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Journal Pre-proof

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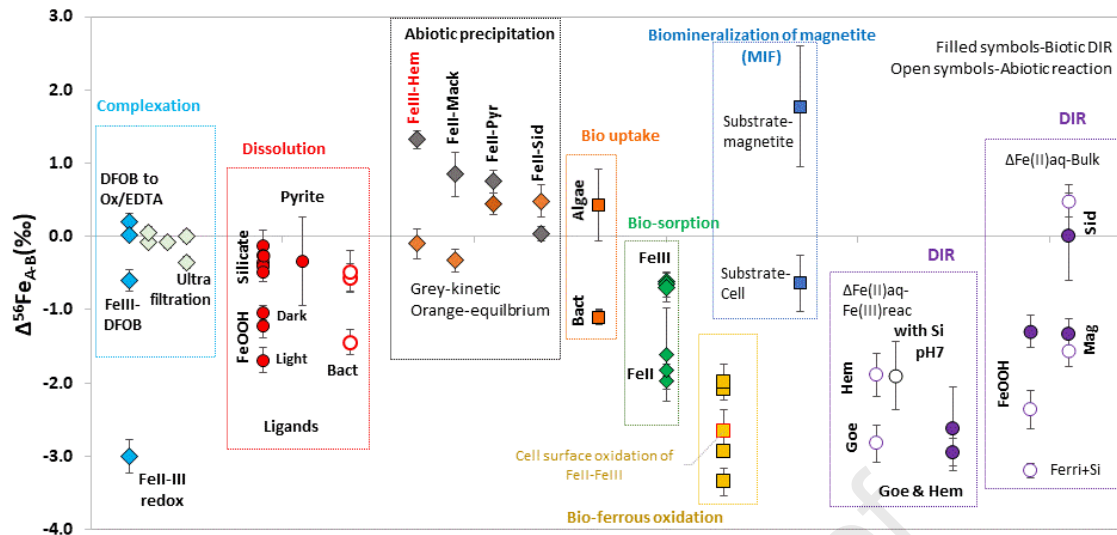
Nang-Htay YIN (Main author, conceptualization, data curation, formal analysis, writing of original draft, editing according to co-authors' input, and final submission)

Pascale LOUVAT (Stable isotope and mass spectrometry expert, supervision, validation, review and editing)

Aubin THIBAUT-DE-CHANVALON (Internal review)

Mathieu SEBILO (The first author has been requested to add this additional co-author by last co-author)

David AMOUROUX (Project leader, Funding acquisition, Revision)



1
 2 Figure 1. Overview of iron isotopes fractionation driven by low-temperature biogeochemical
 3 processes such as redox reaction ($\Delta^{56}\text{Fe}_{\text{II-III}}$), ligand-complexation ($\Delta^{56}\text{Fe}_{\text{III-ligands}}$),
 4 alteration/dissolution ($\Delta^{56}\text{Fe}_{\text{solution-solid}}$), abiotic mineral precipitation ($\Delta^{56}\text{Fe}_{\text{II/III-mineral}}$), bio-
 5 uptake ($\Delta^{56}\text{Fe}_{\text{III-cell}}$), bio-sorption ($\Delta^{56}\text{Fe}_{\text{II-biomass}}$ vs $\Delta^{56}\text{Fe}_{\text{III-biomass}}$), microbial oxidation
 6 ($\Delta^{56}\text{Fe}_{\text{II-III-FeOOH}}$), biomineralization ($\Delta^{56}\text{Fe}_{\text{II or III-mineral/cell}}$) and dissimilatory iron reduction of
 7 iron minerals ($\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIreact}}$ and $\Delta^{56}\text{Fe}_{\text{FeIIaq-BulkMineral}}$). (DFOB-desferrioxamine B, FeOOH-
 8 oxyhydroxide minerals, Bact-bacteria, Hem-hematite, Fer-ferrihydrite, Goe-goethite, Mag-
 9 magnetite, Sid-siderite, Mack-mackinawite, Pyr-pyrite, and DIR- dissimilatory iron reduction).
 10 In the “Dissolution” box, light refers to the presence of light favoring photochemical reductive
 11 dissolution and dark refers to the absence of light favoring oxidative dissolution. The red outline
 12 of one symbol in the "Bio-ferrous oxidation" box corresponds to the calculation of $\Delta^{56}\text{Fe}$
 13 between FeII_{aq} and FeIII on cell surface while the rest of calculation focused between FeII-
 14 FeIII and precipitated FeIII minerals. In the “DIR” box, the open symbol with black outline
 15 corresponds to the abiotic hematite experiment conducted in the presence of Si at pH7 in
 16 comparison to the experiments without Si. Error bars represent 2SD experimental error and.
 17 Detailed values and corresponding references for each process are given in Table 1, 2 and 3

Journal Pre-proof

1 **Iron Isotopic Fractionation Driven by Low-Temperature**

2 **Biogeochemical Processes**

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8 SEBILO^{1,2}, David AMOUROUX¹

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22 64053 Pau, France.

23 Abstract

24 Iron is geologically important and biochemically crucial for all microorganisms, plants and
25 animals due to its redox exchange, the involvement in electron transport and metabolic
26 processes. Despite the abundance of iron in the earth crust, its bioavailability is very limited in
27 nature due to its occurrence as ferrihydrite, goethite, and hematite (Crichton, 2001) where they
28 are thermodynamically stable with low dissolution kinetics in neutral or alkaline environments.
29 Organisms such as bacteria, fungi, and plants have evolved iron acquisition mechanisms to
30 increase its bioavailability in such environments, thereby, contributing largely to the iron cycle
31 in the environment. Biogeochemical cycling of metals including Fe in natural systems usually
32 results in stable isotope fractionation; the extent of fractionation depends on processes involved.
33 Our review suggests that significant fractionation of iron isotopes occurs in low-temperature
34 environments, where the extent of fractionation is greatly governed by several biogeochemical
35 processes such as redox reaction, alteration, complexation, adsorption, oxidation and reduction,
36 with or without the influence of microorganisms. This paper includes relevant data sets on the
37 theoretical calculations, experimental prediction, as well as laboratory studies on stable iron
38 isotopes fractionation induced by different biogeochemical processes.

39 Keywords

40 Iron isotopes, fractionation, biogeochemical processes, microbial uptake, ligands

41 1. Introduction

42 Iron is the fourth most abundant element in the Earth's crust and is present in natural aquatic
43 environment at varying quantities depending upon the background geology and type of
44 waterway; marine, rivers or acid-mine (USEPA 1986). Despite its abundance, iron is mostly
45 present as various iron (oxyhydro)oxides such as ferrihydrite, goethite, and hematite. Their
46 solubility is limited and controlled by other environmental parameters, leading to poor
47 bioavailability in nature. Being an essential element for plants, animals and microorganisms,
48 iron is actively involved in many biogeochemical processes.

49 Iron biogeochemical cycle in nature has an important role over other elemental cycles;
50 notably oxygen, sulfur, mercury, and other transitional elements like zinc, copper, chromium
51 or nickel. For instance, Fe as an essential element for life, bacteria to plants and vertebrates,
52 affect carbon and nitrogen cycles, ocean biological production and organic matter
53 mineralization and production of climate effective biogenic gases. Dissolved FeS and
54 mackinawite mineral affect the degradation of dimethyl-mercury in the ocean, thereby
55 controlling in part biogeochemical cycle of mercury. Likewise, the formation of different Fe
56 (oxyhydro)oxides in oxidative environments is well-known for scavenging transitional metals
57 like Zn, Cu, Ni and Cr via coprecipitation and adsorption, thereby, effecting not only their
58 biogeochemical cycles but also their respective isotopic fractionation. It is important to
59 understand Fe geochemical cycles and its isotopic fractionations as it could help solve the
60 fundamental questions involving other elemental cycles as well.

61 Understanding the biogeochemical transformation of iron in terrestrial and aquatic
62 ecosystems has been promising, thanks to the developments in Multicollector-Inductively
63 Coupled Plasma Mass Spectrometer (MC-ICPMS) and stable iron isotopes. A large number of
64 studies were conducted over the last ~20 years investigating Fe isotope fractionation in various
65 experimental and field studies, as well as many theoretical calculations. Recent literature

66 reviews with larger scope involve iron isotope systematics in both terrestrial and extraterrestrial
67 systems (Dauphas et al., 2016; 2017); iron cycling and isotope fractionation in terrestrial
68 ecosystems (Wu et al., 2019); and iron isotopes in modern surficial world and the ancient earth
69 (Johnson et al., 2020). Therefore, this review article will focus on the available theoretical and
70 experimental data on stable iron isotope fractionation during various low-temperature
71 biogeochemical processes in microenvironments or at a cellular level. The aim is to reorganize
72 the existing data set, to identify the limitations of applying iron isotopes in differentiating the
73 abiotic processes from microbial iron recycling, and to point out future research direction.

74 Our review suggests that significant fractionation of iron isotopes occurs in low-temperature
75 environments, where the extent of fractionation is greatly governed by several biogeochemical
76 processes such as redox reaction, alteration, complexation, adsorption, oxidation and reduction,
77 where overlapping signatures is observed between abiotic and biotic contribution. The
78 influence of microorganisms is isotopic iron fractionation is too weak compared to our
79 understanding/the complexity of iron cycling to use iron isotope as a tracer for biotic influences.
80 Our understanding remains even poorer when it comes to the microbially-driven fractionation
81 at intracellular and extracellular levels, therefore providing a great opportunity for future work.

82 **2. Overview of biogeochemical processes inducing iron isotopes fractionation**

83 Figure 1 represents biogeochemical cycling of iron in nature involving several processes
84 such as redox reactions, ligand-complexation, dissolution (biotic and abiotic), precipitation
85 from FeII or/and FeIII source (abiotic), bioassimilation (bio-uptake, bio-sorption,
86 biomineralization, dissimilatory iron reduction processes). Detailed values and corresponding
87 references for each process are given in Table 1, 2, 3 and 4.

88 The simple redox reactions between FeII and FeIII could induce $\Delta^{56}\text{Fe}_{\text{II-III}}$ of $-3.00\pm 0.23\text{‰}$
89 as calculated by several experimental studies (Table 2), whereas the complexation of FeIII and
90 desferrioxamine B (DFOB) ligand induce less fractionation, $\Delta^{56}\text{Fe}_{\text{III-DFOB}}$ of $-0.60\pm 0.15\text{‰}$

91 (Table 2). During mineral weathering, the fractionation induced by both abiotic and biotic
92 influence is overlapped for all minerals (silicates/phylosilicates and oxyhydroxides).
93 Nonetheless, the fractionation is rather distinguished by the isotopic signatures of the original
94 minerals. The observed fractionation induced by ligand or proton promoted dissolution,
95 $\Delta^{56}\text{Fe}_{\text{sol-solid}}$ is $-0.32\pm 0.13\text{‰}$ for silicates/phylosilicates dissolution, whereas $\Delta^{56}\text{Fe}_{\text{sol-solid}}$ is
96 $-1.22\pm 0.17\text{‰}$ for oxyhydroxide dissolution. Photochemically driven reductive dissolution
97 resulted in more negative $\Delta^{56}\text{Fe}$ values than dark dissolution (in absence of light) as the
98 presence of light activates the photo-reactive functional groups on the oxalic ligands
99 accommodating reductive mechanisms.

100 Abiotic precipitation of oxidized (hematite) or reduced (siderite, mackinawite and pyrite)
101 minerals showed that the extent of Fe isotope fractionation depends strongly on whether kinetic
102 or equilibrium isotope effects are dominant. Degree of saturation, different precipitation rates,
103 dissolution-reprecipitation, mixing interfaces (between mineral surface and solution vs bulk
104 mineral and solution) and the experimental duration could induce significant kinetic isotopic
105 fractionation or could shift the isotopic equilibrium.

106 Variation in iron isotope fractionation is observed during uptake by different
107 microorganisms, i.e. $\Delta^{56}\text{Fe}_{\text{FeIII-algae}}$ is $0.43\pm 0.49\text{‰}$ for algae as well as $\Delta^{56}\text{Fe}_{\text{FeIII-bact}}$ is
108 $-1.10\pm 0.11\text{‰}$ for bacteria. A similar and large variation is observed during the microbial
109 ferrous oxidation, $\Delta^{56}\text{Fe}_{\text{FeII-FeOOH}}$ varies from $-1.99\pm 0.24\text{‰}$ to $-3.35\pm 0.19\text{‰}$ depending on the
110 types of microorganisms involved, different metabolic pathways and different surface
111 functional group on cell, as well as the diversity of end mineral formation. Precipitation could
112 take place either in the periplasm, at the cell surface or extracellularly (exopolysaccharides).
113 On the other hand, the bio-mineralization of magnetite by *Magnetospirillum Magneticum strain*
114 *AMB-1* resulted in a positive $\delta^{56}\text{Fe}$ enrichment of FeII and FeIII substrates¹ with respect to

¹ FeII-lysate or FeIII-lysate substrates

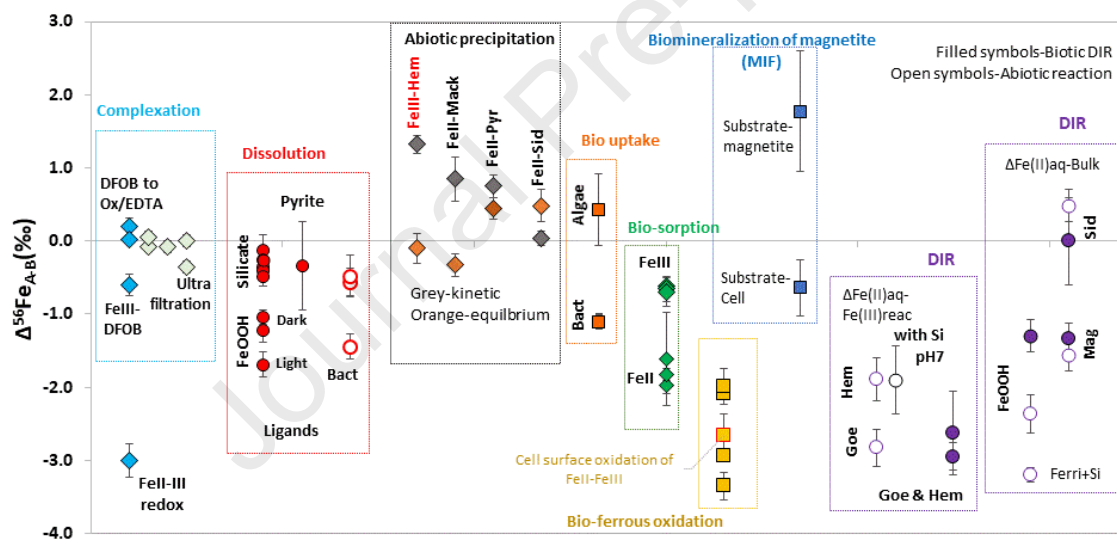
115 magnetite where $\Delta^{56}\text{Fe}_{\text{FeII/III-magnetite}}$ is $1.77\pm 0.82\%$ (Amor et al., 2016). It is also noteworthy
116 that MIF is observed on ^{57}Fe during biomineralization in parallel to other biogeochemical
117 processes.

