An evolving view on biogeochemical cycling of iron

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Abstract

Iron (Fe) biogeochemical cycling is crucial to many environmental processes such as ocean productivity, carbon storage, greenhouse gas emissions and the fate of nutrients and toxic metal(loids). Knowledge of the underlying processes involved in iron cycling has accelerated in recent years along with appreciation of the complex network of biotic and abiotic reactions dictating the speciation, mobility and reactivity of iron in the environment. Recent studies have provided insights into novel processes in the biogeochemical iron cycle such as microbial ammonium oxidation or methane oxidation coupled to Fe(III) reduction, and they revealed that processes in the biogeochemical iron cycle spatially overlap and may compete with each other, and that oxidation and reduction of iron occurs cyclically or simultaneously in many environments. This Review discusses these advances with a particular focus on their environmental consequences, including the formation of greenhouse gases and the fate of nutrients and contaminants.

[H1] Introduction

Almost 200 years ago, the German naturalist Christian Gottfried Ehrenberg looked under the microscope at iron-rich mats from freshwater springs and peatlands[1]. The microbially produced stalk-like iron oxide structures he described are now considered characteristic biosignatures for microaerophilic **[G]** Fe(II)-oxidizing bacteria[2]; yet for much of the 20th century, redox reactions involving iron were presumed to be primarily abiotic processes. In recent years, a great diversity of microorganisms has emerged that harvest energy from iron redox transformations, and our understanding of their physiology, ecology and environmental importance is growing at an ever-increasing pace.

Iron occurs in two main redox states in the environment: ferric iron (Fe(III)), which is poorly soluble at circumneutral pH, and ferrous iron (Fe(II)), which is typically more soluble and therefore more bioavailable. Despite having only two naturally occurring redox states, a complex network of biogeochemical interactions, including a tight interplay of biotic and abiotic reactions[3], dictates the speciation **[G]**, mobility and reactivity of iron in the environment **(Fig. 1)**. The biotic part of iron redox species turnover at circumneutral neutral pH is catalyzed by Fe(III)-reducing as well as microaerophilic, phototrophic and nitrate-reducing Fe(II)-oxidizing bacteria. These can be found in virtually all habitats: terrestrial and aquatic, freshwater and marine, hot and cold, contaminated and pristine, as well as many extreme habitats[4]. Iron reduction and oxidation can even occur cyclically[5] or simultaneously[6], with biotic reactions super-imposed against a backdrop of abiotic reactions[3]. The bulk geochemistry therefore reflects the net effect of all co-occurring reactions. Unravelling the individual contribution of certain biotic or abiotic processes during Fe cycling is extremely challenging[7], despite the availability of various wet-chemical, microscopic, spectroscopic, molecular biological and other analytical methods to follow the abiotic and microbial transformation of dissolved, colloidal and particulate Fe redox species (**Box 1**).

The redox potentials **[G]** of diverse Fe(II)—Fe(III) redox couples lie between those of oxidized and reduced carbon, nitrogen, oxygen and sulfur redox species. Consequently, any redox reactions involving Fe is tightly linked to these major biogeochemical element cycles (**Box 2**). In some cases, this can influence the emission of gaseous products such as N_2O[8] and CH_4[9] that are potent greenhouse gases. Changes in solubility caused by iron redox transformations also indirectly influence the mobility of elements such as phosphorus[10], carbon[11] and metals such as arsenic or cadmium[12,13], with substantial consequences for the fate of nutrients and contaminants in the environment.

Recent advances have revealed the great complexity of the biogeochemical iron cycle. First, much more insight has emerged into the microorganisms and mechanisms behind novel processes in the biogeochemical iron cycle such as microbial ammonium oxidation or methane oxidation coupled to Fe(III) reduction. Second, processes in the biogeochemical iron cycle that were previously thought to be restricted to distinct geochemical gradients actually overlap spatially and may even compete with each other. Third, oxidation and reduction of iron occurs cyclically or simultaneously in many environments, leading to so-called ‘cryptic’ iron cycling which is not necessarily reflected in the bulk geochemistry.

In this Review, we highlight those recent advances in our understanding of the biogeochemical iron cycle with a particular focus on iron reactivity in pH neutral environments, how anaerobic Fe(III)-reducing or O_2-, light- and NO_x-dependent Fe(II)-oxidizing microorganisms transform iron, and how overlapping processes impact the fate of iron and other elements in the environment. We also highlight recent mechanistic insights into metabolisms such as Fe(III)-coupled ammonium and methane oxidation and cryptic iron cycling.

[H1] Iron accessibility in the environment

Iron exists in various aqueous and solid phases in the environment at dissolved concentrations from several nM to mM and in solids from the µg–g range up to high mg–g concentrations, which affects the energy that microorganisms can gain from redox processes. The redox potentials (E_h) of Fe-bearing phases and their reactivity change considerably as a combined function of ligand and mineral identity, concentrations of dissolved Fe(III) (Fe^{3+}_{aq}) and dissolved Fe(II) (Fe^{2+}_{aq}), pH, particle size, and solid-phase Fe(III)/Fe_{total} ratio (**Fig. 2**). Faster microbial reduction rates and reactivity are observed for poorly crystalline ferrihydrite **[G]** over more crystalline Fe(III) (oxyhydr)oxide minerals (including Fe(III) oxyhydroxides such as goethite or Fe(III) oxides such as hematite)[14]; Fe(III) (oxyhydr)oxides over clays[15]; the clay NAu-2 over NAu-1[16]; and smaller Fe(III) (oxyhydr)oxide particles of the same mineralogy[17]. In batch systems, recent innovative electrochemical methods directly showed that the redox potentials of Fe(III) (oxyhydr)oxides decrease with progressive microbial reduction[18]. Some Fe(III)-reducing microorganisms can respond to variations in redox potentials by utilizing metabolic pathways that are best suited to extract the maximum energy yield under any given condition[19]. Besides the redox potential, factors such as activation energies, aqueous speciation and sorption can also affect bioavailability and reduction rates [20]. Complexation of aqueous Fe with natural organic matter **[G]** (NOM) can modify the rate of microbial redox cycling[21], but redox potential values for Fe–NOM complexes are poorly known. NOM complexation is particularly important for Fe(II) oxidation as it can maintain a pool of stable Fe(II) under oxic conditions when it would have otherwise been completely oxidized[22].

Fe minerals also exist in a wide size distribution in nature, including nanoparticles **[G]**, to colloids **[G]** and particulates **[G]** with different reactivities and transport potentials[23]. Colloidal

Fe minerals display a higher potential for being transported than particulates, making them important vectors for mobilization and transport of Fe and associated nutrients and trace metals[24]. Nanoparticles often exhibit the highest reactivity owing to size-dependent quantum confinement effects that enhance solubilities for particles <100 nm (**Fig. 2**). The aggregation of colloids and nanoparticles can substantially affect their reactivity and mobility, as shown for microbial reduction of hematite nanoparticles which showed higher rates for aggregates that are more accessible for the electron-transferring proteins[25].

[H1] Fe(II) oxidation by oxygen

At circumneutral pH, Fe(II) is thermodynamically unstable in the presence of dissolved oxygen (O_2) at air saturation. Oxidation of Fe(II) coupled to the reduction of O_2 will occur, but the presence of organic ligands, Fe(III) (oxyhydr)oxides, Fe(II)-oxidizing bacteria and temperature determine the rates and mechanisms[26].

[H2] Abiotic Fe(II) oxidation.

