

# Photoferrotrophs thrive in an Archean Ocean analogue

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Considerable discussion surrounds the potential role of anoxygenic phototrophic Fe(II)-oxidizing bacteria in both the genesis of Banded Iron Formations (BIFs) and early marine productivity. However, anoxygenic phototrophs have yet to be identified in modern environments with comparable chemistry and physical structure to the ancient Fe(II)-rich (ferruginous) oceans from which BIFs deposited. Lake Matano, Indonesia, the eighth deepest lake in the world, is such an environment. Here, sulfate is scarce ( $<20 \mu\text{mol}\cdot\text{liter}^{-1}$ ), and it is completely removed by sulfate reduction within the deep, Fe(II)-rich chemocline. The sulfide produced is efficiently scavenged by the formation and precipitation of FeS, thereby maintaining very low sulfide concentrations within the chemocline and the deep ferruginous bottom waters. Low productivity in the surface water allows sunlight to penetrate to the  $>100\text{-m}$ -deep chemocline. Within this sulfide-poor, Fe(II)-rich, illuminated chemocline, we find a populous assemblage of anoxygenic phototrophic green sulfur bacteria (GSB). These GSB represent a large component of the Lake Matano phototrophic community, and bacteriochlorophyll *e*, a pigment produced by low-light-adapted GSB, is nearly as abundant as chlorophyll *a* in the lake's euphotic surface waters. The dearth of sulfide in the chemocline requires that the GSB are sustained by phototrophic oxidation of Fe(II), which is in abundant supply. By analogy, we propose that similar microbial communities, including populations of sulfate reducers and photoferrotrophic GSB, likely populated the chemoclines of ancient ferruginous oceans, driving the genesis of BIFs and fueling early marine productivity.

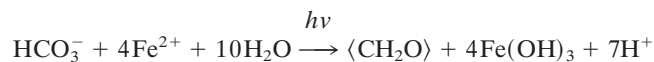
anoxygenic photosynthesis | banded iron formation | green sulfur bacteria | iron oxidation | Lake Matano

Early in Earth's history ( $>2.4$  Ga), ocean chemistry was very different from today. Sulfate concentrations were exceptionally low, and deep ocean waters were oxygen free and contained abundant ferrous Fe [Fe(II)] (1, 2). Oxidation and precipitation of this Fe led to the deposition of Banded Iron Formations (BIFs), conspicuous, Fe-rich, sedimentary deposits formed from 3.8 to 1.8 billion years ago. There has been considerable debate concerning the mechanisms responsible for BIF genesis (3–6). Of particular contention are the processes contributing to the production of the ferric Fe [Fe(III)], which comprises, on average, 40% of total BIF Fe (7). Potential Fe(III) production mechanisms include inorganic and biologically mediated Fe(II) oxidation by oxygen (5, 8, 9), UV-induced Fe(II) oxidation (3), and anoxygenic phototrophic Fe(II) oxidation, termed photoferritrophy (6, 10–13).

There is some debate concerning the exact timing and mechanism of oxygen introduction to the atmosphere and oceans. However, the general consensus, which is largely based on mass independent fractionation of sulfur isotopes, is that oxygen was scarce before 2.4 Ga (14). The scarcity of molecular  $\text{O}_2$  early in the earth's history would have rendered abiotic Fe(II) oxidation exceptionally slow (15). However, biological Fe(II) oxidation at

low oxygen partial pressure is much faster, and this chemoautotrophic metabolism (see review in ref. 1) is well known to occur in modern microaerophilic environments. Provided an adequate supply of  $\text{O}_2$ , chemolithoautotrophy could have been an important source of oxidized Fe to BIFs.

UV photolysis, however, would not have required oxygen. The UV oxidation of Fe(II) has been demonstrated in the laboratory (e.g., ref. 3), but not in complex seawater solutions. Furthermore, this mechanism may have been inhibited by the formation of amorphous Fe-silica gels in the ancient silica-saturated ocean waters from which BIFs deposited (5). Photoferrotrophy also occurs in the absence of oxygen according to the following reaction (16):



Recent studies have explored the population sizes of phototrophic and nonphototrophic Fe-oxidizing bacteria needed to generate the sedimentary Fe(III) fluxes recorded in ancient BIFs (5, 13). These studies conclude that ancient marine settings likely had sufficient light and nutrients to sustain the necessary bacterial population sizes. Because photoferrotrophic Fe(II) oxidation is an anaerobic process, it could have generated Fe(III) for BIFs before the evolution of cyanobacteria and oxygenic photosynthesis. Indeed, the available evidence shows that anoxygenic photosystems evolved before oxygenic photosystems (17). It has also been proposed that photoferrotrophs could have been the most important primary producers of organic matter before the advent of oxygenic photosynthesis (18).

