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REVIEWS

Opinion

Nitrogenase Inhibition Limited Oxygenation of Earth's Proterozoic Atmosphere

John F. Allen,[1,4,5,@,*] Brenda Thake,[2] and William F. Martin[3]

Cyanobacteria produced the oxygen that began to accumulate on Earth 2.5 billion years ago, at the dawn of the Proterozoic Eon. By 2.4 billion years ago, the Great Oxidation Event (GOE) marked the onset of an atmosphere containing oxygen. The oxygen content of the atmosphere then remained low for almost 2 billion years. Why? Nitrogenase, the sole nitrogen-fixing enzyme on Earth, controls the entry of molecular nitrogen into the biosphere. Nitrogenase is inhibited in air containing more than 2% oxygen: the concentration of oxygen in the Proterozoic atmosphere. We propose that oxygen inhibition of nitrogenase limited Proterozoic global primary production. Oxygen levels increased when upright terrestrial plants isolated nitrogen fixation in soil from photosynthetic oxygen production in shoots and leaves.

Highlights

Photosynthesis in cyanobacteria introduced oxygen into Earth's atmosphere, giving the Great Oxidation Event, about 2.4 billion years ago. Atmospheric oxygen concentration then remained puzzlingly low, at most only 10% of its present value, for nearly 2 billion years.

Nitrogen-fixing cyanobacteria that lack protection against oxygen cease to grow when oxygen reaches 10% of its present atmospheric level, the oxygen threshold for nitrogenase inhibition.

In the Proterozoic 'boring billion', oxygen inhibited nitrogen fixation, cell growth, and photosynthesis on a global scale, suppressing any further rise in atmospheric oxygen until the arrival of land plants about 450 million years ago.

Land plants separate aerial photosynthesis from nitrogen fixation in soil.

Earth's Great Oxidation Event

Oxygen gas is a classic example of life changing Earth: from a fully inhabited but anoxic planet to today's green, rich biosphere in an atmosphere containing 21% O_2 by volume. The history of life is closely tied to the history of O_2 accumulation. **Cyanobacteria** (see Glossary), prokaryotes with two photochemical reaction centres acting in series, first generated the O_2 we breathe [1–3]. The history of O_2 accumulation is key to understanding its impact on evolution [4]. Because life on land only goes back about 450 or 500 million years (MY) [5–8], almost 90% of biological evolution took place in subsurface [9] and aquatic environments. Current views of oxygen in Earth's history (Figure 1) depict the first traces of O_2 appearing in the oceans starting about 2.7 to 2.5 gigayears (Gyr) ago [4,9–11]. The **Great Oxidation Event (GOE)**, roughly 2.4 billion years ago [12–15], marked the end of the **Archaean** and began the third Eon of Earth's history, the **Proterozoic**, which lasted until the beginning of the Cambrian period at 541 MY ago. Following the GOE, O_2 rose to only 10% of its present atmospheric level (PAL), and stayed there for most of the Proterozoic. Thus, the Proterozoic atmosphere contained roughly 2% O_2 by volume [1,12,16] or less [4,17]. Isotopic studies indicate that for perhaps 2 billion years following the comparatively sudden GOE, further net O_2 accumulation ceased, with atmospheric levels remaining stable at no more than 10% PAL [12,16] and possibly as little as 0.001% PAL [4].

With atmospheric O_2 low, O_2 in aqueous solution stayed low as well [12,16]. Geochemical evidence suggests that the oceans remained largely anoxic throughout the Proterozoic [4,12,16], with a rise to modern oxygen levels starting around 580 MY ago [18] and in deep oceans perhaps as recently as only 430 MY ago [19] (Figure 1). The emergence of land plants and terrestrial carbon burial are implicated in the eventual increase in atmospheric O_2 near the beginning of the **Phanerozoic** Eon. Today, land plants comprise roughly 97% of Earth's surface biomass [20]. Their ecological success has been linked to the late rise in O_2 because terrestrial sequestration of organic carbon as fibrous biomass protects it from reoxidation to CO_2, curbing O_2 consumption [19,21].

