Coversheet

Experimental comparisons of carbonate-associated sulfate extraction methods

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1   Abstract

Carbonate-associated sulfate (CAS) refers to trace amounts of sulfate incorporated into carbonate minerals during precipitation. CAS has been the most commonly used approach to recover the paleo-seawater sulfate sulfur isotope composition ($\delta^{34}S_{sw}$) as carbonate rocks are more common and occur in less restricted marine environments than alternative sulfate-bearing minerals (such as gypsum and anhydrite). However, uncertainties remain about the reliability of preparation techniques due to the inadvertent inclusion of contaminant sulfurous species. This study applied three oxidative leaching CAS extraction methods and compared their final $\delta^{34}S_{CAS}$ values with those following repeated single leaching in 10% NaCl (aq). The final $\delta^{34}S_{CAS}$ values after sequential leaching by a combined 12% NaOCl, 1% H_2O_2, and 10% NaCl approach were systematically higher by between 0.65‰ and 0.90‰ than rival methods. Our experiments indicate that contamination can affect measured $\delta^{34}S_{CAS}$ even given short reaction times (<30 min). A single leach with standard oxidizing reagents may not entirely eliminate contamination when handling organic and pyrite-rich carbonate samples.

Keywords: Carbonate-associated sulfate; CAS extraction; Contaminant sulfur; Sulfur isotopes

2   Introduction

Carbonate-associated sulfate (CAS) refers to trace sulfate that was incorporated into carbonate minerals during precipitation (Burdett et al., 1989). CAS has been used as a reliable proxy archive for tracing paleo-seawater isotopic composition since the 1980s (Burdett et al., 1989; Staudt and Schoonen, 1995; Kampschulte and Strauss, 2004; Bottrell and Newton, 2006; Tostevin et al., 2017). Compared with sulfate minerals (barite and sulfate evaporite minerals), which generally precipitate from derived seawater, CAS co-precipitates with normal marine carbonate minerals and so $\delta^{34}S_{CAS}$ can serve as a more reliable tracer of the isotopic evolution of seawater sulfate ($\delta^{34}S_{SW}$) and may even be used for chemostratigraphic correlation (Kampschulte and Strauss, 1998; Tostevin et al., 2017; Shi et al., 2018; Edwards et al., 2019; He et al., 2019). Moreover, carbonate deposits occur more widely and in less restricted marine environments than other sulfate minerals, further underlining the potential of CAS to better understand the long-term global sulfur cycle (Marenco et al., 2008b).

Despite such promise, $\delta^{34}S_{CAS}$ might still deviate from $\delta^{34}S_{SW}$ due to post-depositional alteration during meteoric water-rock interaction and recrystallization (Present et al., 2015). Such issues can generally be resolved after careful petrological (crystal size, mineral assemblages) and geochemical screening. For example, recrystallized carbonate crystals are usually larger (>50μm) with lower $\delta^{18}O$ values (Al-Aasm and Clarke, 2004; Zhang et al., 2020) and higher Mn/Sr ratios (Kaufman and Knoll, 1995; Bartley et al., 1998; Lan et al., 2019). Cross-plots of $\delta^{34}S_{CAS}$ against traditional indicators of diagenetic alteration, CAS concentration, Mn/Sr, Mg/Ca and $\delta^{18}O_{carb}$ values are therefore widely used to evaluate carbonate diagenesis and the fidelity of measured $\delta^{34}S_{CAS}$ values (Veizer, 1983; Derry et al., 1994; Goldberg et al., 2005; Guo et al., 2015; He et al., 2019). Nevertheless, it seems unlikely that such alteration proxies can reliably identify deviation from open ocean $\delta^{34}S_{SW}$ values due to mineral precipitation from porewaters affected by early diagenetic microbial sulfate reduction (MSR) (Rennie and Turchyn, 2014; Fike et al., 2015). MSR results in ^{34}S-enrichment in the residual sulfate reservoir, while sulfide oxidation

46   may conversely release ^{34}S-depleted sulfate into porewaters. Although these effects are difficult to assess

47   in individual samples and may lead to uncertainty over the contemporaneous $\delta^{34}S_{SW}$ value, systematic

48   trends and similar correlative $\delta^{34}S_{CAS}$ values are unlikely to result from such localized isotopic

49   fractionation.

50   The thorniest problem surrounding $\delta^{34}S_{CAS}$ studies pertains to the inadvertent extraction of sulfate

51   from the non-carbonate matrix of bulk carbonate rocks, including secondary atmospheric sulfate (SAS),

52   organic-bound sulfur (OSC), disseminated pyrite (Py), and other metastable metal-sulfide minerals, with

53    $\delta^{34}S$ values down to -50‰ (Canfield, 2001; Sim et al., 2011; Hoefs, 2015). It has been demonstrated, in

54   particular, that pyrite oxidation can occur in both strong and weak acids during the CAS extraction process

55   (Marenco et al., 2008b). Such non-CAS sulfate sources may contaminate extracted CAS, resulting in

56   lowered $\delta^{34}S_{CAS}$ values (Lomans et al., 2002; Marenco et al., 2008a; Peng et al., 2014). The extent of

57   deviation from primary values is dependent not only on the amount and isotopic composition of the

58   contaminants but also on the chosen analytical protocols (Wotte et al., 2012; Fichtner et al., 2017). Even

59   working with well-preserved carbonate samples, generating a reliable CAS signal without incorporating

60   non-CAS contaminants is still a key consideration when using the CAS approach.

61   In order to produce reliable CAS signals, two different approaches have been proposed for CAS

62   extraction. The first of these techniques (Aim-I) aims to eliminate soluble salts and SAS, as well as hinder

63   further pyrite oxidation by washing samples in 10% NaCl solution before final extraction. It was first used

64   to isolate structurally substituted sulfate from both sedimentary carbonate and phosphate minerals

65   (Kampschulte and Strauss, 1998; Shields et al., 1999; Kampschulte et al., 2001). A slightly different

66   method was developed by (Shen et al., 2008; Li et al., 2010; Shen et al., 2011), who washed their samples

67   firstly with 10% NaCl for 24 h and then washed them three times with deionized water. Wotte et al. (2011,

68   2012) washed their samples with 10% NaCl until no leachable sulfate was precipitated, following addition

69   of barium chloride. A five times 10% NaCl wash in between washing with deionized water three times

70   has been widely used in recent years (Tostevin et al., 2017; He et al., 2019; Ma et al., 2021). The second

71   approach (Aim-II) aims to eliminate organic sulfur and disseminated pyrite by adding oxidants such as

72   NaOCl or H_2O_2 (Burdett et al., 1989; Hurtgen et al., 2002; Marenco et al., 2008a; Guo et al., 2015). Various

73   comparative experimental studies have been carried out. One slight difference between these methods is

74   the leaching sequence using those solutions. For example, Burdett et al. (1989) soaked the samples with

75   5.25% NaOCl before the final CAS extraction step. Gellatly and Lyons (2005) rinsed their samples with

76   deionized water before adding the 5.25% NaOCl. Gill et al. (2007) added two more deionized water rinse

77   steps after washing with 4% NaOCl. Loyd et al. (2012b) washed samples four times with deionized water

78   before the NaOCl wash, although they consider their data to have been affected by pyrite oxidation. In

79   contrast to Loyd et al. (2012b), Thompson and Kah (2012) switched the order of deionized water and

80   NaOCl washes.

81   Other Aim-I trials to prevent sulfide oxidation have included using N_2 carrier gas under anaerobic

82   conditions to prevent the oxidation of acid volatile sulfides and pyrite (Newton et al., 2004; Bottrell and

83   Newton, 2006; Wei et al., 2020). Other Aim-II trials to use oxidants to remove reduced sulfur included

84   adding 30% H_2O_2 for 48 h to 30-100g sample powder before or after rinsing with 10% NaCl solution and

85   deionized water (Shen et al., 2011; Xiao et al., 2012) and roasting the samples at 600°C for 4 h after a 10%

86   NaCl wash with a subsequent deionized water rinse to isolate phosphate-bound sulfate (Goldberg et al.,

87   2011). Highly concentrated 30% H_2O_2 (aq) is not stable (might cause spontaneous combustion when in

88   contact with organic material) and will cause partial dissolution of the carbonate portions as H_2O_2 (aq) is

89   also a weak dibasic acid. Nevertheless, the addition of salt rinses, after deliberate oxidation, has the

90 potential to guarantee the effective removal of both oxidized sulfide and the remaining oxidizing reagent.

91 Many of these CAS extraction techniques are very similar. Wotte et al. (2012) compared a range of
92 pre-leaching techniques: Aim-I: 1) 10% NaCl (aq); 2) pure water only (aq); and Aim-II: 1) 10% NaOCl
93 (aq) only; 2) 10% NaCl (aq) followed by 10% NaOCl (aq); 3) 10% NaCl (aq) followed by 10% H_2O_2 (aq).
94 The authors noted that pre-leaching with those oxidizing agents was insufficient to remove all non-CAS
95 contamination from even finely ground, bulk carbonate rock powder, while residual oxidants proved
96 difficult to eradicate fully. As a consequence, they recommended a single reagent NaCl pre-leaching
97 method (Aim-I), commenting further that the addition of oxidizing agents (NaOCl and H_2O_2) as the last
98 pre-leaching step could potentially oxidize surviving sulfide minerals in the residue, thus contaminating
99 the extracted CAS sulfate. However, for organic and pyrite-rich carbonate samples, this simplified method
100 might still incorporate contaminant sulfur due to oxidation during the experiment.

101 In this study, we designed and compared three CAS extraction methods in order to eliminate all non-
102 CAS sulfur-bearing phases using oxidizing agents (Aim-II) for bulk carbonate rock CAS extraction. Pure
103 reagents need to be used so as not to add contaminant ions, while methods need to ensure that oxidants
104 are completely removed in the final CAS extraction steps. The three methods are: (1) 10% H_2O_2 (aq)
105 followed by 10% NaCl (aq); (2) 12% NaOCl (aq) followed by 10% NaCl (aq); (3) Combined NaOCl then
106 H_2O_2 followed by 10% NaCl (aq). We compared these three oxidative leaching methods against a refined
107 single reagent 10% NaCl (aq) leaching method (He et al., 2019) in order to identify contaminant sulfur
108 sources and their influence on the measured CAS sulfur isotope values. Here we present the results of
109 those experiments in order to establish a more effective CAS extraction protocol for dealing with
110 problematic organic and/or pyrite-rich carbonate rocks.

111

112 **3 Sample selection, pre-treatment, and analytical methodology**

113

114 We first extracted CAS using a single 10% NaCl method (method 1) for around 80 carbonate samples
115 during the Ediacaran Period in South China and then selected four representative samples to test the
116 oxidative methods. Basic descriptions of the selected samples are given in Table 1, while more detailed
117 descriptions can be found in the Supplementary Materials. The samples were selected based on three
118 criteria: 1) the most common carbonate rock types; 2) a wide range of CAS concentrations and $\delta^{34}S_{CAS}$
119 values; 3) a wide range of pyrite and TOC contents.

120 Hand specimens of these samples were first cleaned with water and cut into small blocks in order to
121 remove weathered surfaces before being heated in an oven at 60°C for 48 hours to dry them out thoroughly.
122 One of the dried blocks was used for making thin sections at University College London (UCL) and
123 studied petrographically. The other blocks were first broken into small pieces, and only the pieces without
124 veins were chosen to be grounded into powder in a tungsten carbide Tema mill and crushed again by hand
125 with an agate mortar to maximize its surface area and expose interstitial pyrite. After the crushing process,
126 the machine was cleaned carefully with DI water and acetone to prevent contamination between samples.

127 For the elemental test, including Ca, Mg, Mn, Sr, Al, K, Fe concentrations, around 20mg of carbonate
128 rock powder was dissolved in 10ml 2% HNO_3 overnight and centrifuged before being analyzed using a
129 *Varian®* 720 ICP-OES at the London Geochemistry and Isotope Centre (LOGIC) at UCL. The Laboratory
130 control solution standard samples were tested after every 20 samples to monitor the drift and precision.
131 The analytical precision for all elemental analyses was better than 5% (1SD).

132 Pyrite density was estimated using a petrographic microscope at UCL. Pyrite crystals were counted
133 within a 1×1 mm^2 area. For each sample, five representative 1×1 mm^2 areas were chosen to estimate the

pyrite density of the sample. The pyrite crystal sizes are relatively homogeneous and range from 7-16μm (See supplementary). The final qualitative pyrite density reflects the average number of pyrite crystals within a 1×1 mm^2 area in each thin section. Samples with pyrite density < 10/mm^2 were labelled as low pyrite density, 10/mm^2 < pyrite density < 100/mm^2 labelled as medium pyrite density, and pyrite density > 100/mm^2 labelled as high pyrite density.

