Iron isotope fractionation during microbial dissimilatory iron oxide reduction in simulated Archaean seawater

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ABSTRACT

The largest Fe isotope excursion yet measured in marine sedimentary rocks occurs in shales, carbonates, and banded iron formations of Neoarchaean and Paleoproterozoic age. The results of field and laboratory studies suggest a potential role for microbial dissimilatory iron reduction (DIR) in producing this excursion. However, most experimental studies of Fe isotope fractionation during DIR have been conducted in simple geochemical systems, using pure Fe(III) oxide substrates that are not direct analogues to phases likely to have been present in Precambrian marine environments. In this study, Fe isotope fractionation was investigated during microbial reduction of an amorphous Fe(III) oxide-silica coprecipitate in anoxic, high-silica, low-sulphate artificial Archaean seawater at 30 °C to determine if such conditions alter the extent of reduction or isotopic fractionations relative to those observed in simple systems. The Fe(III)-Si coprecipitate was highly reducible (c. 80% reduction) in the presence of excess acetate. The coprecipitate did not undergo phase conversion (e.g. to green rust, magnetite or siderite) during reduction. Iron isotope fractionations suggest that rapid and near-complete isotope exchange took place among all Fe(II) and Fe(III) components, in contrast to previous work on goethite and hematite, where exchange was limited to the outer few atom layers of the substrate. Large quantities of low- δ^{56} Fe Fe(II) (aqueous and solid phase) were produced during reduction of the Fe(III)-Si coprecipitate. These findings shed new light on DIR as a mechanism for producing Fe isotope variations observed in Neoarchaean and Paleoproterozoic marine sedimentary rocks.

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INTRODUCTION

Microbial dissimilatory iron reduction (DIR) couples reduction of Fe(III) oxides and hydroxides to the oxidation of organic matter or H₂. This metabolism is deeply rooted in the phylogenetic tree of life on Earth, suggesting that it evolved early in Earth's history (Vargas et al., 1998; Lovley, 2004). DIR has been hypothesized to have played a role in Fe redox cycling in Precambrian marine environments (Canfield et al., 2006), potentially leaving behind geological (Walker, 1984; Nealson & Myers, 1990; Konhauser et al., 2002, 2005; Fischer & Knoll, 2009) and isotopic signatures (Beard et al., 2003; Johnson et al., 2008a; Fischer et al., 2009; Heimann et al., 2010; Tangalos et al., 2010; Craddock & Dauphas, 2011). In modern natural environments, an Fe isotope fingerprint appears to be associated with DIR in the form of low δ^{56} Fe values for aqueous Fe(II) (Fe(II)_{aq}) (Severmann *et al.*, 2006, 2010; Fehr *et al.*, 2008; Teutsch et al., 2009; Tangalos et al., 2010), representing a significant flux of isotopically light Fe(II)_{aq} to the oceans (Severmann *et al.*, 2006, 2010). Low δ^{56} Fe values for Fe(II)_{aq} may also be produced by extensive oxide precipitation (Rouxel et al., 2008; Severmann et al., 2010), where δ^{56} Fe values for residual Fe(II)_{aq} decline rapidly as aqueous Fe concentrations become very low (>80% oxidation). However, previous discussions of DIR versus oxide precipitation as mechanisms for generation of low- δ^{56} Fe values in the rock record have highlighted the importance of considering the relative quantities of Fe produced by these processes (Johnson et al., 2008b), where it has been argued that DIR is a more plausible mechanism to provide large inventories of isotopically light Fe(II) in Fe-rich rocks. Previous experimental work on Fe isotope fractionation produced by DIR has used pure cultures and conditions analogous to freshwater systems (Icopini et al., 2004; Crosby et al., 2005, 2007; Johnson et al., 2005), and to date only one study has looked at the effects of complex media such as the presence of dissolved silica (Wu et al., 2009). No experimental work has investigated DIR-driven Fe isotope fractionation under conditions analogous to natural

marine systems, especially those present in the Archaean and Paleoproterozoic time.

Precambrian ocean chemistry was different from that of modern oceans, due to the primitive atmosphere and, importantly, different controls on the silicon budget. The Archaean oceans are thought to have had low sulphate contents (e.g. Habicht et al., 2002) reflecting generally low atmospheric O₂ concentrations. In addition, seawater in the Proterozoic and Archaean was probably saturated with respect to amorphous silica (Siever, 1992; Maliva et al., 2005) because of the lack of silica-secreting organisms in the Precambrian. It is therefore possible that Fe(III) oxide-silica interactions impacted DIR in the Precambrian in ways not reflected in previous experiments that used simple systems. Aqueous silica exists as monomeric, dimeric or polymeric forms depending on the amount of silica present and the pH of the system (Sigg & Stumm, 1980; Swedlund & Webster, 1999; Davis et al., 2002). At low pH values, monomeric silica dominates, whereas silica polymerization is widespread at alkaline pH (Iler & Knovel, 1979; Svennson et al., 1986; Davis et al., 2001; Hiemstra et al., 2007). Silica polymerization is hypothesized to interfere with DIR at high pH, based on experiments that used hematite as the terminal electron acceptor (Wu et al., 2009). High-silica concentrations may also affect transformation of ferrihydrite to crystalline mineral phases, as the presence of dissolved silica generally inhibits phase transformations (Cornell & Giovanoli, 1987; Cornell et al., 1987; Mayer & Jarrell, 1996; Jones et al., 2009).

In this contribution, we report on Fe isotope fractionation coupled to DIR under conditions that simulated key aspects of Archaean marine conditions, through use of an artificial seawater medium under anaerobic conditions that had low sulphate concentration and high dissolved silica, and use of an amorphous Fe(III) oxide-silica coprecipitate designed to provide a better analogue to Fe(III) phases that probably existed in the Archaean oceans relative to pure Fe(III) oxides (Konhauser et al., 2007; Fischer & Knoll, 2009). This work demonstrates production of large quantities of Fe(II) in aqueous and solid phases that have distinct Fe isotope compositions, thus providing support for DIR as a means for producing large quantities of low- δ^{56} Fe Fe(II), as well as fine-scale Fe isotope heterogeneity in solids as seen in the Fe isotope record in Precambrian marine sedimentary rocks and banded iron formations.

MATERIALS AND METHODS

Experimental overview

Dissimilatory iron reduction experiments were conducted under simulated Archaean conditions using an Fe(III)–Si coprecipitate as the electron acceptor and solution chemistry that mimicked that of an anaerobic low-sulphate, Si-rich Archaean seawater. These conditions are similar to those used by Wu *et al.* (2009), who examined DIR in silica-saturated conditions using hematite as an electron acceptor. Given that the experimental medium was at saturation with respect to silica, net mobilization of silica during microbial reduction, such as that proposed in Fischer & Knoll (2009), was not expected, although it is certainly possible that silica exchange between solid and fluid occurred. Another major goal of this work was to track Fe isotope fractionation as a function of the extent of DIR, which was achieved by varying the amount of organic carbon in the culture medium relative to a fixed electron acceptor concentration.

Electron acceptor

The Fe(III)–Si coprecipitate was created through modification of the procedure for synthesis of goethite from aqueous Fe(II) (Schwertmann & Cornell, 1991). A solution of 100 mM NaHCO₃, 100 mM Na₂SiO₃·9H₂O and 50 mM FeCl₂·4H₂O was prepared and allowed to oxidize in open exchange with the atmosphere. After 2 weeks of continuous stirring or shaking, Fe(III) accounted for 95–100% of total Fe as determined by 0.5 M HCl extraction and *Ferrozine* analysis (see below). The solid was centrifuged and rinsed with distilled water to remove excess salts.