Previously, anoxygenic phototrophic bacteria were identified as sulfide oxidizers, and their known occurrence was restricted to the chemocline of environments such as sulfide-containing microbial mats (19), sulfidic lakes (20), and strongly stratified marine water bodies like the Black Sea (21). The plausibility of photoferrotrophy in ancient ferruginous oceans has been made more tangible by the recent discovery of several extant anoxygenic phototrophs, including a variety of purple bacteria ( $\alpha$  and  $\gamma$  *Proteobacteria*; see review in ref. 22) and a single strain of green sulfur bacteria [*Chlorobium ferrooxidans* (23)] that can pho-

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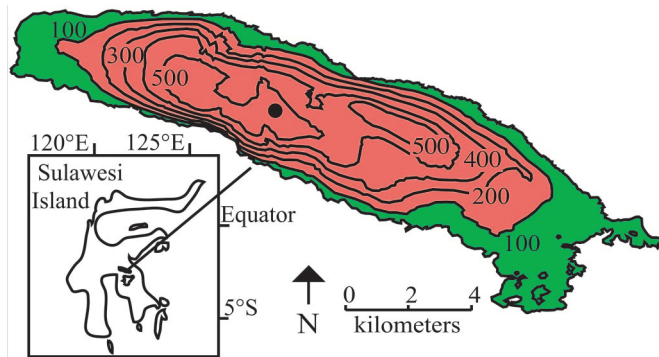
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**Fig. 1.** Map showing the location of Lake Matano on Sulawesi Island, Indonesia (*Inset*), and bathymetric map of Lake Matano. The circle marks a central deep water master station, and the area shaded in red is underlain by anoxic water.

totrophically oxidize dissolved Fe(II). To date, however, all known Fe(II)-oxidizing anoxygenic phototrophs have been cultured from iron-rich springs, ditches, and other shallow, ephemeral environments (6, 10, 12, 23, 24). There have been no good modern analogues with which to evaluate the presence and potential activity of anoxygenic phototrophic communities, or microbial ecosystems in general, under conditions comparable to the early ferruginous oceans (15).

In this article, we highlight the microbial ecology and biogeochemistry of Lake Matano, Indonesia, which exhibits a water column structure and chemistry resembling the ferruginous conditions construed for the Archean and early Proterozoic oceans. In Lake Matano, anoxygenic phototrophs thrive within a ferruginous chemocline, and they are likely sustained by photoferrotrophy.

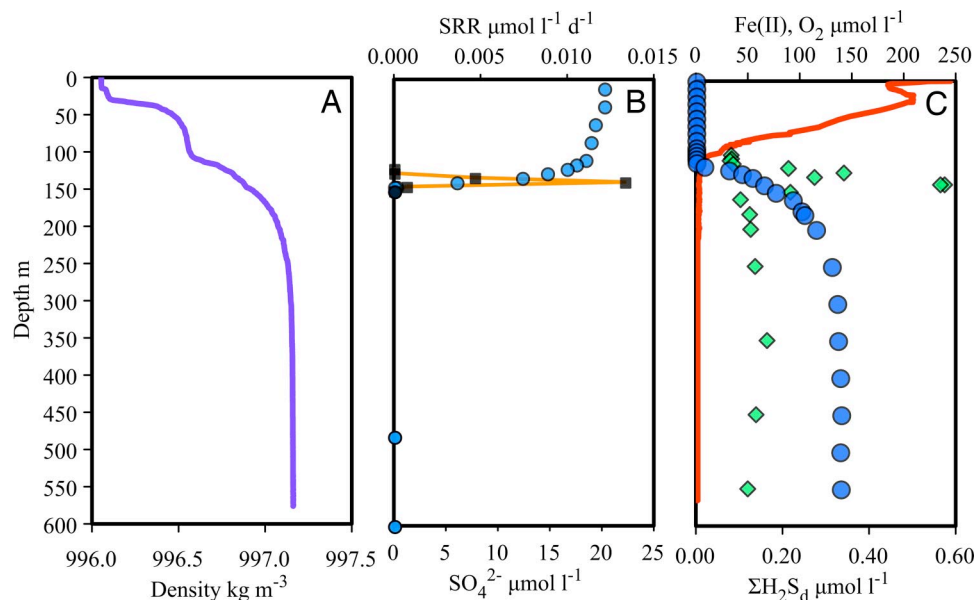
### Physical, Chemical, and Microbial Features of Lake Matano

