Microbial iron-redox cycling in subsurface environments

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Abstract

In addition to its central role in mediating electron-transfer reactions within all living cells, iron undergoes extracellular redox transformations linked to microbial energy generation through utilization of Fe(II) as a source of chemical energy or Fe(III) as an electron acceptor for anaerobic respiration. These processes permit microbial populations and communities to engage in cyclic coupled iron oxidation and reduction within redox transition zones in subsurface environments. In the present paper, I review and synthesize a few case studies of iron-redox cycling in subsurface environments, highlighting key biochemical aspects of the extracellular iron-redox metabolisms involved. Of specific interest are the coupling of iron oxidation and reduction in field and experimental systems that model redox gradients and fluctuations in the subsurface, and novel pathways and organisms involved in the redox cycling of insoluble iron-bearing minerals. These findings set the stage for rapid expansion in our knowledge of the range of extracellular electron-transfer mechanisms utilized by subsurface micro-organisms. The observation that closely coupled oxidation and reduction of iron can take place under conditions common to the subsurface motivates this expansion in pursuit of molecular tools for studying iron-redox cycling communities *in situ*.

Introduction

The importance of iron in biochemistry hardly needs to be emphasized. As a prime example, we know from molecular phylogenetic analyses that haem-bearing cytochromes have played a central role in respiratory energy generation (i.e. electron transport) throughout the evolutionary history of all domains of life (Eukarya, Bacteria and Archaea) on Earth [1]. Of interest in the present brief review is the utilization of iron not for intracellular energy metabolism, but rather as source of extracellular chemical energy (reductant) or electron acceptor (oxidant) for micro-organisms (specifically bacteria) that dwell beneath the Earth's surface, i.e. in subsurface environments. As we shall see, prevailing models for these modes of extracellular iron-redox metabolism themselves rely on the activity of haem-containing cytochromes, making this an iron-on-iron story with obvious implications toward the suspected role of primordial metalloenzymes in the origin of the energy-generating machinery of life [2].

In the simplest of terms, subsurface extracellular iron metabolism is linked to either (Figure 1a): (i) utilization of energy from the oxidation of ferrous iron [Fe(II)], a form of 'chemolithotrophic' metabolism that drives (wholly or in part) cell biosynthesis; or (ii) generation of energy through anaerobic oxidation of organic compounds (or molecular hydrogen) coupled to the reduction of ferric iron [Fe(III)], a form of 'chemo-organotrophic' metabolism. The interested reader may consult one of several recent reviews for details on the various pathways and metabolic modes involved in the extracellular redox transformation of iron [3–6]. A crucial aspect of these metabolisms is that they provide microbial populations and communities with an opportunity to engage in cyclic coupled iron oxidation and reduction at various spatial and temporal scales in Nature. From an ecological perspective, cyclic extracellular iron oxidation and reduction can be viewed as a form of metabolic syntrophy. Although microbial metabolic syntrophy is typically thought of in terms of the utilization of organic compounds [7], the linkage of chemolithotrophic and chemo-organotrophic metabolism through extracellular iron-redox cycling is an excellent example of ecosystem-level 'biochemical co-operation', akin to that known for other redox-active elements such as sulfur and nitrogen [8,9]. Although beyond the scope of the present paper, it is noteworthy that extracellular microbial iron-redox metabolism contributes significantly to sediment carbon and energy flow, and exerts a broad range of effects on the behaviour of natural and contaminant organic and inorganic compounds [10–12].