Aqueous medium

Artificial Archaean seawater (AAS) (Table S1) was prepared based on the artificial seawater recipe of Kester et al. (1967), modified through elimination of Na2SO4 and addition of 0.608 g L⁻¹ Na₂SiO₃·9H₂O to account for high dissolved silica content of the Precambrian oceans. The medium included vitamins and trace elements (Lovley & Phillips, 1988), the latter of which added 200 µM sulphate, comparable to values suggested for Archaean seawater (Habicht et al., 2002). Typical DIR growth medium contains high concentrations of phosphate (≥ 4 mM) (Lovley & Phillips, 1988); the amount of phosphate was reduced to 0.1 mM in AAS in order to avoid production of large quantities of Fe(II)-phosphate phases (e.g. vivianite). The Fe(III)-Si coprecipitate was added (from a concentrated stock slurry) to a final concentration of 95 mmol Fe L⁻¹. The medium was buffered with 30 mm NaHCO₃, and rendered anoxic by bubbling with a 50:50 mix of O₂-free N₂:CO₂ (passed through a reduced copper column to remove any traces of O_2), resulting in a final pH of approximately 6.5. After bubbling, the bottles were capped with a thick rubber stopper to prevent intrusion of atmospheric O₂. The elevated CO₂ was intended to reflect the higher atmospheric CO₂ concentrations of the Archaean atmosphere (Rye et al., 1995; Ohmoto et al., 2004, 2006; Sheldon, 2006), while producing a pH within the physiological limits of modern marine dissimilatory iron-reducing bacteria (6.5–8.5; Brenner et al., 2005). The slightly subneutral pH also minimized potential silica polymerization on Fe(III) oxide surfaces

(Sigg & Stumm, 1980; Davis *et al.*, 2001), which at elevated pH may inhibit DIR (Wu *et al.*, 2009).

Before inoculation, 1.3 mM of FeCl₂ was added as a reducing agent (Shelobolina *et al.*, 2003). To avoid crystallization of the electron acceptor, the medium was not autoclaved. The lack of autoclaving had no effect on bacterial growth compared to previous transfers using similar autoclaved medium, and because the DIR experiments were conducted with a mixed culture (see next section) rather than a pure culture, culture purity was not a concern.

DIR enrichment culture

Many previous studies of DIR have employed pure cultures such as Geobacter sp. or Shewanella sp. (Lovley & Phillips, 1987; Lovley et al., 1987; Nealson & Myers, 1990) in nonmarine freshwater culture medium. Although many modern marine microbes are capable of DIR (Lovley et al., 2004), the experiments reported here involved novel high-silica aqueous chemical conditions, and the need for a microbial culture capable of reducing a Fe(III)-Si coprecipitate. To this end, an enrichment culture was initiated and propagated in AAS medium with the Fe(III)-Si coprecipitate as the electron acceptor. The enrichment was derived from marine mud-flat surface sediment obtained from the shores of Sausalito, CA. The AAS-based culture medium contained 100 mmol L^{-1} of Fe(III)-Si coprecipitate and 20 mM acetate. The culture was transferred when the 0.5 M HCl extractable Fe(II) content reached 60-80% of total Fe in the medium. After each transfer, an aliquot of that generation was frozen at -80 °C for DNA extraction and phylogenetic analysis.

DNA was isolated using the Mo-Bio PowerSoil[®] (Mo-Bio, Carlsbad, CA, USA) DNA Isolation Kit. For denaturing gradient gel electrophoresis (DGGE) analysis, 16S rRNA genes were amplified using PCR primers 1055F and 1392R and established protocols (Ferris *et al.*, 1996). Visual inspection of DGGE gels indicated a stable culture had been established. After 15 generations, 16S rRNA genes were amplified with primers 27F and 338R and pyrosequencing was carried out through facilities at the University of Colorado in Boulder, CO (Hamady *et al.*, 2008).

Experiment configuration

Four experiments were conducted, using medium that contained 1, 5, 10 or 20 mM acetate, which produced molar C/Fe ratios of 0.08, 0.42, 0.84 and 1.68 respectively. A 10% (vol/vol) inoculum was introduced from identical medium that had limiting (1 mM) acetate. Duplicate reactors were prepared for each acetate concentration, and an uninoculated control reactor was sampled along with the inoculated cultures. All cultures were incubated at 30 °C in the dark. This temperature is in line with Archaean marine temperature estimates of 26–35 °C determined by oxygen isotopes in phosphate-containing strata from the Barberton Greenstone Belt (Blake *et al.*, 2010), although considerably lower than some proposed temperatures for the Archaean ocean (e.g. Knauth & Lowe, 2003).

Sampling and extraction procedures

All additions to or removals from the culture bottles were done using syringes which had been flushed with O_2 -free N_2 :CO₂. Subsamples (2 mL) were collected from each bottle and used to separate different pools of Fe for chemical and isotopic analysis. Samples were collected from shaken reactor vessels to ensure homogeneous sampling of the solid suspension. All extractions were done inside an anaerobic chamber (Coy Products, Grass Lake, MI, USA).

Fe(II) and total Fe were determined using 0.5 M HCl extraction, followed by *Ferrozine* analysis (Stookey, 1970) of Fe(II) and total Fe in the extract; Fe(III) contents were determined from the difference of Fe(II) and total Fe. The aqueous component was isolated through centrifugation, extraction and filtration of the supernatant through a 0.2- μ m filter, followed by addition of HCl to a final concentration of 0.5 M. Extraction of the aqueous component in this manner minimized any potential contamination by different Fe pools, although incomplete centrifugation and penetration through the 0.2- μ m filter may have resulted in slight (<0.10 μ mol) contamination with Fe from the bulk aliquot.

Dilute HCl extractions were done to isolate separate Fe pools that may have undergone isotopic exchange during DIR, following approaches in previous work (Crosby et al., 2005; Wu et al., 2009; Tangalos et al., 2010). In this study, we follow the same approach for investigating Fe isotope mass balance. Individual pools of Fe were extracted from the solid-phase Fe through two serial digestions. After removal of aqueous Fe, the solids underwent a 0.1 M HCl digestion for 15 min. The extracts were then centrifuged, and the supernatant filtered through a 0.2-µm filter. Tests were completed using 0.01, 0.05, 0.1, 0.15 and 0.25 M HCl and various extraction times ranging from 5 to 60 min to determine which extraction would produce the greatest amount of Fe(II) relative to Fe(III) in the extract. A partial dissolution (c. 10% of total Fe) with 0.1 M HCl for 15 min resulted in an extract that was 97-100% Fe(II). After the 0.1 м HCl digestion, the remaining Fe in the solid was completely dissolved in 0.5 M HCl before filtration through a 0.2-μm filer.

All samples were analysed in duplicate for Fe(II) and total Fe contents after extraction. Aqueous silica was determined in samples after filtration, and solid-phase silica was determined in the 0.1 and 0.5 M HCl extracts. Silica was analysed colourometrically (Clesceri *et al.*, 1989) and also by ICP–OES. Samples were collected from both duplicate reaction vessels for extraction, where replicate number 1 was used for Fe isotope analysis and solid-phase silica concentration, and replicate 2 was used for aqueous silica determination. In all experiments, microbial Fe(III) reduction had ceased by day 20. Solids were extracted in an anaerobic chamber, rinsed $3\times$ using anaerobic distilled water, and dried under a constant flush of O₂-free N₂. They were examined using a Rigaku Rapid II X-ray diffraction XRD (Madison, WI, USA) and Philips CM200 TEM (Madison, WI, USA).

Fe isotopic analysis

Iron isotope compositions were determined on purified Fe aliquots that were processed through anion-exchange chromatography (Beard *et al.*, 2003), followed by analysis by MC-ICP-MS (Micromass IsoProbe; Micromass Ltd., Wythenshave, Manchester, UK). Yields for 0.1 and 0.5 M HCl extractable components were $95 \pm 5\%$, as determined using colorimetry. However, due to matrix interference during *Ferrozine* measurement of the aqueous fraction, yields were instead determined using ion intensities during MC-ICP-MS analysis, and these averaged $90 \pm 10\%$. Complete yields were confirmed by Fe concentration determination, of the non-Fe fractions from ion-exchange chromatography.

Measured data are reported as the 56 Fe/ 54 Fe ratios relative to the average of igneous rocks using standard δ notation, in units of per mil (${}^{\circ}_{\circ\circ}$):

$$\delta^{56}$$
Fe = [(⁵⁶Fe/⁵⁴Fe)_{sample}/(⁵⁶Fe/⁵⁴Fe)_{IgRx} - 1] × 1000.