Lake Matano is situated on Sulawesi Island, Indonesia (25) (Fig. 1). Its steep bathymetry, great depth (>590 m), and the

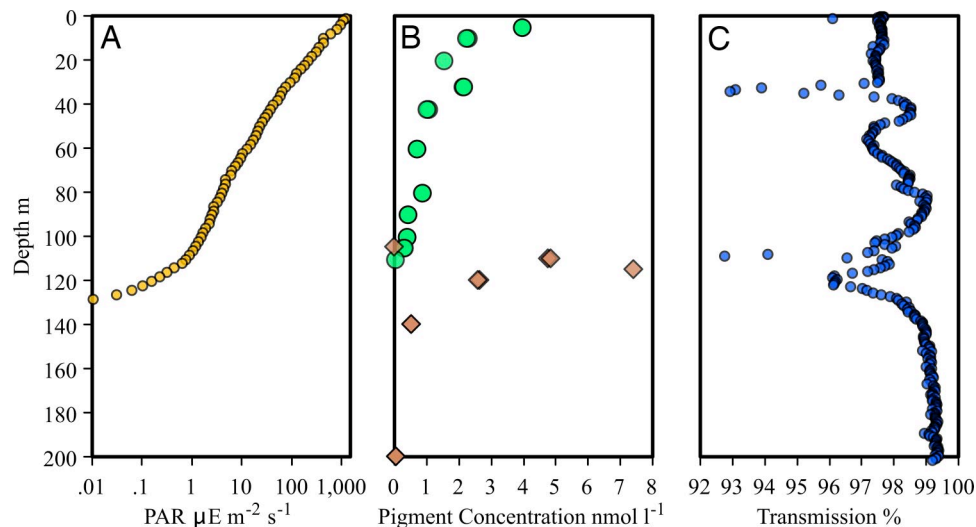
absence of strong seasonal temperature fluctuations maintain a persistent pycnocline at  $\approx 100$  m depth, separating an oxic surface mixed layer from anoxic bottom waters (Fig. 2A). Sulfate concentrations are low ( $< 20 \mu\text{mol}\cdot\text{liter}^{-1}$ ) in the surface mixed layer (Fig. 2B) and sulfate reduction ensues at slow rates in the anoxic waters of the chemocline. Although slow, sulfate reduction rates within the chemocline are sufficient to completely remove sulfate supplied from the surface waters, resulting in deep waters with undetectable sulfate concentrations (Fig. 2B). This produces very low, but measurable, concentrations of sulfide (Fig. 2C), which, according to our standard analytical protocol, includes free sulfide as well as sulfide-containing colloids and larger reactive particles, such as NiS and FeS (26). Voltammetric measurements of sulfur speciation indicate that free sulfide concentrations are on the order of  $0.01\text{--}0.06 \mu\text{mol}\cdot\text{liter}^{-1}$ , much less than the total sulfide values reported in Fig. 2C [see also [supporting information \(SI\) Text](#) and Fig. S1]. This dearth of sulfide allows the accumulation of dissolved ferrous iron to high concentrations (Fig. 2C). Scavenging of phosphate by allochthonous and authigenic iron (hydr)oxides likely limits primary productivity in the surface mixed layer (25), which, together with a low suspended inorganic particulate load, allows light to penetrate well into the anoxic bottom water (Fig. 3A).

We measured the depth distribution of photosynthetic pigment concentrations within the lake. Chlorophyll *a* (Chl *a*) dominates in the surface mixed layer of the lake (Fig. 3B). Its low concentration throughout this layer is consistent with low rates of primary production ( $3.8 \times 10^{-3} \text{ mol C m}^{-2} \text{ d}^{-1}$ ) by oxygen-producing cyanobacteria and algae. The low rates of photosynthetic C fixation measured in Lake Matano are similar to those of ultraoligotrophic, high arctic lakes (27) and are in line with the hypothesis that phosphorus scavenging by iron (hydr)oxides at the chemocline induces nutrient (phosphorus) limitation and restricts primary productivity under ferruginous conditions (28).

The transition from the oxic surface waters to the anoxic bottom waters is accompanied by a shift in the dominant photosynthetic pigment from Chl *a* to bacteriochlorophyll *e* (BChl *e*; Fig. 3B). The latter is a light-harvesting pigment used by brown-colored anoxygenic phototrophic GSB of the family



**Fig. 2.** Vertical physical and chemical profiles collected at a deep-water master station in 2007. (A) The typical vertical distribution of water density in Lake Matano showing a seasonal pycnocline at 32 m depth and the persistent pycnocline at 110–120 m depth during the month of February 2007. (B) The vertical distribution of dissolved sulfate (blue circles) and sulfate reduction rates (SRR) (black dots connected with orange line). (C) The vertical distribution of dissolved oxygen (red line), iron (blue circles) and total dissolved sulfide ( $\Sigma\text{H}_2\text{S}_d$ ) (green diamonds) over the same time period.