Solid inorganic sulfides, operationally defined as acid volatile sulfur (AVS) and chromium reducible sulfur (CRS), were extracted in the Geobiology Laboratory at the University of St Andrews using the chromium reduction method, as previously described (Canfield et al., 1986; Zerkle et al., 2012; Izon et al., 2015). Briefly, 5-10 g of rock powder was placed in a tri-neck flask and sequentially refluxed with 6M HCl to release AVS, followed by acidified 1M $CrCl_2$ to release CRS. The resulting H_2S gas was carried on a stream of oxygen-free N_2 into a solution of $AgNO_3$ and captured as silver sulfide (Ag_2S) for sulfur isotope analyses.

We used deionized water and sulfate-free reagents for cleaning and oxidative leaching in this study (see Method X). The detail of the CAS extraction procedures will be discussed later. After CAS extraction, sulfate was collected as barium sulfate using excess $BaCl_2$ (aq). The $BaSO_4$ from CAS extraction and Ag_2S from AVS, CRS extraction were weighed into tin cups plus vanadium pentoxide catalyst and combusted at 1,080°C to yield SO_2 for sulfur isotope analysis by EA-IRMS (ANCA-GSL/20-20; SerCon®, Crewe, UK) on dried residues of > 0.05mg at the Iso-Analytical Ltd. Laboratory, UK. The reference material used for analysis was IA-R061 (barium sulfate, $\delta^{34}S_{CDT}$ = +20.33‰). IA-R025 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +8.53‰), IA-R026 (silver sulfide, $\delta^{34}S_{V\text{-}CDT}$ = +3.96‰) and IAEA-SO-5 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +0.5 ‰). S isotope values are reported in the δ notation against V-CDT. We also obtained %S data from the total ion beam data recorded by the mass spectrometer, which was then used to calculate the purity of the precipitates. Duplicate samples and reference samples (IA-R061 and IAEA-SO-5) were measured every five samples for quality control. Analytical precision was better than 0.06‰ (1SD).

Total organic carbon (TOC) content was analyzed using a *Leco® CS-200* carbon analyzer at UCL. About 1g of sample powders was weighed and reacted with excess 10% HCl to eliminate carbonate, followed by repeated rinsing with Milli-Q water to remove contamination. The residual was dried at 40°C for 48 hours for the TOC test. Analytical precisions within 1% (1SD).

Table 1: Selected late Ediacaran carbonate samples from South China (QLKS = Qinglinkou section, CC = Zhimaping section, LH = Lianghong section). Pyrite density: Low < 10/mm^2; Medium 10-100/mm^2; High >100/mm^2.

Sample	Geological unit	Lithology	CaCO$_3$ (wt%)	[CAS] (ppm)	Pyrite Density	$\delta^{34}S_{CAS}$ (‰) NaCl rinse
QLKS18	Upper Shibantan Member, Dengying Formation	Grey fine-grained dolomitic limestone rock with white calcite veins	50.36	21.73	3.70/mm^2 Low	35.84
QLKS6	Middle Shibantan Member, Dengying Formation	Dark grey thinly laminated micritic limestone rock with white calcite veins	91.63	27.29	22.50/mm^2 Medium	46.00
CC3	Lower Shibantan Member, Dengying Formation	Dark thin banded micritic limestone rock rich in organic matter.	76.63	111.53	236.10/mm^2 High	36.52
LHA6.5	Member III, Doushantuo Formation	Grey fine-grained banded limestone	84.92	78.84	17.90/mm^2 Medium	20.21

4   Refined NaCl leaching method (method 1)

Many previous studies used large amounts (30-250g or even larger) of samples for CAS extraction (Wotte et al., 2012; Xiao et al., 2012; Shi et al., 2018; Toyama et al., 2020). However, using smaller and more finely ground samples can significantly decrease the risk of contamination by non-CAS components in bulk carbonate powders, especially for deep-time CAS studies. Moreover, incomplete leaching due to large sample sizes can mean that measured CAS values might not represent the whole rock CAS signals. In these regards, the NaCl leaching method (Kampschulte and Strauss, 1998; Wotte et al., 2011; Wotte et al., 2012) (see Fig. 1-A) has been applied increasingly to smaller samples (~10g) for pre-Cambrian and Cambrian studies (Tostevin et al., 2017; He et al., 2019). In this study, we tested around 80 samples before comparing methods and found that 8-10g samples are large enough in this case to extract sufficient CAS for isotopic analysis.

It has been shown by (He et al., 2019; He et al., 2020) that the sulfur concentration in the NaCl-leachates declines dramatically through sequential leaching, reaching near blank levels after the third or fourth leach for most carbonate samples. In our study, rock powder was leached therefore five times in an actively mixed 10% NaCl solution over 24 hours, followed each time by centrifugation to remove the supernatant. Between each leach, the residue was washed three times with deionized water. After the final leach with NaCl solution, the residue was washed five times with deionized water, followed by immediate CAS extraction using 6 M HCl (aq).

After the pre-leaching, we used excess acid (6M HCl) for the final dissolution step (8-10g samples) for all tested CAS extraction methods (Fig. 1-D). Reaction time was kept to no more than 30 minutes to minimize the potential of pyrite oxidation (He et al., 2019). The solution was then filtered using a polyethylene syringe and a 0.2μm polypropylene membrane filter (Wynn et al., 2008). Some previous studies used saturated $BaCl_2$ solution (358g/L, 20°C, ~26.4%) to precipitate barium sulfate at room temperature (Wynn et al., 2008; Li et al., 2017; Tostevin et al., 2017; Edwards et al., 2019; He et al., 2019). Some others used 12% $BaCl_2$ (Wotte et al., 2011; Wotte et al., 2012), 10% $BaCl_2$ (Markovic et al., 2016), or 8.5% $BaCl_2$ (Theiling and Coleman, 2015) to precipitate sulfate. However, adding saturated $BaCl_2$ (aq) into the decarbonated HCl solution may cause $BaCl_2$ to precipitate instead of $BaSO_4$, thus removing Ba^{2+} ions from the solution that might have reacted with SO_4^{2-}. After several tests, we found that adding 1.5 ml 200g/L $BaCl_2$ (aq) improved the purity of the yield (Fig. 1-D). Another option for future studies could be to use sulfate-free barium iodide or barium nitrate.

In this study, 1.5 ml 200g/L (~16.7%) $BaCl_2$ (aq) was added to the filtrate. Once sealed, sample tubes were left at room temperature for 3-4 days in order to precipitate released trace sulfate as barium sulfate. After centrifugation and supernatant removal, the residue was rinsed with 6M HCl and deionized water to remove any remaining acid-soluble components, such as $BaCl_2$ and $BaCO_3$, and dried at 60°C for three days. Finally, precipitates were collected in a tin capsule for $\delta^{34}S$ analysis on EA-IRMS. Although we washed the precipitates beforehand, some samples were still not pure barium sulfate (possibly containing residual $BaCl_2$ or H_2O). We back-calculated the $BaSO_4$ yield based on the %S data.

This method is easy to handle and can remove most water-soluble sulfate and SAS in the carbonate powder. However, the method does not seek to eliminate AVS, CRS, and organic sulfur, which could undergo partial oxidation during CAS extraction. Therefore, this method could potentially generate a different, most likely lower $\delta^{34}S_{CAS}$ value compared with the actual carbonate value, which should approximate the $\delta^{34}S_{sw}$ value of contemporaneous seawater.

5 Three oxidative leaching methods

5.1 H$_2$O$_2$ and NaOCl leaching (methods 2a and 2b)

These two methods were developed from a combined NaCl-NaOCl leach method, as applied by (Gill et al., 2011a; Gill et al., 2011b), in which 10% NaCl solution is first added to the samples for 24h. Subsequently, 5-10 g of the carbonate powders are washed twice with deionized water and then soaked in 4% NaOCl solution for 48h in order to oxidize the non-CAS sulfur. Two additional washes with deionized H$_2$O follow this step. Wotte et al. (2012) argued that this method has the following drawbacks: 1) It cannot ensure the elimination of pyrite; and 2) It cannot totally remove the oxidants before CAS extraction. Consequently, if the powder is not sufficiently fine, interstitial pyrite might be oxidized in the subsequent CAS extraction process by a combination of Fe (III) and any oxidants that remain in the slurry (Equation 5-8).

In our revised single oxidative methods (Fig. 1-B), samples were leached firstly with 10% H$_2$O$_2$ (aq) solution (method 2a) or 12% NaOCl (aq) solution (method 2b) for 24 h in order to remove the SAS (Equation 9, 10), organic sulfur and pyrite (Equations 5-8). As NaOCl (aq) and H$_2$O$_2$ (aq) are not stable at high concentrations, we chose a relatively stable concentration for this study. All the anticipated reactions in the slurry are shown in Equations 1-10. These oxidants have been widely used by, for example (Ohkouchi et al., 1999; Hurtgen et al., 2002; Newton et al., 2004; Shen et al., 2011; Loyd et al., 2012a; Xiao et al., 2012). The leaching procedure was repeated three times for 24h each. In order to disintegrate the powder further, remove the calcium sulfate coating on pyrite crystal surface (Brunner, 2003) and maximize pyrite surface area, an ultrasonic bath was used between vigorous shaking using a rotator. The residue was washed three times with deionized water between each leach to remove the oxidized sulfur in the slurry. Nearly no barium sulfate precipitate was observed after the third leach, after which the residue was washed repeatedly using the refined 10% NaCl (aq) leaching method (Fig. 1-A) to remove the remaining sulfate, SAS, and remaining oxidant in the slurry.

$ClO^- + 2Fe^{2+} + H_2O \rightarrow Cl^- + 2Fe^{3+} + 2OH^-$	Equation 1 (soluble iron oxidation)
$7ClO^- + 2S^- + 2OH^- \rightarrow 2SO_4^{2-} + 7Cl^- + H_2O$	Equation 2 (sulfide oxidation)
$2S^- + 4H_2O_2 \rightarrow SO_4^{2-} + 4H_2O$	Equation 3 (soluble iron oxidation)
$2H^+ + H_2O_2 + Fe^{2+} \rightarrow 2Fe^{3+} + 2H_2O$	Equation 4 (sulfide oxidation)
$2FeS_2 + 15ClO^- + 2OH^- \rightarrow 2Fe^{3+} + 4SO_4^{2-} + 15Cl^- + H_2O$	Equation 5 (Pyrite oxidation)
$2FeS_2 + 9H_2O_2 (aq) + 2H^+ \rightarrow 2Fe^{3+} + 2SO_4^{2-} + 10H_2O$	Equation 6 (Pyrite oxidation)
$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$	Equation 7 (Pyrite oxidation)
$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$	Equation 8 (Pyrite oxidation)
$ClO^- (aq) + SO_2 (g) + 2OH^- \rightarrow SO_4^{2-} + Cl^- + H_2O$	Equation 9 (SAS oxidation)
$H_2O_2 (aq) + SO_2(g) \rightarrow SO_4^{2-} + 2H^+$	Equation 10 (SAS oxidation)

5.2 Combined NaOCl-H$_2$O$_2$ leaching (method 3)

According to the study by (Wotte et al., 2012), it is hard to completely eliminate oxidants in the slurry. The new combined oxidative leaching method (method 3) in this study was designed to solve this issue (Fig. 1-C). After final leaching with NaOCl and Milli-Q water in method 2b, 1% H$_2$O$_2$ (aq) was added to the slurry in order to remove the NaOCl (Equation 11). Using only 1% H$_2$O$_2$ (aq) could minimize the potential for dissolution of our carbonate samples as H$_2$O$_2$ is a weak acid. The absence of bubbles shows

whether the NaOCl was successfully removed from the slurry. Moreover, the addition of hydrogen peroxide will not add further contaminant ions to the slurry, while being unstable, it can be easily removed by heating at 90°C (Equation 12), followed by the refined 10% NaCl (aq) leaching method, as shown in Fig. 1-A.