Abiotic Fe(II) oxidation occurs through two distinct pathways, termed homogeneous and heterogeneous [27]. Homogeneous Fe(II) oxidation **[G]** involves the reaction of dissolved Fe^{2+}_{aq} with O_2. Oxidation of four Fe^{2+}_{aq} ions occurs in four, step-wise, one-electron transfers, and produces the reactive oxygen species **[G]** (ROS) intermediates superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$) (see equations 1–4) :

$$Fe^{2+}_{aq} + O_{2\,aq} \rightarrow Fe^{3+}_{aq} + O_2^{\cdot-}{}_{aq} \qquad (1)$$

$$Fe^{2+}_{aq} + O_2^{\cdot-}{}_{aq} + 2\,H^{+} \rightarrow Fe^{3+}_{aq} + H_2O_{2\,aq} \qquad (2)$$

$$Fe^{2+}_{aq} + H_2O_{2\,aq} \rightarrow Fe^{3+}_{aq} + OH\cdot_{aq} + OH^{-}_{aq} \qquad (3)$$

$$Fe_{aq}^{2+} + OH\cdot_{aq} \rightarrow Fe_{aq}^{3+} + OH_{aq}^{-} \quad (4)$$

Reactions 1 and 3 are rate-determining steps of the pseudo-first order reaction. H_2O_2 and O_2 are the main oxidants of Fe(II) in seawater[28], although the concentrations of reactants govern the observed oxidation rates[29]. The rate of abiotic Fe(II) oxidation by O_2 can be slowed when Fe(II) is stabilized by organic ligands[22].

Precipitation of poorly soluble Fe(III) (oxyhydr)oxide minerals stimulates rapid abiotic surface-catalysed heterogeneous oxidation of sorbed Fe(II) with rates being directly proportional to the concentration of solid Fe[30].

[H2] Microaerophilic Fe(II) oxidation.

Microaerophilic, neutrophilic Fe(II)-oxidizing bacteria grow lithoautotrophically using Fe(II) as an electron donor and O_2 as an electron acceptor (see equation 5)[31]:

$$4Fe^{2+} + 10\,H_2O + O_2 \rightarrow 4Fe(OH)_3 + 8H^+ \quad (5)$$

These bacteria are members of either the freshwater Betaproteobacteria of which known genera include *Gallionella*, *Sideroxydans*, *Ferriphaselus*, *Ferritrophicum* and *Leptothrix*[31], or of the marine Zetaproteobacteria, for example, *Mariprofundus spp.* or *Ghiorsea spp*[31-33].

Microaerophilic Fe(II)-oxidizers live in mostly aquatic oxic–anoxic interfaces with opposing gradients of O_2 and Fe(II). They are found as microbial mats at groundwater seeps, water treatment systems and deep-sea hydrothermal vents[34,35]. They live in freshwater and marine sediments (and also some soils)[36,37]. They colonize oceanic crust, and also live planktonically in redox-stratified water columns[38,39]. Microaerophilic Fe(II)-oxidizers must compete with the rapid abiotic oxidation of Fe(II) by O_2 at circumneutral pH[40]. To do so they inhabit niches where the activity of O_2 is well below air saturation. The rates of microaerophilic Fe(II) oxidation outcompete abiotic oxidation at or below 50 μM O_2 (Ref. [26]). Optimum growth conditions for microaerophilic Fe(II)-oxidizers occur at 5-20 µM O_2 (Ref. [40]), but growth can still occur at sub-micromolar concentrations of O_2 (Refs. [41,42]).

Oxidation of Fe(II) most likely occurs extracellularly to avoid cell encrustation[43]. A putative fused cytochrome–porin, Cyc2, that is encoded in the genome of all isolates[42,44] is currently considered the most promising candidate as an Fe oxidase in microaerophilic Fe(II)-oxidizers. This putative Fe oxidase was first demonstrated in acidophilic bacteria[45], but later observed to be highly expressed in the proteome of the neutrophilic marine Fe(II)-oxidizer *Mariprofundus ferrooxydans* PV-1 during Fe(II) oxidation[52], and recently validated to have an important role in neutrophilic Fe(II)-oxidizing mats by metagenomics and metatranscriptomics[44]. The *cyc2* gene is widespread across many lineages of neutrophilic Fe(II)-oxidizing bacteria. Moreover, *cyc2* is highly transcribed in iron-bearing microbial mats and stimulated by Fe(II) addition[44]. This makes *cyc2* a promising genetic marker for Fe(II) oxidation, although its functionality in neutrophilic Fe(II)-oxidizers is still unproven and it is found in the genomes of organisms which have not been described to oxidize Fe(II). There is much still to learn before *cyc2* could be used as a marker gene. The current hypothesis is that electrons from oxidation of Fe(II) at the cell surface are transported to periplasmic cytochromes (Cyc1 or others) to a cbb3-type cytochrome oxidase for the reduction of O_2, creating a proton motive force for the generation of ATP[43]. Another possibility is the transfer of electrons from periplasmic cytochromes to another cytochrome and a quinone pool in the inner membrane to finally produce reducing power in the form of NADH[43,46].

Another potential Fe oxidase gene (*mtoA*) is present in *Sideroxydans lithotrophicus* ES-1[46], but this gene was found only in a few other genomes of Fe(II)-oxidizers[47]. Therefore, Fe(II) oxidation by Cyc2 is presumably more widespread amongst microaerophilic Fe(II)-oxidizing bacteria[47].

Some microaerophilic Fe(II)-oxidizing bacteria direct extracellular Fe biomineralization onto twisted stalks, tubular sheaths, granular or dreadlock-like structures[31,32] that consist of poorly crystalline ferrihydrite, lepidocrocite **[G]** , goethite **[G]** or akageneite **[G]** [37,48] and an organic matrix (probably acidic polysaccharides and saturated aliphatic chains of organic carbon)[37]. Besides preventing cell encrustation, the extracellular biominerals were also suggested to fulfill different functions depending on their morphology. Twisted stalks help position bacteria at optimum growth conditions within concentration gradients of O_2 and Fe(II) and to anchor them to surfaces[49]. Dreadlock-like structures are easily shed from cells to help planktonic Fe(II)-oxidizers stay suspended in water columns[41].

Microaerophilic Fe(II)-oxidizing bacteria greatly affect Fe(II) oxidation rates, either directly by their metabolism or indirectly by producing Fe(III) (oxyhydr)oxides that form a surface catalyst for heterogeneous Fe(II) oxidation **[G]** [32]. Microaerophilic Fe(II)-oxidizers primarily influence the environment by forming unique microenvironments such as microbial mats influencing and forming gradients of O_2 and Fe(II)[2]. The biomineral mixture of poorly crystalline Fe(III) (oxyhydr)oxides and organic constituents functions as carbon and energy sources for Fe(III)-reducing bacteria or other bacteria[31], especially because nutrients, organic matter and heavy metals can also adsorb or co-precipitate with those highly reactive biominerals[37,50].

[H2] Parsing abiotic from biotic circumneutral Fe(II) oxidation.

Biotic and abiotic Fe(II) oxidation reactions occur in parallel, which makes identifying the occurrence and quantitative contribution of microaerophilic bacteria to overall Fe(II) oxidation challenging. Microbial Fe(II) oxidation can account for up to 50-80% of the total Fe(II) oxidation over a wide range of microoxic conditions[31,32]. Voltammetric microelectrodes **[G]** have been applied to field settings to identify zones where Fe(II) and O_2 concentrations should support

Fe(II)-oxidizing bacteria[35]. Gradient tubes have long been used to enrich Fe(II)-oxidizing bacteria, but can also be used to distinguish biotic from abiotic Fe(II) oxidation by comparing Fe(II) concentrations measured with voltammetric microelectrodes in gradient tubes inoculated with or without Fe(II)-oxidizers[26,51]. Recently, a liquid culture microcosm approach was applied to quantify the effect of heterogeneous Fe(II) oxidation on biotic versus abiotic Fe(II) oxidation at varying O_2 concentrations[40].