What Limited Oxygen Accumulation during the Boring Billion?

A major puzzle of Earth's history is why O_2 rose so late, that is, why atmospheric and marine O_2 levels remained low for almost 2 billion years, fully half of life's history, despite the existence of cyanobacteria, which were capable of producing enough O_2 to change the planet. What held cyanobacteria back, and why did O_2 not accumulate linearly following the GOE? The search for the cause of persistent low oxygen during the Proterozoic, or 'the boring billion' as it is sometimes called [22], is a search for the factors or mechanisms that limited O_2 accumulation.

Geochemists have long recognised that Proterozoic O_2 stasis presents a problem and have proposed a number of explanations to account for the delayed oxygen rise. The proposals fall broadly into three

[1]Research Department of Genetics, Evolution and Environment, Darwin Building, University College London, Gower Street, London WC1E 6BT, UK

[2]School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK

[3]Institute of Molecular Evolution, Heinrich-Heine-Universitaet Duesseldorf, Universitaetsstr. 1, 40225 Duesseldorf, Germany

[4]https://jfallen.org

[5]https://www.ucl.ac.uk/biosciences/people/john-allen

@Twitter: @ProfJohnAllen (J.F. Allen).

*Correspondence: j.f.allen@ucl.ac.uk

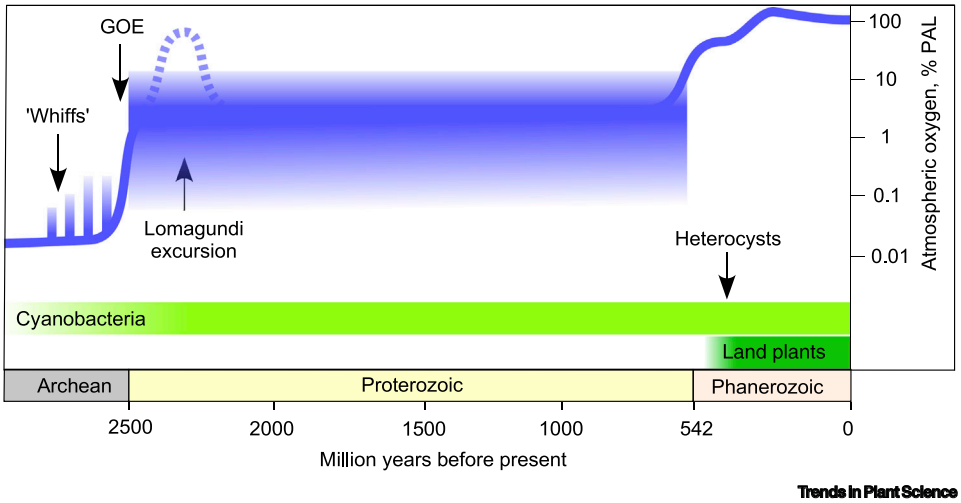

Figure 1. Schematic Summary of O_2 Accumulation in Earth History.
Modified after references [1,4,12,16,19]. For most of the Proterozoic Eon, free O_2 was much less abundant than it is today. Lyons *et al.* [4] estimate Proterozoic O_2 in the atmosphere as low as 0.01% of present atmospheric level (PAL), Holland [12] estimates atmospheric O_2 at around 10%–20% PAL, Stolper and Keller [16] estimate mid-Proterozoic deep ocean dissolved O_2 concentrations at about 11 μM or roughly 6% the present value of 178 μM. 'Whiffs' refer to isotope signatures for evidence of local O_2 before the Great Oxidation Event (GOE) [1,4]. The Lomagundi excursion is dotted because it is included in the summary of [4] but not in that of [1,12]. Heterocysts refer to differentiated cells produced by some cyanobacteria to protect nitrogenase from inactivation by O_2. Their relevance is that cyanobacteria have an ancient fossil record, but the oldest fossil heterocysts [84] are younger than land plants, suggesting that cyanobacteria evolved this mechanism of O_2 protection in response to Phanerozoic O_2 accumulation.

