Intensive cryptic microbial iron cycling in a low iron environment

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SIGNIFICANCE STATEMENT

Microbial iron cycling in environments low in iron and high in sulfide has largely been ignored until now. Here we show that tightly coupled microbial iron oxidation and reduction processes can lead to extremely high “cryptic” iron turnover in a low iron environment, with a significant impact on the local carbon cycle. Moreover, iron sulfides may not necessarily be the ultimate sink for iron in sulfidic water columns, as some microorganisms are capable of recycling the FeS in the presence of light. Given the widespread occurrence of seasonally stratified lakes, microbial iron cycling may be much more globally important for the biogeochemical iron and carbon cycles than previously believed.

SUMMARY

Iron redox reactions play an important role in carbon remineralization, supporting large microbial populations in terrestrial and aquatic sediments where iron is abundant. Stratified water columns with comparably low iron concentrations are globally widespread, but microbial iron cycling in these systems has largely been ignored. We found evidence for unexpectedly high turnover rates of iron in the low (1-2 $\mu\text{mol}\cdot\text{l}^{-1}$) iron Lake Cadagno. Light dependent iron oxidation rates of 1.4-13.8 $\mu\text{mol}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ were observed, which is even higher than rates in ferruginous lakes with well-studied microbial iron cycles. This microbially-mediated iron oxidation by photoferrotrophs may account for up to 10% of total primary production in the chemocline. We could identify the organisms responsible for this intense iron cycling in 16S rRNA gene amplicon libraries and by the cultivation of some of these bacteria. Based on our combined results, we propose a model in which iron is oxidized by photoferrotrophs and microaerophiles, and iron oxides are immediately reduced by heterotrophic iron reducers, resulting in a cryptic iron cycle. We hypothesize that

microbial iron cycling in water column redoxclines with low iron content, especially those within the photic zone, may be much more widespread than previously believed.

INTRODUCTION:

Although iron (Fe) is the fourth most abundant element in the earth's crust, the majority of it is bound in mineral form and therefore not readily accessible to living organisms. Iron is an extremely redox-sensitive element, oxidizing rapidly in the presence of oxygen to form insoluble iron-oxides or reacting with sulfide to form FeS and FeS₂ precipitates. Solid-phase iron therefore accumulates in sediments where large pools of reactive iron can be actively maintained through biotic and abiotic benthic iron cycling at redox transitions zones. Dissolved iron in porewaters can reach hundreds of micromolar, and reactive solid phase iron may be several 100-fold higher (e.g. (Thamdrup et al., 1994)). Concentrations of dissolved iron in water columns are generally much lower, from a few micromolar in lacustrine systems depending on inflow source (e.g. Davison et al. (1982); Nagai et al. (2007)) to subnanomolar in the open ocean gyres (e.g. Johnson et al. (2003)). Exceptions are stratified, ferruginous lakes such as Lake Matano, Indonesia and Lake La Cruz, Spain which contain hundreds of micromolar dissolved Fe(II) in the anoxic bottom waters (Crowe et al. (2008); Walter et al. (2014)). Although these environments are rare today, they represent model systems for early Earth's ferruginous Archaean Ocean (Konhauser et al. (2002)) and have therefore been subject of intense investigations.

The prokaryotes known to catalyze iron transformations are extremely diverse in phylogeny and metabolism. So far, microbial iron oxidation has been demonstrated with O₂, NO₂⁻, NO₃⁻, and N₂O in cultures and in the environment (e.g. (Emerson and Moyer, 1997), (Straub et al., 1996), (Benz et al., 1998)). The lithotrophic iron oxidizers belong mainly to the phylum *Proteobacteria*, such as

Leptothrix spp. (Fleming et al., 2013) or *Siderooxydans* spp. (Weiss et al., 2007). In addition, some anoxygenic phototrophic bacteria such as *Chlorobium ferrooxidans* (Heising et al., 1999) and *Rhodomicrobium vannielli* (Heising and Schink, 1998) can oxidize iron using light as an energy source (Widdel et al., 1993). Organisms with photoferrotrophic potential are common in aquatic environments but their ecological role and quantitative contribution to iron cycling remain unknown. The microorganisms capable of iron reduction are also diverse (e.g. *Geobacter* spp., *Shewanella* spp., and *Geothrix* spp.) and can utilize H₂ or simple organic carbon compounds as substrates ((Lovley et al., 1993), (Kostka et al., 1996), (Coates et al., 1999)). Up to 50% of carbon remineralization in marine sediments can be driven by microbial iron reduction (Thamdrup (2000)), and this number is expected to be even higher in low-sulfate, freshwater systems. All the microbes involved in iron redox transformations compete with the spontaneous oxidation of iron by O₂, NO₂⁻ and MnO_x and reduction by organic acids and sulfide (Thamdrup et al. (1994)). Determining whether biotic or abiotic reactions control iron redox chemistry in different environments is still a major challenge.

The redox transformation of iron is a one electron transfer process, which means large quantities of iron must be oxidized or reduced for microbes to gain sufficient energy for growth. It is therefore expected that microbial iron cycling predominantly occurs in environments with large pools of available iron. The importance of microbial iron cycling in stratified anoxic lakes with low iron content, which are globally widespread, is largely unknown. Provided that turnover rates are sufficiently rapid, however, coupled iron oxidation and reduction could recycle even a small iron pool enough times to significantly contribute to the energy flux and element cycling in a system. This type of closed microbial iron cycle has previously been demonstrated in the laboratory where

heterotrophic iron reduction is directly coupled to iron oxidation with nitrate (Straub et al. (2004); Coby et al. (2011)) and/or light (Melton et al. (2012)). It has also recently been shown that microscale microbial iron cycling occurs in coastal marine sediments (Laufer et al. (2015)). It is currently unknown whether such cryptic iron cycling can occur at oxic-anoxic interfaces in stratified lakes where iron concentrations are low and oxidized Fe(III) may immediately be reduced to Fe(II) and vice versa.

We investigated potential for microbial iron cycling in the permanently stratified Lake Cadagno where steep gradients of physicochemical parameters, nutrients, and trace metals point to high fluxes and thus high microbial activity. This lake has been cited as an Archean ocean analogue (Canfield et al., 2010) for the study of sulfur cycling processes due to its low ($1\text{--}2\text{ mmol}\cdot\text{l}^{-1}$) sulfate concentrations relative to the modern oceans. However, sulfate is still sufficient to fuel sulfate reduction in the sediments and monolimnion. The $\sim 2\text{ m}$ thick chemocline of Lake Cadagno remains both sulfide- and oxygen-free due to the activity of large populations of anoxygenic phototrophs (Tonolla et al. (1999); Storelli et al. (2013)). In the absence of measureable nitrate and nitrite (Halm et al. (2009); Milucka et al. (2015)) iron and manganese are the next likely alternative electron acceptors/donors for bacteria in the chemocline. We investigated the microbial potential for iron cycling in Lake Cadagno because it is representative of many present-day lakes which develop seasonal stratification with sulfidic bottoms waters (see ref Holmer and Storkholm (2001) for review). Manganese is discussed here alongside iron because of its tight link to the iron cycle. By combining results from chemical flux measurements, molecular analyses, and cultivation experiments, we created a model of the iron cycle in aquatic, low iron environments.

RESULTS & DISCUSSION

***In situ* fluxes and rates**

We measured *in situ* chemical profiles in Lake Cadagno both in Aug 2013 and Aug 2014 over the span of several days and under different light conditions to monitor potential changes in iron chemistry and related physico-chemical parameters over time. The representative example night and day profiles from August 2014 (Fig 1) reveal strong stratification of the lake with opposing gradients of oxygen and sulfide separated by a distance of ~2 m. The chemocline, defined as this 2 m region between the oxycline and the sulfidic monolimnion, coincided with a turbidity maximum where the total microbial cell numbers were on the order of 10^6 - 10^7 cells $\cdot$ ml $^{-1}$. Conservative parameters such as conductivity (Ω) were constant throughout the layer indicating that it is well mixed. This relatively homogenous physico-chemical structure has been attributed to bacterially-driven mixing (Wüest (1994)). The evident vertical up and down movement of this layer over time may be a response to light conditions in addition to surface wind-driven internal waves (Egli et al. (1998)).