118 Bio-sorption of both FeII and FeIII onto the microbial biomass can be expected in parallel
119 other microbial processes. The extent of fractionation due to bio-sorption is $-1.81\pm 0.18\%$ for
120 $\Delta^{56}\text{Fe}_{\text{FeII-cell}}$ and $-0.66\pm 0.04\%$ for $\Delta^{56}\text{Fe}_{\text{FeIII-cell}}$ during the incubation of planktonic
121 cyanobacteria (*Gloeocapsa sp.*, *Synechococcus sp.*, and *Planthothrix sp.*) in FeII and FeIII
122 substrates. Fractionation is presumably linked to changes of the Fe coordination chemistry
123 between the aqueous phase and cells surface. The surface carboxyl and phosphoryl groups
124 could interact with both FeII and FeIII through covalent binding; therefore, reducing chances
125 of FeIII polymerization and preventing subsequent oxyhydroxides precipitation.

126 Biotic or abiotic reduction of iron minerals can be expected whenever anoxic and anaerobic
127 conditions are favored in soil, groundwater, and in sediments. Studies have confirmed that the
128 negative $\delta^{56}\text{Fe}$ values for aqueous FeII in natural systems reflects dissimilatory iron reduction
129 process (DIR). The second largest range observed was for $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIreact}}$, which includes
130 both biotic and abiotic reduction of goethite and hematite ($-1.68\pm 0.08\%$ to $-2.83\pm 0.23\%$),
131 while the largest range was observed for microbial ferrous iron oxidation ($-1.99\pm 0.24\%$ to
132 $-3.35\pm 0.19\%$). "The extent of fractionation could be shifted further by the presence of dissolved
133 Si, different solution pHs, the natural organic matter or the changes in the mineral structures
134 when these impurities are incorporated. The main mechanism inducing fractionation is due to
135 the isotopic exchange between aqueous FeII and reactive FeIII on the oxide surface for both
136 abiotic and biotic experiments where the role of bacteria was to catalyze the isotopic exchange
137 via electron pumping to the oxide surface. Microbial reduction of ferrihydrite, goethite and
138 hematite could lead to the formation of biogenic oxides and carbonates minerals such as

139 magnetite and siderite via reprecipitation. The extent of fractionation is primarily controlled by
 140 the source signature of the original mineral rather than the biotic or abiotic contribution.

141 In summary, a wide range of iron isotope fractionation, $\Delta^{56}\text{Fe}_{\text{A-B}}$ can be observed across
 142 each process (Fig 1 and Table 2, 3, 4); redox reaction between FeII and FeIII (-3.00%),
 143 complexation (-0.60% to 0.20%), dissolution (-1.44% to 0.48%), precipitation (-0.33% to
 144 1.30%), biouptake (-1.1% to 0.4%), biosorption (-2% to -0.6%), bio-oxidation (-3.35% to
 145 -2.15%), mineralization (-0.64% to 1.77%), dissimilatory iron reduction (-2.95% to
 146 -1.68%). One process can be a subsidiary of another and more than one process may take place
 147 simultaneously, where iron isotopes can be useful as a tool to identify which family of
 148 geochemical processes is dominant in place, but not biotic influence from abiotic one.



149
 150 Figure 1. Overview of iron isotopes fractionation driven by low-temperature biogeochemical
 151 processes such as redox reaction ($\Delta^{56}\text{Fe}_{\text{II-III}}$), ligand-complexation ($\Delta^{56}\text{Fe}_{\text{III-ligands}}$),
 152 alteration/dissolution ($\Delta^{56}\text{Fe}_{\text{solution-solid}}$), abiotic mineral precipitation ($\Delta^{56}\text{Fe}_{\text{II/III-mineral}}$), bio-
 153 uptake ($\Delta^{56}\text{Fe}_{\text{III-cell}}$), bio-sorption ($\Delta^{56}\text{Fe}_{\text{II-biomass}}$ vs $\Delta^{56}\text{Fe}_{\text{III-biomass}}$), microbial oxidation
 154 ($\Delta^{56}\text{Fe}_{\text{II-III-FeOOH}}$), biomineralization ($\Delta^{56}\text{Fe}_{\text{II or III-mineral/cell}}$) and dissimilatory iron reduction of
 155 iron minerals ($\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIreact}}$ and $\Delta^{56}\text{Fe}_{\text{FeIIaq-BulkMineral}}$). (DFOB-desferrioxamine B, FeOOH-
 156 oxyhydroxide minerals, Bact-bacteria, Hem-hematite, Fer-ferrihydrite, Goe-goethite, Mag-

157 magnetite, Sid-siderite, Mack-mackinawite, Pyr-pyrite, and DIR- dissimilatory iron reduction).
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163 corresponds to the abiotic hematite experiment conducted in the presence of Si at pH7 in
164 comparison to the experiments without Si. Error bars represent 2SD experimental error and.
165 Detailed values and corresponding references for each process are given in Table 1, 2 and 3.

166 **3. Detailed overview on Fe isotopes fractionation by different biogeochemical processes**

167 This section represents several experimental studies detailing on each biogeochemical
168 process where abiotic vs biotic contribution, as well as kinetic vs equilibrium Fe isotope
169 fractionation, is further addressed. Experimental fractionation is further compared to the
170 calculated or theoretical fractionation where relevant. It is noteworthy that each geochemical
171 process can be either biotic or abiotic, during which isotope fractionation could be either
172 kinetic-driven, at equilibrium or a convergence of both.

173 **3.1. Redox reactions (abiotic, kinetic and equilibrium)**

174 The iron isotopes are fractionated during redox reactions (oxidation or reduction), either
175 kinetically or at equilibrium. Equilibrium and kinetic iron isotope fractionation between
176 aqueous FeII and FeIII was reported over a range of salinity (0, 11, 110mM Cl^-) and
177 temperatures (0 and 22°C). The average measured equilibrium fractionation; $\Delta^{56}\text{Fe}_{\text{II-III}}$ is
178 $-3.00\pm 0.23\text{‰}$ at 22°C whereas it becomes lower, $-3.57\pm 0.38\text{‰}$ at 0°C for all salinity,
179 indicating that fractionation is a function of temperature rather than salinity (Welch et al., 2003).
180 These fractionations are slightly different from the study by Johnson et al. (2002) as they were
181 corrected from experimental errors related to separation and recovery, or partial reaction of FeII

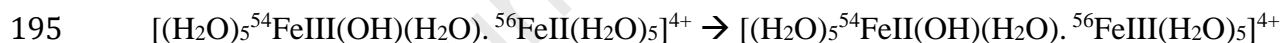
182 and FeIII during precipitation. Kinetics of iron isotope exchange can be described by a second-
 183 order rate equation where the rates are affected by temperature and salinity. The kinetic
 184 exchange rates are about an order of magnitude slower at 0°C compared to rapid exchange at
 185 22°C, and 40% slower at the salinity of 11mM Cl⁻ compared to the rates at 0mM or 100mM
 186 Cl⁻. The redox exchange between FeII and FeIII is a simple homogenous exchange reaction
 187 with a net exchange of one electron between two high-spin Fe atoms. Electron exchange takes
 188 place in three steps (Wehrli, B., 1990): the formation of a precursor complex, electron transfer
 189 (across a bridging hydroxyl, water, or chlorite ion), and dissociation of the complex, where a
 190 series of reactions can be written as follows:

191 Overall isotopic exchange during redox, $^{56}\text{Fe}^{2+} + ^{54}\text{Fe}^{3+} \rightarrow ^{54}\text{Fe}^{2+} + ^{56}\text{Fe}^{3+}$

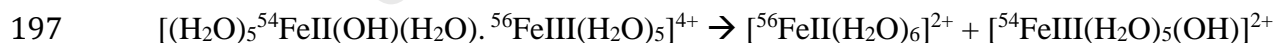
192 Precursor formation,



194 Electron transfer,



196 Dissociation of complex,



198 **3.2. Complexation of Fe and organic matter (sub process of every process)**

199 Complexation of Fe to organic molecules could be one of the initial, however, influential
 200 processes taking place within each biogeochemical process in nature, where Fe could be either
 201 in dissolved, colloidal or particulate form and organic molecules of different sizes (organic
 202 matter like humic substances, biological byproducts, bioligands). As an example, the
 203 equilibrium Fe isotope fractionation induced by complexation of the reference organic ligand,
 204 desferrioxamine B (DFOB) to inorganic FeIII species resulted in a negative $\Delta^{56}\text{Fe}_{\text{FeIII-DFOB}}$ of

205 $-0.60 \pm 0.15\%$ as given in Fig 2A (Dideriksen et al., 2008). Fe isotope effects can also be
 206 driven by changes in Fe coordination that involve the exchange between different organic
 207 ligands. For instance in Fig 2A, the measured equilibrium Fe isotope fractionation between
 208 FeIII-DFOB (desferrioxamine B) to FeIII-Ox (Oxalate), and to FeIII-EDTA
 209 (ethylenediaminetetraacetic acid) resulted in $\Delta^{56}\text{Fe}_{(\text{FeIII-DFOB})-(\text{FeIII-Ox})}$ of $0.20 \pm 0.11\%$ and
 210 $\Delta^{56}\text{Fe}_{(\text{FeIII-DFOB})-(\text{FeIII-Ox})}$ of $0.02 \pm 0.11\%$ (Morgan et al., 2010). In these studies, preferential
 211 complexation of heavy Fe isotopes by OM and/or hydroxyl ligands to form stronger chemical
 212 bonds is reported (Roe et al., 2003; Dideriksen et al., 2008; Morgan et al., 2010).

213 A similar study on Fe complexation with organic matter at pH 6.5 reported that the relative
 214 distribution of Fe and OM depends on different particle size fractions by ultrafiltration (Lotfi-
 215 Kalahroodi et al., 2019). Significant Fe isotope fractionation was observed for the smallest
 216 particles. As shown in Fig 2A, at circumneutral pH 6.5, 43% of Fe and 57% of OM were in the
 217 $<0.2 \mu\text{m}$ fraction and only 0.8% of Fe and 20% of OM were in the $<30 \text{ kDa}$ fraction. However,
 218 the isotope fractionation was only $\Delta^{56}\text{Fe}_{\text{TotFe}-<0.2\mu\text{m}}$ $-0.07 \pm 0.08\%$ for the larger particle size
 219 while it was $\Delta^{56}\text{Fe}_{\text{TotFe}-<30 \text{ kDa}}$ $-0.35 \pm 0.08\%$ for the smallest size. As a budget, 42% of Fe and
 220 38% of OM were found in the $0.2 \mu\text{m} - 30 \text{ kDa}$ fraction with $\Delta^{56}\text{Fe}_{\text{TotFe}-(0.2\mu\text{m}-30 \text{ kDa})}$ of
 221 $-0.08 \pm 0.08\%$ (Lotfi-Kalahroodi et al., 2019)². Fractionation of Fe isotope can be significant
 222 depending upon particles' size during Fe complexation with oxyhydroxide/OM to form
 223 nanoaggregates at pH 6.5, natural range for aquatic environments and soil pore waters.

224 In complement to experiments, quantum chemical calculations allow to better understand
 225 the isotopic effects of Fe speciation and complexation. Reduced partition function ratios
 226 between ^{56}Fe and ^{54}Fe , $\beta^{56/54}$ factor³ (or $10^3 \text{Ln}\beta^{56/54}$ at 25°C in Fig 2B), depict the magnitude of

² The $\Delta^{56}\text{Fe}_{\text{A-B}}$ is recalculate based on the $\delta^{56}\text{Fe}$ values given in the Table 5 of the publication (Lotfi-Kalahroodi et al., 2019)

³ Iron reduced partition isotopic function ratio (β factor) is commonly used to calculate magnitude and direction of equilibrium iron isotope fractionation in several theoretical calculation using density functional theory (DFT) modeling and ab-initio calculation. One can say that $10^3 \text{Ln}\beta^{56/54}$ in calculation studies is similar to $\delta^{56}\text{Fe}$ measured in MC-ICPMS and $10^3 \text{Ln} \alpha_{\text{A-B}}$ will be equivalent to the big delta $\Delta_{\text{A-B}}$, respectively. Please refer to SI for details Nomenclature and Comparability.

227 the equilibrium isotopic fractionation between different Fe redox species, organic and inorganic
 228 ligands, in respect to the bond lengths of the molecules formed. Three different basic
 229 fractionations can be expected: (1) redox reaction of Fe within the same phosphate complex
 230 ($\text{Fe}^{\text{II}}\text{HPO}_4(\text{H}_2\text{O})_5$ & $\text{Fe}^{\text{III}}\text{HPO}_4(\text{H}_2\text{O})_5^+$), (2) the exchange between different inorganic ligands
 231 but the same Fe redox ($\text{Fe}^{\text{III}}\text{HPO}_4(\text{H}_2\text{O})_5^+$ & $\text{Fe}^{\text{III}}\text{CO}_3(\text{H}_2\text{O})_4^+$), (3) the exchange between
 232 inorganic and organic ligands but the same Fe redox ($\text{Fe}^{\text{III}}(\text{cit})_2\text{OH}_4^-$ & $\text{Fe}^{\text{III}}\text{Cl}_2(\text{H}_2\text{O})_4^+$). It is
 233 also noteworthy that β factor is inversely proportional to the bond length and that heavy isotopes
 234 form shorter bonds than light isotopes. Calculations allows us to predict the order of magnitude
 235 Fe isotope fractionation during ligands exchange, and show the expected diversity according to
 236 the reactions and bonds involved. Experimental studies to address/verify such theoretical data
 237 are however very limited.

238 **3.3. Dissolution (abiotic vs biotic)**

239 Different organic ligands (oxalic acid, acetic acid, citric acid, desferrioxamine mesylate-
 240 DFAM) are used to study abiotic dissolution of iron-bearing minerals. Minerals involved in the
 241 studies were hornblende (iron silicates), phyllosilicates (biotite/cholorite), pyrites (reduced
 242 minerals) and goethite (amorphous mineral). Soil bacteria, *Bacillus mycooides* and *Streptomyces*
 243 *sps* were cultured with the minerals in order to better understand their influence on continental
 244 weathering, but also mineral and soil formation (Brantley et al., 2001a,b; 2004, Wiederhold et
 245 al., 2006; Jang et al., 2008; Kiczka et al., 2010a; Wolfe et al., 2016).

