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Ecosystem functioning from a geomicrobiological perspective – a conceptual framework for biogeochemical iron cycling

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Environmental context. Microbial ecosystems are characterised by the interplay of various microorganisms with their biotic and abiotic environment. Biogeochemical niches host adapted microbial communities that are in constant competition for substrates and nutrients. Their natural distribution, interactions and responses to fluctuating environmental conditions are often impossible to simulate in laboratory studies. Using biogeochemical iron redox cycling as an example, we suggest the application of a conceptual framework to improve our understanding of the principal functioning of (geo)microbial ecosystems.

Abstract. Our knowledge on how microbial ecosystems function profits from the support of biogeochemical concepts which describe the cycling of elements through various geochemical gradients. Using the example of the iron cycle in freshwater sediments, we propose a theoretical framework that describes the dynamic interactions between chemical and microbial Fe^{II} oxidation and Fe^{III} reduction, their spatial location and how they are affected by changing environmental conditions. This contribution emphasises the complexity ecological research faces when dealing with heterogeneous and dynamic natural systems. Our concept aims to provide further insights into how flows of energy and matter are controlled during microbial and chemical Fe redox transformations and how various key variables, such as substrate availability and competition as well as thermodynamic and kinetic parameters, affect flow directions.

Additional keywords: bioenergetics, biogeochemistry, micro-ecology.

Ecosystem functioning – the need for concepts

Essential questions in ecology are focused on how ecosystems are assembled and how they function as a whole or as the sum of interconnected sub-systems. A major goal is to understand how energy and matter flow through an ecosystem and how these fluxes are controlled by biotic and abiotic processes. The physicochemical conditions microorganisms are exposed to control their competitiveness and viability, and thereby their abundance and distribution (Fig. 1). Microorganisms compete, for example, for energy sources and nutrients, such that species found in a specific ecological niche can be considered to be well adapted to their environment. Species niches can also widen because of phenotypic plasticity, and can vary even purely behaviourally, as an immediate response to an altered resources or species structure. However, even slight environmental variations (e.g. electron donor and acceptor concentrations or intensity of light radiation) might shift the pattern of microbial activity and their abundance (Fig. 1).[1] The chances of survival of a particular microbial population is therefore increased by exhibiting a high metabolic flexibility, which includes the ability to use different electron donors and acceptors (e.g. facultative aerobes) or exploit various energy sources (i.e. phototrophic *v.* chemotrophic growth).

In order to increase the habitability of their living space, microorganisms can actively change their immediate environment through their own metabolic activity (e.g. exopolysaccharide production, local pH changes, release of siderophores) or, if they are able to move towards more favourable conditions. Moreover, microorganisms that functionally complement each other or cooperate increase their efficiency and energetic yield by exploiting resources that were otherwise unfavourable for them. In contrast, microorganisms can also have the ability to limit or prevent survival of other life forms that compete for their niche occupation. Therefore, geochemistry and microbiology should not be considered separately when trying to resolve the chemical and biological structure of an ecosystem.

Bulk investigations of the interrelation of different microbial, chemical and physical processes certainly contribute to a better understanding of the functioning of ecosystems. However, the micrometre-scale conditions in natural ecosystems and their effect on microbial niches are often impossible to replicate under laboratory studies and hard to resolve with bulk chemical approaches. We therefore developed a conceptual framework that shows how different microbial and biogeochemical processes are interconnected and where they are located throughout environmental gradients. As an example we focused on the biogeochemical cycling of iron in freshwater sediments.