Both manganese and iron were present almost exclusively in the dissolved, reduced form in all profiles. Iron below 13 m depth (day profile; Fig 1) was likely in the form of FeS due to the presence of sulfide. The total iron concentrations (1 - 2 μ mol $\cdot$ l $^{-1}$) in Lake Cadagno were only a fraction of those measured in ferruginous lakes (up to 200 μ mol $\cdot$ l $^{-1}$) where active microbial iron cycling is expected (Crowe et al. (2008)) or has been demonstrated (Walter et al. (2014)). The night profile (Fig 1), taken after a full 10 hours of darkness, was characterized by a redox zonation with electron acceptors (O_2 , Mn, Fe, and SO_4^{2-}) apparently consumed in succession according to decreasing energy yield. In the night profile, both manganese and iron gradients correlated with conductivity suggesting that the distribution of these metals is determined by turbulent mixing

within the chemocline. Dissolved manganese and iron disappeared at the oxycline as they were oxidized with O_2 . However, in the profile taken at midday, gradients of manganese and iron indicated their consumption well below the oxycline (Fig 1). In this case, the independent behavior of manganese and iron from conductivity, which showed no gradient, indicates that these metals must be consumed faster than the rate of mixing in the layer. The calculated iron fluxes in Lake Cadagno (Table 1) were extremely high, ranging from 0.027 - $0.392 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$, using the most and least conservative turbulence coefficients (D) reported for Lake Cadagno, respectively (Wüest (1994)). Interestingly, these calculated fluxes were comparable to the reported iron fluxes in the ferruginous Lake La Cruz and Lake Matano, which have 100-fold higher iron concentrations ((Crowe et al., 2008); Walter et al. (2014); see also Table 1). This is likely due to the steep gradients with a consumption zone over cm rather than m, and relatively high turbulence (large D values). This high flux implies high turnover rates and a potential for intensive biotic and abiotic cycling of iron.

Steep gradients of dissolved metals suggest that manganese and iron are consumed below the oxycline via oxidation processes generating particulate metal that tend to sink in the water column. The oxidation of iron and manganese can be driven biotically or abiotically with oxygen. It is also possible that manganese serves as an oxygen carrier, forming MnO_x particles at the oxycline that rapidly oxidize iron and thus regenerating Mn(II) . We tested this abiotic potential for iron oxidation by MnO_x by incubating lake water with added hydrous MnO_2 under alternating light and dark conditions (Fig S1). The addition of MnO_2 at 5-fold higher concentrations than lake water iron caused a complete oxidation of iron within the 15 min time period between MnO_2 addition and taking the first sample (t_0). Indeed, it is known that the reaction of MnO_2 with Fe(II)

is almost instantaneous at $> 1 \mu\text{M}$ concentrations and circumneutral lake water pH (Lovley and Phillips (1988b); Myers and Nealson (1988)). Thus, manganese could be partially responsible for iron oxidation in the Lake Cadagno chemocline.

Light is an important factor regulating iron speciation by stimulating *in situ* oxygen production, driving anoxygenic phototrophic iron oxidation, or by direct photooxidation of iron (Braterman et al. (1983)). In the day profile, the zone of iron consumption between 11 and 11.5 m coincided with the sharp decrease in light intensity from 3.2 to $0.5 \mu\text{E m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR). Given that the photolysis of Fe^{2+} has only been shown to occur with UV light at $\text{pH} > 6.5$ (Braterman et al. (1983)), this process is unlikely to contribute significantly to iron turnover in Lake Cadagno). The lower threshold for oxygenic photosynthesis in freshwater is estimated to be $0.09 \mu\text{E m}^{-2} \text{s}^{-1}$ (Gibson (1985); Raven et al. (2000)) and therefore *in situ* oxygen production could fuel microaerophilic iron oxidation. Indeed, oxygenic photosynthesis in the upper part of the anoxic zone has previously been shown to play a major role in methane removal (Milucka et al. (2015)). Because oxygen concentrations remained below detection limits, we could not assess the contribution of microaerophilic iron oxidizing bacteria based only on the chemical profiles. It has been shown that photoferrotrophs can thrive by oxidizing iron at comparably low light intensities (Lliros et al 2015). It is likely that at these organisms play an important role in the chemocline considering the abundance of anoxygenic phototrophs in this zone. Photoferrotrophy is consistent with our observations that iron was consumed about 1 m below the oxycline during daylight hours, while iron and oxygen gradients intersected during the night (Fig 1). In sum, mainly light-driven processes are likely responsible for the oxidation of iron during the day, whereas

oxidation of iron appears to be primarily driven by downwards-diffusing oxygen and/or Mn oxides in the dark.

Integrating the iron flux over the steep gradient region just below the oxycline, we obtained iron oxidation rates of 1.393-13.064 $\mu\text{mol Fe}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ during the day and 0.648-6.070 $\mu\text{mol Fe}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ at night. These light oxidation rates are very similar to rates of phototrophic iron oxidation estimated from Lake La Cruz (Table 1). The iron-oxidizing anoxygenic phototrophs use the following reaction to drive CO_2 fixation (Ehrenreich and Widdel, 1994):

Assuming that photoferrotrophs account for all of the daylight iron oxidation in the chemocline, we calculated the amount of CO_2 fixed using the stoichiometry in equation (1). The estimated contribution of phototrophic iron oxidation to the total daytime carbon fixation processes in the chemocline of Lake Cadagno is 0.348-3.267 $\mu\text{mol C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$. With respect to average light carbon fixation rates of 23.7 $\mu\text{mol C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ measured in 2014, photoferrotrophic activity could account for up to 10 % of the total primary production. Considering that phototrophic sulfide oxidation is responsible for up to 85% (Musat et al. (2008)) of total carbon fixation in the chemocline, iron cycling could make up a large fraction of the remaining carbon fixation processes.

The failure to detect any oxidized manganese or iron species suggests that oxidation and reduction processes must be tightly coupled. In other words, no net oxidation could be observed because oxides were reduced as rapidly as they were formed. Oxidized manganese and iron can be reduced by bacteria utilizing simple organic carbon compounds or H_2 (Lovley and Phillips, 1988a). High primary productivity at and above the chemocline (Del Don et al., 2001) likely supplies ample organic compounds for bacterial iron reduction. Alternatively, sulfide can

chemically reduce iron and manganese resulting in the formation of FeS and S⁰ and various other products (Canfield (1989); Thamdrup et al. (1994)). According to Canfield et al. (2010), the highest rates of sulfate reduction in the water column occurred within the mixed bacterial layer, although no free H₂S was detected there. Because it is possible that any sulfide produced is immediately consumed, we used the same sensitive radiotracer method as (Canfield et al., 2010) to measure sulfate reduction rates at various depths. In contrast to (Canfield et al., 2010), we did not detect any sulfate reduction in the chemocline above the sulfidic zone (data not shown). However, sulfide could also be formed from sulfur reduction or disproportionation. Low concentrations (0.6 µmol·l⁻¹) of reduced sulfur could occasionally be measured within the chemocline in the dark (Fig S2). Sulfide reacts rapidly with Fe (III) or Fe(II) to form FeS (cite), which is detected as total sulfide by the Cline (1969) method. It is therefore possible that when photosynthetic sulfide oxidation ceases in the dark, heterotrophic bacteria compete with abiotic Fe(III) reduction by sulfide.

Overall, our results confirm that zones of iron reduction and sulfate reduction in Lake Cadagno are spatially separated. This is in contrast to recent findings by (Hansel et al., 2015) that even in sulfate-poor environments, sulfur cycling largely drives iron cycling in a manner inverse to thermodynamic predictions. Hansel *et al.* did not observe microbial iron respiration until sulfate in the system was depleted by sulfate-reducing microorganisms. In Lake Cadagno, iron reduction is not fueled by sulfate reduction despite the ~1 mmol·l⁻¹ available sulfate in the chemocline. It is therefore not possible to generalize that sulfur cycling is the dominant force in iron cycling, but the environmental factors governing the competition between iron-reducing and sulfate-reducing bacteria remain to be investigated.

