Automation of boron chromatographic purification for δ¹¹B analysis of coral aragonite

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Rationale: To detect the small changes in past pH, the boron isotope ratio of coral carbonates, expressed as the δ¹¹B value, needs to be both precise and accurate (2sd << 1‰). Boron measurements by Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS) require the boron to be carefully purified before analysis, which is time consuming, and requires specialist training. Here, we use the prepFAST-MC method that enables the automatic extraction of B (up to 25 ng load) from a CaCO₃ matrix.

Methods: Samples were purified using the prepFAST-MC automated system with a ~25-μL column of Amberlite IRA743 resin. Boron isotope measurements were performed by MC-ICPMS. The effects of matrix load, speed of sample loading onto the column, and blank contamination were tested to evaluate the effects on the purification process. The optimised protocol was tested on various standards and samples of aragonite corals.

Results: The blank contribution for the approach is ~60 pg and is negligible given our sample size (<0.2% sample size). Efficiency of matrix removal is demonstrated with the addition of up to 1.6 mg of dissolved low-B calcium carbonate to NIST SRM 951 with no impact on the accuracy of δ¹¹B values. The Japanese Geological Survey Porites reference material JCp-1, boric acid standard NIST SRM 951, and seawater, all processed on the prepFAST-MC system, give δ¹¹B values within error of literature values (δ¹¹BJCp-1 = 24.31 ± 0.20‰ (2sd, n = 20); δ¹¹BNIST 951 = −0.02 ± 0.15‰ (2sd, n = 13) and δ¹¹BSeawater = 39.50 ± 0.06‰ (2sd, n = 2)). Results obtained from the coral Siderastrea siderea purified with the prepFAST-MC system show an average offset from the manual ion-exchange protocols of Δδ¹¹B = 0.01 ± 0.28‰ (2sd, n = 12).

Conclusions: Our study demonstrates the capacity of the prepFAST-MC method to generate accurate and reproducible δ¹¹B values for a range of materials, without fractionation, with efficient matrix removal and with negligible blank contribution.
1 | INTRODUCTION

Measurements of atmospheric carbon dioxide (CO₂) over the last century have shown a significant increase with concentrations during the pre-industrial period (pre-mid 19th century) of around 280 ppm, reaching >400 ppm in 2015. This CO₂ rise has caused a decrease in surface seawater pH of ~0.1 unit on average due to the absorption of anthropogenic CO₂ into the ocean, and a strengthening of the greenhouse effect causing a rise in global mean surface temperature. The pH decrease and temperature rise have impacted the health of some marine calcifying organisms, including coral reefs, that have experienced bleaching and decrease in skeletal extension or density (e.g.2-5). However, instrumental records of pH are scarce and only go back a few decades. One way to reconstruct environmental parameters further back in time is to use indirect measurement of pH using, for example, the boron isotope ratio pH proxy in corals. This method has been used to capture existing records of surface pH change and to extend the historical pH record (e.g.6,7) but often at back a few decades. One way to reconstruct environmental parameters further back in time is to use indirect measurement of pH using, for example, the boron isotope ratio pH proxy in corals. This method has been used to capture existing records of surface pH change and to extend the historical pH record (e.g.6,7) but often at limited spatial and temporal resolution. To accurately and precisely evaluate environmental changes, pH and climate records are needed at high temporal resolution (e.g. a millennial resolution for geological time scales and sub-annual for historic timescales). The principal reason for the scarcity in boron isotope data to date is the labour-intensive laboratory processes required during sample preparation for accurate boron analysis including material collection, clay and organic matter removal and, for some methodologies, time-consuming boron purification by skilled users in a boron-free clean laboratory.

Several mass spectrometry techniques are in common usage for measuring the isotopic composition of boron in marine carbonates: Negative and Positive Thermal Ionisation Mass Spectrometry (NTIMS8-11 and PTIMS12-14) and, more recently, Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS; e.g.15). The techniques require varying quantities of boron (2–5 ng for NTIMS; 1 μg for PTIMS, and 10–20 ng for MC-ICPMS), have different sample preparation protocols, and each is associated with different B isotope ratio precisions (from 0.1 to 0.7‰,16 roughly equivalent to 0–0.1 pH unit). The MC-ICPMS technique in general has arguably significantly improved the measurement of many isotopic systems.17 This approach offers many advantages when measuring dual isotope systems like boron because of the stability of the mass fractionation (although it is large: e.g. 16% per m/z unit for boron17) and efficient ionisation (boron: 60–90%). The B isotopic composition of a sample is commonly expressed in the palaeoceanographic literature as:

\[ \delta^{11}B = \left( \frac{^{11}B_{sample}}{^{11}B_{NIST 951}} \right) \times 1000 \]