246 During the abiotic and biotic dissolution of iron silicates (hornblende and phyllosilicates)
 247 given in Figure 2C, the measured offset $\Delta^{56}\text{Fe}_{\text{sol-solid}}$ is $-0.36 \pm 0.25\text{‰}$ (DFAM), $-0.25 \pm 0.18\text{‰}$
 248 (oxalic acid), $-0.13 \pm 0.21\text{‰}$ (acetic acid), $-0.40 \pm 0.21\text{‰}$ (citric acid), $-0.56 \pm 0.19\text{‰}$ (*Bacillus*
 249 *mycooides*) and $-0.48 \pm 0.29\text{‰}$ (*Streptomyces*) respectively. Variation in fractionation among
 250 different ligands and different types of soil bacterial strains is insignificant within error, and
 251 with overlapping offsets between abiotic and biotic contribution. Fe isotope fractionation is

252 presumably induced during hydrolysis of surface complexes by ligands where the strength of
253 Fe-ligands complex is greater than that of bonds retaining Fe in the mineral lattice, accentuating
254 the irreversibility of the hydrolysis.

255 On the other hand, the abiotic and biotic dissolution of goethite (Figure 2C) led to
256 $\Delta^{56}\text{Fe}_{\text{sol-solid}}$ of $-1.04\pm 0.10\text{‰}$ (DFAM), $-1.2\pm 0.17\text{‰}$ (oxalic-dark), $-1.7\pm 0.17\text{‰}$ (oxalic-light)
257 and $-1.44\pm 0.17\text{‰}$ (*Bacillus mycoides*). Please note that $\Delta^{56}\text{Fe}$ are recalculated from the $\Delta^{57}\text{Fe}$
258 assuming that all the measured data points follow a mass-dependent fractionation. The
259 fractionation observed in oxalic-light experiments indicated photochemical reductive
260 dissolution, promoting electron transfer at the mineral surface that access the crystallographic
261 sites, normally not reactive in ligand controlled (oxalic dark) dissolution (Wiederhold et al.,
262 2006). Similarly, Fe release was enriched in light isotopes in proton promoted dissolution of
263 pyrite by HCl at pH 4-6 with $\Delta^{56}\text{Fe}_{\text{sol-solid}}$ of $-0.34\pm 0.61\text{‰}$ (Wolfe et al., 2016). During the
264 proton promoted dissolution of silicates, the exfoliation of the silicate structure is triggered by
265 the release of potassium, which favors the dissolution of an octahedral layer from the edges
266 inwards and leaves the tetrahedral sheets less affected (Kiczka et al., 2010a; Wolfe et al., 2016).

267 **3.4. Mineral precipitation (Abiotic, kinetic and equilibrium)**

268 Only few studies explored the precipitation of iron to minerals via abiotic experiments;
269 hematite at 98°C (Skulan et al., 2002) extrapolated to 25°C (Beard et al., 2010), ferrihydrite at
270 25°C (Johnson et al., 2002), siderite at 25°C (Wiesli et al., 2004), sulfide between 2 and 40°C
271 (Guilbaud et al., 2010) and goethite at 25°C (Handler et al., 2009). These studies have
272 demonstrated how different rates of mineral precipitation can influence kinetic iron isotope
273 fractionation. High degree of saturation induced high precipitation rates that will induce almost
274 no kinetic fractionation, whereas moderately fast precipitation rates induce significant kinetic
275 fractionation (Johnson et al., 2002; Skulan et al., 2002; Jimenez-Lopez and Romanek, 2004;

276 Wiesli et al., 2004). But if precipitation rates are small enough, each portion of mineral
277 precipitated is in isotopic equilibrium with aqueous Fe, resulting in a global equilibrium.

278 ***Iron oxyhydroxide precipitation:*** Johnson et al., (2002) demonstrated that extremely rapid
279 precipitation within seconds produces no significant iron isotopic fractionation between
280 aqueous FeII in solution and colloidal FeIII oxyhydroxide.

281 ***Hematite precipitation:*** Skulan et al., (2002) demonstrated that moderately rapid
282 precipitation within hours could produce the largest kinetic isotopic fractionation between FeIII
283 and hematite. Beard et al. (2010) extrapolated the precipitation study at 98°C by Skulan et al.
284 (2002) to 25°C. The extrapolated kinetic fractionation, $10^3 \text{Ln}^{56} \alpha_{\text{FeIIIaq-hematite_kinetic}}$ is
285 $1.32 \pm 0.12\text{‰}$ at 25°C where the extrapolated equilibrium fractionation remains close to zero;
286 $10^3 \text{Ln}^{56} \alpha_{\text{FeIIIaq-hematite_equilibrium}}$ is $-0.15 \pm 0.20\text{‰}$ at 25°C (Figure 2D). The moderately rapid
287 precipitation rates are more likely to be associated with kinetic isotope fractionation, or largest
288 fractionation observed in the experiment.

289 ***Siderite precipitation:*** Wiesli et al., (2004) also mentioned that super-saturation with high
290 precipitation rate produce almost no fractionation ($\Delta^{56}\text{Fe}_{\text{FeIIaq-siderite_kinetic}}$ of $0.04 \pm 0.10\text{‰}$) in
291 agreement with Johnson et al., (2002). Contrastingly, at moderate precipitation rate Wiesli et
292 al. (2004) mention larger kinetic isotope fractionation, which extend depends on the formed
293 mineral size ($\Delta^{56}\text{Fe}_{\text{FeIIaq-siderite_10H}}$ of $2.06 \pm 0.10\text{‰}$ for $>10 \mu\text{m}$ siderite, vs $0.93 \pm 0.10\text{‰}$ for 0.45-
294 $10 \mu\text{m}$ siderite). The proposed equilibrium fractionation factor between FeII and siderite from
295 regression of all the experimental data is $0.48 \pm 0.22\text{‰}$ ($10^3 \text{Ln}^{56} \alpha_{\text{FeIIaq-siderite_equilibrium}}$).

296 ***Mackinawite precipitation:*** Butler et al. (2005) and Guilbaud et al. (2010) reported the
297 kinetic isotope effects where the isotopic exchange is likely to happen via dissolution-
298 precipitation between the mineral surface and the solution rather than the bulk mineral and the
299 solution. The proposed kinetic isotope fractionation, $\Delta^{56}\text{Fe}_{\text{FeIIaq-mackinawite_kinetic}}$ is $0.85 \pm 0.30\text{‰}$ at

300 25°C. Guilbaud et al., (2011a) observed that the apparent equilibrium precipitation of
 301 mackinawite, $\Delta^{56}\text{Fe}_{\text{FeIIaq-mackinawite_equilibrium}}$ is $-0.33\pm 0.16\text{‰}$ at 25°C (Figure 2D).

302 **Sulfide precipitation:** Redox cycling of Fe coupled to pyrite precipitation from sources with
 303 either negative FeII_{aq} composition or positive FeIII_{aq} composition display the largest $\delta^{56}\text{Fe}$
 304 variation ranging from -4‰ to $+4\text{‰}$ in nature due to its multiple precipitation pathways. The
 305 first pathway is via H_2S or polysulfide pathway where formation of aqueous FeS clusters
 306 $(\text{FeS}_{\text{aq}})^4$ occurs under sulfide-rich conditions (Rickard et al., 2001), the second pathway is via
 307 formation of intermediate phase, greigite $(\text{Fe}_3\text{S}_4)^5$ and the third pathway is via the ferric-
 308 hydroxide-surface (FHS) formation (Peiffer et al., 2015; Wan et al., 2017). As given in Figure
 309 2D, the apparent equilibrium isotopic fractionation between FeII and pyrite, $\Delta^{56}\text{Fe}_{\text{FeSx-Pyrite}}$ is
 310 $0.54\pm 0.21\text{‰}$ via H_2S pathway, ranging between $0.41\pm 0.14\text{‰}$ and $0.59\pm 0.25\text{‰}$ via polysulfide
 311 pathway, and ranging between $0.41\pm 0.23\text{‰}$ and $0.63\pm 0.04\text{‰}$ via greigite as intermediate
 312 (Mansor and Fantle et al., 2019). Therefore, the average equilibrium isotopic fractionation
 313 between FeS_x and pyrite, where FeS_x includes FeS , FeII_{aq} and greigite; $\Delta^{56}\text{Fe}_{\text{FeSx-Pyrite_equilibrium}}$
 314 of $0.51\pm 0.22\text{‰}$, which is identical within error irrespective of precipitation pathways. The
 315 kinetic isotope effect is visible during early stages of precipitation where the proposed
 316 fractionation, $\Delta^{56}\text{Fe}_{\text{FeSx-Pyrite_kinetic}}$ is $0.75\pm 0.15\text{‰}$. Early-stage nucleation vs later-stage crystal
 317 growth also contribute to such isotopic variations, where it is difficult to distinguish nucleation
 318 effects from kinetic effect.

319 **3.5. Microbial assimilation (uptake, oxidation, mineralization)**

320 Iron is an essential nutrient for all microorganisms, different groups use iron as an electron
 321 donor or acceptor to carry out numerous metabolic reactions, thereby contributing to the
 322 microbial share of iron redox transformations in nature. Microbial influence could induce iron

⁴ FeS clusters are the first form of sulfide precipitates where the long polysulfide chain involves one or more groups of sulfur linked together via covalent bonds

⁵ Greigite is has a similar inverse spinel structure where atoms are closely packed cubic array, therefore much more stable.

323 isotope fractionation in several ways: (1) the direct oxidation of FeII to FeIII, (2) sorption of
324 both FeII and FeIII on to biomass either dead or alive, (3) partial sorption of iron onto microbial
325 precipitated oxyhydroxides, and (4) Fe chelated by bacterial byproducts. Microbial assimilation
326 of iron by microorganisms is the primary process from which several specific processes can be
327 identified and subdivided into bio-uptake, bio-oxidation and bio-mineralization. In addition,
328 bio-sorption, a lateral or indirect process, can take place in parallel to microbial assimilation.
329 Note that the microbial processes in this section 3.5 are taking place in aerobic environments
330 while the following section 3.6 will focus on microbial assimilation in anaerobic environments.

331 **Bio-uptake:** Studies have shown that the extent of fractionation due to bacteria is larger
332 compared to that of algae (Figure 2E). The bacterial Fe uptake by the soil bacterium,
333 *Azotobacter vinelandii* gave the fractionation of $-1.10 \pm 0.06\text{‰}$ (Wasylenki et al., 2007),
334 different from the algae uptake by *Chlorella pyrenoidosa* ($0.08 \pm 0.09\text{‰}$), and *Chlamydomonas*
335 *reinhardtii* ($0.78 \pm 0.09\text{‰}$) respectively (Sun and Wang, 2018). The difference in sign for algae
336 (+) and bacteria (-) of $\Delta^{56}\text{Fe}_{\text{III-cell/algae}}$, is worth further discussion regarding their differences in
337 the uptake mechanisms (phytoplankton vs. bacteria). The algae depicted contrasting Fe isotope
338 compositions in intracellular and extracellular fractions. The intracellular Fe have heavy Fe
339 isotope composition in relative to the initial FeII substrate, whereas the extracellular Fe is
340 isotopically variable hosting a mixture of FeII with light signature and FeIII enriched in heavy
341 isotopes. The oxidation of adsorbed FeII in the extracellular fraction and the selective transport
342 of such isotopically heavy Fe into the cell could account for the fractionation observed in the
343 studies with algae (Sun and Wang, 2018). Bacterium, *A. vinelandii* is known to produce high
344 affinity and stronger Fe chelator, dihydroxybenzoic acid, compared to the Fe-citrate substrate
345 present in the medium. The equilibrium shift between the two pools of Fe is thus responsible
346 for isotopically light Fe-citrate and a heavy Fe bound to stronger chelator, and for the overall
347 fractionation observed between substrate and cell (Wasylenki et al., 2007). It is hard to pinpoint

348 the exact fractionation mechanisms in these bio-uptake experiments as the overall fractionation
 349 is resulting from FeII-FeIII redox, adsorption onto microbial biomass, possible formation of
 350 thin oxyhydroxides coating, and complexation with biologically-produced Fe chelating ligands.

351 **Bio-oxidation:** Variations based on type of bacterial strain can be observed in Figure 2F;
 352 $-2.09 \pm 0.05\text{‰}$ for fresh water photoautotrophs, *Thiodictyon strain F4* (Croal et al., 2004),
 353 $-2.94 \pm 0.05\text{‰}$ for nitrate reducer *Acidovorax sp. BoFeNI* (Kappler et al., 2010), $-1.99 \pm 0.24\text{‰}$
 354 for marine photoferrotrophs *Rhodovulum iodosum* (Swanner et al., 2015), and $-3.35 \pm 0.19\text{‰}$
 355 for marine cyanobacterium *Synechococcus PCC7002* (Swanner et al., 2017). The precipitates
 356 observed are a mixture of many FeIII oxyhydroxide phases such as ferrihydrite, goethite, and
 357 lepidocrocite, where amorphous ferrihydrite was initially precipitated and then into more
 358 crystalline minerals over time (Croal et al., 2004; Kappler et al., 2004 and 2010; Swanner et al.,
 359 2015). A study on a natural hydrothermal spring depositing Fe oxy(hydr)oxide with and without
 360 cyanobacterial biofilm showed $\Delta^{56}\text{Fe}_{\text{sol-solid}}$ of $-0.54 \pm 0.08\text{‰}$ (abiotic) and of $-0.32 \pm 0.10\text{‰}$
 361 (biotic) (Mulholland et al., 2015b).

362 Kappler et al., (2010) confirmed a two-step equilibrium-kinetic microbial ferrous oxidation
 363 process where equilibrium fractionation between $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIaq}}$ produces -3.0‰ and the
 364 kinetic fractionation between $\Delta^{56}\text{Fe}_{\text{FeIIIaq-FeIIIsolid}}$ gives 1.0‰ , making the final net fractionation
 365 $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIsolid}}$ of -2.0‰ in agreement to the Rayleigh trend of -2.0‰ . Swanner et al., (2017)
 366 pointed out that the effect of adsorption of FeIII onto the cell surface, recovered by washing
 367 with sodium acetate, $\delta^{56}\text{Fe}$ of $-0.28 \pm 0.14\text{‰}$ where the measured fractionation between aqueous
 368 FeII and adsorbed FeIII on cell surface can produce $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIacetate}}$ of -1.84‰ . Different
 369 net $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIsolid}}$ observed in several experiments can be related to the proportions of
 370 aqueous FeII and FeIII(OH)₃ precipitates at different time points, the extent of isotope exchange
 371 between the two Fe pools, i.e. a combination of equilibrium which also records initial and
 372 variable kinetic fractionation as well as potential changes in crystallinity of iron minerals. If Fe

373 isotope fractionation is induced by FeIII precipitation outside, the kinetic Fe isotope
374 fractionation will be dominant throughout the redox cycling outside the cell in addition to
375 equilibrium Fe isotope fractionation being dominant inside the periplasm. It is less likely that
376 isotopic exchange will occur between aqueous Fe and FeIII(OH)₃ precipitate, as compared to a
377 restricted environment of periplasm with a smaller volume of precipitates with higher surface
378 area (Miot et al., 2009).

