Molecular oxygen (O$_2$) began to accumulate in the atmosphere and surface ocean ca. 2,400 million years ago (Ma), but the persistent oxygenation of water masses throughout the oceans developed much later, perhaps beginning as recently as 580–550 Ma. For much of the intervening interval, moderately euxinic surface waters lay above an oxygen minimum zone (OMZ) that tended toward euxinia (anoxic and sulfidic). Here we illustrate how contributions to primary production by anoxygenic photoautotrophs (including physiologically versatile cyanobacteria) influenced biogeochemical cycling during Earth’s middle age, helping to perpetuate our planet’s intermediate redox state by tempering O$_2$ production. Specifically, the ability to generate organic matter (OM) using sulfide as an electron donor enabled a positive biogeochemical feedback that sustained euxinia in the OMZ. On a geologic time scale, pyrite precipitation and burial governed a second feedback that moderated sulfide availability and water column oxygenation. Thus, we argue that the proportional contribution of anoxygenic photosynthesis to overall primary production would have influenced oceanic redox and the Proterozoic O$_2$ budget. Later Neo-proterozoic collapse of widespread euxinia and a concomitant return to ferruginous (anoxic and Fe$^{2+}$ rich) subsurface waters set in motion Earth’s transition from its prokaryote-dominated middle age, removing a physiological barrier to eukaryotic diversification (sulfide) and establishing, for the first time in Earth’s history, complete dominance of oxygenic photosynthesis in the oceans. This paved the way for the further oxygenation of the oceans and atmosphere and, ultimately, the evolution of complex multicellular organisms.

Over the past decade, paleoenvironmental insights from iron speciation (1–7), sulfur isotopes (3, 8–12), Mo systematics (13, 14), and organic geochemistry (15) have converged on a view of Proterozoic oceans (16, 17). With the global cessation of iron formations $\sim$1,840 million years ago (Ma) (5), euxinic water masses expanded beneath an oxygenated surface mixed layer. We do not know whether the oxygen minimum zone (OMZ) was always and everywhere euxinic (14, 18), but existing data suggest that euxinia was both widespread and persistent for an interval at least 1,000 Ma in duration (14). Deep ocean chemistry is less certain; mid-Proterozoic bottom waters have been modeled variously as sulfidic, anoxic but not sulfidic, and dysoxic (10, 13, 16, 19) — possibly all three existed in varying proportions. What mattered most for Proterozoic life (and, in fact, for the partial pressure of atmospheric oxygen; P$_{O2}$), however, was the general state of waters within the photic zone and immediately beneath the oxygenated surface ocean. How the world persisted in this seemingly static state, distinct from both Archean and Phanerozoic biospheres, for a billion years (20) remains largely unknown.

Oxygenic photosynthesis provides Earth’s only major source of molecular oxygen (O$_2$). In oxygenic cyanobacteria, photosystem I (PSI) strips electrons from chlorophyll to generate energy and reductants (ATP and NADPH), while a second photosystem (PSII), assisted by a Mn-based catalytic complex, replenishes the electron pool by oxidizing H$_2$O to O$_2$ (21). In contrast, green and purple sulfur bacteria (anoxygenic photoautotrophs) commonly use sulfide to drive primary production with PSI- and PSII-like machinery, respectively (22, 23). In this case, the production of oxidized sulfur compounds (S$^0$ or SO$_4$), rather than O$_2$, balances the formation of OM. Similarly, in the presence of sulfide, many cyanobacteria down-regulate PSII and obtain proportionally fewer (or no) electrons from water, instead oxidizing S$^2$ to S$^0$, much like green S bacteria (24). Such versatile cyanobacteria are often observed where sulfide intrudes in the photic zone (25), not surprising insofar as the oxidizing potential required to extract electrons from sulfide is significantly lower than that for water (21, 26). Unlike most anoxygenic photoautotrophs, however, cyanobacteria can quickly reinstate PSI and O$_2$ generation when returned to an oxic environment (27).

