Proterozoic Ocean Chemistry and Evolution: A Bioinorganic Bridge?

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Recent data imply that for much of the Proterozoic Eon (2500 to 543 million years ago), Earth's oceans were moderately oxic at the surface and sulfidic at depth. Under these conditions, biologically important trace metals would have been scarce in most marine environments, potentially restricting the nitrogen cycle, affecting primary productivity, and limiting the ecological distribution of eukaryotic algae. Oceanic redox conditions and their bioinorganic consequences may thus help to explain observed patterns of Proterozoic evolution.

On the present-day Earth, O_2 is abundant from the upper atmosphere to the bottoms of ocean basins. When life began, however, O_2 was at best a trace constituent of the surface environment. The intervening history of ocean redox has been interpreted in terms of two long-lasting steady states: anoxic oceans (or nearly so) that persisted for some 2000 million years, followed by essentially modern oceans of comparable duration. Here, we review recent evidence pointing to the presence of "intermediate" oceans—oxic at the surface but anoxic and sulfidic at depth—that may have persisted for more than 1000 million years, originating some time after ~1800 million years ago (Ma). Aspects of the evolutionary pattern recorded by fossils of Proterozoic eukaryotes may be explained by the scarcity of biologically essential trace metals in such sulfidic seas, suggesting a bioinorganic bridge between environmental and biological evolution.

Sulfidic Deep Oceans

The classical argument that the deep oceans became oxidized at ~1800 Ma is based principally on the disappearance of banded iron formations (BIFs; Fig. 1A). BIFs are massive, laterally extensive and globally distributed chemical sediment deposits that consist primarily of Fe-bearing minerals and silica. Their formation seems to require anoxic deep waters to deliver hydrothermally derived Fe^{2+} to locations where deposition took place [e.g., (1–3)]. Oxygenation of the oceans would produce Fe^{3+}, which readily hydrolyz-es and forms insoluble Fe-oxyhydroxides, thus removing Fe and precluding BIF formation. This reading of the stratigraphic record made sense because independent geochemical evidence indicates that the partial pressure of atmospheric oxygen (PO_2) rose substantially about 2400 to 2000 Ma (4–7).

Because the solubility of Fe-sulfides is also low, however, the disappearance of BIF can alternatively be taken to indicate that the deep oceans became sulfidic, rather than oxic, after 1800 Ma. According to this scenario, recently advanced by Canfield (8), deep sea water became more reducing rather than more oxidizing at this time despite the rise in atmospheric oxygen. Ocean anoxia might have persisted into the Neoproterozoic (9), when C and S isotopic data indicate another increase in the oxidation state of Earth surface environments (10–13).

At first blush, sulfidic oceans appear counterintuitive in the face of contemporaneous atmospheric oxygenation. However, simple modeling of ocean redox suggests that deep waters would have remained anoxic if PO_2 had been <0.07 atm and if biological productivity, which delivers reduced C to the deep sea, was at all comparable to that of modern oceans (8). It is likely that PO_2 did not approach modern values until the Neoproterozoic (14, 15). Sulfidization follows from the fact that the concentration of hydrogen sulfide (H_2S) in seawater is affected by the supply of both organic C and sulfate (SO_4^{2-})—which constitute a source of H_2S when their reaction is catalyzed by dissimilatory bacterial sulfate reduction (BSR)—and by the availability of O_2, which acts as a reactive sink for H_2S and inhibits BSR. Today, pervasive O_2 limits H_2S concentrations, as has generally been the case for the Phanerozoic Eon. In the Archean and early Paleoproterozoic, the low solubility of reduced S minerals in igneous and sedimentary rocks during weathering under a nearly anoxic atmosphere limited the SO_4^{2-} supply, keeping H_2S concentrations low. In contrast, weathering under a moderately oxidizing mid-Proterozoic atmosphere would have enhanced the delivery of SO_4^{2-} to the anoxic depths. Assuming biologically productive oceans, the result would have been higher H_2S concentrations during this period than either before or since (8).