categories. The first posits a steady supply of geochemical reductants from within the Earth, such as Fe^{2+} or S^{2-}. In this proposal these reductants continuously consumed the O_2 produced by cyanobacteria, keeping O_2 low [23,24], or they sustained anoxygenic phototrophs that out-competed oxygenic cyanobacteria for light or for nutrients such as phosphorus [25]. The second category posits nutrient limitations imposed by trace elements, such as molybdenum, that are required for cyanobacterial (or eukaryotic algal) growth: scarce trace nutrients limited O_2 production by limiting photosynthetic biomass [26,27]. One component of this category is trace element limitation of biologically available nitrogen, thereby limiting both oxygen accumulation and eukaryotic diversification [28]. A third category invokes biotically induced changes affecting the degree of mixing between nutrient-rich reservoirs and the photic zone, for example, through animal burrowing activity [29,30] or by grazing activity of early animals to improve light penetration into the photic zone, increasing O_2 production [31].

The three kinds of explanation, in addition to others [4,11,15,17], might apply in some areas of the ocean, or might apply for some phases of Earth's history. But how and why they should act in concert to produce low O_2 across the planet for almost 2 billion years is yet unresolved. Accounting for limited O_2 accrual on a global scale for 2 billion years poses a substantial challenge [4,11,15,17,32]. All three proposals limit the rate of O_2 accumulation, as opposed to determining its final value.

Reductant (the initial electron donor) for photosynthesis cannot have been the factor limiting O_2 accumulation. When cyanobacteria invented **oxygenic photosynthesis** [33–35], the biosphere gained access to unlimited reductant: water. That was a monumental change in the nature of limiting principles for primary production. Prior to the advent of photosynthesis, geochemical H_2 was the reductant for autotrophic growth and its availability limited primary production [9,36,37]. The advent of **anoxygenic photosynthesis** with only one type of photochemical reaction centre offered cells access to compounds other than H_2 as a reductant to generate reduced ferredoxin for CO_2 fixation [35]. But

Glossary

Anoxygenic photosynthesis: conversion of radiant energy into chemical potential energy using an electron donor other than water and without production of free, molecular oxygen.
Archaean: also 'Archean'. The second geological Eon of Earth's history, from 4.0 to 2.5 Ga before present.
Cyanobacteria: prokaryotes able to perform oxygenic photosynthesis.
Diazocyte: a multifilament structure formed by some filamentous cyanobacteria, such as *Trichodesmium*.
Great Oxidation Event (GOE): the rise in atmospheric oxygen at 2.4 to 2.3 at Gigayears (Ga) before present.
Heterocyst: a nitrogen-fixing, specialised, and morphologically distinct cell of filamentous cyanobacteria.
Lomagundi excursion: a carbon isotopic anomaly at 2.3 Ga taken to indicate a global, transient rise in atmospheric oxygen.
Nitrogenase: the enzyme catalysing the conversion of nitrogen gas, N_2, to ammonia, NH_3.
Oxygenic photosynthesis: conversion of radiant energy into chemical potential energy using water as the initial electron donor and thereby producing free, molecular oxygen.
Phanerozoic: the fourth, and current, geological Eon of Earth's history, from 541 Ma to the present day.
Proterozoic: the third geological Eon of Earth's history, from 2.5 Ga to 541 Ma before present.

even in the presence of anoxygenic photosynthesis, reductants such as H_2S and Fe^{II} remained limiting for primary production. The cooperation of two distinct types of photochemical reaction centre at the origin of water-splitting, oxygenic photosynthesis 2.5 billion years ago fundamentally changed the nature of primary production: the novel reductant, water, could never be limiting in aquatic environments. If reductant was no longer limiting, what was?