All δ^{56} Fe data relative to igneous rocks are reported in data tables in the Supporting Information, whereas in Tables S3 and S7, the δ^{56} Fe values have been normalized to a 'system' δ^{56} Fe value of 0.00% to facilitate comparison to other studies via the equation:

$$\delta^{56}$$
Fe_{norm} = δ^{56} Fe_{IgRxScale} + 0.53,

which reflects the δ^{56} Fe value of -0.53% for the Fe(III)–Si coprecipitate on the igneous rock scale (see raw isotope data). Isotopic data plotted in all figures have been normalized to a system value of zero. External precision of δ^{56} Fe values is estimated to be $\pm 0.06\%$ (2σ) based on replicate analysis of ultrapure standards, synthetic samples made with known Fe isotope composition iron and samples. The measured Fe isotope composition of the IRMM-014 Fe isotope standard was δ^{56} Fe = -0.08 ± 0.02 (2 SD, n = 5) relative to the igneous rock scale. Iron isotope compositions of additional inhouse standards are: HPS I: δ^{56} Fe = 0.49 ± 0.04 (2 SD, n = 6), HPS II: δ^{56} Fe = 0.44 ± 0.05 (2 SD, n = 3), J-M Fe: δ^{56} Fe = 0.25 ± 0.04 (2σ , n = 4) (Beard *et al.*, 2003). In total, 101 samples were analysed and 26 samples were duplicated with an average reproducibility of $\pm 0.06\%$.

The accuracy of the Fe isotope analyses was evaluated by analysis of five test solutions that contained 2 mL of AAS and 10, 20, 25, 50 or 75 µg of Fe of a known isotopic composition. After sample processing, the results were within error of the pure HPS-I, at δ^{56} Fe = 0.49 ± 0.04 (2 SD, *n* = 5).

Isotopic fractionation between two phases A and B is expressed using standard notation:

$$\Delta^{56} \mathrm{Fe}_{\mathrm{A-B}} = \delta^{56} \mathrm{Fe}_{\mathrm{A}} - \delta^{56} \mathrm{Fe}_{\mathrm{B}}$$

X-ray diffraction and transmission electron microscopy analysis

X-ray diffraction (XRD) analyses were carried out on solids collected before and after microbial reduction. Samples were dried under a stream of O_2 -free N_2 environment and placed in thin-wall glass capillaries. Diffraction data were acquired using a Rigaku Rapid II XRD system with a two-dimensional image plate detector (Mo K α radiation). The two-dimensional images were integrated to produce conventional patterns using Rigaku's 2DP software.

Solids before and after microbial reduction (20 mM acetate cultures) were examined with a FEI Titan 80-200 (FEI, Hillboro, OR, USA) aberration corrected scanning/transmission electron microscope equipped with a high resolution energydispersive X-ray spectroscopy (EDS) detector and Gatan image filtering system (Gatan Inc., Pleasanton, CA, USA), and operated at 200 kV. Samples were prepared by drying under a stream of O2-free N2 and stored under N2 until analysis. The solids were imaged in both transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) mode. EDS in STEM mode was used to determine whether or not Fe, Si and O were co-associated at the nanometre level as opposed to being present as separate Fe-O and Si-O containing phases; point analyses were made at 65 spots with an approximately 1-Å beam. Elemental mapping via electron energy loss spectroscopy (EELS) was used to determine whether Fe:O ratios were consistent within different regions of the coprecipitate. A three-window technique was used for background removal while acquiring the elemental maps for Fe and O.

RESULTS

Enrichment culture

Denaturing gradient gel electrophoresis analysis of 16S rRNA genes indicated that the microbial composition of the enrichment culture stabilized by the 11th generation (Fig. S1). Pyrosequencing of 16S rRNA genes from the 15th generation showed that the enrichment culture was dominated by organisms from the family *Desulfuromonaceae* (Table S4). Ninety-eight per cent (out of 1354 reads) were sequences corresponding to this one family within the *Deltaproteobacteria*. These organisms belong to the same order as *Geobacteraceae*, and are probably related to *Desulfuromonas acetoxidans*,

a marine acetate-oxidizing, Fe(III)-reducing relative of *Geobacter* (Roden & Lovley, 1993).

Fe(III)-Si coprecipitate structure and composition

X-ray diffraction (Fig. 1) and TEM with selected area electron diffraction (SAED) analysis (Fig. 2A) revealed that the Fe(III)–Si coprecipitate was an amorphous solid that had a typical particle size of <50 nm in diameter. EDS analysis in STEM mode showed no evidence of localized Fe- or Si-rich domains (data not shown), suggesting that the material was a homogeneous coprecipitate at the nanometre scale. Bulk dissolution using 0.5 \mbox{M} HCl, followed by analysis by ICP-OES indicated a molar Fe:Si ratio of 0.56 (Table S2), which is equivalent to a nominal stoichiometry of FeSi_(1.78)(OH)_{10.12}. For convenience, the coprecipitate will be simplified to a stoichiometry of FeSi₂(OH)₁₁ for the discussions below.

Isotopic homogeneity of the Fe(III)-Si coprecipitate was assessed by dissolving aliquots of the material using HCl of different strengths (10, 20, 50 or 100 mM) for 5 min and analysing the δ^{56} Fe values of the extracts. The most easily extractable component (10 mM HCl extraction) had a slightly higher δ^{56} Fe value than the least easily extractable component (Table S3). However, the maximum range in δ^{56} Fe values for all partial extractions was fairly small (0.49%). Thus, if this range in δ^{56} Fe values represents true isotopic heterogeneity of the Fe(III)-Si coprecipitate, it is negligible relative to the much larger range in isotopic fractionation observed in the DIR experiments (see below). Moreover, because the results shown below indicate that the Fe(III)-Si coprecipitate is highly exchangeable, it is possible that the small range in δ^{56} Fe values measured in the partial extractions reflects re-equilibration between residual Fe(III)-Si coprecipitate and dissolved Fe, and we consider this to be the most likely explanation for the small range in δ^{56} Fe values measured.



Fig. 1 XRD patterns for solids obtained from experiments after day 20; also shown is the XRD pattern for the starting Fe(III)–Si coprecipitate. No significant peaks are present, indicating no formation of crystalline mineral products during DIR.

Extent and end products of Fe(III) reduction

The extent of reduction of the Fe(III)–Si coprecipitate varied strongly as a function of acetate concentration. Cultures that contained 20 mM acetate reduced 79% of the coprecipitate by the end of the 20-day experiment. The 10 mM acetate culture reduced slightly less (76%), and the 1 and 5 mM acetate cultures reduced the least (15% and 36% respectively). The pH of the growth medium increased slightly to approximately 6.7 during bacterial reduction. XRD analysis of the reduction end products did not detect production of any crystalline phases during bacterial reduction (Fig. 1). TEM/SAED analysis of reduced (20 mM acetate cultures) solids showed that the coprecipitate remained amorphous during microbial reduction (Fig. 2B), although some minor localized zones of partial transformation to a primitive smectite-like phase (indicated by the very weak rings at 1.5, 2.5 and 4.5 Å in the SAED pattern)



Fig. 2 (A) TEM image and SAED pattern of unreduced Fe(III)–Si coprecipitate. The material is amorphous as indicated by the SAED pattern. (B) HRTEM image and SAED pattern of reduced (20 mm acetate) Fe(III)–Si coprecipitate. The reduced material showed evidence of localized zones of partial transformation to a primitive smectite-like phase (indicated by the white arrows) that were detectable by SAED (rings 1, 2 and 3, corresponding to 1.5, 2.5 and 4.5 Å diffraction lines respectively).



Fig. 3 Temporal variation in aqueous Si and Fe(II) concentrations. The grey bar indicates the amount of aqueous Si in the uninoculated control (2 mm).

were evident. Based on TEM analysis, these zones comprised <0.5% of the Fe(III)–Si coprecipitate after reduction, and therefore have no influence on the isotopic results. Multipoint EDS analysis under STEM mode showed that Fe, Si and O were co-associated at the nanometre level, and EELS mapping confirmed that Fe and O were essentially homogeneously distributed within the reduced solid (data not shown). These results indicate that microbial reduction of the Fe(III)–Si coprecipitate did not lead to formation of separate Fe–O and Si–O bearing phases, i.e. no discrete SiO₂ phases were produced.