Is there any evidence for such a world? Canfield and his colleagues have developed an argument based on the S isotopic composition of biogenic sedimentary sulfides, which reflect SO_4^{2-} availability and redox conditions at their time of formation (16–18). When the availability of SO_4^{2-} is strongly limited (SO_4^{2-} concentration < ~1 mM, ~4% of that in present-day seawater), H_2S produced by BSR is depleted in ^{34}S by ~ <5 per mil (‰) relative to dissolved SO_4^{2-}. Fractionation increases to as much as ~45‰ when SO_4^{2-} is more freely available. Larger fractionations (45 to 70‰) appear to require a cyclical process in which ^{34}S-depleted sulfides are reoxidized to elemental sulfur (S^0), followed by bacterial disproportionation of S^0 to produce extremely ^{34}S-depleted H_2S (19, 20). Hence, S isotope fractionation > ~45‰ between sedimentary sulfides and sulfates may indicate increased oxygenation of the environment (21).

Several changes in S isotope systematics are seen in the Precambrian geological record (Fig. 1B) (22). BSR appears to have been in place by at least ~3470 Ma, as suggested by a fractionation of up to 21‰ (mean ~11‰) between S in evaporitic barite deposits and sulfide inclusions found within these sediments (23). However, in rocks older than 2400 Ma, $\Delta^{34}S$ (the difference in $\delta^{34}S$ between marine sulfate minerals—which record $\delta^{34}S$ of seawater SO_4^{2-}—and co-occurring sulfides) is typically ≪20‰. From this time until 800 to 600 Ma, $\Delta^{34}S$ reaches ~40‰, near the maximum associated with BSR, but rarely exceeds this value. Only in later Neoproterozoic and Phanerozoic rocks does $\Delta^{34}S$ approach the modern maximum of ~65‰.

The biogeochemical record of S is thus consistent with SO_4^{2-}-poor Archean oceans giving way to modest SO_4^{2-} concentrations, and consequent global enhancement of BSR, in the Paleoproterozoic. Presumably, the rise of a moderately oxidizing atmosphere facilitated, for the first time, the delivery of large quantities of SO_4^{2-} to the oceans (8, 24). The observation that $\Delta^{34}S$ < 45‰ during the mid-Proterozoic

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suggests that the oceans were oxygenated only to shallow depths during this time, and that more extensive oxygenation did not occur until late in the eon (12).

If sulfidic conditions were common through much of the Proterozoic Eon, independent geochemical redox indicators should provide evidence of anoxia. In addition, seawater SO_4^{2-} concentrations in the Proterozoic, although greatly elevated over Archean values, should have been lower than at present; this prediction can be tested by looking for "reservoir" effects in pyrite $\delta^{34}S$ within individual sedimentary basins (25).

Both predictions are met in superbly preserved black shales deposited ~1730 and 1640 Ma during maximum flooding of the Tawallah and McArthur basins, respectively, in northern Australia. Two geochemical indicators reliably differentiate sediments formed beneath oxic and sulfidic waters in the Black Sea (26–28) and in Mesozoic marine basins (29). The ratio of "highly reactive" Fe (Fe present in pyrite or oxides/hydroxides) to total Fe tends to be higher in sediments deposited beneath sulfidic waters than in sediments deposited beneath an oxic water column. Similarly, the "degree of pyritization" (the proportion of reactive Fe incorporated into pyrite) tends to be substantially higher beneath sulfidic bottom waters. Both indicators show that Tawallah and McArthur shales accumulated beneath sulfidic waters (30). Moreover, relative to the Black Sea, Cariaco Basin, and other modern sulfidic settings (27, 31), sedimentary pyrites in Tawallah and McArthur shales are markedly ^{34}S-enriched, suggesting that BSR strongly depleted the SO_4^{2-} reservoir in deep waters of these basins. Given reasonable estimates of primary production in surface waters, this indicates a seawater SO_4^{2-} reservoir as much as 90% lower than today's (30).

Elsewhere, large and systematic stratigraphic variation in $\delta^{34}S$ of sedimentary pyrites (>20‰) over tens to hundreds of meters of section) is seen in 1470- to 1440-Ma rocks from the Belt Supergroup, Montana, suggestive of reservoir effects. This finding provides evidence of episodic SO_4^{2-} limitation in another mid-

Proterozoic basin, as might be expected in a low-SO_4^{2-} ocean (32, 33).