Nitrogenase Is an Autoregulator of Oxygen Levels

Here we propose Proterozoic limitation of primary production by a macronutrient: fixed nitrogen. We propose that feedback inhibition of a single enzymatic activity limited O_2 accumulation for almost 2 billion years: O_2 inhibition of **nitrogenase**. We further propose that this negative feedback accounts for the delayed oxygen rise during the Proterozoic in a manner that is robust to local or global fluctuations in geochemical O_2 consumption, intermittent trace element limitation, or ocean water mixing.

Carbon and nitrogen enter the biosphere in distinct chemical reactions catalysed by specific enzymes. For carbon there are six pathways of CO_2 assimilation that differ in age, oxygen tolerance, and CO_2 reducing enzymes [38,39]. Cyanobacteria use the Benson-Calvin cycle [40]. Its primary step is a carboxylation reaction catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Although O_2 competes with CO_2 for the Rubisco active site [41], O_2 does not inactivate Rubisco, and the Benson-Calvin cycle has no intrinsically O_2-sensitive enzyme. For nitrogen the situation is different. There is only one entry point of N_2 into metabolism: nitrogenase [42–44]. Nitrogenase catalyses direct reduction of nitrogen to ammonia [45,46] using electrons provided by an iron sulphur protein, ferredoxin [47,48], which also readily reduces oxygen [49]. Nitrogenase is widespread among cyanobacteria [43,50]. There are Mo-, Fe-, and V-containing isoforms of the enzyme [42,51] that all share a common ancestor and share the same active site [52–54]. All known nitrogenases are inactivated by O_2 [32,43,55–58].

The nitrogenase active site is replete with metals (Figure 2) and harbours a metal-coordinated carbide carbon atom, unique among all enzymes known so far [53]. Nitrogenase traces back to the universal common ancestor of all cells [59] and represents life's only catalytic tool capable of accessing N_2 for primary production. Like a blacksmith, nitrogenase uses ancient but robust technology. Nitrogenase has an obligatory H_2-producing side reaction, requires 8 electrons and 16 ATP per N_2 fixed, and ATP is consumed at steps that alter the redox potential of FeS clusters via conformational change [44]. Nitrogenase requires numerous assembly factors [51] and has been neither replaced nor improved during evolution, which tells us that the solution that life found to fix N_2 is the only one readily attainable in 4 billion years of physiological engineering by microorganisms. Nitrogenase is a limiting factor. Nitrogen fixation is thus inhibited by molecular oxygen [32,43,55–58] in the feedback loop depicted in Figure 3 (Key Figure). We propose that this simple property alone limited O_2 accumulation over geological time.

Nitrogenase feedback inhibition operates as follows. By dry weight, cells are about 50% carbon and about 10% nitrogen [60–62]. Cyanobacteria had water as unlimited reductant for CO_2 fixation, but, for net growth to occur, N_2 incorporation had to keep pace. Nitrogenase is inhibited by oxygen, but there is a threshold of oxygen concentration below which nitrogenase remains active and above which nitrogen fixation ceases completely. Diazotrophic (nitrogen-fixing) cyanobacteria such as *Plectonema* appear to lack mechanisms to protect nitrogenase from O_2. When such cultures are maintained with N_2 as the sole N source, and with sufficient CO_2 and light, then they grow and accumulate no more than 2% oxygen in their gas phase [32,55,57,58,63–67]. The 2% O_2 remains constant during prolonged cell culture because it is the O_2 partial pressure beyond which nitrogenase activity limits growth. With greater O_2, nitrogenase is inactivated and there is no fixed N to support further biomass accumulation. With less O_2, nitrogenase outpaces CO_2 fixation until the latter catches up, returning O_2 to 2% in the culture. In cyanobacteria, CO_2 fixation means O_2 production. Oxygen inhibition of nitrogenase makes it an O_2-sensing autoregulator of N availability (Figure 3).