Previous studies have shown that MC-ICPMS allows the precise and accurate determination of \( \delta^{11}B \) values, in some instances to better than 0.25‰ (at 95% confidence), on as little as 10–20 ng of B.16

The principal requirement of the MC-ICPMS methodology, however, is the need to purify the analyte prior to analysis to avoid interference and differential ionisation or instrumental mass fractionation between samples and the bracketing standard used to correct for it (e.g.17,18). Thus, a requirement of accurate B isotope analysis by MC-ICPMS is to remove the CaCO₃ matrix and isolate boron before analysis, while simultaneously avoiding isotopic fractionation. Current boron purification methods rely on manual gravity columns, which are slow (~10 samples purified in one day) and require specific training in column chemistry, the use of gravity columns and low-blank methods to avoid blank contamination and/or fractionation by the operator.

Here we present a new protocol that facilitates this step through the automation of boron purification using the prepFAST-MC (prepFAST Multi Collector, developed by Elemental Scientific, ESI, Omaha, NE, USA). This method has been used successfully for other isotopic systems measured in various materials, including Sr and Ca isotopes,19 Cu isotopes in biological samples,20 separation of Sr, Pb and Nd isotopes from marine sediments,21 U and Th isotopes in corals,22 and Nd.23 Here for boron isotopes, we optimise the blank contribution and matrix wash-out and test the method on a variety of standards and matrices including coral samples, seawater, and carbonate/boric acid reference materials or standards. The reader is referred to the literature19,21,22 and Figure S1 (supporting information) for a more complete description of the prepFAST-MC system and schematic of the set-up used here.

2 | EXPERIMENTAL

2.1 | Reagents and materials

Reagents used for sample cleaning, dissolution and boron purification [nitric acid (HNO₃), hydrochloric acid (HCl), 30% hydrogen peroxide (H₂O₂), ammonium hydroxide (NH₄OH), sodium acetate (NaCH₃CO₂) and acetic acid (CH₃CO₂H)] were all reagent grade to minimise boron contamination. HNO₃ and HCl were further distilled using a DST1000 sub-boiling still (Savillex, Eden Prairie, MN, USA; <60 °C), and these and other solutions were diluted to the required concentration with Milli-Q water (>18.2 MΩ/cm; Millipore, Burlington, MA, USA) dispensed with a Qguard B-pack. All laboratory procedures were carried out in an overpressurised box with HEPA-filtered (high-efficiency particulate air) boron-free air within a purpose-made class-100 boron-free laboratory. All PFA (Perfluoroalkoxy from Savillex) vials used for sample preparation were pre-washed in HNO₃ (24 h in 7 M HNO₃ and 24 h in 0.3 M HNO₃), and plastic-ware was washed in 3 M HCl for 24 h.

2.2 | Samples

In order to validate the accuracy of \( \delta^{11}B \) values using the prepFAST-MC method, several reference materials and samples have been processed including a biogenic coral reference material (JCP-124; \( \delta^{11}B = 24.3 \pm 0.18% \) (2sd), see Foster et al.,16 Table S1 (supporting information), and other literature reports5,25-27, seawater
The principle of boron purification with the prepFAST-MC system (described and illustrated further in Figure S1a, supporting information) remains similar to the standard method detailed above but with several modifications to adapt it to automation. Samples were first manually dissolved and buffered in the same way but in the prepFAST-MC system samples were introduced with a probe that takes up the buffered sample and dispenses it through an online column (CF-MC-B-25: Figure S1c, supporting information) filled with 25 μL of hand-crushed Amberlite resin identical to the gravity columns in the manual method. The sample matrix was then rinsed with a succession of Milli-Q water rinses and the boron fraction was eluted with HNO₃. Five elutions of 100 μL 0.5 M HNO₃ were sufficient to collect all the boron from the prepFAST-MC column as shown on the cumulative elution curve in Figure 1. Complete collection of boron (100% yield) is necessary for measurements by MC-ICPMS₁⁴ and was obtained after 300 μL with the prepFAST-MC system. Considering that most boron is collected after 300 μL, the volume of eluent used can be adjusted depending on the equipment used and the volume required for the measurement of the $\delta^{11}$B value by MC-ICPMS.₁⁵,₁₆