Gypsum and anhydrite ($CaSO_4$ minerals) are common in rocks that formed along the margins of ~1200-Ma carbonate platforms in the Bylot Supergroup, North America, but are scarce in older rocks. $\delta^{34}S$ in these minerals varies by up to 10‰ over 300 m of section (34). The appearance of extensive $CaSO_4$ deposits indicates that SO_4^{2-} inventories began to rise at this time, but the observed isotopic variability, not seen in Phanerozoic sulfate minerals (35), again suggests a smaller mid-Proterozoic global ocean SO_4^{2-} reservoir. Seawater SO_4^{2-} may have remained well below present-day levels until the end of the Proterozoic Eon (36).

As yet, detailed stratigraphic analyses are too few to demonstrate unequivocally the global nature of sulfidic deep waters in mid-Proterozoic oceans. However, available data point to globally extensive BSR in low-SO_4^{2-} oceans, ocean oxygenation insufficient to support S^0 disproportionation, and sulfidic bottom waters in at least some marine basins during this time. We must therefore take seriously the proposition that the deep oceans were persistently sulfidic for much of our planet's middle age.

Biology of Mid-Proterozoic Oceans

What was biology like in mid-Proterozoic oceans? Hints come from C isotopes and fossils, which show distinctive stratigraphic trends that correlate broadly with the inferred redox history.

Secular variation of C isotopes in marine carbonates ($\delta^{13}C_{carb}$) reflects changes in the ratio of organic to inorganic C removed from the oceans during burial in sediments (37). This ratio (and hence $\delta^{13}C_{carb}$) may increase when enhanced tectonic activity increases the opportunities for organic C burial (38). Enhanced tectonic activity may also affect this ratio by increasing the supply of P to the oceans (39), stimulating primary production where N is not limiting.

Unusually large variations in $\delta^{13}C_{carb}$ characterize rocks that formed at the beginning and the end of the Proterozoic Eon (Fig. 1C), both times of widespread glaciation and increasing oxidation of the biosphere (5, 10, 40–42). The intervening interval is equally striking for its lack of variation; $\delta^{13}C_{carb}$ varies only within the limits of $0 \pm 2‰$ between ~1850 and 1250 Ma (Fig. 1C), documenting unique long-term stability of the C cycle (43–45). This stasis gave way to moderate variation (similar to that seen in Phanerozoic carbonates) after ~1250 Ma (45–47), before the onset of large-amplitude $\delta^{13}C_{carb}$ variations at ~800 Ma.
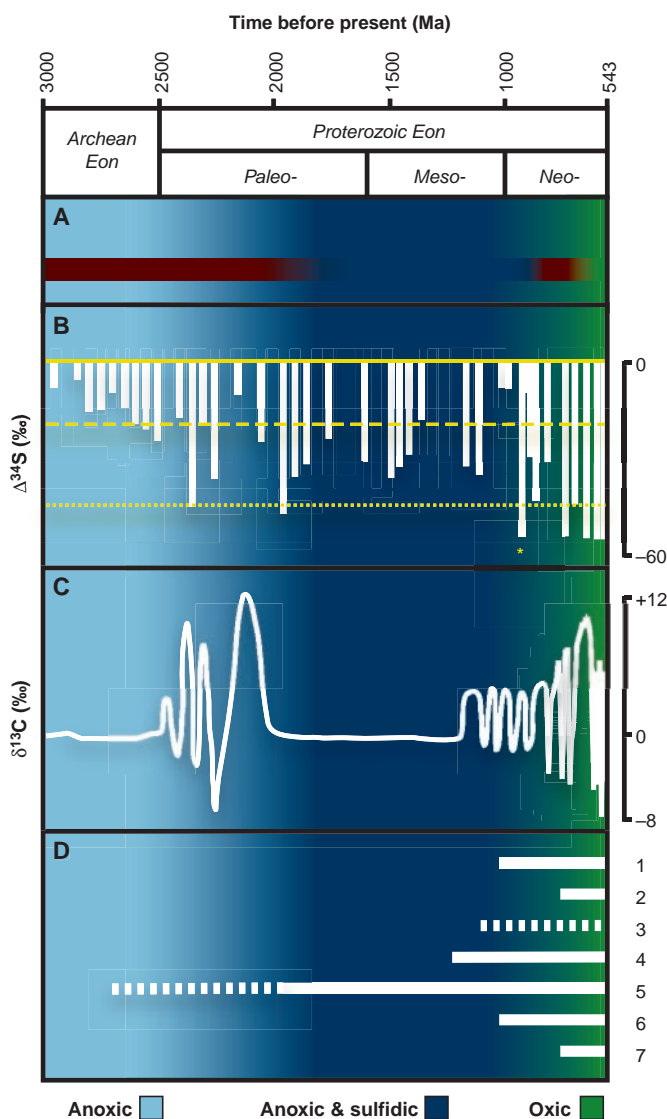
Fig. 1. Biological and geochemical changes during the Proterozoic Eon. Color gradations denote postulated changes in deep sea redox. (**A**) Periods of deposition of banded iron formations. (**B**) Range of values of $\Delta^{34}S$, the difference in $\delta^{34}S$ between coeval marine sulfides and sulfates. Dashed line: $\Delta^{34}S = 20‰$, the maximum Archean value. Dotted line: $\Delta^{34}S = 45‰$, the maximum fractionation associated with single-step BSR. Asterisk: $\Delta^{34}S$ determined from a single sample, and thus not well constrained. (**C**) Range of values of $\delta^{13}C_{carb}$ (after a compilation by A. J. Kaufman). The frequency and magnitude of variations in the Paleoproterozoic are somewhat uncertain. (**D**) Eukaryotic evolution, as indicated by the first appearances of body fossils (solid lines) and molecular biomarkers (dotted lines), including chlorophytes (1), ciliates (2), dinoflagellates (3), rhodophytes (4), eukaryotes of unknown affinities, possibly stem groups (5), stramenopiles (6), and testate amoebae (7). See text for geochemical references. Fossil distributions from (147).

