

## The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle

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**Abstract** | Many iron (Fe) redox processes that were previously assumed to be purely abiotic, such as photochemical Fe reactions, are now known to also be microbially mediated. Owing to this overlap, discerning whether biotic or abiotic processes control Fe redox chemistry is a major challenge for geomicrobiologists and biogeochemists alike. Therefore, to understand the network of reactions within the biogeochemical Fe cycle, it is necessary to determine which abiotic or microbially mediated reactions are dominant under various environmental conditions. In this Review, we discuss the major microbially mediated and abiotic reactions in the biogeochemical Fe cycle and provide an integrated overview of biotic and chemically mediated redox transformations.

### Fe speciation

Refers to the redox state of iron (Fe) and the identity of its ligands. The two most common environmental Fe redox species are Fe(II) and Fe(III).

The environmental abundance of iron (Fe) and its possession of electrons in *d* orbitals with  $\pi$ -character, which can form complexes with carbon (C), oxygen (O), nitrogen (N) and sulphur (S) species, make it an essential element for nearly all living organisms. Fe occurs in two main redox states in the environment: oxidized ferric Fe (Fe(III)), which is poorly soluble at circumneutral pH; and reduced ferrous Fe (Fe(II)), which is easily soluble and therefore more bioavailable. Fe speciation and bioavailability are dynamically controlled by the prevalent changing redox conditions. Redox reactions of Fe with C, N, O and S drive global biogeochemical cycles, as the redox potential of the Fe(III)–Fe(II) redox couple lies between the redox potentials of the major C, N, O or S species redox couples. Although microbial Fe oxidation had been described by the early nineteenth century<sup>1</sup>, the prevailing view in the early twentieth century was that Fe cycling was mainly mediated abiotically by chemical reactions with molecular oxygen (O<sub>2</sub>), nitrite (NO<sub>2</sub><sup>-</sup>), divalent and tetravalent manganese (Mn), various S species and organic C. Since the discovery of neutrophilic Fe-metabolizing bacteria<sup>2–5</sup>, we have entered a ‘golden age’ of Fe geomicrobiology. All redox processes that were previously assumed to be purely abiotic (for example, oxidation of Fe(II) by O<sub>2</sub> or photochemical oxidation of Fe(II)) are now known to also be microbially mediated. However, the contribution of microorganisms alone may not be sufficient to interpret the observed spatial

and temporal distribution of Fe redox species in some environments, such as heterogeneous sediments, plant rhizospheres and microbial mats<sup>6</sup>, and abiotic Fe transformations with biologically produced intermediates must be taken into account<sup>7,8</sup>.

Despite our growing knowledge of the widespread environmental occurrence and the importance of microbial Fe redox cycling for the degradation and preservation of C and the fate of many nutrients and contaminants<sup>9–11</sup>, we still lack a comprehensive understanding of the mechanisms and pathways of electron transfer in Fe(II)-oxidizing and Fe(III)-reducing bacteria. Unlike electron transfer between other major inorganic redox species, electron transfer to and from Fe can be mediated by various cellular pathways. The genes that are involved in gaining energy from oxidizing Fe(II) or reducing Fe(III) are only known for a few bacteria, such as *Rhodopseudomonas palustris* strain TIE-1 and *Shewanella oneidensis* strain MR-1 (REFS 12–14). Gene families that encode proteins with a potential role in Fe(II) oxidation or Fe(III) reduction within different microbial taxa generally have low sequence identity, although some homologues of key genes have recently been identified<sup>15–18</sup>.

The increased understanding of the genetic and mechanistic pathways that are involved in Fe geomicrobiology over the past 25 years has now converged with the understanding of the abiotic chemical pathways

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Microbially mediated reactions

<p><b>Microaerophiles</b></p> $4\text{Fe}^{2+} + 10\text{H}_2\text{O} + \text{O}_2 \rightarrow 4\text{Fe}(\text{OH})_3 + 8\text{H}^+$ <i>Gallionella</i> spp., <i>Leptothrix</i> spp., <i>Mariprofundus</i> spp., <i>Sideroxydans</i> spp.
<p><b>Photoferrotrophs</b></p> $\text{HCO}_3^- + \text{Fe}^{2+} + 10\text{H}_2\text{O} \xrightarrow{h\nu} (\text{CH}_2\text{O}) + 4\text{Fe}(\text{OH})_3 + 7\text{H}^+$ <i>Rhodospseudomonas palustris</i> TIE-1 <i>Rhodobacter</i> sp. SW2 <i>Chlorobium ferrooxidans</i> (KoFox) <i>Thiodictyon</i> sp. F4
<p><b>NO<sub>3</sub><sup>-</sup>-reducing Fe(II)-oxidizers</b></p> $10\text{Fe}^{2+} + 2\text{NO}_3^- + 24\text{H}_2\text{O} \rightarrow 10\text{Fe}(\text{OH})_3 + \text{N}_2 + 18\text{H}^+$ <i>Acidovorax</i> spp., KS, 2002 <i>Thiobacillus denitrificans</i>
<p><b>Fe-ammox</b></p> $\text{NH}_4^+ + 6\text{FeOOH} + 10\text{H}^+ \rightarrow \text{NO}_2^- + 6\text{Fe}^{2+} + 10\text{H}_2\text{O}$ Unknown
<p><b>Fe(III)-reducing organic C and/or H<sub>2</sub>-oxidizers</b></p> $4\text{FeOOH} + \text{CH}_3\text{CHOHCOO}^- + 7\text{H}^+ \rightarrow 4\text{Fe}^{2+} + \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 6\text{H}_2\text{O}$ $2\text{Fe}(\text{OH}) + \text{H}_2 \rightarrow 2\text{Fe}^{2+} + 2\text{H}_2\text{O}$ <i>Geobacter</i> spp., <i>Shewanella</i> spp., <i>Albidiferax ferrireducens</i> , <i>Geothrix</i> spp.

Chemically mediated reactions

<p><b>ROS</b></p> <p><b>Oxidation</b></p> $\text{Fe}^{2+} + \text{O}_2 \rightarrow \text{Fe}^{3+} + \text{O}_2^{\cdot-}$ $\text{Fe}^{2+} + \text{O}_2^{\cdot-} + 2\text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O}_2$ $\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{OH}^+ + \text{H}_2\text{O}$ $\text{Fe}^{2+} + \text{OH}^+ + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O}$
<p><b>Reduction</b></p> $\text{O}_2^{\cdot-} + \text{Fe}(\text{III}) \rightarrow \text{O}_2 + \text{Fe}^{2+}$
<p><b>Light reactions</b></p> <p><b>Reduction</b></p> $\text{Fe}(\text{III})\text{-L} \xrightarrow{h\nu} \text{Fe}(\text{II})\text{-L}$
<p><b>Nitrogen species</b></p> <p><b>Oxidation</b></p> $4\text{Fe}^{2+} + 2\text{NO}_2^- + 5\text{H}_2\text{O} \rightarrow 4\text{FeOOH} + \text{N}_2\text{O} + 6\text{H}^+$ $\text{Fe}^{2+} + \text{NO}_2^- + 2\text{H}^+ \rightarrow \text{FeOOH} + \text{NO} + \text{H}_2\text{O}$ $\text{Fe}^{2+} + \text{NO} + \text{H}^+ \rightarrow \text{FeOOH} + \frac{1}{2}\text{N}_2\text{O} + \frac{1}{2}\text{H}_2\text{O}$
<p><b>Manganese</b></p> <p><b>Oxidation</b></p> $2\text{Fe}^{2+} + \text{MnO}_2 + 2\text{H}_2\text{O} \rightarrow \text{Mn}^{2+} + 2\text{FeOOH} + 2\text{H}^+$
<p><b>HumS</b></p> <p><b>Reduction</b></p> $\text{Fe}(\text{III}) + \text{HumS}^- \rightarrow \text{HumS} + \text{Fe}(\text{II})$
<p><b>Sulphur species</b></p> <p><b>Reduction</b></p> $2\text{FeOOH} + 3\text{H}_2\text{S} \rightarrow 2\text{FeS} + \text{S}^0 + 4\text{H}_2\text{O}$