### TABLE 1 Standards and samples processed on the prepFAST column to assess accuracy of $\delta^{11}$B values, with corresponding matrix.

| Standard or sample | Type of matrix        | Typical amount of buffer (μL) | Approximate amount of carbonate or standard used to yield ~20 ng of B | Approximate B/Ca ratio (μmol/mol) | $\delta^{11}$B (%o) long-term average (or range for carbonate samples) |
|--------------------|-----------------------|------------------------------|------------------------------------------------------------------|---------------------------------|-------------------------------------------------------------------|
| JCP-1              | Calcium carbonate (aragonite) | 100-150                      | 0.5-1 mg JCP-1 powder                                             | 500 (n = 50)                    | 24.25 (n = 50)                                                    |
| Seawater           | Ionic                 | 10                           | 5 μL                                                             | -                               | 39.6±²⁸                                                           |
| NIST 951           | Boric acid only, no matrix | 40                           | 20 μL of 1 ppm                                                   | -                               | 0                                                                 |
| S. siderea         | Calcium carbonate (aragonite) | 200                         | 1 mg                                                             | 460 (n = 12)                    | ~23 to 26⁷                                                        |

($\delta^{11}$B = 39.6 ± 0.2%,²⁸), matrix-free boric acid NIST SRM 951 ($\delta^{11}$B = 0%,²⁹), and samples of corals *Siderastrea siderea* (hereafter *S. siderea*) from the forereef of the Mesoamerican barrier reef system (core FR-02, Belize³⁷). The sample size, B/Ca ratio³⁰ (when applicable), indicative of boron content in a given biogenic carbonate, and the long-term average $\delta^{11}$B values of these samples and reference materials are summarized in Table 1.

The *S. siderea* corals were sampled at annual resolution and cleaned and dissolved using established protocols.³¹ The samples were oxidised with H₂O₂ (30% by weight), buffered with 0.1 M NH₄OH (3.4% of peroxide in final oxidative mix) to remove organic matter, placed in a hot bath for 3 × 5 min and separated by brief ultrasonication. The samples were leached in 0.0005 M HNO₃ for 30 s to remove remaining cations and contaminants, and dissolved in ~0.15 M HNO₃. The samples were then split into two aliquots with one processed with the standard manual method¹⁵ (see section 2.3) and the other with the prepFAST-MC system using the protocol established in this study following optimisation tests on in-house standards and reference materials.

All prepFAST-MC data are compared with the respective long-term average at Southampton (for the reference materials) and the $\delta^{11}$B value of the same sample processed manually using the approach detailed elsewhere¹⁵,¹⁶ and briefly described below.
system. A further sixth elution (the ‘elution check’) was also collected and measured to monitor complete boron recovery (as is also done in the manual method).

The volume and flow rate through the prepFAST-MC column were controlled through a set of syringe pumps. Contrary to the gravity column, this enables the user to precisely define the optimized flow rate of reagent going through the column at each stage. A rotary multivalve enables different solutions (sample, Milli-Q water and acid) and air to be loaded and dispensed through the column. Each solution is temporarily stored in a coil before being dispensed to the location of interest (column, probe or waste). All steps are controlled by software and parameterised by the user.

### 2.4 Boron isotope and elemental analysis

Boron isotope analyses were carried out on a Neptune MC-ICPMS system (Thermo Fisher, Bremen, Germany) with $10^{12}$ Ω amplifier resistors for Faraday cups H3 ($^{11}$B) and L3 ($^{10}$B) at the University of Southampton using bracketing standards of NIST SRM 951 following methods described extensively elsewhere.$^{15,16}$

![FIGURE 1](image)

**FIGURE 1** Elution curve for a 25 ng B size of SRM NIST 951. The boron concentration was measured by direct reading of the $^{11}$B intensity (in V) on MC-ICPMS; a 10% error is applied to the measurement. The red dotted line represents the 100% yield target. [Color figure can be viewed at wileyonlinelibrary.com]