NaOCl (aq) + H$_2$O$_2$ (aq) → NaCl (aq) + O$_2$ (g) + H$_2$O   Equation 11 (NaOCl removal)

2H$_2$O$_2$ (aq) → 2H$_2$O + O$_2$ (g)   Equation 12 (H$_2$O$_2$ removal)
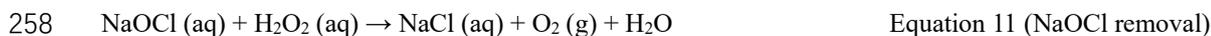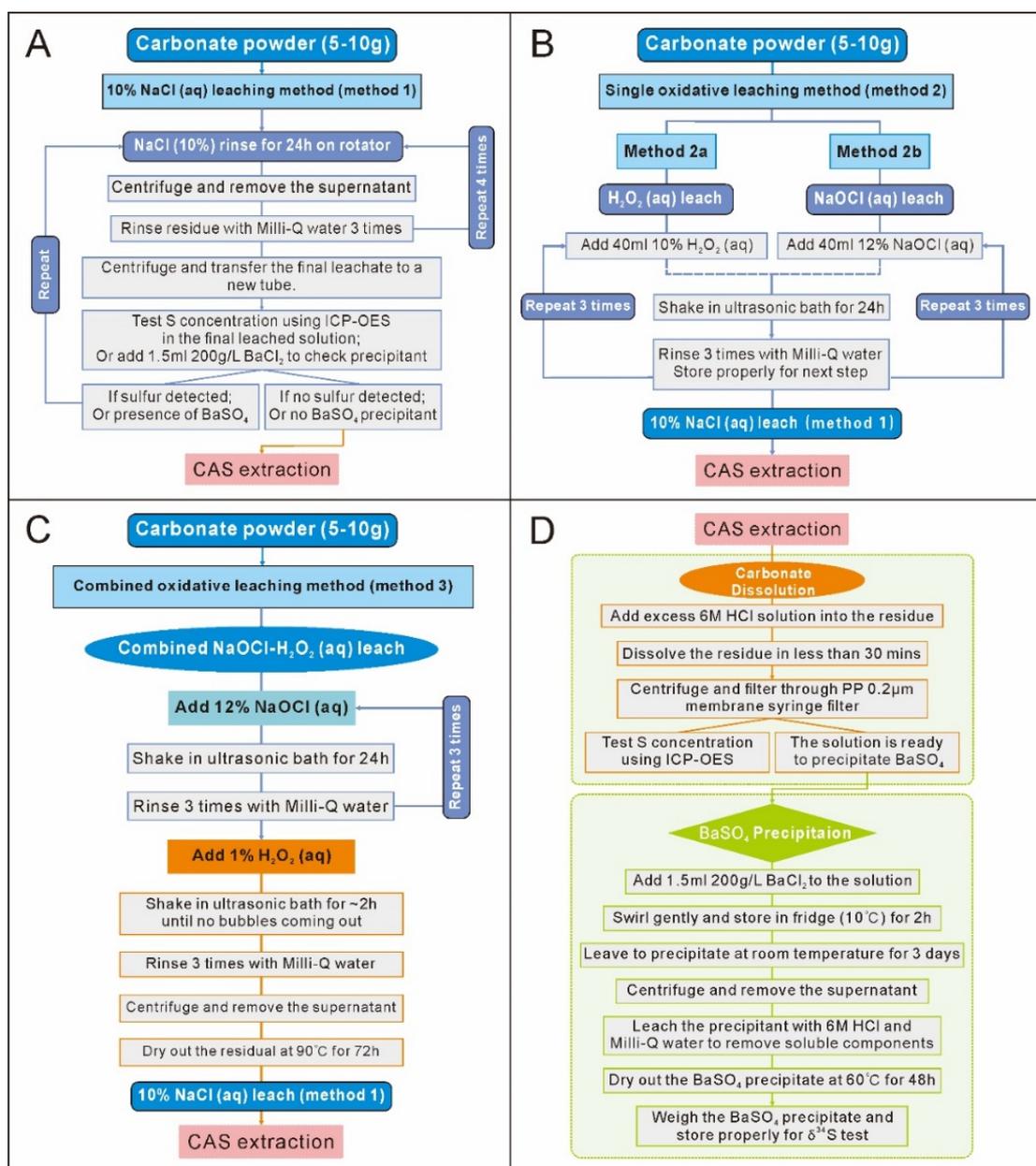
Fig. 1. New designed and tested carbonate-associated sulfate (CAS) pre-leaching and extraction protocols in this study.

A: Refined 10% NaCl (aq) pre-leaching method (method 1). B: Single oxidative-NaCl pre-leaching methods: 10% H$_2$O$_2$ (aq) leach (method 2a) and 12% NaOCl leach (aq) (method 2b). C: Combined NaOCl-H$_2$O$_2$-NaCl pre-leaching method (method 3). D: Refined CAS extraction protocols.

6 Results

Leachate precipitate weights, sulfur isotope values, and final CAS sulfur isotope values are shown in Table 2. Because precipitates are rarely pure barium sulfate, we back-calculated barium sulfate weights based on the purity data obtained during isotopic analysis. The leachate precipitate sulfur isotope values are shown as $\delta^{34}S_{SL}$, and the final CAS sulfur isotope values are shown as $\delta^{34}S_{CAS}$. For method 2a, as H_2O_2 (aq) is a weak dibasic acid, it will cause partial dissolution of samples. Moreover, H_2O_2 (aq) is not stable and might cause spontaneous combustion at concentrations higher than 8% in contact with organic material, so we have not tested the leachate sulfur isotope value of all samples using method 2a. As QLKS18 is a dolostone sample with a low sulfate concentration (Table 1), we failed to get the final CAS sulfur isotope data. All the elemental test data are shown in the Supplementary Information (Table S1). Sample LHA6.5 is a pure limestone with very low pyrite and TOC content, so we did not obtain AVS and CRS sulfur isotope values from this sample.

Table 2. Leachate precipitate weight, sulfur isotope composition, and final CAS sulfur isotope values from different pre-leaching methods together with the TOC content, AVS, CRS concentration, and its sulfur isotope values of the samples.

"--" means too low to get a reliable value. SL = solution. S isotope values are reported in the δ notation against V-CDT.

Sample	Method	Powder weight (g)	Rinse Times	BaSO₄ Corrected (mg)	Leachate $\delta^{34}S_{SL}$ (‰)	CAS $\delta^{34}S_{CAS}$ (‰)	Method 1 $\delta^{34}S_{CAS}$ (‰)	AVS S (ppm)	AVS $\delta^{34}S_{AVS}$ (‰)	CRS S (ppm)	CRS $\delta^{34}S_{CRS}$ (‰)	TOC (wt%)
QLKS18	2a	7.59	1	1.39								
	2a		2	1.84	--	--						
	2a		3	1.09								
	2b	8	1	0.08	20.65		35.84	4.00	--	9.75	30.47	0.022
	2b		2	0.63	--	--						
	2b		3	0.33	--							
	3	8	4	0.12	--	--						
QLKS6	2a	7.5	1	0.34								
	2a		2	0.77	--	--						
	2a		3	0.17								
	2b	8	1	0.12	31.82		46.00	1.41	--	90.50	37.16	0.14
	2b		2	0.15	20.28	46.37						
	2b		3	0.05	--							
	3	8	4	0.03	--	46.79						
CC3	2a	7.52	1	133.37								
	2a		2	2.42	--	--						
	2a		3	0.16								
	2b	8	1	134.24	24.15		36.52	16.05	24.26	1769.16	22.19	2.53
	2b		2	0.84	20.45	36.91						
	2b		3	0.04	18.88							
	3	8	4	0.04	--	37.42						
LHA6.5	2a	7.51	1	0.13								
	2a		2	1.09	--	--						
	2a		3	0.29								
	2b	8	1	0.08			20.21	1.37	--	13.77	--	0.013
	2b		2	0.33	--	20.72						
	2b		3	0.11								
	3	8	4	0.08	--	20.86						

7 Discussion

7.1 Evaluation of the three oxidative leaching methods

NaOCl leachates with a pink to brown color show how after adding oxidants to the slurry, iron (likely in the form of pyrite) and possibly TOC, have been oxidized (supplementary Fig. S1). Fig. 2-A, B shows that the final barite precipitate (back-calculated) weight from the leached solutions using all these three tested methods shows a similar trend towards zero after the third leach. Only sample CC3, which is dark limestone with the highest pyrite density and TOC content, shows a continuously decreasing trend of the precipitate weight (grey line). The other samples show that the second leachate has the most sulfate. The third and fourth leachates have similar precipitate weights almost below the lower limit for S isotope analysis (Fig. 2-C), which confirms that nearly all the contaminant S has been removed by leach steps 3 or 4.

All leachates have significantly lower sulfur isotope $\delta^{34}S_{SL}$ values compared with the final $\delta^{34}S_{CAS}$ values (~ 13-26‰ lower) (Table 2), indicating that the leaching process removes contaminant sulfur-bearing species (atmospheric sulfate or SAS, organic sulfur, and pyrite) rather than CAS. The first leachates from samples QLKS18, QLKS6, and CC3, all from the Shibantan Member of the Dengying Formation, have the highest sulfur isotope values with $\delta^{34}S_{SL}$ of 20.65‰, 31.82‰, and 24.15‰ respectively, whereas second and third leachates of those samples have consistent $\delta^{34}S_{SL}$ values around 20‰.

Fig. 2-D shows the final $\delta^{34}S_{CAS}$ value using the different methods. Compared with a single reagent 10% NaCl (aq) pre-leaching method, our methods 2b and 3 show incrementally higher final $\delta^{34}S_{CAS}$ values for the same sample. For samples QLKS6, CC3, and LHA6.5, there is a systematic difference of 0.79‰, 0.90‰, and 0.65‰, respectively, between the NaCl leaching method and the combined oxidative method 3. These relative differences (see Fig. 2-E) mirror the samples' visually estimated pyrite density (17.9, 22.5, and 236.1) and measured AVS + CRS contents, respectively (Table 2). Our experiment reveals method 3 to be the favoured method in the sense that it will more likely result in pristine CAS sulfur isotope values for pyrite-rich samples. Although systematic in direction, the differences are relatively minor, due perhaps in part to the high $\delta^{34}S_{SL}$, $\delta^{34}S_{CRS}$, and $\delta^{34}S_{AVS}$ values, relative to $\delta^{34}S_{CAS}$.

7.2 Contaminant sulfur evaluation

Atmospheric SO_2 can potentially influence final CAS sulfur isotope values but is relatively hard to evaluate (Peng et al., 2014). Moreover, SAS sulfur is likely to be sorbed onto the surfaces of carbonate crystals (Edwards et al., 2019) and so ought to be leached out with repeated rinsing with 10% NaCl (aq). In this study, therefore, we mainly tested the S isotope values of AVS and CRS (pyrite) in each sample and the TOC content in order to evaluate the potential influence of other sulfur-bearing contaminants on our final CAS sulfur isotope values. Sample CC3, which has the highest pyrite and TOC content, exhibits the greatest difference between CAS sulfur isotope values. Although LHA6.5 and QLKS6 have similar TOC contents, sample QLKS6, which has a higher CRS concentration, shows a greater change in the final CAS value. Our results confirm therefore that TOC content and/or pyrite content can significantly influence measured $\delta^{34}S_{CAS}$ values (Fig. 2-E).

Fig. 2-F shows that except for sample CC3, leachate $\delta^{34}S_{SL}$ values are lower than $\delta^{34}S_{CRS}$. As pyrite sulfur and organic sulfur compounds (OSC) are the two main sulfur sources in TOC-rich carbonate samples (Werne et al., 2003), we consider therefore that OSC might lower the leachate sulfur isotope

values. The origin and composition of the organic sulfur compounds are still not fully understood, but it has been reported that organic sulfur isotope values are at least 8‰ lower than ambient seawater sulfate (Siedenberg et al., 2018), while kerogen sulfur was reported to range widely between -32.6‰ and 30.3‰ in Archean stromatolite samples (Bontognali et al., 2012). The first leachate precipitate weight of sample CC3 is around 100 times higher than the highest values from the other samples, even though its AVS + CRS concentration is only 20 times higher, indicating perhaps that the difference between $\delta^{34}S_{SL}$ and $\delta^{34}S_{CRS}$ values was caused by oxidizing other sulfur sources, possibly the OSC.

We note that $\delta^{34}S_{SL}$ values show a decreasing trend and that leachates 2 and 3 have relatively stable isotope values of around 20‰. It seems that the first leachate is more complex, whereby the first leachate of sample CC3 has nearly the same sulfur isotope value as AVS: 24.15‰ and 24.26‰, respectively. As SAS is unlikely to influence leachate sulfur isotope values, the first leachate seems more likely to represent a combination of both AVS and CRS. However, leachate 2, and especially leachate 3 might be more influenced by the oxidation of organic sulfur.