Iron-redox cycling subsurface environments

Iron-redox cycling takes place across a wide range of subsurface and near-surface environments, potentially encompassing millimetre-to-decametre (or more) spatial scales. A common ingredient in all such environments is the presence of a redox transition zone (or boundary environment) where input of an oxidant such as oxygen or nitrate drives Fe(II) oxidation, thereby generating Fe(III) [e.g. in the form of Fe(III) oxyhydroxides at circumneutral pH] that can be utilized by Fe(III)-reducing organisms (Figure 1b). In most

Key words: iron, metabolism, microbe, redox cycling, subsurface environment.
Abbreviations used: FeOB, Fe(II)-oxidizing bacteria; FeRB, Fe(III)-reducing bacteria; OM, outer membrane.
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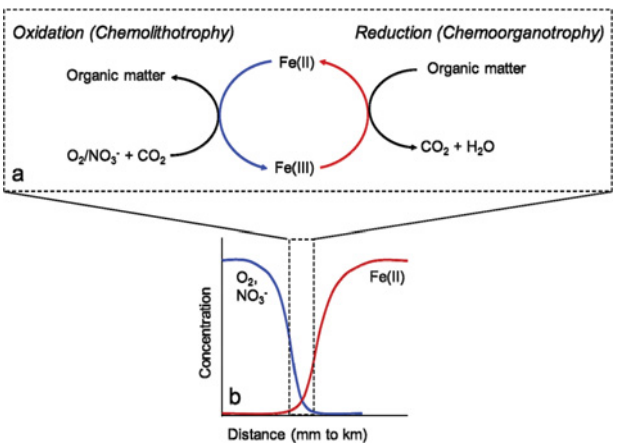
Figure 1 | **Microbial iron-redox cycling**

(**a**) Conceptual model for coupled microbial Fe(II) oxidation and Fe(III) reduction, a form of microbial metabolic syntrophy. (**b**) Diagrammatic depiction of a redox interfacial environment within which microbial iron-redox cycling can take place.



situations, the Fe(II) undergoing oxidation is of biological origin, i.e. it arises from microbial Fe(III) reduction. Even in situations (see below) where an external source of Fe(II) input is involved in the overall iron cycle, internal iron cycling processes are likely to occur. Metre-to-decametre-scale iron-redox cycling has been documented in a variety of stream and aquifer systems (e.g. [13–15]), where both natural and contaminant organic compounds provide the driving force for biogenic Fe(II) production that sets up the iron-redox cycle. An interesting decametre-to-centimetre-scale redox boundary was recently identified within the Hanford Formation in Eastern Washington [16], where both aqueous and solid-phase (e.g. clay minerals) Fe(II) phases are likely to be involved in iron-redox cycling. Centimetre-to-millimetre-scale redox cycling is well known in both acidic and neutral pH aquatic sediments [12], and, in a few instances, the potential role of FeOB [Fe(II)-oxidizing bacteria] and FeRB [Fe(III)-reducing bacteria] has been assessed directly (e.g. [17–19]). Groundwater iron-seep systems represent another type of centimetre-to-millimetre-scale near-surface environment where microbial iron-redox cycling has been documented extensively (e.g. [20–23]). Although not direct analogues to sedimentary environments, groundwater iron seeps [where Fe(II)-rich fluids emerge to the Earth's surface] provide a convenient natural laboratory for studies of microbial iron-redox cycling [24]. In particular, mat-like structures in groundwater seeps display redox gradients analogous to those present in myriad aquatic surface sediments and physically/chemically heterogeneous aquifer materials (e.g. [25,26]). Controlled laboratory incubation experiments have also provided valuable insight into the mechanisms and microbial populations that catalyse *in situ* extracellular redox cycling in natural redox transition environments [23,24,27–30]. Results from our work in iron seeps and laboratory studies of nitrate-driven iron-redox cycling are used below to address key biochemical aspects of how extracellular iron-redox metabolism may be manifested in subsurface environments. It should be noted that the contribution by Shi et al. [31] in this issue of *Biochemical Society Transactions* provides an additional perspective on what appears to be a common theme of multihaem cytochrome catalysis of extracellular iron oxidation and reduction.