As shown for a peatland drainage and groundwater discharge channel, the *in-situ* rates of microbial Fe(II) oxidation depend on water flow leading to advection and turbulent mixing[52], and those reports emphasize that *ex-situ* experiments in laboratory settings risk underestimating the oxidation rates that actually occur in nature[52]. Rates have recently been quantified by modeling Fe concentrations as a function of transit time through a small stream, revealing seasonal differences in the contribution of biotic processes to Fe(II) oxidation[53]. Long-range correlations of temporal fluctuations of redox potential can also distinguish bacterial from abiotic Fe(II) oxidation in incubation of field samples[54].

[H1] Light-induced Fe redox reactions

Iron redox cycling often occurs at oxic–anoxic interfaces, but light-driven reactions can also drive iron cycling, even under anoxic conditions. These processes are relevant to aquatic systems, as photosynthetically active radiation (PAR) penetrates >100 m in water or 5-6 mm in sediments[55].

[H2] Microbial phototrophic Fe(II) oxidation.

Photoautotrophic Fe(II)-oxidizing bacteria (photoferrotrophs) are primary producers that use light energy and electrons from Fe(II) to fix bicarbonate into organic carbon (see equation 6):

$$HCO_3^- + 4Fe^{2+} + 10H_2O \overset{h\nu}{\rightarrow} \langle CH_2O \rangle + 4Fe(OH)_3 + 7H^+ \qquad (6)$$

They were first described by Widdel et al.[56], but the existence of such a metabolism was previously hypothesized and suggested to have predated the origin of oxygenic photosynthesis[57]. Photoferrotrophy has thus been implicated as an oxygen-independent mechanism for Fe(II) oxidation and deposition of Precambrian-aged banded iron formations (BIF) from the oceans[56], as well as in primary productivity before oxygenic photosynthesis[58].

Isolated photoferrotrophs comprise three taxonomic groups. Purple-sulfur bacteria (PSB) fall within the bacterial phylum Gammaproteobacteria, represented by *Thiodictyon sp.*[59]. Purple non-sulfur bacteria (PNSB) are Alphaproterobacteria and include *Rhodobacter ferrooxidans* SW2[60], *Rhodopseudomonas palustris* TIE-1[61], and two marine strains (*Rhodovulum iodosum* and *R. robiginosum*)[62]. Green-sulfur bacteria (GSB) are encompassed completely within the bacterial family *Chlorobiaceae*, and are represented by *Chlorobium ferrooxidans* strain KoFox, the dominant member of an enrichment culture[63], the first pelagic isolate *C. phaeoferrooxidans*[64], and the marine *Chlorobium* sp. strain N1[65].

Two protein-encoding operons are known to catalyze Fe(II) oxidation in PNSB. The *pioABC* operon is required by *R. palustris* strain TIE-1 for phototrophic Fe(II) oxidation[66]. *pioB* encodes a putative outer membrane porin that may transport Fe(II) into, or Fe(III) out of the periplasm. PioA, a periplasmic decaheme *c*-type cytochrome, forms a complex with the outer membrane porin PioB and facilitates uptake of extracellular electrons across the outer membrane[67]. PioC, a high potential ion sulfur protein is thought to subsequently shuttle electrons to the photosynthetic reaction center[79]. The *foxEYZ* gene cluster, which is not a *pioABC* homolog, stimulates light-dependent Fe(II) oxidation in *R. ferrooxidans* SW2 (Ref. [59]).

Fe(II) is thought to be transported by an inner membrane protein encoded by *foxZ*, whereas *FoxY* is likely to have a role in electron transfer. FoxE is a diheme cyctochrome *c* suggested to function as iron oxidoreductase[68], and it is required for light-dependent Fe(II) oxidation[59].

The GSB *C. phaeoferroxidans* as well as the recently isolated *Chlorobium* strain N1 *both* encode *cyc2*[69,70], an outer-membrane protein whose homologs in oxygen-dependent Fe(II)-oxidizing bacteria are thought to directly accept electrons from Fe(II)[43] (see above).

Photoferrotrophs produce poorly crystalline ferric oxyhydroxide minerals, which mature into goethite or lepidocrocite[71]. Photoferrotrophs do not seem to become encrusted in minerals and do not form elaborate structures as microaerophilic Fe(II)-oxidizers do[72]. Proposed strategies to localize precipitation away from the cell surface include lowering pH around the cell[73], using lipopolysaccharide fibers to template biomineralization[71], or secretion of organic iron-binding ligands, such as extracellular polymeric substances **[G]** (EPS), that help to bind and/or transport Fe(III)[74].

[H2] Photochemically induced Fe cycling.

Photochemical Fe(III) reduction has a major role for Fe availability in sunlit aquatic and sedimentary environments by converting Fe into more reactive and potentially more bioavailable phases [75]. Fe(III) photoreduction occurs by two major mechanisms: either by direct ligand-to-metal charge transfer (LMCT) [76] or indirectly by photochemically produced radicals[77]. The mechanism depends on the speciation of Fe, whereas rates and extent of Fe(III) photoreduction depend on the wavelength and intensity of light, pH, temperature and the ionic strength[78].

At circumneutral pH, most dissolved Fe(III) is complexed by organic ligands [Fe(III)–L] in the form of colloids (0.02-0.4 µm)[75], which drastically increases Fe(III) solubility. The organic ligand pool in natural waters may contain polysaccharides, humic substances **[G]** or siderophores **[G]** with different functional groups[79]. Fe(III)-organic complexes containing an α-hydroxy carboxylic acid group can undergo light-induced LMCT reactions[80] – those ligands can also cause light-induced dissolution of Fe(III) colloids[81]. The LMCT reaction produces Fe(II), oxidizes organic ligand to CO_2 and/or organic molecules with altered binding properties[82].

Alternatively, Fe(III) can be reduced by superoxide produced from photochemical reactions of NOM with O_2[83]. Photochemical reactions of NOM are the primary pathway for ROS production in sunlit surface waters[83]. The relative importance of LMCT reactions versus superoxide-mediated Fe(III) photoreduction in the environment is still a subject of debate[84], and the contributions of either process are likely to be determined by the dominant Fe(III) species and the type of NOM[82,84]. Photolabile Fe(III)-organic complexes favor LMCT reactions, whereas Fe(III) bound to photostable complexes or present as Fe(III) (oxyhydr)oxides favors superoxide-mediated Fe(III) reduction[84].

Fe(III) can also be reduced by photic zone-dwelling phytoplankton. Marine phytoplankton produce extracellular superoxide[85], leading to ROS-driven Fe(III) reduction, although superoxide is also produced by diverse bacteria below the photic zone[86]. Cyanobacteria are able to reduce Fe(III) to Fe(II) enzymatically[87], in addition to a superoxide-mediated pathway[84]. These mechanisms likely enhance Fe bioavailability to cyanobacteria, and their contribution to Fe(II) production in the photic zone of Fe-rich waters may be substantial[88].

[H2] Integrating light-dependent abiotic and microbial iron oxidation.

Despite fast Fe(II) oxidation kinetics in oxygenated, circumneutral pH waters, Fe(III) photoreduction leads to increased Fe(II) concentrations in sunlit surface waters following diel cycles[89] as well as increased Fe(II) concentrations in the upper millimeters of light-illuminated sediments[90,91]. Photoreduction can provide a source of Fe(II) to photoferrotrophs and other Fe(II)-oxidizing bacteria[92], which in turn provide Fe(III) for microbial Fe(III) reduction[93]. Light-driven iron cycling is ultimately limited by light penetration depth. Photosynthetic organisms stratify according to light quantity and quality: oxygenic phototrophs generally need about 1% of surface PAR[94], whereas photoferrotrophic PSB and PNSB as well as GSB can thrive with less light and prefer anoxic conditions[95]. GSB photoferrotrophs should live deepest, as they use shorter wavelengths than PSB and PNSB, and they are adapted to extreme light-limitation[96].