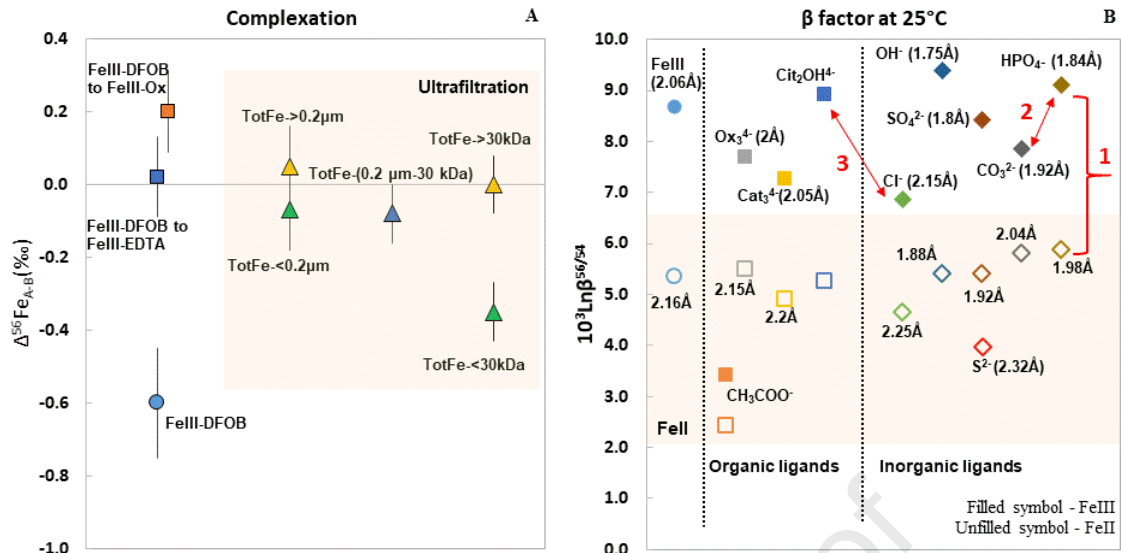
379 **Bio-mineralization (MDF vs MIF):** Amor et al. (2016) determined mass-dependent and
380 mass-independent fractionation of Fe isotope during intracellular biomineralization of
381 magnetite nanoparticles by magnetotactic bacterium, *Magnetospirillum Magneticum strain*
382 *AMB-1* (Figure 2F). The magnetite is strongly enriched in light isotopes relative to initial Fe
383 sources (FeII ascorbate and FeIII quinate) with a net fractionation of $1.39 \pm 0.19\%$ for
384 $\Delta^{56}\text{Fe}_{\text{FeII-magnetite}}$ and $2.15 \pm 0.18\%$ for $\Delta^{56}\text{Fe}_{\text{FeIII-magnetite}}$. Bacterial lysate $\delta^{56}\text{Fe}$ values were
385 enriched in the heavy isotopes relative to initial Fe sources with a net fractionation of
386 $-0.80 \pm 0.08\%$ for $\Delta^{56}\text{Fe}_{\text{FeII-Lysate}}$ and $-0.49 \pm 0.17\%$ for $\Delta^{56}\text{Fe}_{\text{FeIII-Lysate}}$ where Fe in the lysates is
387 more likely to be present as FeIII. Iron initially present as FeII or FeIII, was later incorporated
388 into the cell and stored as FeIII in ferritin. FeIII was partially reduced for trafficking to
389 magnetosomes and FeII was precipitated as magnetite in processes involving cytochromes or
390 similar proteins. Such magnetite precipitation in AMB-1 is in line with Fe isotope fractionations
391 determined in FeIII-reducing bacteria and in abiotic magnetite precipitation.

392 Mass-independent isotope fractionation (MIF) is observed in the odd isotope of Fe (⁵⁷Fe)
393 but not in even isotopes (⁵⁴Fe, ⁵⁶Fe, and ⁵⁸Fe), highlighting a magnetic isotope effect. The initial
394 Fe sources had $\Delta^{57}\text{Fe}$ of $0.00 \pm 0.04\%$ within uncertainties and the reported mass-independent
395 fractionation of Fe isotopes, $\Delta^{57}\text{Fe}_{\text{FeSources-magnetite}}$ ranges from 0.00% to -0.26% , while the
396 average isotope mass balance gives $\Delta^{57}\text{Fe}$ of $0.00 \pm 0.04\%$, confirming isotope anomalies. The
397 observed MIF is induced by magnetic isotope effects (MIEs) due to the nuclear spins and

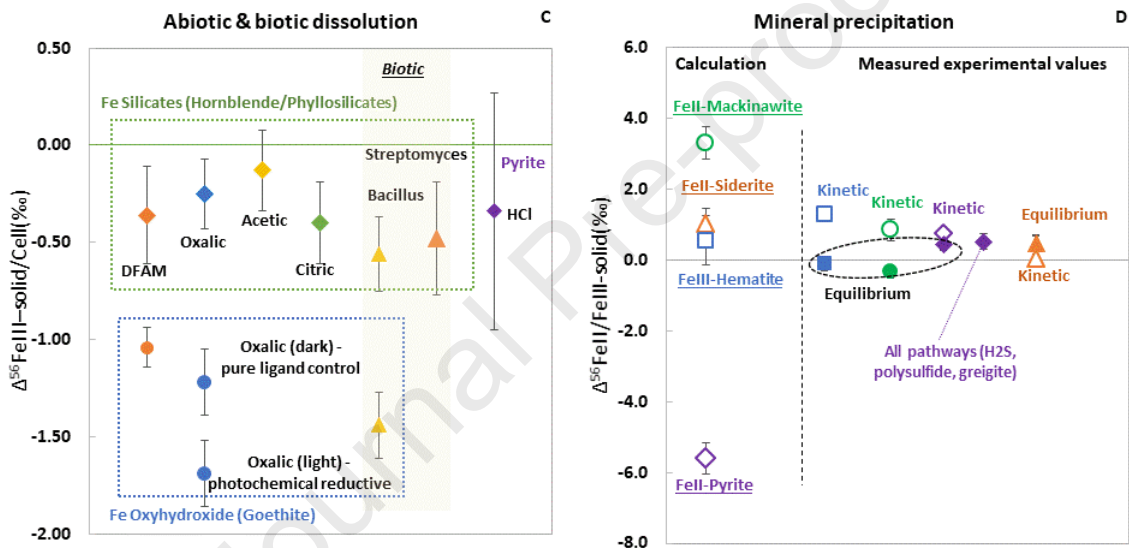
398 nuclear magnetic moments. MIEs are usually common in reactions involving free radicals and
399 paramagnetic species, affecting odd isotopes. As FeII is in high spin configuration and FeIII is
400 paramagnetic, ^{57}Fe would behave differently from ^{54}Fe , ^{56}Fe , and ^{58}Fe . An extra experiment
401 with ^{58}Fe was conducted to determine potential isotope anomalies on ^{58}Fe in comparison to
402 ^{57}Fe ; $\Delta^{58}\text{Fe}$ is $\sim 0\text{‰}$ within uncertainties, confirming only ^{57}Fe is affected by MIF and MIEs.

403 **Bio-sorption:** Significant Fe isotope fractionation is observed during the adsorption of FeII
404 and FeIII onto the dead biomass of cyanobacteria (Figure 2E); *Gloeocapsa sp.*, *Synechococcus*
405 *sp.*, and *Planthothrix sp.* (Mulholland et al., 2015b). Reported net fractionation, $\Delta^{56}\text{Fe}_{\text{FeII-cell}}$ is
406 $-1.62\pm 0.64\text{‰}$ (*Gloeocapsa sp.*), $-1.97\pm 0.12\text{‰}$ (*Planthothrix sp.*) and $-1.83\pm 0.08\text{‰}$
407 (*Synechococcus sp.*). Meanwhile, the reported $\Delta^{56}\text{Fe}_{\text{FeIII-cell}}$ is $-0.65\pm 0.17\text{‰}$ (*Gloeocapsa sp.*),
408 $-0.62\pm 0.07\text{‰}$ (*Planthothrix sp.*) and $-0.70\pm 0.20\text{‰}$ (*Synechococcus sp.*). The discrepancy
409 observed between the FeII-biomass pair compared to the FeIII-biomass pair, indicates the redox
410 state of Fe playing an important role during the adsorption. Swanner et al. (2017) have proven
411 further that the fractionation can be as light as $-2.66\pm 0.29\text{‰}$ when the adsorbed FeII is
412 simultaneously oxidized into FeIII at the cell surface of marine cyanobacterium,
413 *Synechococcus*. The fractionations are presumably linked to changes in Fe coordination
414 chemistry between the aqueous phase and cells surface. The preferential enrichment of heavy
415 isotopes on the cell surface can be due to the stronger covalent metal-ligand bonding (Fe-O-C-
416 P) where Fe is octahedrally coordinated with phosphoryl or carboxyl groups on the cell wall,
417 leaving behind the light isotopes of Fe aqua-complexes (O-Fe-O) in the solution.

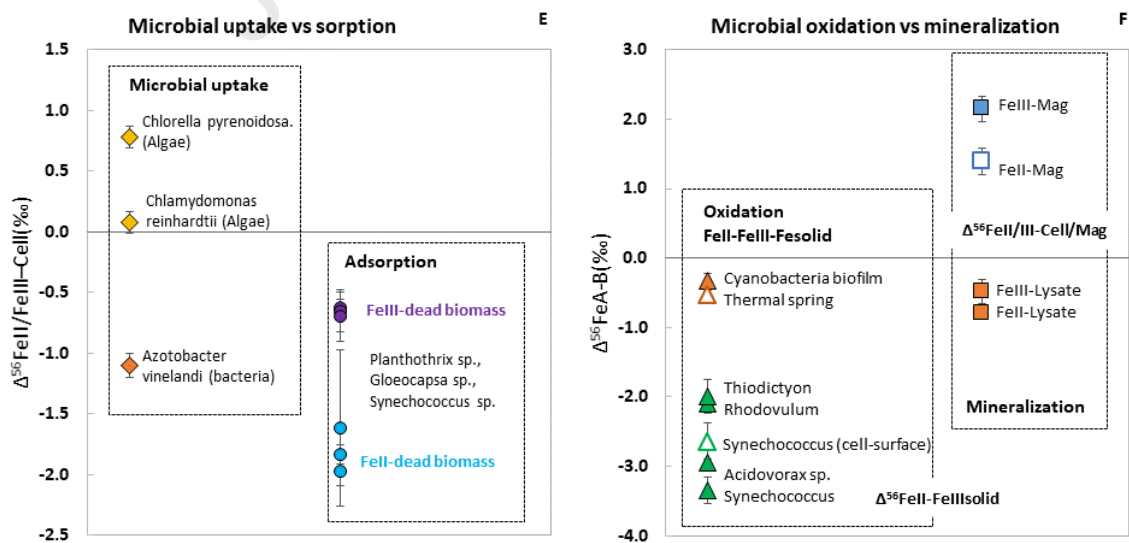
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421 Figure 2. Iron isotope fractionation due to (A) Complexation of Fe and ligands (experimental),
422 (B) complexation of Fe with different organic and inorganic ligands (theoretical calculation),
423 (C) dissolution, (D) precipitation, (E) microbial uptake vs bio-sorption of FeII and FeIII onto
424 biomass, and (F) microbial oxidation and mineralization (Mag-magnetite). In Fig (F), the green
425 outline of one symbol in "Bio-ferrous oxidation" box corresponds to the calculation of $\Delta^{56}\text{Fe}$
426 between FeII-FeIII and cell surface while the rest of the calculation focused on FeII-FeIII and
427 precipitated FeIII minerals. Error bars represent 2SD experimental replicates. Detailed values
428 and corresponding references for Fig 2A, C, D, E, F are given in Table 2 and those for Fig 2B
429 in Table 3.

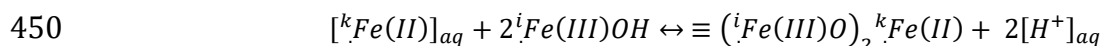
430 3.6. Dissimilatory iron reduction (abiotic vs biotic)

431 Iron reducing microorganisms complete the iron redox cycle by dissimilative reduction of
432 FeIII to FeII in neutrophilic environments under anaerobic conditions. Microorganisms from
433 both the Archaea and Bacteria domains are capable of metabolically exploiting the favorable
434 redox potential of iron. They respire FeIII as its terminal electron acceptor coupled to oxidation
435 of organic carbon/hydrogen into assimilation of biomass. Several studies have confirmed Fe
436 isotope fractionation during dissimilatory reduction of Fe minerals such as goethite, hematite,
437 ferrihydrite, magnetite, siderite by both strictly anaerobic bacterium, *Geobacter* and facultative
438 anaerobic bacterium, *Shewanella* species. There are three different types of fractionation
439 reported in these studies (1) equilibrium fractionation between aqueous FeII and bulk goethite,
440 (2) fractionation between aqueous FeII and sorbed FeII, and (3) fractionation between aqueous
441 FeII and reactive FeIII in the solid surface. These studies have concluded that Fe isotope
442 fractionation was largely driven by electron and atom exchange between FeII_{aq} and a reactive
443 FeIII layer ($\text{FeIII}_{\text{reac}}$) produced on the Fe mineral surface.

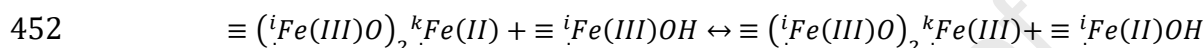
444 Coupled electron transfer-atom exchange at the iron mineral surface is described in three
445 consecutive steps: (1) adsorption of FeII_{aq} onto FeIII mineral surface, (2) the electron transfer

446 from the adsorbed FeII into the structural FeIII of iron mineral, followed by the atom-exchange
 447 between the two, and (3) the subsequent release of FeII from the ferric structure into the solution
 448 (Mikutta et al., 2009).