Diversity of putative iron-oxidizing and -reducing bacteria

We investigated the diversity of putative iron-oxidizing and -reducing bacteria by 16S rRNA gene amplicon sequencing from the chemocline in 2013 and 2014. A total of 213,726 sequences were recovered from 2013, which clustered into 8,395 operational taxonomic units (OTUs) at 98% sequence identity, whereas the 159,032 sequences recovered from 2014 clustered into 7,221 OTUs. Taxonomic classification based on closest BLAST hit revealed that majority of 16S rRNA gene sequences, ~63 % from libraries from both 2013 and 2014, affiliated with the purple sulfur bacteria from the genus *Chromatium* (Fig 2). Among the remaining 16S rRNA gene sequences, we identified putative Fe(II)-oxidizers and Fe(III)-reducers based on close sequence affiliation with known iron-oxidizing and -reducing organisms. Approximately 6-7% of all sequences affiliated with known Fe(III)-oxidizing bacteria, and 1% affiliated with known Fe(III)-reducers. It is likely that the full diversity of iron cycling bacteria was underestimated here because many sequences affiliated with groups containing no cultured representatives and as such their metabolism is not yet known.

The fraction of bacteria capable of iron reduction was comprised of heterotrophs capable of reducing iron directly (e.g. *Geobacter*, *Geothrix*) or indirectly (e.g. *Pseudomonas*, *Clostridium*). The bacteria capable of iron oxidation were comprised of both phototrophic bacteria (e.g. *Chlorobium*, *Rhodobacter*) and microaerophilic as well as nitrate-reducing chemotrophic bacteria (e.g. *Leptothrix*, *Leptospirillum*). Sequences from microaerophilic Fe(II)-oxidizers accounted for less than 10% of the sequences of putative Fe(II)-oxidizing bacteria. A few of the bacteria identified are capable of biomineralizing iron without utilizing it for energy gain (e.g. *Magnetospirillum*). These magnetotactic bacteria contribute to the fate of iron by manufacturing magnetosomes composed of mixed valence iron, either as magnetite (Fe_3O_4) or greigite (Fe_3S_4).

Cryptic iron cycling in a co-culture of iron-oxidizing and -reducing bacteria

Although the 16S rRNA gene amplicons revealed a potential for microbial iron cycling in the water column of Lake Cadagno, it remained to be demonstrated that these bacteria were truly capable of actively oxidizing or reducing iron. We set up enrichment cultures using water collected in August 2013 to investigate which of the organisms might be involved in iron cycling in Lake Cadagno. Fe(II) and acetate were added in a 10:1 ratio to lake water in anoxic serum bottles which were incubated under light of wavelength >780 nm to prevent oxygenic photosynthesis. After 3 months, a pink-to-orange coloration developed, suggesting the formation of iron oxide and/or photopigments. 16S rRNA gene clone libraries of this enrichment culture revealed 4 relevant groups of bacteria: *Rhodomicrobium* sp., *Rhodoferax* sp., *Geothrix* sp., and *Chlorobium* sp. (Table S1). Cells tended to form aggregates in which close relatives of the known photoferrotroph *Rhodomicrobium vannielii* could be easily identified under the microscope based on its conspicuous stalk-like morphology (Fig S3a). Active iron oxidation by *R. vannielii*-like cells was evidenced in culture by cell encrustation in Fe-P oxides (Fig S3b,c,d). It is unlikely that such encrustation occurs in the water column where *in situ* iron and phosphate concentrations are much lower, and encrustation and aggregation of bacteria would cause them to sink out of the photic zone. Another member of the enrichment culture was closely related to *Rhodoferax ferrireducens*, which can reduce iron with acetate (Finneran et al., 2003) and was among the most abundant sequence (51.7%) of iron-reducers recovered from *in situ* samples in 2014. *Geothrix fermentans* and other *Geothrix/Geobacter* species are also capable of reducing iron with acetate, formate, and other short-chain fatty acids (Coates et al., 1999) (Lovley et al 1993, Caccavo et al 1994). The remaining sequences from the enrichment belonged to anaerobic *Firmicutes* which

were presumably growing by mixed fatty acid fermentation. In 2014 we were able to enrich bacteria closely related to *Chlorobium ferrooxidans* and *Rhodopseudomonas pseudopalustris*, the most abundant photoferrotrophs identified in our *in situ* 16S rRNA gene amplicon libraries, by adding Fe(II) and acetate (Table S1). It was thus apparent that although in our enrichment we targeted iron-oxidizing bacteria with the addition of Fe(II), we had simultaneously enriched Fe(III)-reducers. We speculate that the iron reducing bacteria in our cultures thrived on oxidized iron produced by phototrophic iron oxidizers. Our data suggest that these consortia were originally present in the lake water and readily re-established in our *in vitro* incubations.

To monitor whether iron cycling processes were linked to microbial growth, we set up incubations with iron and ^{13}C -DIC or ^{13}C -acetate using the enrichment culture dominated by *R. vannielii*- and *R. ferrireducens*-like bacteria. Fe(II)-sulfate was added to the cultures which had accumulated Fe(III) over time, resulting in a 50:50% mixture of Fe(II):Fe(III) and ultimately targeting the activity of both the iron oxidizers and reducers. During the 48 h incubation period, the iron speciation fluctuated slightly under both light and dark conditions as compared to the killed control (Fig 3a). This could be due to the delicate balance between oxidation and reduction processes, or the heterogeneous distribution of bacterial aggregates in replicate tubes. Overall, in dark incubations, we observed that Fe(II) concentrations continued to increase after 2 to 3 weeks and only 10% of Fe(III) remained. On the contrary, no net phototrophic iron oxidation could be observed in the light, presumably because heterotrophs immediately consumed Fe(III) products thus generating a cryptic iron cycle.

As with our *in situ* measurements, it was not possible to demonstrate microbial iron oxidation activity in our incubations based on changes in iron speciation alone. However, by measuring ^{13}C -

CO₂ uptake into biomass (Fig 3b) we could detect light-dependent CO₂ fixation which was likely performed by the only potential autotroph in our cultures, the *Rhodomicrobium vannielii*-related bacteria. The carbon fixation rate was not linear, possibly due to heterogeneous activity of bacteria within aggregates, as heavily iron-encrusted bacteria (Fig S3b) were likely not active. Because of the variability in carbon assimilation in our time series, we calculated both a minimum carbon fixation rate (linear regression through t₀, t₂, t₃, and t₄; r² = 0.952) and a maximum carbon fixation rate (linear regression through t₀, t₁, and t₆; r² = 0.714), giving rates of 1.2 μmol C l⁻¹·d⁻¹ and 7.6 μmol C l⁻¹·d⁻¹, respectively. Based on these rates and using the stoichiometry from equation 1, we determined iron oxidation rates in the range of 4.9-30.6 μmol Fe l⁻¹·d⁻¹. These rates are well within the range of volumetric iron oxidation rates experimentally determined for purple sulfur and non-sulfur bacteria (100-200 μmol Fe l⁻¹·d⁻¹) at light intensities similar to our experiments (Hegler et al. (2008)). Contrary to our expectations, the iron oxidation rates determined from our incubations were only slightly higher than the rates calculated from iron gradients in the lake (1.3-13.1 μmol Fe l⁻¹·d⁻¹). We anticipated the *in vitro* rates to be much higher because iron cycling bacteria were highly enriched in our cultures and because we conducted experiments at higher than *in situ* light intensities. It has been shown that microbial iron oxidation rates are dependent on both light quality and light intensity (Straub et al. (1996)), and the light level at the top of the chemocline is on average 1-2% of surface radiation (see PAR profile, Fig 1), or much lower than in our incubations. One explanation for these comparable iron oxidation rates is that the use of a light filter >750 nm halted oxygenic photosynthesis, which may in part contribute to iron oxidation *in situ*. In fact, this indicates that photosynthetic oxygen production is coupled to microaerophilic iron oxidation to a large extent, despite the low relative sequence

abundance of microaerophilic Fe(II)-oxidizers compared to anaerobic Fe(II)-oxidizers in our 16S rRNA gene amplicon libraries.