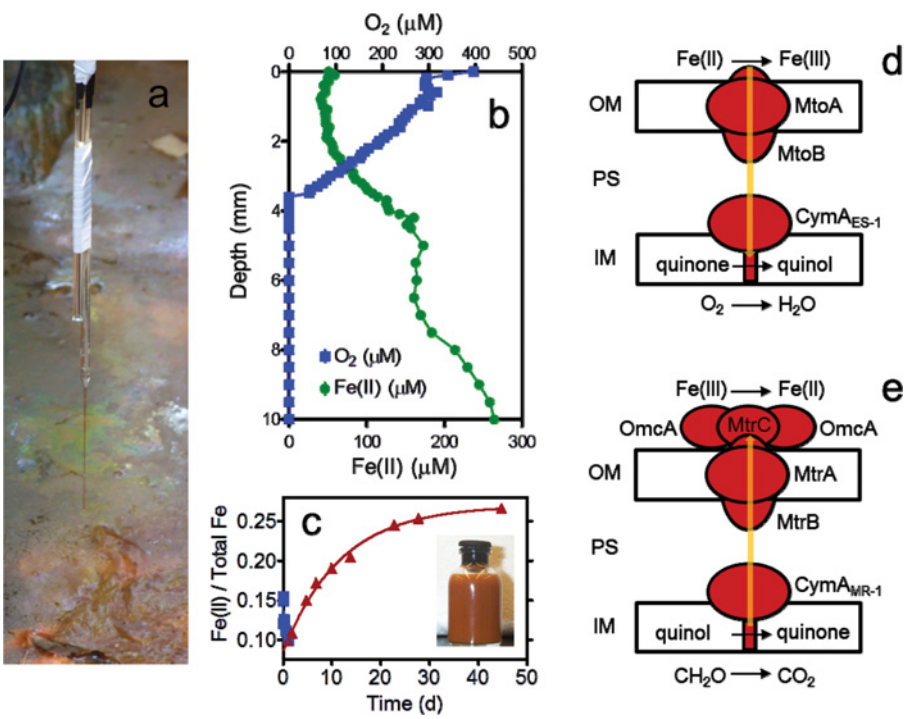
Extracellular iron-redox cycling at the aerobic/anaerobic interface

A considerable diversity of bacteria are capable of oxidizing Fe(II) with oxygen as the electron acceptor at either acidic or neutral pH [32]. Under acidic conditions, Fe(II) [as well as Fe(III)] is stable in solution and hence virtually all of the chemical energy from Fe(II) oxidation is available for enzymatic utilization. Note, however, that, even under conditions where Fe(II) and Fe(III) are stable in solution, Fe(II) oxidation by organisms such as *Acidithiobacillus* or *Leptospirillum* takes place at or near the cell surface rather than within the cytoplasm [33,34]. At circumneutral pH, the abiotic reaction of Fe(II) with oxygen is rapid [the half-life for dissolved Fe(II) in air-saturated solution is <5 min], and FeOB must therefore compete with abiotic oxidation. To cope with this problem, neutrophilic FeOB thrive under 'microaerophilic' conditions, where the dissolved oxygen concentration is considerably lower (typically $\leqslant$10%) than atmospheric saturation (see [32] for a review) (Figure 1b). Under these conditions, rates of abiotic Fe(II) reaction with oxygen are two to three orders of magnitude lower than under full oxygen saturation, whereas the amount of free energy available for the oxidation of Fe(II) [e.g. to amorphous Fe(III) oxyhydroxides] remains virtually unchanged [11]. Experimental studies have shown that FeOB can catalyse a large fraction of overall Fe(II) oxidation under microaerophilic conditions, up to 90% or more in diffusion-limited Fe(II)–O_2 opposing gradients [35]. The beauty of this process is that the amorphous Fe(III) oxides {and, possibly, aqueous or colloidal Fe(III) compounds [35]} generated during microbial Fe(II) oxidation are excellent substrates for dissimilatory FeRB which may thrive within and below (i.e. on the anoxic side of) the redox boundary. This phenomenon has been demonstrated experimentally with pure cultures [17], leading to the development of a conceptual model for microbial iron-redox cycling in which FeOB and FeRB can be juxtaposed at millimetre spatial scales [11].