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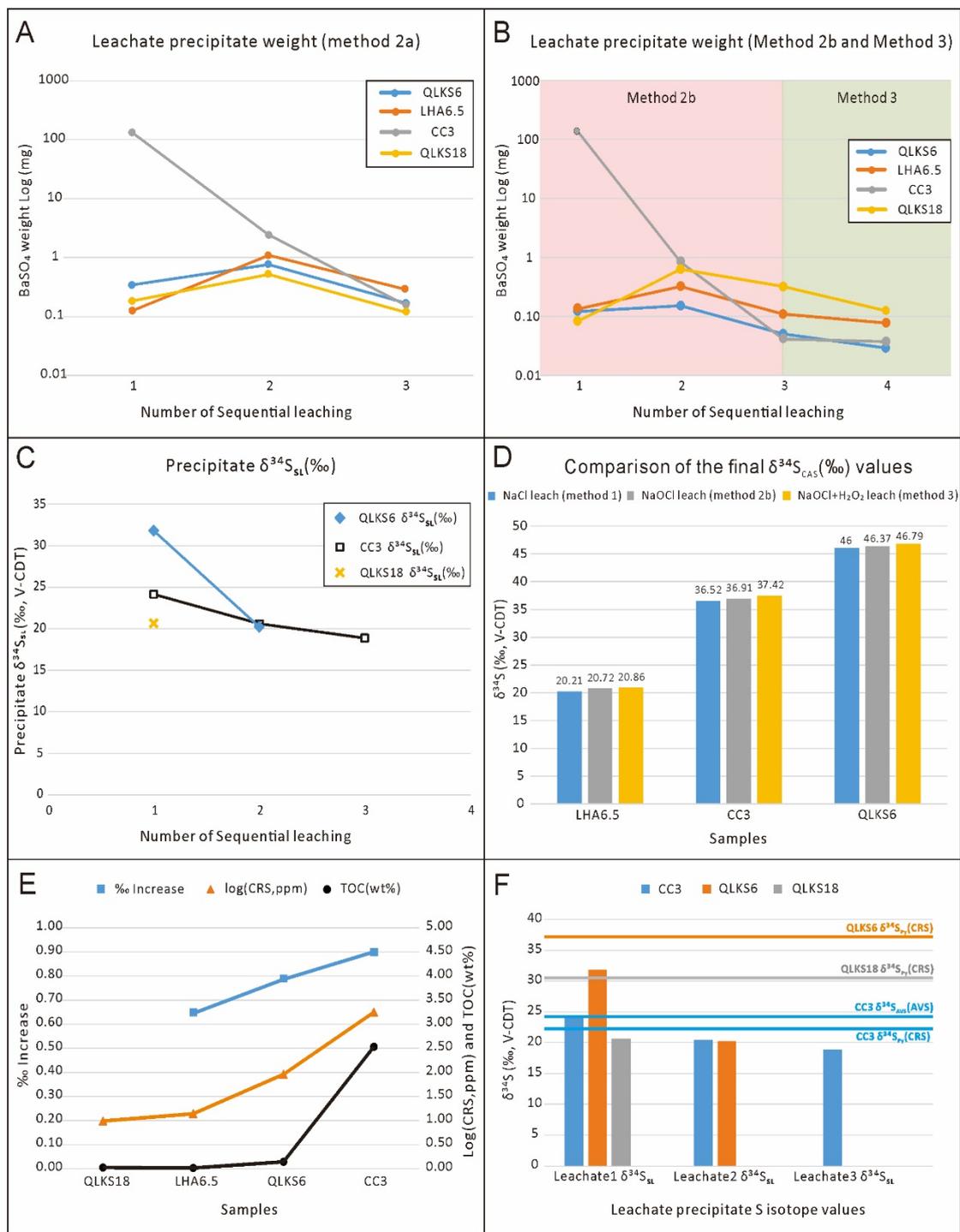
Fig. 2. Comparison and evaluation of four methods result in this study. A: Barite precipitate weight from 3 leachates using method
2a. B: Barite precipitate weight from 3-4 leachates using method 2b (shaded in pink) and 3 (shaded in green). C: Leachate sulfate
sulfur isotope values $\delta^{34}S_{SL}$. D: Comparison of the final CAS sulfur isotope value $\delta^{34}S_{CAS}$ using three different methods (NaCl only,
methods 2b, and method 3). E: Comparison between the gradient of CAS $\delta^{34}S_{CAS}$ increase from NaCl pre-leaching to method 3 and
samples' CRS concentration and TOC content (The ‰ increase refers to the increase in the $\delta^{34}S_{CAS}$ values from method 1 to
method 3). F: Comparison of the leachate precipitate S isotope value with pyrite S isotope value showing the leachate S isotope
values are consistently lower than the coeval pyrite S isotope values. Sample CC3 (dark limestone with the highest pyrite density
and AVS + CRS concentration) has the highest % increase of the final $\delta^{34}S_{CAS}$ value, then QLKS6 (dark grey limestone), then
LHA6.5 (grey limestone).

7.3   Comparisons with published CAS data

This study investigated the sulfur isotope systematics of three carbonate samples from the Shibantan Member, Dengying Formation (CC3, QLKS6, QLKS18), and one sample from Doushantuo Member III (LHA6.5) (supplementary Fig. S2). In the Yangtze Gorges area, the Dengying Formation is subdivided into three members, which from bottom to top are the siliceous dolostone Hamajing Member, dark banded limestone Shibantan Member, and light grey dolostone Baimatuo Member. These three members can be correlated with the Algal Dolomite Member, Gaojiashan Member, and Beiwan Member, respectively, in the southern Shaanxi Gaojiashan area (Chen et al., 2015; Cui et al., 2016). At the Gaojiashan section, CAS sulfur isotope values do not change much (average 42.40 ± 3.74‰), Further afield, the Dengying Formation is often correlated with the late Ediacaran Nama Group in Namibia (Tostevin et al., 2017). For sample LHA6.5, with the carbon isotope value -8.1‰ (Lu et al., 2013), it can be correlated with relatively stable values (-8.9‰) from the upper Doushantuo member III (~ 120–140 m) at the Jiulongwan section (Shi et al., 2018).

The CAS sulfur isotope data and $\Delta\delta^{34}S$ data from this study and published equivalent samples' data are summarized in Table 3. Our average $\delta^{34}S_{CAS}$ using method 3 is 40.02‰, which is comparable with stratigraphically equivalent samples from the Gaojiashan Member, Gaojiashan section (42.40‰) (Cui et al., 2016) but higher than values from the Gaojiashan Member, Lianghekou section (36.1‰) (Chen et al., 2015) and the correlative Nama group (34.13‰) (Tostevin et al., 2017). Although we only tested three samples from the Shibantan Member for this study, one sample yielded a $\delta^{34}S_{CAS}$ value of 46.8‰, which is higher than all published values from either the Gaojiashan Member or the Nama Group. Our results suggest that the improved method 3 here could significantly prevent the inclusion of contaminant sulfur (up to 15.2‰ lower than resultant CAS, Table 2) and generate more reliable $\delta^{34}S_{CAS}$ values. Moreover, by oxidizing the contaminant sulfur, our new method 3 could prevent the incorporation of contaminant O from water H_2O or dissolved oxygen O_2 (Equation 7, 8), which could potentially generate more reliable CAS $\delta^{18}O_{CAS}$ too.

In summary, our experiments outlined here demonstrate that method 3 is a potentially superior method for handling organic-rich (high TOC content) and pyrite-rich limestone samples. However, If the sample is pure limestone with low pyrite density and TOC content, repeated pre-leaching with 10% NaCl is still shown to be both straightforward and relatively reliable.

7.4   Rethinking the 'Superheavy Pyrite'

The phenomenon of high sulfide sulfur isotope values (higher than contemporaneous sulfate sulfur isotope values) has been referred to as the 'superheavy pyrite' problem (Ries et al., 2009). However, sedimentary pyrite sulfur $\delta^{34}S_{PY}$ can seldom if ever be isotopically heavier than its ambient sulfate reservoir $\delta^{34}S_{sulfate}$, which has puzzled geochemists for decades (Canfield, 2001; Fike et al., 2015; Cui et al., 2018). Superheavy pyrite has been reported from Neoproterozoic successions around the world, but in particular from the late Ediacaran Nama Group (Ries et al., 2009). Data from Tostevin et al. (2017) indicate, however, a much smaller $\Delta\delta^{34}S_{CAS-PY}$ in those same successions of only less than 1‰ (-0.72 ± 5.68‰, Table 3, supplementary Fig. S3), with only two samples having higher pyrite sulfur $\delta^{34}S_{PY}$ than $\delta^{34}S_{CAS}$. In this regard, it may be significant that $\delta^{34}S_{CRS}$ values from our study, although high, up to 37.2‰, are consistently lower than $\delta^{34}S_{CAS}$ values from the same sample. It may still be argued therefore that 'superheavy pyrite' might just be 'heavy pyrite', which can be achieved in a low sulfate environment via

Rayleigh distillation (Chen et al., 2008; Ries et al., 2009; Wood et al., 2015). Cui et al. (2018) proposed that post-depositional TSR might generate high pyrite sulfur $\delta^{34}S_{PY}$. However, hydrothermal activity is a local process, and not all isotopically heavy pyrite has been subject to hydrothermal influence (Wang et al., 2019). Moreover, isotopically heavy pyrite might also form in shallow, high sedimentation rate environments, which involves partial oxidation of the sulfide during frequent sedimentary reworking (Fike et al., 2015). Until more reliable isotopic data come out, the origins or even existence of 'superheavy pyrite' (see also Wang et al., 2019) will likely remain obscure.

Table 3. Comparisons between CAS sulfur isotope values $\delta^{34}S_{CAS}$ and $\Delta\delta^{34}S_{CAS-PY}$ from this study and published data. 'n' indicates the sample number. Errors are one standard deviation.

Lithostratigraphy	Samples	$\delta^{34}S_{CAS}$ highest	$\delta^{34}S_{CAS}$ lowest	$\delta^{34}S_{CAS}$ mean	$\Delta\delta^{34}S_{CAS-Py}$
Middle Dengying Formation (~550-546 Ma)	This study Shibantan member Qinglinkou section (Method 3)	46.79‰	35.84‰	40.02 ± 5.92‰ (n=3)	10.08 ± 4.95‰ (n=3)
	Cui et al. (2016) Gaojiashan member Gaojiashan section	45.8‰	32.5‰	42.4 ± 3.7‰ (n=42)	34.7 ± 9.6‰ (n=26)
	Chen et al. (2015) Gaojiashan member Lianghekou section	37.8‰	33.1‰	36.1 ± 2.2‰ (n=4)	--
	Tostevin et al. (2017) Nama Group Namibia	45.43‰	21.87‰	34.13 ± 5.34‰ (n=51)	-0.72 ± 5.68‰ (n=11)
Doushantuo Member III (~570-551 Ma)	This study Upper Doushantuo Lianghong section (Method 3)	20.86‰	20.86‰	20.86‰	--
	McFadden et al. (2008) Upper Doushantuo Jiulongwan section	43.5‰	6.9‰	20.7 ± 8.6‰ (n=50)	22.8 ± 10.0‰ (n=18)
	Shi et al. (2018) Upper Doushantuo Jiulongwan section	34.3‰	13.5‰	20.2 ± 6.3‰ (n=14)	24.8 ± 11.8‰ (n=13)

8   Conclusions

In this study, we designed and compared three oxidative CAS leaching methods with the refined NaCl leaching method in order to establish a robust protocol for bulk carbonate rock CAS extraction. Our experiments demonstrate that:

(1) The combined oxidizing method '12% NaOCl + 1% H_2O_2 followed by repeated 10% NaCl leaching' is, by comparison, the most suitable method to handle samples with high pyrite and organic contents. If the sample is pure limestone with low pyrite density, repeated pre-leaching with 10% NaCl is more straightforward and relatively reliable.

(2) Our new method 3 could prevent the incorporation of contaminant O from the water H_2O and/or dissolved oxygen O_2, which could potentially generate more reliable CAS $\delta^{18}O_{CAS}$.

(3) The final CAS sulfur isotope values ($\delta^{34}S_{CAS}$) using the combined 12% $NaOCl$ + 1% H_2O_2 method were between 0.65‰ and 0.9‰ higher than rival methods.

(4) Even with short reaction times (<30 min) to extract CAS, non-CAS contaminants (organic sulfur, and especially pyrite sulfur) still have the potential to lower final CAS sulfur isotope compositions $\delta^{34}S_{CAS}$.

(5) Our experiment confirms that a single leaching step with oxidizing reagents is commonly not enough to remove all contaminant sulfate.

(6) By adding oxidizing agents $NaOCl$ and H_2O_2, we successfully oxidized pyrite and OSC. The first leachate likely represents a combination of both AVS and CRS. However, leachates 2 and 3 are more likely to represent the organic sulfur isotope values $\delta^{34}S_{OSC}$.

(7) Our study suggests that organic sulfur isotope values $\delta^{34}S_{OSC}$ can be significantly different from, and in this case, lower than the coeval pyrite sulfur isotope value $\delta^{34}S_{PY}$.

(8) Our data fit well with published data from South China and Namibia but show consistently high $\delta^{34}S_{CAS}$ values.

(9) With only ~0.7‰ difference between CAS sulfur and pyrite sulfur isotope values $\Delta\delta^{34}S_{CAS-Py}$ in published 'superheavy pyrite' studies, it is worthwhile to revisit such cases with improved CAS extraction methods in future studies.

Acknowledgments

References

Al-Aasm, I.S., Clarke, J.D., 2004. The effect of hydrothermal fluid flow on early diagenetic dolomitization: an example from the Devonian Slave Point Formation, northwest Alberta, Canada.

Bartley, J.K., Pope, M., Knoll, A.H., Semikhatov, M.A., Petrov, P., 1998. A Vendian-Cambrian boundary succession from the northwestern margin of the Siberian Platform: stratigraphy, palaeontology, chemostratigraphy and correlation. Geol Mag, 135(4): 473-94.