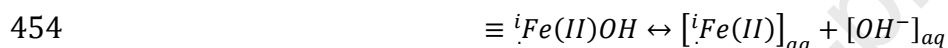
449 Adsorption



451 Electron transfer



453 Desorption



455 where subscripts denote different isotopes of Fe where $i > k$ (e.g., $i = {}^{56}\text{Fe}$, $k = {}^{54}\text{Fe}$)

456 **Dissimilatory iron reduction of goethite:** The reported fractionation between the FeII_{aq} and
 457 bulk goethite, $\Delta^{56}\text{Fe}_{\text{FeII}_{aq}\text{-goethite}}$ ranges from -0.52‰ to -1.61‰ (average of $-0.89 \pm 0.33\text{‰}$) in
 458 presence of *Shewanella putrefaciens* and *Geobacter sulfurreducens* (Crosby et al., 2005 &
 459 2007; Icopini et al., 2004). In parallel abiotic experiments, FeII_{aq} adsorption onto goethite
 460 shifted $\Delta^{56}\text{Fe}_{\text{FeII}_{aq}\text{-goethite}}$ by -0.80‰ (Icopini et al., 2004). Similarly, different particle size of
 461 goethite could also affect the overall fractionation, $\Delta^{56}\text{Fe}_{\text{FeII}_{aq}\text{-goethite}}$ as $-1.05 \pm 0.08\text{‰}$ for micro-
 462 goethite and $-1.22 \pm 0.08\text{‰}$ for nano-goethite (Beard et al., 2010; Friedrich et al., 2014a, Reddy
 463 et al., 2015).

464 Fractionation between aqueous and sorbed FeII, $\Delta^{56}\text{Fe}_{\text{FeII}_{aq}\text{-FeII}_{sorbed}}$ ranges from -0.58‰ to
 465 -1.18‰ for *Shewanella putrefaciens* and *Geobacter sulfurreducens* (average $-0.86 \pm 0.18\text{‰}$,
 466 Crosby et al., 2005 & 2007). However, no significant difference is observed between nano and
 467 micro-goethite, with $\Delta^{56}\text{Fe}_{\text{FeII}_{aq}\text{-FeII}_{sorbed}}$ of $-1.24 \pm 0.14\text{‰}$ in abiotic experiment (Beard et al.,
 468 2010). Greater quantities of FeII are sorbed to nano-goethite relative to micro-goethite due to

469 larger surface area, with maximum sorption at pH 6.5-7.5 and minimum at lower pH, effecting
 470 the initial kinetic isotope effects (Beard et al., 2010; Reddy et al., 2015). Over time, these
 471 variable kinetic isotope fractionation merged slowly towards the equilibrium Fe(II)_{aq}-goethite
 472 fractionation (Frierdich et al., 2014a).

473 Fractionation between FeII_{aq} and surface reactive FeIII or "FeIII_{react}", $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIreact}}$
 474 ranges from -2.49‰ to -3.89‰ for *Shewanella putrefaciens* and *Geobacter sulfurreducens*,
 475 where the average is $-2.62\pm 0.64\text{‰}$ (Crosby et al., 2005 & 2007). Beard et al. (2010) also
 476 reported significant fractionation, $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIreact}}$ as $-1.68\pm 0.08\text{‰}$ for micro-goethite and
 477 $-2.10\pm 0.48\text{‰}$ for nano-goethite. The overall isotopic fractionation FeII_{aq} and FeIII_{react} should
 478 be $\sim -3.0\text{‰}$ if it reflects equilibrium fractionation at room temperature.

479 Jang et al. (2008) claimed that isotopic composition of FeII_{aq} is largely controlled by
 480 sorption of FeII onto goethite and there is a kinetic fractionation associated with electron
 481 transfer from adsorbed FeII to structural FeIII of goethite, producing FeIII with higher δFe than
 482 FeII_{aq} and initial goethite. Different particle size can also further contribute to the difference in
 483 surface area, crystal edges, corners or faces creating different energetic sites hosting FeIII_{react}.

484 **Dissimilatory iron reduction of hematite:** The reported fractionation between the FeII_{aq}
 485 and bulk hematite, $\Delta^{56}\text{Fe}_{\text{FeIIaq-hematite}}$ is ranging from -0.97‰ to -2.07‰ for *Shewanella*
 486 *putrefaciens* and *Geobacter sulfurreducens* (average $-1.56\pm 0.28\text{‰}$, Crosby et al., 2005 &
 487 2007). Frierdich et al. (2014b & 2015) confirmed Fe isotope exchange was a function of particle
 488 size using an enriched ^{57}Fe -tracer in a parallel abiotic experiment. Experimental
 489 $\Delta^{56}\text{Fe}_{\text{FeIIaq-hematite}}$ ranges from $-2.77\pm 0.37\text{‰}$ for fine-grained hematite ($60\text{ m}^2\text{g}^{-1}$) to
 490 $-3.1\pm 0.36\text{‰}$ coarse-grained hematite ($7\text{ m}^2\text{g}^{-1}$) (average $-2.83\pm 0.25\text{‰}$, Frierdich et al., 2019).

491 The reported fractionation between the FeII_{aq} and sorbed FeII, $\Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIsorbed}}$ ranges
 492 from -0.07‰ to -0.53‰ for both bacteria strains (average $-0.30\pm 0.15\text{‰}$, Crosby et al., 2005
 493 & 2007). A similar fractionation of $-0.48\pm 0.07\text{‰}$ is observed in experiment without Si, at pH

494 7 and it shifted to $-0.20 \pm 0.13\%$ with Si (Wu et al., 2009). Formation of Fe-Si gel was reported
 495 at pH 8.7 where the fractionation between FeII_{aq} and Fe-Si gel, $\Delta^{56}\text{Fe}_{\text{FeII}_{\text{aq}}-\text{FeIISiGel}}$ is
 496 $-0.51 \pm 0.18\%$, which is indistinguishable from the average fractionation, $\Delta^{56}\text{Fe}_{\text{FeII}_{\text{aq}}-\text{FeIISorbed}}$ at
 497 pH 7. In a parallel abiotic experiment, $\Delta^{56}\text{Fe}_{\text{FeII}_{\text{aq}}-\text{FeIISorbed}}$ ranges from $-0.74 \pm 0.45\%$ for fine-
 498 grained hematite ($60 \text{ m}^2\text{g}^{-1}$) to $-0.83 \pm 1.08\%$ for coarse-grained hematite ($7 \text{ m}^2\text{g}^{-1}$) (reported
 499 average $-0.77 \pm 0.17\%$ for all particle size, Friedrich et al., 2019).

500 A larger range of $\Delta^{56}\text{Fe}_{\text{FeII}_{\text{aq}}-\text{FeIII}_{\text{reac}}}$ is reported; -2.51% to -4.01% for both bacteria strains
 501 (average $-2.95 \pm 0.19\%$, Crosby et al., 2005 & 2007), comparable to $-2.64 \pm 0.19\%$ without Si
 502 at pH 7 (Wu et al., 2009). Presence of dissolved Si could favor the heavy isotopic signature of
 503 surface reactive FeIII, for example, the average fractionation $\Delta^{56}\text{Fe}_{\text{FeII}_{\text{aq}}-\text{FeIII}_{\text{reac}}}$ is $-1.90 \pm 0.47\%$
 504 with Si at pH 7 and $-2.64 \pm 0.19\%$ without Si at pH 7. However, higher pH could favor light
 505 signature in surface reactive FeIII even in the presence of Si (average $-2.66 \pm 0.18\%$ with Si at
 506 pH 8.7 and $-1.76 \pm 0.21\%$ without Si, at pH 8.7). At elevated pH (8.7) the hematite reduction
 507 by *G. sulfurreducens*, reduced the size of the total reactive Fe pool and FeII existed exclusively
 508 as sorbed FeII (Wu et al., 2009).

509 Although kinetic effect could occur during rapid sorption of FeII onto hematite,
 510 recrystallization of hematite will govern the overall fractionation process. If homogeneous
 511 recrystallization occurs, where the mineral continuously equilibrates with the fluid, the isotopic
 512 values of FeII_{aq} linearly will approach steady state, depicting equilibrium fractionation. If
 513 heterogeneous recrystallization occurs, where portion of the mineral no longer interacts with
 514 the fluid due to FeII oxidative growth, the isotopic composition of FeII_{aq} will approach the same
 515 value as hematite, in apparent violation of equilibrium fractionation effect (Friedrich et al.,
 516 2019).

517 ***Goethite vs Hematite vs particle sizes:*** Particle size dependent Fe isotope fractionation is
 518 observed during the interaction between FeII_{aq} and goethite, as well as FeII_{aq} and hematite. In

519 general, $\Delta^{56}\text{Fe}_{\text{FeIIaq-goethite}}$ is more negative for smaller particle size, whereas the opposite trend
520 is observed for $\Delta^{56}\text{Fe}_{\text{FeIIaq-hematite}}$ with more positive values. Difference between fractionation
521 for nano- and micro-goethite is 0.2‰ compared to that between nano- and micro-hematite
522 which is 0.5‰. Therefore, the effect of particle size is more visible on the extent of isotopic
523 exchange between FeII_{aq} and hematite than in goethite. Larger Fe isotope fractionation in
524 smaller particle size of goethite could reflect a higher proportion of surface atoms that
525 preferentially concentrate the heavy Fe isotopes in high energy surface bonds (Beard et al.,
526 2010; Frierdich et al., 2014a). However, the opposite trend of larger Fe isotope fractionation in
527 large particle sizes of hematite could indicate different fractionation mechanisms. If kinetic
528 isotope fractionation were the governing process, light isotopes of Fe can be expected to
529 preferentially partition into hematite during rapid sorption, leaving a heavy isotopic signature
530 in aqueous FeII. However, the opposite trend of light isotopic signature in aqueous FeII rules
531 out the kinetic isotope effect.

532 ***Dissimilatory iron reduction of ferrihydrite:*** Hydrous ferric oxide (HFO or ferrihydrite) is
533 quickly precipitated in neutral environments, making it widespread in soils and sediments.
534 Fractionation between the FeII_{aq} and bulk ferrihydrite, $\Delta^{56}\text{Fe}_{\text{FeIIaq-ferrihydrite}}$ is $-1.30\pm 0.22\text{‰}$ in
535 the presence of *Shewanella alga* (*Strain BrY and Strain BCMB*, given in Figure 3C) where the
536 fractionation is invariant with the choice of growth media and strains (Beard et al., 1999 &
537 2003). In parallel, in abiotic studies, the reported equilibrium fractionation between FeII_{aq} and
538 pure HFO without impurities, $\Delta^{56}\text{Fe}_{\text{FeIIaq-HFO}}$ is $-3.2\pm 0.10\text{‰}$. When dissolved Si is present,
539 $\Delta^{56}\text{Fe}_{\text{FeIIaq-HFO+Si}}$ is $-3.17\pm 0.08\text{‰}$. However, it is completely shifted if Si is coprecipitated with
540 HFO, thereby embedded inside the HFO structure (molar Si:Fe =1), with $\Delta^{56}\text{Fe}_{\text{FeIIaq-Si-HFO}}$ of
541 $-2.58\pm 0.14\text{‰}$ (Wu et al., 2011). Similarly, when HFO is coprecipitated with Suwannee River
542 natural organic matter (SRNOM, molar C:Fe=1.2), the reported equilibrium fractionation,

543 $\Delta^{56}\text{Fe}_{\text{FeIIaq-C-HFO}}$ is $-2.36\pm 0.26\text{‰}$ (Chanda et al., 2020). Unfortunately, there is no data available
544 for the estimated equilibrium $\Delta^{56}\text{Fe}_{\text{FeII-ferrihydrite}}$ using β factor.

545 It has been difficult to determine equilibrium Fe isotope fractionation between aqueous FeII
546 and HFO due to the fast transformation of the latter to more stable minerals (Wu et al., 2011).
547 The presence of C and Si in ferrihydrite structure could inhibit the rapid transformation of
548 ferrihydrite to more stable iron oxides upon interaction with aqueous FeII (Chanda et al., 2020).
549 The presence of C and Si also increased the extent of Fe isotope exchange, shifting the overall
550 fractionation. Dissolved Si could block the reactive surface sites by sorption via inner-sphere
551 complexes, inhibiting atom exchange between FeII and FeIII of HFO. Liberation of Si from Si-
552 HFO coprecipitate into solution presumably freed reactive surface sites, thus promoting
553 isotopic exchange between FeII and the Si-HFO coprecipitate. Si mass transfer from Si-HFO
554 into solution was accompanied by Fe transfer from aqueous FeII_{aq} into Si-HFO solid,
555 facilitating both electron transfer as well as atom exchange. Similarly, coprecipitation with
556 organic C changes Fe bonding environment, where the coordination number of Fe-Fe bonds in
557 FeO_6 octahedral structure decreases along with the decline in crystal size, resulting in increased
558 numbers of defects and a more disordered surface, in turn influencing the equilibrium
559 fractionation factor (Wang et al., 2016, Zhou et al., 2018; Chanda et al., 2020).

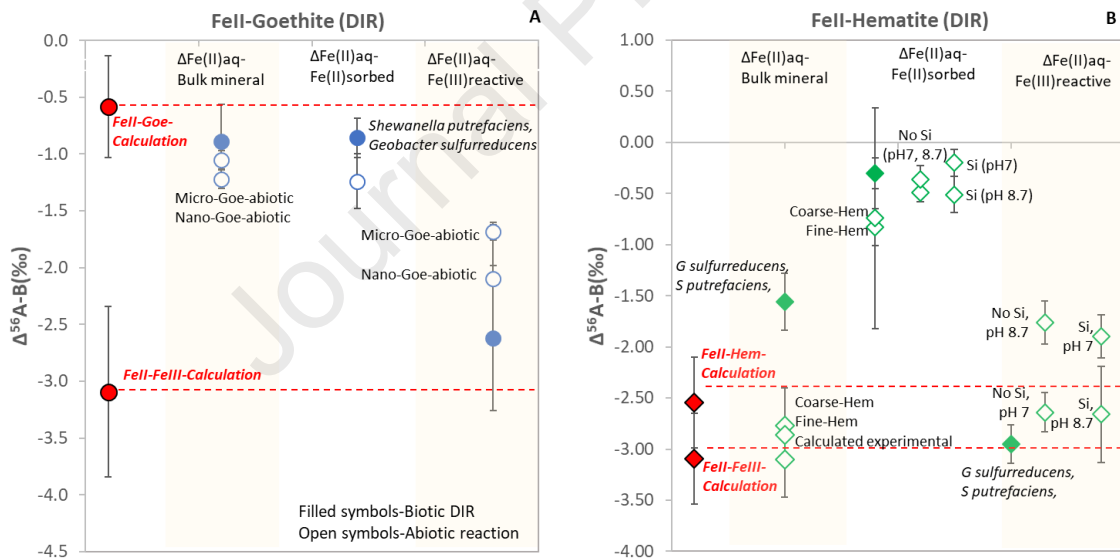
560 ***Dissimilatory iron reduction of siderite:*** Johnson et al. (2005) conducted a study on
561 biogenic siderite formation via dissimilatory reduction of hydrous ferric oxides by *Geobacter*
562 *sulfurreducens*, where the estimated equilibrium fractionation between FeII_{aq} and siderite,
563 $\Delta^{56}\text{Fe}_{\text{FeIIaq-siderite}}$ is $0.0\pm 0.22\text{‰}$, near zero for pure siderite and $\sim 1\text{‰}$ for Ca-substituted siderite
564 (Figure 3C). Kinetic isotope effects are responsible for large fractionation observed in an initial
565 period, where $\Delta^{56}\text{Fe}_{\text{FeIIaq-siderite}}$ can be as large as 1.2‰ for pure siderite and 2.2‰ for Ca-
566 substituted siderite. Wiesli et al. (2004) have assessed the equilibrium fractionation factor
567 between FeII_{aq} and siderite by performing abiotic experiments where the reported

568 $\Delta^{56}\text{Fe}_{\text{FeIIaq-siderite}}$ at 20°C is $0.48\pm 0.22\text{‰}$ based on slow carbonate precipitation experiments. No
569 discernable isotopic fractionation was observed at extremely rapid synthesis of siderite (a few
570 seconds). Johnson et al. (2003) initially predicted FeII_{aq} and siderite fractionation to lie in the
571 range of -1.7‰ to 0.3‰ at 25°C. The net fractionation increases with decreasing mole fraction
572 of Fe from siderite to ankerite, indicating carbonate stoichiometry exerting a substantial control
573 on Fe isotope fractionations. Bonding changes and distortions in the crystal lattice due to Ca
574 substitution in siderite could be the reason behind such large Fe isotope effects, a similar
575 fractionation could be expected in Mn and Mg substitution.