It is difficult to conduct *in situ* process-based studies of coupled iron oxidation and reduction in complex soil and sedimentary environments. As mentioned above, groundwater iron seeps can provide insight into how iron-redox cycling may be manifested in such systems, as illustrated by recent studies in a groundwater seep in Indiana [24]. Voltammetric microelectrode profiles of dissolved oxygen and Fe(II) within the upper centimetre of a Fe(III) oxide mat (Figures 2a and 2b) provided *in situ* evidence for ongoing iron-redox cycling: the shape of the Fe(II) profile, where significant Fe(II) concentrations are maintained in the

Figure 2 | **Iron-redox cycling at the aerobic–anaerobic interface in a groundwater seep**

(**a**) Photo of voltammetric microelectrode deployment in the Bloomington, IN, U.S.A., groundwater seep microbial mat described in [24]. (**b**) Profiles of dissolved oxygen and Fe(II) in the upper 1 cm of the mat. (**c**) Fe(III) reduction in materials from the microbial mat; symbols show the average ratio of Fe(II) to total iron in 0.5 M HCl extracts from duplicate suspensions. (**d** and **e**) Proposed models for Fe(II) oxidation by *Sideroxydans lithotrophicus* ES-1 and *Shewanella putrefaciens* MR-1 respectively, based on the MtoAB/MtrAB and CymA *c*-type cytochrome system (see the text for details). Abbreviations: PS, periplasmic space; IM, inner membrane; CH_2O, generic unit of organic carbon. (**a**–**c**) Figure as originally published in Roden, E.E., McBeth, J.M., Blöthe, M., Percak-Dennett, E.M., Fleming, E.J., Holyoke, R.R., Luther, III, G.W., Emerson, D. and Schieber, J. (2012) The microbial ferrous wheel in a neutral pH groundwater seep. Front. Microbiol. **3**, 172. doi:10.3389/fmicb.2012.00172. (**d**) Previously published in: Liu, J., Wang, Z., Belchik, S.M., Edwards, M.J., Liu, C., Kennedy, D.W., Merkley, E.D., Lipton, M.S., Butt, J.N., Richardson, D.J., Zachara, J.M., Fredrickson, J.K., Rosso, K.M. and Shi, L. (2012) Identification and characterization of MtoA: a decaheme *c*-type cytochrome of the neutrophilic Fe(II)-oxidizing bacterium *Sideroxydans lithotrophicus* ES-1, Front. Microbiol., **3**, 37. (**e**) Reprinted with kind permission from Dr L. Shi. Reproduced with permission from Liang Shi, David J. Richardson, Zheming Wang, Sebastien N. Kerisit, Kevin M. Rosso, John M. Zachara and James K. Fredrickson, The roles of outer membrane cytochromes of *Shewanella* and *Geobacter* in extracellular electron transfer, Environmental Microbiology Reports: John Wiley and Sons, © 2009 Society for Applied Microbiology and Blackwell Publishing Ltd.



aerobic surface layer (0–5 mm), can only be explained by an internal source of Fe(II) from Fe(III) reduction. In support of this explanation, the mat materials were shown to contain significant numbers of culturable FeRB (comparable with the abundance of culturable FeOB), and 16S rRNA gene clone libraries revealed the presence of known FeRB related to *Rhodoferax ferrireducens* [36], together with characteristic FeOB from the *Gallionella*/*Sideroxydans* group. In addition, *in vitro* incubation experiments revealed the potential for immediate Fe(III) reduction upon isolation of the mat materials (Figure 2c), suggesting that FeRB were present and active in the mat. Fe(III) reduction was likely to be fuelled by decay of chemolithoautotrophic biomass as well as organic inputs from the surrounding terrestrial landscape (deciduous forest). Previous experimental and modelling studies in an analogous groundwater seep suggested that internal iron-redox cycling could significantly increase iron throughput, leading to the conclusion that microbial iron-redox cycling is likely to take place in virtually all redox interfacial environments [23], including those in complex soil and sedimentary environments where Fe(II) and oxygen may come into contact at various spatial and temporal scales.