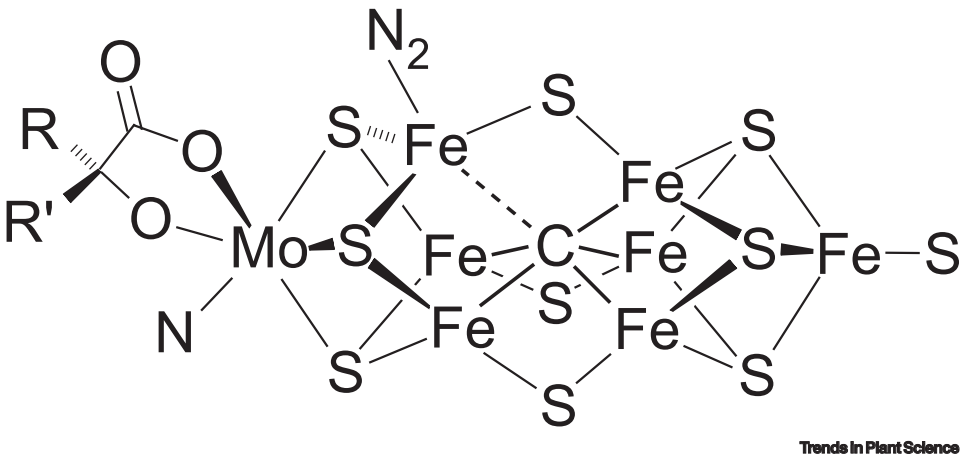

Figure 2. Model of the Oxygen-Sensitive Catalytic Site (M Cluster) of Nitrogenase.
Redrawn from [52] with the proposed binding site for N_2 [52] and the alternative substrate C_2H_2 [109]. There are three FeS-containing clusters in nitrogenase [51,109].

Because there is no biochemical alternative to nitrogenase for fixing N_2, because there are no O_2 tolerant nitrogenases known, and because reductant for CO_2 fixation was not limiting for cyanobacteria, this feedback loop would have operated, on a planetary scale, for 2 billion years or more. While primary production using H_2S instead of H_2O, as in *Oscillatoria limnetica* [33,68–72], would not have been subject to O_2-feedback inhibition, it would have been limited by the availability of reductant [37] at levels far below that of oxygenic photosynthesis. Furthermore, water oxidation at photosystem II is inhibited by sulphide [69,70]. Nitrogenase was an O_2-inhibited sensor that kept environmental O_2 low throughout the Proterozoic.

The Late Arrival of the Heterocyst

Nitrogen fixation is inhibited by molecular oxygen and cyanobacteria have evolved several mechanisms to deal with this inhibition [73,74]. In some filamentous cyanobacteria, **heterocysts** are

Key Figure

Inhibitory Feedback at Nitrogenase

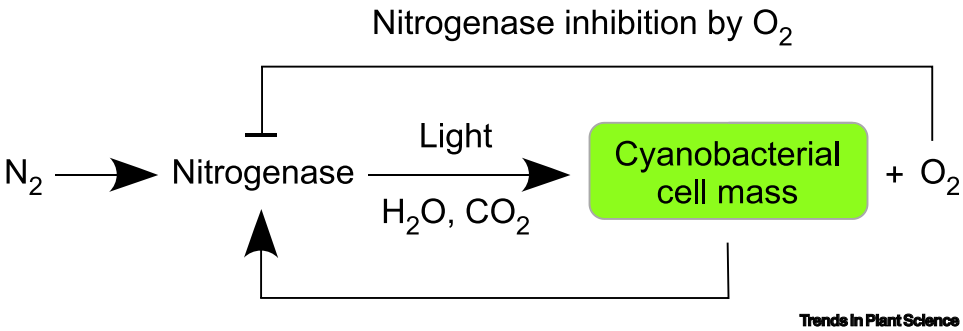

Figure 3. O_2 inhibits nitrogenase, which is required for cell growth and O_2 production in photosynthesis. During the Proterozoic Eon of Earth's history this negative feedback produced a steady state in which environmental O_2 levels did not exceed 10% of present atmospheric level (PAL).