[H1] Fe(II) oxidation by nitrogen species

As a consequence of environmental nitrogen (N)-cycling processes, and amplified by intensive fertilizer use over the past decades, nitrate co-occurs with Fe(II) in many habitats such as in aquifers, stratified water bodies or in the top few anoxic millimeters and centimeters of sediments. Microbial and abiotic redox reactions between dissolved and solid-phase Fe(II) species and the oxidized N-compounds nitrate and nitrite can facilitate nitrate removal and enhance the production of the greenhouse gas N_2O.

[H2] Microbially mediated nitrate-reducing Fe(II) oxidation.

Oxidation of Fe(II) coupled to reduction of nitrate to N_2 (see equation 7) or to ammonium (dissimilatory nitrate reduction to ammonium, DNRA; see equation 8) under anoxic conditions was first described in 1996 (Ref. [97]).

$$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+ \quad (7)$$

$$8\,Fe^{2+} + \ NO_3^- + 21\,H_2O \rightarrow 8\,Fe(OH)_3 + NH_4^+ + 14\,H^+ \quad (8)$$

Several strains capable of catalyzing nitrate reduction coupled to Fe(II) oxidation (NRFeOx) have been reported but, in recent years, it has become evident that only a minority represent chemolithoautotrophic **[G]** NRFeOx[98], wherein this case Fe(II) oxidation is coupled to energy generation by nitrate reduction and to CO_2 fixation for biomass production[99]. Chemolithoautotrophic NRFeOx has been demonstrated unambiguously for the enrichment culture KS[99], for an enrichment culture obtained from a nitrate-contaminated groundwater aquifer[100] as well as in marine sediments[101]. However, in most cases nitrate reduction is instead coupled to oxidation of background or cell-stored carbon, and Fe(II) oxidation is catalysed by nitrite and other reactive N-species produced as by-products of heterotrophic denitrification in a process termed 'chemodenitrification'[98]. Some of the published strains may also be true mixotrophs and oxidize both Fe(II) and organic compounds enzymatically with an energetic benefit from Fe(II) oxidation. Many heterotrophic denitrifiers produce reactive N-species that lead to abiotic Fe(II) oxidation and N_2O formation[102], and this is likely to be environmentally important, including for the greenhouse gas budget[103,104].

For several studies published with isolated strains and environmental samples, the extent of enzymatic Fe(II) oxidation and chemodenitrification remains unclear. As both microorganisms catalyzing NRFeOx and chemodenitrifiers use the same enzymatic pathways for nitrate reduction, they cannot be distinguished based on genomic characteristics. The only genomic indicator could be the presence of an Fe(II) oxidase. However, the mechanism responsible for the oxidation of iron is controversial even in the most well-studied chemolithoautotrophic culture growing by NRFeOx, that is culture KS, and cannot be used as a marker for iron oxidation[105]. Recent attempts to analyze N- and O-isotope composition in oxidized and reduced N-species, before and after reaction with Fe(II) in the presence of active microorganisms have shown potential for disentangling the complex network of coupled biotic and abiotic Fe-N redox reactions[106], but more work is needed to solve this conundrum.

[H2] Microbial mineral oxidation with nitrate.

Oxidation of iron(II) sulphide (FeS) and pyrite (FeS_2) minerals has been shown to be coupled to reduction of nitrate[107]. *Thiobacillus denitrificans*, various members of the *Acidovorax* and *Geothrix* genera, and a Marinobacter-related isolate have been suggested to catalyse these reactions[107,108]. However, at least some of the observed FeS and FeS_2 oxidation can probably be attributed to either abiotic pyrite oxidation by microbially produced nitrite during acidic extraction of the Fe species (when nitrite is formed from nitrate reduction coupled to oxidation of reactive elemental sulphur or organic carbon; Box 3)[109], or to oxidation by Fe(III) formed from oxidation of small amounts of Fe^{2+}_{aq} (Ref. [110]).

Additionally, microbial enzymatic nitrate reduction can be coupled to oxidation of Fe(II) in clays (for example, illite[111], smectites[112] or biotite[113]), although a contribution of abiotic oxidation of the Fe(II) in the clays by nitrite or by Fe(III) cannot be ruled out in these cases.

[H2] Microbial mineral formation by nitrate-reducing Fe(II)-oxidizing bacteria.

Oxidation of dissolved Fe^{2+}, Fe(II) complexed by organic matter, or Fe(II) minerals (for example, vivianite or siderite) by bacteria catalyzing NRFeOx at neutral pH leads to the formation of poorly soluble Fe(III)[98]. This was shown to precipitate, depending on the geochemical and physical conditions in the growth medium (for example, the presence of NOM, ions, mineral nucleation sites, pH, temperature), as poorly crystalline ferrihydrite-like Fe(III) oxyhydroxide, as Fe(III)-phosphate, as more crystalline goethite or mixed-valent Fe(II)–Fe(III)-containing green rust[114,115]. Depending on the bacterial strain or strains involved and the resultant extent of either enzymatic, chemolithoautotrophic Fe(II) oxidation[116] or abiotic Fe(II) oxidation by chemodenitrification[71], these minerals were found in close association with the cells forming cell-mineral aggregates. In many cases the minerals were associated with EPS, on the cell surface or even in the cell periplasm. Occasionally minerals even completely encrust the cells. The formation of nanoparticulate Fe(III) minerals with high surface areas and binding capacities by nitrate reduction coupled to iron oxidation can have important implications for the fate of nutrients and pollutants[117].

[H1] Fe(II) oxidation by manganese(IV)

Manganese is present in the environment as reduced, dissolved Mn(II) or in the form of Mn(IV) oxide minerals. The importance of Mn(III) as an intermediate in Mn redox cycling was recently revealed[118]. Mn often co-occurs with iron. These elements influence each other's redox speciation and reactivity with consequences for other biogeochemical cycles[119]. Fe(II) is abiotically oxidized by Mn(IV) oxides through a surface controlled inner-sphere electron transfer process (see equation 9)[120] (**Fig. 1**):

$$MnO_2 + 2\ Fe^{2+} + 2\ H_2O \rightarrow Mn^{2+} + 2\ FeOOH + 2\ H^+ \qquad (9)$$

Because of its more positive redox potential, Mn(IV) reduction is generally expected to occur first, then Fe(III) reduction and, as a consequence, reduction of Mn(IV) minerals is spatially separated from reduction of Fe(III) minerals (for example, in stratified lake sediments[121]). However, in many environments Mn and Fe cycling are tightly coupled even on very small spatial scales, for example in rice paddies where steep redox gradients exist on small scales[122].

[H1] Iron(III) reduction

[H2] Microbial iron(III) reduction.

Microbial Fe(III)-reducing bacteria couple the reduction of ferric iron with the oxidation of organic or inorganic electron donors. This capability has been demonstrated for different microorganisms in almost every anoxic environment on Earth. Examples of Fe(III)-reducers **[G]** include *Geobacter* spp.[123], *Shewanella* spp.[124], *Albidoferax ferrireducens*[125], *Geothrix fermentans*[126] and hyperthermophilic archaea[127].

Electron donors used by Fe(III)-reducers include fatty acids, carbohydrates, amino acids, aromatic compounds and dihydrogen (H_2)[20]. Fe(III)-reducers can use complexed dissolved Fe(III) as electron acceptor. At circumneutral pH and in the absence of organic ligands, Fe(III) is more typically present as either short-range ordered mineral phases (for example, ferrihydrite), or as crystalline minerals (for example, goethite, hematite and magnetite). Although a number of Fe(III) mineral phases have been shown to function as electron acceptors for *G. sulfurreducens* including hematite, lepidocrocite, feroxyhyte, akageneite and schwertmannite[14], the energy gained by such electron transfer varies depending on the mineral (**Fig. 2**).