Parameters controlling the distribution of chemical compounds and consequences for metabolic energy yield

In sedimentary systems, high-resolution geochemical and redox profiles can be measured as a function of depth. The distribution of chemical compounds in sediments is physically

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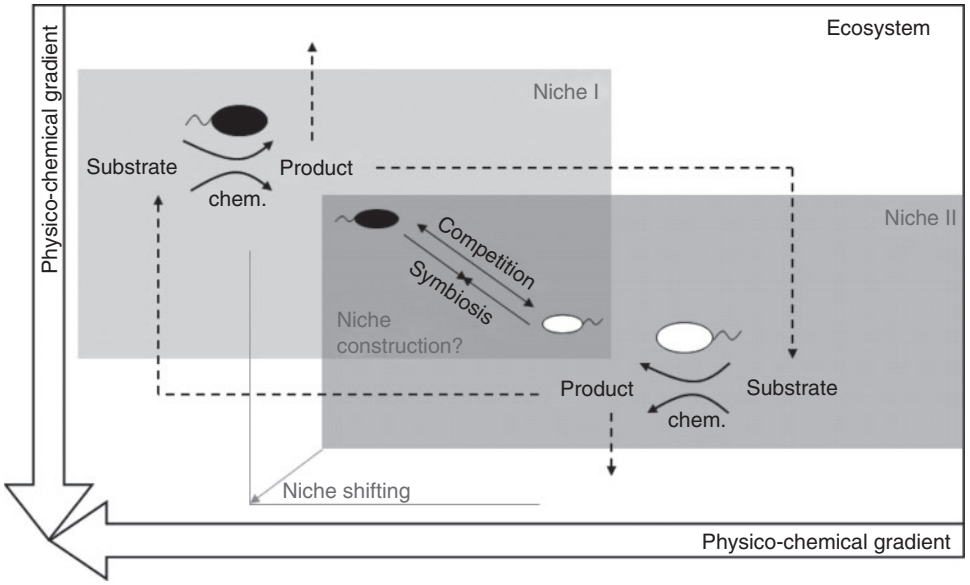

Fig. 1. Schematic representation of a microbial ecosystem. The controlling parameters that affect microbial distribution and process stratification within the ecosystem are indicated by the different arrows. The substrate conversion can either proceed biotically (indicated by the bacteria) or abiotically (indicated by the abbreviation 'chem.'). Symbiosis refers to mutualistic, parasitic or commensal biological interactions.

controlled by diffusion and biologically, as well as chemically, by consumption and production. The sediment surface is exposed to atmospheric oxygen, which in the upper layers, where light penetrates,[2] can also be produced by photosynthesis. Oxygen is usually used up within the first millimetres of the sediment, depending on the presence of biomass and the extent of heterotrophic growth, with oxygen as the terminal electron acceptor. With increasing depth the sediment becomes more reduced as a result of abiotic and biotic processes. The developing redox zones determine microbial niche occupation and with that the distribution of microbial ecotypes. With decreasing redox potential the energetic yield per transferred electron (ΔG_{e^-}) of a specific biogeochemical process systematically decreases. If the geochemistry (concentrations of electron donors and acceptors) of an ecosystem is known, the harvestable energy can be calculated for each electron donor and acceptor pair and associated microbial transformation process.

In order to provide energy for anabolic processes, microorganisms need to gain at least 15–20 kJ mol^{-1}, the smallest amount of metabolically convertible energy for an ion transported across the cytoplasmic membrane, equivalent to one-third of an adenosine triphosphate (ATP) unit.[3,4] To increase the energy yield, microorganisms have to increase the rate of substrate conversion per unit time (multiple repetition of metabolic reaction within a defined fraction of time), which results in a higher substrate requirement. Hence, the survival of microorganisms is not only limited by the overall energetic yield of biogeochemical reactions, but is also constrained by the availability (quality and quantity) of substrate.

