but more Sr incorporated into *A. kudakajimensis* as more rapid growth at lower temperature (Figure 1). It is possible that temperature is likely to be the predominant control on Sr concentrations in the cultured samples which may be also affected by shell growth rates in a particular species. A previous study of two *Globorotalia* species, *G. inflata* and *G. truncatulinoides*, from the North Atlantic has revealed a positive correlation between sea surface temperature and Sr/Ca ratios, which ranged from  $\sim 1.2$  to  $\sim 1.5$  mmol mol $^{-1}$ , but





*G. ruber* and *G. bulloides*, and *P. obliquiloculata* do not show any temperature dependence (Cl eroux et al., 2008). Although interspecific differences in Sr concentration are small, we would expect that Sr incorporation in natural foraminifera tests is affected by other processes, including growth rate and water chemistry (Cl eroux et al., 2008).

Because salinity was constant during our experiment, the question we addressed was whether temperature and growth rate control Na concentration. The effect of temperature needs to be solved when applying Na/Ca salinometer (Mezger et al., 2016). The concentrations of Na show considerable interspecific differences. Na concentrations of both species were unaffected by temperature changes. Although test growth was highly variable between individuals, the changes did not appear to be systematically related to changes of Na content in either species (Figure 1). The Na/Ca ratio of benthic and planktonic tests has been proposed as a proxy for ambient salinity (Wit et al., 2013; Mezger et al., 2016), but Na/Ca ratios in cultured planktonic foraminiferal calcite tests do not vary while the Na/Ca ratio of the culture solution changes ~2.5-fold at a constant Ca concentration (Delaney et al., 1985). The results showed a large biological modulation of Na incorporation into foraminifera tests. Recent

culture experiment by Hauzer et al. (2018) demonstrated that the sensitivity of Na/Ca ratios of *O. ammonoides* to changes in Ca<sup>2+</sup> concentrations is greater than salinity control. This fact is very important for understanding Na partitioning if LBFs modify chemical compositions at the site of crystal formation by active transport of Ca<sup>2+</sup>. Although the type of Ca<sup>2+</sup>-transporters that are used by foraminifera are still unknown, Ca<sup>2+</sup>-ATPases, H<sup>+</sup>/Ca<sup>2+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> antiporters might be responsible for test calcification (de Nooijer et al., 2014).

*Amphisorus kudakajimensis* and *C. gaudichaudii* belong to the respective orders miliolida and rotaliida. These orders have different test structures (porcelaneous and perforate, respectively) which are precipitated via different calcification mechanisms (Figure 2). *A. kudakajimensis* secretes porcelaneous imperforate tests. Porcelaneous species precipitate needle-shaped micro crystals within cytoplasmic vesicles, then the high-Mg calcite crystals are released to the organic matrices to form a chamber wall (Figure 2A; Erez, 2003; de Nooijer et al., 2009). *C. gaudichaudii* precipitates hyaline perforate tests. In their calcification, seawater vacuoles are formed by endocytosis, and modified seawater vacuoles are then exocytosed into a delimited biomineralization space and mixed with seawater which is directly transported to the space (Figure 2B; Erez, 2003; de Nooijer et al., 2009). The amount of vesicles at the site of calcification of perforate foraminifera *Ammonia beccarii* increases during calcification, thus plays a key role in transporting substances for calcification (Nagai et al., 2018). Seawater vacuoles serve as the main ion source not only for the calcification process but also for metabolic processes. Therefore, active uptake of Na<sup>+</sup> from seawater vacuoles for cellular processes lowers its concentrations in the calcifying solution. Another possible explanation is the selective Na removal by Na<sup>+</sup>/Ca<sup>2+</sup> antiporter. From these point of views, the lower Na contents of *A. kudakajimensis* reflects lowered Na/Ca ratios in the vacuoles. On the other hand, the higher Na contents of *C. gaudichaudii* are considered to be affected by the mixing of exocytosed "modified" seawater vacuoles with directly transported seawater (Figure 2B). Therefore, it is proposed that the Na contents is a function of relative strength of cellular Na<sup>+</sup> transport versus mass fluxes of seawater vacuolization, which is related to calcification mechanisms of foraminifera groups.