In some present day stratified lakes, which often contain shallow chemoclines, anoxygenic photoautotrophs can dominate primary production (up to 83%) (26). Anoxic marine basins commonly have much deeper chemoclines; nonetheless, anoxygenic photosynthesis can still contribute to overall primary production (28). In mid-Proterozoic oceans, lower P$_{O2}$ and warmer temperatures (reducing O$_2$ solubility) would have made sulfide much more available for anoxygenic photoautotrophy, enhancing their potential contribution to overall primary production. Increasing the relative proportion of anoxygenic photosynthesis would have decreased the direct link between OM burial and O$_2$ generation (Fig. 1). With this in mind, we explore the biogeochemical consequences of mixed oxygenic and anoxygenic photosynthesis in the oceans of Earth’s middle age.

In Proterozoic surface waters underlain by an anoxic OMZ, fixed N may have exerted fundamental control on total primary productivity (17, 29). Thus, when considering the aggregate of oxygenic and anoxygenic photosynthesis, the Proterozoic nitrogen (N) cycle must be taken into account simultaneously. In the modern oligotrophic ocean, upwelling of remineralized inorganic NO$_3$ provides most of the nutrient N (30) for photoautotrophs. In OMZs, however, N$\text{P}$ often falls well below the Redfield ratio of 16:1, suggesting net N loss via biological denitrification and anammox (31). In mid-Proterozoic oceans with a strong redoxcline, a microbial nutrient gauntlet would have developed as nutrient N (NO$_3$$^-$/and/or NH$_4^+$) ascended from deep waters toward the photic zone (29). Collectively, denitrification and anammox reactions would have removed upwardly advecting bioavailable N (31–33), and when the OMZ fell within the photic zone, anoxygenic photoautotrophs would have consumed much or all of remaining fixed N before it reached obligately oxygenic photoautotrophs in surface waters.
Thus, a persistent fixed-N deficiency throughout the OMZ and photic zone (17, 31) becomes likely and would have conferred ecological advantage on photoautotrophs able to fix N2. In the Proterozoic ocean, then, both impinging sulfide and a scarcity of fixed-N would have favored diazotrophic (N2-fixing) photoautotrophic bacteria over eukaryotic algae. Molecular fossils of pigments derived from anoxygenic phototrophs provide direct evidence for photic zone euxinia in Proterozoic oceans (15); such data, however, remain limited.

Widespread OMZ euxinia thus would have exerted a strong influence on the nature of primary producers in mid-Proterozoic oceans. Trace metal scarcity in Proterozoic oceans might further have limited the amount of primary production, via its effects on certain key enzymes (17, 34). The effect of trace metal limitation on primary production in Proterozoic seas remains an area of active debate and experimentation (35–37), but regardless of its resolution, the predominant influence on marine redox conditions in mid-Proterozoic oceans was the proportional contribution of anoxygenic photosynthesis to overall primary production.

Regardless of the source of OM, for energetic reasons, oxygen would still be the favored oxidant for OM remineralization; and as is true today, the propensity toward water column anoxia would scale with the amount of exported OM. Importantly, however, it is the fraction of OM escaping aerobic respiration that would set limits on anoxygenic photosynthesis (38). In Proterozoic oceans, with low overall NO3− and Fe3+ availability, SO42− reduction would have been the principal reductive metabolism after aerobic respiration, generating sulfide within oxygen-depleted OMZs. An increase in photosynthetic electron donation from this sulfide source (rather than from H2O) would depress surface O2 concentrations further, simultaneously enhancing the potential for N2-fixation. This would increase both primary and, presumably, export production—an overall positive feedback on OMZ euxinia (39) (Fig. 2A) that would limit rather than foster P O2 accumulation. That is, when primary production includes a nontrivial contribution from anoxygenic photoautotrophy, the generation of organic matter, in principle, exceeds the generation of oxygen available to complete the carbon cycle. This would increase the probability that the OMZ will become euxinic and, in consequence, that sulfide conditions will encourage the potential for N2-fixation. This photic zone sulfide is available for further anoxygenic photosynthesis—establishing the feedback loop.