What is the biochemical basis for the extracellular iron-redox cycling activity observed in the seep materials? The answer to this question must ultimately relate to the specific machinery used to carry out Fe(II) oxidation or Fe(III) reduction exterior to the cell surface. In the case of Fe(III) reduction, although the exact mechanism of Fe(III) reduction by *Rhodoferax* has not yet been elucidated, the genome for *R. ferrireducens* contains numerous multihaem *c*-type

cytochromes analogous to those known to be involved in Fe(III) reduction by well-known FeRB such as *Geobacter* and *Shewanella* [37]. One *c*-type cytochrome gene in *R. ferrireducens* (Rfer_0244) is a homologue of OmcE, an OM (outer membrane) *c*-type cytochrome that is essential for Fe(III) oxide reduction in *Geobacter sulfurreducens*. It therefore seems safe to assume that Fe(III) oxide reduction was catalysed (at least in part, the possible involvement of bacterial nanowires [38–41] notwithstanding) by OM *c*-type cytochromes, expressed by organisms such as *Rhodoferax* thriving in anoxic microenvironments in a manner analogous to that observed in FeOB/FeRB co-cultures [11,17]. The fact that FeRB such as *Shewanella* [42], *Geobacter* [43] and *Rhodoferax* [36] are capable of survival and growth under aerobic conditions is consistent with their ability to persist in redox interfacial environments where periodic exposure to oxygen may take place.

Until very recently, the mechanisms by which neutral-pH aerobic chemolithotrophic FeOB oxidize Fe(II) was unknown. However, findings for anoxygenic phototrophic FeOB suggested a likely role for OM *c*-type cytochromes in Fe(II) oxidation [44,45]. The PioAB system identified in *Rhodopseudomonas palustris* [45] appears to be analogous to the MtrAB system in *Shewanella oneidensis* strain MR-1, in which the decahaem *c*-type cytochrome MtrA traverses the OM (connecting to additional OM proteins MtrC and OmcA) through the β-barrel protein MtrB, thereby serving as a conduit for the transfer of electrons to the external environment [46,47]. A recent study by Shi and colleagues [48] identified homologues (dubbed MtoA and MtoB) to the MtrAB and PioAB systems in the neutral-pH FeOB *Sideroxydans lithotrophicus* strain ES-1, a chemolithoautotrophic organism capable of growth with Fe(II) as the sole energy source [49]. The oxidized form of purified MtoA (obtained by cloning and expression in *S. oneidensis* MR-1) was shown to have the capacity to oxidize various forms of aqueous Fe(II). In addition to MtoAB, the *S. lithotrophicus* ES-1 genome also contains a homologue (dubbed $CymA_{ES\text{-}1}$) of CymA in *S. oneidensis* MR-1, a tetrahaem *c*-type cytochrome which is known to participate in electron transfer from the quinone/quinol pool in the inner membrane to MtrA in the OM either directly or indirectly via other periplasmic proteins [47]. On the basis of these results, the authors came to the remarkable conclusion that the basic mechanism for Fe(II) oxidation in *S. lithotrophicus* is directly analogous to that for Fe(III) reduction in *S. oneidensis*, with the exception that the direction of MtoAB/$CymA_{ES\text{-}1}$-mediated electron transfer during Fe(II) oxidation is opposite to that of MtrABC/CymA-mediated reactions during Fe(III) reduction [47]! This proposition is consistent with the suggestion that MtrAB and analogous *c*-type cytochrome-based electron-transfer systems represent a fundamental biochemical 'module' for extracellular electron transport [46], which can either transfer electrons from low-E_h electron donors (e.g. organic compounds) within the cell to high-E_h electron acceptors outside of the cell [e.g. Fe(III) oxides], or from low-E_h electron donors [e.g. Fe(II)] outside the cell to high-E_h electron acceptors (e.g. O_2) inside the cell (Figures 2d and 2e). Studies with proteoliposomes showed that the MtrAB module is in fact able to move electrons either way across a lipid bilayer, depending on the orientation of oxidant and reductant relative to the bilayer [46]. Whether or not this basic module (or variations upon it) is conserved across diverse FeOB and FeRB taxa remains to be determined, but one can speculate that this general arrangement makes perfect sense, given that iron-redox transformations were likely to be involved in the assembly of primitive electron-transport systems in the last common ancestor of all living cells on Earth [1]: as primitive cells incorporated iron into their respiratory chains, why not co-opt analogous proteins for the purpose of generating energy from extracellular iron-redox metabolism? The concept of extracellular iron-redox metabolism as an early form of cellular metabolism on Earth is consistent with the fact that iron was abundant at or near the surface of the early Earth [50], and that both Fe(II)-oxidizing and Fe(III)-reducing organisms are present on the deepest branches of the 16S rRNA gene-based universal phylogenetic tree [27].