| TABLE 2 | Protocol of boron purification for the standard manual method (a) and optimised protocol for the prepFAST automated method (b). When not indicated, the direction of flow through the column is forward. See Figure S1 (supporting information) for reference to forward and reverse direction of flow. |
| --- | --- |
| **a.** | **Reagents and volumes** |
| **Step** | **Clean column** | 1 x fill the column with 0.5 M HNO₃ |
| | 1 x 1 mL 0.5 M HNO₃ |
| **Condition column (to pH ~5)** | 2 x 1 mL Milli-Q water |
| **Load buffered sample** | Buffered sample |
| **Wash out matrix** | 4 x 200 μL Milli-Q water around the rim of the column to wash the walls |
| | 4 x 200 μL Milli-Q water |
| **Elute boron** | 5 x 110 μL 0.5 M HNO₃ |
| **Collect 6th elution (elution check)** | 1 x 110 μL 0.5 M HNO₃ |
| **Clean column** | 1 x fill the column with 0.5 M HNO₃ |
| | 1 x 1 mL 0.5 M HNO₃ |
| **Wash acid and store column** | 2 x 1 mL Milli-Q water |
| **b.** | **Volume (μL)** | **Reagent** | **Flow rate (μL/min)** |
| **Clean column** | 3 x 1000 | 0.5 M HNO₃ | 2000 |
| **Condition column** | 2 x 1000 | Milli-Q water | 2000 |
| **Load sample** | Sample volume | Buffered sample | 100 |
| **Matrix wash out configuration 2** | [1000 Forward 1000 Reverse] x 2 1000 Reverse x 4 | Milli-Q water | 10 000 |
| **Elute boron** | 5 x 100 | 0.5 M HNO₃ | 500 |
| **Elute tail** | 1 x 100 | 0.5 M HNO₃ | 500 |
The reported external uncertainty is calculated based on a fit to long-term reproducibility of JCP-1 processed by gravity columns at the University of Southampton at different concentrations (between 1 and ~25 ng), typically <0.25‰ (2σ).

The Na intensity was measured on the Neptune mass spectrometer prior to analysis on a six-fold-diluted aliquot (20 μL) of the purified sample to assess the level of sodium acetate buffer left in the sample, here used as an indicator of the efficiency of total matrix removal (i.e. Na and dissolved CaCO₃ sample). The Na contents are reported in gram units estimated from the Na peak height and an Na intensity of 170 mV per ppb.

Following previous studies, elemental analysis was also undertaken on a diluted aliquot of the dissolved carbonate samples (prior to boron purification) on Element ICPMS and X-series ICPMS instruments (Thermo Fisher) at the University of Southampton. This ensured that the carbonate cleaning was efficient (e.g. 32), in particular by assuring that the Al/Ca ratios, indicator of clay, were low (<100 μmol/mol) since B adsorbs to clay and excessive clay contamination can adversely affect the measured δ¹¹B values. 40, 41

2.5 | prepFAST-MC method development

2.5.1 | Matrix wash-out and matrix effect

Two configurations of matrix wash-out were examined by varying the volume, flow rate and direction of flow of Milli-Q water through the prepFAST column to determine the protocol with highest matrix wash-out efficiency (Table 3). The direction of flow was set in the forward direction (with respect to sample loading and elution, see Figure S1a, supporting information) for configuration 1 or alternated (forward and reverse) for configuration 2. The flow rate was also varied by washing out the matrix slowly (1000 μL/min, configuration 1) or rapidly (10 000 μL/min, configuration 2). As a comparison, the flow rate on the gravity column is unidirectional and depends on the hydraulic head in the column (averaging at 100 μL/min). We tested the influence of wash-out flow rate on matrix removal on NIST 951 (with Na-acetate buffer) for configuration 1 and JCP-1 (with Na-acetate buffer) for configuration 2.

In the gravity column methodology described previously 15, 16 the efficiency of the matrix wash-out is routinely assessed after boron purification, with measurements of Na intensity by MC-ICPMS as an indicator of the sample carbonate and buffer matrix (see section 2.4).

Here an aliquot taken of six JCP-1 samples before and after purification was also measured on the ICPMS X-series to assess the levels of Na and Ca in the purified sample and to test how reliable Na is as an indicator of matrix removal.

To explore the effect of varying amount of carbonate matrix on boron separation, boron-free (B content was below blank levels) natural carbonates were added to NIST 951. These carbonates were dissolved stalagmite from diverse caves from five countries with a wide range of trace element concentration (e.g. Mg/Ca ranging from 1 to 60 mmol/mol). The carbonates were added to the NIST 951 standard such that the Ca level ranged from 0 to 0.6 mg and then processed with the prepFAST-MC system. This range is equivalent to 0 to 1.6 mg of CaCO₃ and is typical of what is encountered for samples of biogenic carbonates with B/Ca ranging from 100 to 400 μmol/mol.

2.5.2 | Flow rate of sample loading

The influence of the flow rate during sample loading was explored through altering the sample loading speed by varying the flow rate from 100, 200 and 500 μL/min. These tests were conducted with carbonate (JCP-1) and seawater samples as well as boric acid, to evaluate the influence of matrix type on the loading speed and the measured δ¹¹B value.