**Fig. 3.** Profiles illustrating the transmission of light and the vertical distribution of microorganisms and particles at a deep-water master station in 2007. (A) Photosynthetically active radiation profile collected on a sunny day in February 2007. (B) The distribution of photosynthetic pigments (green circles, Chl *a*; brown diamonds, BChl *e*) collected, respectively, in February and March 2007. (C) Light transmission (beam attenuation) profile collected in February 2007.

*Chlorobiaceae*, which are especially well adapted to low light conditions (29). BChl *e* has *in vivo* light absorption maxima at 461 and 715 nm (29). A peak in BChl *e* concentrations between 115 and 125 m delineates a maximum in the population size of anoxygenic phototrophs and coincides with the maximum attenuation of light (Fig. 3C). The depth-integrated quantity of BChl *e* ( $10.8 \text{ nmol}\cdot\text{cm}^{-2}$ ) in the anoxic bottom water is nearly as large as that of Chl *a* ( $14 \text{ nmol}\cdot\text{cm}^{-2}$ ) in the oxic surface water, indicating that GSB are an important component of the phototrophic community in Lake Matano. Furthermore, volume-based rates of total carbon fixation at 118 m depth ( $0.65 \mu\text{g}\cdot\text{liter}^{-1}\cdot\text{h}^{-1}$ ) are as high as rates of photosynthetic carbon fixation in the surface water ( $0.66 \mu\text{g}\cdot\text{liter}^{-1}\cdot\text{h}^{-1}$ ). The depth of the BChl *e* peak coincides with the depth ( $120 \pm 5 \text{ m}$ ) at which soluble, reactive phosphorus reaches appreciable ( $>0.35 \mu\text{mol}\cdot\text{liter}^{-1}$ ) levels (see *SI Text* and Fig. S2) indicating that microorganisms dwelling within the chemocline likely benefit from a supply of P from the underlying anoxic waters.

The specific cell content of BChl *e* in GSB growing under low light conditions can range from 50 to  $200 \mu\text{g}$  of BChl *e*  $\text{mg}^{-1}$  protein (30) which yields a protein content of  $100\text{--}400 \mu\text{g}\cdot\text{liter}^{-1}$  for the GSB community at 118 m depth in Lake Matano. A typical range for bacterial cell protein contents of between 0.24 and  $3.5 \times 10^{-7} \mu\text{g}$  (31) translates to a relatively high GSB cell density of  $0.3\text{--}16 \times 10^9$  cells per liter in Lake Matano.

Molecular fingerprinting by using the 16S rRNA gene revealed the presence of a mixed bacterial community between 110 and 120 m depth, including several phylogenetically distinct members of *Chlorobiaceae* (Fig. 4). Most known *Chlorobiaceae* are obligate photolithoautotrophs that use the reverse citric acid cycle to fix carbon with sulfide as an electron donor. As mentioned above, *C. ferrooxidans* is a notable exception that uses ferrous iron as an electron donor (23).