Respiration of added ^{13}C -acetate and ^{13}C -formate, used as a proxy for heterotrophic activity, was measured as $^{13}\text{CO}_2$ production. As expected, $^{13}\text{CO}_2$ concentrations increased linearly in a light-independent manner (Fig 3c). The calculated CO_2 production rate was $5.8 \mu\text{mol CO}_2 \text{ l}^{-1}\cdot\text{d}^{-1}$. The respiration of acetate and formate with iron are stoichiometrically different. The oxidation of acetate to CO_2 coupled to iron reduction proceeds according to equation (2):

Using the CO_2 production rate above, the iron reduction rate with acetate would be $23.2 \mu\text{mol Fe}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$. Alternatively, *Geothrix fermentans* and related organisms are known to utilize formate as an electron acceptor for iron reduction (Coates et al. (1999)) with the following stoichiometry:

This converts to an iron reduction rate in our cultures of $11.6 \mu\text{mol Fe}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$. Therefore, we can approximate that the true iron reduction rate lies somewhere within the range of $11.6\text{-}23.2 \mu\text{mol Fe}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, depending on the ratio of organic carbon compounds respired. These calculated *in vitro* iron reduction rates were very similar to the *in vitro* iron oxidation rates ($4.9\text{-}30.6 \mu\text{mol Fe l}^{-1}\cdot\text{d}^{-1}$) and this close balance between iron oxidation and reduction rates might explain why no net changes in iron speciation could be observed in our light incubations. This tight coupling of oxidation and reduction processes may also regulate iron speciation in the lake. There, the predominance of Fe(II) species indicates that Fe(III) is limiting because it is consumed before it can be detected, and thus iron reduction proceeds more rapidly than iron oxidation.

A fraction of the labeled organic compounds was also assimilated into biomass for growth. Evidently, uptake of ^{13}C -labeled fatty acids occurred both in the dark and the light (Fig 3d), but the much higher ^{13}C assimilation in the light can most likely be attributed to photoassimilation of ^{13}C -acetate by *Rhodomicrobium* sp. It has previously been shown that phototrophic growth of *R. vannielii* with Fe(II) as the electron donor was stimulated by the addition of acetate (Heising and Schink, 1998).

Microbial FeS oxidation

Due to the scavenging of iron by sulfides, significant iron cycling is not expected to occur in euxinic lakes where FeS particulates settle out of the water column. The periodic appearance and disappearance of FeS in the Lake Cadagno chemocline suggests the potential for rapid microbial recycling of FeS rather than removal via sedimentation. Because bacteria in the lake are most likely recurrently exposed to FeS, we investigated the capacity for microbial FeS utilization by setting up enrichment cultures with FeS as the sole electron donor. Cultures were also provided with a light source $>750\text{ nm}$. After about 6 months of continuous incubation, cultures were dominated by purple sulfur bacteria morphologically similar to *Thiodictyon* sp. that appeared to contain gas vacuoles allowing them to float at the liquid surface and sequences of *Thiodictyon* sp. were also recovered in our 16S rRNA clone library (Table S2). *Thiodictyon* sp. have been shown capable of oxidizing FeS to Fe(III) in culture (Kappler and Newman (2004)). Our enrichment culture also contained sequences closely related to *Ectothiorhodospira* sp., *Rhodoferax* sp., *Rhodomicrobium* sp., and some *Flavobacteria*- and *Cytophaga*-related sequences with low similarity (80-90%) to any cultured representatives (Table S2). Active FeS oxidation could be observed from the change in coloration of the medium from black, immediately after FeS

addition, to colorless within a few days. We set up incubations with stable isotopes to monitor autotrophic and heterotrophic ^{13}C uptake linked to FeS cycling processes. Throughout the experiment, the percentage of reduced iron remained stable in the light, dark, and killed control conditions (Fig 3e). However, phototrophic sulfide oxidation occurred, as evidenced by the steady decrease in sulfide (Fig 3f) and a concomitant uptake of CO_2 (Fig 3g) into biomass in the light, but not in the dark. Our cultures appeared to preferentially oxidized sulfide, presumably due to the higher energy gain of S^{2-} relative to iron oxidation. Still, the recovery of many *Rhodospirillum rubrum* sp. sequences suggests that after the depletion of sulfide, iron may be oxidized and serves as a substrate for this heterotrophic iron reducer. ^{13}C -organic carbon (Fig 3h) was assimilated into biomass in the light. We did not, however, observe any organic carbon assimilation in the dark, nor did we observe any $^{13}\text{CO}_2$ production (from ^{13}C -organic carbon respiration) in the light or the dark. This implies that heterotrophs were not active in the presence of FeS as they lacked an electron acceptor. Instead, the organic carbon uptake in the light most likely resulted from photoassimilation of acetate by *Thiodictyon* sp. or *Ectothiorhodospira* sp. ((Imhoff and Trüper, 1981; Peduzzi et al., 2012)). We recovered not only sequences of *Thiodictyon* from our *in situ* 16S rRNA gene libraries, but also sequences of other bacteria such as *Rhodobacter* and *Chromatium* previously shown to oxidize FeS (Ehrenreich and Widdel, 1994). Overall, our experiments imply that FeS_x is not the ultimate sink for iron (nor sulfide), as bacteria in the Lake Cadagno chemocline can rapidly recycle this compound.

CONCLUSIONS

Because the redox transformation of iron involves the transfer of a single electron, it has generally been assumed that high iron concentrations are a prerequisite for substantial microbial energy

gain from iron metabolism. Microbial iron-cycling may therefore seem negligible in a system like Lake Cadagno with only several micromoles per liter of reactive iron, especially because concentrations of oxygen, methane, and sulfide are orders of magnitude higher. Here we show that it is not the absolute concentration of the iron that is important, but rather its rate of turnover. High fluxes of iron in the Lake Cadagno chemocline are evidence for high rates of microbial iron oxidation and reduction. Remarkably, these fluxes and rates are on the same order of magnitude as iron fluxes in ferruginous lakes where microbial iron cycling has previously been studied. It appears that microbial redox transformations of iron are important in terms of energy generation and carbon turnover in Lake Cadagno, supporting diverse populations of iron metabolizing bacteria. Moreover, distinct populations of chemotrophic and phototrophic iron oxidizers coexist on this limiting substrate although it is not clear if these different microbes are in direct competition with one another or if they are adapted to specific light and chemical conditions. Light is an important driver in the Lake Cadagno iron cycle, intensifying microbial iron cycling either through direct stimulation of photoferrotrophy or indirectly fueling microaerophilic iron oxidation by stimulation of *in situ* oxygen production by photosynthetic algae.

Our profiles indicate that Mn may also be intensively recycled at the chemocline of Lake Cadagno and strongly impacts the iron cycle. The flux of manganese ($0.049\text{--}0.458\ \mu\text{mol Mn}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$) was even higher than the iron flux, revealing a large potential for microbial activity. Traditionally, manganese species have been measured as dissolved [Mn(II)] and particulate [Mn(IV)] fractions due to methods limitations in distinguishing these fractions directly. Recently, the more unstable Mn(III) species was shown to be an important intermediate in Mn redox cycles at oxic-anoxic interfaces (e.g. Trouwborst et al. (2006); Madison et al. (2013)). Using new methods to detect

Mn(III) photometrically (Camacho et al. (2009)), it may be possible to elucidate a microbially-driven manganese cycle in Lake Cadagno as well.

Finally, our enrichment cultures served as a model for processes occurring in Lake Cadagno with co-existing populations of bacteria simultaneously oxidizing and reducing iron. This tight coupling between the oxidative and reductive reactions generates an invisible or “cryptic” iron cycle (Fig 4). Whereas iron oxidation in our *in vitro* incubations ceased in the dark, nighttime iron oxidation in the lake could be continuously driven by oxygen diffusing downwards from the oxycline or by sinking manganese oxides. These laboratory observations help to explain the *in situ* profiles of ferrous iron and emphasize that the failure to detect any reaction intermediates or net changes over time is not evidence of zero activity. Moreover, we must revise the common assumption that active iron cycling is negligible in stratified waters containing low iron concentrations. Given that such shallow, seasonally stratified, low-iron lakes are common, cryptic iron cycling may be a widespread process on modern Earth and not restricted to the ferruginous Archaean ocean.