2.5.3 | Boron blank

In a similar way to the manual method, total procedural blanks (TPBs) were processed with each batch of samples with the prepFAST-MC method and interspaced with samples of size 15–20 ng B to check if there is any carry-over effect. The TPBs are prepared with the same reagents used to prepare regular samples, i.e. Milli-Q water and 0.5 M HNO₃, and buffered with sodium acetate/acetic acid, but without introducing any sample material. Measuring the boron content of the resulting eluent therefore allowed a quantification of the level of blank contamination related to the reagents used to prepare and process the sample: buffer, 0.5 M HNO₃ (for column cleaning and elution), and Milli-Q water (for column conditioning and matrix wash out) as well as the blank associated with the handling and sample exposure and sample carry-over. It should be noted that the configurations used on the automated system pass a larger volume of

| Matrix wash-out configuration | Volume (μL) | Reagents     | Flow rate (μL/min) | Na (ng)   |
|------------------------------|------------|--------------|-------------------|-----------|
| pF-configuration 1           | 8 x 1000   | Milli-Q water| 1000              | 15.2 (n = 2) |
| pF-configuration 2           | [1000 forward + 1000 reverse] x 2 + 1000 reverse x 4| Milli-Q water    | 10 000          | 1.6 (n = 20) |
| Gravity column               | 8 x 200    | Milli-Q water| <250 μL/min       | 0.8 (n = 50) |

Table 3: Configurations of two types of matrix wash-out on the prepFAST-MC (pF) tested on boron reference materials, standards and carbonate samples (for typically ~1–2 mg CaCO₃ and ~250 μL of buffer) and Na content post-purification (undiluted) to assess matrix removal efficiency. The Na levels with the wash-out on the prepFAST range between 6.5 ng (pF-configuration 1) and 0.7 ng (pF-configuration 2). The wash-out with the standard gravity column method gives an average Na concentration of 0.3 ng. See Figure S1 (supporting information) for reference to the forward and reverse direction of flow. When not indicated, the direction of flow is forward.
Milli-Q water (8 mL) through the column during the matrix wash-out stage than in the manual method (1.6 mL) which may elevate the TPB for the prepFAST-MC method.

The eluent was collected in autosampler vials with a small aspect ratio (~1 cm² surface in contact with the air) and capped immediately to avoid evaporation and excessive blank contamination from fall-in (airborne boron). At Southampton we observe ~0.02‰ lighter fractionation per hour as a result of both evaporation and boron fall-in. During the duration of this study, we observed evaporation of ~10 μL/h and boron fall-in blank contamination of 8 pg/h. To give acceptable levels of evaporation and contamination, and given the sample size, it is therefore possible to leave a sample open for up to 5 h, although this may vary between laboratories.

3 | RESULTS

3.1 | Efficiency of matrix wash-out

The different configurations of matrix wash-out (Table 3) were tested using NIST 951 and JCp-1. The forward and low flow rate configuration 1 tested on NIST 951 boric acid showed elevated levels of Na (~15.2 ng), significantly higher than the range found when using gravity columns (~0.8 ng). Wash configuration 2 shows a more efficient removal with Na of ~1.6 ng, demonstrating the need for a fast flow rate when washing off the sample and buffer matrix and alternating flow for the most efficient removal. Overall, the Na level measured by the prepFAST-MC method using configuration 2 is consistently around twice that of the manually processed data. Although this indicates that the matrix is less effectively removed with the prepFAST-MC system, it is important to note that matrix contamination levels of <1 ppm are not associated with significant matrix effects for δ¹¹B values obtained by MC-ICPMS. Based on these results, configuration 2 was used for subsequent carbonate samples and standards.

The viability of Na as an indicator of matrix wash-out efficiency is confirmed by measurements of the Na and Ca concentrations prior to and after purification (Figure 2) using wash-out configuration 2. The Na and Ca contents post-purification are both low (median of 0.2 μg for both Na and Ca, corresponding to an approximately 20,000 and 1000 times matrix reduction, respectively) suggesting efficient wash-out and the suitability of Na measured by MC-ICPMS as a proxy for Ca removal.

To test potential matrix effects, NIST 951 was also processed with variable amounts of boron-free carbonate matrix (Figure 3). The results show no correlation between the load of matrix onto the column (0 to 0.6 mg Ca loaded, which is equivalent to a range of 0–1.5 mg of pure CaCO₃) and the δ¹¹B values, ruling out any
influence of Ca matrix and sample B/Ca ratio (within the range 100–500 μmol/mol for a 20–25 ng B target) on the measured δ¹¹B values.

