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Highlight

Cryptic biogeochemical cycles: unravelling hidden redox reactions

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Cryptic element cycling

The turnover of organic matter in the environment is controlled by a series of (often connected) biogeochemical cycles, mainly involving the transformation of C, N, S, O, Fe and Mn under both oxic and anoxic conditions (Druschel and Kappler, 2015). Typically, in order to quantify the extent and rates of turnover, the geochemical end products or reaction intermediates are quantified over time (e.g. sulfide and sulfate or Mn(II) and Mn(IV)). In many cases, the absence of a certain species is used to exclude the relevance of the respective biogeochemical cycle. Recently, however, more evidence has appeared which shows that very reactive, sometimes short-lived intermediates at low concentrations with poorly understood redox properties can play key roles in biogeochemical cycles. For example, redox-active humics are important in carbon cycling, nitrous oxide in the nitrogen cycle and polysulfides in the sulfur cycle (Hansel *et al*., 2015). The concentrations of these intermediates represent steady-state concentrations arising from the balance between continuous oxidation and reduction. This leads to so-called 'cryptic element cycles' where changes in concentrations of a certain redox species cannot be measured but rapid turnover (connected to other element cycles) means that they are key components of the biogeochemical processes that are occurring. In recent years, for example, the importance of cryptic cycling of sulfur has become increasingly clear. The main driver of the sulfur cycle is microbial sulfate reduction which ultimately produces sulfide (H_2S). However, this is not a direct process and a large diversity of reactive sulfur species are formed during the six intermediate oxidation states between sulfate and sulfide which are themselves suitable for microbial redox reactions (Zopfi *et al*., 2004). These reactive intermediates are often below detection limit in the environment, yet are thought to play very important roles in biogeochemical cycling of both sulfur and associated redox species (Holmkvist *et al*., 2011). Additionally, sulfide can be rapidly recycled making the detection of sulfate reduction difficult. For example, Canfield *et al*. (2010) found that diverse communities of sulfur cycling microorganisms were present in oxygen minimum zones off the Chilean coast despite very low sulfate and sulfide concentrations. These sulfur cycling communities were sustained through rapid recycling of the H_2S which is also linked to nitrogen cycling in these regions. Although cryptic cycling has been described in detail for sulfur, and other elements to a small extent, almost nothing is known about cryptic iron cycling (Hansel *et al*., 2015).

Microbially mediated iron redox transformations

The one-electron transfer involved in iron redox reactions gives the false impression that iron cycling is a simple process when compared to, for example, sulfur or carbon cycling. In fact, there are both biotic and abiotic reactions involving both dissolved and solid-phase Fe species, including very reactive mixed-valent Fe(II)-Fe(III) minerals such as magnetite (Fe_3O_4) and green rust which contains layers of both Fe(II) and Fe(III) interspaced with anions such as CO_3^{2-}, Cl^- or SO_4^{2-}. The wide diversity of biotic and abiotic reactions act to cycle iron between its oxidized and reduced forms and make both Fe(II) and Fe(III) dynamic in most environments (reviewed in Melton *et al*., 2014). Figure 1 summarizes the main microbial processes involved in iron redox transformations. Fe(III) is reduced to Fe(II) by Fe(III)-reducing microbes which use organic carbon or hydrogen as an electron donor. As Fe(III) is poorly soluble at circumneutral pH, Fe(III)-reducing microbes may also use novel strategies to access the poorly soluble substrate, such as by the production of conductive cell appendages (nanowires) or electron shuttle molecules

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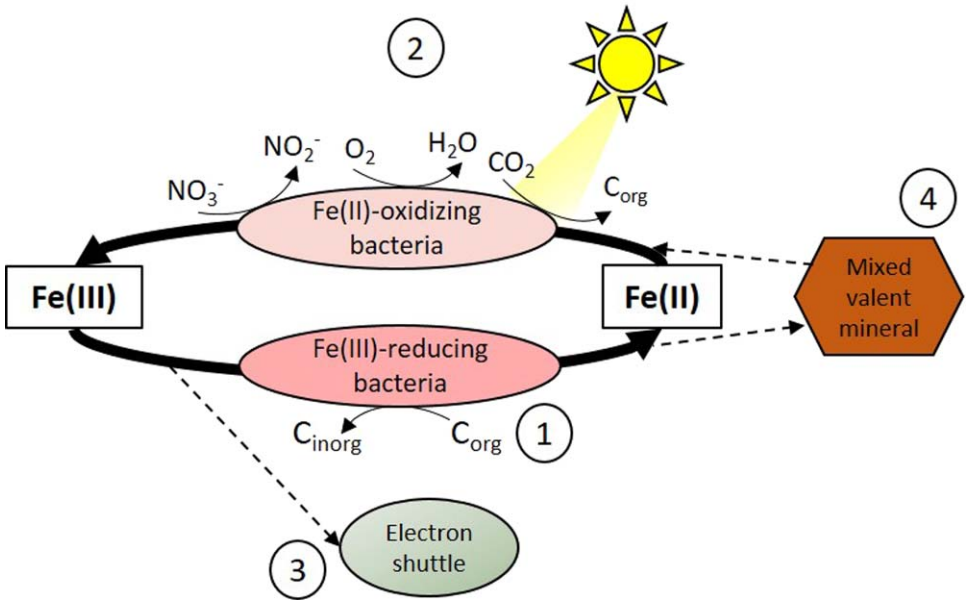