EXPERIMENTAL PROCEDURES

Sampling

Lake Cadagno is a meromictic alpine lake located in the Piora valley, Switzerland. Samples were taken on field trips in August 2013, September 2013, and August 2014. For sampling and *in situ* measurements a profiling ion analyzer (see Kirf et al. (2014) for description) was lowered from a platform anchored at the deepest part of the lake (20.7 m). Conductivity, turbidity, depth (pressure), temperature and pH were measured with a multi-parameter probe (XRX 620, RBR). Dissolved oxygen was recorded online with two micro-optodes with detection limits of 125 nM (normal) and 20 nM (trace) (types PSt1 and TOS7, PreSens). Samples for Winkler titration were

taken from the lake surface to calibrate the oxygen sensors. Water samples for chemical analyses and cell counts were taken with a rosette syringe sampler equipped with twelve 60-ml syringes which were triggered online at the appropriate depths. The night profile from August 2014 was measured with a SBE 19 plus V2 CTD probe (Sea-Bird Electronics, WA, USA) with attached sensors for pressure, temperature and conductivity, and with additional sensors for turbidity (WET Labs Eco), oxygen (SBE 43), pH (18-I) and two fluorescence wavelengths (WET Labs ECO-AFL, FL, USA). Samples were obtained using a pump-cast system, pumping water through a tube attached to the CTD and filling 60-ml syringes on board after rinsing twice with the pumped water.

Filtered (0.22 μm pore size) samples for iron speciation were fixed immediately with ferrozine reagent. Filtered (0.45 μm pore size) and unfiltered samples for total manganese and iron were added to vials containing HNO_3 to a final concentration of 0.1M. Sulfide samples were fixed with Zn-acetate to a final concentration of 0.1 % (w/v). Water samples for incubation experiments and molecular analyses were pumped through clean tubing attached to the CTD and collected in 1 L Duran bottles. Bottles were stored at 4°C in the dark until further processing. Samples for catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) analysis were immediately fixed with 2% (v/v) formaldehyde. Samples for DNA analysis were filtered onto polycarbonate filters (0.22 μm pore size) on site and frozen at -20°C until further processing.

Analytical Techniques

Iron and sulfide were measured spectrophotometrically using the ferrozine method (Stookey (1970)) and the Cline (1969) methods, respectively. Total and dissolved iron and manganese were measured by inductively coupled plasma optical emission spectrometry (Optima 3300 R ICP-OES;

PerkinElmer, Waltham, MA, USA). Sulfate was measured on a 761 Compact ion chromatograph (Metrohm, Filderstadt, Germany) equipped with a Metrosep A SUPP 5 column.

Sulfate reduction rates were measured in duplicates at four depths: 12, 13, 14 and 15 m. The radiotracer $^{35}\text{SO}_4^{2-}$ (5 MBq) was added to lake water incubated anoxically in 50-ml glass syringes. The $\sim 20 \mu\text{mol}\cdot\text{l}^{-1}$ background zero-valent sulfur served as a trap for any H_2^{35}S that might be re-oxidized. At each sampling point, 10 ml of sample was dispensed into 5 ml of 20% (w/v) Zn-acetate. Reduced sulfur species (e.g. sulfur and sulfide as ZnS) were separated out via the chromium distillation method described in Kallmeyer et al. (2004) and the radioactivity per sample was determined via scintillation counting (Packard 2500 TR).

Flux Calculations

Turbulent fluxes (J) of iron and manganese within the mixed chemocline were calculated assuming steady state by applying Fick's first law: $J = -D\partial C/\partial x$. The maximum concentration change of total dissolved iron (∂C_{Fe}) was computed over 30 cm and 41.25 cm (∂x) in light and dark profiles (Fig 1), respectively, at the top of the chemocline where steep gradients exist. The change in total dissolved manganese (∂C_{Mn}) was calculated over 150 cm and 41 cm in light and dark profiles, respectively. Reported turbulent diffusion coefficients for the Lake Cadagno water column (that is, Eddy diffusivity D) are $1.6 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ at the chemocline boundaries and $1.5 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ within the chemocline (Wüest (1994)). We used these values as the minimum and maximum turbulence coefficients to calculate the upper and lower limits to iron fluxes.

***In situ* water incubations**

The 1-L Duran bottles with water collected from chemocline were bubbled with N_2 for 15 min to remove any contaminant O_2 . Experiments to monitor the interaction of Fe and Mn were

performed by supplementing water with hydrous MnO_2 , prepared according to Golden et al. (1987). Water was distributed anoxically into 50-ml glass Winkler bottles and sealed with no headspace. Bottles in the incubation series were maintained at 4-6°C in a water bath and exposed to approximately 6 h darkness, 12 h light, and then 4 h darkness. At each sampling time point a bottle was sacrificed and sampled for iron, manganese, and sulfide as described above. CO_2 fixation rates were assessed separately by adding a ^{13}C -DIC solution to anoxic chemocline water to a final concentration of 200 μM (or 10% ^{13}C -DIC). The incubation series was performed in Winkler bottles at 4-6°C for 12 h in the light. Bottles were sampled destructively at each time point by concentrating ^{13}C -labeled biomass onto precombusted (6 h at 600 °C) GF/F filters (Whatman, St Louis, MO, USA). At the beginning of the experiment, a water sample was sterile-filtered into a glass HPLC vial to verify the ^{13}C -DIC labeling percentage.

Enrichment cultures

Water from the chemocline was distributed within an anoxic glove box (Mecaplex, Grenchen, Switzerland) into serum bottles containing $\text{N}_2:\text{CO}_2$ (90:10) headspace. To target Fe(II)-oxidizing bacteria, a sterile, anoxic solution of FeSO_4 was added to a final concentration of approximately 1 $\text{mmol}\cdot\text{l}^{-1}$. To target FeS-oxidizing bacteria, a FeS solution was prepared by mixing equimolar concentrations of FeSO_4 and Na_2S and then added to Lake Water at concentrations of approximately 0.5 $\text{mmol}\cdot\text{l}^{-1}$. Both cultures were periodically supplemented with 0.1 $\text{mmol}\cdot\text{l}^{-1}$ Na-acetate. Cultures were maintained at 10°C under a filter excluding light <750 nm to prevent the growth of oxygenic phototrophs. After 2-3 months, Fe(II) cultures developed a faint reddish color, possibly due to purple bacterial pigments or the formation of ferric iron precipitates. The FeS cultures were colored black by the addition of FeS and turned clear after several days. Cultures

were transferred to new artificial freshwater medium (see below) every 2-4 weeks for 1 year before experiments were performed.

Isotope labeling experiments

Freshwater medium was prepared according to the recipe of Pfennig (1991) for cultivation of purple and green non-sulfur bacteria using a concentration of 1-2 mmol·l⁻¹ HCO₃⁻ to match *in situ* DIC concentrations. For incubations with Fe(II), approximately 0.5 mmol·l⁻¹ FeSO₄ was added to medium mixed with enrichment culture to a ratio of 1:1. To monitor photoautotrophic iron oxidation, cultures received 2% ¹³C-DIC and plus 100 μM acetate and 100 μM formate before being aliquoted anoxically as described above into Hungate tubes and sealed with rubber stoppers. Half of these tubes were incubated at room temperature in the light (~300 lux) and half of these were incubated in the dark. To monitor heterotrophic iron reduction, cultures received a mixture of 100 μM of 10% ¹³C-labeled acetate (¹³CH₃¹³CO₂) and 100 μM of 10% ¹³C-labelled sodium formate (H¹³COONa) before being aliquoted anoxically into Hungate tubes and sealed with rubber stoppers. Half of these tubes were incubated in the light and half of these were incubated in the dark. After 0, 5, 11, 25, 31, and 50 hours a tube from each condition was sampled destructively for iron and carbon analyses. Iron species were measured immediately and diluted in 0.5 M HCl when necessary. Samples for ¹³C incorporation into biomass were filtered as described above and parallel samples for ¹³CO₂ production measurements were sterile-filtered directly into glass HPLC vials and sealed with no headspace.

For incubations with FeS, approximately 0.5 mmol·l⁻¹ FeS was added to medium mixed with enrichment culture to a 1:1 ratio. The resulting iron and sulfide concentrations measured were 450 μM and 350 μM, respectively, or a 100 μM excess of Fe(II). The incubation setup and sampling

was identical to that described above, except that only 100 μM of 10% ^{13}C -labelled acetate was added. Additional samples were also taken for sulfide determinations by fixing in Zn-acetate to a final concentration of 1% (w/v).