3.2 Influence of sample loading flow rate

The effect of sample loading flow rate on the measured δ¹¹B values was examined using boric acid NIST 951, JCp-1 and seawater (Figure 4; and Table S2, supporting information). NIST 951 which has no sample matrix (but is mixed with a Na-acetate/acetic acid buffer) appears to be unaffected by the flow rate during sample loading whereas JCp-1 coral and seawater yield lower isotope ratios when loaded at faster flow rates (0.2 and 1‰ lower for 250 and 500 μL/min, respectively). Thus, the accuracy of the method using standards with increasingly complex matrices is sensitive to the loading flow speed. Given that the δ¹¹B values for all samples at 100 and 250 μL/min agree within error and that the values produced are in agreement with the long-term average, we infer that samples must be loaded with a flow rate no greater than 200 μL/min. A flow rate of 100 μL/min was used for all samples.

3.3 Level of blank contamination

TPBs processed on the prepFAST-MC system ranged between 7 and 60 pg of boron (4 replicates, Figure 5) for the standard matrix wash-out configuration 2 (Table 3). These values are similar to published values in the literature for the standard manual approach using gravity columns but tend to be slightly elevated compared with the standard manual approach currently in use at Southampton (ranging from below detectability to 40 pg). Nevertheless, this level of blank represents less than 0.2% of the sample boron concentration (for a 20 ng sample) and therefore requires no correction on the δ¹¹B value. The consistent and low TPB values (interspaced by 15–20 ng B samples) demonstrate little or no effect of carryover from one sample to another.

3.4 Optimised method for marine carbonates

Based on our experiments, the optimal method (Table 2b) yielding the most efficient matrix wash-out and accurate isotope ratio was used to test long-term reproducibility by application to coral samples from Belize. Our optimised method utilises a sample loading flow rate of 100 μL/min and configuration 2 for the matrix wash-out stage. One sample is processed fully automatically in approximately 60 min. New resin, freshly loaded onto the column, was thoroughly cleaned with multiple sequences of 0.5 M HNO₃ rinses (total 30 mL) before use for samples. The volumes of reagents necessary for the purification need to be regularly topped up after every ~10 samples. Storage of the column is done in line with Milli-Q water by calling a specific program at the end of the last sample processed.

3.5 Reproducibility and accuracy of the prepFAST-MC system with the optimised method

3.5.1 Reproducibility and external precision

Using the optimised method, results for JCp-1 and NIST 951 (for a 20 ng B sample size) show good reproducibility (Figure 6A) with...
\( \delta^{11}\text{B}_{\text{JCp-1}} = 24.31 \pm 0.20 \) \( \% \) (2sd, \( n = 20 \)) and \( \delta^{11}\text{B}_{\text{951}} = -0.02 \pm 0.15 \) \( \% \) (2sd, \( n = 13 \)) \( (\delta^{11}\text{B}_{\text{951}} \) refers here to NIST 951 processed through the column, not the bracketing normalising NIST 951 on MC-ICPMS). Seawater processed by the prepFAST method yielded \( \delta^{11}\text{B}_{\text{seawater}} = 39.50 \pm 0.06 \) \( \% \) (2sd, \( n = 2 \)), in agreement with the long-term \( \delta^{11}\text{B} \) value of 39.61 \( \pm 0.21 \) \( \% \) (2sd).²⁸

Our data demonstrate that the prepFAST-MC method generates highly reproducible data for JCp-1, NIST 951 and seawater, similar to the manual gravity column method (long-term average at Southampton and previous studies, Table 2), with no significant mass fractionation induced by the automated B purification.

3.5.2 | Accuracy

To further test the prepFAST-MC method on real samples, purifications were performed with samples of the coral S. siderea and the results compared with those for replicates processed with gravity columns. A \( \delta^{11}\text{B} \) cross-plot between the two methods shows an average offset of 0.01\% \( \pm 0.25 \) (2sd) for S. siderea (Figure 6B). A compilation of the coral results, along with standards and reference materials processed on the prepFAST-MC system (Figure 6C), shows that they have an average offset of 0.02 \( \pm 0.25 \)\% (2sd) given a sum of squares propagation of the associated uncertainties. These results illustrate a high degree of accuracy for the prepFAST-MC purification method for all the matrices examined here.