576 ***Dissimilatory iron reduction of magnetite:*** Magnetite is well-known for housing both
577 redox species of Fe; one FeIII ion on the tetrahedral site (A site), and one FeII and one FeIII
578 ion on the octahedral site (B site), posing great challenge for experimental studies. Johnson et
579 al. (2005) estimated fractionation between FeII_{aq} and magnetite, $\Delta^{56}\text{Fe}_{\text{FeIIaq-magnetite}}$ is
580 $-1.34\pm 0.22\text{‰}$ from magnetite precipitation during microbial Fe reduction of ferrihydrite by
581 *Geobacter sulfurreducens* (Figure 3D). Friedrich et al. (2014b) conducted abiotic experiment
582 using ^{57}Fe enriched tracer, three-isotope method and multi-directional approach to equilibrium.
583 The extrapolated equilibrium fractionation between FeII_{aq} and magnetite, $\Delta^{56}\text{Fe}_{\text{FeIIaq-magnetite}}$ is
584 $-1.56\pm 0.22\text{‰}$ at 22°C regardless of distinct initial isotope signatures. An additional experiment
585 was conducted to study the effect of phase transformation on Fe isotope exchange during
586 magnetite synthesis by reacting FeII_{aq} and ferrihydrite, with final $\Delta^{56}\text{Fe}_{\text{FeIIaq-magnetite}}$ of
587 $-1.61\pm 0.22\text{‰}$, identical within errors to other experiments. It is also relevant to compare the
588 intracellular biomineralization of magnetite nanoparticles by *Magnetospirillum Magneticum*
589 *strain AMB-1*, inducing a much higher $\Delta^{56}\text{Fe}_{\text{FeIIsubstrate-magnetite}}$ of $1.39\pm 0.19\text{‰}$ (Amor et al.,
590 2016, See the details in section 3.4).

591 Fe isotope exchange between FeII_{aq} and magnetite could reflect two possible mechanisms
592 (i) an initial kinetic isotope effects during rapid dissolution and reprecipitation (ii) site-specific

593 isotope exchange such as octahedral vs. tetrahedral sites (Friedrich et al., 2014b). Relative
 594 heavy $\delta^{56}\text{Fe}$ of FeII_{aq} reflect the kinetic isotope transport where light FeII_{aq} being transferred
 595 via boundary layer and attaching to the magnetite surface, kinetic isotope effects were later
 596 erased by an approach to equilibrium at greater extents of exchange. One third of Fe in
 597 magnetite bound as FeIII in tetrahedral site (A site) and two thirds of Fe bound as FeII+FeIII
 598 in octahedral site (B site). Based on the different β factors available for each site in magnetite
 599 ($10^3\ln\beta^{56/54}_{\text{SiteA}}$ as 7.5‰ and $10^3\ln\beta^{56/54}_{\text{SiteB}}$ as 5.7‰⁶), calculated tetrahedral site A hosting
 600 FeIII should have $^{56}\text{Fe}/^{54}\text{Fe}$ ratio 1.8‰⁷ higher at 25°C (Personal communication and Polyakov
 601 et al., 2007). Isotope selective exchange experiment and Mössbauer spectroscopy by Gorski et
 602 al. (2012) showed no indication of preferential exchange of Fe isotope based on tetrahedral or
 603 octahedral site.



⁶ Via personal communication, updated polynomial coefficients of $^{57/54}\text{Fe}$ for magnetite :

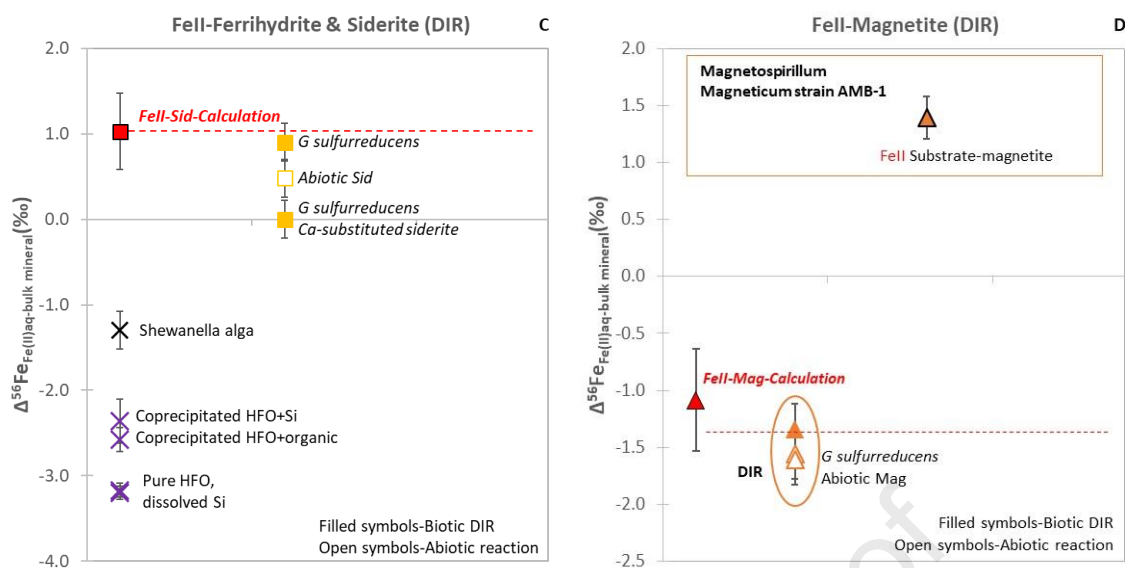
$$10^3\ln\beta_A = 1.0168 X - 2.946 \times 10^{-3} X^2 + 1.2032 \times 10^{-5} X^3$$

$$10^3\ln\beta_B = 0.7668 X - 1.678 \times 10^{-3} X^2 + 5.3232 \times 10^{-6} X^3$$

$$10^3\ln\beta = 0.8502 X - 2.101 \times 10^{-3} X^2 + 7.5595 \times 10^{-6} X^3$$

Conversion to $10^3\ln\beta^{56/54}\text{Fe}$, multiply $10^3\ln\beta^{57/54}\text{Fe}$ by $(2/3) \cdot (57/56)$

⁷ Previous reported value is 3.5‰ as reported by Friedrich et al., 2014b using erroneous β factors of Polyakov and Mineev(2000).



605

606 Figure 3. Dissimilatory iron reduction processes or microbial iron reduction of (A) Goethite,
 607 (B) Hematite, (C) Ferrihydrite and Siderite, (D) Magnetite in comparison to abiotic reduction
 608 reaction and available calculation data. Error bars represent 2SD experimental replicates.

609 4. Comparison between experimental studies and theoretical ones

610 In parallel to several experimental studies, spectroscopy studies and pure theoretical
 611 calculations were done to determine reduced partition function ratios (β factor) in order to
 612 evaluate the equilibrium fractionation factors of iron isotopes, $10^3\text{Ln}\beta^{56/54}$. Mössbauer
 613 spectroscopy and inelastic nuclear resonant X-ray scattering (INRXS) in synchrotron radiation
 614 experiments were used to investigate β factor of different iron minerals (Polyakov and Mineev,
 615 2000; Polyakov et al., 2007; Polyakov and Soultanov., 2011; Polyakov et al., 2013). Density
 616 functional theory (DFT) and ab-initio calculations were applied to determine the β factor of
 617 iron with different organic and inorganic species in aquatic media (Anbar et al., 2005; Domagal-
 618 Goldman and Kubicki, 2008; Hill et al., 2009; Ottonello and Zuccolini, 2009; Fujii et al., 2014).
 619 For example, the equilibrium iron isotope fractionation due to redox reaction between FeII and
 620 FeIII can be calculated using the β factor ($10^3\text{Ln}\beta^{56/54}$) obtained from the study of Fujii et al.
 621 (2014) and is given in Table 1.

622 $10^3\text{Ln}\alpha^{56/54}_{\text{FeII-FeIII}} = 10^3\text{Ln}\beta^{56/54}_{\text{FeII}} - 10^3\text{Ln}\beta^{56/54}_{\text{FeIII}} = 5.10 - 8.07 = -2.98\text{‰}$

623 The calculated $10^3\text{Ln}\alpha^{56/54}_{\text{FeII-FeIII}}$ varies up to 0.75‰ depending on the variations of the
 624 individual $10^3\text{Ln}\beta^{56/54}_{\text{FeII}}$ and $10^3\text{Ln}\beta^{56/54}_{\text{FeIII}}$ from different studies (Table 1). Different
 625 equilibrium fractionation factors, $10^3\text{Ln}\alpha^{56/54}_{\text{A-B}}$, for each biogeochemical process are
 626 calculated and given in Table 2 in order to compare with the values obtained in experimental
 627 studies. During redox reactions, the average $10^3\text{Ln}\alpha^{56/54}_{\text{FeII-FeIII}}$ obtained using the reduced
 628 partition function ratios (β factor) is $-3.09\pm 0.75\text{‰}$, in agreement with the equilibrium
 629 experimental values, $\Delta^{56}\text{Fe}_{\text{FeII-FeIII}}$: $-3.00\pm 0.23\text{‰}$ obtained in experimental studies (Johnson et
 630 al., 2002; Welch et al., 2003).

631 **Precipitation:** the experimental equilibrium fractionation during hematite precipitation
 632 remains close to zero ($\Delta^{56}\text{Fe}_{\text{III-Hematite_Equilibrium}}$ $-0.15\pm 0.20\text{‰}$ at 25°C) while it was estimated at
 633 $0.29\pm 0.70\text{‰}$ from Mössbauer spectroscopy studies and density functional theory (Fujii et al.,
 634 2014 & Polyakov et al., 2007). Similarly, the experimental equilibrium fractionation factor
 635 between FeII and siderite, $\Delta^{56}\text{Fe}_{\text{II-Siderite_Equilibrium}}$ of $0.48\pm 0.22\text{‰}$, is smaller than the theoretical
 636 prediction, $10^3\text{Ln}\alpha^{56}_{\text{FeII-Siderite}}$ of $0.89\pm 0.45\text{‰}$ (Fujii et al., 2014 and Blanchard et al., 2009).
 637 Using very old spectroscopic data for Mackinawite led to great uncertainties and huge
 638 discrepancy where a much larger fractionation, $10^3\text{Ln}\alpha^{56}_{\text{FeII-Mackinawite}}$ of $3.17\pm 0.45\text{‰}$ (Fujii et
 639 al., 2014; Polyakov and Soultanov., 2011) is predicted in comparison to the experimental
 640 values, $\Delta^{56}\text{Fe}_{\text{II-Mackinawite_Equilibrium}}$ of $-0.33\pm 0.16\text{‰}$ (Butler et al., 2005; Guilbaud et al., 2010;
 641 Guilbaud et al., 2011a). In Pyrite precipitation, predicted equilibrium isotopic fractionation
 642 between FeII_{aq} and pyrite⁸, $10^3\text{Ln}\alpha^{56}_{\text{FeIIaq-Pyrite}}$ is -5.74‰ (Fujii et al., 2014; Polyakov and
 643 Soultanov, 2011), is significantly different from the experimental fractionation factor,
 644 $\Delta^{56}\text{Fe}_{\text{II-Pyrite_Equilibrium}}$ $0.44\pm 0.15\text{‰}$ (Mansor and Fantle et al., 2019).

⁸ please note that only β factor of pyrite from Polyakov and Soultanov, (2011) is only considered due to experimental validation in conjunction with sulfur isotopes.

645 **Bio-oxidation and Bio-mineralization:** During the microbial oxidation of FeII to FeIII, the
 646 oxidized FeIII precipitated into a mixture of FeIII minerals such as ferrihydrite, goethite, and
 647 lepidocrocite. The experimental fractionation between FeII-FeIII_{solids}, $\Delta^{56}\text{Fe}_{\text{FeII-FeIII-Solid}}$
 648 varies for each bacteria strain as seen in Table 2. Nonetheless, these experimental values can
 649 be further compared to the estimated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$ between FeII, FeIII, goethite and
 650 lepidocrocite initially proposed by Beard and Johnson (2004), Johnson et al. (2005), and
 651 Dauphas and Rouxel (2006) as follows:

$$652 \quad \Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIsolid}} \rightarrow \Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIaq}} + \Delta^{56}\text{Fe}_{\text{FeIIaq-FeIIIsolid}}$$

653 The calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$ on FeII-FeIII-minerals deviated from experimental values, with a
 654 smaller net value of <1‰ ($10^3\text{Ln}^{56}\alpha_{\text{FeII-FeIII-Goethite}} -0.72\%$ for FeII-FeIII-Goethite and
 655 $10^3\text{Ln}^{56}\alpha_{\text{FeII-FeIII-lepidocrocite}} -0.34\%$ for FeII-FeIII-lepidocrocite). The more negative values
 656 (-1.99‰ and -3.35‰ in Table 2) observed for $\Delta^{56}\text{Fe}_{\text{FeII-FeIIaq-FeIIIsolid}}$ in experimental studies
 657 could be related to several possible mechanisms: (1) equilibrium exchange between FeII and
 658 FeIII species, (2) additional equilibrium isotope shift control by bioligands, (3) formation of a
 659 mixture of final FeIII oxyhydroxide minerals (ferrihydrite, goethite, lepidocrocite) and their
 660 subsequent transformation of one mineral to another, (4) similar colloidal Fe phases bound to
 661 additional inorganic ligands (SiO_4^{4-} and PO_4^{4-}), (5) a kinetic isotope effect produced by such
 662 precipitation over the equilibrium exchange between existing Fe species and inorganic/organic
 663 ligands, (6) difference in specific surface area of bacteria (*Synechococcus cyanobacteria* vs
 664 *Rhodovulum*) to sorb iron, favoring additional isotope shift, and (7) specific metabolic pathway
 665 of each microbial strain, for instance, FeII oxidation by *Acidovorax sp.* strain taking place in
 666 the periplasm where equilibrium is attained via coupled electron and atom exchange between
 667 FeIIaq and FeIIIprecipitates in the periplasm.