When we deal with complex reaction networks, it is challenging to find a comparable unit for the constraints that control the occurrence of specific biotic or abiotic processes. In most cases semiquantitative comparisons that account for physico-chemical as well as for physiological properties can be performed in order to understand the architecture of reaction networks and the substrate and energy fluxes in between networking parts. Physiological properties include the tolerance to chemical inhibitors (i.e. low levels of oxygen for anaerobes), metabolic flexibility or the adaptation to different wavelengths and low light intensities.[5] The additional use of classical thermodynamic computations represents a promising approach to evaluate microbial niche occupation and their spatial distribution. The general mathematical expression for the determination of the energy for chemotrophic reactions is:

$$\Delta G = \Delta G^\circ + \mathrm{R}T \ln Q \qquad (1)$$

with ΔG, Gibbs free energy; ΔG°, standard Gibbs free energy; R, universal gas constant (8.314 J mol^{-1} K^{-1}); T, temperature (K); and Q, reaction coefficient. The energy yield (as presented in figures) is defined as the absolute value of the determined Gibbs free energy. The Gibbs free energy can also be calculated from the redox potentials:

$$\Delta G = -n\mathrm{F} \cdot \Delta E \text{ or } \Delta G^\circ = -n\mathrm{F} \cdot \Delta E^\circ \qquad (2)$$

with F, Faraday constant (96 4858 C mol^{-1}); E, redox potential; and n, the number of electrons that are involved in the reaction.

For phototrophic processes the energetic yield can be calculated as follows:

$$\Delta G = \mathrm{N_A} \cdot \mathrm{h} \cdot c \cdot \lambda^{-1} \qquad (3)$$

with $\mathrm{N_A}$, Avogadro's number (6.023×10^{23} mol^{-1}); h, Planck's constant (6.63×10^{-34} J s); c, the speed of light (2.99×10^8 m s^{-1}); and λ, the wavelength (nm). However, this equation only accounts for the quality of light (wavelength) and omits the importance of the quantity of light (irradiance) which predominantly limits and constrains the respective phototrophic processes, similar to how concentrations of substrates limit chemotrophic processes.

Another key parameter that controls the availability of substrates for microbial processes (that require the same electron donors and acceptors) is the speed of reaction that is

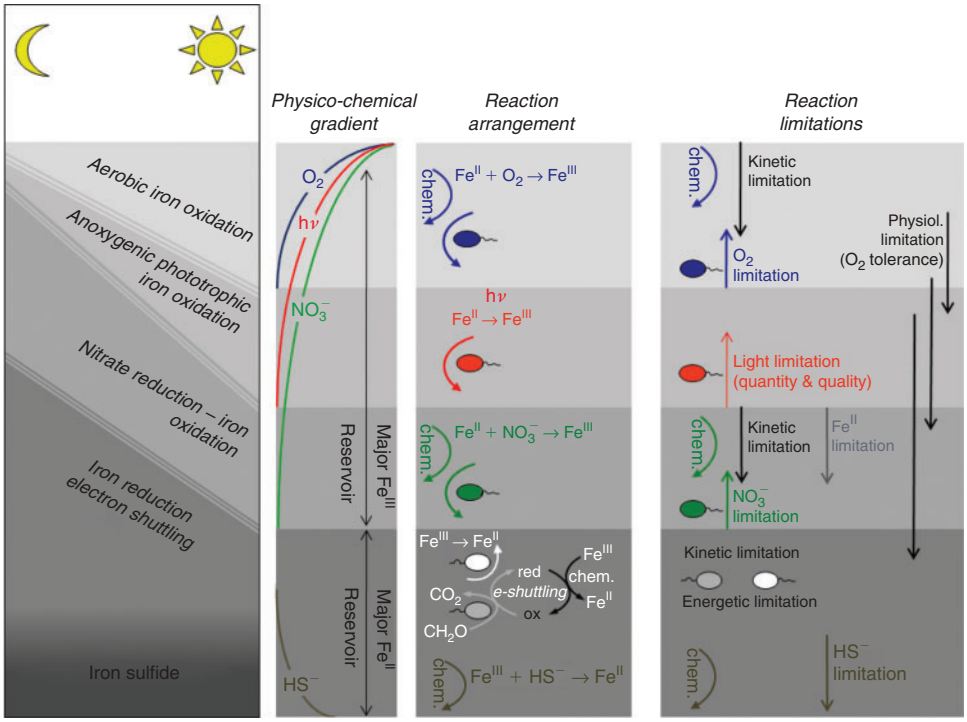

Fig. 2. Schematic representation of the spatio-temporal distribution of biogeochemical processes associated with iron redox cycling in freshwater sediments. Shown are geochemical gradients, competition between processes and the physico-chemical constraints of the different microbial metabolisms. The figure shows the classical layering of biogeochemical processes alongside redox gradients in freshwater sediments. However, microhabitats with steep redox gradients caused by microbial activity and aggregate formation might be distributed throughout the entire sediment column, resulting in e.g. anoxic, strongly reduced micro-environments even within well oxygenated sediment layers.