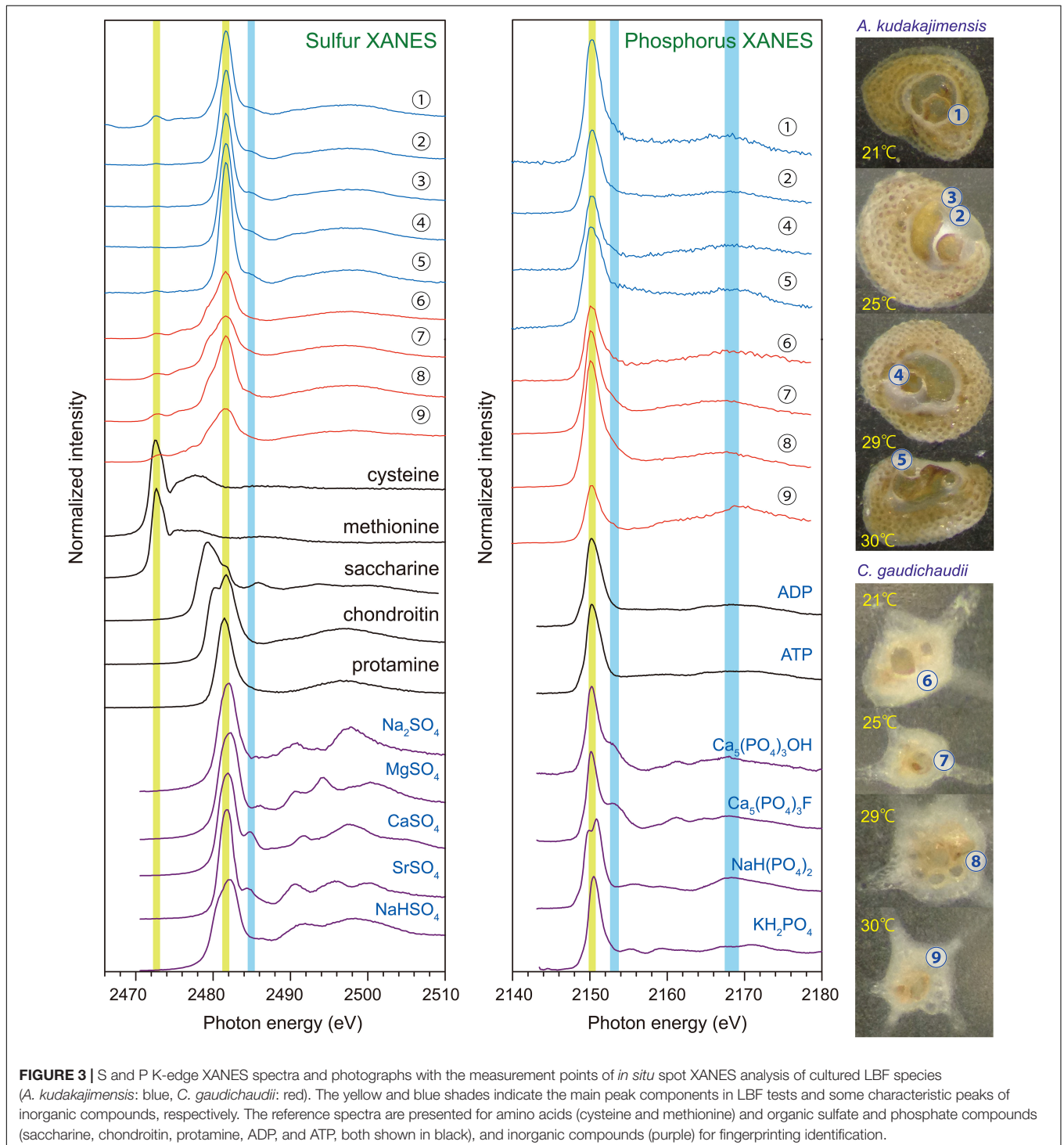
## Anion Incorporation: Sulfur and Phosphorus