Over geologic time scales, OM burial permits O2 accumulation (40), but only to the extent that primary production is driven by oxygenic photoautotrophs. OM burial in anoxic sediments is usually accompanied by significant pyritization, a net oxidative process relative to sulfide, as it effectively combines H2S with S0. Here, the S0 produced by anoxygenic photoautotrophy (26, 41–43) would pair with H2S and Fe2+, satisfying the electron balance required for pyrite formation (Fig. 2B). Export of OM-associated S0 to sediments, at potentially significant sinking velocities (26), could have served as ballast before fecal pellets came to play this role. Alternatively, iron sulfides may have been produced in the water column, as sinking S0 would react with dissolved sulfide to produce polysulfides, an important precursor to pyrite production (44). Either mechanism would facilitate loss of sulfur to sedimentary burial at a rate no greater than twice Fe delivery, ameliorating the potential for runaway sulfide production (Fig. 2). Finally, as OM burial and pyritization are both electron sinks, they would increase the overall oxidation state of ambient seawater (45). This is not, however, equivalent to increasing the O2 concentration of the ocean-atmosphere system, especially if a fraction of buried carbon derives from anoxygenic species.

In mid-Proterozoic oceans, then, the cycle of primary production and remineralization would have established a system in which two conjoined feedback loops worked to perpetuate OMZ euxinia and maintain moderate, but not high, levels of O2 (Fig. 2). These biologically mediated feedbacks link P O2 and OMZ euxinia, whereas the burial of reducing potential (over geological time scales) allows the accumulation of enough oxidizing capacity to avoid return to a largely anoxic fluid Earth like that of the Archean.

The relative contribution of anoxygenic photoautotrophy to mid-Proterozoic PO2 need not have been large for their presence to be felt. Primary production in the modern ocean is 1.5 × 1015 mol C/year, with an organic carbon burial rate of 5.33 × 1012 mol C/year (46, 47). When primary production is 100% oxygenic, the maximum rate at which O2 can accumulate is equivalent to the rate of carbon burial (≈5 × 1012 mol O2/year). Because the theoretical maximum
For calculation details).

thesis to total modern primary production at the burial of anoxygenically produced carbon is not strictly coupled to residual genic (by cyanobacteria, purple S bacteria, and/or green S bacteria) contribu-
der of the system. The presence of sulfide increases the likelihood of anoxy-
of net sulfide generation (2.62 equal to integrated rates of sulfate reduction (38), the modern rate
Fig. 2. A schematic view of feedbacks that acted to sustain Proterozoic environments on both short and long geologic time scales (A and B, respectively). The point of entrance into this cycle is the establishment of sulfidic conditions at ~1,840 Ma (5) and possibly earlier. Dashed green and solid red arrows note the direction of the feedback. If an increase in one quantity is followed by a decrease in the next, the connecting arrow is red (a negative feedback). If an increase in one quantity leads to an increase in the next, then the connecting arrow is green (a positive feedback). For example, if we begin in A with an increase in OMZ sulfide, PO2 correspondingly decreases (thus a red arrow preceding the PO2 ellipse), propagating responses through the remain-
der of the system. The presence of sulfide increases the likelihood of anox-
genic (by cyanobacteria, purple S bacteria, and/or green S bacteria) contribu-
tions to primary productivity, which would then produce less overall O2, encourage N2 fixation, increase primary production and carbon export, and increase the degree of euxinia (a positive feedback). (B) A sulfide-rich ocean in which S²⁻ is an oxidant byproduct of primary producers and provides sedi-
mentary conditions conducive to burial of both pyrite and carbon, although the burial of anoxogenically produced carbon is not strictly coupled to residual O2 (no O2 left behind). The loss of sulfide through pyrite burial dampens the extent of ocean euxinia (a negative feedback). The result is a system that maintains both oxygenic and anoxygenic photosynthesis.

can estimate a rate of diminished O2 production of 0.4% PO2 per 10 million years, even with the relative contribution of anoxygenic photosynthesis at only ~1% of the total production. When con-
sidering that Proterozoic O2 was likely much lower today, perhaps 1–10% of modern O2 (or PO2 ~0.2–2%), it is clear that even these humble contributions from anoxygenic photosynthesis, integrated across geologic time scales, would have impacted Earth’s surface oxygen budget. Although our arguments are framed in terms of water column budgets, they also apply to microbial mat systems, which were widespread on Proterozoic seafloors (5).

In contrast to the Proterozoic Eon, when euxinia was persistent, more recent episodes of euxinia in the Phanerozoic oceans have been transient, presumably because euxinia cannot not be sustained over multimillion year time scales in the face of the greater PO2 and, thus, the buffering capacity of the Phanerozoic atmosphere (SI Text).