Aqueous/solid-phase Fe(II) and Si partitioning

Aqueous Fe(II) concentrations produced by DIR in our experiments were generally $\leq 1 \text{ mM}$ (Fig. 3; Table S5). The low levels of Fe(II)_{aq} in the 1 and 5 mM acetate cultures reflect an overall lower level of Fe(III) reduction, as higher Fe(II)_{aq} contents were measured in cultures that had higher levels of acetate and a greater extent of reduction. The highest Fe(II)_{aq} levels were reached in all cultures between days 3 and 5, and these gradually decreased to nearly half of the peak value by day 20, indicating incorporation of Fe(II) into solid or sorbed components. Aqueous Si generally remained constant as bacterial reduction proceeded (Fig. 3; Table S5).

Sequential HCl extraction of the solids identified distinct pools of solid-phase Fe. The 0.1 \times HCl extraction released approximately 10% of total Fe, 97 ± 3% of which was Fe(II). Operationally, we infer this component to be Fe(II) that was weakly bound or 'sorbed' to the Fe(III)–Si coprecipitate. The 0.5 \times HCl extraction recovered the remaining approximately 90% of the total Fe in the system. The proportions of Fe(II) and Fe(III) in the 0.5 M HCl extracts varied greatly as a function of the extent of bacterial reduction (Fig. 4).

Analysis of HCl extracts for their Si contents showed incomplete (<10%) Si recovery (data not shown). In particular, the 0.5 M HCl extractions had a residual translucent white pellet after extraction. This material was likely amorphous SiO₂, as it rapidly dissolved in 1 M NaOH. Because TEM analysis indicated that there was no formation of distinct amorphous SiO₂ phases during microbial reduction, it likely that amorphous SiO₂ precipitated in the HCl extract itself as Fe was dissolved.

Fe isotope analyses

Isotopic compositions

Initial $\delta^{56}Fe$ values for $Fe(II)_{aq}~(\delta^{56}Fe_{Fe(II)aq})$ and 0.1 ${\rm M}~HCl$ extractable Fe(II) (δ^{56} Fe_{Fe(II)0.1 MHCl}) were negative, reflecting rapid coupled Fe atom electron exchange (see Discussion) between the initial Fe(II) pool [comprised of the added FeCl₂ and the Fe(II) introduced with the bacterial inoculum] and the Fe(III)–Si coprecipitate. Large changes in δ^{56} Fe_{Fe(II)aq} and δ^{56} Fe_{Fe(II)0.1 M HCl} subsequently occurred during progressive microbial reduction of the Fe(III)-Si coprecipitate (Fig. 5, Table 1). In contrast, δ^{56} Fe values for Fe obtained by 0.5 M HCl extraction (δ^{56} Fe_{Fe0.5 M HCl}) were relatively constant with time, reflecting the fact that the majority of the Fe was contained in this component. Initially, $\delta^{56}Fe_{Fe(\rm II)0.1\,\,{\tiny M}\,HCl}$ values were -2.3% in the 1 and 5 mm acetate experiments, and these rose to -1.5% or -1%, respectively, by day 7. The δ^{56} Fe_{Fe(II)aq} component was highly variable in the 1 mM culture, which can be attributed to slight contamination of the aqueous phase with finegrained solids. During removal of the aqueous phase, physi-



Fig. 4 Temporal variation in solid-phase Fe(II) and Fe(III) concentrations. Fe(III)_{0.1 M HCI} concentrations fell within the analytical error (<0.30 mm) and are not shown.

Fig. 5 Temporal variation in δ^{56} Fe values for aqueous and solid-phase Fe pools. The δ^{56} Fe values for Fe(II) and Fe(III) in the 0.5 $\scriptstyle\rm M$ HCl extractions were extrapolated as described in the Results section. Error bars are smaller than the symbols unless visible.

cal contact with residual Fe could be responsible for introduction of a small amount of residual Fe to the aqueous component (<0.10 µmol). Although care was taken to minimize physical contamination; contamination effects would be the most problematic in the 1 mM culture due to the very low amount of Fe(II)_{aq} produced in the experiment. In the 5 mM acetate cultures, $\delta^{56} Fe_{Fe(II)aq}$ values followed the trend for Fe(II)_{0.1 M HCl}, where the greatest changes occurred early in the experiment, and then reached relatively stable values

at around day 5. In the 10 and 20 mM acetate experiments, $\delta^{56}Fe_{Fe(II)0.1\,M\,HCl}$ and $\delta^{56}Fe_{Fe(II)aq}$ values were initially approximately -2.5%, and then gradually increased over time to approximately -0.5%.

To determine the Fe isotope composition of Fe(II) and Fe(III) pools in the 0.5 M HCl extracts, we followed an isotopic mass-balance approach (Crosby *et al.*, 2007; Wu *et al.*, 2009), in which the overall δ^{56} Fe of the system can be represented as

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Table 1 Iron pools and measured $\delta^{56}F$	e values
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Time (days)	Acetate (тм)	Aqueous Fe(II)		0.1 M HCl extraction			0.5 м HCl extraction		
		Mole proportion*	$\delta^{56}Fe_{aq}{}^{\dagger}$	Mole proportion	Fe(II)/total Fe	δ^{56} Fe _{0.1 M HCl}	Mole proportion	Fe(II)/total Fe	δ ⁵⁶ Fe _{0.5 м HCl}
0	1	0.001	-2.45	0.03	0.95	-2.26	0.96	0.07	0.15
1	1	0.004	-1.24	0.02	0.98	-2.12	0.97	0.12	0.16
3	1	0.003	-2.85	0.05	0.99	-1.95	0.95	0.14	0.20
5	1	0.004	-2.86	0.04	1.00	-1.86	0.96	0.18	0.15
7	1	0.003	-1.11	0.05	0.96	-1.59	0.95	0.17	0.17
10	1	0.003	-2.79	0.06	0.98	-1.65	0.94	0.12	0.27
20	1	0.003		0.07	0.97	-1.51	0.93	0.32	0.19
0	5	0.001	-2.39	0.02	0.92	-2.22	0.98	0.11	0.13
1	5	0.002	-2.68	0.01	1.01	-2.10	0.99	0.13	0.09
2	5	0.008	-1.92	0.06	0.98	-1.44	0.93	0.26	0.20
3	5	0.010	-1.70	0.06	0.97	-1.12	0.93	0.40	0.17
5	5	0.010	-1.78	0.06	0.97	-1.10	0.93	0.41	0.11
7	5	0.007	-1.92	0.05	0.98	-1.11	0.94	0.43	0.11
10	5	0.009	-1.91	0.08	0.98	-1.11	0.91	0.47	0.06
20	5	0.008	-2.04	0.12	0.98	-1.01	0.87	0.38	0.12
0	10	0.002	-2.50	0.01	0.90	-2.13	0.99	0.13	0.14
1	10	0.003	-2.76	0.02	0.95	-2.09	0.98	0.12	0.11
2	10	0.008	-2.08	0.07	1.00	-1.42	0.92	0.28	0.18
3	10	0.011	-2.82	0.07	0.97	-1.13	0.92	0.44	0.08
5	10	0.010	-1.38	0.05	0.98	-0.78	0.94	0.37	0.04
7	10	0.006	-0.88	0.05	0.98	-0.60	0.95	0.55	0.06
10	10	0.007	-0.72	0.10	0.99	-0.51	0.90	0.67	0.13
15	10	0.008	-0.67	0.10	0.99	-0.42	0.90	0.70	0.08
20	10	0.008	-0.73	0.09	0.98	-0.43	0.90	0.74	0.07
0	20	0.002	-1.20	0.01	0.87	-2.30	0.98	0.19	0.07
1	20	0.002	-1.60	0.03	0.98	-2.26	0.97	0.15	0.17
2	20	0.008	-2.29	0.05	0.99	-1.60	0.94	0.26	0.18
3	20	0.013	-1.65	0.04	0.98	-1.40	0.95	0.51	0.02
5	20	0.010	-0.89	0.08	0.99	-0.85	0.91	0.54	0.10
7	20	0.008	-1.08	0.10	0.98	-0.74	0.90	0.61	0.10
10	20	0.008	-0.79	0.06	0.99	-0.52	0.93	0.70	0.04
20	20	0.006	-0.54	0.05	0.99	-0.36	0.94	0.77	0.01

 $*mol_{Fe(pool)}/mol_{Fe(total)}$.