mathematically determined applying the laws of kinetics. Although a reaction can be favourable in terms of energy yield, the substrate competition with a faster chemical or biological process might limit the actual substrate availability and therefore, effectively the associated energy yield.

In the following we discuss the above described aspects with respect to biogeochemical iron cycling in freshwater sediments but the general concept can be extended to redox transformations of other elements in different environments.

Biogeochemical element cycling – the case of iron

The elemental cycle of iron comprises complex microbiological and abiotic interactions and competitions throughout geochemical gradients. First thought to be an abiotic phenomenon, the visual observation of microorganisms in 1836 that were oxidising iron[6] fostered hypotheses on the involvement of biotic processes in the iron redox cycle and extended geochemical research questions to biogeochemical ones.[7]

Under circumneutral conditions the biogeochemical cycling of iron comprises oxic and anoxic habitats (Fig. 2) where iron is either occurring in its oxidised (Fe^{III}) or reduced (Fe^{II}) redox state, as dissolved (aq) or precipitated species. The current knowledge of different iron converting processes, their spatial arrangement and interconnection in ecosystems is mainly restricted to aerobic and anaerobic processes.[8–11] Research conducted in order to answer this question will have to consider processes that compete for the same electron donor (Fe^{II}_{aq}), but rely on different energy sources (chemical and light energy) or different electron acceptors (O_2, NO_3^-).

In oxic environments the oxidation of Fe^{II}_{aq} by molecular oxygen is the predominant iron converting process. Aerobic iron-oxidising microbes are restricted to micro-oxic niches where chemical oxidation rates decrease allowing microorganisms to successfully compete with abiotic oxidation.[12–14] It was shown that at 50 µM O_2 the contribution of biotic Fe^{II}_{aq} oxidation is ~20%, increasing to >80% with decreasing O_2 levels (15 µM).[12] Following the redox gradient towards more reduced (anoxic) conditions, Fe^{II}_{aq} is oxidised by anoxygenic phototrophic[15] and nitrate-reducing (autotrophic and mixotrophic) iron-oxidising microorganisms.[16,17] Their tolerance to low levels of oxygen[18,19] allows them to harvest substrate and energy along, and partly across, the oxic–anoxic interface, where competition with aerobic iron oxidation could occur. At environmentally relevant oxygen, nitrate and Fe^{II}_{aq} concentrations, autotrophic nitrate-reducing iron oxidation yields less energy than the aerobic oxidation pathway (per mole Fe^{II}_{aq}) (Fig. 3a) favouring the dominance of aerobic iron-oxidisers. However, the metabolic flexibility of mixotrophic nitrate-reducing iron-oxidising bacteria, which allows most of them to switch between different electron acceptors (e.g. nitrate and oxygen)[18] enables them to occupy various niches across the redox gradient to exploit multiple electron acceptors as substrate. Competition with chemical Fe^{II}_{aq} oxidation by nitrite is only relevant in low pH environments and at elevated nitrite concentrations (>0.3 mM).[20,21]

In light exposed sediments, in which anoxygenic phototrophic iron oxidation occurs, the spatial distribution and extension of iron-converting processes appears to be more complex. The distribution of anoxygenic phototrophs is