668 During intracellular biomineralization of magnetite nanoparticles by *Magnetospirillum*
 669 *Magneticum strain AMB-1*, the net equilibrium isotopic fractionation $\Delta^{56}\text{Fe}_{\text{FeII-magnetite}}(\text{‰})$ is

670 $1.39\pm 0.19\text{‰}$ and $\Delta^{56}\text{Fe}_{\text{FeIII-magnetite}}(\text{‰})$ is $2.15\pm 0.18\text{‰}$ (Amor et al., 2016). A much closer
 671 equilibrium isotopic fractionation is calculated for $10^3\text{Ln}^{56}\alpha_{\text{FeIII-magnetite}}(\text{‰})$, $1.75\pm 0.70\text{‰}$
 672 compared to $10^3\text{Ln}^{56}\alpha_{\text{FeII-magnetite}}(\text{‰})$, $-1.22\pm 0.45\text{‰}$ (Fujii et al., 2014; Polyakov, personal
 673 communication, 2020).

674 ***Dissimilatory iron reduction:*** During the microbial reduction of goethite, the predicted
 675 equilibrium isotope fractionation, $10^3\text{Ln}^{56}\alpha_{\text{FeII-Goethite}}$ is $-0.72\pm 0.45\text{‰}$ (Fujii et al., 2014;
 676 Polyakov & Mineev., 2000), in agreement with fractionation obtained from biotic experiment,
 677 $\Delta^{56}\text{Fe}_{\text{FeIIaq-Goethite}}$ of $-0.89\pm 0.33\text{‰}$ (Crosby et al., 2005 & 2007; Icopini et al., 2004), given in
 678 Table 4. However, it is slightly higher than the abiotic experimental fractionation factors;
 679 $-1.05\pm 0.08\text{‰}$ for micro-goethite and $-1.22\pm 0.08\text{‰}$ nano-goethite (Beard et al., 2010; Frierdich
 680 et al., 2014; Reddy et al., 2015). The Beard et al. (2010) extrapolated $\Delta^{56}\text{Fe}_{\text{FeIII-Hematite}}(\text{‰})$ from
 681 the study of Skulan et al. (2002)⁹ to a temperature of 20°C to be -0.15‰ . In combination with
 682 the study of Welch et al. (2003) where $\Delta^{56}\text{Fe}_{\text{FeII-FeIII}}(\text{‰})$ is -3.01‰ , the calculated experimental
 683 $\Delta^{56}\text{Fe}_{\text{FeII-hematite}}(\text{‰})$ between relevant experimental studies is -2.86‰ in a very close vicinity to
 684 the values obtained by Frierdich et al. (2019). In comparison, the direct estimated equilibrium
 685 $10^3\text{Ln}^{56}\alpha_{\text{FeII-Hematite}}(\text{‰})$ obtained from β factors of $[\text{FeII}(\text{H}_2\text{O})_6]^{2+}$ and hematite; -2.68‰ which
 686 is slightly lower (Fujii et al., 2014; Polyakov et al., 2007). Theoretical predictions obtained the
 687 equilibrium fractionation, $10^3\text{Ln}^{56}\alpha_{\text{FeII-Pyrite}}(\text{‰})$ of 0.89‰ (Blanchard et al., 2009; Rustad et al.,
 688 2010; Fujii et al., 2014), a much closer values to the experimental values, $\Delta^{56}\text{Fe}_{\text{FeII-Pyrite}}(\text{‰})$ of
 689 $1.00\pm 0.22\text{‰}$ obtained in Ca-substituted siderite (Johnson et al., 2005). In comparison, the
 690 predicted equilibrium factor, $10^3\text{Ln}^{56}\alpha_{\text{FeIIaq-magnetite}}(\text{‰})$ is $-1.22\pm 0.45\text{‰}$ (Fujii et al., 2014;
 691 Polyakov, personal communication, 2021), lower than the equilibrium experimental values.

692 ***Discrepancy between experimental fractionation and theoretical predictions:*** It is
 693 important to reconcile the discrepancies and inconsistencies observed between predicted and

⁹ Skulan et al. (2002) initially conducted the experiment at 98°C, the measured fractionation is -0.10‰ .

694 experimentally determined equilibrium fractionation factors. The main issue in comparison is
695 that calculation can be accounted for single step or simple process while several processes can
696 take place simultaneously in experimental studies which resulted in fractionation. It is hard to
697 constrain Fe reactivity experimentally in order to isolate specific processes and to decipher
698 respective fractionation.

699 It is possible to estimate the extent of discrepancy between experimental and theoretical
700 values especially for redox reactions by considering all absolute values obtained and the
701 respective standard deviation: the estimated discrepancy range is between 0.43‰ to 0.61‰.
702 The type of model (gas vs aqueous) used for the calculations (some theoretical studies ignoring
703 the effects of extended hydration spheres), the experimental design/set-up (iron speciation such
704 as nitrate, chloride, salinity, temperature), final iron separation and recovery, experimental
705 artifacts (formation of unwanted colloids), kinetic isotope effect on the determined final
706 equilibrium fractionation, mathematical methods of calculation and overall analytical
707 uncertainties, these could all be accounted for the discrepancy estimated between experimental
708 and theoretical values in the case of redox reactions.

709 The theoretical vs experimental comparison is harder to do for biogeochemical processes
710 other than redox reactions, due to fractionation values that envelop several complexities in the
711 experimental studies, while seeming over-simplified in theoretical ones. For instance, in
712 hematite precipitation experiment (Table 4), the kinetic isotope effect between FeIII and
713 hematite is significantly much bigger than the equilibrium isotope effect, while the theoretical
714 calculation failed to distinguish between the two. The limitation in theoretical studies is due to
715 the type of model used as mentioned before, as well as to the purity, age, particle size,
716 crystallinity of the minerals modelled, and for in spectroscopy studies it is mainly due to
717 analytical uncertainties. Likewise, several complexities in the experiments include the diverse
718 formation pathways of iron minerals (as in redox reactions), their sensitivity to pH and

719 temperature changes, their crystallinity and stability, the incorporation of impurities, the
720 bonding environment, the influence of biological factors (bio-uptake, bio-sorption, their
721 different metabolic pathways, the complexation with several metabolites and bio-products).
722 Despite how arbitrary it could seem to be, we estimate the discrepancy range between iron
723 fractionation determined experimentally vs theoretically: it lies between 0.43‰ and 1.59‰ for
724 kinetic fractionation and between -0.94‰ and 0.06‰ for equilibrium fractionation.

725 It is also possible that one or more geochemical processes can take place at the same time
726 such as dissolution and recrystallization, and formation of thin mineral crust and subsequent
727 adsorption, which could modify the Fe isotope signatures and will add more complexity to the
728 matter. Therefore, predicted equilibrium isotope fractionation from theoretical calculation or
729 spectroscopy studies does not account for such issues. Nonetheless, it is reliable and consistent
730 to compare predicted and observed equilibrium fractionations between fluid–fluid or mineral–
731 mineral fractionations, rather than for fluid–mineral fractionations.

732

733

734 Table 1. Reduced partition function ratios (β factor- $10^3\text{Ln}\beta^{56/54}$) obtained in spectroscopy
 735 studies and theoretical calculations for different iron minerals vs redox Fe species (FeII and
 736 FeIII). The Schauble et al. (2001) calculation is not considered because the $10^3\text{Ln}\beta^{56/54}\text{FeIII}$ is
 737 a significant outlier due to spectroscopic data issues.

References	Iron minerals	$10^3\text{Ln}\beta^{56/54}$ at 25°C
Polyakov and Mineev, 2000	Goethite	5.82
	Lepidocrocite	5.44
Polyakov et al., 2007	Hematite	7.78
Polyakov & Soultanov., 2011	Pyrite	10.84
Polyakov & Soultanov., 2011	Mackinawite	1.92
Blanchard et al., 2009	Siderite	4.20
<i>Polyakov (personal communication)</i>	Magnetite	6.32
References	$10^3\text{Ln}\beta^{56/54}$ for $[\text{FeII}(\text{H}_2\text{O})_6]^{2+}$	$10^3\text{Ln}\beta^{56/54}$ for $[\text{FeIII}(\text{H}_2\text{O})_6]^{3+}$
Anbar et al., 2005	5.69	8.75
Domagal-Goldman & Kubicki., 2008	5.77	8.62
Otonello & Zuccolini., 2009	4.86	9.33
Rustad et al., 2010	4.66	7.69
Moynier et al., 2013	5.32	7.50
Fujii et al., 2014	5.10	8.07

738

739 Table 2. Comparison of the equilibrium iron isotope fractionation obtained in experimental
 740 studies ($\Delta^{56}\text{Fe}_{\text{A-B}}$) to those obtained in spectroscopy studies and theoretical calculations
 741 ($10^3\text{Ln}\beta^{56/54}$) for redox reactions, complexation, dissolution, precipitation, bio-oxidation, bio-
 742 mineralization.

References	Fe Pairs	Scope of studies	$\Delta^{56}\text{Fe}_{\text{A-B}}(\text{‰})$
Redox Equilibration			
Johnson et al., 2002; Welch et al., 2003	Abiotic FeII-FeIII	$\Delta^{56}\text{Fe}_{\text{FeII-FeIII}}$	$-3.00 \pm 0.23\text{‰}$
Anbar et al., 2005; Domagal-Goldman & Kubicki., 2008; Ottonello & Zuccolini., 2009; Rustad et al., 2010; Moynier et al., 2013; Fujii et al., 2014	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-FeIII}}$	$-3.09 \pm 0.75\text{‰}$
Complexation (Abiotic/experimental)			
Dideriksen et al., 2008	Abiotic FeIII-DFOB	$\Delta^{56}\text{Fe}_{\text{FeIII-DFOB}}$	$-0.60 \pm 0.15\text{‰}$
Morgan et al., 2010	Abiotic FeIII-DFOB to FeIII-Ox	$\Delta^{56}\text{Fe}_{(\text{FeIII-DFOB})-(\text{FeIII-Ox})}$	$0.20 \pm 0.11\text{‰}$
	Abiotic FeIII-DFOB to FeIII-EDTA	$\Delta^{56}\text{Fe}_{(\text{FeIII-DFOB})-(\text{FeIII-EDTA})}$	$0.02 \pm 0.11\text{‰}$
Lotfi-Kalahroodi et al., 2019	Abiotic organic matter binding to FeIII at pH 6.5	$\Delta^{56}\text{Fe}_{\text{TotFe}<0.2\mu\text{m}}$	$-0.07 \pm 0.08\text{‰}$
		$\Delta^{56}\text{Fe}_{\text{TotFe}>0.2\mu\text{m}}$	$0.05 \pm 0.08\text{‰}$
		$\Delta^{56}\text{Fe}_{\text{TotFe}<30\text{ kDa}}$	$-0.35 \pm 0.08\text{‰}$
		$\Delta^{56}\text{Fe}_{\text{TotFe}>30\text{ kDa}}$	$0.00 \pm 0.08\text{‰}$
		$\Delta^{56}\text{Fe}_{\text{TotFe-(0.2 }\mu\text{m-30 kDa)}}$	$-0.08 \pm 0.08\text{‰}$
Dissolution (Abiotic)			
Brantley et al., 2001a	<i>Bacillus mycoides</i> & <i>Streptomyces</i> sps.	$\Delta^{56}\text{Fe}_{\text{solution-Silicates}}$	$0.48 \pm 0.29\text{‰}$
Brantley et al., 2001b; Brantley et al., 2004; Kiczka et al., 2010	Abiotic Silicates + ligands		$-0.32 \pm 0.13\text{‰}$
Brantley et al., 2004	<i>Bacillus mycoides</i> & <i>Streptomyces</i> sps.	$\Delta^{56}\text{Fe}_{\text{solution-Goethite}}$	$-1.44 \pm 0.17\text{‰}$
Wiederhold et al., 2006; Jang et al., 2008	Abiotic Goethite + ligands		$-1.32 \pm 0.34\text{‰}$
Wolfe et al., 2016	Abiotic Pyrite + ligands	$\Delta^{56}\text{Fe}_{\text{solution-Pyrite}}$	$-0.34 \pm 0.61\text{‰}$
Precipitation (Abiotic)			
Skulan et al., 2002; Beard et al., 2010	Abiotic experiment	$\Delta^{56}\text{Fe}_{\text{III-Hematite-Kinetic}}$	$1.30 \pm 0.12\text{‰}$
		$\Delta^{56}\text{Fe}_{\text{III-Hematite-Equilibrium}}$	$-0.15 \pm 0.20\text{‰}$
Fujii et al., 2014; Polyakov et al., 2007	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeIII-Hematite}}$	$0.29 \pm 0.70\text{‰}$
Wiesli et al., 2004	Abiotic experiment	$\Delta^{56}\text{Fe}_{\text{II-Siderite-Kinetic}}$	$0.04 \pm 0.10\text{‰}$
		$\Delta^{56}\text{Fe}_{\text{II-Siderite-Equilibrium}}$	$0.48 \pm 0.22\text{‰}$
Fujii et al., 2014; Blanchard et al., 2009	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Siderite}}$	$0.89 \pm 0.45\text{‰}$