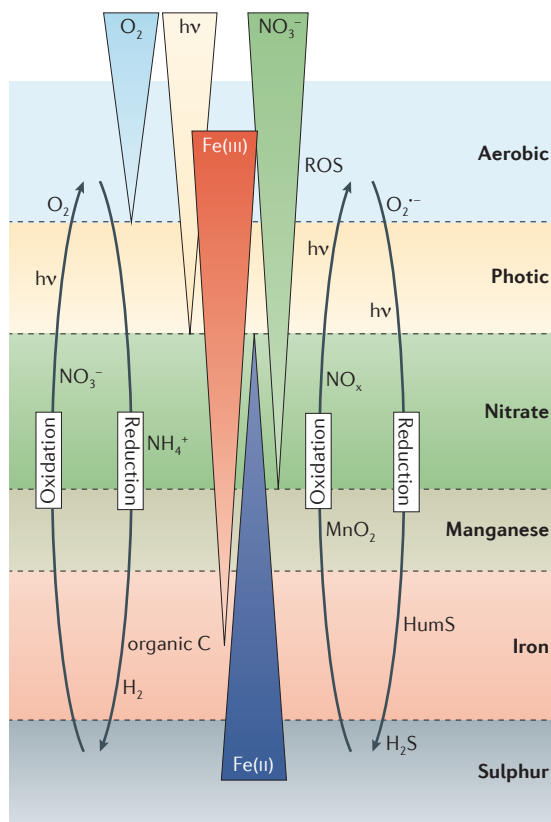


Figure 1 | Microbially and chemically mediated reactions that form the biogeochemical Fe cycle. Microbially mediated iron (Fe) redox reactions are shown on the left-hand side and abiotic Fe redox transformations are shown on the right-hand side, listed in a thermodynamic order (although some of these reactions may overlap in the natural environment). Within the oxic zone, microaerophilic Fe(II) oxidizers oxidize ferrous Fe (Fe(II)) using oxygen (O<sub>2</sub>). Reactive oxygen species (ROS) can oxidize Fe(II), and superoxide (O<sub>2</sub><sup>·-</sup>) can abiotically reduce ferric iron (Fe(III)). Within the photic zone, phototrophic microorganisms oxidize Fe(II) and photochemical reactions reduce Fe(III) that is bound to organic ligands (L). Mixotrophic and autotrophic nitrate (NO<sub>3</sub><sup>-</sup>)-dependent Fe(II) oxidation is restricted to anoxic conditions in the denitrification zone. NO<sub>3</sub><sup>-</sup>-reducing Fe(II)-oxidizing bacteria use Fe(II) as an electron donor. Fe-ammox bacteria couple the oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) to Fe(III) reduction. Fe(II) can be chemically oxidized via chemodenitrification by reactive nitrogen (N) species. Fe(II) is abiotically oxidized by manganese (Mn) via surface-catalysed reactions. Fe(III)-reducing microorganisms can reduce Fe(III) coupled to the oxidation of various electron donors such as organic carbon (C) and H<sub>2</sub>. Electron-rich (that is, reduced) humic substances (HumS) abiotically reduce Fe(III) to Fe(II). Fe(III) is chemically reduced by hydrogen sulphide (H<sub>2</sub>S) to ferrous sulphide (FeS) species. The different gradients of O<sub>2</sub>, light, NO<sub>3</sub><sup>-</sup> and Fe(II) and Fe(III) in a redox-stratified environmental system are shown, as well as where the different biotic and abiotic Fe redox transformations are expected to take place.

Microaerophilic

A term used to describe microbial metabolism that requires oxygen (O<sub>2</sub>) concentrations to be very low; for example, microaerophilic ferrous iron Fe(II)-oxidizing bacteria function when the O<sub>2</sub> concentration is below 50 μM.

Lithotrophic

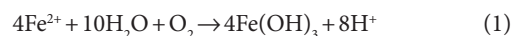
A term used to describe microbial metabolism that uses inorganic substrates as electron donors.

that are involved in environmental Fe cycling. So far, many studies have focused on either abiotic or biotic processes; however, these processes are interconnected and cannot be studied separately if we are to truly understand environmental biogeochemical Fe cycling. Therefore, a current challenge is to discern abiotic from microbially mediated Fe redox processes and to estimate their overall contributions to the Fe biogeochemical cycle. In this Review, we discuss the most important microbially mediated and abiotic reactions and address their interactions within the biogeochemical Fe cycle.

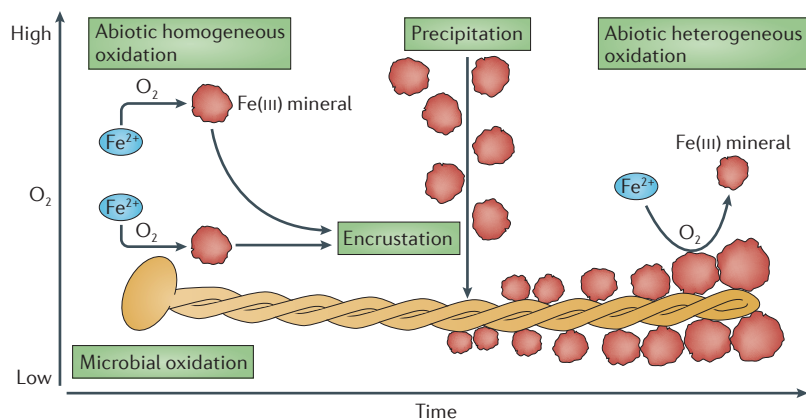
**Fe(II) oxidation by O<sub>2</sub>**

The high redox potential of O<sub>2</sub> and its one-electron transfer derivatives readily initiates the exergonic abiotic oxidation of Fe(II) (FIG. 1).

**Microbially mediated Fe(II) oxidation by O<sub>2</sub>.** Microaerophilic Fe(II) oxidizers are lithotrophic bacteria that oxidize Fe(II) with O<sub>2</sub> according to the following stoichiometric equation<sup>5</sup> (FIG. 1):



Phylogenetically, these bacteria belong to the phylum Proteobacteria, which includes the freshwater genera *Leptothrix*<sup>19</sup>, *Gallionella*<sup>20</sup> and *Sideroxydans*<sup>21</sup> and the marine genus *Mariprofundus*<sup>22</sup>. Microaerophilic Fe(II) oxidizers are common in many freshwater and marine environments that are exposed to O<sub>2</sub> (REFS 5,23,24), and their growth is severely retarded under anoxic conditions, with the exception of the nitrate (NO<sub>3</sub><sup>-</sup>)-reducing Fe(II)-oxidizing co-culture KS<sup>25</sup> that contains a *Sideroxydans* spp. relative. A recent study showed that the ecological niche

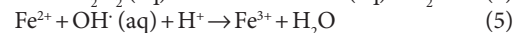
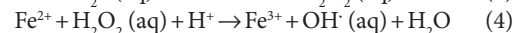
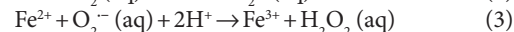
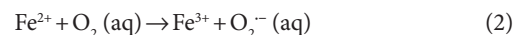


**Figure 2 | Microbially and chemically mediated Fe(II) oxidation by O<sub>2</sub>.** Different stages of abiotic and microbially mediated ferrous iron (Fe(II)) oxidation by microaerophilic Fe(II) oxidizers occurs at low oxygen (O<sub>2</sub>) concentrations, and homogeneous abiotic Fe(II) oxidation occurs simultaneously at high O<sub>2</sub> concentrations. Ferric Fe (Fe(III)) mineral products are formed, followed by precipitation and encrustation (for example, at the twisted stalks of *Gallionella* spp. and *Mariprofundus* spp. strains). The precipitated Fe(III) minerals function as a surface catalyst for further chemical Fe(II) oxidation (that is, heterogeneous Fe(II) oxidation), which ensues in parallel to microbial and homogeneous Fe(II) oxidation.

of the stalk-forming Gallionellales is in waters that have a low organic C content and steep redoxclines, whereas the sheath-forming *Leptothrix ochracea* is abundant in waters that contain much organic C, Fe and Mn and that have gentle redoxclines<sup>26</sup>.