#### Lake Matano as an Ancient Ocean Analogue

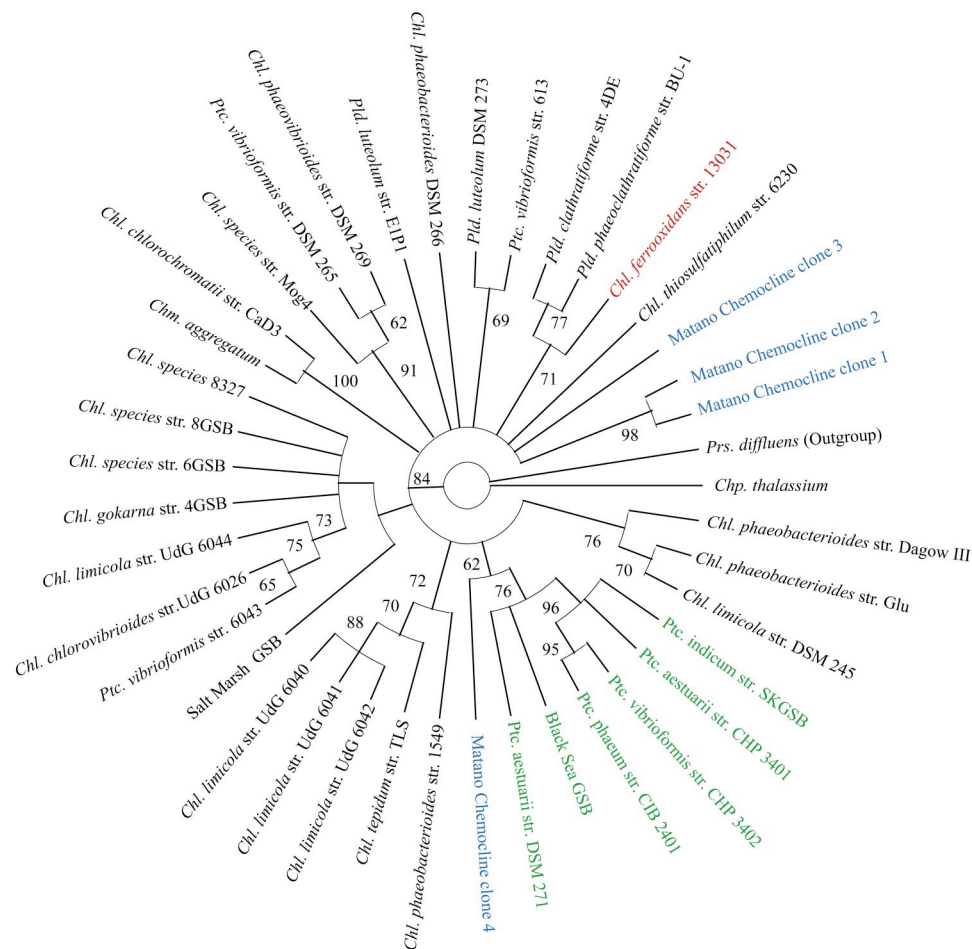
Overall, the high ferrous iron concentrations, combined with low sulfate, deep light penetration and other physical and chemical characteristics (Table 1) make Lake Matano an excellent modern analogue for the chemistry and biology of Archean and early Proterozoic oceans. Our results document measurable water column sulfate reduction at exceptionally low sulfate concentrations and at rates sufficient to completely remove sulfate within the chemocline. Sulfate concentrations in the

Archean were  $<200 \mu\text{mol}\cdot\text{liter}^{-1}$  (32) and perhaps even as low as the  $20 \mu\text{mol}\cdot\text{liter}^{-1}$  we find in Lake Matano. By analogy to Lake Matano, it seems likely that sulfate reduction occurred in the water column of early ferruginous oceans leading to substantial or even complete sulfate removal directly within the water column. This means that pyrite associated with BIFs and other deep-water sediments likely formed partially or even wholly within the water column. In contrast, sedimentary sulfate reduction and pyrite formation may have been largely restricted to shallower environments within or above the chemocline depth.

Most importantly, our results document that a populous and diverse assemblage of anoxygenic photosynthetic GSB inhabits the chemocline of Lake Matano. Ferrous iron concentrations are up to  $30 \mu\text{mol}\cdot\text{liter}^{-1}$  within the depth interval (100–120 m) in which BChl *e* concentrations are highest (Figs. 2C and 3B, respectively). In this depth interval, the midday light intensity (Fig. 3A) varies from  $1 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  at the top of the chemocline (100 m) where Fe(II) is undetectable ( $<0.5 \mu\text{mol}\cdot\text{liter}^{-1}$ ) to  $\approx 0.13 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  at 120 m, immediately below the depth exhibiting the maximum BChl *e* concentrations. These light intensities exceed those supporting substantial anoxygenic photosynthesis by low-light-adapted *Chlorobiaceae* in the Black Sea (30). Unlike the Black Sea, however, the Lake Matano chemocline has extremely low dissolved sulfide concentrations, suggesting that the populous assemblage of GSB is sustained by using the abundant supply of Fe(II) as an electron donor.

Given the small, but measurable, concentrations of free sulfide in the chemocline of Lake Matano (Fig. 2C), we must consider whether the GSB in the lake could be sustained by using this sulfide as an electron donor. The lowest known half-saturation constant for sulfide oxidation by green sulfur bacteria is  $0.8 \mu\text{mol}\cdot\text{liter}^{-1}$  (33), a factor of 13 to 80 greater than the free sulfide concentrations in Lake Matano. Unless the Lake Matano GSB are much more proficient at using sulfide than similar organisms studied to date, sulfide concentrations are too low to support sulfide-driven anoxygenic phototrophy. Thus, we are led to conclude that the Lake Matano GSB population is largely sustained by photoferrotothrophy.