Nitrate-driven anaerobic iron-redox cycling

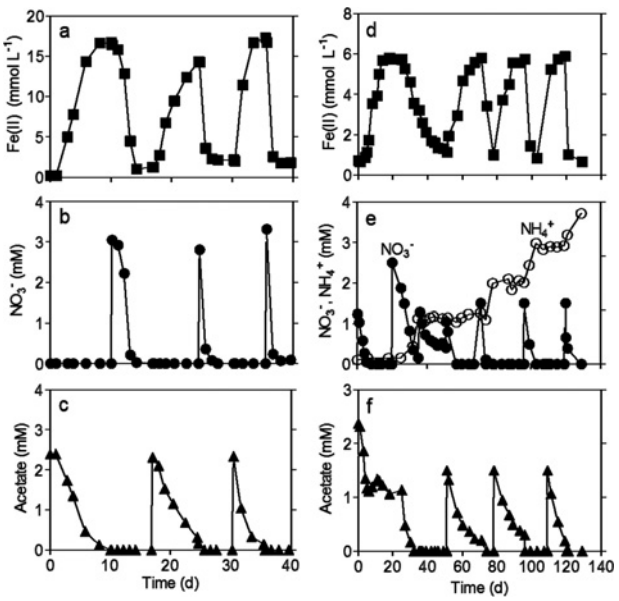
Oxidants capable of receiving electrons from Fe(II) under anoxic conditions include anoxygenic photosynthetic machinery, manganic [Mn(IV)] oxides, and oxidized forms of nitrogen (e.g. nitrate or nitrite) [51]. Photosynthesis is not a relevant process for the subsurface, and there are no known organisms that couple Fe(II) oxidation to Mn(IV) oxide reduction {note, however, that Mn(IV) oxides are potent abiotic oxidants for Fe(II), and may thus play a significant role in subsurface iron-redox cycling [52,53]}. In contrast, the potential for microbially catalysed nitrogen-driven Fe(II) oxidation and attendant iron-redox cycling in sediments is well recognized (see discussion and references in [29]). In general terms, anaerobic nitrogen-driven iron-redox cycling is analogous to cycling at the aerobic/anaerobic interface, where nitrate or other oxidized nitrogen forms replace oxygen as the oxidant. However, there are two important exceptions, the first being that nitrate does not react spontaneously with Fe(II) under normal Earth-surface conditions, with the result that nitrate-reducing FeOB do not need to compete with abiotic oxidation pathways. The second exception, discussed below, relates to the metabolic capacity of certain FeRB to enzymatically oxidize Fe(II) with nitrate, thus playing a role in both sides of the iron-redox cycle.

A wide variety of denitrifying bacteria have the capacity to oxidize Fe(II). In contrast with most aerobic FeOB, however, most of these organisms are not autotrophic; instead they function as 'mixotrophs', oxidizing Fe(II) (presumably) to gain energy while utilizing organic compounds for cell biosynthesis as well as energy generation (see [54] for a recent discussion of such organisms). To date, only three autotrophic Fe(II)-oxidizing nitrate-reducing cultures are known: the Archaeon *Ferroglobus placidus* [55], the Betaprotobacterium *Pseudogulbenkiania* sp. strain 2002 [56] and an enrichment culture reported by Straub and colleagues [57], which is