At circumneutral pH, Fe(III) exists as poorly soluble ferric oxyhydroxides and, therefore, microbial Fe(II) oxidation is likely to be carried out by an outer cell membrane Fe(II)-oxidizing protein to prevent Fe(III) mineral precipitation inside the cell. The decahaem *c*-type cytochromes MtoA, MtoB and CymA<sub>ES-1</sub> potentially form a conductive pathway for electron transfer from extracellular Fe(II) to a quinone pool in the inner membrane of *Sideroxydans* strain ES-1 (REF. 17). *Gallionella* strain ES-2 contains homologues of MtoA and MtoB, but instead of a CymA homologue, it contains an additional multihaem *c*-type cytochrome that has weak homology to MtrD in *S. oneidensis* strain MR-1 (REF. 18). Although the ES-1 and ES-2 strains share the MtoA- and MtoB-encoding genes, differences in the homology and synteny of neighbouring genes suggest that these genes may function differently in each strain. Moreover, the genes that encode MtoA and MtoB also share homology with the genes that encode MtrA and MtrB (decahaem *c*-type cytochromes) in the Fe(III)-reducing *S. oneidensis* strain MR-1 (REFS 14, 16) and the genes that encode PioA and PioB (a periplasmic cytochrome and an outer membrane porin) in the anoxygenic phototroph *R. palustris* strain TIE-1 (REF. 15). There are no gene homologues to MtrA and MtrB or PioA and PioB in the genome of the marine microaerophilic Fe(II) oxidizer *Mariprofundus ferrooxydans* strain PV-1. However, a molybdopterin oxidoreductase Fe<sub>3</sub>S<sub>4</sub> protein is highly expressed when PV-1 oxidizes Fe(II)<sup>22</sup>. Similar orthologous gene ‘neighbourhoods’ of the PV-1 ‘Mob gene’ were also found in the genomes from other metal-oxidizing and -reducing Proteobacteria<sup>22</sup>.

**Chemical Fe(II) oxidation by O<sub>2</sub>.** When Fe(II) and O<sub>2</sub> are simultaneously present at neutral pH, Fe(II) is readily chemically oxidized by O<sub>2</sub> (REF. 27). In homogeneous abiotic Fe(II) oxidation, both Fe(II) and O<sub>2</sub> are in the dissolved form. The reactions can be summarized as follows<sup>27</sup>:



in which the first step (see equation 2) is rate limiting<sup>28</sup>. Thus, in oxygenated aquatic environments, Fe(II) is oxidized by O<sub>2</sub> and secondary oxidants (the reactive oxygen species (ROS) O<sub>2</sub><sup>-</sup>, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and OH<sup>•</sup>) are produced during the stepwise reduction of O<sub>2</sub> (FIG. 1). The precipitated Fe(III) oxyhydroxides (Fe(OH)<sub>3</sub>) function as a surface catalyst for further chemical Fe(II) oxidation — this is termed heterogeneous Fe(II) oxidation (also known as auto-oxidation)<sup>13</sup>. The oxygenation of Fe<sup>2+</sup> ions in pH-neutral solutions is therefore accelerated by the reaction product — for example, Fe(III) oxyhydroxides (FIG. 2).

**Integrating abiotic and microbial Fe oxidation.** Microbial and abiotic Fe(II) oxidation by O<sub>2</sub> generally occurs in opposed gradients of Fe(II) and O<sub>2</sub> concentrations, which enables microaerophilic Fe(II) oxidizers to occupy a niche in which the enzymatic oxidation of Fe(II) can outcompete the kinetics of the homogeneous abiotic reaction<sup>29</sup> (BOX 1; FIG. 2). In addition, the rate of abiotic Fe(II) oxidation by O<sub>2</sub> at neutral pH has an initially linear temperature dependency according to the Arrhenius equation, which enables the temperature dependence of reaction rates to be calculated<sup>30</sup>, whereas the rate of microbial Fe(II) oxidation depends on the optimum temperature of the enzymes that are used by the individual species. As *Gallionella ferruginea* displays growth optima at temperatures of 20–25 °C (REF. 20), this difference in temperature-dependent Fe(II) oxidation optima between microbial and abiotic oxidation could provide a kinetic advantage for neutrophilic microaerophilic Fe(II) oxidizers over the competing abiotic reaction at temperatures around 20 °C (REF. 30). This is only true for the initial homogeneous reaction; once Fe(III) mineral products provide surface sites for autocatalytic heterogeneous Fe(II) oxidation, the abiotic reaction will be more favourable (FIG. 2). Therefore, it is not trivial to produce a time- and temperature-dependent universal Fe(II) oxidation rate equation that takes into account sequential and parallel microbial, homogeneous and heterogeneous Fe(II) oxidation (BOX 1). Such an equation would be widely applicable in Fe biogeochemical research and would enable the prediction of Fe oxidation rates under dynamic fluctuating environmental conditions.

**Biogenic mineral formation.** The Fe(III) that is produced by microaerophilic Fe(II) oxidizers rapidly precipitates as Fe(III) minerals. Oxidation and precipitation occur in two steps, and members of the *Gallionella*

#### Redoxclines

The interfaces between two spatially distinct areas that differ in chemical composition and redox potential; they are usually used to describe a transition from oxic to anoxic conditions.

#### *c*-type cytochromes

Small haem-containing proteins that have an important role in respiratory electron transfer reactions.

#### Reactive oxygen species (ROS)

Oxygen-containing species that have unpaired electrons, making them highly reactive towards transition metals such as iron and copper.

#### Heterogeneous Fe(II) oxidation

Oxidation of ferrous iron (Fe(II)), where dissolved Fe(II) is adsorbed to a mineral surface, which functions as a catalyst, and the oxidant is in a different physical phase; for example, dissolved oxygen.

**Box 1 | Limits in formulating a universal rate equation for O<sub>2</sub>-dependent Fe(II) oxidation**

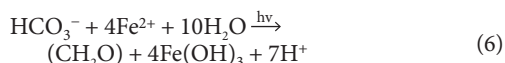
The rate of ferrous iron (Fe(II)) oxidation by oxygen (O<sub>2</sub>) strongly depends on pH, salinity, temperature, pressure and oxygen concentration<sup>27</sup>. In simplified rate expressions, the acceleration of O<sub>2</sub>-dependent oxidation of Fe<sup>2+</sup> ions by the reaction product (Fe(III) oxyhydroxides) by surface catalysis is often neglected. Precipitated Fe(III) oxyhydroxides function as an adsorbent for dissolved Fe(II), which is then heterogeneously oxidized by oxygen, with the reductant and oxidant existing in different physical phases. When homogeneous or microbial Fe oxidation generates a ferric mineral surface, aqueous Fe(II) can sorb and undergo subsequent heterogeneous Fe(II) oxidation. This means that the term that describes the heterogeneous reaction in a mathematical rate equation increases exponentially while Fe(II) and O<sub>2</sub> remain in solution. The reaction rates of heterogeneous and microbial Fe(II) oxidation are significantly higher than that of homogeneous Fe(II) oxidation<sup>6</sup>. As a consequence, the biogenic mineral product is a substrate competitor for microbial Fe(II) oxidation<sup>6</sup>. In order to evaluate the contribution of biotic reactions to total Fe(II) oxidation, Schmidt *et al.*<sup>145</sup> established an approach in which Fe(II) oxidation rates were calculated for either heterogeneous or homogeneous Fe(II) oxidation as a function of the ambient O<sub>2</sub> and dissolved Fe(II) concentrations as well as Fe(III) mineral formation over time. Although such an approach accounts for the co-occurrence of homogeneous and heterogeneous oxidation, it does not take into account that natural Fe(III) oxyhydroxides often contain impurities, such as organic matter and silicon, that affect the surface activity and subsequently the reaction rates<sup>146</sup>. In addition, a limitation of any kinetic Fe(II) oxidation model is the uncertainty in the role of organic ligands (including exopolysaccharides (EPSs)) that change the number of surface sites and strongly affect the sorption capacity of the mineral<sup>145</sup>. Microbial reaction kinetics depend on the enzymatic activity of the respective microorganisms and are described by Monod or Michaelis–Menten kinetics. Microbial Fe(II) oxidation rate constants need to be experimentally determined for each specific strain under well-defined geochemical conditions. However, abiotic and biotic Fe(II) oxidation are difficult to distinguish, as even under optimal conditions for microaerophilic Fe(II)-oxidizing bacteria, abiotic heterogeneous Fe oxidation will proceed and falsify microbial oxidation rates.

and *Mariprofundus* species purposefully direct Fe(II) oxidation to secreted, extracellular organic structures that form twisted stalks. Wetland Fe(II)- and Mn(II)-oxidizing *Leptothrix* spp. and *Sphaerotilus* spp. generate mineralized organic sheaths<sup>19</sup> to avoid the encrustation of the cell by Fe mineral precipitates. At least in some of these strains (such as *M. ferrooxydans* strain PV-1 and the Gallionellales strain R-1), the cell surfaces are hydrophilic and have a near-neutral surface charge that prevents encrustation<sup>31</sup>, whereas mineral precipitation on negatively charged organic stalks leads to characteristic structures that can be used as biosignatures<sup>32</sup>. The minerals formed by microaerophilic Fe(II) oxidizers include two-line ferrihydrite, lepidocrocite<sup>32</sup> and akaganeite<sup>33</sup>. Recent work with marine microbial mats suggests that the presence of C and/or silicon (Si) during precipitation inhibits mineral polymerization and results in phases with lower structural order than two-line ferrihydrite<sup>34</sup>.