We can test the potential contribution of photoferrotothrophy to Fe(II) oxidation in Lake Matano by evaluating whether the irradiance at 110 m depth is sufficient to phototrophically drive the Fe(II) flux through the chemocline. Physical transport within



**Fig. 4.** A tree generated by using maximum parsimony and displaying the phylogenetic relationships between Lake Matano clones and representative members of the Chlorobiaceae family in addition to *Persicobacter diffluens* as an outgroup. Bootstrap support is indicated at the branch points of the tree. Lake Matano clones are highlighted in blue, marine isolates in green, and *C. ferrooxidans*, a known photoferrotoph, in red. Lake Matano clones have up to 95% sequence similarity to *C. ferrooxidans*.

Lake Matano's chemocline is largely due to small-scale eddy diffusion, and thus, chemical fluxes can be calculated by using Fick's first law (flux =  $-K_z dC/dx$ ) (25). By using a  $K_z$  of 600–4,900  $\text{cm}^2\text{d}^{-1}$  (Table 2) and the measured concentration gradients, we calculate an upward flux of Fe(II) through the chemocline of between 0.034 and 0.27  $\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$  (Table 2). The light incident at the top of the chemocline, 1  $\mu\text{mol}$  quanta  $\text{m}^{-2}\text{s}^{-1}$  (Fig. 3A), translates into a quantum flux of 2.2  $\mu\text{mol}$

quanta  $\text{cm}^{-2}$  per day for a conservative estimate of 6 daily hours of sunlight. With a typical GSB requirement of 1 to 2 moles quanta per mole of electrons transferred [one electron is transferred to oxidize Fe(II) to Fe(III)] (34), this light flux is sufficient to phototrophically oxidize the entire flux of Fe(II) through the chemocline (Table 2).

These rates of Fe(II) oxidation are also consistent with the metabolic capacities of known photoferrotophs. The vertical

**Table 1. Comparing Lake Matano with the Archean Ocean**

Surface mixed layer	Lake Matano 100 m		Archean Ocean >200 m (13)
	Mixed layer	Anoxic layer	
Fe(II)		140 $\mu\text{mol}\cdot\text{liter}^{-1}$	40–120 $\mu\text{mol}\cdot\text{liter}^{-1}$ (1)
O <sub>2</sub>	Saturated	<1 $\mu\text{mol}\cdot\text{liter}^{-1}$	<0.08% PAL* (1)
SO <sub>4</sub> <sup>2-</sup>	<20 $\mu\text{mol}\cdot\text{liter}^{-1}$	<0.1 $\mu\text{mol}\cdot\text{liter}^{-1}$	<200 $\mu\text{mol}\cdot\text{liter}^{-1}$ (32)
PO <sub>4</sub> <sup>3-</sup>	<0.025 $\mu\text{mol}\cdot\text{liter}^{-1}$ (25)	9 $\mu\text{mol}\cdot\text{liter}^{-1}$ (25)	0.03–0.29 $\mu\text{mol}\cdot\text{liter}^{-1}$ (28)
SiO <sub>2(aq)</sub>	300 $\mu\text{mol}\cdot\text{liter}^{-1}$ (25)	420 $\mu\text{mol}\cdot\text{liter}^{-1}$ (25)	670–2200 $\mu\text{mol}\cdot\text{liter}^{-1}$ (7)
pH	8.6 (25)	7.00 (25)	>6.5 (28, 50)
Euphotic zone		<130 m	<150 m (13)
Ionic strength	300 $\text{mmol}\cdot\text{liter}^{-1}$ (25)	4.6 $\text{mmol}\cdot\text{liter}^{-1}$ (25)	?
T		25–28°C (25)	≈40°C (7)

\*PAL, present atmospheric levels.

**Table 2. Fe<sup>2+</sup> fluxes and rates of oxidation in Lake Matano**

$K_z^*$	Fe <sup>2+</sup> gradient	Fe <sup>2+</sup> flux	Fe oxidation rate <sup>†</sup>
600–4900 cm <sup>2</sup> ·d <sup>-1</sup>	$5.6 \times 10^{-5}$ μmol·cm <sup>-4</sup>	0.034–0.27 μmol·cm <sup>-2</sup> ·d <sup>-1</sup>	0.034–0.27 μmol·liter <sup>-1</sup> ·d <sup>-1</sup>

\*A range of  $K_z$  values within the chemocline was estimated from the Thorpe size of overturning eddies (48). A previously published value of 3,900 cm<sup>2</sup>·d<sup>-1</sup> (25) and a value (2,000 cm<sup>2</sup>·d<sup>-1</sup>) estimated from sulfate reduction rates and sulfate profiles are both within this range (see *SI Text*).