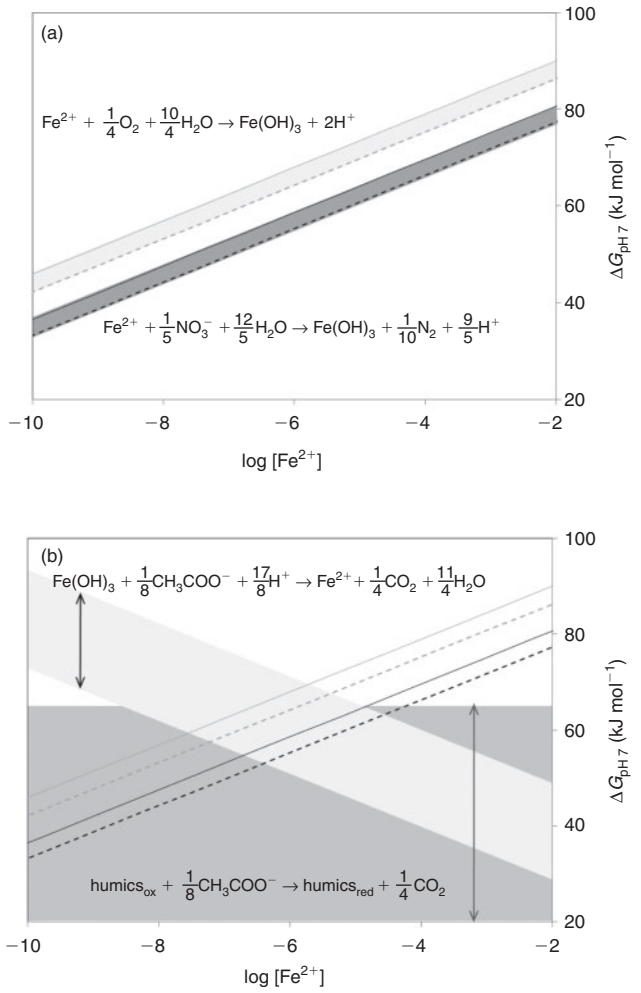

Fig. 3. Energetic yield (absolute value of Gibbs free energy) for chemolithotrophic iron oxidation and iron reduction per mol electron donor. (a) Circumneutral energetic yield for aerobic and nitrate-reducing iron oxidation as a function of Fe^{II}_{aq} (log scale; determined from moles per litre Fe^{2+}) and environmentally relevant NO_3^- (dark grey: range 1–1000 µM (dashed and solid line)) and O_2 (light grey: range 1–500 µM (dashed and solid line)) concentrations. (b) Energetic yield for aerobic and nitrate-reducing iron oxidation as presented in (a) compared with direct iron (ferrihydrite) reduction ($E^{\circ\prime}_h$ ferrihydrite/$Fe^{2+} = -100$ to $+100$ mV; acetate/$CO_2 = -287$ mV) (light grey shading) and electron shuttling by humic substances ($E^{\circ\prime}_h$ humics $= -300$ to $+400$ mV; acetate/$CO_2 = -287$ mV) (dark grey shading). Calculations were performed for circumneutral conditions (pH 7). The microbial reduction of magnetite using formate as substrate is not included in this figure as the energetic yield is substantially lower than for the displayed reactions.