**Fe redox reactions by photochemical processes**

Light can penetrate approximately 5–6 mm in sandy sediments, depending on the grain size<sup>35</sup>, and penetrate up to a depth of 100 m through a water column<sup>36</sup>. The light can function as an energy source for microorganisms and, at the same time, can initiate a series of abiotic reactions that lead to Fe redox transformations.

**Microbial phototrophic Fe(II) oxidation.** Photoautotrophic Fe(II)-oxidizing microorganisms that live in near-surface environments require light energy, bicarbonate as the electron acceptor and a C source and Fe(II) as the electron donor, according to the following stoichiometric equation<sup>3</sup> (FIG. 1):



It has been suggested that photoferrotrophs were a major contributor to the deposition of Precambrian banded iron formations, which are the largest Fe deposits present on Earth today<sup>3,36,37</sup>.

Photoferrotrophs can oxidize Fe(II) using light energy and produce poorly crystalline ferric oxyhydroxides, which mature into goethite or lepidocrocite<sup>38,39</sup>. This metabolic group includes, among other species, the green sulphur bacterium *Chlorobium ferrooxidans*<sup>40</sup>, the purple sulphur bacterium *Thiodictyon* sp.<sup>41</sup> and the purple non-sulphur bacteria *Rhodobacter ferrooxidans*<sup>42</sup> and *R. palustris*<sup>43</sup>. Although they are common in many aquatic environments<sup>23,37</sup>, the ecological role of these phototrophic Fe(II) oxidizers in environmental Fe redox cycling and their quantitative contribution to Fe cycling are mostly unknown.

Genes encoding proteins that catalyse phototrophic Fe(II) oxidation have been identified in *R. palustris* strain TIE-1 and *R. ferrooxidans* SW2 (REFS 15,44). *R. palustris* TIE-1 requires a three-gene-containing operon (*pioABC*) for phototrophic Fe(II) oxidation<sup>15</sup>: *PioA* is a periplasmic decahaem c-type cytochrome, *PioB* is an outer membrane porin and *PioC* is a periplasmic high-potential Fe–S protein. *PioA* and *PioC* are likely to be involved in electron transfer from Fe(II) to the cytoplasmic electron transport chain, whereas *PioB* might function in Fe(II) transport into, or Fe(III) transport out of, the periplasm. In *R. ferrooxidans* SW2, the operon *foxEYZ* was found to stimulate light-dependent Fe oxidation<sup>44</sup>. *FoxE* is predicted to be a dihaem cytochrome c and to function as an Fe oxidoreductase<sup>45</sup>, *FoxY* is predicted to function as a pyrroloquinoline quinone-binding protein and *FoxZ* is predicted to function as an inner membrane transport protein. Although the three-gene-containing *pioABC* operon of the TIE-1 strain and the *foxEYZ* operon of the SW2 strain function in phototrophic Fe(II) oxidation, they are not homologues. Even so, an obligate photoferrotroph has not yet been found.

**Homogeneous Fe(II) oxidation**

A chemical reaction in which both ferrous iron (Fe(II)) and the oxidant are in the same physical phase; for example, dissolved.

**Two-line ferrihydrite**

A nano-scale ferric iron (Fe(III)) oxyhydroxide mineral with an average primary crystallite size of 2–3 nm and a formula of Fe<sub>10</sub>O<sub>14</sub>(OH)<sub>2</sub>. Two-line refers to the two diffraction signals observed by X-ray diffraction.

**Lepidocrocite**

An orange-coloured FeOOH polymorph (γ-FeOOH); it is a ferric iron oxyhydroxide mineral.

**Akaganeite**

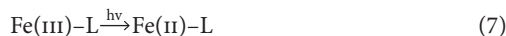
A FeOOH polymorph (β-FeOOH); it is a ferric iron oxyhydroxide mineral that is yellowish-brown in color and typically occurs in saline environments.

**Goethite**

An FeOOH polymorph (α-FeOOH); it is a ferric iron oxyhydroxide mineral. It is yellow to dark brown depending on the crystal size.

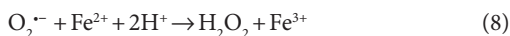
**Photochemical Fe(III) reduction.** Abiotic photochemical reactions have a large influence on the biogeochemical Fe cycle in sunlit aquatic environments<sup>46,47</sup>. The photo-induced reduction of Fe(III) minerals and colloids has been linked to the increase and stabilization of Fe(II) concentrations in natural surface waters<sup>48</sup>. Most Fe(II) in the photic zone originates from photochemical reactions and constitutes a substantial fraction of the total dissolved Fe in surface waters<sup>49</sup>.

Modelling and Fe speciation analyses have predicted that only 0.03% of the total Fe pool in seawater exists as purely hydrolysed Fe, whereas more than 99% of dissolved Fe in seawater is complexed to organic ligands of biological origin, such as siderophores<sup>50</sup>. Ligand (L) to metal charge transfer occurs in metal–ligand complexes when the ligand absorbs a photon, and consequently, an electron is excited to a higher energy state that overlaps with empty orbitals in the metal<sup>51</sup>. The electron is subsequently transferred from the ligand orbital to the oxidized Fe (FIG. 1):

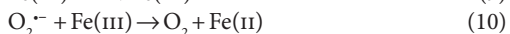


Oxidation or loss of functional groups in the ligand molecule can weaken the bond with Fe. Importantly, for natural environments, photoreduction of Fe(III) L complexes also occurs when bacterially produced siderophores are the ligand<sup>52</sup>, which highlights the interplay between abiotic and microbial reactions. Ultraviolet light drives photoreduction of Fe(III) L complexes<sup>52</sup> and penetrates deeper into the water column than visible light. Therefore, ligand to metal charge transfer may be as important to Fe(II) production as photoreduction by superoxide ( $\text{O}_2^{\cdot-}$ )<sup>52</sup> (see below), which is promoted at longer wavelengths and restricted to shallow waters<sup>48</sup>.

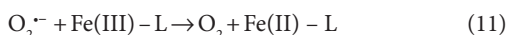
**$\text{O}_2^{\cdot-}$ -mediated Fe redox reactions.**  $\text{O}_2^{\cdot-}$  is formed abiotically in the environment via photochemical reactions of  $\text{O}_2$  with natural organic matter (NOM) in the photic zone<sup>53</sup> or via the reduction of  $\text{O}_2$  by  $\text{Fe}^{2+}$  (see equation 2). In addition, extracellular  $\text{O}_2^{\cdot-}$  production has been documented for phytoplankton<sup>54</sup>, fungi<sup>55</sup>, heterotrophic bacteria<sup>56</sup> and plants<sup>57</sup>. The biological production of  $\text{O}_2^{\cdot-}$  in aquatic systems<sup>56</sup> suggests that  $\text{O}_2^{\cdot-}$ -mediated Fe cycling may not be restricted to the photic zone. Fe functions as a sink for  $\text{O}_2^{\cdot-}$ -radicals, via both abiotic oxidation and reduction.  $\text{O}_2^{\cdot-}$  can be reduced by inorganic Fe(II):



or oxidized by inorganic Fe(III) after dissociation from its ligands:



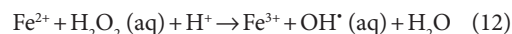
or it is oxidized directly by complexed Fe(III):



However, the rate of Fe(III) reduction with  $\text{O}_2^{\cdot-}$  is faster than that of Fe(II) oxidation by  $\text{O}_2^{\cdot-}$  or  $\text{O}_2$  (REF. 58), and therefore  $\text{O}_2^{\cdot-}$ -mediated Fe(III) reduction is dominant in marine environments<sup>48</sup>. It is unlikely that  $\text{O}_2^{\cdot-}$  is

a major contributor to the oxidation of complexed Fe(II) in marine waters<sup>59</sup>, and  $\text{O}_2$  and  $\text{H}_2\text{O}_2$  probably have a much larger role.