†Calculated assuming a 10-m interval for Fe<sup>2+</sup> oxidation.

distribution of BChl *e* in Lake Matano suggests that most of the phototrophic Fe(II) oxidation occurs within a 10-m depth interval (110–120 m), yielding average volume-based Fe(II) oxidation rates of 0.034–0.27 μmol·liter<sup>-1</sup>·d<sup>-1</sup> (Table 2). From laboratory studies, the Fe(II) oxidation rate of photoferrotrophic purple bacteria has been estimated at 14 μmol·liter<sup>-1</sup>·d<sup>-1</sup> for a spectral quality of light corresponding to 100-m water depth in the ocean and a light level of 3 μmol quanta m<sup>-2</sup> s<sup>-1</sup>, or 300% of the light level measured at the top of the Matano chemocline (13). For comparison, if we assume that the rate of phototrophic Fe(II) oxidation varies linearly with light levels at low light intensities, we can scale down the experimental rates (13) by a factor of 3, yielding a rate of 4.7 μmol·liter<sup>-1</sup>·d<sup>-1</sup>. This rate is still more than one order of magnitude higher than our estimates of Fe(II) oxidation rates. Hence, the rates of Fe(II) oxidation observed in the Lake Matano chemocline are well within the physiological capabilities of known photoferrotrophs.

### Conclusions and Extensions

Our results from Lake Matano, Indonesia, provide the first glimpse at a microbial ecosystem developed in a stable, ferruginous aquatic environment. We note that ferruginous conditions likely restrict primary production in oxic waters because the scavenging of P by Fe (hydr)oxides. We also document the complete removal of the lake's low sulfate content within the chemocline, allowing the accumulation of high iron concentrations at depth. Even where sulfate reduction is most active, free sulfide accumulates to <0.1 μmol·liter<sup>-1</sup>, likely rendering it unavailable to anoxygenic phototrophs. In contrast, concentrations of Fe(II) are high in the chemocline, and oxidation of this Fe(II) likely drives the metabolism of a prominent chemocline GSB population. Therefore, our results demonstrate that anoxygenic phototrophic GSB are not restricted to anoxic sulfidic lakes and euxinic marine basins like the Black Sea, but also flourish under ferruginous conditions that favor photoferrotrophy. Because Lake Matano shares many chemical and physical characteristics (Table 1) with ancient ferruginous oceans, we propose that anoxygenic phototrophic communities populated those ancient water columns as well and, through their metabolic activities, contributed to water column iron oxidation.

Recent laboratory experiments have shown that the high silica concentrations likely present in the Archean oceans preclude photolytic iron oxidation (7). In the presence of such high silica concentrations, the kinetics of ferrous silicate mineral precipitation are more rapid than photolytic oxidation (7). Therefore, the oxidation of ferrous Fe in ancient silica-rich ferruginous oceans would have required an oxidative mechanism more rapid than UV photolysis. One possibility would be the oxidation of Fe<sup>2+</sup> either biologically or abiotically with O<sub>2</sub>. However, as constrained by sulfur isotope studies (35), the O<sub>2</sub> levels of the Archean atmosphere were <10<sup>-5</sup> of present levels, which would yield air-saturated surface-water O<sub>2</sub> concentrations of <0.003 μmol·liter<sup>-1</sup> at 25°C (36). Such low O<sub>2</sub> levels would limit abiological Fe<sup>2+</sup> oxidation (37), and would also restrict O<sub>2</sub> availability for biological oxidation. This leaves photoferrotrophy as the most likely mechanism of Fe-oxide production from the water column of ancient Fe-rich oceans. Modern photoferrotrophs have only been cultured from shallow environments, yet

the sedimentary features of BIFs demonstrate deposition in relatively deep water below the storm wave base under low-light conditions. Our discovery of GSB in ferruginous Lake Matano bolsters the hypothesis that deep-water photoferrotrophy contributed to BIF formation.

Our results also support the theory of an ancient dynamic global carbon cycle driven by photoferrotrophy before the evolution of oxygenic photosynthesis (18). Indeed, calculations reveal that photoferrotrophy could have driven global primary production rates up to 10% of present-day values (18). Based on these calculations, photoferrotrophy would have been, by far, the most active primary producing metabolism before the dawn of oxygenic photosynthesis (18).