The XANES measurements clarified that both inorganic and organic sulfur coexist in LBF tests. The main peaks of the S K-edge XANES spectra undergo large shifts toward higher energies with increasing oxidation state, from 2473 eV for amino acids to 2482 eV for sulfate (yellow line in Figure 3). The spectrum of protamine sulfate, a model organic sulfate compound, showed a single broad hump at ~2498 eV, ranging from 2490 to 2510 eV, in the post-edge region, similar to the LBF spectra of 6, 7, and 9 (Figure 3). In contrast, post-edge peaks in the LBF spectra could be confirmed on the high-energy

side of the main peak at  $\sim 2484$  eV in the inorganic carbonate (Perrin et al., 2017) and  $\text{CaSO}_4$  spectrum, an indication that inorganic sulfate was also present in the LBF tests (blue line in **Figure 3**). The present results indicate mixed S speciation, with dominant contributions from both inorganic and organic sulfate and a minor contribution from reduced sulfur and amino acids (**Figure 3**). A change in relative peak heights of

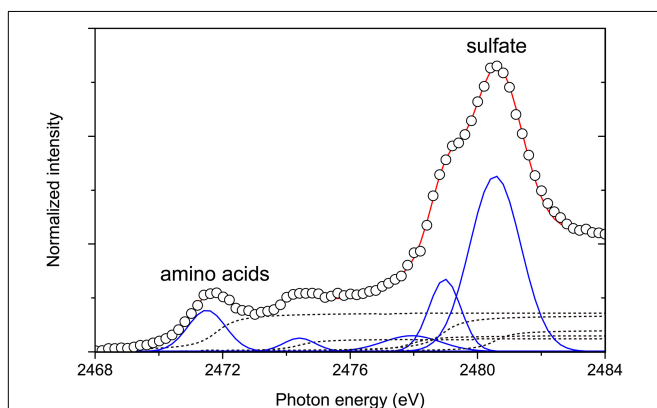
the inorganic sulfate was observed between *A. kudakajimensis* and *C. gaudichaudii*, and inorganic sulfate were evident in *A. kudakajimensis* tests (spectra #1–5 in **Figure 3**).

Similar relationships were apparent in the P-XANES. The spectral shapes of most LBF samples were very similar, with a broad peak at  $\sim 2169$  eV. Both the shape and the peak are distinguishing features of the organic reference compounds ADP



and ATP. Overall, phosphorus is predominantly present as an organic phosphate. Changes in the positions of the post-edge peaks were apparent for P-XANES spectrum #9, and there was a weak shoulder peak on the high-energy side of the main peak in some LBFs and also in inorganic phosphate standards (Figure 3).