**Fe(II) oxidation by  $\text{H}_2\text{O}_2$ : the Fenton reaction.**  $\text{H}_2\text{O}_2$  is formed by the abiotic photochemical oxidation of NOM, via comproportionation of two hydroperoxyl radicals ( $\text{HOO}^{\cdot}$ ) or two  $\text{O}_2^{\cdot-}$  radicals or via the reaction of Fe(II) or Fe(II) bound to a ligand with  $\text{O}_2^{\cdot-}$  (see equation 8) (REF. 60).  $\text{H}_2\text{O}_2$  oxidizes Fe(II) via the Fenton reaction, which produces hydroxyl radicals ( $\text{OH}^{\cdot}$ ) by  $\text{H}_2\text{O}_2$  disproportionation<sup>61</sup>:



The reaction proceeds faster with NOM as a ligand for Fe(II)<sup>62</sup>, and the radical yield is dependent on the functional groups and the structure of the NOM<sup>63</sup>. The oxidation of Fe(II) by nanomolar concentrations of  $\text{H}_2\text{O}_2$  (see equation 4) is faster than by micromolar concentrations of  $\text{O}_2$  (REF. 59) (see equation 2). However, Fe reactions via photo-redox cycling are most likely to be a minor pathway for  $\text{O}_2^{\cdot-}$  and  $\text{H}_2\text{O}_2$  production in surface waters, although it could have an important role at oxic–anoxic interfaces<sup>28</sup>. Production of  $\text{H}_2\text{O}_2$  is not restricted to the photic zone<sup>64</sup>, and biotic and abiotic dark reactions between Fe and  $\text{H}_2\text{O}_2$  or  $\text{O}_2^{\cdot-}$  are an emerging research theme.

#### Integrating abiotic and microbial Fe(II) photooxidation.

There are several areas of potential overlap between the photochemical and the microbially mediated reactions in the biogeochemical Fe cycle. Although photochemically driven Fe reduction and photochemically produced  $\text{H}_2\text{O}_2$  that initiates Fe oxidation are primarily driven by ultraviolet light, visible light can also contribute to these processes. By contrast, anoxygenic photosynthetic Fe(II) oxidation is restricted by the penetration depths of visible light and  $\text{O}_2$  into the sediment or water column. Fe(II) for photosynthesis primarily originates from diffusion from underlying sediments, but anoxygenic photosynthetic Fe(II) oxidation could also use photochemically produced Fe(II). Photosynthetic pigments such as chlorophyll and bacteriochlorophyll, which absorb visible light, could also mediate photochemical Fe reduction if they are present in the environment. This implicates cyanobacteria and plants in facilitating photochemical Fe reduction and  $\text{H}_2\text{O}_2$  production by contributing to the organic ligand pool. Although light absorption by organic compounds could also lead to decreased light penetration through to the anoxic photic zone, photochemically degraded organic ligands can be used as electron donors by photoreducers instead of Fe<sup>65</sup>, as they display a lot of metabolic flexibility<sup>42,66</sup>. Phytoplankton produce  $\text{O}_2$ , which drives Fenton-type reactions, and many phytoplankton indiscriminately produce  $\text{O}_2^{\cdot-}$  radicals<sup>56,57,67</sup>, which can chemically reduce Fe(II)<sup>58</sup>. As a consequence, the interplay between the phototrophic Fe-metabolizing microorganisms and the abiotic photochemical reactions could involve the entire aquatic community within the photic zone.

#### Colloids

Particles that are dispersed in a liquid or gas within a size fraction ranging from 1 nm to 1  $\mu\text{m}$  in diameter.

#### Siderophores

Microbially produced organic molecules that are excreted in order to complex ferric iron (Fe(III)) ions, so the Fe can be taken up into the cells in a dissolved phase.

#### Comproportionation

A chemical reaction in which two reactants of the same element with a different oxidation state react to create a product with a single oxidation state.

#### Disproportionation

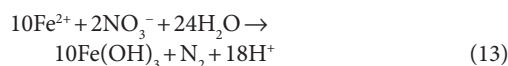
A chemical reaction in which a reactant is split into two species of the same chemical element with different oxidation states: one more oxidized and the other more reduced.

**Fe(II) oxidation by N species**

Fe has a role in the chemically and microbially mediated redox transformations of N species, including the production of the greenhouse gas N<sub>2</sub>O. Therefore, a thorough understanding of the possible redox reactions between reactive N species and Fe will contribute to a better understanding of the link between the Fe and N cycles in natural environments.

**Microbially mediated NO<sub>3</sub><sup>-</sup>-reducing Fe(II) oxidation.**

The ability to oxidize Fe(II) is common in known NO<sub>3</sub><sup>-</sup>-reducing Proteobacteria<sup>4,68</sup>. Mixotrophic NO<sub>3</sub><sup>-</sup>-dependent Fe(II) oxidation is restricted to anoxic conditions and may have a key role in the microbially mediated oxidation of Fe(II) to Fe(III) in the upper few millimetres of sedimentary or terrestrial environments<sup>69</sup>. The microbial reaction between Fe(II) and NO<sub>3</sub><sup>-</sup> adheres to the following stoichiometry (FIG. 1):

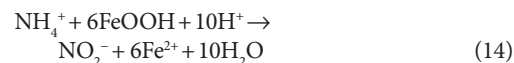


Most NO<sub>3</sub><sup>-</sup>-reducing Fe(II)-oxidizing bacteria require an organic co-substrate such as acetate to continually oxidize Fe(II) to Fe(III)<sup>70</sup>, with the exception of the purely lithotrophic mixed culture KS<sup>25</sup> and, potentially, *Pseudogulbenkiania* sp. strain 2002 (REF. 71). Most NO<sub>3</sub><sup>-</sup>-reducing Fe(II) oxidizers can also reduce NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> and further denitrification intermediates and products, including the gaseous species NO, N<sub>2</sub>O and N<sub>2</sub>, using an organic electron donor<sup>4,70,72</sup>. As NO<sub>2</sub><sup>-</sup> is an intermediate of denitrification (see below) and also a potent chemical oxidant for Fe(II)<sup>73</sup>, it is possible that the observed NO<sub>3</sub><sup>-</sup>-dependent Fe(II) oxidation is, at least to some extent, a by-product of microbial denitrification, whereby the produced NO<sub>2</sub><sup>-</sup> chemically oxidizes Fe(II)<sup>8</sup>. Genetic, transcriptional and physiological studies on the NO<sub>3</sub><sup>-</sup>-reducing Fe(II)-oxidizing bacterium *Thiobacillus denitrificans* did not reveal any evidence for *c*-type cytochromes in the electron transfer from Fe(II) to NO<sub>3</sub><sup>-</sup>, nor did the observed link between Fe oxidation and NO<sub>3</sub><sup>-</sup> reduction result in energy conservation or growth of *T. denitrificans*<sup>74</sup>. These findings suggest that Fe(II) oxidation by *T. denitrificans* is a predominantly indirect abiotic process caused by the reactive NO<sub>2</sub><sup>-</sup> intermediate that is formed during denitrification by this strain. This contradicts a recently postulated general mechanism for NO<sub>3</sub><sup>-</sup>-dependent Fe(II) oxidation<sup>7</sup>, whereby electron transfer from Fe(II) to the quinone pool was suggested to be mediated by the cytochrome *bc<sub>1</sub>* complex. Thus, although cell growth of denitrifying populations is increased in the presence of Fe(II)<sup>70,75</sup>, and in some cases, Fe(II) oxidation was induced by precultivation in the presence of Fe(II)<sup>72</sup>, genetic evidence for the existence of an enzymatic pathway that couples Fe(II) oxidation to NO<sub>3</sub><sup>-</sup> reduction is still lacking<sup>8,68</sup>.

**NO<sub>3</sub><sup>-</sup>-dependent pyrite oxidation.** The S and Fe cycles are linked by microbially mediated NO<sub>3</sub><sup>-</sup> reduction coupled to ferrous sulphide (FeS) and pyrite (FeS<sub>2</sub>) oxidation<sup>76,77</sup>. However, it is unclear to what extent FeS<sub>2</sub> oxidation in these studies was due to abiotic oxidation

by microbially produced NO<sub>2</sub><sup>-</sup> during acidic extraction of the Fe species. Two reactions for FeS<sub>2</sub> oxidation coupled to denitrification have been proposed: denitrification to N<sub>2</sub> or to NO<sub>2</sub><sup>-</sup> (REFS 78,79). An environmental isolate that has been shown to catalyse these reactions is *T. denitrificans* and, moreover, members of the *Acidovorax* and *Geothrix* genera, as well as a Marinobacter-related isolate, have also been suggested to couple FeS<sub>2</sub> oxidation to NO<sub>3</sub><sup>-</sup> reduction<sup>24,76,80</sup>.