Butler et al., 2005; Guilbaud et al., 2010; Guilbaud et al., 2011a	Abiotic experiment	$\Delta^{56}\text{Fe}_{\text{II-Mackinawite-Kinetic}}$	$0.85 \pm 0.30\%$
		$\Delta^{56}\text{Fe}_{\text{II-Mackinawite-Equilibrium}}$	$-0.33 \pm 0.16\%$
Fujii et al., 2014; Polyakov & Sultantov., 2011	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Mackinawite}}$	$3.17 \pm 0.45\%$
Mansor and Fantle et al., 2019	Abiotic experiment	$\Delta^{56}\text{Fe}_{\text{II-Pyrite-Kinetic}}$	$0.75 \pm 0.15\%$
		$\Delta^{56}\text{Fe}_{\text{II-Pyrite-Equilibrium}}$	$0.44 \pm 0.15\%$
Fujii et al., 2014; Polyakov & Sultantov., 2011	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Pyrite}}$	$-5.74 \pm 0.45\%$
Bio-oxidation & Bio-mineralization			
Croal et al., 2004	<i>Thiodictyon strain F4</i>	$\Delta^{56}\text{Fe}_{\text{FeII-FeIII-FeIIISolid}}$	$-2.09 \pm 0.05\%$
Kappler et al., 2010	<i>Acidovorax sp. BoFeN1</i>		$-2.94 \pm 0.05\%$
Swanner et al., 2015	<i>Rhodovulum iodosum</i>		$-1.99 \pm 0.24\%$
Swanner et al., 2017	<i>Synechococcus PCC7002</i>		$-3.35 \pm 0.19\%$
Fujii et al., 2014; Polyakov and Mineev, 2000	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$\Delta^{56}\text{Fe}_{\text{II-III-Goethite}}$	$-0.72 \pm 0.45\%$
		$\Delta^{56}\text{Fe}_{\text{II-III-Lepidocrocite}}$	$-0.34 \pm 0.45\%$
Amor et al., 2016	<i>Magnetospirillum Magneticum strain AMB-1 (MDF)</i>	$\Delta^{56}\text{Fe}_{\text{II-Magnetite}}$	$1.39 \pm 0.19\%$
		$\Delta^{56}\text{Fe}_{\text{III-Magnetite}}$	$2.15 \pm 0.18\%$
		$\Delta^{56}\text{Fe}_{\text{II-Lysate}}$	$-0.80 \pm 0.08\%$
		$\Delta^{56}\text{Fe}_{\text{III-Lysate}}$	$-0.48 \pm 0.17\%$
	<i>Magnetospirillum Magneticum strain AMB-1 (MIF)</i>	$\Delta^{57}\text{Fe}_{\text{II-Magnetite}}$	$-0.06 \pm 0.08\%$
		$\Delta^{57}\text{Fe}_{\text{III-Magnetite}}$	$-0.24 \pm 0.04\%$
		$\Delta^{57}\text{Fe}_{\text{II-Lysate}}$	$-0.02 \pm 0.03\%$
		$\Delta^{57}\text{Fe}_{\text{III-Lysate}}$	$-0.04 \pm 0.00\%$
Fujii et al., 2014; Polyakov (personal communication)	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Magnetite}}$	$-1.22 \pm 0.45\%$
		$10^3\text{Ln}^{56}\alpha_{\text{FeIII-Magnetite}}$	$1.75 \pm 0.70\%$

744 Table 3. Theoretical calculations of ($10^3\text{Ln}\beta^{56/54}$) for different redox of Fe complex to different organic and inorganic ligands in respect to bond
 745 length (Å) when available.

	Bond length (Å)	$10^3\text{Ln}\beta^{56/54}$ for FeIIcomplex		Bond length (Å)	$10^3\text{Ln}\beta^{56/54}$ for FeIIIComplex
$[\text{Fe}_{\text{II}}(\text{H}_2\text{O})_6]^{2+}$	2.16	5.4	$[\text{Fe}_{\text{III}}(\text{H}_2\text{O})_6]^{3+}$	2.06	8.7
$=[(\text{CH}_3\text{COO})\text{Fe}_{\text{II}}]^+$	n.a	2.42	$=[(\text{CH}_3\text{COO})\text{Fe}_{\text{III}}]_2^+$	n.a	3.42
$[\text{Fe}_{\text{II}}(\text{Ox})_3]^{4-}$	2.15	5.50	$[\text{Fe}_{\text{III}}(\text{Ox})_3]^{3-}$	2.00	7.70
$[\text{Fe}_{\text{II}}(\text{Cat})_3]^{4-}$	2.20	4.91	$[\text{Fe}_{\text{III}}(\text{Cat})_3]^{3-}$	2.05	7.27
$\text{Fe}_{\text{II}}(\text{cit})_2\text{OH}^{5-}$	n.a	5.25	$\text{Fe}_{\text{III}}(\text{cit})_2\text{OH}^{4-}$	n.a	8.93
$\text{Fe}_{\text{II}}\text{Cl}_2(\text{H}_2\text{O})_4$	2.25	4.64	$\text{Fe}_{\text{III}}\text{Cl}_2(\text{H}_2\text{O})_4^+$	2.15	6.87
$\text{Fe}_{\text{II}}(\text{OH})_6^{2-}$	1.88	5.41	$\text{Fe}_{\text{III}}(\text{OH})_3(\text{H}_2\text{O})_3$	1.75	9.39
$\text{Fe}_{\text{II}}\text{SO}_4(\text{H}_2\text{O})_5$	1.92	5.40	$\text{Fe}_{\text{III}}\text{SO}_4(\text{H}_2\text{O})_5^+$	1.80	8.42
$\text{Fe}_{\text{II}}\text{CO}_3(\text{H}_2\text{O})_4$	2.04	5.81	$\text{Fe}_{\text{III}}\text{CO}_3(\text{H}_2\text{O})_4^+$	1.92	7.85
$\text{Fe}_{\text{II}}\text{HPO}_4(\text{H}_2\text{O})_5$	1.98	5.88	$\text{Fe}_{\text{III}}\text{HPO}_4(\text{H}_2\text{O})_5^+$	1.84	9.12
Fe-II-HS	2.32	3.96			
(1) redox reactions of Fe within the same phosphate complex ($\text{Fe}_{\text{II}}\text{HPO}_4(\text{H}_2\text{O})_5$ & $\text{Fe}_{\text{III}}\text{HPO}_4(\text{H}_2\text{O})_5^+$), $\Delta^{56}\text{Fe}_{\text{A-B}}(\text{‰})$ is -3.24‰					
(2) the exchange between different inorganic ligands but the same Fe redox ($\text{Fe}_{\text{III}}\text{HPO}_4(\text{H}_2\text{O})_5^+$ & $\text{Fe}_{\text{III}}\text{CO}_3(\text{H}_2\text{O})_4^+$), $\Delta^{56}\text{Fe}_{\text{A-B}}(\text{‰})$ is 1.27‰					
(3) the exchange between inorganic and organic ligands but the same Fe redox ($\text{Fe}_{\text{III}}(\text{cit})_2\text{OH}_4^-$ & $\text{Fe}_{\text{III}}\text{Cl}_2(\text{H}_2\text{O})_4^+$), $\Delta^{56}\text{Fe}_{\text{A-B}}(\text{‰})$ is 2.06‰					
(References: Fujii et al., 2014; Ottonello and Zuccolini, 2008; Moynier, et al., 2013)					

746

747 Table 4. Comparison of the equilibrium iron isotope fractionation obtained in experimental studies ($\Delta^{56}\text{Fe}_{\text{A-B}}$) to those obtained in spectroscopy
 748 studies and theoretical calculations ($10^3\text{Ln}\beta^{56/54}$) for dissimilatory iron reduction.

Dissimilatory iron reduction					
References	Scope of studies	Fe Pairs	$\Delta^{56}\text{Fe}_{\text{IIaq-Bulk}}$ (‰)	$\Delta^{56}\text{Fe}_{\text{IIaq-FeII sorbed}}$ (‰)	$\Delta^{56}\text{Fe}_{\text{IIaq-FeII reac}}$ (‰)
Crosby et al., 2005 & 2007; Icopini et al., 2004	<i>Shewanella putrefaciens</i>	$\Delta^{56}\text{Fe}_{\text{FeIIaq-Goethite}}$	$-0.89 \pm 0.33\text{‰}$	$-0.86 \pm 0.18\text{‰}$	$-2.62 \pm 0.64\text{‰}$
Beard et al., 2010; Friedrich et al., 2014; Reddy et al., 2015	Abiotic-micro-goethite	$\Delta^{56}\text{Fe}_{\text{FeIIaq-Micro-Goethite}}$	$-1.05 \pm 0.08\text{‰}$	$-1.24 \pm 0.14\text{‰}$	$-1.68 \pm 0.08\text{‰}$
	Abiotic-nano-goethite	$\Delta^{56}\text{Fe}_{\text{FeIIaq-Nano-Goethite}}$	$-1.22 \pm 0.08\text{‰}$	$-1.24 \pm 0.14\text{‰}$	$-2.10 \pm 0.48\text{‰}$
Fujii et al., 2014; Polyakov and Mineev, 2000	Calculated $10^3\text{Ln}\beta^{56}_{\text{A-B}}$	$10^3\text{Ln}\beta^{56}_{\alpha_{\text{FeII-Goethite}}}$	$-0.72 \pm 0.45\text{‰}$	-	-
Crosby et al., 2005; 2007 & Wu et al., 2009	<i>Shewanella putrefaciens</i> , <i>Geobacter sulfurreducens</i>		$-1.56 \pm 0.28\text{‰}$	$-0.30 \pm 0.15\text{‰}$	$-2.95 \pm 0.19\text{‰}$
Wu et al., 2009	Abiotic experiment without Si, pH7		-	$-0.49 \pm 0.09\text{‰}$	$-2.64 \pm 0.19\text{‰}$
	Abiotic experiment with Si, pH7		-	$-0.20 \pm 0.13\text{‰}$	$-1.90 \pm 0.47\text{‰}$
	Abiotic experiment without Si, pH 8.7	$\Delta^{56}\text{Fe}_{\text{FeIIaq-bulk-Hematite}}$	-	$-0.36 \pm 0.13\text{‰}$	$-1.76 \pm 0.21\text{‰}$
	Abiotic FeIIaq and Fe-Si gel, pH 8.7		-	$-0.51 \pm 0.18\text{‰}$	$-2.66 \pm 0.18\text{‰}$
Wu et al., 2010; Friedrich et al., 2019; Skulan et al., 2002; Welch et al., 2003	Abiotic-Coarse-hematite ($7 \text{ m}^2\text{g}^{-1}$)		$-3.10 \pm 0.36\text{‰}$	$-0.83 \pm 1.08\text{‰}$	-
	Abiotic-Fine-hematite ($60 \text{ m}^2\text{g}^{-1}$)		$-2.77 \pm 0.37\text{‰}$	$-0.74 \pm 0.45\text{‰}$	-
Fujii et al., 2014; Polyakov et al., 2007	Calculated $10^3\text{Ln}\beta^{56}_{\text{A-B}}$	$10^3\text{Ln}\beta^{56}_{\alpha_{\text{FeII-Hematite}}}$	$-2.68 \pm 0.45\text{‰}$	-	-
Beard et al., 1999 & 2003	<i>Shewanella alga</i> (Strain BrY and Strain BCMB)		$-1.30 \pm 0.22\text{‰}$	-	-
Wu et al., 2011	Abiotic pure HFO	$\Delta^{56}\text{Fe}_{\text{FeIIaq-Ferrihydrite}}$	$-3.20 \pm 0.10\text{‰}$	-	-
	Abiotic HFO + Si		$-3.17 \pm 0.08\text{‰}$	-	-
	Abiotic Si+HFO		$-2.58 \pm 0.14\text{‰}$	-	-

Chanda et al., 2020	Abiotic C+HFO		$-2.36 \pm 0.26\text{‰}$	-	-
n.a	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Ferrihydrite}}$	n.a	-	-
Johnson et al., 2005	<i>Geobacter sulfurreducens</i>	$\Delta^{56}\text{FeII-Siderite}$	$0.00 \pm 0.22\text{‰}$	-	-
	<i>Geobacter sulfurreducens</i> + Ca-Sid		$1.00 \pm 0.22\text{‰}$	-	-
Wiesli et al., 2004	Abiotic experiment		$0.48 \pm 0.22\text{‰}$	-	-
Fujii et al., 2014; Blanchard et al., 2009	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Siderite}}$	$0.89 \pm 0.45\text{‰}$	-	-
Johnson et al., 2005	<i>Geobacter sulfurreducens</i>		$-1.34 \pm 0.22\text{‰}$	-	-
Frierdich et al., 2014b	Abiotic experiment	$\Delta^{56}\text{FeFeII(aq)-bulk-Magnetite}$	$-1.56 \pm 0.22\text{‰}$	-	-
	Abiotic Magnetite from HFO		$-1.61 \pm 0.22\text{‰}$	-	-
Fujii et al., 2014; Polyakov (personal communication)	Calculated $10^3\text{Ln}^{56}\alpha_{\text{A-B}}$	$10^3\text{Ln}^{56}\alpha_{\text{FeII-Magnetite}}$	$-1.22 \pm 0.45\text{‰}$	-	-

749

750 5. Outlook

751 A very limited number of studies addressed iron isotope fractionation during microbial
752 uptake. Microbial ferrous oxidation and adsorption are areas where our understanding remains
753 rather poor given the large discrepancies observed; it could be due to microbial diversity, cell
754 structure, metabolic pathways, the mechanisms involved, as well as to experimental and
755 analytical artifacts arisen from sample preparation. In addition, the role of microbial secreted
756 ligands such as siderophores and how it contributes to the overall iron isotope fractionation in
757 aquatic environment is still unknown. Several abiotic experimental studies have focused on
758 using three-isotope-techniques to mathematically achieve the equilibrium isotope fractionation
759 from the kinetic driven experiment, as a way to decipher kinetic and equilibrium isotope effects.
760 However, it is still unknown how the presence of microorganisms could influence and shift
761 such isotope effects.

762 Our review suggests that significant fractionation of iron isotopes occurs in low-temperature
763 environments, where the extent of fractionation is greatly governed by several biogeochemical
764 processes such as redox reaction, alteration, complexation, adsorption, oxidation and reduction,
765 with or without the influence of microorganisms. Many challenges remain to untangle the
766 isotope signature when all of these processes are taking place at the same time; to decipher the
767 biotic influence from abiotic one, to adequately differentiate kinetic fractionation from
768 equilibrium fractionation, to understand the microbial fractionation at a cellular level, and how
769 the microbial diversity, cell structure and different metabolic pathway contributes to the overall
770 fractionation. A great deal of work is also still required to disentangle the abiotic iron mineral
771 precipitation from biogenic mineral formation. Therefore the mineral transformation pathway
772 coupled to the iron isotope fractionation pathway could be a way to a better comprehension of
773 the iron mineral cycle in nature. This review thus presents several opportunities for future work
774 on iron isotope fractionation in low-temperature biogeochemical environment.

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Highlights

- Extent of Fe isotope fractionation depends on different geochemical processes
- Source signature of the original Fe minerals can be the primary control
- Limitations due to overlapping signatures between abiotic and biotic influences
- Reconciling the discrepancies observed between predicted and experimental values
- Microbial driven fractionation at inter/extracellular levels for future direction

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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