Bontognali, T.R. et al., 2012. Sulfur isotopes of organic matter preserved in 3.45-billion-year-old stromatolites reveal microbial metabolism. Proc Natl Acad Sci U S A, 109(38): 15146-51.

Bottrell, S.H., Newton, R.J., 2006. Reconstruction of changes in global sulfur cycling from marine sulfate isotopes. Earth-Science Reviews, 75(1-4): 59-83.

Brunner, B., 2003. The Sulfur cycle: From bacterial microenvironment to global biogeochemical cycles, ETH Zurich.

Burdett, J.W., Arthur, M.A., Richardson, M., 1989. A Neogene Seawater Sulfur Isotope Age Curve from Calcareous Pelagic Microfossils. Earth and Planetary Science Letters, 94(3-4): 189-198.

Canfield, D.E., 2001. Biogeochemistry of Sulfur Isotopes. Reviews in Mineralogy and Geochemistry, 43(1): 607-636.

Canfield, D.E., Raiswell, R., Westrich, J.T., Reaves, C.M., Berner, R.A., 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chemical geology, 54(1-2): 149-155.

Chen, X., Li, D., Ling, H.-F., Jiang, S.-Y., 2008. Carbon and sulfur isotopic compositions of basal Datangpo Formation, northeastern Guizhou, South China: Implications for depositional environment. Progress in Natural Science, 18(4): 421-429.

Chen, Y., Chu, X., Zhang, X., Zhai, M., 2015. Carbon isotopes, sulfur isotopes, and trace elements of the dolomites from the Dengying Formation in Zhenba area, southern Shaanxi: Implications for shallow water redox conditions during the terminal Ediacaran. Science China Earth Sciences, 58(7): 1107-1122.

Cui, H. et al., 2016. Environmental context for the terminal Ediacaran biomineralization of animals. Geobiology, 14(4): 344-63.

Cui, H. et al., 2018. Questioning the biogenicity of Neoproterozoic superheavy pyrite by SIMS. American Mineralogist, 103(9): 1362-1400.

Derry, L., Brasier, M., Corfield, R.e.a., Rozanov, A.Y., Zhuravlev, A.Y., 1994. Sr and C isotopes in Lower Cambrian carbonates from the Siberian craton: a paleoenvironmental record during the 'Cambrian explosion'. Earth and Planetary Science Letters, 128(3-4): 671-681.

Edwards, C.T., Fike, D.A., Saltzman, M.R., 2019. Testing carbonate-associated sulfate (CAS) extraction methods for sulfur isotope stratigraphy: A case study of a Lower–Middle Ordovician carbonate succession, Shingle Pass, Nevada, USA. Chemical Geology, 529.

Fichtner, V. et al., 2017. Diagenesis of carbonate associated sulfate. Chemical Geology, 463: 61-75.

Fike, D.A., Bradley, A.S., Rose, C.V., 2015. Rethinking the Ancient Sulfur Cycle. Annual Review of Earth and Planetary Sciences, 43(1): 593-622.

Gellatly, A.M., Lyons, T.W., 2005. Trace sulfate in mid-Proterozoic carbonates and the sulfur isotope record of biospheric evolution. Geochimica et Cosmochimica Acta, 69(15): 3813-3829.

Gill, B.C., Lyons, T.W., Jenkyns, H.C., 2011a. A global perturbation to the sulfur cycle during the

Toarcian Oceanic Anoxic Event. Earth and Planetary Science Letters, 312(3-4): 484-496.

Gill, B.C., Lyons, T.W., Saltzman, M.R., 2007. Parallel, high-resolution carbon and sulfur isotope records of the evolving Paleozoic marine sulfur reservoir. Palaeogeography, Palaeoclimatology, Palaeoecology, 256(3-4): 156-173.

Gill, B.C. et al., 2011b. Geochemical evidence for widespread euxinia in the later Cambrian ocean. Nature, 469(7328): 80-3.

Goldberg, T., Poulton, S.W., Strauss, H., 2005. Sulphur and oxygen isotope signatures of late Neoproterozoic to early Cambrian sulphate, Yangtze Platform, China: diagenetic constraints and seawater evolution. Precambrian Research, 137(3-4): 223-241.

Goldberg, T., Shields, G.A., Newton, R.J., 2011. Analytical Constraints on the Measurement of the Sulfur Isotopic Composition and Concentration of Trace Sulfate in Phosphorites: Implications for Sulfur Isotope Studies of Carbonate and Phosphate Rocks. Geostandards and Geoanalytical Research, 35(2): 161-174.

Guo, H. et al., 2015. Sulfur isotope composition of carbonate-associated sulfate from the Mesoproterozoic Jixian Group, North China: Implications for the marine sulfur cycle. Precambrian Research, 266: 319-336.

He, T. et al., 2020. An enormous sulfur isotope excursion indicates marine anoxia during the end-Triassic mass extinction. Sci Adv, 6(37): eabb6704.

He, T. et al., 2019. Possible links between extreme oxygen perturbations and the Cambrian radiation of animals. Nat Geosci, 12(6): 468-474.

Hoefs, J., 2015. Stable Isotope Geochemistry. Springer.

Hurtgen, M.T., Arthur, M.A., Suits, N.S., Kaufman, A.J., 2002. The sulfur isotopic composition of Neoproterozoic seawater sulfate: implications for a snowball Earth? Earth and Planetary Science Letters, 203(1): 413-429(1): 413-429.

Izon, G. et al., 2015. Multiple oscillations in Neoarchaean atmospheric chemistry. Earth and Planetary Science Letters, 431: 264-273.

Kampschulte, A., Bruckschen, P., Strauss, H., 2001. The sulphur isotopic composition of trace sulphates in Carboniferous brachiopods: implications for coeval seawater, correlation with other geochemical cycles and isotope stratigraphy. Chemical Geology, 175(1-2): 149-173.

Kampschulte, A., Strauss, H., 1998. The isotopic composition of trace sulphates in Paleozoic biogenic carbonates: implications for coeval seawater and geochemical cycles. Min. Mag. A, 62: 744-745.

Kampschulte, A., Strauss, H., 2004. The sulfur isotopic evolution of Phanerozoic seawater based on the analysis of structurally substituted sulfate in carbonates. Chemical Geology, 204(3-4): 255-286.

Kaufman, A.J., Knoll, A.H., 1995. Neoproterozoic variations in the C-isotopic composition of seawater: stratigraphic and biogeochemical implications. Precambrian Res, 73(1-4): 27-49.

Lan, Z. et al., 2019. An integrated chemostratigraphic (δ13C-δ18O-87Sr/86Sr-δ15N) study of the Doushantuo Formation in western Hubei Province, South China. Precambrian Research, 320: 232-252.

Li, C. et al., 2017. Uncovering the spatial heterogeneity of Ediacaran carbon cycling. Geobiology, 15(2): 211-224.

Li, C. et al., 2010. A stratified redox model for the Ediacaran ocean. Science, 328(5974): 80-3.

Lomans, B., Van der Drift, C., Pol, A., Den Camp, H.O., 2002. Microbial cycling of volatile organic sulfur compounds. Cellular and Molecular Life Sciences CMLS, 59(4): 575-588.

Loyd, S.J., Berelson, W.M., Lyons, T.W., Hammond, D.E., Corsetti, F.A., 2012a. Constraining pathways of microbial mediation for carbonate concretions of the Miocene Monterey Formation using carbonate-associated sulfate. Geochimica et Cosmochimica Acta, 78: 77-98.

Loyd, S.J. et al., 2012b. Sustained low marine sulfate concentrations from the Neoproterozoic to the Cambrian: Insights from carbonates of northwestern Mexico and eastern California. Earth and Planetary Science Letters, 339: 79-94.

Lu, M. et al., 2013. The DOUNCE event at the top of the Ediacaran Doushantuo Formation, South China: Broad stratigraphic occurrence and non-diagenetic origin. Precambrian Research, 225: 86-109.

Ma, H. et al., 2021. Sulfur and oxygen isotopic compositions of carbonate associated sulfate (CAS) of Cambrian ribbon rocks: Implications for the constraints on using CAS to reconstruct seawater sulfate sulfur isotopic compositions. Chemical Geology, 580.

Marenco, P.J., Corsetti, F.A., Hammond, D.E., Kaufman, A.J., Bottjer, D.J., 2008a. Oxidation of pyrite during extraction of carbonate associated sulfate. Chemical Geology, 247(1-2): 124-132.

Marenco, P.J., Corsetti, F.A., Kaufman, A.J., Bottjer, D.J., 2008b. Environmental and diagenetic variations in carbonate associated sulfate: An investigation of CAS in the Lower Triassic of the western USA. Geochimica et Cosmochimica Acta, 72(6): 1570-1582.

Markovic, S., Paytan, A., Li, H., Wortmann, U.G., 2016. A revised seawater sulfate oxygen isotope record for the last 4 Myr. Geochimica et Cosmochimica Acta, 175: 239-251.

McFadden, K.A. et al., 2008. Pulsed oxidation and biological evolution in the Ediacaran Doushantuo Formation. Proc Natl Acad Sci U S A, 105(9): 3197-202.

Newton, R.J., Pevitt, E.L., Wignall, P.B., Bottrell, S.H., 2004. Large shifts in the isotopic composition of seawater sulphate across the Permo–Triassic boundary in northern Italy. Earth and Planetary Science Letters, 218(3-4): 331-345.

Ohkouchi, N. et al., 1999. Sulfur isotope records around Livello Bonarelli (northern Apennines, Italy) black shale at the Cenomanian-Turonian boundary. Geology, 27(6): 535-538.

Peng, Y. et al., 2014. Widespread contamination of carbonate-associated sulfate by present-day secondary atmospheric sulfate: Evidence from triple oxygen isotopes. Geology, 42(9): 815-818.

Present, T.M., Paris, G., Burke, A., Fischer, W.W., Adkins, J.F., 2015. Large Carbonate Associated Sulfate isotopic variability between brachiopods, micrite, and other sedimentary components in Late Ordovician strata. Earth and Planetary Science Letters, 432: 187-198.

Rennie, V.C., Turchyn, A.V., 2014. The preservation of $\delta SSO434$ and $\delta OSO418$ in carbonate-associated sulfate during marine diagenesis: A 25 Myr test case using marine sediments. 395: 13-23.

Ries, J.B., Fike, D.A., Grotzinger, J.P., Pratt, L.M., Lyons, T.W., 2009. Superheavy pyrite ($\delta34Spyr > \delta34SCAS$) in the terminal Proterozoic Nama Group, southern Namibia: A consequence of low seawater sulfate at the dawn of animal life. Geology, 37(8): 743-746.

Shen, B. et al., 2011. Carbon, sulfur, and oxygen isotope evidence for a strong depth gradient and oceanic oxidation after the Ediacaran Hankalchough glaciation. Geochimica et Cosmochimica Acta, 75(5): 1357-1373.

Shen, B. et al., 2008. Stratification and mixing of a post-glacial Neoproterozoic ocean: evidence from carbon and sulfur isotopes in a cap dolostone from northwest China. Earth and Planetary Science Letters, 265(1-2): 209-228.

Shi, W. et al., 2018. Sulfur isotope evidence for transient marine-shelf oxidation during the Ediacaran Shuram Excursion. Geology, 46(3): 267-270.

Shields, G.A., Strauss, H., Howe, S.S., Siegmund, H., 1999. Sulphur isotope compositions of

sedimentary phosphorites from the basal Cambrian of China: implications for Neoproterozoic-Cambrian biogeochemical cycling. Journal of the Geological Society, 156(5): 943-955.

Siedenberg, K., Strauss, H., Podlaha, O., van den Boorn, S., 2018. Multiple sulfur isotopes (δ34S, Δ33S) of organic sulfur and pyrite from Late Cretaceous to Early Eocene oil shales in Jordan. Organic Geochemistry, 125: 29-40.

Sim, M.S., Bosak, T., Ono, S., 2011. Large sulfur isotope fractionation does not require disproportionation. Science, 333(6038): 74-77.

Staudt, W.J., Schoonen, M.A., 1995. Sulfate incorporation into sedimentary carbonates. ACS Publications.

Theiling, B.P., Coleman, M., 2015. Refining the extraction methodology of carbonate associated sulfate: Evidence from synthetic and natural carbonate samples. Chemical Geology, 411: 36-48.

Thompson, C.K., Kah, L.C., 2012. Sulfur isotope evidence for widespread euxinia and a fluctuating oxycline in Early to Middle Ordovician greenhouse oceans. Palaeogeography, Palaeoclimatology, Palaeoecology, 313: 189-214.

Tostevin, R. et al., 2017. Constraints on the late Ediacaran sulfur cycle from carbonate associated sulfate. Precambrian Research, 290: 113-125.

Toyama, K., Paytan, A., Sawada, K., Hasegawa, T., 2020. Sulfur isotope ratios in co-occurring barite and carbonate from Eocene sediments: A comparison study. Chemical Geology, 535.

Veizer, J., 1983. Trace elements and isotopes in sedimentary carbonates. 11: 265-300.