spatially constrained by opposing gradients of light and dissolved Fe^{II}_{aq}. Phototrophic iron-oxidisers require a minimum (threshold) light irradiance of a specific wavelength (controlled from light attenuation on surface), as well as a minimum (threshold) concentration of substrate (Fe^{II}_{aq}) (controlled from the reduced zone located below). In the case of a low photon flux, within the minimum photon requirement limitation, phototrophic metabolisms would slow down, but still account for significant iron oxidation.[22] An absolute minimum photon requirement of 0.005 µmol quanta $m^{-2}\,s^{-1}$ has been determined for green sulfur bacteria *Chlorobiaceae*,[23] of which some species can also oxidise iron phototrophically (e.g. *C. ferrooxidans* sp. KoFOx).[24] In terms of electron donor requirements, photoferrotrophs compete with nitrate-reducing iron-oxidisers. The photoferrotroph's effective exploitation of electron donor and acceptor and carbon sources is directly controlled by physical parameters, such as changes in light intensity as a result of meteorological or diurnal fluctuations.[25] During high irradiation light penetrates several millimetres into the sediment,[2] possibly reaching the anoxic zone where it can drive anoxygenic photoferrotrophic processes. Assuming a surface photon flux of 200–2000 photons $m^{-2}\,s^{-1}$ (400 to 700 nm) at midday,[26] ~10% of the photons will reach the oxic–anoxic transition zone located at a sediment depth of 4 to 6 mm. This is still sufficient to drive low-photon-requiring phototrophic microbial processes (Table 1) (even if surface light will be partly attenuated by the water column and overlaying microbial biofilms). However, because of strongly varying light conditions photoferrotrophs are forced into a flexible metabolic 'lifestyle' (e.g. switching to chemoorganoheterotrophic growth[27] or sulfide and thiosulfate oxidation (*Rhodovulum iodosum* and *Rhodovulum robiginosum*)[28]), a dormant state or to actively move towards better illuminated regions. Under such conditions nitrate-reducing iron-oxidising bacteria are no longer in competition for Fe^{II}_{aq}, which might allow them to either enlarge their habitat or to increase their substrate turnover rate.

Anoxygenic photoferrotrophs would, therefore, most probably find their niche close to the oxic–anoxic and light–dark interface, between the zones of aerobic iron oxidation and anaerobic nitrate-reducing iron oxidation. However, because of environmental heterogeneities (including local micro-redox gradients, short-term fluctuating environmental conditions (i.e. light restriction, substrate delivery) or generally the lack of a specific substrate (i.e. nitrate-depleted environments)), the microbial arrangement might deviate from the above suggested scenario. In environments that lack nitrate, the boundaries and the predominant spatial location of photoferrotrophs (and anaerobic iron-oxidisers) will only be driven by the light reach and oxygen penetration level.

If the nitrate reduction zone reaches deep into the sediment, the location of photoferrotrophic and nitrate-reducing iron-oxidising microorganisms might be spatially more separated. On a stoichiometric basis 5 moles of Fe^{II}_{aq} are required to reduce 1 mole of nitrate. Therefore, nitrate-reducing iron-oxidisers will find optimal growth conditions closest to the Fe^{II}_{aq} source where they can still reach sufficient nitrate levels. On the other hand photoferrotrophs prefer to extend their habitat towards the sediment surface, where light is more readily available. Although this would result in less direct substrate competition with nitrate-reducing iron-oxidising bacteria, the depletion of Fe^{II}_{aq} by nitrate-reducing iron-oxidising bacteria in deeper sediment layers also reduces the upward-flux of Fe^{II}_{aq} which decreases the electron donor availability for photoferrotrophs (Fe^{II}_{aq} requirement per reduction of 1 mol of CO_2 is 4 mol).

Moreover, nitrate-reducing iron-oxidising bacteria compete with heterotrophic denitrifying microorganisms for electron acceptors. Denitrifiers will be located in sediment layers that are enriched in nitrate and organic matter, located close to the oxic–anoxic interface. In order to escape competition while still reaching sufficient iron and nitrate concentrations, autotrophic and mixotrophic nitrate-reducing iron-oxidising bacteria will occupy niches below the nitrate reduction zone.

To close the biogeochemical iron cycle, Fe^{III}, which is mostly trapped in various barely soluble minerals, has to be reduced in order to provide Fe^{II} for re-oxidation (Fig. 2). Fe^{III} is either reduced chemically by sulfide[29] or biologically through

Table 1. Circumneutral standard energetic yield for microbial iron oxidation and reduction processes

Chemical compounds in parentheses are the respective electron acceptors and donors for the considered oxidation and reduction process. The energetic yield (absolute value of Gibbs free energy) for photoferrotrophic metabolisms was determined considering the required wavelengths (per photon, hν). The values for half light saturation indicate at which photon flux light dependent iron oxidation proceeds at maximum rates at the respective wavelength. Iron reduction: E'_h −100 to +100 mV ferrihydrite/Fe^{2+}; e^- shuttling (humics): E'_h −300 to +400 mV acetate/CO_2