It has been hypothesized that mid-Proterozoic $\delta^{13}C_{carb}$ stasis reflects tectonic quiescence during this time, in contrast to major continental rifting and orogenesis at the beginning and end of the eon (*44*). However, Phanerozoic-scale $\delta^{13}C_{carb}$ variations might still be expected before 1250 Ma, as the mid-Proterozoic was not a period without variations in tectonic activity (*48*). Hence, $\delta^{13}C_{carb}$ stasis appears to call for attenuation of the link between tectonism and primary production. Such attenuation might follow naturally if P were not the limiting nutrient during this time. Increased availability of P in the mid-Proterozoic oceans compared to the Archean and Paleoproterozoic goes hand-in-hand with the end of BIF deposition and the advent of sulfidic oceans at ~1800 Ma because Fe oxides are a sink for dissolved P (*49*), and because P is released from organic-rich sediments under sulfidic conditions (*50, 51*).

A changed nutrient regime in mid-Proterozoic oceans is consistent with suggestions of lower overall productivity at this time as compared to the Paleoproterozoic, Neoproterozoic, and Phanerozoic. The evidence again comes from C isotopes. First, the average value of $\delta^{13}C_{carb}$ in mid-Proterozoic carbonates appears to be ~1.5‰ lower than in Paleoproterozoic, Neoproterozoic, and Phanerozoic carbonates (*38, 43–45, 52, 53*), as would be expected from a decrease in the proportion of carbon buried as organic C due to depressed mid-Proterozoic primary productivity. Second, the export of ^{13}C-depleted organic C from surface waters reflects rates of primary production (*54*). In consequence, one would expect low productivity to be accompanied by a relatively small depth gradient in the isotopic composition of dissolved inorganic C. Data from several Mesoproterozoic basins are consistent with this notion (*43, 44*). In contrast, gradients for both earlier Paleoproterozoic and later Neoproterozoic oceans are larger (*55, 56*). The apparent absence of extensive continental ice sheets during the long interval between the large ice ages of the early Paleoproterozoic (*42*) and later Neoproterozoic (*57*) is also consistent with nutrient limitation of the biological C pump, although other factors undoubtedly contributed to the long-term maintenance of a mid-Proterozoic greenhouse.

Like C isotopes, fossils of presumed eukaryotes show distinct mid- and late Proterozoic distributions (Fig. 1D). Eukaryotic fossils appear in the geologic record as early as 1800 to 2100 Ma (*58, 59*), and recent organic geochemical studies indicate that at least stem eukaryotes diverged as early as 2700 Ma (*60*). Despite this early differentiation, photosynthetic protists appear to have played a limited role in mid-Proterozoic ecosystems. Fossil diversity is low (*59*), and eukaryotic biomarker molecules are limited in both abundance and diversity (*61*). Moreover,

photosynthetic eukaryotes appear to have been most abundant and diverse in shoreline environments, despite the stresses that fluctuating salinity and temperature impose on such habitats (*62*).