specialised nitrogen-fixing cells [43,75] that have no oxygen-evolving photosystem II. Heterocysts fix nitrogen using ATP from photosystem I cyclic photophosphorylation together with electrons from substrates imported from neighbouring vegetative cells [43,75–77]. Heterocysts therefore permit cyanobacterial nitrogen fixation under aerobic conditions. Some cyanobacteria without heterocysts can fix nitrogen, but do so only in darkness, temporally separating nitrogen fixation from photosynthesis [78,79], or under low oxygen conditions [64,65,71], often using sulphide instead of water as the inorganic electron donor [68,71]. Yet other cyanobacteria without heterocysts can fix nitrogen, but do so only at low oxygen partial pressures [32,55,57,58,63–67] because they have not evolved special O_2 protection mechanisms. Non-nitrogen-fixing cyanobacteria obtain nitrogen from ammonia, nitrate, nitrite, or urea [80], and control photosystem stoichiometry to match these substrates' differing requirements for ATP relative to electrons from reduced ferredoxin [81,82].

Cyanobacterial nitrogen fixation is ancient [83] (Figure 4). Nevertheless, fossil heterocysts have not been seen in rocks predating the Devonian period [72]. The cyanobacterial lineages that today possess heterocysts also possess spore-like structures termed akinetes [84,85]. Though fossil akinetes have a record that extends into the Proterozoic [85,86], heterocysts themselves are lacking in the otherwise well-documented Precambrian cyanobacterial fossil record [11,85–87]. The oldest uncontroversial fossil heterocysts trace to land ecosystems of the Rhynie chert [84], a mere 408 MY old [88]. A Devonian origin of heterocysts (Figure 4) suggests that they arose as cells dedicated to protection of nitrogen fixation from an oxygenated atmosphere and in response to late (Figure 1) oxygen accumulation.

Cyanobacteria have evolved mechanisms besides heterocysts to avoid nitrogenase inhibition by oxygen [43,50], including N_2 fixation in the dark [89], or filament bundles, as in *Trichodesmium* [55,58,90]. Critics might counter that such mechanisms could have bypassed O_2 feedback inhibition during the Proterozoic. There are, however, two problems with this objection. First, the mechanisms that cyanobacteria use to deal with modern O_2 levels appear in phylogenies to have arisen independently in diverse lineages (Figure 4), not at the base of cyanobacterial evolution when water oxidation had first emerged [50,91]. Second, despite cyanobacteria having a fossil record that extends into the Archaean [92], there is no palaeontological trace of Proterozoic heterocysts [93,94]. The oldest uncontroversial fossil heterocysts are merely Devonian in age [84], younger than the first land plants and contemporary with the late atmospheric O_2 increase (Figure 1). We propose that filamentous cyanobacterial lineages are ancient, while their heterocysts are not, and that heterocysts emerged to protect nitrogenase from the high atmospheric levels of oxygen generated by land plants. Indeed, a recent phylogenomic investigation of trait evolution in cyanobacteria also indicates that heterocysts arose late in cyanobacterial evolution [95].

Critics might also interject that cyanobacteria were under intense selection to overcome the O_2 feedback inhibition and so attain higher levels of primary production. There are two strong rebuttals of this point. First, evolution operates without foresight and within unconditional chemical constraints. Organisms cannot know what the future might hold for them if only they can make a particular innovation, in the same way that scientists do not know what is possible with optoelectronics, semiconductors, or gene technology until discovery is made and the new technology is functional. In the boring billion, everything was (boringly) stable from the standpoint of microbes. Cyanobacteria had their preferred amount of O_2 and N, and there was enough oxygen around in the photic zone to make prokaryotic respiratory chains with terminal oxidases useful. Predominantly anoxic oceans remained inhabited by anaerobic lineages that had existed prior to the GOE and that continue to thrive and evolve to this day. Low oxygen also permitted the establishment of mitochondria that employed both terminal oxidases and O_2-independent redox balance pathways, allowing the diversification of aerobic, anaerobic, and facultatively anaerobic eukaryotic lineages [21,96,97] during the Proterozoic. The second rebuttal uncovers evidence in favour of a late origin of nitrogenase protection mechanisms. In the light, the oxygen content of a photosynthesizing cyanobacterial cell has two components: oxygen that diffuses in from the surroundings and oxygen that is produced by the cell's own photosynthetic membrane. The relative contributions of each have been reported [98] for *Synechococcus*, which possesses thylakoids, and *Gloeobacter violaceus*, which does not. For *Synechococcus*,