Fig. 1. Schematic overview of microbial iron transformations in the environment. (1) Reduction of Fe(III) minerals or dissolved Fe(III) complexes coupled to the oxidation of organic matter. (2) Microbial oxidation of Fe(II) with oxygen, nitrate or bicarbonate (via photosynthesis) as electron acceptor. (3) Reduction of Fe(III) by an electron shuttling molecule (4) Transfer of electrons from iron redox reactions either on to or away from mixed-valent iron minerals.

which alleviate the requirement for direct cell-mineral contact (Shi *et al.*, 2016). The oxidation of Fe(II) can be coupled to the reduction of oxygen, to the reduction of nitrate, and by the action of anoxygenic photoferrotrophs which couple Fe(II) oxidation to the reduction of bicarbonate using light as an energy source for photosynthesis. It has also been shown that the above-mentioned mixed-valent minerals magnetite (Byrne *et al.*, 2015) or green rust (Zegeye *et al.*, 2012) can facilitate iron cycling by acting as an electron source during Fe(II) oxidation and as an electron sink during Fe(III) reduction.

In addition to the diverse microbial processes influencing iron speciation, the redox state of iron in the environment is also influenced by an equally diverse range of abiotic reactions such as reactions with reactive oxygen and nitrogen species, sulfide, manganese oxides and humic substances (Fig. 2). Collectively, biotic and abiotic iron redox transformations result in a connection to most of the major biogeochemical cycles on earth; those of oxygen, carbon, sulfur, nitrogen and manganese.

Given the array of reactions which can initiate iron redox transformations it seems likely that iron would be

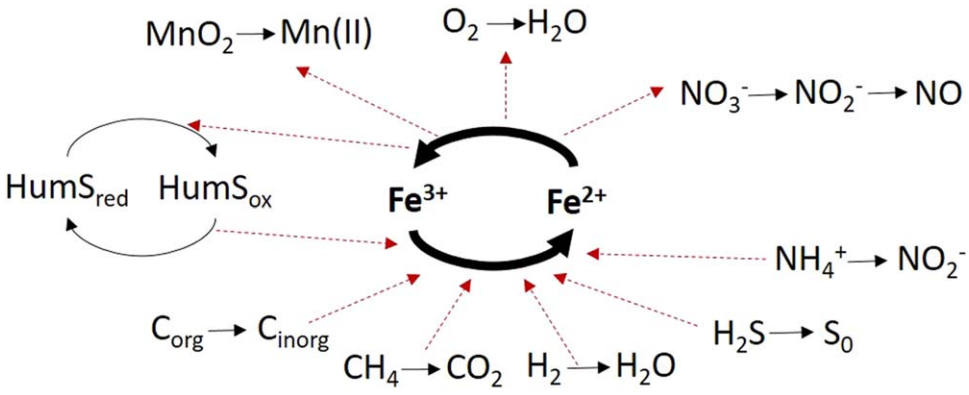

Fig. 2. Role of iron redox transformations in other biogeochemical cycles. Red dashed arrows indicate flow of electrons into and out of the iron cycle, i.e. arrow pointing in means electrons are accepted by the iron cycle, arrows pointing out means electrons are donated by the iron cycle.