Wang, P. et al., 2019. Large accumulations of 34S-enriched pyrite in a low-sulfate marine basin: The Sturtian Nanhua Basin, South China. Precambrian Research, 335.

Wei, H., Tang, Z., Qiu, Z., Yan, D., Bai, M., 2020. Formation of large carbonate concretions in black cherts in the Gufeng Formation (Guadalupian) at Enshi, South China. Geobiology, 18(1): 14-30.

Werne, J.P., Lyons, T.W., Hollander, D.J., Formolo, M.J., Sinninghe Damsté, J.S., 2003. Reduced sulfur in euxinic sediments of the Cariaco Basin: sulfur isotope constraints on organic sulfur formation. Chemical Geology, 195(1-4): 159-179.

Wood, R.A. et al., 2015. Dynamic redox conditions control late Ediacaran metazoan ecosystems in the Nama Group, Namibia. Precambrian Research, 261: 252-271.

Wotte, T., Shields-Zhou, G.A., Strauss, H., 2012. Carbonate-associated sulfate: Experimental comparisons of common extraction methods and recommendations toward a standard analytical protocol. Chemical Geology, 326-327: 132-144.

Wotte, T., Strauss, H., Sundberg, F.A., 2011. Carbon and sulfur isotopes from the Cambrian Series 2–Cambrian Series 3 of Laurentia and Siberia. Museum of Northern Arizona Bulletin, 67: 43-63.

Wynn, P.M., Fairchild, I.J., Baker, A., Baldini, J.U.L., McDermott, F., 2008. Isotopic archives of sulphate in speleothems. Geochimica et Cosmochimica Acta, 72(10): 2465-2477.

Xiao, S. et al., 2012. Integrated chemostratigraphy of the Doushantuo Formation at the northern Xiaofenghe section (Yangtze Gorges, South China) and its implication for Ediacaran stratigraphic correlation and ocean redox models. Precambrian Research, 192-195: 125-141.

Zerkle, A.L., Claire, M.W., Domagal-Goldman, S.D., Farquhar, J., Poulton, S.W., 2012. A bistable organic-rich atmosphere on the Neoarchaean Earth. Nature Geoscience, 5(5): 359-363.

Zhang, X., Zhou, X., Hu, D., 2020. High-resolution paired carbon isotopic records from the Meishucun section in South China: Implications for carbon cycling and environmental changes during the Ediacaran-Cambrian transition. Precambrian Research, 337.

Experimental comparisons of carbonate-associated sulfate extraction methods

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Supplementary Information:

Table S1. Elemental concentrations of the acid-soluble fractions of the samples measured by ICP-OES. Carbonate content (%): weight percentages of acid-leachable content ($CaCO_3$ + $MgCO_3$).

Sample	Ca (%)	Mg (ppm)	Al (ppm)	K (ppm)	Fe (ppm)	Mn (ppm)	Sr (ppm)	Mn/Sr	Mg/Ca	Sr/Ca	Fe/Ca	Carbonate content (%)
QLKS18	20.14	122301	154.37	43.42	38.59	155.81	43.42	3.59	0.61	0.00022	0.00019	93.16
QLKS6	36.65	11917.94	47.71	23.85	4.77	32.92	1125.95	0.029	0.033	0.0031	0.000013	95.80
CC3	30.65	8433.85	121.60	325.88	525.29	14.11	3706.23	0.0039	0.028	0.012	0.0017	79.57
LHA6.5	33.97	3398.95	130.19	91.19	1940.90	333.85	207.06	1.61	0.010	0.00061	0.0057	86.11
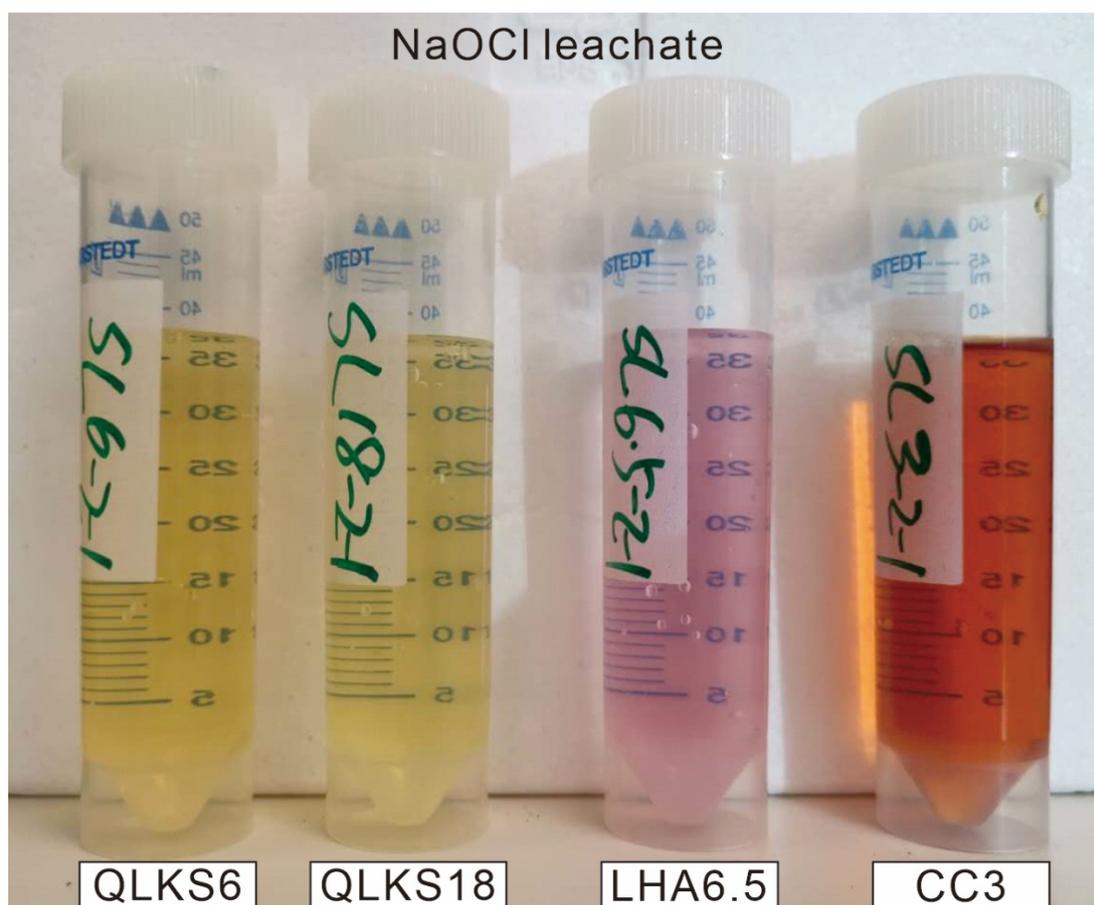
Fig. S1. NaOCl leachate with pink to brown colour showing the pyrite and possibly TOC has been oxidised.
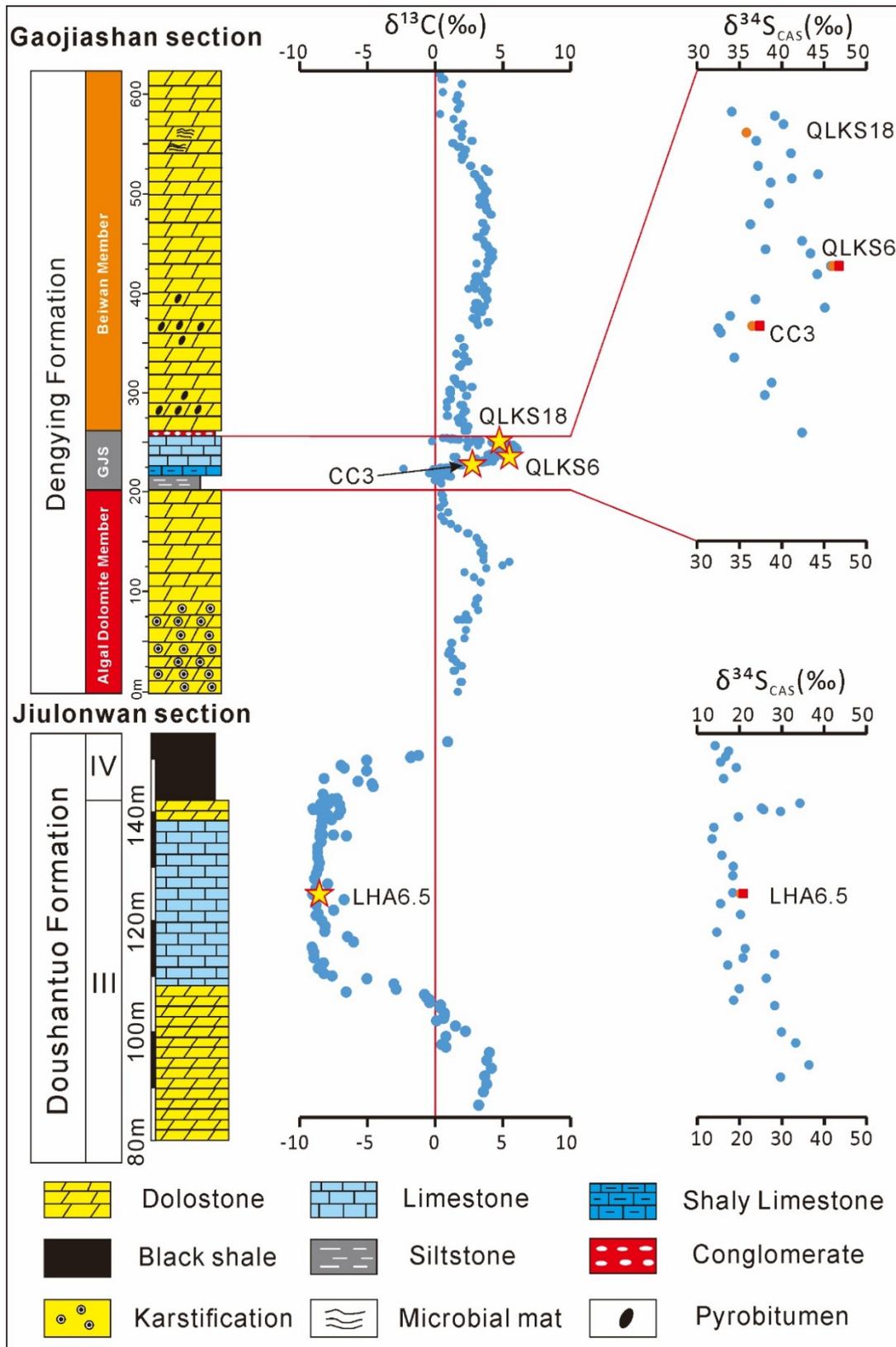
Fig. S2. Comparison of the published δ³⁴S$_{CAS}$ (‰) and the data from this study using method 1 (orange circles) and method 3 (red square). Sample LHA6.5 is from the Lianghong section. Sample CC3 is from the Zhimaping section. Sample QLKS6 and QLKS18 are from the Qinglinkou section. The blue dots are published data from (Cui et al., 2016; Shi et al., 2018). GJS = Gaojiashan Member.
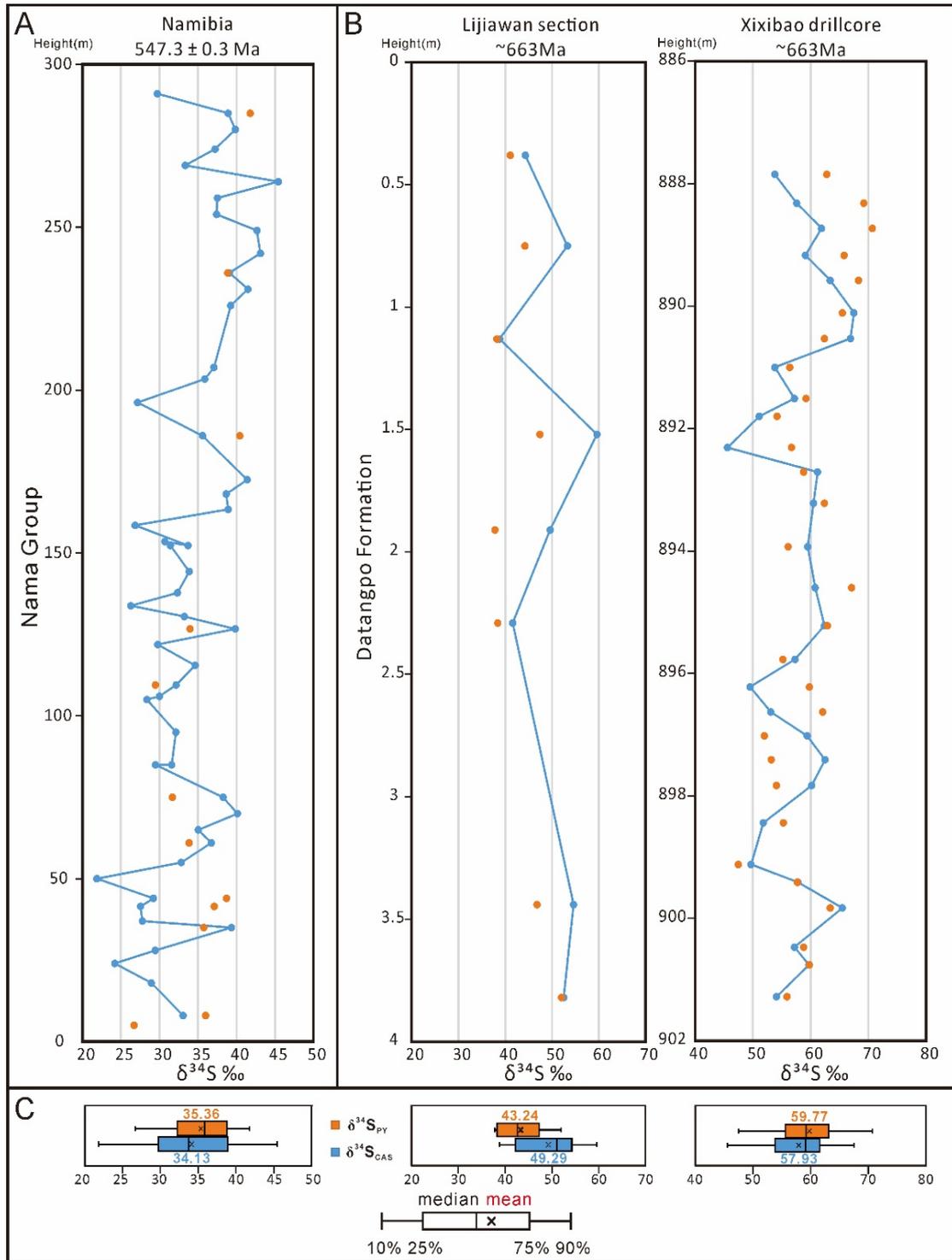
Fig. S3. Comparison of published $\delta^{34}S_{CAS}$ (blue color) and $\delta^{34}S_{PY}$ (orange color) data measured from 'superheavy pyrite' sections. A: Nama Group (~547.3 ± 0.3 Ma) data from (Tostevin et al., 2017). B: Datangpo Formation (662.9 ± 4.3 Ma to 654.5 ± 3.8 Ma) data from (Wang et al., 2019a). C: Box plots of corresponding data. The black lines and cross symbols in each box show the median and mean values, respectively.