The Na concentrations of diluted aliquots of each of these purified samples were also measured to confirm the low level of Ca and Na contamination (from the sample and buffer, respectively). As with the tests performed with standards (with wash-out configuration 2, Table 3), the prepFAST-MC method is associated here with consistently higher levels of Na (3 vs 0.5 ng on gravity columns); however, there is no significant relationship between Na and the offset in the \( \delta^{11}\text{B} \) value from certified or long-term average values from gravity columns \( (r^2 = 0.04, \ p = 0.20, \) Figure S3, supporting information).

4 | DISCUSSION

4.1 | Effect of sample loading speed

Tests conducted with different sample loading speeds show that samples containing a carbonate or strong ionic matrix seem to be more sensitive to the flow rate than buffered boric acid (Figure 4).

FIGURE 6  A, \( \delta^{11}\text{B} \) reproducibility of standards and reference materials NIST 951, JCp-1 and seawater processed on the prepFAST column. All points agree within error with their certified value or long-term average (red dotted line, see text). B, Cross plot of \( \delta^{11}\text{B} \) prepFAST vs. manual columns for corals S. siderea. All data agree within error with the 1:1 line. C, Compilation of various standards, reference materials and S. siderea samples processed on the prepFAST column and compared with their long-term average or column \( \delta^{11}\text{B} \) data. The [prepFAST-column] offset is within error of the zero line (red dashed line) [Color figure can be viewed at wileyonlinelibrary.com]
Since the observed fractionation is towards isotopically lighter (i.e. more negative $\delta^{11}$B) values, it appears that the heavy isotope ($^{11}$B) is preferentially lost when the flow rate of the analyte over the resin bed is too fast. This fractionation occurs although our sample yield did not reveal a significantly reduced boron content at the higher flow speeds ($r^2 = 0.0006$, $p = 0.96$; Figure S4a, supporting information) within the precision of these yield measurements ($\pm 10\%$).

Lemarchand et al. observed that the very first fractions of the elution of boron from Amberlite 743 are enriched in the heavy boron isotope (i.e. more positive $\delta^{11}$B values), and the later elutions are isotopically lighter. Thus, an incomplete removal of the boron from the column, for instance missing the later stages of the elution curve, would result in an eluted sample being isotopically heavier, which is contrary to observations here, suggesting that the fractionations with high flow speed result from some other process. This is also consistent with a similar concentration of boron in the "elution-check" across the samples and this not correlating with loading speed ($r^2 = 0.24$, $p = 0.21$; Figure S4b, supporting information), as would be expected under an incomplete elution scenario. Furthermore, the loss of boron during the matrix wash-out step is also unlikely because of the high partition coefficient between the resin and B at the pH of that step (Milli-Q water pH ~5, section 2.3). Instead we propose that the fractionation with high flow speed is associated with the inefficient exchange of boron onto the Amberlite 743 during loading at fast rates.

Seawater is a complex matrix composed of various anions and cations and shows the largest offset when loaded rapidly (500 $\mu$L/min). JCp-1, which is a simpler matrix (calcium carbonate and minor trace metals), shows a smaller offset when loaded at 500 $\mu$L/min. A complex matrix, particularly one that contains other anions such as $\text{SO}_4^{2-}$, could reduce the surface of resin available for the boron to bind to during the sample loading. As the Amberlite is an anion-exchange resin, the species of aqueous boron to which it binds is the borate ion $\text{B(OH)}_4^-$. At the pH of our Na-acetate/acetic acid buffer (~pH 5.5), the boron is almost entirely in the boric acid form, which is isotopically heavier than the small quantities of borate ion. Furthermore, during sample loading it is likely that kinetic effects would result in isotopically lighter borate ions (e.g. $^{10}\text{B(OH)}_4^-$ rather than $^{11}\text{B(OH)}_4^-$) bonding preferentially to the resin. Also, any kinetic effect associated with the conversion from $\text{B(OH)}_3$ into $\text{B(OH)}_4^-$ onto the resin could lead to $\text{B(OH)}_2$ not converting quickly enough into $\text{B(OH)}_4^-$ and thus escaping the column during loading at a fast rate. It is conceivable, therefore, that any boron not bound to the resin due to rapid flow rates and/or due to a competing effect with matrix anions and kinetics effect, would be isotopically heavy (heavy B(OH)$_3$ and $^{11}$B(OH)$_4^-$), driving the remaining resin-bound boron isotopically lighter. Competition between borate and other anions may then be responsible for the effect being larger in an ionically complex media.