rapidly cycled in any environment where redox gradients exist. This is especially true when we consider that multiple types of iron-metabolizing bacteria are known to co-exist within the same environment. For example, nitrate-reducing Fe(II)-oxidizers and heterotrophic Fe(III)-reducers have been found together in freshwater sediments (Coby *et al*., 2011; Melton *et al*., 2012), photoferrotrophs and Fe(III)-reducers co-exist in the ferruginous waters of Kabuno Bay (Llirós *et al*., 2015) and all four types have previously been found to co-exist in marine sediments (Laufer *et al*., 2016). Whilst the freshwater and marine sediments in these previous studies represent typical sedimentary iron concentrations (approximately 100 μM Fe(II) in pore waters), all previous studies of microbial iron cycling in water columns have focussed on unusual Fe(II)-rich habitats. Typically lake waters have dissolved Fe(II) concentrations around 1-2 μM (Davison *et al*., 1982) whilst oceanic iron concentrations are in the nano molar range (Moore *et al*., 2013). Due to the low energy yield expected from iron redox reactions, microbial iron cycling is typically considered negligible at such low concentrations. As a result, discussion of the role of microbial iron oxidation in water bodies, and photoferrotrophy in particular, is regularly confined to the early earth literature and to rare, ferruginous Archean analogues on the Earth today (reviewed in Koeksoy *et al*., 2015).

However, as has become increasingly appreciated in the cryptic sulfur cycle over recent years, a small amount of substrate recycled quickly enough can have a big impact on element cycling and microbial community composition and dynamics. A cryptic iron cycle would be one in which iron transformations occurred without any appreciable change in Fe(II) or Fe(III) concentrations, as measured by conventional techniques. What are the requirements for such a cryptic iron cycle? Firstly we require a mechanism of Fe(II) oxidation. Microbial Fe(II) oxidation would be expected where there are micro-oxic conditions favouring microaerophilic Fe(II) oxidation, where light and anoxic conditions co-exist for photoferrotrophy, or where nitrate is present. Secondly, for cryptic cycling, we need a way to immediately reduce oxidized Fe(III) back to Fe(II). For this to be instantaneous and thus for no accumulation of Fe(III) to be measurable, the Fe(III) reduction rate must not be limited by electron donor. Therefore, it could be expected that cryptic iron cycling would occur in any environment with conditions favouring microbial Fe(II) oxidation with abundant organic carbon or hydrogen present that could simultaneously sustain Fe(III) reduction. Conversely, in low organic environments with favourable conditions for Fe(II) oxidation, we may expect iron reduction to proceed without any measurable increase in Fe(II) as this will immediately be oxidized (abiotically or biotically) back to Fe(III). In general, we could expect iron cycling to occur without measurable changes in iron redox state in any environment where iron is the rate-limiting factor of either the oxidation or the reduction reaction.

Evidence for a cryptic iron cycle

Despite the fact that a cryptic iron cycle would make sense in many environments, until now we had little detailed evidence of this in the field. In a very recent ground-breaking study, Berg *et al*. (2016) determined iron turnover rates, in particular biological iron oxidation rates, and calculated iron fluxes in the low iron, redox-stratified Lake Cadagno by determining the change in dissolved total iron as a function of depth. In the daytime, iron was seen to be consumed well below the oxycline. The fact that the iron profile was independent from the conductivity profile indicates that Fe(II) oxidation occurs faster than the rate of mixing in this layer. The fluxes calculated are of a similar order of magnitude to those calculated for ferruginous lakes studied previously (Crowe *et al*., 2008). This is a surprising result as the lake contains only 1–2 μM of dissolved Fe(II), compared to ca. 100-times higher concentrations in these ferruginous lakes, and no measurable Fe(III), whilst oxygen, sulfide and methane concentrations are an order of magnitude higher.

This intensive iron turnover appears to support diverse populations of iron-metabolizing bacteria as revealed by 16S rRNA amplicon sequencing, which includes both purple and green-sulfur bacteria, microaerophilic Fe(II)-oxidizers such as *Leptothrix*, nitrate-reducing Fe(II)-oxidizers, magnetotactic bacteria and Fe(III)-reducing heterotrophs. In addition to the field studies, the authors conducted laboratory experiments with enriched co-cultures of Fe(II)-oxidizing and Fe(III)-reducing bacteria from Lake Cadagno in both dark and light conditions. Fe(III) was reduced over time in dark incubation; however in the light, which favours phototrophic Fe(II) oxidation, no discernible change in Fe(II)/Fe(III) ratio could be seen since the Fe(II) formed by Fe(III) reduction was rapidly re-oxidized to Fe(III). Additionally, incubations with ^{13}C-labelled CO_2 and ^{13}C-labelled organic substrates provided clear evidence for CO_2 fixation in the light and organic matter oxidation in the dark respectively. In summary, these experiments with enrichments cultures further emphasize the potential for cryptic iron cycling in Lake Cadagno.