Diagenetic Evaluation

Samples with calcium carbonate content >70% and Mg/Ca <0.025 are shown in Fig. S4-A as limestones (LM), calcium carbonate content >70% and Mg/Ca >0.025 are dolomitic limestones (DL) and calcium carbonate content <70% are dolostones (DM). On this basis, Fig. S4-A shows that sample QLKS18 is near stoichiometric dolomite, while the others are limestones.

Cross-plots of Mn/Sr, Mg/Ca, Sr/Ca, and Fe/Ca are commonly used to evaluate the extent of diagenetic alteration, as dissolution and recrystallisation can raise Mn, Fe, and Zn contents and lower Sr, Na contents (Bartley et al., 1998; Kaufman and Knoll, 1995; Lan et al., 2019). Mn/Sr lower than 2, Sr/Ca higher than 0.001, Mg/Ca lower than 0.257 and Fe/Ca lower than 0.01 indicate that the limestone samples in this study have undergone limited diagenetic alteration (Fig. S4). The low CAS yield from sample QLKS18 is perhaps unsurprising due to dolomitisation, which typically occurs under sulphate-reducing conditions (Fichtner et al., 2017; Staudt and Schoonen, 1995; Swart, 2015); however, we note that several published studies report dolomite CAS isotopic data, implying that carbonate-associated sulfate can withstand burial diagenesis and potentially record the paleo seawater $\delta^{34}S_{CAS}$ signature (Fichtner et al., 2017; Guo et al., 2015; Shi et al., 2018; Tostevin et al., 2017). Although we tried several times with sample QLKS18, we failed to get enough $BaSO_4$ precipitate for isotopic analysis.
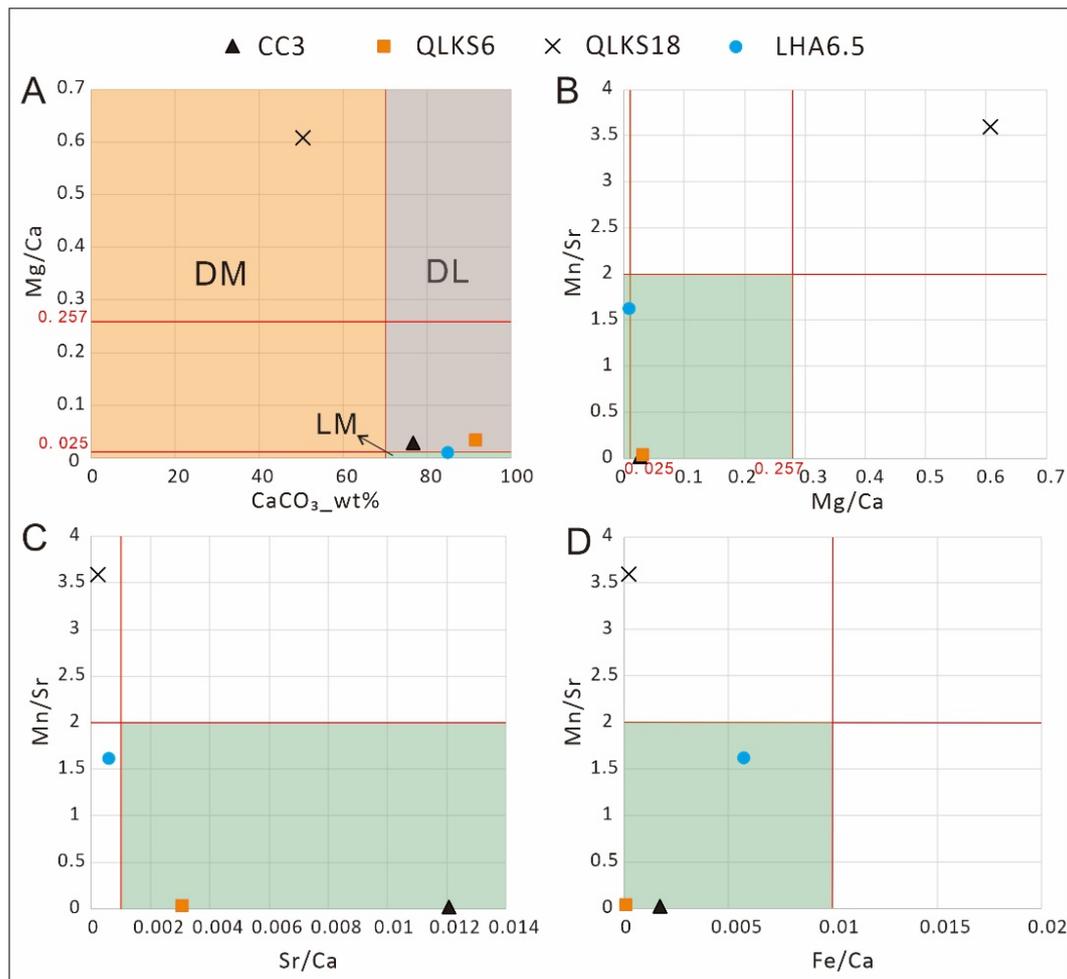
Fig. S4.: Cross-plot of the selected samples. DM: dolomite (calcium carbonate content < 70%); DL: dolomitic limestone (calcium carbonate content >70%; Mg/Ca > 0.025); LM: limestone (calcium carbonate content >70%; Mg/Ca < 0.025). Thresholds for the green shaded area (best-preserved samples) are Mn/Sr < 2, Sr/Ca > 0.001, Mg/Ca < 0.257 and Fe/Ca < 0.01.