Still, some important questions remain. Although photoferrotrophy is the most likely metabolism for Lake Matano GSB, we have yet to isolate an Fe(II)-oxidizing GSB in pure culture. Such a culture would aid us in better understanding the physiology and overall metabolic capabilities of the GSB in the lake. Also, our work does not rule out the possibility that nonphototrophic, aerobic, or nitrate-dependent Fe(II) oxidation might also be a quantitatively important process in the chemocline. Indeed, we have noted the presence of cells with conspicuous morphologies characteristic of the Fe(II) oxidizer *Leptothrix*. Finally, our understanding of the Fe cycle in Lake Matano is still incomplete. In particular, it would be important to better constrain the spatial and temporal relationships between Fe(II) oxidation and Fe reduction in supplying Fe(II) and nutrients (e.g., P) to the Fe(II)-oxidizing populations. Each of these issues stand as important research opportunities to better understand the microbial ecology and biogeochemistry of element cycling in Lake Matano, and by extension, the dynamics of element cycling in ancient ferruginous oceans.

### Materials and Methods

Water density and ferrous iron concentrations were determined as previously described (25). Sulfide concentrations were measured by using the Cline spectrophotometric method with a 10-cm cell (38). Replicate profiles of light transmission were collected *in situ* by using a Wet Labs C-Star transmissometer with a 25-cm pathlength operated at a wavelength of 660 nm. Voltammetric analyses and sulfide speciation measurements were conducted on water samples, recovered with Niskin bottles, by using Au/Hg amalgam microelectrodes as previously described (39). Sulfate reduction rates were determined by using the <sup>35</sup>S radiotracer technique (40). Sulfate concentrations were determined by measuring total sulfur concentrations by using inductively coupled plasma-optical emission spectrometry after 10-fold preconcentration by evaporation and subtracting sulfide from total sulfur (detection limit = 60 nmol·liter<sup>-1</sup>). Photosynthetic pigments were extracted in a mixture of methanol, acetone and *N,N*-dimethylformamide (41). The low concentrations of pigments were determined by using a 1-m pathlength capillary cell interfaced to a fiber-optic spectrometer and a UV-visible light source (42). The profile of photosynthetically active radiation (PAR) in Fig. 2 was obtained at midday on a representative, cloudless day by using a LI-COR 1400-LI light meter.

Rates of C fixation were measured *in situ* at 32 (0.35 μg C liter<sup>-1</sup> h<sup>-1</sup>) and 118 m depth (0.65 μg C liter<sup>-1</sup> h<sup>-1</sup>) by using the H<sup>13</sup>CO<sub>3</sub> labeling technique (43). Total primary production was calculated by normalizing the rate of C fixation at 30 m depth to the chlorophyll *a* concentrations at the same depth. Carbon fixation rates at 10-m intervals were determined by multiplying this factor by the chlorophyll *a* concentrations at the corresponding depth interval. Total primary production was then calculated by summing the C fixation rates for these 10-m intervals within the entire 100-m-deep surface oxic layer.

Cellular material was collected on 0.2- $\mu\text{m}$  cellulose acetate or 0.7- $\mu\text{m}$  glass fiber filters. Filters were preserved in ethanol (cellulose acetate filters) or at  $-80^\circ\text{C}$  (glass fiber filters). DNA was extracted by using a Mo-Bio PowerSoil DNA extraction kit. Small subunit (SSU) rDNA was amplified by using PCR with either the "universal" bacterial primers 27f and 1492r (cellulose acetate filters) or primers specific for Chlorobiaceae GC341f and GSB822r (glass fiber filters) (44). The PCR products were cloned into the TOPO cloning vector and transformed into chemically competent One Shot TOP10 *Escherichia coli*. Plasmid DNA was purified by using a PureLink Quick plasmid Miniprep Kit. Forward and reverse sequencing of the purified DNA was performed by Macrogen. Contiguous sequences were assembled by using the assembly function in Geneious and sequence alignments were conducted online by using the Greengenes alignment tool (45). Chimeric sequences were identified with Bellerophon (version 3) and eliminated from the dataset (45). A subset of the "Hugenholtz" database (46) and the Lake Matano clone sequences were exported to PAUP version 4.0b10 for MacIntosh, in which phylogenetic trees were constructed by using neighbor-joining, maximum parsimony, and maximum likelihood methods (47). Branching topologies were compared for all methods and verified by performing 1,000 bootstrap replications by using the

maximum parsimony method. Lake Matano clone sequences were submitted to GenBank (accession nos. EU275404–EU275407).

Fluxes were calculated by using Fick's first law. The vertical eddy diffusivity coefficient was calculated from the Thorpe size of overturning eddies (48), which was determined from replicate measurements by using a free-falling conductivity, temperature, depth probe. The values obtained bracket the value of  $3,900\text{ cm}^2\text{d}^{-1}$  obtained previously by using a phenomenological relationship with the Brunt–Väisälä frequency (25), and a value of  $2,000\text{ cm}^2\text{d}^{-1}$ , which was estimated by comparing sulfate reduction rates with the rate of sulfate diffusion into the chemocline (49).

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