**Microbially mediated Fe-ammox.** A new metabolic pathway that has recently been suggested to be present in anoxic wetland soils links N and Fe cycles via Fe(III) reduction coupled to ammonium (NH<sub>4</sub><sup>+</sup>) oxidation<sup>81–83</sup> (FIG. 1), although the responsible microorganisms have not yet been identified:



The endproduct of this anaerobic Fe-ammox reaction can be NO<sub>2</sub><sup>-</sup> or N<sub>2</sub>. As N<sub>2</sub> is a gaseous N species, this reaction could lead to a substantial loss of N in environments that are rich in Fe(III) oxyhydroxides<sup>83,84</sup>.

**Chemical Fe(II) oxidation by NO<sub>2</sub><sup>-</sup>.** NO<sub>2</sub><sup>-</sup>, which is produced by denitrification, can oxidize Fe(II) abiotically in a process called chemodenitrification<sup>73</sup> (FIG. 1):



The abiotic reaction of dissolved Fe(II) with NO<sub>3</sub><sup>-</sup> is generally much slower than with NO<sub>2</sub><sup>-</sup>. However, NO<sub>3</sub><sup>-</sup> can oxidize Fe(II) in the presence of Cu<sup>2+</sup> ions that function as a catalyst<sup>85</sup>. Chemodenitrification is also stimulated in the presence of a crystalline Fe(III) oxyhydroxide or at cell surfaces, owing to heterogeneous surface catalysis in geochemical systems, which results in the production of N<sub>2</sub>O (REFS 8,73,85).

The stability of NO<sub>2</sub><sup>-</sup> in the environment depends on the prevailing geochemical conditions, including pH, reduced cation concentrations, such as Fe(II), and the presence of organic matter<sup>85</sup>. Fe(II) and NO<sub>3</sub><sup>-</sup> do not accumulate in the same redox zones and must diffuse towards each other; therefore, the oxidation of Fe(II) by NO<sub>2</sub><sup>-</sup> has an important role at the interface between anoxic and oxic redox zones<sup>86</sup>.

**The role of microbially produced reactive N species.**

Chemical and microbial reactions within the NO<sub>3</sub><sup>-</sup>-reducing redox zone are mainly mediated by the chemical oxidation of Fe by microbially produced reactive N species. Denitrifying and Fe-ammox-performing microorganisms produce such reactive N species as intermediates and endproducts during their metabolisms<sup>8,81</sup>, and NO<sub>2</sub><sup>-</sup> and NO are particularly powerful chemical oxidants for Fe(II)<sup>8,73</sup>. Further work is needed to distinguish chemical from putative biologically catalysed reactions in processes such as NO<sub>3</sub><sup>-</sup>-dependent Fe(II) oxidation.

**Fe(II) oxidation by MnO<sub>2</sub>**

Mn and Fe co-occur in many anoxic environments and are often studied in parallel, as each influences

**Mixotrophic**

A term used to describe microbial metabolism that uses an organic substrate as a carbon source and an inorganic compound as electron donor.

**Heterogeneous surface catalysis**

A reaction in which the catalyst that facilitates the reaction of liquids or gases is present in the solid state.

the speciation and reactivity of the other (for example, see REF. 87). Mn exists as reduced, dissolved Mn(II) or Mn(III), or in the form of Mn(IV) oxides<sup>88,89</sup>. Abiotic oxidation of Fe(II) by Mn oxides occurs via a surface-controlled non-enzymatic chemical reaction<sup>90</sup> (FIG. 1):



Reduction of Mn by Fe(II) has an important role in the formation of redox zones in stratified marine and freshwater water columns and porewaters<sup>89,90</sup>. Owing to its higher redox potential, the zone of Mn reduction

is generally spatially separated from Fe(II). However, Fe(II) oxidation by Mn(IV) may occur in micro-oxic and anoxic freshwater and marine sediments that are subjected to mechanical mixing and bioturbation<sup>87</sup>. Recent advances in the detection of Mn(III) in the environment have led to the inclusion of this species in sedimentary pore-water redox profiles<sup>89</sup>.

### Fe(III) reduction

Fe(III) can be reduced abiotically and by Fe(III)-reducing microorganisms. Fe(III) reduction couples the Fe, C and S cycles and occurs in almost all environments in nature.

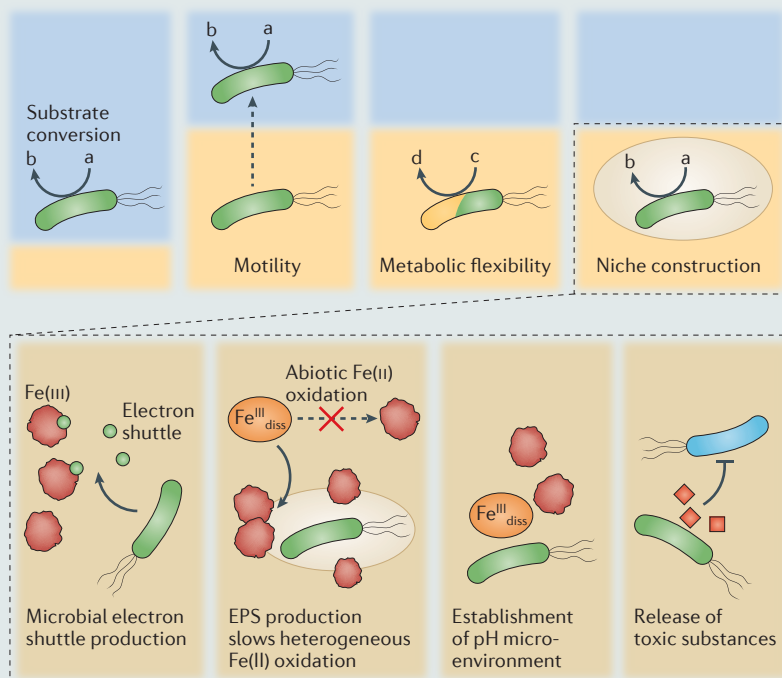
## Box 2 | Survival strategies under environmental fluctuations

The environmental distribution of microorganisms is controlled by their physiological requirements, substrate availability, tolerance towards changing physico-chemical conditions (for example, levels of oxygen and light) and their interplay with other members of the microbial community. The microbial and metabolic diversity at different spatial and temporal positions within an environment is therefore a function of the ability of the microorganisms to compete with each other for substrate and living space.

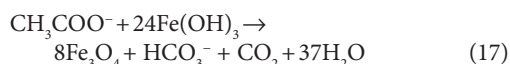
Microorganisms have evolved strategies to overcome social pressure within their community and to adapt to short- and long-term physico-chemical variations (including adaptation to diurnal and seasonal

variations in light and nutrients or substrates). These strategies include motility, starving, switching to alternative metabolisms and niche construction (see the figure, upper panel). The choice for the adequate survival strategy is based on the balance between minimum energy investment and maximum energy yield. It has been hypothesized that microorganisms could experience increasing social pressure and substrate competition from other community members owing to spatial shifting of the oxic–anoxic interface<sup>6</sup>. As an alternative to active displacement, microorganisms can switch their metabolism to improve their chances of survival in environments in which electron donors and electron acceptors rapidly change with environmental fluctuations<sup>9,66</sup>. With respect to the biogeochemical iron (Fe) cycle, examples of metabolic flexibility include the ability of purple non-sulphur bacteria to switch between photoferrotorophy and chemoheterotrophy<sup>43,65,66,147</sup>, and *Geobacter metallireducens* can alternate between ferric Fe (Fe(III)) reduction and nitrate (NO<sub>3</sub><sup>-</sup>)-dependent ferrous Fe (Fe(II)) oxidation<sup>9,105</sup>.