dominated by a putative chemolithoautotrophic organism related to the *Gallionella/Sideroxydans* group [28]. Several other organisms (including the known chemolithoautotroph *Thiobacillus denitrificans*) have been shown to carry out nitrate-dependent Fe(II) oxidation in the absence of added organic compounds [57], although sustained autotrophic growth of such organisms with Fe(II) as the energy source has not been demonstrated. To our knowledge, no solid evidence is yet available as to the molecular mechanism(s) of Fe(II) oxidation in either these or the various mixotrophic nitrate-reducing FeOB. However, in the case of the Straub culture [57], the close phylogenetic relationship between the *Gallionella/Sideroxydans* relative in the culture and organisms such as *S. lithotrophicus* ES-1 suggest that an oxidation mechanism analogous to that depicted in Figure 2(d) is possible, with nitrate replacing oxygen as the intracellular electron sink. The genome for *T. denitrificans* has numerous genes coding for *c*-type cytochromes, although none of these has direct homology with the MtrAB/MtoAB systems discussed above [58].

The ability of nitrate-reducing FeOB to engage in iron-redox cycling has been nicely demonstrated in co-culture experiments with *G. sulfurreducens* and the Straub culture [28] (Figures 3a–3c). The results suggested that the partnering of nitrate-reducing FeOB and dissimilatory FeRB represents a syntrophic interaction, as periodic removal of accumulated Fe(III) oxides [and regeneration of Fe(II) as an energy source] via Fe(III) reduction was shown to facilitate sustained Fe(II) oxidation activity. An analogous syntrophic interaction between nitrate-driven Fe(II) oxidation and dissimilatory Fe(III) oxide reduction has also been documented for natural freshwater sediment microbial communities [27,29] (Figures 3d–3f). An interesting outcome of the latter experiments was that the main reduced nitrogen end-product of nitrate-driven Fe(II) oxidation was ammonium (Figure 3e), in contrast with denitrification-linked pathways utilized by various autotrophic (including the Straub culture) and mixotrophic FeOB. The results were suggestive of possible Fe(II) oxidation by FeRB that are also capable of nitrate reduction. Experiments with *Geobacter metallireducens*, which reduces nitrate to ammonium during organotrophic growth [59] proved that this was possible [27]. Aklujkar et al. [60] speculated that the product of the *ppcF* gene in *G. metallireducens*, which is related to a periplasmic trihaem *c*-type cytochrome involved in Fe(III) reduction in *G. sulfurreducens* [61], may permit electron transfer to nitrate reductase from extracellular electron donors, i.e. in this case Fe(II). These results are consistent with the idea that FeRB were involved in nitrate-driven Fe(II) oxidation in the iron cycling experiments, with nitrogen mass balance calculations indicating that ~75% of Fe(II) oxidation was coupled to nitrate reduction to ammonium [29]. Taxa related to both known betaproteobacterial nitrate-reducing FeOB and *Geobacter* were dominant in the reactors, which suggests microbial populations specifically adapted to take advantage of the energy available proliferated during iron–nitrogen redox oscillations.

Figure 3 | **Nitrate-driven iron-redox cycling**
(**a**–**c**) Iron-redox cycling by a co-culture of *G. sulfurreducens* and the Fe(II)-oxidizing nitrate-reducing enrichment culture described in [57]. Results are means from triplicate cultures. Reproduced with permission from Applied and Environmental Microbiology, Nov. 2009, pp. 6937–6940 Vol. 75, No. 21, doi:10.1128/AEM.01742-09. © 2009, American Society for Microbiology. (**d**–**f**) Iron-redox cycling by natural freshwater sediment bacteria. Results are means from duplicate cultures. In both experiments, iron-redox cycling was induced by periodic addition of acetate to drive Fe(III) oxide reduction, followed by addition of nitrate to trigger Fe(II) oxidation. Reproduced with permission from Applied and Environmental Microbiology, Sept. 2011, pp. 6036–6042 Vol. 77, No. 17, doi:10.1128/AEM.00276-11. © 2011, American Society for Microbiology.