†Samples 100% Fe(II).

$$\begin{split} \delta^{56} \mathrm{Fe}_{\mathrm{system}} &= X_{\mathrm{Fe(II)aq}} \delta^{56} \mathrm{Fe}_{\mathrm{Fe(II)aq}} \\ &+ X_{\mathrm{Fe(II)0.1 M HCl}} \delta^{56} \mathrm{Fe}_{\mathrm{Fe(II)0.1 M HCl}} \\ &+ X_{\mathrm{Fe(II)0.5 M HCl}} \delta^{56} \mathrm{Fe}_{\mathrm{Fe(II)0.5 M HCl}} \\ &+ X_{\mathrm{Fe(III)0.5 M HCl}} \delta^{56} \mathrm{Fe}_{\mathrm{Fe(III)0.5 M HCl}} \end{split}$$
(1)

where X_i is the Fe mole fraction for a specific component. All quantities in equation 1 are measured and normalized to a system value of 0, except $\delta^{56}\text{Fe}_{\text{Fe}(II)0.5 \text{ M} \text{ HCl}}$ and $\delta^{56}\text{Fe}_{\text{Fe}(III)0.5 \text{ M} \text{ HCl}}$, which can be calculated using several approaches. The isotopic mass balance for the 0.5 M HCl extract is given by:

$$\delta^{56} Fe_{0.5 M HCl} = X_{Fe(II)0.5 M HCl} \delta^{56} Fe_{Fe(II)0.5 M HCl} + (1 - X_{Fe(II)0.5 M HCl}) \delta^{56} Fe_{Fe(III)0.5 M HCl},$$
(2)

where the mole fractions for Fe(II) and Fe(III) are determined by *Ferrozine* analysis of the 0.5 M HCl extract.

Equation 2 is a linear mixing relationship on a plot of δ^{56} Fe versus $X_{Fe(II)0.5 \text{ M} \text{HCl}}$ (Fig. S2). Following the approach of Crosby *et al.* (2005, 2007) and Wu *et al.* (2009), we start by assuming that the δ^{56} Fe value of Fe(II) in the 0.1 M HCl extract was equivalent to Fe(II) in the 0.5 M HCl extract. This assumption (whose validity is evaluated below) allows calculation of δ^{56} Fe_{Fe(III)0.5 M HCl} by extrapolation to $X_{Fe(II)0.5 \text{ M} \text{HCl}} = 0$ (Fig. S2), which was done using ISOPLOT (Ludwig, 1991) to allow for full propagation of errors. The calculated δ^{56} Fe_{Fe(III)0.5 M HCl} values have a large range of uncertainties (Table S7), which are a function of $X_{Fe(II)0.5 \text{ M} \text{HCl}}$.

To test the assumption that $\delta^{56}Fe_{Fe(II)0.1\ M\ HCl}$ is equal to $\delta^{56}Fe_{Fe(II)0.5\ M\ HCl}$, we evaluate the Fe(II)_{0.1\ M\ HCl}-Fe(III)_{0.5\ M\ HCl} fractionation for samples whose calculated $\delta^{56}Fe_{Fe(II)0.5\ M\ HCl}$ have an error of 0.25% or less; this produces a $\Delta^{56}Fe_{Fe(II)0.1\ M\ HCl-Fe(III)0.5\ M\ HCl}$ fractionation of $-2.35\pm0.16\%$ (see Table S6), using a weighted average and error propagation from ISOPLOT. This allows us to define a second $\delta^{56}Fe_{Fe(III)0.5\ M\ HCl}$ as:

$$\delta^{56} Fe_{Fe(III)0.5\,M\,HCl} = \delta^{56} Fe_{Fe(II)0.1\,M\,HCl} + 2.35. \eqno(3)$$

Substituting equation 3 into equation 2 and casting in terms of $X_{\text{Fe(III)0.5 M HCl}}$ produces:

- /

$$\delta^{56} Fe_{0.5 M HCl} = (1 - X_{Fe(III)0.5 M HCl}) \delta^{56} Fe_{Fe(II)0.5 M HCl} + X_{Fe(III)0.5 M HCl} (\delta^{56} Fe_{Fe(II)0.1 M HCl} + 2.35).$$
(4)

Equation 4 defines a linear mixing relationship in terms of $X_{\rm Fe(III)0.5~M~HCl}$ in the 0.5 M HCl extract that allows for calculation of δ^{56} Fe_{Fe(II)0.5 M HCl} values (Fig. S3, Table S6), given the assumption of a constant Fe_{Fe(II)0.1 M HCl}-Fe_{Fe(III)0.5 M HCl} fractionation factor. Extrapolated errors for δ^{56} Fe_{Fe(II)0.5 M HCl} were also calculated using ISOPLOT. The Fe_{Fe(II)0.1 M HCl}-Fe_{Fe(II)0.1 M HCl}-Fe_{Fe(II)0.1 M HCl}-Fe_{Fe(II)0.1 M HCl}-Fe_{Fe(II)0.5 M HCl} fractionation factor is defined as:

$$\begin{split} &\Delta^{56} Fe_{Fe(II)0.1 M HCl-Fe(II)0.5 M HCl} \\ &= \delta^{56} Fe_{Fe(II)0.1 M HCl} - \delta^{56} Fe_{Fe(II)0.5 M HCl}. \end{split}$$

Calculating a weighted average that includes uncertainties in $\delta^{56} Fe_{Fe(II)0.5\ M\ HCl}$ produced a $\Delta^{56} Fe_{Fe(II)0.1\ M\ HCl-Fe(II)0.5\ M\ HCl}$ fractionation of +0.27 \pm 0.18‰ (Fig. S4, Table S6). This result suggests that there is a slight isotopic contrast between $\delta^{56} Fe_{Fe(II)0.1\ M\ HCl}$ and $\delta^{56} Fe_{Fe(II)0.5\ M\ HCl}$, at least in the Fe(III)–Si coprecipitate system used in the current study. Revised $\delta^{56} Fe_{Fe(III)0.5\ M\ HCl}$ values can be calculated by combining equations 2 and 5:

$$\delta^{56} Fe_{0.5MHCl} = X_{Fe(II)0.5MHCl} (\delta^{56} Fe_{Fe(II)0.1MHCl} - 0.27) + (1 - X_{Fe(II)0.5MHCl}) \delta^{56} Fe_{Fe(III)0.5MHCl}$$
(6)

and then rearranging and solving for δ^{56} Fe_{Fe(III)0.5 M HCl}:

$$\delta^{56} \text{Fe}_{\text{Fe}(\text{III})0.5\text{MHCl}} = [-X_{\text{Fe}(\text{II})0.5\text{MHCl}} (\delta^{56} \text{Fe}_{\text{Fe}(\text{II})0.1\text{MHCl}} - 0.27) + \delta^{56} \text{Fe}_{0.5\text{MHCl}}]/(1 - X_{\text{Fe}(\text{II})0.5\text{MHCl}}).$$
(7)

This mixing relationship is illustrated in Fig. S5, and all revised fractionation factors discussed below use these values for δ^{56} Fe_{Fe(III)0.5 M HCl}. A summary of the updated δ^{56} Fe values as a function of time is shown in Fig. 5.

In the 1 and 5 mM acetate experiments, the revised $\delta^{56}Fe_{Fe(III)0.5\ {\rm M}\ {\rm HCl}}$ values (equation 7) are fairly similar to the initial $\delta^{56}Fe_{Fe(III)0.5\ {\rm M}\ {\rm HCl}}$ values (equation 2), but they diverge significantly in the 10 and 20 mM acetate experiments, where the revised values are more positive. The $\Delta^{56}Fe_{Fe(III)0.1\ {\rm M}\ {\rm HCl}-Fe(III)0.5\ {\rm M}\ {\rm HCl}}$ fractionations using the revised $\delta^{56}Fe_{Fe(III)0.5\ {\rm M}\ {\rm HCl}}$ values produce a weighted average of $-2.42 \pm 0.12\%$. Based on the discussion above, a four-component mass-balance equation that includes terms for aqueous Fe(II), 0.1\ {\rm M}\ {\rm HCl}-extracted\ Fe(II) and 0.5 ${\rm M}\ {\rm HCl}$ extractable Fe(II) and Fe(III) is needed to explain the behaviour of this system.