If Earth’s middle age was self-sustaining, what drove its demise? Canfield and colleagues (4) recently reported that anoxic subsurface waters of later Neoproterozoic oceans returned to an iron-rich state more characteristic of Archean seas. This reversion resulted from the long-term removal of sulfur by the subduction of pyrite-rich Proterozoic marine sediments (52) (Fig. 2) and an increase in the proportional input of Fe to S into the ocean (53, 54). The loss of photic zone euxinia terminated quantitatively important contrib-
utions from sulfide-driven anoxygenic photosynthesis, thus ending control of the “sulfur world” on the oxidation state of the ocean-atmosphere system (Fig. 3). This switch removed two direct inhib-
itors of eukaryotic evolution: Sulfide, which is toxic to most eukaryotes, and low available N, as eukaryotic photoautotrophs cannot fix N2 (55–57). More work is required to determine whether N cycling (assimilatory and dissimilatory) and availability (balance of sources and sinks) would increase in a ferruginous ocean, relaxing N stress and favoring algal diversification, or whether continued anoxia, despite the loss of euxinia, would keep available N low.

Overall, these observations are consistent with the geologic record, as the oldest well-characterized eukaryotic microfossils occur in near-shore environments (58), where OMZ sulfide incursion was least likely. Similarly, the oldest eukaryotic fossils attrib-
utable to an extant phylum, bangiophyte red algae in ca. 1200 Ma rocks from Canada, lived on a tidal flat (59). Broadly coincident with the late Neoproterozoic geochemical transition, microfossils associated with N₂-fixing cyanobacteria (Nostoccales) decline strongly (60), diverse protists appear (56, 61, 62), and organic-rich sediments begin to record increases in sterane abundances (63) that herald the rise of green algae to ecological prominence.

We do not discount the possibility that tectonic circumstances also contributed to the perpetuation of Earth’s middle age, and we note that factors such as the low bioavailability of trace metals (14, 17, 34, 64, 65) may further have dampened the responsi-

In this formulation, O₂ production is sensitive to: (i) The mag-
nitude of overall primary production and burial fluxes and (ii) their respective ratios to overall sulfate reduction rates modified by the efficiency with which sulfide becomes available to autotrophs. Much of the sulfide produced in today’s ocean will not be available for photoautotrophic oxidation, because the sulfide is produced deep within the marine realm, most commonly within sediments. However, in mid-Proterozoic oceans, although the magnitude of primary production may have been smaller (17), sulfate reduction rates would have been similar or higher, as a greater fraction of primary organic matter was not aerobically respir ed (49). When combined with increased sulfide availability near or within the photic zone (15, 50), these conditions enhanced the likelihood that photosynthetic sulfide oxidizers would moderate oxygen levels due to positive feedbacks (Fig. 2). Simply, as the ratio of export production to sulfate reduction approaches unity, the potential for anoxygenic photoautotrophy to buffer PO2 increases. Thus, using approximations for Proterozoic sulfate reduction (reference 49 and SI Text) and keeping a modern burial efficiency (which likely underestimates Proterozoic burial due to low deep-water O2), we
marked by OMZ euxinia (such as in reference 15), but rare thereafter. And, consistent with this, our model predicts that the timing of Neoproterozoic eukaryotic diversification, as recorded in both paleontological and biomarker records, will be linked stratigraphically to the demise of euxinic OMZs. These geological predictions should be addressed in light of experimental research aimed at constraining contributions from Fe\(^{2+}\) using anoxygenic photoautotrophs (67) and, possibly, cyanobacteria (68) to Neoproterozoic primary production, an avenue yet to be explored.

Together, these perspectives issue a challenge: How do we quantify the interplay between rates of mixed primary production, summing oxygenic and anoxygenic photosynthesis, and remineralization efficiencies (presuming differing availabilities of O\(_2\), Fe\(^{3+}\), and SO\(_4^{2-}\) through time) in a world where OM burial is not strictly linked to P\(_{O2}\), the ultimate source of electron acceptors? In the end, we may find that the three long lasting states of Earth’s biosphere — broadly, the anoxic Archean, intermediate Proterozoic, and fully oxygenated Phanerozoic — will find relatively straightforward explanation in primary production that was largely anoxic in the Archean, oxygenic in the Phanerozoic, and mixed in between.

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