The identity of the minerals produced by microbial iron(III) reduction depends upon a number of factors including temperature, reduction rate, and presence of anions such as bicarbonate or phosphate, and can lead to the formation of siderite, vivianite, magnetite, green rust or, via Fe(II)-catalyzed transformation of ferrihydrite, even to goethite[128]. Equation 10 indicates the formation of magnetite formed by the reduction of an Fe(III) oxyhydroxide coupled to the oxidation of acetate as is typical of *Geobacter sulfurreducens*.

$$CH_3COO^- + 24\ FeOOH \rightarrow 8\ Fe_3O_4 + 2HCO_3^- + 12\ H_2O + H^+ \quad (10)$$

Another well-studied group of Fe(III)-reducing bacteria are members of the *Shewanellacae*, in particular strain *Shewanella oneidensis* MR-1, which was isolated in the 1990s (Ref. [124]) and can reduce ferric iron with H_2, formate or lactate as electron donor.

[H2] Electron transfer strategies.

Fe(III)-reducers, notably *Shewanella* and *Geobacter* species, face the challenge of poor solubility of their electron acceptor. This prevents uptake of iron into the cells and requires them to use various electron transfer mechanisms for dissimilatory iron(III) reduction (**Fig. 3**), which are described below. The first mechanism involves direct contact between proteins associated with the outer cell wall and the Fe(III) mineral surface. This mechanism relies on electrons that originate from intracellular catabolism to be transferred to *c*-type cytochromes **[G]** localized on the cell-surface which then mediate extracellular electron transfer to Fe(III) (oxyhydr)oxides [129]. The differences reported between the electron transport pathways of *S. oneidensis* and *G. sulfurreducens*[130], and even within the *Geobacter* species[131], suggest that there are several biochemical pathways available for direct contact iron(III) mineral reduction.

The second mechanism requires the use of conductive organic pili-like structures (microbial nanowires) to transfer electrons to the surface of the Fe(III) minerals[132]. Extracellular, conductive structures are thought the be constructed by many bacteria and even archaea[133]. The most widely studied are the electrically conductive pili of the *Geobacter* species *Geobacter sulfurreducens* and *Geobacter metallireducens*. *G. sulfurreducens* constructs conductive pili from the type IV pilin monomer protein, PilA[134-136]. There is a substantial and growing body of evidence that these pili in *G. sulfurreducen*s (and the related *G. metallireducens*) facilitate transfer of electrons over distances of around 20 µm to extracellular electron acceptors, including Fe(III) oxides[137]. *Shewanella* species can also transfer electrons across similar distances using extracellular appendages formed by extensions of the outer membrane and periplasm, facilitated by multi-heme cytochromes[138,139] Considerable advancements have been made in recent years in establishing the molecular underpinnings of electron transfer via these appendages; however it remains the subject of lively debate[133].

In addition, redox-active electron shuttles such as dissolved or solid-phase NOM (including humic substances), redox-active mineral particles, sulphur (S) compounds, self-made redox mediators, or mediators produced by other microorganisms can be used to transfer electrons between the intracellular electron transfer chain and the distant solid mineral phases[140-142] (**Fig. 3**). The principle behind this mechanism is that the microorganisms first reduce the electron shuttle (for example, oxidized NOM or oxidized S-species) in an enzymatic reaction, the shuttle becomes reduced (reduced NOM or reduced S-species) and then transfers the electron to the terminal electron acceptor, for example poorly soluble Fe(III) minerals, in an abiotic reaction. The electron shuttles become re-oxidized during this second abiotic part of the process and can serve again as an electron acceptor for the microorganisms thus sustaining the cyclic electron shuttling process.

For electron shuttling, microorganisms have been shown to use dissolved and solid-phase NOM[125,140]. This includes microorganisms with different physiology (for example, fermenters, methanogens, sulphate reducers and halorespirers) from diverse environments such as lake and marine sediment, pristine and contaminated wetland sediments[125,143,144] ultimately making all of them indirect Fe(III)-reducers[145]. In addition to the abiotic reactions of Fe(III) minerals with NOM, reduced S-species such as sulphide are also able to reduce Fe(III) (oxyhydr)oxides abiotically[146].

S. oneidensis excretes self-made redox-active mediators (flavins) as electron shuttles[147,148]. Other Shewanella strains such as *S. alga* strain BrY were shown to also use Fe(III) chelators[149], thereby facilitating the use of Fe(III) as an electron acceptor. Flavins may even enhance direct electron transfer[129], and have an effect on the measured redox potential in sediments[150]. It was recently shown that anthraquinone-2-6-disulfonic acid (AQDS), a model compound for redox-active moieties in NOM, could support long range electron transfer by at least 2 cm through a combination of AQDS molecular diffusion and electron hopping from reduced to oxidized AQDS molecules[151,152]. Iron reducers have also been shown to harness the electron-accepting capabilities of the mixed-valent iron oxide magnetite to replace biological electron-transfer proteins[153]. In that study, a wild-type strain of *G. sulfurreducens* exhibited lower expression of a specific multiheme *c*-type cytochrome OmcS, which is responsible for electron transfer, when incubated with nanoscale magnetite compared to without magnetite. This observation suggests that solid iron minerals such as magnetite might be able to function in a manner similar to cytochromes in microbial extracellular electron transfer

The final mechanism describes nonreductive dissolution of Fe(III) (oxyhydr)oxides by microbial secretion of organic ligands (Fe(III)-chelators), which leads to the release of more readily reducible soluble Fe(III)-complexes[154].

[H2] Microbial Fe(III) reduction coupled to methane and ammonium.

In recent years, the role of Fe(III) reduction in promoting the oxidation of methane and ammonium has received increasing attention. Oxidation of these compounds is most thermodynamically favourable with O_2 as an electron acceptor, but anaerobic methane oxidation can occur in anoxic environments coupled to the reduction of Fe(III), Mn(IV), nitrate or sulphate as electron acceptors[155].

Fe(III)-dependent anaerobic oxidation of methane (Fe-AOM) (see equation 11) has been inferred from geochemical and isotopic evidence in freshwater[156] and marine sediments[9], paddy fields[157], stratified lakes[158] and contaminated aquifers[159,160]. Given the abundance of Fe(III) in the environment, AOM coupled to Fe(III) reduction can represent a substantial methane sink[9].

$$CH_4 + 8Fe(OH)_3 + 16H^+ \rightarrow CO_2 + 8Fe^{2+} + 22H_2O \qquad (11)$$

Anaerobic methane-oxidizing archaea (ANME) similar to the ANME-2 lineage have been identified to be responsible for Fe(III) reduction[161]. ANME archaea may utilize conductive nanowires resembling pili-like structures formed by *Geobacter* consortia[162].

In freshwater sediment bioreactors, *Ca. Methanoperedens nitroreducens* was shown to reduce Fe(III)-citrate coupled to AOM[163]. More recently, *Ca. Methanoperedens ferrireducens* was shown to conduct AOM in an Fe(III)-dependent manner, and may use multiheme cytochromes to facilitate extracellular dissimilatory Fe(III) reduction[164].

Microbial Fe(III) reduction can also be coupled to ammonium oxidation and is colloquially known as Fe-ammox (see equation 12).

$$NH_4^+ + 6\ FeOOH + 10\ H^+ \rightarrow NO_2^- + 6\ Fe^{2+} + 10\ H_2O\ (12)$$

This process occurs in anoxic, iron-rich and water-saturated systems such as riparian[165], forested[166] and coastal wetlands[167], and in rice paddy soils[168]. Although it has also been described in forest soils[169] and sewage sludge[170]. To date only one microorganism, *Acidimicrobiaceae* sp. A6, has been isolated that oxidizes NH_4^+ to NO_2^- under Fe(III)-reducing conditions[171]. Fe-ammox can also result in the conversion of NH_4^+ to NO_3^-[172] or, in the most thermodynamically favourable option, to N_2 gas[169]. However, the individual microorganism or consortium responsible for this are as yet unknown[155]. In addition to driving transformation of Fe(III), Fe-ammox can be responsible for substantial production of gaseous N species such as N_2, N_2O or NO and thus contribute to nitrogen loss and greenhouse gas emissions[173].