Fe isotope fractionation factors

Isotopic fractionation factors between aqueous Fe(II) and the extractable components were calculated using data from the 5, 10 and 20 mM acetate experiments (Fig. 6, Table 2). Overall, these fractionation factors indicate that minor to zero fractionation occurred between Fe(II) components $[\Delta^{56}\text{Fe}_{Fe(II)aq-Fe(II)0.1 \text{ M HCl}} = -0.41\%]$, whereas large fractionations occurred between Fe(II) and Fe(III) phases $[\Delta^{56}\text{Fe}_{Fe(II)aq-Fe(III)0.1 \text{ M HCl}} = -2.82\%]$. These fractionations were established quickly in all experiments, indicating that isotopic exchange was rapid. All experiments show minor



Fig. 6 Temporal variation in Δ^{56} Fe_{Fe(II)aq-Fe(II)0.1 MHCI} (\bigtriangledown), and Δ^{56} Fe_{Fe(II)aq-Fe(II)0.5 MHCI} (\blacktriangle) based on final δ^{56} Fe_{Fe(II)0.5 MHCI} and δ^{56} Fe_{Fe(III)0.5 MHCI} values (see Results section). Weighted average for all experiments are represented by shaded grey bars; averages for each experiment are given in Table 2.

Study	Acetate (тм)	$\Delta^{56}Fe_{Fe(II)aq-Fe(II)0.1M}HCI$	$\Delta^{56}Fe_{Fe(II)aq-Fe(III)0.5M}HCI$	$\Delta^{56}Fe_{Fe(II)aq-Fe(II)0.5M}HCI$	Δ^{56} Fe _{Fe(II)0.1M} HCl–Fe(III)0.5M HCl	∆ ⁵⁶ Fe _{Fe(II)0.5м} нс⊢ғе(III)0.5м нсI
This study	1				-2.47 ± 0.18	-2.74 ± 0.18
·	5	-0.64 ± 0.21	-2.99 ± 0.14	-0.37 ± 0.21	-2.41 ± 0.19	-2.65 ± 0.19
	10	-0.56 ± 0.35	-2.91 ± 0.43	-0.29 ± 0.35	-2.30 ± 0.35	-2.53 ± 0.31
	20	-0.01 ± 0.51	-2.38 ± 0.36	0.28 ± 0.5	-2.52 ± 0.38	-2.78 ± 0.40
	Average	-0.41 ± 0.22	-2.82 ± 0.19	-0.14 ± 0.22	-2.42 ± 0.12	-2.68 ± 0.12
Crosby et al. (2007)	No Si	$-0.30 \pm 0.08*$	-2.95 ± 0.19			
Wu et al. (2009)	No Si	$-0.48 \pm 0.07*$	-2.70 ± 0.36			
Wu <i>et al.</i> (2009)	+Si	$-0.20 \pm 0.16^*$	-2.60 ± 0.19			

 Table 2
 Calculated
 fractionation factors and overall weighted averages for various Fe pools

*Values for fractionation between Fe(II)_{aq} and sorbed Fe(II) recovered by pH 5 Na-acetate (Crosby et al., 2005) or 0.05 M HCI (Wu et al., 2009).

fractionation variation in the first few days, yet after a few days, all isotopic fractionations remained constant with time, suggesting attainment of equilibrium isotope fractionation.

DISCUSSION

Nature and reducibility of the Fe(III)-Si coprecipitate

Extensive reduction of the Fe(III)-Si coprecipitate in the presence of excess acetate may be attributed to its amorphous nature, as documented by XRD and TEM, and the lack of production of crystalline end products (e.g. magnetite), which in some cases prevents complete or near-complete reduction (Zachara et al., 2002). Synthetic 2-line ferrihydrite formed through Fe(II) oxidation in the presence of moderate Si, similar to this work, has been shown to have broad XRD peaks consistent with an amorphous structure (Karim, 1984). Doelsch et al. (2000) documented formation of disorganized Fe(III) oxyhydroxide precipitates that had variable Si/Fe ratios when prepared through FeCl₃ hydrolysis in high-Si solutions. It is important to note that these Fe(III)-Si coprecipitates did not consist of separate, nanometre-size Fe and Si phases, but were composed of a single Fe-Si-O network (Doelsch et al., 2001). FTIR and ²⁹Si solid-state NMR spectra of coprecipitates that had molar Si:Fe ratios greater than unity indicate that Fe polymerization decreases with increasing Si, accompanied by an increase in Si-O bonding (Doelsch et al., 2001). It is inferred that the presence of Si-O-Fe bonds hinders phase transformations to crystalline oxyhydroxides (Vempati & Loeppert, 1989; Doelsch et al., 2000, 2003). Zeng (2003) proposed a structure for Si-rich Fe(III) oxyhydroxide solids that involves bonding between Si(OH)4 and Fe(III). We propose a slight modification on this structure, with the 2:1 Si:Fe ratio represented as a ferric-silicate type chemical composition:

This species could then readily form siloxane linkages between $Si(OH)_4$ molecules, creating small domains represented by:

OH

$$Fe^{III}-O-Si-O-Si(OH)_3$$

$$|$$

$$Fe^{III}-O-Si-O-Si(OH)_3$$

$$|$$

$$Fe^{III}-O-Si-O-Si(OH)_3$$

$$|$$

$$Fe^{III}-O-Si-O-Si(OH)_3$$

$$|$$

$$OH$$

Despite uncertainties about the nature of the amorphous reduction end products, our findings demonstrate an important distinction between the behaviour of the Fe(III)-Si coprecipitate and Si-free synthetic ferrihydrite during microbial reduction: synthetic ferrihydrite typically undergoes transformation to siderite, magnetite and/or green rust during the early stages of microbial reduction (e.g. Fredrickson et al., 1998, 2003; Johnson et al., 2005), end products which can be readily detected by XRD and TEM/SEM. Kukkadapu et al. (2004) found that silica-ferrihydrite coprecipitates (1-5 mol% Si) underwent up to 90% reduction by Shewanella putrefaciens strain CN32 in high phosphate medium, but green rust and vivianite formed under these conditions. The absence of crystalline Fe(II)-bearing mineral formation in our experiments is probably related to the very high abundance of Si in the Fe(III)-Si coprecipitate (66 mol% Si), which presumably inhibited mineral formation in a manner analogous to its retardation of Fe(III) oxide crystallization. This conclusion is supported by the results of mineral saturation state calculations conducted using the geochemical modelling software Phreeqc (Parkhurst & Appelo, 1999). The LLNL thermodynamic database (Delany & Lundeen, 1990) was employed for these calculations. At pH 6.7 with a dissolved Fe(II) concentration of approximately 1 mM (comparable to conditions observed in the cultures; see Fig. 1), the calculations indicated that the system was oversaturated with respect to siderite (saturation index = 0.85, *c*. 10-fold supersaturation). The degree of supersaturation increased to a factor of 100–1000 when the total amount of Fe(II) in the system was increased to values comparable to total Fe(II) measured in the cultures (10–80 mM). These results clearly indicate that conditions were favourable for the formation of large quantities of siderite, as well as magnetite in the cultures, as observed in numerous previous studies of Si-free amorphous Fe(III) oxide reduction, and the absence of these phases thus points directly to an inhibitory impact of Si on mineral precipitation.