Process	$\Delta G_{pH\,7}$ (kJ mol^{-1} e^- acceptor)		$\Delta G_{pH\,7}$ (kJ mol^{-1} e^-)
Aerobic iron oxidation	422 (O_2)		105 (O_2)
Nitrate-reducing iron oxidation	475 (NO_3^-)		95 (NO_3^-)
Iron reduction[16]	144 to 301 (CH_3COO^-)		18 to 38 (CH_3COO^-)
e^- shuttling (AQDS)[39]	82 (CH_3COO^-)^A		10 (CH_3COO^-)^A
e^- shuttling (humics)[38]	−8 to 532 (CH_3COO^-)^A		−1 to 66 (CH_3COO^-)^A
Phototrophic iron oxidation	Absorption max λ (nm)	$\Delta G_{h\nu}$ (kJ mol^{-1} hν)	Half light saturation[19] (μmol hν m^{-2} s^{-1})
Thiodictyon sp. (F4)	362; 490–512	330; 243–234	7
Rhodobacter ferrooxidans (SW2)	430–517	278–231	2.3
Chlorobium ferrooxidans (KoFOx)	435; 462; 491	258; 167	0.22

^{A}Energetic yield for electron shuttling microorganisms (only accounts for biotic reaction part: e^- shuttle reduction).

microorganisms. It has been described that some bacteria reduce Fe^{III} by direct contact between the cell and mineral surface by outer membrane proteins[30] or potentially even by conductive pili, so-called 'nanowires'.[31] Alternatively, microbial Fe^{III} mineral reduction can be facilitated through the production of Fe^{III}-solubilising organic ligands (e.g. *Shewanella oneidensis*).[32,33] Finally, iron can be reduced by a biotic–abiotic reaction cycle (electron shuttling),[34–42] in which bacteria gain energy from the reduction of an organic electron shuttling compound (e.g. humics or flavins[34,37,38,40–42]), that in turn reduces Fe^{III} chemically. Since the redox potential of electron shuttles must be in between the redox potential of the electron donor redox couple (e.g. acetate/CO_2) and the terminal electron acceptor (Fe^{III}), the theoretical microbial energy yield is smaller compared to direct biotic iron reduction (Table 1). As long as sufficient quantities of oxidised electron shuttles are present the microbial shuttle reduction will proceed from a thermodynamic perspective, even if the second reaction step – the chemical reaction of the reduced shuttle with iron – becomes less favourable (Fig. 3b). It has been shown by using humic substances as electron shuttles that the reduction of Fe^{III} is seven times faster than pure biotic iron reduction.[37] As these reactions can be repeated multiple times within the same time interval, the provided energy of the single reaction multiplies to a maximum amount. Electron shuttling is, therefore, a more favourable process for iron-reducing bacteria despite the lower energy yield per transferred electron.[36] As redox-active humics are ubiquitous in natural systems either as solid aggregates (particulate organic matter)[42] or in dissolved forms, electron shuttling might be the dominant reaction mechanism in the iron cycle. Recently, it has been suggested that a network of bacteria stimulate an electron flow across the redox gradient from deeper sediments to the surface,[43] which triggers stratification and spatial extension of single redox zones.[43,44] The observed electron flow might be caused for example by electron shuttling through dissolved and solid phase humics,[42] a mechanism in which iron-reducing strains (e.g. *Geobacter* sp., *Shewanella* sp.) are involved. Microbial iron reduction might, therefore, play an important role in the overall sedimentary electron transfer network.