[H1] Balancing iron oxidation and reduction

Previous sections have detailed the myriad of processes that make up the biogeochemical iron cycle. However, these processes are not separated in natural environments, where oxidation and reduction reactions occur cyclically or even simultaneously. For example, during redox cycling of a tropical forest soil, the Fe(III) (oxyhydr)oxides formed during oxic periods became progressively less crystalline across repeated redox cycles, which facilitated even more rapid Fe(III) reduction with every reducing cycle[174]. Oxidative cycles need not only be initiated by oxygen, but can also be promoted by nitrate under anoxic conditions[175].

Iron redox cycling does not always lead to mineral phase transformation, (for example, from ferrihydrite to siderite) but can occur within a single mineral phase. For instance, some mixed-valent iron minerals (that is, containing Fe(II) and Fe(III)) such as magnetite can function as both electron donors and acceptors, and therefore function as recyclable ‘biogeobatteries’ without transformation[5]. This process is size-dependent with oxidation confined to the surface and reduction enabling bulk electron transfer though the entire mineral[5].

Given the diversity of reactions that can cycle iron in the environment it is not uncommon that they can spatially overlap[176]. In these cases, a cryptic cycling scenario can emerge in which turnover is so rapid that the product of iron oxidation or reduction cannot be measured with standard analytical techniques measured (**Fig. 4**). This cryptic cycling was observed in Lake Cadagno, Switzerland, where the re-reduction of Fe(III) in the stratified water column was so rapid that it masked the contribution of a population of Fe(II)-oxidizing bacteria[6]. A similar process was observed in laboratory incubations, when the activity of Fe(II)-oxidizing phototrophic bacteria was masked by the light-induced reduction of Fe(II) in Fe–organic matter complexes[92]. This cryptic iron cycle may also be closely tied to the even more enigmatic processes in the sulfur cycle, with sulphate reduction hypothesized to drive Fe(III) reduction even when sulphide concentrations remain low[177]. The interactions between Fe and nitrogen are also prime candidates for potential cryptic interactions in the iron cycle as the reactive N species produced are highly reactive and short lived, thus unlikely to be accurately reflected in standard aqueous geochemical measurements. For example, in microbial nitrate-dependent Fe(II) oxidation by *Acidovorax* sp. BoFeN1, Fe(II) is not oxidized directly by the microorganisms, but by short-lived denitrification intermediates such as NO_2^- and NO[178]. These react so quickly with Fe(II) that they may never accumulate in solution despite contributing substantially to Fe(II) oxidation. Rapid reactions between Fe and N species can promote incorporation of inorganic nitrogen into organic nitrogen, fundamentally altering soil nitrogen pools[179].

[H1] Conclusions

Since the discovery of the first iron-metabolizing bacteria, we have come a long way in our understanding of the diversity, physiology, ecology and environmental influence of the microorganisms who transform iron — and iron biogeochemistry remains a fascinating and complex subject of study. We are only just beginning to appreciate the complexity of iron transformations in the environment and are increasingly adopting new tools (**Box 2**) that enable us in future research to observe and unravel the competing and co-occurring iron cycling processes. It also has become obvious that Fe biogeochemical cycling is linked to future changes in Earth's climate via CO_2 formation and/or CH_4 oxidation by Fe(III)-reducing bacteria[9,123], CO_2 fixation by autotrophic Fe(II)-oxidizing bacteria[56], and N_2O formation by bacteria linking the Fe and N cycles[103]. Further indirect effects on climate by Fe-metabolizing bacteria are caused by iron mineral-precipitating and -dissolving microorganisms that lead to mobilization or stabilization of organic carbon or nutrients[50,180,181], as well as by microorganisms that are involved in changing the bioavailability of iron species in oceans, thus influencing primary productivity[75]. This leads us into an exciting new decade of iron biogeochemistry and opens up various future research directions.

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Acknowledgements

The authors acknowledge funding for several research grants from the German Research Foundation (DFG), in particular the Collaborative Research Center CAMPOS (DFG, Grant Agreement SFB 1253/1) and the Priority Program EARTHSHAPE.

Author contributions

A.K and C.B initiated the manuscript, designed the content, wrote the manuscript, created some of the figures, and compiled and revised all content. E.D.S, M.M, U.L and J.M.B wrote the manuscript and created some of the figures.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Microbiology thanks J. Gralnick, who co-reviewed with A. Jain, J. Senko and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

<u>Publisher's note</u>

Figure 1. **The biogeochemical Fe cycle**. All iron (Fe) redox reactions that occur at circumneutral pH are listed in thermodynamic order, with microbially-mediated reactions on the left and abiotic reactions on the right. The central panel depicts gradients of O2, light, NO_3^-, Fe(II) and Fe(III) that are typical of redox stratified environments. The coloured panels indicate the sequence in which the different biotic and abiotic reactions are expected to take place based on thermodynamics, however these often overlap in the real environment. Fe-ammox = Fe-dependent ammonium oxidation. ROS = reactive oxygen species. HumS = humic substances. Figure adapted from Ref. 3.

Figure 2.**Redox potentials of diverse Fe(II)—Fe(III) redox couples**. Redox potential (E_h) of the Fe(III)–Fe(II) pair for Fe-bearing phases at 25°C, pH 7 and constant $[Fe^{2+}] = 10^{-5}$ M (unless otherwise specified) showing that iron-bearing minerals and complexes do not have a defined E_h but exist within a range depending on geochemical parameters and their physical properties. The $[Fe^{2+}] = 10^{-5}$ M concentration was chosen as an environmentally relevant and representative concentration, typically found in aquatic systems such as sediments (depending on the specific conditions, this value can be higher or lower in nature). The figure is divided into four sections, with E_h values calculated according to the specified variation in ($[Fe^{3+}L]$/ $[Fe^{2+}L]$ of ligand-complexed Fe species **(a),** $[Fe^{2+}{}_{aq}]$ concentration (colored boxes with solid outlines) and pH (unfilled boxes with dashed outlines; numbers indicate the pH boundary) **(b)**, particle radius **(c)** and solid-phase Fe(III)/Fe_{total} **(d)**. Exact ranges of E_h values are specified at the top part of the figure and color-coded. Shaded horizontal areas correspond to the E_h range of key Fe-oxidoreductase enzymes[68,182-184]. Additional information for the E_h determination are as follows: **(a)** 1:1 Fe:L complexation and nondissociative reduction were assumed. Standard redox potentials (Eh^0) were calculated from the stability constant of the one-electron reduction of Fe^{3+} to Fe^{2+} (log K = 13) and the stability constants of the respective $Fe^{3+}L$ and $Fe^{2+}L$ complexes, as obtained from the Thermochimie v10a database[185] (for EDTA, NTA, cit and oxa) or from ref[186] (for CLC and SRFA; conditional log K values at pH 8.1 in seawater). **(b)** Eh^0 obtained or calculated from standard Gibbs free energy of formation (ΔG_f^0) values from refs[187-189] and converted to E_h as a function of $[Fe^{2+}]$ (at constant pH 7) or pH (at constant $[Fe^{2+}] = 10^{-5}$ M). For schwertmannite, assume constant $[SO_4^{2-}] = 10^{-4}$ M. **(c)** Eh^0 corrected for increased surface energy of small particles through published mineral-specific values of enthalpies of hydrated surfaces (ΔH_s^h)[187,190] and molar volumes[191], assuming geometric surface area. **(d)** E_h values determined directly at pH 7-7.5 through electrochemistry for 10-20 nm magnetite particles and various clay reference materials (NAu-2, NAu-1, SWa-1, SWy-2, MX-80)[192,193]. EDTA, ethylenediaminetetraacetic acid; NTA, nitriloacetic acid; cit, citrate; oxa, oxalate; CLC, NOM extract from sugar cane; SRFA, Suwannee River fulvic acid; Sch, schwertmannite; Fer, feroxyhyte; Lep, lepidocrocite; Aka, akageneite; Goe, goethite; Hem, hematite; Magh, maghemite; Magn, magnetite; Fh, ferrihydrite.