The authors propose a model for cryptic iron cycling in Lake Cadagno which is largely driven by light. In the daytime iron cycling in the chemocline is stimulated by the activity of photoferrotrophic bacteria and by production of oxygen via oxygenic photosynthesis, which in turn stimulates the abiotic reaction of Fe(II) with oxygen and the activity of microaerophilic Fe(II)-oxidizing bacteria. In this

environment, both phototrophic and microaerophilic Fe(II)-oxidizers co-exist and may or may not compete for this limited substrate. In addition to light-induced reactions, the authors also suggest that MnO_x formation at the oxycline could play a role in rapid Fe(II) oxidation, in particular for Fe(II) oxidation at night or in the dark.

Despite much evidence for intense Fe(II) oxidation processes in Lake Cadagno, Berg *et al*. were unable to measure any Fe(III) in the chemocline. This is likely a result of high productivity supplying abundant organic carbon substrates as electron donor for Fe(III)-reducing bacteria, yet low total iron concentrations result in Fe(III) limitation of this reaction. As a result, any oxidized Fe(III) is instantaneously reduced back to Fe(II) after sinking of the Fe(III)-containing cell-mineral aggregates into the anoxic part of the lake.

Recently it has been suggested by Hansel *et al*. (2015) that iron reduction, and therefore indirectly also iron cycling, is driven by sulfate reduction even when sulfate is low. This is due to the production of sulfides which readily reduce Fe(III) abiotically. However, contrary to this, no sulfate reduction is detected above the sulfate zone in Lake Cadagno. It is possible that Fe(III) reduction could proceed via the abiotic reduction of Fe(III) by sulfide produced via sulfur reduction or disproportionation. However, the authors conclude that this is only significant in the dark when sulfide is not readily consumed by photosynthetic sulfide oxidation. Even when Fe(III) is reduced by sulfide, the resultant FeS may not be the final sink for either the iron or sulfide. Through a set of complimentary microcosm experiments, Berg *et al*. demonstrated the potential for rapid FeS recycling by chemocline communities.

Impacts on understanding iron biogeochemistry

This contribution from Berg *et al*. expands our appreciation of the dynamic factors controlling iron cycling in the environment and suggests that intensive iron cycling may be significantly more abundant and more important than previously realized, even in habitats with low iron concentrations. It still remains to be assessed how widespread this cryptic iron cycling is in the environment, and future research efforts should surely pursue this goal. However, Lake Cadagno is not an unusual environment. Seasonally stratified lakes are globally widespread and there are no special circumstances to suggest this would be a unique phenomenon at this site. There also does not appear to be many reasons why a cryptic iron cycle would not be expected in any environment with potential for both Fe(II)-oxidizing and Fe(III)-reducing metabolisms. It could be envisioned that in soil, for example, where organic carbon is abundant, where there is nitrate and oxygen available, as well as light in the top few millimetres, that both Fe(II)-oxidizers and Fe(III)-reducers could efficiently cycle iron with important impacts on the nitrogen and carbon cycles.

From a microbial ecology perspective, this study calls to mind the familiar saying: where there is a will, there is a way. Just because the photoferrotrophic microorganisms found in this study have only micromolar concentrations of substrate available to them, they still have some substrate. If there is energy available, there will be organisms there to use it. The photoferrotrophs in Lake Cadagno not only find enough substrate to survive, but are able to gain enough energy to account for up to 10% of the total carbon fixation. However, little trace of their activity could be predicted from the geochemistry alone. This highlights a particular strength gained by combining methodologies from both the geochemical and biological sciences when interpreting biogeochemical cycles. Even when chemical species cannot be measured, we can gain clues of their importance in the organisms which use them.

The implications of this study for our understanding of the activity of photoferrotrophs in particular are very important. These organisms are very often thought of as relics of the ancient Earth. Once great contributors to the Earth system in the ferruginous oceans of the Archean, they are typically thought to be minor components of modern habitats. This study suggests a much more widespread role for photoferrotrophs in modern environments than may be expected and highlights, in general, that concentration of substrate alone may not be the deciding factor in shaping the microbial community.

To conclude, as we continue to explore biogeochemical cycling we become increasingly aware of the subtle influences shaping these processes. To hope to understand the element cycles which shape our planet we must appreciate that anything we can measure is the outcome of a myriad of extremely complex and interrelated processes, and we must find new methods to understand these hidden redox reactions.

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