The levels of Fe(II)_{aq} produced in our experiments are lower than those documented in other studies of amorphous Fe(III) oxide reduction, which have found up to several mM of Fe(II)_{aq} accumulation (Fredrickson et al., 2003; Tangalos et al., 2010). The simplest explanation for this result is that Fe(II) produced during bacterial reduction became physically bound to, or incorporated into, the Fe(III)-Si coprecipitate. The fact that maximum Fe(II)_{aq} concentrations occurred during the early stages of reduction in our experiments, and declined thereafter, suggests that some Fe(II)_{aq} eventually readsorbed to the coprecipitate surface. Nevertheless, that distinct Fe(II) and Fe(III) components could be extracted during HCl treatment suggests different degrees of lability for the Fe components in the coprecipitate. We consider the reduced materials produced in our experiments to be analogous to precursor phases that are inferred for siderite and magnetite formation in marine sedimentary rocks such as banded iron formations (e.g. Klein, 2005).

Fe isotope fractionations

The mechanism of Fe isotope fractionation produced by DIR has been previously investigated using partial, protonpromoted acid extractions to isolate subcomponents of the solids that underwent isotopic exchange with Fe(II) (Crosby et al., 2005; Tangalos et al., 2010). The isotopic fractionations appear to reflect coupled exchange of electrons and atoms between aqueous and sorbed Fe(II) and a reactive Fe(III) surface phase [Fe(III)_{reac}]. Studies using hematite and goethite (Crosby et al., 2005) have shown that to account for isotopic mass balance, the outer layer of the oxide becomes enriched with isotopically heavier Fe(III) during DIR, balancing the isotopically light Fe(II)_{aq}. The average Fe(II)_{aq}-Fe(III)_{reac} fractionation for goethite during DIR was $-2.62 \pm 0.57\%$ in ⁵⁶Fe/⁵⁴Fe (Crosby *et al.*, 2005, 2007). This lies within the range of the Fe(II)_{aq}-Fe(III)_{reac} fractionation of approximately -2% estimated for goethite by Beard et al. (2010), but is distinct from the equilibrium $Fe(II)_{aq}$ goethite fractionation of $-1.08 \pm 0.08\%$ determined by Beard et al. (2010) for bulk goethite. Crosby et al. (2005, 2007) determined a Fe(II)_{aq}-Fe(III)_{reac} fractionation during microbial hematite reduction of -2.95 ± 0.19 %, which is

consistent with (i) the equilibrium Fe(II)aq-hematite fractionation of -3.1% at room temperature inferred from equilibrium Fe(II)_{ag}-Fe(III)_{ag} and Fe(III)_{ag}-hematite (Skulan et al., 2002) fractionations; and (ii) the fractionation between $Fe(II)_{aq}$ and $Fe(III)_{reac}$ of $-2.87 \pm 0.19\%$ in abiological experiments with hematite at pH 7 in the absence of Si (Wu, 2010). Studies of DIR using ferrihydrite have shown that the Fe(II)_{aq} that is produced has low- δ^{56} Fe values (Beard et al., 1999; Johnson et al., 2005), similar to the results obtained with goethite and hematite. It was difficult, however, to determine the isotopic mass balance among reactive components in these previous experiments because ferrihydrite is not readily amenable to partial acid extraction. Moreover, solid-phase conversion of pure ferrihydrite during DIR in simple systems adds complexity to interpreting the isotopic data, as does production of Fe(II)-bearing secondary minerals such as siderite and magnetite or amorphous Fe(II) solids (e.g. Johnson et al., 2005).

Calculated fractionation factors for the DIR experiments reported here are broadly comparable to ranges obtained during microbial hematite reduction experiments with and without Si (Table 2). The measured Δ^{56} Fe_{Fe(II)ag-Fe(II)0.1 M HCl} fractionation $-0.41 \pm 0.22\%$ compares well with previously reported fractionations between aqueous and sorbed Fe(II) produced during hematite reduction (Table 2). A key aspect of the current study is the documentation of Fe isotope fractionation across a wide range of Fe(III) reduction, particularly extending to very high degrees of reduction compared to previous studies. Most previous studies of Fe isotope fractionation coupled to DIR have employed crystalline Fe(III) oxides such as hematite and goethite, which undergo only minor reduction. The only other study to observe a broadly similar extent of Fe(III) reduction (Tangalos et al., 2010) had greater quantities of $Fe(II)_{aq}$ but $\delta^{56}Fe$ values similar to those observed in this work.

In Fig. 7, results from previous studies of Fe isotope fractionation during DIR are compared in terms of per cent Fe(III) reduction, the Fe isotope composition of total and aqueous Fe(II), and the ratio of aqueous to total Fe(II). The results of this study occupy a zone of very high reduction and low δ^{56} Fe_{Fe(II)} values not captured in prior studies. An important aspect of the current work is the very large extent of isotopic exchange among Fe(II) and Fe(III) components, which is required to produce large shifts in δ^{56} Fe values for Fe(II) at large extent of reduction; this contrasts with the shifts observed during small extents of reduction of goethite and hematite substrates, which involved isotopic exchange with only the outer few atom layers of the oxide (e.g. Crosby *et al.*, 2007; Wu *et al.*, 2009).

Implications for Precambrian geochemical cycles

This is the first study to examine microbial reduction of an amorphous Fe(III)–Si coprecipitate analogous to the Si-rich



Fig. 7 Fe isotope composition and abundance of Fe(II)_{aq} as a function of per cent Fe(III) reduction for various DIR studies: δ^{56} Fe values for all Fe(II) species (A), concentration of Fe(II)_{aq} (B), and the per cent of total Fe(II) present as Fe(II)_{aq} (C). The results of this study are compared to those from prior studies of microbial reduction of hematite and goethite in the absence of Si (Crosby *et al.*, 2007), microbial reduction of hematite in the presence and absence of Si (Wu *et al.*, 2009), and microbial reduction of natural amorphous Fe(III) oxides (Tangalos *et al.*, 2010). All δ^{56} Fe values were normalized to the isotopic composition of (i) per cent Fe(III) reduction, and (ii) Fe(II) isotope composition and aqueous/solid-phase abundance which could result in intra-sediment heterogeneity; the dark grey box represents a combination of these parameters which could result in basin-scale segregation of Fe isotopes via transport of Fe(II)_{aq}.

precipitates thought to have formed, and been deposited to sediments, in Precambrian oceans (Konhauser et al., 2002; Kesler & Ohmoto, 2006). The highly reducible nature of the Fe(III)-Si coprecipitate provides support for arguments that DIR was important in biogeochemical cycling in Archaean marine environments (Baur et al., 1985). In addition to promoting substrate reducibility, the presence of Si probably led to production of Si-bearing products during DIR. Klein (1974) first speculated that greenalite present in BIFs was due to diagenesis of precursor amorphous Fe-Si gels, similar to those produced in this research, although Klein envisioned that such diagenesis occurred abiologically. Although no definitive phase transformations were seen in these experiments, the end products of microbial reduction of the coprecipitate are analogues to precursor phases of crystalline minerals that would ultimately be produced during burial diagenesis and possible metamorphism at elevated temperature and pressure. We thus suggest that formation of greenalite could occur indirectly via the following overall reaction for microbial Fe(III) reduction coupled to organic carbon oxidation:

$$\begin{split} 3 FeSi_2(OH)_{11} + 0.75CH_2O &\rightarrow Fe_3Si_2O_5(OH)_4 \\ &+ 0.75HCO_3^- + 4SiO_2 + 14.5H_2O + 0.75H^+. \end{split}$$

This reaction would produce amorphous SiO_2 or chert due to silica in excess of that required to form greenalite. In addition to greenalite, we hypothesize that microbial reduction of a Fe(III)–Si substrate could also lead to production of nonsilicate mineral end products, such as magnetite and siderite via reactions such as:

$$\begin{split} 3 \text{FeSi}_2(\text{OH})_{11} + 0.25 \text{CH}_2\text{O} &\rightarrow \text{Fe}_3\text{O4} + 0.25 \text{HCO}_3^- \\ &+ 6 \text{SiO}_2 + 16.5 \text{H}_2\text{O} + 0.25 \text{H}^+ \end{split}$$

$$FeSi_{2}(OH)_{11} + 0.25CH_{2}O + 0.75HCO_{3}^{-} + 0.75H^{+}$$

$$\rightarrow FeCO_{3} + 2SiO_{2} + 6.5H_{2}O.$$

Finally, pyrite may be produced via reaction of a DIR-generated Fe(II)–Si phase with sulphide