Figure 3: **Electron transfer mechanisms from microorganisms to Fe(III) minerals.** This schematic summarizes the strategies utilized by Fe(III) reducers to access solid Fe as an electron acceptor. Over short distances, they can directly transfer electrons to surfaces with which they are in contact. Alternatively, they can utilize chelating agents or microbial/environmental redox-active electron shuttles to facilitate electron transfer. Within biofilms, they may assemble electrically conductive pili or outer-membrane extensions to transfer electrons, or they can pass the electrons through the biofilm via redox co-factors in a process termed electron hopping. In a combination of diffusion of soluble redox-active shuttles and electron hopping via insoluble (solid-phase) shuttles, microorganisms can facilitate Fe(III) reduction over long distances (cm). Figure adapted from Ref. 3.

Figure 4: **Overview of processes that can over-lap and lead to cryptic Fe cycling.** Fe(II) is oxidized abiotically by molecular oxygen (O_2) formed during oxygenic photosynthesis, by reactive oxygen species (ROS) or by MnO_x formed during microbial Mn^{2+} oxidation (Mn cycle). Fe(II) is microbially oxidized either by phototrophic or by microaerophilic Fe(II)-oxidizing bacteria. Fe(II) can also be microbially oxidized by nitrate (NRFeOx) and form nitrite which abiotically oxidizes Fe(II), is further transformed via denitrification (producing NO, N_2O or N_2), or can transform to ammonium or dissolved organic nitrogen (DON). Fe(III) can be rapidly re-reduced by Fe(III)-reducing bacteria, by Fe(III) photoreduction, especially if Fe(III) is organically complexed (Fe(III)-organic matter), by superoxide or by sulphide (H_2S) leading either to precipitation of FeS minerals or to the formation of intermediate sulphur species (S^0, S_n^{2-}, $S_2O_3^{2-}$) that themselves are converted back to sulphide or to sulphate (SO_4^{2-}) (cryptic S cycle).

Box 1: Analyzing Fe biogeochemistry

Various wet-chemical, microscopic, spectroscopic, molecular biological and other analytical methods are used to follow the abiotic and microbial transformation of dissolved, colloidal and particulate Fe redox species.

Mineral identity

- **Mössbauer spectroscopy**. ^{57}Fe-specific absorption of gamma-rays provides identification of the Fe redox state, identity of minerals, and information about mineral crystallinity and particle size.
- **X-ray diffraction (XRD)**. Diffraction of X-rays at the crystal lattice enables the identification and provides information about crystallite size and crystallinity of minerals.
- **X-ray absorption spectroscopy (XAS)**. Synchrotron-based XAS is used to identify the Fe redox state and binding environment (including Fe-complexes and minerals).
- **Sequential extraction**. Different combinations of acids, reducing agents and complexing agents provide information about mineral identity and crystallinity.
- **Fourier-transform infrared (FTIR) and Raman spectroscopy**. Absorption of specific wavelengths (energy) by certain bonds enables the identification of minerals.

Mineral properties and cell mineral associations

- **Zetasizer**. The movement of particles and aggregates in an electrical field provides information about their charge.
- **Wet-chemical titrations**. pH measurements following addition of acids or bases enables the calculation of the charge of particles and aggregates.
- **Laser scattering**. Laser light diffractions are used to determine the size of mineral particles and aggregates.
- **Transmission electron microscopy [G] (TEM) and scanning electron microscopy [G] (SEM)**. An electron beam is used in scanning or transmission mode to characterize the morphology, size and structure of mineral aggregates, particles, individual crystals and cell-mineral associations. Use in combination with electron diffraction can aid mineral identification.
- **Fluorescence microcopy**. The use of specific fluorescence dyes enables localization, quantification and identification of specific microorganisms and parts of cell-mineral aggregates
- **BET (Brunauer–Emmett–Teller method).** Calculation of a specific mineral surface area by quantification of sorption of gas molecules or organic compounds.
- **X-ray photoelectron spectroscopy (XPS).** Elemental composition at mineral surfaces (~10 nm) is obtained from the kinetic energy of electrons released after irradiation with X-rays
- **Electrochemical measurements**. Quantification of currents flowing at different redox potentials applied at electrodes enables the calculation of mineral and cell redox potentials.
- **Microscopy**. Many kinds of microscopy can be used to image cell–mineral associations depending on the size range. Light or fluorescence microscopy will capture larger particles, whereas electron microscopy methods will provide high levels of detail. Careful sample preparation may be required to preserve cells.

Compounds associated with minerals:

- **Total and dissolved organic carbon (TOC and DOC, respectively).** TOC provides the total amount of carbon present. Following chemical dissolution of iron minerals, a DOC analyser can measure co-eluted carbon.
- **Trace metal and nutrients.** After mineral dissolution, metals, P and S co-extracted with the minerals can be quantified, for example, by an inductively coupled plasma mass spectrometer.
- **X-ray fluorescence.** Indicates elemental composition of solid samples.
- **X-ray absorption spectroscopy (XAS)**. Information about identity, redox state, binding environment and location of mineral associated organic compounds and metal ions.
- **Energy-dispersive X-ray spectroscopy (EDX) in SEM and TEM**. Radiation released as a consequence of electrons interacting with the minerals enables the identification and/or quantification of elements.
- **Nanoscale secondary ion mass spectrometry**. A primary ion beam (for example, Cs^+) is used to release secondary ions from the specimen in high (nm) resolution and to identify and/or quantify them in a mass spectrometer.

Transformation processes:

- **Geochemical, spectroscopic and mineralogical analyses of field samples, laboratory batch and/or column incubations (microcosms)**. Such analyses, potentially in combination with Fe isotope analyses, can quantify and identify dissolved, colloidal and mineral Fe species and thus provide quantitative information about rates and extent of Fe transformation.
- **Liquid-cell TEM**. Application of electrons in liquid-TEM cells enables monitoring of mineral transformation in real-time.

Microorganisms involved in biogeochemical cycling:

- **Fluorescence microscopy or flow cytometry.** Cells stained with fluorescent dyes can be quantified.
- **Most-probable number quantification.** Cultivation-based quantification of living Fe(II)-oxidizing or Fe(III)-reducing microbial cells.
- **Quantitative PCR.** Analysis of genes involved in Fe(III) reduction and Fe(II) oxidation enables the identification and estimation of Fe-metabolizing microbial activities.
- **Fluorescence in-situ hybridization.** The application of specific DNA-binding fluorescent probes enables the localization and quantification of specific microorganisms in laboratory and environmental samples.

Box 2: Impact of iron transformation on other biogeochemical cycles

Despite only one electron being transferred during Fe(II) oxidation or Fe(III) reduction, iron has a disproportionate impact on other major biogeochemical element cycles due to a combination of redox reactions, as well as sorption and co-precipitation.

The redox potentials of the Fe(II)–Fe(III) redox couples lie between that of couples of environmentally relevant carbon (C), nitrogen (N), oxygen (O) and sulfur (S) species, which means that iron redox reactions directly influence the redox state of C, N, O or S (Fig. 1). This has important environmental implications, for example by Fe(III) reduction contributing to the mineralization of carbon, or Fe(II) mitigating toxic nitrate and nitrite in wastewaters. Greenhouse gases such as N_2O can be emitted as a consequence of Fe(II) oxidation[8], and Fe(III)-dependent anaerobic oxidation of methane (AOM) can attenuate CH_4 fluxes[9,194]. Iron redox reactions can also be harnessed for remediation purposes[195]. For instance, the reducing capacity of Fe(II) can be exploited to transform several organic and inorganic contaminants such as hydrocarbons, pesticides, explosives, azides or heavy metals[192,196].