Sample Descriptions
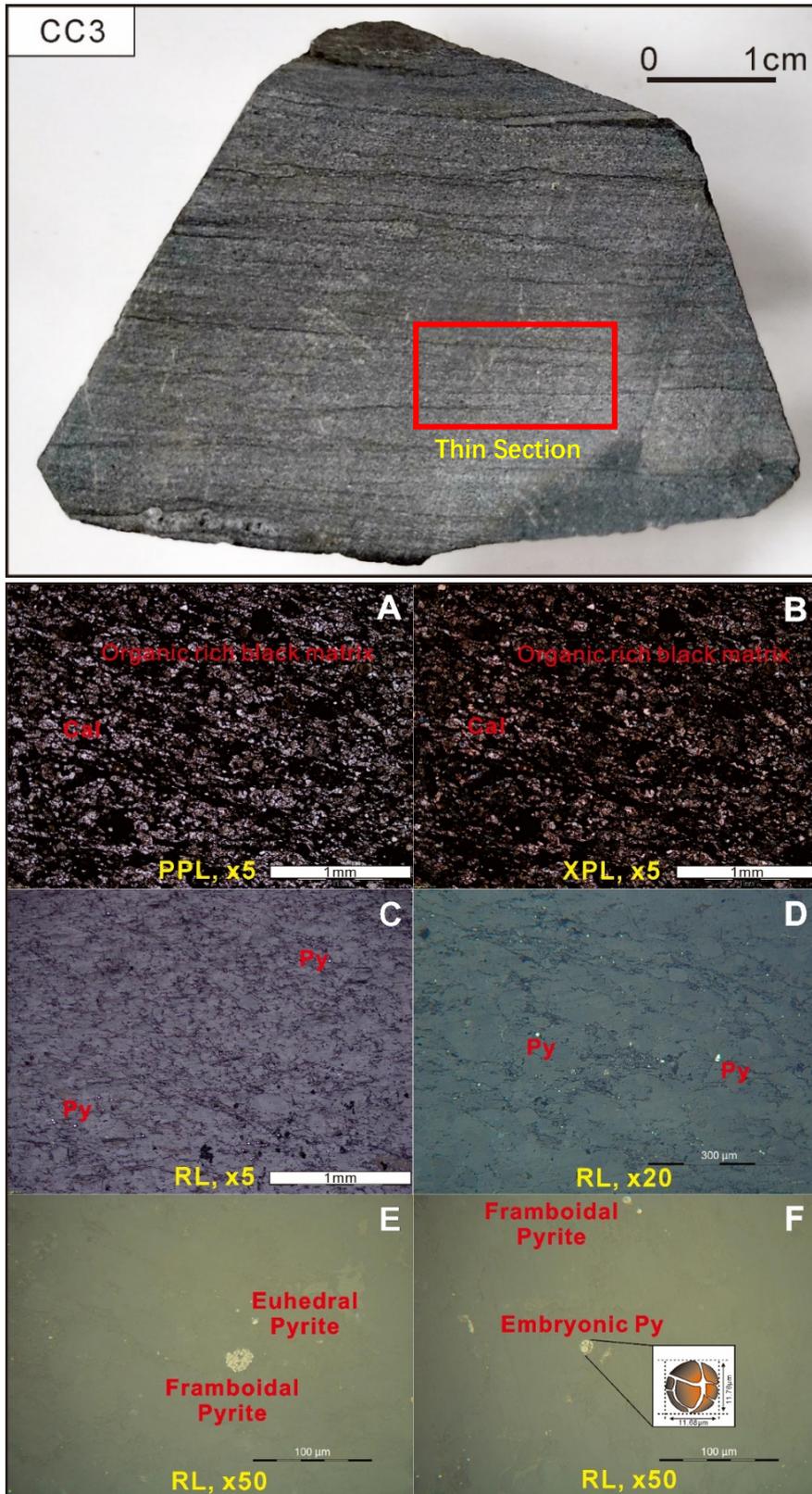
Fig. S5. Sample CC3: Dark thin banded micritic limestone rock rich in organic matter. Microphotographs show the rock is dark grey banded fine-grained limestone (grain size ~0.028mm). A: Sparry calcite grains (PPL, x5). B: Organic-rich black mud matrix (XPL, x5). C: Seminated pyrite (RL, x5). D: Euhedral pyrite (RL, x20). E: Framboidal pyrite (RL, x50). F: Pyrite with the shape of a metazoan (RL, x50). PPL = plane-polarized light; XPL = cross-polarized light; RL: reflected light; Py = pyrite; Cal = Calcite.
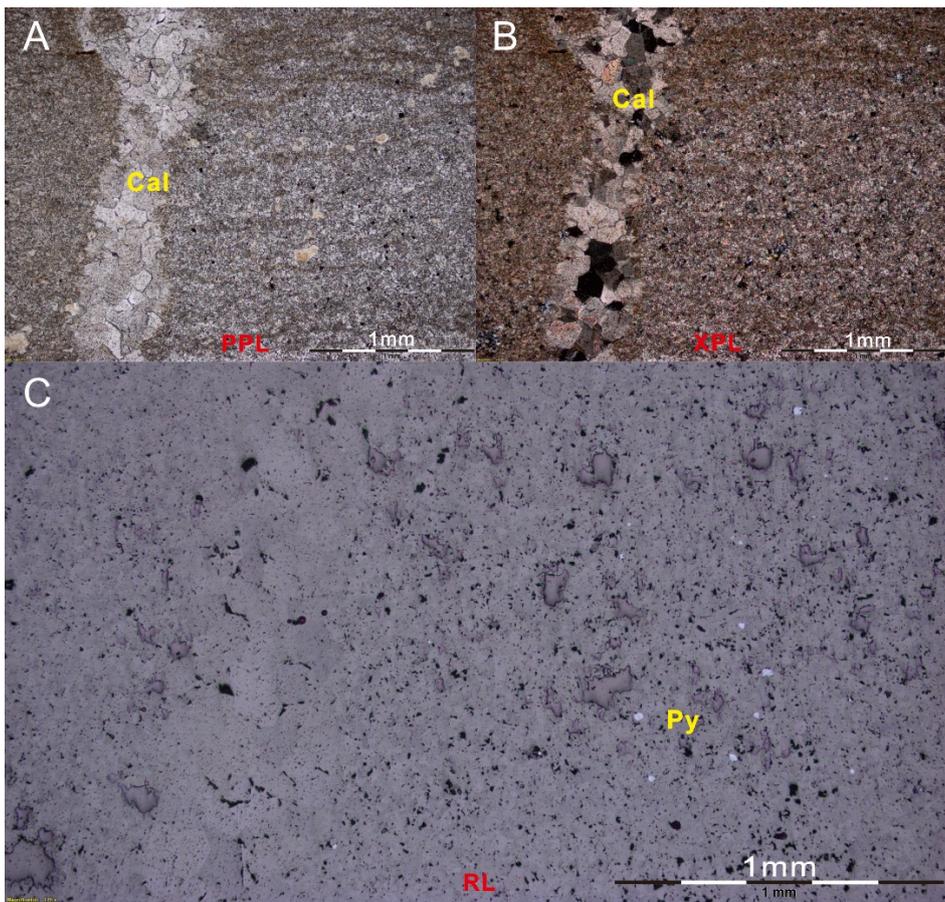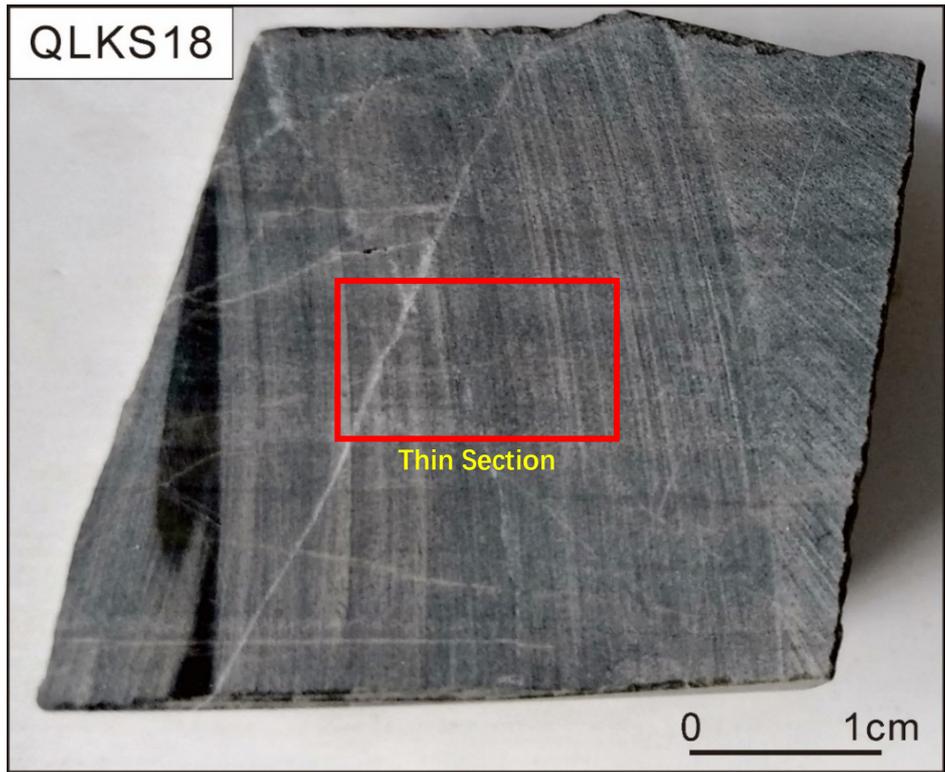
Fig. S6. Sample QLKS18: The rock here is a fine-grained dolomitic limestone rock with some white calcite veins cut through. Photomicrograph shows the rock is limestone (grain size ~0.019mm). A: Sparry calcite grains (PPL, x5). B: Calcite dyke cut through the thin section (XPL, x5). C: Seminated euhedral pyrite (RL, x5).
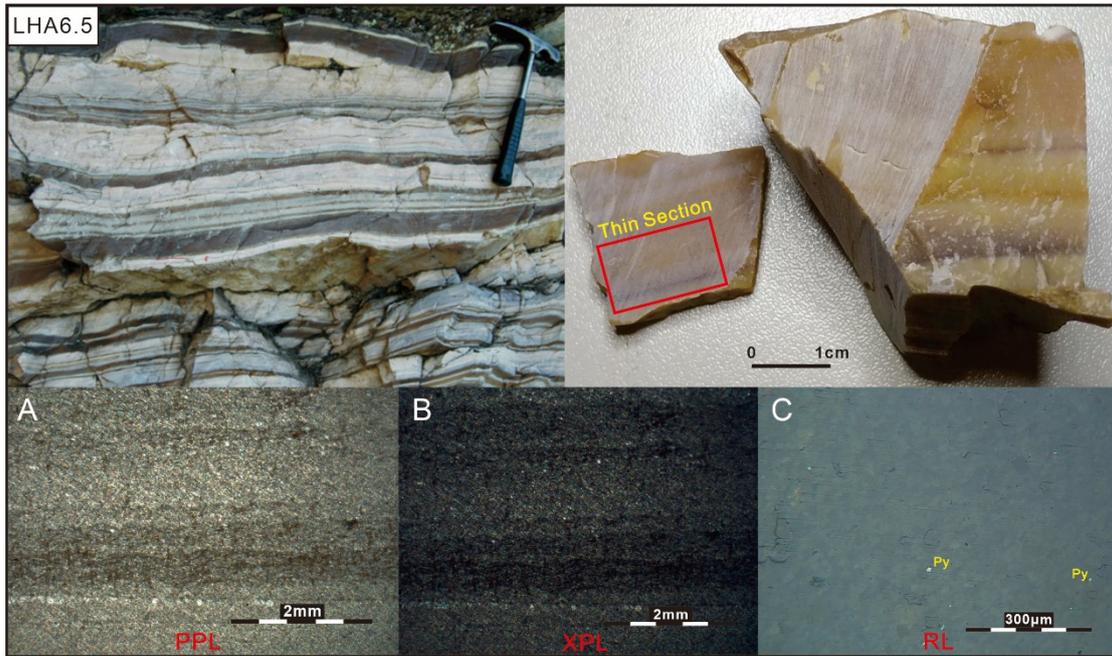
Fig. S7. Sample LHA6.5: The rock here is a grey fine-grained banded limestone (grain size ~0.022mm) from Doushantuo member III, Lianghong section, Southern Sichuan. A-B: thin beded limestone (x2.5). C: euhedral pyrite (RL, x20)
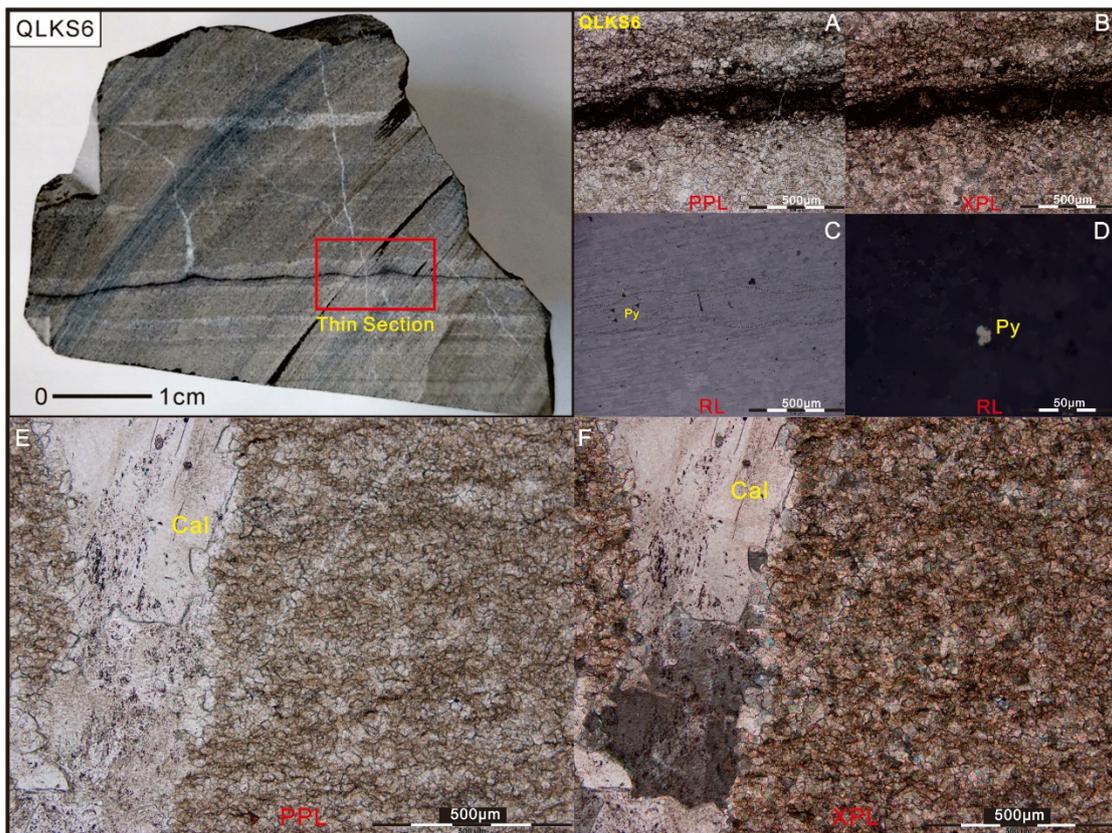
Fig. S8. Sample QLKS6: This sample is dark grey thinly laminated micritic limestone rock (grain size ~0.027mm) with white calcite veins cut through. Sample from Shibantan member, Dengying Formation, Qinglinkou Section. Microphotographs show the rock is fine-grained limestone. A-B: Organic rich thin layer interbedded with the carbonate layers (x10). C: Seminated pyrite (RL, x10). D: Framboidal pyrite (RL, x100). E-F: Sparry calcite grains inside the calcite vein (x10).

References

Bartley, J.K., Pope, M., Knoll, A.H., Semikhatov, M.A., Petrov, P., 1998. A Vendian-Cambrian boundary succession from the northwestern margin of the Siberian Platform: stratigraphy, palaeontology, chemostratigraphy and correlation. Geol Mag, 135(4): 473-94.

Cui, H. et al., 2016. Environmental context for the terminal Ediacaran biomineralization of animals. Geobiology, 14(4): 344-63.

Fichtner, V. et al., 2017. Diagenesis of carbonate associated sulfate. Chemical Geology, 463: 61-75.

Guo, H. et al., 2015. Sulfur isotope composition of carbonate-associated sulfate from the Mesoproterozoic Jixian Group, North China: Implications for the marine sulfur cycle. Precambrian Research, 266: 319-336.

Kaufman, A.J., Knoll, A.H., 1995. Neoproterozoic variations in the C-isotopic composition of seawater: stratigraphic and biogeochemical implications. Precambrian Res, 73(1-4): 27-49.

Lan, Z. et al., 2019. An integrated chemostratigraphic (δ13C-δ18O-87Sr/86Sr-δ15N) study of the Doushantuo Formation in western Hubei Province, South China. Precambrian Research, 320: 232-252.

Shi, W. et al., 2018. Sulfur isotope evidence for transient marine-shelf oxidation during the Ediacaran Shuram Excursion. Geology, 46(3): 267-270.

Staudt, W.J., Schoonen, M.A., 1995. Sulfate incorporation into sedimentary carbonates. ACS Publications.

Swart, P.K., 2015. The geochemistry of carbonate diagenesis: The past, present and future. Sedimentology, 62(5): 1233-1304.

Tostevin, R. et al., 2017. Constraints on the late Ediacaran sulfur cycle from carbonate associated sulfate. Precambrian Research, 290: 113-125.

Wotte, Strauss, Fugmann, Garbe-Schönberg, 2012, Geochemica et Cosmochemica Acta 85, 228-253.