### 4.2 Matrix wash-out and blank contamination

Although it does not influence the measured $\delta^{11}$B value, there is a significant difference in the efficiency of matrix wash-out between the standard gravity method and the prepFAST-MC approach, with an average $\sim$0.5 ng of Na for the standard method compared with 3 ng (configuration 2 in Table 3) for the prepFAST-MC method. This is despite the use of a significantly greater volume of Milli-Q water to wash out the matrix with the prepFAST-MC method (8 mL) than in the gravity column approach (1.6 mL). This less efficient wash-out for the prepFAST-MC can probably be ascribed to: (i) the geometry of the column on the prepFAST-MC system being long and narrow ($\sim 30 \times 1$ mm) compared with the wider standard gravity columns ($\sim 5 \times 5$ mm); and (ii) the greater wetted-volume of the prepFAST-MC column ($\sim 0.1 \times 70$ cm of tubing). The matrix, which is not chemically bound to the resin, needs to be physically flushed out of the column and the rapid and hence turbulent Milli-Q water flow rate used in configuration 2 appears to improve the removal of Na and matrix.

The TPBs processed with configuration 2 (Figure 5) show levels comparable with published values and at the high end of what is typically observed on gravity columns at Southampton (0–40 pg). This result is attributed to the requirement to use a significantly greater volume of Milli-Q water to remove the matrix with the prepFAST-MC method. The boron concentration of Milli-Q water is estimated to be $\sim$10 ppt or less; therefore, the 6 mL of extra matrix washing in the prepFAST-MC method can easily account for the elevated blank (an extra 60 pg). Even at this level of blank contamination however, given our sample sizes (10 to 20 ng B), no correction is required.

### 4.3 Matrix effect

Despite documenting a matrix effect during the sample loading stage (increasing fractionation toward lower delta values when loading complex matrices - e.g. seawater - at high loading flow rate, Figure 4), the matrix addition tests conducted on NIST 951 with our effectively B-free CaCO$_3$ (Figure 3) showed no significant effect of increasing CaCO$_3$ matrix (0 to 0.6 mg Ca, equivalent to B/Ca of 100–400 $\mu$L/mol for a 20 ng B target) on the $\delta^{11}$B values. Unlike seawater that contains other anions, dissolved CaCO$_3$ principally contains cations (Ca$^{2+}$) that are not expected to interact with the anion-exchange resin. Hence, despite significant calcium loadings, at the low loading speed established in the optimised method (100 $\mu$L/min), all the boron binds to the resin.

### 4.4 Resin lifetime

The analyses here were performed using two separate resin beds. Although no systematic study of resin lifetime was attempted, based on the accuracy found here, and the consistently low amount of boron in the elution check, the resin bed lifetime is judged to be a
minimum of 60 samples (for a boron and CaCO₃ load of 20 ng and 1 mg on average, respectively) and we recommend regular checking of resin performance with carbonate reference materials or standards such as JCp-1 or NIST 951 and monitoring of the elution check. This level of performance is consistent with that obtained with gravity columns.

5 | CONCLUSIONS

Here we show that the prepFAST-MC system can automatically separate boron from a variety of matrices without significant isotopic fractionation. We find the following principal results. (i) The δ¹¹B value is accurate and reproducible for boric acid, seawater and coral CaCO₃ provided that the sample is loaded slowly (100–200 μL/min) onto the column (Figures 4 and 6). (ii) For the majority of samples examined here, the matrix was washed off less efficiently using the prepFAST-MC method than with the standard manual gravity column method (Table 3); however, the excess of matrix monitored with Na did not impact the accuracy of the δ¹¹B values (Figure 6; and Figure S3, supporting information). Furthermore, switching to a buffer that does not contain significant cations (e.g. ammonium acetate) may alleviate this contamination issue. (iii) The amount of CaCO₃ matrix loaded onto the column demonstrates no correlation with the accuracy of the δ¹¹B values (within the range 0–1.5 mg; Figure 3). (iv) The level of blank during boron purification was low (<60 pg, Figure 5), within the range of published values for which no correction was needed.

Further work is needed to develop a prepFAST-MC method suitable for other carbonate materials commonly used in paleoceanographic studies (in particular foraminifera). But importantly, the prepFAST-MC method requires less training than for gravity columns and makes it possible to process one sample automatically every 60 min. The methodology described here will therefore speed up the sample throughput considerably for boron isotope analysis in carbonates applied to different areas such as paleoclimatology, oceanography and environmental sciences.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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