The redox-dependent solubility of iron at neutral pH also has a strong impact on other element cycles through sorption and co-precipitation. For example, phosphorus strongly binds to Fe(III) (oxyhydr)oxides, thus the mobility of phosphorus is tied to the precipitation and dissolution of iron minerals in sediments, soils and freshwater habitats[10]. Iron minerals are also thought to have a stabilizing effect on organic matter in soils[197] and marine sediments[11]. Iron can also strongly bind trace metals and both organic as well as inorganic contaminants[195], a function widely exploited in remediation technologies. For example, the ability of iron minerals to sorb arsenic has been widely utilized in drinking water purification in countries such as Vietnam and Bangladesh[198], but such processes are also useful to trap Ni, Cu, Zn and Pb[199].

Box 3: Pitfalls in iron analyses

Most studies of iron cycling rely to some extent on wet-chemical extractions to dissolve minerals and to stabilize Fe(II) and Fe(III) concentrations for subsequent analyses. Numerous extraction protocols exist, many involving dissolution with varying strengths of acid[200]. However, care must be taken to avoid common pitfalls related to sample acidification. For example, solubilisation of Fe(III) (oxyhydr)oxides under acidic conditions can lead to electron transfer from reduced species such as hydrogen sulfide or natural organic matter (NOM) to aqueous Fe^{3+} which has a more positive redox potential, leading to Fe(III) reduction and an over-estimation of the Fe(II) content of the sample.

Problems can also occur in the acidification of samples containing nitrite. Nitrite becomes protonated to nitrous acid at low pH, and further decomposes to NO_2 and NO that rapidly oxidize Fe(II)[178]. This results in under-estimation of dissolved Fe(II) concentrations. In this case sulfamic acid, which quenches nitrite, has been proposed to be more suitable for sample preservation[178]. However, this will be inadequate to preserve the redox state of carbonate-rich samples due to pH buffering. A combination of sulfamic acid and hydrochloric acid is suggested to preserve Fe(II)/Fe(III) ratios in high nitrite, high carbonate samples[201].

Although Fe(II)–Fe(III) ratios are generally considered to be stable at low pH, oxidation by O_2 occurs within minutes in 6M HCl at 70°C but is not seen in 1M HCl at ambient temperatures[202]. This is because Fe–HCl complexes are rapidly oxidized at increased temperatures. Acidity and temperature therefore need to be factored into any decision about whether to conduct extractions under oxic or anoxic conditions.

Strong acid extractions aim to dissolve minerals. Acid extraction of adsorbed Fe(II) on Fe(III) (oxyhydr)oxides is generally accomplished by incubation with 1 M sodium acetate at pH 4.85 or 0.5 M hydrochloric acid, with the latter extractant able to dissolve some of the solid[203]. However, 1 M sodium acetate will also slightly dissolve Fe carbonates[204], which would overestimate adsorbed Fe in carbonate-bearing samples. Using independent techniques (for example, X-ray diffraction or Mössbauer spectroscopy) to verify the types of Fe minerals can minimize unexpected results.

Even if the redox state can be accurately preserved during acid extraction, numerous compounds interfere with spectrophotometric methods typically used for iron quantification.

These methods involve the use of complexing agents that form stable, coloured complexes with dissolved Fe(II) such as ferrozine[205] or phenanthroline[206]. The reaction must be well-buffered, as the absorption of the Fe(II)–ferrozine complex is attenuated below pH 4 and above pH 10 (Ref. [205]). Reduction of Fe(III) complexed with organic matter by hydroxylamine can also be incomplete, in the presence of humic substances, leading to underestimation of total iron and therefore inaccurate Fe(II)–Fe(III) ratios[207]. In this case, an alternate quantification method for total Fe may be warranted. The accuracy of the ferrozine assay is also strongly impacted by heavy metals like copper and cobalt[205] that also form complexes with ferrozine. For well characterized samples, relevant metals should be included in the standards.

Table of content:

In this Review, Kappler and colleagues highlight recent advances in our understanding of the biogeochemical iron cycle, exploring the great complexity of the processes involved and novel mechanistic insights that have been gained.

Glossary

Chemolithoautotrophic. Microorganisms that use energy from a chemical reaction of inorganic compounds (for example oxidation of iron(II)) to fix carbon from CO_2 *into biomass.*

Microaerophilic. Microorganisms that oxidize Fe(II) at O_2 *concentrations in the tens of µM range are microaerophilic Fe(II)-oxidizers.*

Speciation. Describes the oxidation state and the identity of the coordinating ligands, for example organic matter, chloride or sulfide.

Redox potential (E_h*). Redox potential (in millivolts) indicates the thermodynamic driving force for reduction or oxidation, for example of an Fe(III)–Fe(II) pair.*

Natural organic matter (NOM). Mixture of organic compounds resulting in nature from the degradation of biopolymers (proteins, lipids, lignin, polysaccharides, etc.) stemming from plants, microorganisms, and animals.

Nanoparticles. Particles that are <100 nm in size in at least one dimension.

Colloids. Particles <1,000 nm in size in at least one dimension that are dispersed in a substance of another physical state, for example mineral particles in liquid.

Particulates. Particles that are >1,000 nm in size in all dimensions.

Reactive oxygen species (ROS). Very reactive compounds with unpaired electrons forming from molecular O_2*.*

Lepidocrocite. A ferric iron oxyhydroxide polymorph (γ-FeOOH) with a yellow to reddish brown color.

Goethite. A ferric iron oxyhydroxide polymorph (α-FeOOH) known for its use as paint pigment and named after the poet Johann Wolfgang von Goethe.

Akaganeite. A ferric iron oxyhydroxide polymorph (ß-FeOOH) typically forming in marine environments.

Ferrihydrite. A poorly crystalline Fe(III) oxyhydroxide mineral with a nm-sized primary particle diameter and a resulting large surface area and high reactivity.

Homogeneous Fe(II) oxidation. This term describes the oxidation of reduced iron (Fe(II)) by an oxidant that is in the same physical state (for example oxidation of dissolved Fe^{2+} *by dissolved* O_2*).*

Heterogeneous Fe(II) oxidation. This term describes the oxidation of iron (Fe(II)) by an oxidant that is in a different physical state, for example oxidation of sorbed Fe(II) by dissolved O_2*.*

Voltammetric microelectrodes. Electrodes with tip diameters in the µm range – the potential at the working electrode is varied and the resulting current is recorded. Such electrodes can be used to identify and quantify iron redox species with high spatial resolution, for example in sediments.

Extracellular polymeric substances (EPS). Organic molecules consisting of polysaccharides and proteins, but also DNA and lipids, purposefully released by microorganisms into the environment, for example during biofilm formation

Humic substances. Stable organic molecules that are redox-active and thought to form by humification, that is the transformation of biomolecules (including lignin, proteins and polysaccharides) – this formation theory has been questioned and is being gradually replaced by a soil continuum model.

Mixotrophic. Microorganisms using an inorganic electron source (for example Fe(II)) in addition to an organic compound for their metabolism are termed mixotrophic microorganisms.

Siderophores. Organic compounds produced and released by microorganisms in order to make otherwise poorly soluble Fe(III) ions bioavailable for the cells and to facilitate its uptake.

Fe(III)-reducers. Microorganisms that specialize in gaining energy by coupling Fe(III) reduction with the oxidation of an electron donor (for example, an organic compound).

c-type cytochromes. Proteins that contain heme as prosthetic group and are involved in oxidation and reduction reactions inside and outside the microbial cell.

Scanning electron microscopy (SEM). A microscope technique using a beam of electrons to scan the surface of a specimen to obtain information about the morphology, topography and surface structure – applied for example to characterize cell-mineral structures of iron-metabolizing microorganisms.

Transmission electron microscopy (TEM). An imaging technique using a beam of electrons transmitted through a thin specimen to obtain an image of the specimen up to atomic resolution, applied in physical, chemical and biological sciences – can be used for example to characterize nanoparticles formed by iron-metabolizing microorganisms.