Mass spectrometry isotope uptake analysis

Heterotrophic respiration of ^{13}C -acetate and ^{13}C -formate was measured as production of $^{13}\text{CO}_2$. For this, 1 ml of culture filtrate in HPLC vials was transferred into a 12 ml Exetainer (Labco Limited, High Wycombe, UK) and the headspace was exchanged with He. Samples were then acidified with the addition of $\sim 100\ \mu\text{l}$ concentrated H_3PO_4 so the outgassed $^{13}\text{CO}_2$ could be analyzed using a GasBench II coupled to a Delta Plus isotope ratio mass spectrometry (IRMS; Thermo Finnigan, Dreieich, Germany). The incorporation of ^{13}C from $^{13}\text{CO}_2$ or ^{13}C -labelled organic compounds into biomass was measured by combustion of the particulate organic carbon fraction on GF/F filters. Filters were first decalcified by incubation with 37% fuming HCl overnight, dried in an oven at 60°C , and packed into tin capsules for combustion analysis. The C-isotopic composition of particulate organic carbon in these experiments was determined by an automated elemental analyser (Thermo Flash EA, 1112 Series) coupled to a Delta Plus XP IRMS (Thermo Finnigan, Dreieich, Germany). Instrument accuracy and precision were estimated at 1.06589 ± 0.00079 ^{13}C atom% based on the mean and 3 standard deviations of caffeine standards measured in parallel with the samples.

SEM-EDS

Samples for SEM were fixed with 2% (v/v) formaldehyde for no more than 24 hours at 4°C and pelleted by centrifugation at 5000 RCF for 3 min. Cell pellets were washed with MilliQ water before dehydration in graded ethanol, followed by critical point drying (CPD). Because the harsh

chemical treatment used in CPD tends to leach compounds from cells, samples for EDS analysis were simply fixed with formaldehyde, filtered onto gold-palladium sputtered filters, and washed with MilliQ. Dried aggregates or filter pieces were mounted on electrically conductive, adhesive stubs (Leit-Tab; Plano GmbH, Wetzlar, Germany). Imaging was performed with a FEI environmental field emission SEM Quanta 250 FEG (FEI, Eindhoven, Netherlands) at an electron energy of 2 keV using the Everhart-Thornley secondary electron detector (ETD). EDS measurements were performed at an electron energy of 10 keV with an EDS system Quantax 400 with an XFlash 6/30 double detector system (Bruker Nano GmbH, Berlin, Germany). The detectors have an energy resolution of < 123 eV at MnK α .

CARD-FISH

Samples were fixed with 2 % (v/v) formaldehyde for 24 hours at 4°C and filtered onto 0.2 μ m polycarbonate filters (Millipore). CARD-FISH was performed on filter pieces following the protocol of Pernthaler et al. (2002). Filters were first embedded in 0.1 % (w/v) agarose to prevent cell detachment, then incubated for 10 min at room temperature in 0.01 M HCl to inactivate endogenous peroxidases. Cells were permeabilized by digestion with lysozyme (Sigma-Aldrich), 10 mg ml⁻¹, for 30 min at 37°C. The probe Cmok453 was used to target *Chromatium okenii* Tonolla et al. (1999). Hybridization was performed at 46°C for 2.5 h using 2 μ l of horseradish peroxidase(HRP)-labeled oligonucleotide probe mix per 600 μ l of hybridization buffer containing 35% formamide. Filters were washed in 50 ml wash buffer at 48°C for 15 min and 1xPBS for 10 min. The probe signal was amplified with the tyramide Oregon Green 488 (1 μ l ml⁻¹) for 20 min at 37°C, and then washed in 1xPBS for 10 min, and then MilliQ. Filter pieces were counterstained with 4',6-diamidino-2-phenylindole (DAPI, 1 μ g ml⁻¹) and embedded in a 4:1 mix of

Citifluor/Vectashield and mounted onto glass slides. Cell numbers were enumerated with a grid ocular of an epifluorescence microscope (Nikon ECLIPSE Ci) by counting a minimum of 1000 total cells in randomly selected fields of view.

DNA extraction, sequencing, and analysis

DNA from the 2013 enrichment cultures was extracted by centrifuging 15 ml of culture and processing the cell pellet with the PowerSoil DNA Kit (MoBio Laboratories, Carlsbad, USA) according to the accompanying protocol with the following modification: the bead beading step was reduced to 30 sec followed by incubation on ice for 30 sec, and this was repeated a total of 4 times. The 16S rRNA genes were amplified using oligonucleotide primers GM3 (5'-AGAGTTTGATCMTGGC-3') and GM4 (5'-TACCTTGTTACGACTT-3') (Muyzer et al 1993) under standard PCR conditions (see SI). PCR products were purified by centrifugation through Multi Screen 96-well plates (Millipore, Billerica, MA, USA) filled with Sephadex G-50 Superfine powder (GE Healthcare Bio-science, Göteborg, Sweden). Cleaned PCR products were ligated to the vector pCR™4-TOPO® TA and cloned into the chemically competent *Escherichia coli* strain TOP10 using the TOPO-TA cloning kit (Thermo Fisher Scientific) following the manufacturer's protocol. Clones were screened by PCR for correct-sized inserts, re-purified on Sephadex plates, and sequenced with the BigDye Terminator cycle sequencing kit, version 3.1 (Applied Biosystems, Darmstadt, Germany), with vector primers M13R (5'-TTCACACAGGAAACAGCTATGACC-3') and M13F (5'-ACGACGTTGTAAAACGACGGCCAG-3') using PCR conditions specified in the SI.

DNA from the 2014 enrichment culture and environmental DNA was extracted from polycarbonate filters with the MoBio PowerSoil DNA kit as described above. Primers 341F and 805R (Herlemann et al. 2011) were used to target the V3- V4 region of the 16S rRNA gene. The

forward primers were modified to introduce a sample-specific barcode at the 5' end for multiplex sequencing. Ten parallel PCR reactions were run as specified in the SI. All replicate reactions were pooled and DNA was concentrated using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's protocol, with a final elution step in 30 µl TE buffer. The DNA was then gel-purified using SYBR Green I Nucleic Acid Gel Stain (Invitrogen) and the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturers' protocol. DNA concentration was determined fluorometrically at 260 nm, using the Qubit 2.0 Fluorometer and the Qubit dsDNA HS Assay KIT (Invitrogen). PCR products were pooled in equimolar concentrations and sent to the Max Planck-Genome Centre (Cologne, Germany) for sequencing. Bacterial 16S rRNA gene amplicons were sequenced by Illumina MiSeq (Illumina Inc., USA) paired end (2x 300 bp) sequencing following a TruSeq library preparation. Paired end reads were merged with the software package BBmap v4.2 (overlap >50 bases). Merged reads were quality trimmed (q30) and split by barcodes with MOTHUR (Schloss et al 2009) and uploaded to SilvaNGS (Quast et al. (2013)). Reads shorter than 200 bp, reads containing >2% homopolymers or ambiguities, and reads with an alignment identity <50% were excluded from analyses. The remaining reads were de-replicated and clustered at 98% sequence identity. Taxonomic assignment was based on a local nucleotide BLAST (Camacho et al. (2009)) search against the SILVA 16S rRNA SSU reference database, release 119.

Nucleotide sequence accession numbers

All nucleotide sequences from this study have been deposited in the NCBI nucleotide database under GenBank accession numbers *pending*. The 16S rRNA gene Illumina tag sequences can be retrieved from the ENA Sequence Read Archive under study accession number PRJEB13589/

ERP015149 (not public; accessible to reviewers upon request). Additional fasta files for review are submitted alongside this manuscript.