Bangiophyte red algae occur in silicified tidal flat carbonates deposited around 1200 Ma (*63*), and conspicuously ornamented acritarchs also occur in rocks this age or older (*64–66*). Only in the latest Proterozoic, however, did morphologically complex, larger eukaryotic phytoplankton and branching macroalgal benthos diversify markedly in open shelf settings (*67–71*). Thus, the fossil record of algal diversification parallels, at least broadly, the history of ocean oxidation inferred from S isotopes (Fig. 1).

Trace Metals and the Nitrogen Cycle

If widespread sulfidic conditions were a unique feature of the mid-Proterozoic oceans, as suggested by S isotopes and other indicators, it is perhaps not surprising that C isotopes and fossils also mark this period as unique with respect to C cycling and evolution. But what might relate these seemingly unrelated phenomena?

The connection may lie in the effect of sulfidic conditions on the availability of redox-sensitive bioessential metals in the oceans (*72, 73*). In particular, Fe and Mo, important for biological N_2 fixation (the reduction of N_2 to biologically useful ammonia) and nitrate (NO_3^-) assimilation, are removed from solution in H_2S-bearing waters. These metals, therefore, directly couple ocean redox conditions to N bioavailability—leading us to question the common assumption that biological N_2 fixation precludes N limitation of the biosphere on geologic time scales.

Fe is effectively removed from solution in both oxic and sulfidic conditions. In the anoxic Archean oceans, the Fe concentration may have been as high as 50 μM (*2*), as opposed to concentrations more than three orders of magnitude lower in both modern oxygenated seawater (*74*) and sulfidic deep waters of the chemically stratified Black Sea (*75*)—the closest modern analog to a sulfidic ocean. Hence, Fe availability surely declined from the Archean to the mid-Proterozoic, whether the deep sea became more oxidized or reduced (*76*).

Mo forms the highly mobile molybdate anion (MoO_4^{2-}) under oxidizing conditions. Hence, today Mo is the most abundant transition metal in the oceans, with a concentration of 105 nM and an ocean residence time of ~8 × 10^5 years (*77–79*). In the presence of H_2S, Mo is readily removed to sediments by reduction to insoluble sulfides or conversion to particle-reactive thiomolybdate (MoS_4^{2-}) (*80*). Therefore, in the Black Sea, Mo concentrations fall from ~40 nM in oxygenated surface waters to ~3 nM below the chemocline (*81*). Such removal

would have limited the Mo concentration in mid-Proterozoic seawater (*82*). Sulfidic waters cover only ~0.3% of the sea floor today, localized in areas of restricted circulation and high productivity, but may account for as much as ~40% of Mo removal (*77, 79*). We infer that Mo surface concentrations in the late Paleoproterozoic and early Mesoproterozoic oceans were less than 10% of present levels if sulfidic conditions covered >10% of the sea floor (*83*).

A similar set of arguments can be made for some other bioessential metals that are also less available under sulfidic conditions, such as Cu, Zn, and Cd (*84*). The mid-Proterozoic interval may thus be the only extended period in Earth history during which Fe, Mo, and some other redox-sensitive, bioessential metals were simultaneously scarce in the oceans (Fig. 2) (*85*).

If so, the consequences for biology would have been profound. The energy-intensive process of N_2 fixation, a capability limited to some bacteria and archaea, can be catalyzed by three known nitrogenase metalloenzyme systems. Each requires Fe, in the form of Fe-S clusters. The best studied nitrogenase, present in all known diazotrophs, also requires Mo as part of a Fe_7MoS_9 cluster (*86*). In a number of organisms, two "alternative" nitrogenases—genetically distinct but clearly homologous with MoFe-nitrogenase—use V and Fe, or Fe alone, but not Mo (*87, 88*). The mechanism by which these enzyme systems reduce N_2 remains elusive (*89*). The specific activity of MoFe-nitrogenase for N_2 reduction appears to be ~1.5 times that of VFe-nitrogenase at ~30°C (*90*), and it is at least this much more efficient than Fe-nitrogenase (*87*), which helps to explain the prevalence of MoFe-nitrogenase in the modern environment (*91*).