Although iron reduction is expected to be located beneath the oxygen and nitrate reduction zone in the classical redox cascade, iron-reducing microorganisms have been found in similar abundance in various redox zones of freshwater and marine sediments.[38] Fe^{III} might also serve as an electron acceptor in oxic environments when nitrous and anoxic microhabitats form within soil–microbe-aggregates that lead to a tight coupling of oxidative and reductive iron transformation processes on the micrometre scale. It has been demonstrated that the iron-reducing strain *Geobacter* sp. is also able to oxidise Fe^{II}_{aq} using nitrate as an electron acceptor in anoxic freshwater wetland sediments.[45] An estimation of $\Delta G_{pH\,7}$ shows that iron reduction becomes energetically less favourable with increasing Fe^{II}_{aq} (which reflects increasing ferrous and reducing conditions in a geochemical profile), decreasing below the energetic gain of iron oxidation processes in the range of 10^{-6} to 10^{-5} M Fe^{II}_{aq} (Fig. 3b). Slight variations in Fe^{II}_{aq}, which might be caused by microbial production and consumption, could therefore shift the (dominant) metabolic activity from reduction to oxidation (or vice versa). In terms of energy yield, microorganisms that are capable of switching between Fe^{II} oxidation, Fe^{III} and humics reduction could, therefore, exploit zones at redox transitions to a maximum. To date, this issue remains an interesting area for future exploration, as no experimental evidence for the existence of microorganisms that unite all three metabolic capabilities has been provided so far.

Metabolic flexibility constitutes a key selective advantage for microorganisms that thrive in fluctuating environments with steep redox gradients. They can exploit various niches to secure their survival, which might explain their scattered distribution throughout an entire sediment profile. The iron reducer *Geobacter sulfurreducens* for example also shows facultative heterotrophic growth with oxygen as a terminal electron acceptor.[46] Similar to *Geobacter* members of the facultative anaerobic genus *Shewanella* sp. have been described to respire Fe^{III}, as well as a wide range of other inorganic and organic electron acceptors (including fumerate, oxygen, nitrate, nitrite, manganese, thiosulfate, dimethyl sulfoxide (DMSO), dissolved and solid-phase humics).[42,47] In addition, behavioural aspects, motility, chemotaxis and the regulation of gene expression,

determine the successful survival of bacteria and the maintenance of populations.

The natural distribution, spatio-temporal variations and the biogeochemical constraints of iron converting processes in freshwater sediments are summarised in Fig. 2.

Implications – from concept to reality

The goal of setting up a theoretical framework is to understand how a biogeochemical cycle is assembled, how substrates and energy flow through different reservoirs and how microbial processes are spatio-temporally arranged and constrained. This approach is necessary to better comprehend the importance of elemental cycling within a certain ecosystem, from the substrate sources (i.e. reaction and metabolic products), by intermediates of different stability to their sinks. The described approach will also help to better understand how elemental cycles are connected with each other and how the cycling of redox-active elements affects the stability, mobility and transformation of organic and inorganic compounds. The described conceptual framework aims to demonstrate interactions in the biogeochemical iron cycle that are challenging to simulate in laboratories. It represents a theoretical foundation which requires empirical techniques to validate what has conceptually been suggested. Combinations of state-of-the-art high-resolution techniques (such as the combination of microsensors and fluorescence in situ hybridisation (FISH),[2,48] scanning transmission X-ray microscopy,[49,50] nano secondary ion mass spectrometry[51] and spectroscopic imaging and quantification of microbial pigments[52]) will improve our understanding of the distribution of chemical and microbial species and their associations and interactions with microorganisms. The coupling of in situ microbial abundance profiles (i.e. using real-time polymerase chain reactions (PCR), and FISH-based cell counts) with geochemical (i.e. by microelectrode measurements or gel probe techniques) and solid phase analysis (i.e. by micro-X-ray diffraction, Mössbauer spectroscopy and synchrotron-based X-ray absorption spectroscopy techniques) at resolutions relevant to microbial processes will provide information on electron donor and acceptor defined boundaries of microbial life. In addition, microbial genomics will allow a better understanding of the functional potential and metabolic flexibility of microorganisms and their phenotypic plasticity. The combination of these techniques with an elaborate theoretical framework of biogeochemical element cycling as described here will advance our knowledge of microbial ecosystem structure and functioning under environmental perturbations.

Acknowledgements

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