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REFERENCES

- Benz, M., Brune, A., and Schink, B. (1998) Anaerobic and aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria. *Archives of Microbiology* **169**: 159-165.
- Braterman, P.S., Cairns-Smith, A.G., and Sloper, R.W. (1983) Photo-oxidation of hydrated Fe²⁺;—significance for banded iron formations. *Nature*. **303**: 163-164
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. (2009) BLAST+: architecture and applications. *BMC bioinformatics* **10**: 421.
- Canfield, D.E. (1989) Reactive iron in marine sediments. *Geochimica et Cosmochimica Acta* **53**: 619-632.
- Canfield, D.E., Farquhar, J., and Zerkle, A.L. (2010) High isotope fractionations during sulfate reduction in a low-sulfate euxinic ocean analog. *Geology* **38**: 415-418.
- Cline, J.D. (1969) spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* **14**: 454-458.
- Coates, J.D., Ellis, D.J., Gaw, C.V., and Lovley, D.R. (1999) *Geothrix fermentans* gen. nov., sp. nov., a novel Fe (III)-reducing bacterium from a hydrocarbon-contaminated aquifer. *International Journal of Systematic and Evolutionary Microbiology* **49**: 1615-1622.
- Coby, A.J., Picardal, F., Shelobolina, E., Xu, H., and Roden, E.E. (2011) Repeated anaerobic microbial redox cycling of iron. *Applied and Environmental Microbiology* **77**: 6036-6042.
- Crowe, S.A., Jones, C., Katsev, S., Magen, C., O'Neill, A.H., Sturm, A. et al. (2008) Photoferrotrophs thrive in an Archean Ocean analogue. *Proceedings of the National Academy of Sciences* **105**: 15938-15943.
- Davison, W., Woof, C., and Rigg, E. (1982) The dynamics of iron and manganese in a seasonally anoxic lake; direct measurement of fluxes using sediment traps. *Limnology and Oceanography* **27**: 987-1003.
- Del Don, C., Hanselmann, K.W., Peduzzi, R., and Bachofen, R. (2001) The meromictic alpine Lake Cadagno: orographical and biogeochemical description. *Aquatic Sciences* **63**: 70-90.
- Egli, K., Wiggli, M., Klug, J., Gerss, J., and Bachofen, R. (2004) Spatial and temporal dynamics of a plume of phototrophic microorganisms in a meromictic alpine lake using turbidity as a measure of cell density. *Aquatic Microbial Ecology* **35**: 105-113.
- Ehrenreich, A., and Widdel, F. (1994) Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Applied and Environmental Microbiology* **60**: 4517-4526.
- Emerson, D., and Moyer, C. (1997) Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Applied and Environmental Microbiology* **63**: 4784-4792.

- Finneran, K.T., Johnsen, C.V., and Lovley, D.R. (2003) *Rhodoferrax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe (III). *International Journal of Systematic and Evolutionary Microbiology* **53**: 669-673.
- Fleming, E.J., Davis, R.E., McAllister, S.M., Chan, C.S., Moyer, C.L., Tebo, B.M., and Emerson, D. (2013) Hidden in plain sight: discovery of sheath-forming, iron-oxidizing Zetaproteobacteria at Loihi Seamount, Hawaii, USA. *FEMS Microbiology Ecology* **85**: 116-127.
- Gibson, C. (1985) Growth rate, maintenance energy and pigmentation of planktonic cyanophyta during one-hour light: dark cycles. *British Phycological Journal* **20**: 155-161.
- Golden, D., Chen, C., and Dixon, J. (1987) Transformation of birnessite to buserite, todorokite, and manganite under mild hydrothermal treatment. *Clays and Clay Minerals* **35**: 271-280.
- Halm, H., Musat, N., Lam, P., Langlois, R., Musat, F., Peduzzi, S. et al. (2009) Co-occurrence of denitrification and nitrogen fixation in a meromictic lake, Lake Cadagno (Switzerland). *Environmental Microbiology* **11**: 1945-1958.
- Hansel, C.M., Lentini, C.J., Tang, Y., Johnston, D.T., Wankel, S.D., and Jardine, P.M. (2015) Dominance of sulfur-fueled iron oxide reduction in low-sulfate freshwater sediments. *The ISME Journal* **9**: 2400-2412.
- Hegler, F., Posth, N.R., Jiang, J., and Kappler, A. (2008) Physiology of phototrophic iron (II)-oxidizing bacteria: implications for modern and ancient environments. *FEMS Microbiology Ecology* **66**: 250-260.
- Heising, S., and Schink, B. (1998) Phototrophic oxidation of ferrous iron by a Rhodomicrobium vannielii strain. *Microbiology* **144**: 2263-2269.
- Heising, S., Richter, L., Ludwig, W., and Schink, B. (1999) *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a “*Geospirillum*” sp. strain. *Archives of Microbiology* **172**: 116-124.
- Holmer, M., and Storkholm, P. (2001) Sulphate reduction and sulphur cycling in lake sediments: a review. *Freshwater Biology* **46**: 431-451.
- Imhoff, J.F., and Trüper, H.G. (1981) *Ectothiorhodospira abdelmalekii* sp. nov., a new halophilic and alkaliphilic phototrophic bacterium. *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene: I Abt Originale C: Allgemeine, Angewandte und Ökologische Mikrobiologie* **2**: 228-234.
- Johnson, K.S., Elrod, V.A., Fitzwater, S.E., Plant, J.N., Chavez, F.P., Tanner, S.J. et al. (2003) Surface ocean-lower atmosphere interactions in the northeast Pacific Ocean gyre: Aerosols, iron, and the ecosystem response. *Global Biogeochemical Cycles* **17**.
- Kallmeyer, J., Ferdelman, T.G., Weber, A., Fossing, H., and Jørgensen, B.B. (2004) A cold chromium distillation procedure for radiolabeled sulfide applied to sulfate reduction measurements. *Limnol Oceanogr Methods* **2**: 171-180.

- Kappler, A., and Newman, D.K. (2004) Formation of Fe (III)-minerals by Fe (II)-oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta* **68**: 1217-1226.
- Kirf, M.K., Dinkel, C., Schubert, C.J., and Wehrli, B. (2014) Submicromolar oxygen profiles at the oxic–anoxic boundary of temperate lakes. *Aquatic Geochemistry* **20**: 39-57.
- Konhauser, K.O., Hamade, T., Raiswell, R., Morris, R.C., Ferris, F.G., Southam, G., and Canfield, D.E. (2002) Could bacteria have formed the Precambrian banded iron formations? *Geology* **30**: 1079-1082.
- Kostka, J.E., Stucki, L.J.W., Nealson, K.H., and Wu, J. (1996) Reduction of structural Fe (III) in smectite by a pure culture of the Fe-reducing bacterium *Shewanella putrefaciens* strain MR-1. In *Clays and Clay Minerals*.
- Laufer, K., Nordhoff, M., Røy, H., Schmidt, C., Behrens, S., Jørgensen, B.B., and Kappler, A. (2015) Co-existence of microaerophilic, nitrate-reducing, and phototrophic Fe (II)-oxidizers and Fe (III)-reducers in coastal marine sediment. *Applied and Environmental Microbiology* **82**(5):1433-1447.
- Lovley, D.R., and Phillips, E.J. (1988a) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology* **54**: 1472-1480.
- Lovley, D.R., and Phillips, E.J. (1988b) Manganese inhibition of microbial iron reduction in anaerobic sediments. *Geomicrobiology Journal* **6**: 145-155.
- Lovley, D.R., Giovannoni, S.J., White, D.C., Champine, J.E., Phillips, E., Gorby, Y.A., and Goodwin, S. (1993) *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Archives of Microbiology* **159**: 336-344.
- Madison, A.S., Tebo, B.M., Mucci, A., Sundby, B., and Luther, G.W. (2013) Abundant porewater Mn (III) is a major component of the sedimentary redox system. *Science* **341**: 875-878.
- Melton, E.D., Schmidt, C., and Kappler, A. (2012) Microbial iron (II) oxidation in littoral freshwater lake sediment: the potential for competition between phototrophic vs. nitrate-reducing iron (II)-oxidizers. *Frontiers in Microbiology* **3**.
- Milucka, J., Kirf, M., Lu, L., Krupke, A., Lam, P., Littmann, S. et al. (2015) Methane oxidation coupled to oxygenic photosynthesis in anoxic waters. *The ISME journal*.
- Musat, N., Halm, H., Winterholler, B., Hoppe, P., Peduzzi, S., Hillion, F. et al. (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proceedings of the National Academy of Sciences* **105**: 17861-17866.
- Myers, C.R., and Nealson, K.H. (1988) Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochimica et Cosmochimica Acta* **52**: 2727-2732.
- Nagai, T., Imai, A., Matsushige, K., Yokoi, K., and Fukushima, T. (2007) Dissolved iron and its speciation in a shallow eutrophic lake and its inflowing rivers. *Water Research* **41**: 775-784.