Chemostat experiments (*92*) have shown that nitrogenase expression is regulated by Mo concentration. Alternative nitrogenase expression begins when Mo < 100 nM, and MoFe-nitrogenase is not expressed when Mo < 25 nM, about one-fourth the concentration in modern seawater. Hence, it seems likely that the less efficient alternative nitrogenases had prominent roles in global N cycling until the oceans were thoroughly oxygenated.

Redox-sensitive metals are also important in other parts of the N cycle. Mo, as part of a molybdopterin cofactor, is found in the nitrate reductase enzymes used for NO_3^- assimilation by eukaryotes and some prokaryotes, and in the nitrate reductases used by some prokaryotes in NO_3^- respiration ("denitrification") (*93–95*). Chemoautotrophs that oxidize ammonia ("nitrification") use the Mo enzyme nitrite oxidoreductase (*94*) and probably also use Cu in ammonia monooxygenase (*96*). Cu is also used in both nitrite and N_2O reductases (*97*). Fe appears to be necessary for all these processes as well as for NO reduction (*93, 96, 97*).

It follows that the development of sulfidic Proterozoic oceans would have initiated a period of exceptional N stress for the biosphere. Before this development, in the Fe-rich Archean and Paleoproterozoic oceans, biological N_2 fixation dominated by Fe-nitrogenase probably accounted for most of the fixed N supply (98). MoFe-nitrogenase was likely unimportant because, as with S, input of Mo from weathering would have been limited under an atmosphere with only trace O_2. Regardless of metal abundances, nitrification and denitrification were

(102). At the same time, rising PO_2 likely led to biological nitrification and denitrification becoming important on a global scale. These processes, although hampered by metal scarcity, would have converted NH_4^+ in surface seawater to NO_3^-, NO_2^-, N_2, and N_2O. Although NO_3^- and NO_2^- are bioavailable forms of fixed N today, their utility would have been limited in the mid-Proterozoic because of the Mo (and Fe) requirement of the various nitrate reductase enzymes. Because N_2 and N_2O are volatile

shorter than ocean mixing times, which are on the order of 1000 years. In contrast, the residence time of dissolved P is >10,000 years in oxygenated oceans (51, 105, 106) and perhaps longer in sulfidic oceans. Therefore, the effect of a typical orogenic episode on the availability of bioessential metals, and hence on ocean productivity, would have been local or regional and variable with time, rather than global and persistent (as is the case with P today). Lower overall productivity in the mid-Proterozoic, inferred from $\delta^{13}C_{carb}$, is also consistent with N stress and with the scarcity of other micronutrients (e.g., Zn, Cd) (107).

Fe scarcity apparently limits N_2 fixation in parts of the modern, oxygenated oceans [e.g., (108–110)], but the global bioavailability of fixed N is probably less constrained today for three reasons. First, because of the higher specific activity of MoFe-nitrogenase compared to the alternative nitrogenases, and the heavy Fe requirement of all the nitrogenases, the availability of Mo in oxygenated oceans may substantially reduce the impact of Fe limitation on biospheric N fixation rates. Second, Mo availability facilitates assimilation of NO_3^- as an N source, as well as exploitation of NO_3^- reduction to drive metabolism in suboxic environments with abundant NO_3^-. Third, the Phanerozoic development of a vigorous terrestrial N cycle introduced a new source of fixed N, largely unconstrained by metal availability, to the oceans. Although the N budget is not tightly quantified, at least as much N_2 is fixed each year on land as in the oceans (111). The transfer of even a small fraction of this fixed N from land to sea may have an important impact on the ocean N budget.