The Fe isotope effects of the above reactions will depend upon the extent of reduction and mobilization (via diffusion– dispersion and/or advection) of isotopically light Fe(II). For example, under conditions of partial reduction, all evidence indicates that greenalite, siderite or pyrite would incorporate isotopically light Fe(II) that was produced by partial DIR. Partial reduction would produce residual Fe(III) that would ultimately be transformed to more crystalline oxides and hydroxides with heavier δ^{56} Fe values relative to the reduced minerals. Mixed Fe(II)–Fe(III) oxides such as magnetite might have positive or negative δ^{56} Fe values, depending upon the δ^{56} Fe values of the Fe(II) and Fe(III) components. In addition, reduction of residual, isotopically heavy Fe(III) left behind after mobilization of light Fe(II) would produce Fe(II)-bearing minerals that have high δ^{56} Fe values. Heimann et al. (2010) and Craddock & Dauphas (2011), for example, found high- δ^{56} Fe siderite in 2.5 Ga banded iron formations that was ascribed to DIR based on C and O isotope compositions that indicate siderite did not form in isotopic equilibrium with seawater. The end result of these processes would be a section of sediment that contained a large range in Fe isotope compositions for Fe(II)-bearing phases over relatively small spatial scales and thickness. As discussed by Johnson et al. (2008a), deposition of Fe-rich sediments suggests high concentration of aqueous Fe(II) in the oceans, which in turn requires a long residence time for Fe. Equilibrium precipitation of Fe(III) oxyhydroxides from the reservoir of Fe(II) is not predicted to give rise to significant fine-scale Fe isotopic heterogeneity in the underlying sediments. Our results demonstrate that solid Fe(II) and Fe(III) components produced during DIR may have very different Fe isotope compositions, providing an explanation for the fine-scale Fe isotope heterogeneity seen in Neoarchaean and Paleoproterozoic BIFs (Johnson et al., 2008a; Heimann et al., 2010; Craddock & Dauphas, 2011).

Mobilization of low- δ^{56} Fe aqueous Fe(II) produced during DIR may occur through diffusion-dispersion and advection prior to diagenesis. Despite the relatively low concentrations of aqueous Fe(II) recovered in this study, it is important to consider the potential for sorbed, isotopically light Fe(II) to become mobilized during the formation of crystalline phases during early diagenesis and concomitant changes in surface area and pH. Such mobilization could account for Fe isotope segregation within a sediment section (Tangalos et al., 2010). Based on the range of substrates and extents of reduction involved in various DIR experiments, we suggest that conditions where moderately negative δ^{56} Fe values for Fe(II)_{ag} are produced (c. -1% to -2%) during generation of high absolute and relative [to total Fe(II)] amounts of Fe(II)_{aq} (dark grey boxes in Fig. 7) are conditions most likely to have led to basin-scale Fe isotope variations (e.g. Severmann et al., 2008, 2010; Czaja et al., 2010). Although Rouxel et al. (2005) suggested that a low extent of reduction of DIR would produce limited quantities of light Fe(II)_{aq}, Johnson et al. (2008b) noted that a sustained state of partial reduction in a basin, supplied by a constant flux of iron oxides/hydroxides and organic carbon from the photic zone, could produce significant basinscale Fe isotope segregation. By contrast, conditions where DIR produced a high extent of reduction but low absolute and relative $Fe(II)_{aq}$ (i.e. the conditions in the current study) would most likely have led to fine-scale Fe isotope heterogeneities rather than basin-scale Fe isotope variations.

Because crystalline Fe(II) minerals were not produced in our experiments, a direct link cannot be drawn between the results of this study and the mineralogy found in ancient marine sedimentary rocks, where conversion of poorly crystalline Fe(II)-bearing precursors to crystalline minerals presumably took place during moderate burial metamorphism. It is not yet known the extent to which DIR-generated Fe isotope compositions may be changed by heating and re-equilibration among phases. It seems likely, however, that fine-scale Fe isotope heterogeneity, produced before or during diagenesis and possible low-to-moderate grade metamorphic processes, will remain stable on geologic time scales, thus allowing such minerals to be distinguished from those that formed in equilibrium with seawater. As noted by Heimann *et al.* (2010) and Craddock & Dauphas (2011), supporting isotopic data, such as C and O, can be used to distinguish minerals that formed in equilibrium with seawater and those that formed authigenically or diagenetically in the sediment.

CONCLUSIONS

An amorphous Fe(III) oxide–Si coprecipitate was highly susceptible to dissimilatory microbial reduction under chemical conditions that simulated Archaean marine conditions. Very large quantities of low- δ^{56} Fe aqueous, surface-associated, and bulk solid-phase Fe(II) were produced, far more than in previous experiments that investigated Fe isotope fractionations produced during microbial reduction of goethite or hematite. Although the efficiency of DIR as a mechanism for producing large quantities of low- δ^{56} Fe Fe(II) has been questioned (e.g. Rouxel *et al.*, 2005; Anbar & Rouxel, 2007), these results demonstrate that DIR may be a very efficient pump for producing isotopically light Fe(II).

The measured Fe isotope fractionations probably reflect near-complete isotopic equilibrium among all Fe(III) and Fe(II) components early in the experiments, as well as the changing proportions of Fe(II) and Fe(III) during DIR. Essentially complete isotopic exchange is required to produce significantly negative δ^{56} Fe values for Fe(II) at high extents of reduction. The high extent of Fe(III) reduction and Fe isotope exchange are both presumably related to the small particle size and high reactivity of the amorphous coprecipitate. This contrasts with studies of crystalline goethite and hematite reduction, in which minor degrees of Fe(III) reduction were associated with isotopic exchange with only the outer few atom layers of the oxide substrate (Crosby *et al.*, 2007; Wu *et al.*, 2009).

The majority of low- δ^{56} Fe Fe(II) produced in our experiments was contained in a poorly crystalline solid phase that was in intimate contact with high- δ^{56} Fe residual Fe(III). This finding provides insight into how fine-scale Fe isotope variations that have been observed in the rock record (e.g. Johnson *et al.*, 2008a; Heimann *et al.*, 2010) may have been generated via DIR followed by burial diagenesis and metamorphism. Such variations are difficult to explain by direct precipitation from seawater, and the new experimental results provide evidence that such variations may reflect the effects of DIR within the

soft sediment prior to lithification. The connection between the experimental results and the rock record lies in further understanding the pathways involved in transformation of poorly crystalline phases produced by DIR into Fe(II)-bearing minerals such as greenalite, magnetite, siderite and pyrite under conditions of increased in temperature and pressure.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. DGGE analysis of 16S rRNA gene amplicons retrieved from different generations of Fe(III)–Si coprecipitate reducing enrichment cultures.

Fig. S2. Mixing lines used to estimate $\delta^{56}Fe$ values for the Fe(III) component of the 0.5 $_{M}$ HCl extracts calculated assuming that $\delta^{56}Fe_{Fe(II)0.5\,M\,HCl} = \delta^{56}Fe_{Fe(II)0.1\,M\,HCl}$.

Fig. S3. Mixing lines used to estimate $\delta^{56} Fe$ values for the Fe(II) component of the 0.5 ${\rm M}$ HCl extracts.

Fig. S4. Plot of estimated δ^{56} Fe_{Fe(II)0.5 M HCI} calculated values versus measured δ^{56} Fe_{Fe(II)0.1 M HCI} values.

Fig. S5. Mixing lines employed to resolve δ^{56} Fe_{Fe(III)0.5 M HCI} Fe(III)_{0.5 M HCI} given the constraints of Fe(II)_{0.5 M HCI} expressed in equation 7 from the text.

Table S1. Artificial Archaean seawater composition.

 Table S2.
 Fe:Si ratio of Fe–Si coprecipitate.

 Table S3. Fe isotope composition of partial dissolutions of the Fe–Si coprecipitate.

Table S4. Phylogenetic association of the most common 16S rRNA gene sequences from 454 pyrosequencing.

Table S5. Aqueous silica and Fe concentrations in DIR experiments.

Table S6. Determination of isotopic composition of $0.5\,{\rm m}$ HCl extractable Fe(II) and Fe(III).

Table S7. Raw Fe isotope data.

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