In biogenic carbonates, sulfur is present as a major component of S-containing amino acids and sulfated sugars (Dauphin et al., 2013). For example, *in situ* analysis has revealed heterogeneous S distribution pattern in mollusk shells; intraprismatic organic matrices and outer membranes are enriched in organic sulfates, whereas S-bearing amino acids are present in low concentrations (Dauphin et al., 2003a,b). Cuif et al. (2008) have revealed sulfate-rich bands in the wall of *Globorotalia* sp., which is interpreted predominantly as sulfated polysaccharides. However, a low signal-to-noise ratio in the XANES analyses of *Globorotalia* sp. seems difficult to address the conclusive evidence of whether the average local structural and compositional environment of sulfate are accompanied only by variations in sulfated polysaccharides. On the basis of the spectral profiles confirmed by *in situ* XANES, we concluded that the sulfur speciation in the LBF tests is more complex and the sulfur is present in oxidized to reduced forms. The main peaks of the sulfur compounds show large shifts toward higher energies with an increasing oxidation state. From the observations on mixed S-species in LBFs, an example of deconvolution of the spectrum of S  $\mu$ -XANES measurement point #8 is shown in Figure 4. The experimental XANES spectrum was fitted using Gaussian fitting. We observed five peaks at 2471.8, 2474.4, 2477.9, 2479.0, and 2480.6 eV in the near-edge region of the S *K*-edge spectrum. These correspond to three sulfur components with intermediate oxidation states between the most reduced and oxidized sulfur (Figure 4). This spectral feature is similar to insoluble conchiolin sheets of a freshwater mussel *Margaritifera laevis* (Tamenori and Yoshimura, 2018). Note that some characteristic peaks were evident at pre- post-edge shoulders and in the post-edge region of each S compound



**FIGURE 4** | Peak deconvolution of the near-edge region of the sulfur XANES spectrum obtained at the measurement point-8 of Figure 3. The open circles and red line represent measured data and the best-fit line. The blue and dashed lines represent fitted Gaussian peaks and arctangent functions that represent the transition of ejected photoelectrons, respectively.

(Yoshimura et al., 2013), thus the deconvoluted peak areas do not simply reflect relative abundance of compounds of different oxidation states.

There was a clear, inter-species difference in anion concentrations. *A. kudakajimensis* tests were enriched in both S and P. As for the structural  $\text{SO}_4^{2-}$  substitution, an increased activity of sulfate ions relative to carbonate ions in the calcifying fluid would have affected sulfate concentrations in the skeletons [the sulfate-carbonate link of Berry (1998) and co-workers]. A growth-related change in the carbonate ion concentration in the calcifying space affects the relative activity of sulfate ions to carbonate ions, which would be recorded as sulfate concentrations in the biogenic carbonates (Yoshimura et al., 2014). The impact of seawater  $[\text{CO}_3^{2-}]$  on S/Ca ratios is recently re-evaluated by van Dijk et al. (2017); S incorporation increases with decreasing  $[\text{CO}_3^{2-}]$ , and high S bands in the test of *Amphistegina lessonii* coincide with elevated Mg concentrations. There were considerable differences between the two genera in total sulfur contents of individual tests (Figure 1). Neither temperature nor growth rate parameters affected S and P incorporation significantly. Incorporation of S and P in LBFs is apparently insensitive to differences in the conditions under which calcification takes place, i.e., the  $\text{CaCO}_3$  accumulation rate or calcifying fluid chemistry. Therefore, the organic matrix of the tests is the primary candidate for controlling total S and P contents in modern LBF tests. Extracting reproducible paleoceanographic records, such as carbonate parameters and nutrient cycling, from LBFs will require intensive cleaning of organic matrices or selective measurements of specific ultrastructures at high spatial resolution.

## CONCLUSION

In this study, the Mg, Sr, Na, S, and P concentrations of two LBF species grown under four different temperature conditions are measured. The temperature dependent Mg incorporation is apparent in individual tests of *C. gaudichaudii*, but that of *A. kudakajimensis* did not show a linear relationship. The variation of Sr contents in both species showed a clear negative correlation with temperature. Mg and Sr contents of the two species fall in the similar range. In contrast, Na, S and P concentrations of LBF tests highlight considerable interspecific differences, which is critical to consider for cellular ion transport and organic matrices during calcification. The chemical environments of S and P were analyzed using *in situ* XANES, and they are predominantly present as sulfate and phosphate groups. We observed five sulfur components with variable oxidation states.

## AUTHOR CONTRIBUTIONS

AM, AS, KF, and HK contributed to conception and design of the culture experiments. TY and YT performed the X-ray analysis. TY wrote the first draft of the manuscript. AM wrote

the sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

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