Implications for Eukaryote Evolution

Compared with autotrophic bacteria and archaea, photosynthetic eukaryotes are poorly equipped to cope with N-limited oceans in at least three ways. First, and most obviously, eukaryotes lack the capacity for biological N_2 fixation and must assimilate fixed N from their surroundings. Mo and Cu scarcity in sulfidic oceans would have exacerbated this problem by limiting the ability of eukaryotes to assimilate NO_3^- and NO_2^-. Second, red algae and most green algae (the two algal clades with chloroplasts descended directly from cyanobacterial endosymbionts) and all multicellular members of these groups secrete cellulosic cell walls that preclude ingestion of N-bearing organic particles. Third, in living cyanobacteria, NH_4^+ depletion induces formation of a transcriptional regulator, which in turn unleashes a battery of enzymes that efficiently scavenge bioavailable N from seawater (112, 113). Eukaryotes lack this ability.
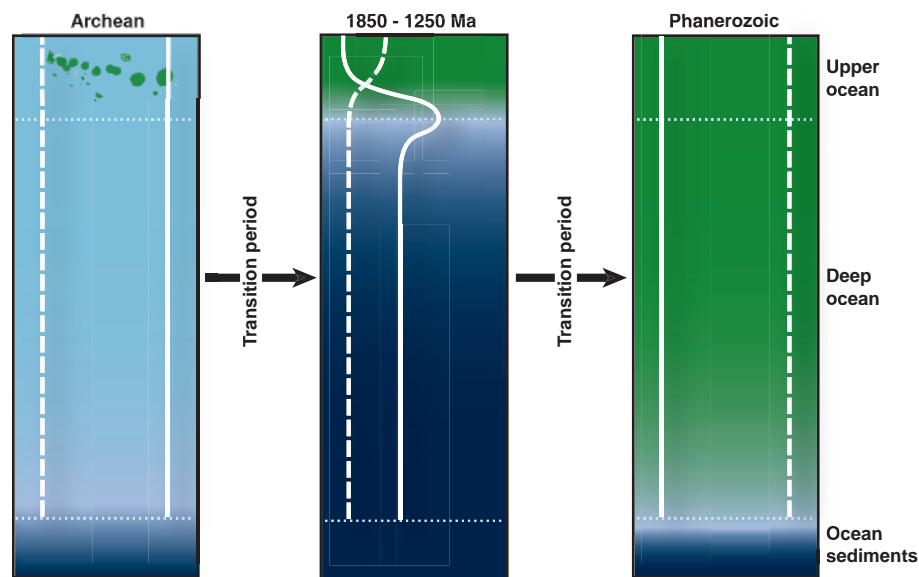
Fig. 2. Schematic depiction of effects of changing ocean redox conditions on the depth distributions of Mo (dashed lines) and Fe (solid lines). Influences of nutrient-type depletion and aeolian inputs on surface seawater concentrations are omitted for simplicity. Color gradations are the same as in Fig. 1. During the Archean, oceans are anoxic but not sulfidic. Significant O_2 is only associated with cyanobacterial "blooms." Mo is scarce because it is not readily mobilized from crustal rocks during weathering under low PO_2. Fe is abundant in the absence of O_2 and H_2S. From 1850 to 1250 Ma, moderate PO_2 oxygenates surface waters but sulfidic deep waters develop. Mo is scarce because of rapid removal in sulfidic waters. Mo is somewhat elevated at the surface because of upper ocean oxygenation and enhanced oxidative weathering. Fe, as in the modern Black Sea, is depleted in sulfidic deep waters, severely depleted in oxic surface waters, and enriched near the redoxcline where both O_2 and H_2S are scarce. During the Phanerozoic, O_2 penetrates to the sediment-water interface. Mo and Fe distributions are similar to today's. See text for details and references.

likely of minor importance before oxygenation of the surface ocean (99). Hence, denitrification as a pathway for loss of fixed N was minor as long as PO_2 remained low. Fixed N may have been relatively abundant in the form of the ammonium ion (NH_4^+) and was probably lost primarily by burial of organic N in sediments and loss of volatile NH_3 to the atmosphere. Phosphate was likely the limiting nutrient (49).

The situation would have changed after ~1800 Ma. Global rates of N_2 fixation presumably decreased because of the decrease in ocean Fe, particularly if—in contrast to modern oceans—the use of the more efficient MoFe-nitrogenase was limited by Mo scarcity (100, 101). The antagonistic effect of O_2 on nitrogenase activity may also have been important before the development of compensatory strategies

and much less soluble than NH_3, their formation would have accelerated loss of fixed N from the oceans. Thus, it seems likely that the ocean inventory of fixed N, as well as NH_4^+ levels in surface waters, decreased after ~1800 Ma. Relevant N isotope data are sparse and difficult to interpret, but they suggest a qualitative change in the global N budget at about this time, consistent with oxygenation of the upper ocean (103, 104).