Microorganisms can also actively modify and optimize their immediate geochemical surroundings, such as by the mobilization of nutrients for uptake and increasing the accessibility of electron acceptors for energy generation (known as potential niche construction; see the figure, lower panel) to facilitate assimilation and dissimilation. Examples of this with respect to Fe biogeochemistry include the excretion of electron shuttles by Fe(III)-reducing microorganisms such as *Shewanella* sp. to overcome the low solubility of Fe minerals<sup>123,124</sup>, slowing down abiotic, heterogeneous Fe(II) oxidation rates by excretion of EPS (exopolysaccharide)<sup>30,68</sup>, the establishment of a pH microenvironment by photoferrotorophic organisms to alter the solubility of mineral precipitates in order to prevent cellular mineral encrustation<sup>148</sup> and the active control of oxygen levels in Fe(II)-oxidizing communities<sup>29,34</sup>. In addition, it is conceivable that Fe-metabolizing microorganisms release toxic compounds to kill or outcompete contending bacteria, similarly to other microorganisms<sup>149</sup>. Thus, microbial metabolisms and their specific survival strategies strongly affect the local geochemical composition, the metabolic diversity and the selective pressure within the community, which thus creates an ecological inheritance for successor organisms.



**Microbial Fe(III) reduction.** Many microorganisms can reduce Fe(III) using an assortment of electron donors, such as acetate, lactate and H<sub>2</sub>. The most notable examples include: *Geobacter* spp.<sup>2,91</sup>, *Shewanella* spp.<sup>92–94</sup>, *Albidoferax ferrireducens*<sup>95</sup>, *Geothrix fermentans*<sup>96</sup> (FIG. 1) and various hyperthermophilic archaea<sup>97–99</sup>. *Geobacter* spp. were among the first dissimilatory Fe(III) reducers shown to reduce Fe(III) minerals to the mixed valence mineral magnetite (Fe<sub>3</sub>O<sub>4</sub>) using short chain fatty acids<sup>100</sup>, monoaromatic compounds — such as toluene or benzene<sup>101,102</sup> — or hydrogen as the electron donor<sup>91</sup>.



Together with fermenting bacteria, *Geobacter* spp. completely mineralize organic C to CO<sub>2</sub> and are ubiquitously present in reducing environments<sup>91</sup>. They exhibit chemotactic behaviour towards Fe(II) (which is the product of Fe(III) mineral reduction) as a mechanism to locate the Fe(III) mineral source and can attach to mineral surfaces using pili<sup>103,104</sup>. In addition to Fe(III)

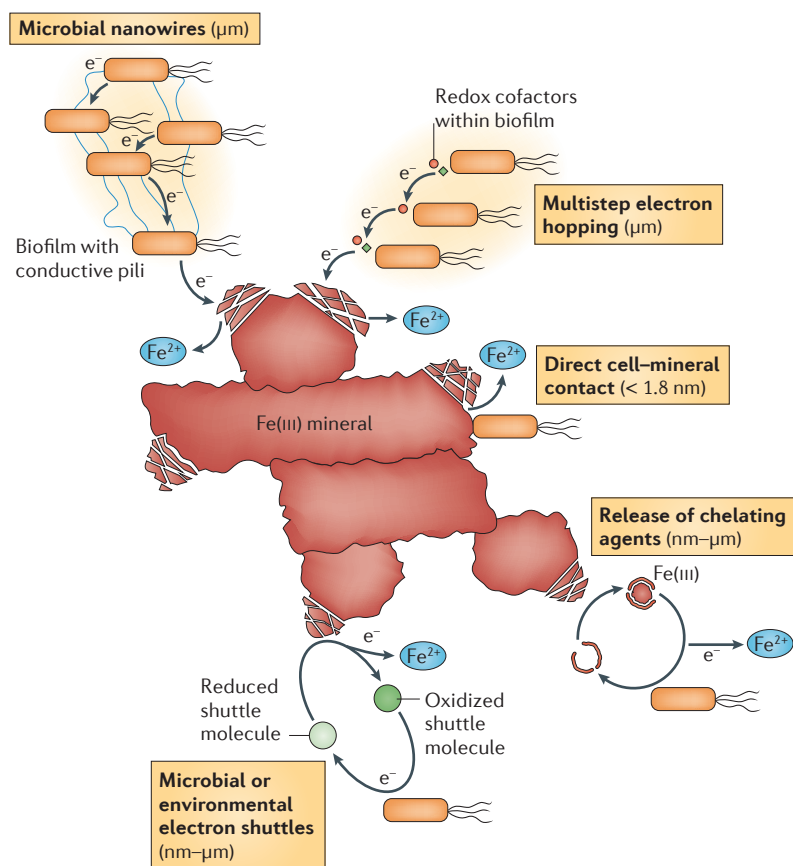
reduction, *Geobacter sulfurreducens* can also couple NO<sub>3</sub><sup>-</sup> reduction to ammonium with Fe(II) oxidation in the presence of acetate<sup>104,105</sup> (BOX 2).

Another well-studied group of Fe(III)-reducing bacteria are members of the Shewanellaceae family, in particular *S. oneidensis* strain MR-1, which was characterized in the 1990s<sup>94</sup> and can reduce Fe(III) with H<sup>+</sup>, formate or lactate (FIG. 1):



Genome sequence information from both *Shewanella* spp.<sup>106</sup> and *Geobacter* spp.<sup>104</sup> has aided the identification of the genes that are involved in Fe(III) reduction pathways. Electrons that originate from intracellular catabolism are transferred to cell surface-localized *c*-type cytochromes, which catalyse the extracellular electron transfer for the reduction of Fe(III) and Mn(IV) oxides<sup>107</sup>. In *S. oneidensis* MR-1, the outer membrane-associated decahaem cytochromes MtrC, MtrF and OmcA are thought to function in the binding to, and reduction of, Fe minerals<sup>107–111</sup>. The outer membrane cytochromes are connected to respiratory electrons of the intracellular quinone pool by outer membrane porin–cytochrome complexes, such as MtrA, MtrB and CymA<sup>112,113</sup>. Homologues of MtrA and MtrB may be phylogenetically conserved among several classes of the Proteobacteria, including the *Shewanella*, *Geobacter* and *Rhodopseudomonas* genera<sup>14–16</sup>. Many members of the genus *Geobacter* secrete extracellular cytochromes<sup>114</sup>; for example, the hexahaem OmcS in *G. sulfurreducens* has been reported to be associated with electrically conductive pili nanowires<sup>114,115</sup>, which mediate the conduction of current along the length of the wire or function as a contact point for mineral Fe(III) reduction<sup>116</sup>. In addition to cytochromes, *G. sulfurreducens* requires the outer membrane porin OmpJ for Fe reduction<sup>117</sup>. Although the currently described electron transport pathways in *S. oneidensis* and *G. sulfurreducens* contain similar components, they are considerably different<sup>118</sup>. Moreover, different *Geobacter* species seem to even have different complements of cytochromes<sup>119</sup>, which suggests that the necessary electron transport for Fe(III) reduction can occur via many different biochemical pathways.

Owing to the poor solubility of Fe(III) oxyhydroxides and the fact that the largest distance that an electron can ‘hop’ between cytochromes is 2.0 nm (REF. 120), explanations for microbial Fe(III) mineral reduction generally invoke strategies for extracellular electron transfer from the microorganism onto the Fe(III) mineral, other than those that require direct cell contact<sup>121</sup>. Several mechanisms have been proposed for the extracellular transfer of electrons from the microorganism to solid surfaces that can transfer electrons from micrometres and millimetres<sup>14,121</sup> up to centimetres in distance<sup>122</sup> (FIG. 3). In low-Fe(III) environments, *S. oneidensis* secretes redox-active electron shuttles (for example, flavins) that transport electrons between the cells and Fe(III) minerals<sup>123,124</sup>, or uses Fe(III) chelators<sup>125</sup>, thereby facilitating the use of Fe(III) as an electron acceptor (BOX 2). Redox-active pili (known as nanowires) have also been implicated in



**Figure 3 | Mechanisms of electron transfer from microorganisms to Fe(III) minerals.** Schematic representation of metabolic strategies by microbial Fe(III) reducers to reduce Fe(III) minerals. Direct contact between the bacterial cell and Fe(III) minerals facilitates Fe(III) reduction over short distances. Bacteria secrete chelating agents or exploit microbial or environmental redox-active electron shuttles (such as flavins or dissolved and solid-state humic substances, respectively) to facilitate electron transfer over short (nm) and long (µm) distances. Electrically conductive pili and multistep electron hopping via redox cofactors that are present in biofilms have been implicated in long-distance extracellular electron transfer.