- Peduzzi, S., Storelli, N., Welsh, A., Peduzzi, R., Hahn, D., Perret, X., and Tonolla, M. (2012) Candidatus "*Thiodictyon syntrophicum*", sp. nov., a new purple sulfur bacterium isolated from the chemocline of Lake Cadagno forming aggregates and specific associations with *Desulfocapsa* sp. *Systematic and Applied Microbiology* **35**: 139-144.
- Pernthaler, A., Pernthaler, J., and Amann, R. (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology* **68**: 3094-3101.
- Pfennig, N., Trueper H. (1991) *The family Chromatiaceae. In The Prokaryotes*, 2nd edn. Berlin: Springer.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**: D590-D596.
- Raven, J., Kübler, J., and Beardall, J. (2000) Put out the light, and then put out the light. *Journal of the Marine Biological Association of the UK* **80**: 1-25.
- Stookey, L.L. (1970) Ferrozine –a new spectrophotometric reagent for iron. *Analytical Chemistry* **42**: 779-781.
- Storelli, N., Peduzzi, S., Saad, M.M., Frigaard, N.-U., Perret, X., and Tonolla, M. (2013) CO₂ assimilation in the chemocline of Lake Cadagno is dominated by a few types of phototrophic purple sulfur bacteria. *FEMS microbiology ecology* **84**: 421-432.
- Straub, K.L., Benz, M., Schink, B., and Widdel, F. (1996) Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Applied and Environmental Microbiology* **62**: 1458-1460.
- Straub, K.L., Schönhuber, W.A., Buchholz-Cleven, B.E., and Schink, B. (2004) Diversity of ferrous iron-oxidizing, nitrate-reducing bacteria and their involvement in oxygen-independent iron cycling. *Geomicrobiology Journal* **21**: 371-378.
- Thamdrup, B. (2000) Bacterial manganese and iron reduction in aquatic sediments. In *Advances in Microbial Ecology*: Springer, pp. 41-84.
- Thamdrup, B., Fossing, H., and Jørgensen, B.B. (1994) Manganese, iron and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Geochimica et Cosmochimica Acta* **58**: 5115-5129.
- Tonolla, M., Demarta, A., Peduzzi, R., and Hahn, D. (1999) In situ analysis of phototrophic sulfur bacteria in the chemocline of meromictic Lake Cadagno (Switzerland). *Applied and environmental microbiology* **65**: 1325-1330.
- Trouwborst, R.E., Clement, B.G., Tebo, B.M., Glazer, B.T., and Luther, G.W. (2006) Soluble Mn (III) in suboxic zones. *Science* **313**: 1955-1957.

Walter, X.A., Picazo, A., Miracle, M.R., Vicente, E., Camacho, A., Aragno, M., and Zopfi, J. (2014) Phototrophic Fe (II)-oxidation in the chemocline of a ferruginous meromictic lake. *Frontiers in Microbiology* **5**.

Weiss, J.V., Rentz, J.A., Plaia, T., Neubauer, S.C., Merrill-Floyd, M., Lilburn, T. et al. (2007) Characterization of Neutrophilic Fe (II)-Oxidizing Bacteria Isolated from the Rhizosphere of Wetland Plants and Description of *Ferritrophicum radicolica* gen. nov. sp. nov., and *Sideroxydans paludicola* sp. nov. *Geomicrobiology Journal* **24**: 559-570.

Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B., and Schink, B. (1993) Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature* **362**: 834-836.

Wüest, A. (1994) Interactions in lakes: Biology as source of dominant physical forces. *Limnologica Jena* **24**: 93-104.

TABLE & FIGURE LEGENDS

Figure 1: Chemical profiles measured after a full dark period, just before sunrise (left) and during the day (right). Sulfide is a sum of total species $\text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$. Only total Mn and Fe profiles are shown, because these were identical to Mn(II) and Fe(II). Values of conductivity shown are to a factor of 10^{-1} . The region of constant conductivity, circa 11-14.5 m depth, corresponds to the well-mixed chemocline and turbidity maximum (shaded gray region).

Figure 2: Bacterial 16S rRNA gene amplicon libraries from the chemocline of Lake Cadagno in 2013 and 2014. Sequences were classified based on best BLAST hit results, and putative iron metabolizing bacteria were identified based on phylogenetic affiliation with known iron oxidizers and reducers.

Figure 3: Top four panels from incubations performed with enrichment cultures grown on Fe(II) show iron speciation (a) and parallel carbon uptake measurements: CO_2 assimilation (b) in the presence of $^{13}\text{C}\text{-HCO}_3^-$, production of $^{13}\text{CO}_2$ (c) and assimilation of ^{13}C into biomass (d) in the presence of ^{13}C -acetate and ^{13}C -formate mixture. Lower four panels from incubations

performed with enrichment cultures grown on FeS show iron speciation (e), sulfide (f), and parallel carbon uptake measurements: CO₂ assimilation (g) in the presence of ¹³C-HCO₃⁻, and assimilation of ¹³C into biomass (h) in the presence of ¹³C-acetate. Symbols indicate light (○), dark (●) and the killed control + light (●) conditions.

Figure 4: Schematic representation of the proposed cryptic Fe cycle in the Lake Cadagno water column. Only reactions relevant to the lake are shown. Shaded purple region represents the chemocline.

Table 1: Calculated ferrous iron fluxes and oxidation rates and fluxes in Lake Cadagno chemocline for each sampling period. The maximum and minimum turbulence coefficients experimentally determined in Uhde (1992) and Wüest (1994).*Lake La Cruz values from Walter et al. (2014).

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	$D \text{ cm}^2 \text{ s}^{-1}$	Fe(II) flux $\mu\text{mol cm}^{-2} \text{ s}^{-1}$	Fe(II)-oxidation $\mu\text{mol l}^{-1} \text{ d}^{-1}$
Lake Cadagno day	0.016-0.15	0.042-0.392	1.386-13.756
Lake Cadagno night	0.016-0.15	0.027-0.250	0.648-6.070
*Lake La Cruz	0.0005-0.004	0.031-0.244	0.174-1.396
**Lake Matano	0.0069-0.057	0.034-0.270	0.034-0.270

Figure S1: Manganese and iron speciation in *in situ* incubations supplemented with hydrous MnO₂. Note that all Fe was oxidized between MnO₂ addition and the first sampling point.

Figure S2: Lake Cadagno profile from 2013 taken 4 h after sunset. Where sulfide and Fe overlap, Fe is most likely speciated as FeS.

Figure S3: Scanning electron micrograph (SEM) of a bacterial aggregate in an Fe(II) enrichment culture. The photoferrotroph *Rhodomicrobium vanielii* can be distinguished based on its stalk-like morphology. Scale bar is 5 μm . SEM-EDX images of a bacterial aggregate in an aged culture. *Rhodomicrobium* and other rod-shaped bacteria are visibly encrusted in minerals identified as iron (blue), phosphorus (yellow), and oxygen (red). Scale bars are 2.5 μm .

Table S1: Table of sequences identified in 2013 and 2014 Lake Cadagno enrichment cultures with added Fe(II) and acetate. Taxonomic identity of 16S rRNA gene sequences was assigned based on best BLAST hit identity. Sequences are listed in decreasing order of abundance as determined for the 2013 enrichment.

Closest relative	Sequence abundance	
	enrichment 2013 (n _{sequences} =37)	enrichment 2014 (n _{sequences} =30,809)
<i>Rhodoferax sp.</i>	62%	0.2%
other Bacteria	30%	26.0%
<i>Rhodomicrobium sp.</i>	3%	0.1%
<i>Geothrix sp.</i>	3%	-
<i>Chlorobium sp.</i>	3%	60.2%
<i>Rhodopseudomonas sp.</i>	-	12.8%
<i>Sulfurospirillum sp.</i>	-	0.7%

Table S2: Table of sequences identified in a 2013 Lake Cadagno enrichment culture with added FeS. Taxonomic identity of 16S rRNA gene sequences was assigned based on best BLAST hit identity. Sequences are listed in decreasing order of abundance as determined for the 2013 enrichment.

Closest relative	Sequence abundance (n _{sequences} =33)
<i>Rhodoferax sp.</i>	76%
other Bacteria	12%
<i>Ectothiorhodospira sp.</i>	6%
<i>Thiodictyon sp.</i>	3%
<i>Rhodomicrobium sp.</i>	3%