N stress of the mid-Proterozoic biosphere is consistent with $\delta^{13}C_{carb}$ stasis because, in contrast to P, continental weathering is a minor source of N to the oceans. Although the supplies of many redox-sensitive metals are ultimately controlled by tectonic activity, in the postulated sulfidic mid-Proterozoic oceans the residence times of many of these elements would probably have been much

Under conditions of nutrient limitation, therefore, eukaryotic algae compete poorly against cyanobacteria (*114*). Indeed, eukaryotic algae with larger cells (i.e., the seaweeds and net plankton most likely to be recognized as eukaryotic in the fossil record) compete most effectively when N availability exceeds their immediate metabolic needs, allowing them to store fixed N in intracellular vacuoles; NH_4^+ is not easily stored. Thus, in modern oceans the growth of larger algae is facilitated by high NO_3^- levels (*115, 116*). As a consequence of these limitations, mid-Proterozoic eukaryotic algae would likely have fared best in coastal and estuarine habitats where proximity to riverine metal sources minimized the effects of metal limitation, and where upwelling of NH_4^+-bearing deep waters could have provided an adequate source of bioavailable N.

Greatly enhanced weathering associated with the extensive Grenville orogeny at ~1250 Ma may have increased the supply of metals to the oceans. Enhanced burial of organic C initiated at this time may also have led to a modest rise in PO_2 (*45*). Together, these effects could have eased N limitation, facilitating limited eukaryotic diversification as the Neoproterozoic Era began (Fig. 1D). The contemporaneous termination of $\delta^{13}C$ stasis (*45*), suggestive of an intensification of the link between primary production and tectonics, is consistent with this scenario. However, only with the later Neoproterozoic appearance of more fully oxic oceans would the increased availability of Mo and Cu have facilitated the biological assimilation of NO_3^- and NO_2^-. Such assimilation would have greatly expanded the pool of bioavailable N, returned the oceans to a phosphate-limited regime, and enabled algae to diversify throughout the marine realm.

The known fossil record of eukaryotic algae is subject to preservational biases but is consistent with this scenario (*62, 67–71, 117–119*). Latest Proterozoic animal diversification, itself likely influenced by renewed oxygenation, would further have facilitated algal diversification via ecological interactions (*59, 69*).

Conclusions and Future Directions

Earth's "middle age" is emerging as neither a direct extension of its youth nor a simple prelude to its current state. At present we know just enough about this period to develop intriguing hypotheses connecting life and environments. The hypothesis presented here, consistent with available data, provides a compelling explanation for observed patterns of early eukaryote evolution. However, in view of the limitations of available data, it should be regarded primarily as a new lens through which to focus research.

In the geosciences, this hypothesis should help to motivate further investigations into Pro-terozoic environments and biology. Specifically, further work is needed to substantiate the inferences drawn from $\delta^{34}S$, to better constrain the timing and mechanism(s) of redox transitions, to determine the effects of these transitions on ocean biogeochemistry, and to tease more paleobiological information from the geologic record. New approaches may be helpful, including study of redox-sensitive metal abundances in sediments (*31, 120*), $\delta^{34}S$ of carbonate-associated sulfate (*36, 121*), mass-independent S isotope effects (*7*), and mass-dependent fractionation of Mo isotopes (*122*). Development of a reliable proxy for marine N isotopes would help to shed light on perturbations of the N cycle. Molecular biosignatures can provide an improved perspective on the relative abundances of prokaryotes and eukaryotes in Proterozoic oceans. Progress will come most rapidly if these and other techniques are applied by multidisciplinary teams working in an integrated manner on stratigraphic sequences of paleoenvironmental importance.

The hypothesis articulated here also suggests that bioinorganic chemistry, broadly defined, can provide unique insights into the coevolution of life and environment (*72, 73*). Integrated study of genetic diversity, (metallo-)enzyme gene expression, and elemental bioavailability in natural systems may be useful in unraveling life's history on Earth (and evaluating the prospects for life elsewhere). This should be fertile ground for research as the nascent subdisciplines of geobiology and astrobiology unfold.

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~100 m deep, and a riverine Mo flux comparable to the modern. There is no evidence of substantially reduced ocean circulation during this time. Although physical stratification and large fluxes of organic C are prerequisites to generating anoxic waters today, this would not have been the case under an atmosphere containing only a fraction of present-day PO_2. Even lower Mo concentration is expected if, as seems likely, the riverine Mo flux was lower under an atmosphere with lower PO_2.
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