**Humic substances**

(HumS). Organic molecules that are present in terrestrial and aquatic environments with a wide variety of structures that result from the degradation and polymerization of biopolymers, such as lignin, lipids, proteins, and polysaccharides. Diverse functional groups within the humic substance molecules are redox-active and have electron-donating or -accepting capacities.

**Humic and fulvic acids**

Humic acids are humic substances that are insoluble at low pH values, partially soluble at neutral pH and completely soluble at alkaline pH. Fulvic acids are humic substances that are soluble at all pH values.

**First order kinetics**

A term used to describe the kinetics of a reaction in which the concentration of one of the reactants is linearly related to the reaction rate.

extracellular electron transfer in *Shewanella* and *Geobacter* species<sup>126,127</sup>. Moreover, *Shewanella* and *Geobacter* species can transfer electrons to an Fe(III) mineral that is distal to the location of the cell<sup>128</sup> via a non-local electron transfer strategy<sup>129</sup> that involves redox-active molecules, such as S compounds that are present in some anoxic environments<sup>130</sup>, multistep electron hopping via redox-active cofactors that are present in a biofilm<sup>131</sup>, or natural organic matter such as redox-active humic substances (HumS; see below). *Geobacter* species might also use Fe oxides as conductors for interspecies electron transfer<sup>132</sup>.

**Abiotic Fe(III) reduction by HumS electron shuttling.**

The microbial reduction rate of Fe(III) minerals can be stimulated by redox-active dissolved and solid-phase HumS<sup>133–135</sup>. The first part of the reaction involves microbially mediated electron donation to the HumS, which involves humic and fulvic acids, and the second part of the reaction is the abiotic electron donation from the reduced HumS to the Fe(III) mineral. The ability to reduce HumS is not constrained to metal-respiring organisms: many bacterial groups, including fermenting bacteria, methanogens, sulphate reducers and halorespirers, in diverse environments, such as lake and marine sediments and pristine and contaminated wetland sediments, were shown to be able to transfer electrons to HumS<sup>133,136–138</sup>. Therefore, the abiotic reduction of Fe(III) with HumS as electron shuttles could be a widespread

and important pathway that could potentially extend biogeochemical Fe redox transformations to microorganisms that do not have the enzymatic machinery to directly reduce Fe(III)<sup>139</sup>.

**Abiotic Fe(III) reduction by S species.** At neutral pH, hydrogen sulphide (H<sub>2</sub>S) can abiotically reduce Fe(III) oxyhydroxides<sup>140,141</sup> (FIG. 1):



The reduction rate is dependent on the mineral surface area, as well as on the pH, and displays first order kinetics with respect to the H<sub>2</sub>S and Fe(III) oxide concentrations<sup>141</sup>. H<sub>2</sub>S reactions with Fe are especially important in marine environments, where high sulphate concentrations and microbial S reduction lead to pronounced H<sub>2</sub>S production. These reactions are important as the volatile, gaseous H<sub>2</sub>S species is precipitated in this reaction as S<sup>0</sup>, so to a certain extent, Fe minerals control the distribution of toxic H<sub>2</sub>S by preventing its release from the sediments into the overlying waters<sup>88,140</sup>.

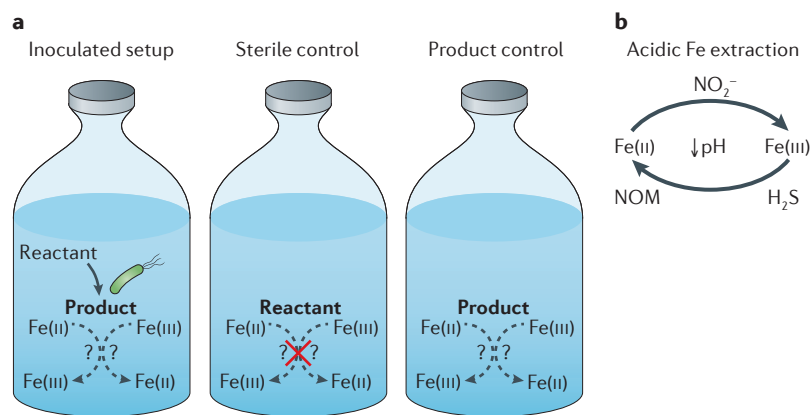
**Future directions**

The reactivity of Fe and its reaction network with many other biogeochemically important elements challenges scientists to separate biotic from abiotic processes. New techniques will enable the detection of extremely low concentrations of highly reactive species such as O<sub>2</sub><sup>-</sup>, which will enable scientists to investigate the mechanisms of Fe redox transformations in both laboratory and complex environmental systems.

Future experimental designs should emphasize distinguishing the relative contributions of abiotic and enzymatically catalysed reactions. Although the contribution of biotic processes can be determined in traditional (that is, sterile) control experiments, the potential involvement of an abiotic process that is induced by a microbially mediated reaction cannot be addressed (for example, the biotic formation of H<sub>2</sub>S or NO<sub>2</sub><sup>-</sup>, followed by the abiotic reduction of Fe(III) by H<sub>2</sub>S or oxidation of Fe(II) by NO<sub>2</sub><sup>-</sup>) (FIG. 4). Adapting current standard experimental approaches by including further controls will not only facilitate the estimation of the contribution of both chemical and microbial reactions but will also provide insights into their interactions.

Another challenge in studying Fe redox processes is the susceptibility of Fe(II) to oxidation. Anoxic extractions can prevent Fe(II) oxidation by O<sub>2</sub> at strongly acidic pH<sup>142</sup>. However, components within the samples, such as NO<sub>2</sub><sup>-</sup>, can also function as potent Fe(II)-oxidizing agents at circumneutral pH, and even more so at acidic pH<sup>8</sup>. Fe(II)-oxidation artefacts during acidic extraction in the presence of NO<sub>2</sub><sup>-</sup> could be prevented by using sulphamic acid instead of hydrochloric acid to acidify samples and stabilize Fe(II) for analysis<sup>8</sup>.

Techniques that hold promise for distinguishing abiotic reactions from enzymatic reactions include characterizing the stable isotope fractionation of non-Fe substrates — for example, N isotopic fractionation in NO<sub>3</sub><sup>-</sup>-dependent Fe(II) oxidation. Ongoing investigations of the genetic underpinnings of electron transfer



**Figure 4 | Designing the proper controls and recognizing pitfalls during acidification for Fe-extraction procedures.** **a** | Microbially mediated transformation of reactants into a product (for example, sulphate to sulphide or nitrate to nitrite), which can initiate the redox transformation of iron (Fe) (left-hand panel). From this experiment, it will not be clear whether the Fe redox transformation was microbially or chemically induced. A typical sterile control contains the reactant and Fe but lacks bacteria and thus the product (sulphide or nitrite) (centre panel). The absence of a redox transformation of Fe could lead to the incorrect conclusion that the Fe redox reaction is microbially mediated. To address the possibility that the microbially generated product could be used in a chemical reaction, an additional sterile control should be added (right-hand panel). In this case, the bacteria are omitted, but instead of the reactant, the product (sulphide or nitrite) and Fe (ferric Fe (Fe(III)) or ferrous Fe (Fe(II))) are added to the setup. If the Fe redox transformation is microbially mediated, no Fe redox reaction will occur; however, if the Fe redox transformation is driven by an abiotic process, a reaction will take place. **b** | When samples are acidified (for example, for Fe extractions) chemical Fe(II) oxidation by nitrite or Fe(III) reduction by reduced sulphur (S) species or natural organic matter (NOM) can be facilitated. In particular, the solubilization of Fe(III) oxyhydroxides at acidic pH can enable electron transfer from reduced compounds to the aqueous Fe(III) that has a much more positive redox potential.

mechanisms to and from metal oxides by genetic and emerging 'omic' techniques<sup>143,144</sup> will be crucial to distinguish active microbial catalysis from purely chemical reactions in complex environments.

Finally, small environmental fluctuations can shift the balance between a reaction being microbially or chemically mediated, as well as between Fe oxidation or reduction processes (BOX 2). Further work is required to gain a deeper understanding of the dynamic balance between

abiotic and biotic Fe redox processes, and this will reveal how microorganisms adapt to changing geochemical conditions and adopt alternative metabolic strategies for survival. Exploring the relationship between geochemical fluctuations and the shift in Fe-metabolizing communities in more detail will provide a more comprehensive understanding of local ecological structures and will also provide insights into the metabolic flexibility and survival strategies of Fe-metabolizing bacteria.

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### Competing interests statement

The authors declare no competing interests.