The central conclusion from the above experimental studies is that microbial iron cycling communities are likely to be present and active in anoxic soil and sedimentary environments experiencing shifts in organic carbon and nitrate input, analogous to those known to be present in aerobic–anaerobic interfacial environments. The fact that ammonium can be a major end-product of nitrate-dependent Fe(II) oxidation during anaerobic iron-redox cycling has important implications for the fate of nitrogen in sediments, as ammonium so produced may eventually be returned to surface waters where it could serve as a nutrient for algal and higher plant production. This is in contrast with the conversion of nitrate to N_2 which effectively represents a loss of nitrogen from the sedimentary nitrogen cycle [62].

Solid-state iron-redox cycling

Solid-phase Fe(II) compounds are generally abundant in sediments, and such phases have the potential to serve as a significant source of energy for lithotrophic microbial metabolism. The importance of solid-associated Fe(II) in iron-redox cycling was illustrated in the experiments depicted

in Figure 3, where aqueous Fe(II) accounted for less than 20% of total Fe(II) production and oxidation in the Fe(III)-reducing and iron cycling cultures (results not shown). In these experiments, Fe(II) sorbed or precipitated on to residual Fe(III) oxide phases was likely to be the dominant form of solid-phase Fe(II). X-ray diffraction analyses showed that, upon oxidation, the iron was converted back into the same insoluble Fe(III) oxide (goethite) from which it was originally derived. Other solid-phase Fe(II) compounds may also be subject to oxidation, thus triggering the potential for subsequent iron-redox cycling. For example, Bosch et al. [63] recently demonstrated the ability of *T. denitrificans* to oxidize the nanometre-sized grains of the iron sulfide mineral pyrite (FeS_2), which is abundant in many types of aquatic and subsurface environments. The potential for anaerobic microbial redox cycling of structural iron in phyllosilicate minerals was demonstrated experimentally in pure culture studies with *Desulfitobacterium frappieri* [64], as well as in recent experiments with novel FeOB isolated from subsurface sediments in Wisconsin [30] and Washington state (E.S. Shelobolina, J. Benzine, M.Y. Xiong, D.W. Kennedy, J.P. McKinley and E.E. Roden, unpublished work). These findings provide verification of the potential for nitrate-driven phyllosilicate–iron-redox cycling that had been inferred previously from *in situ* studies of iron–nitrogen geochemistry in clay-rich subsurface environments [65,66].

The concept of structural Fe(II) cycling in clays is intriguing given that, under normal soil/sediment conditions, clays remain virtually insoluble, such that cyclic oxidation and reduction represents a true solid-state iron-redox cycle [64]. Another potential form of microbially driven solid-state iron-redox cycling has recently been recognized, wherein lithotrophic oxidation of structural Fe(II) in the primary iron-silicate mineral biotite was demonstrated for the Straub culture [67], and the potential for organotrophic reduction of structural Fe(III) in biotite was demonstrated for *S. oneidensis* (D.R. Brookshaw, V.S. Coker, J. Lloyd, R.A.D. Pattrick and D.J. Vaughan, personal communication). The fundamentally extracellular nature of iron-redox metabolism in these organisms is entirely consistent with their apparent ability to utilize insoluble structural iron in clays and other phyllosilicate minerals for energy metabolism, i.e. through utilization of one or another configuration of the basic biochemical module for extracellular electron transport discussed above. The potential for enzymatically driven interconversion of iron in such phases has major implications for the behaviour of a wide variety of redox-sensitive organic and inorganic contaminants [68], as well as long-term weathering processes whereby iron and other mineral-associated nutrients (e.g. potassium) are released to surface and subsurface environments [30,67].

Conclusions

The purpose of the present brief review was to highlight basic aspects and recent discoveries related to the biochemical basis for extracellular iron-redox metabolism, specifically in the context of soil/sedimentary environments. A key realization emerging from the analysis is that our understanding of the redox behaviour and cycling of iron in natural environments, although by no means complete, is far ahead of our knowledge of the diversity and mechanistic details of extracellular iron-redox metabolism in the organisms responsible for mediating iron-redox transformations. Uncovering these details represents an exciting frontier in microbial physiology and biochemistry, one which will almost certainly reveal new aspects of environmental iron-redox cycling, and along the way facilitate development of molecular tools to study microbially driven iron cycling *in situ* [69].

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