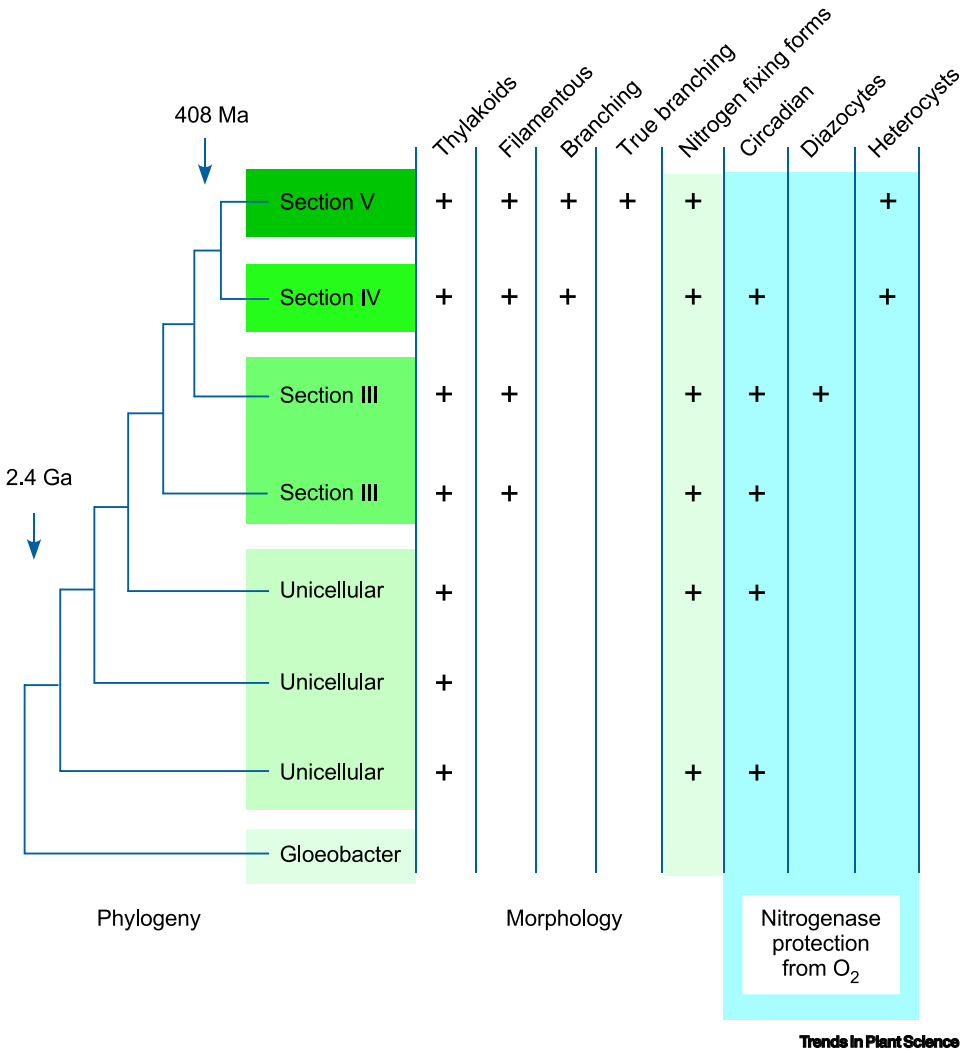

Figure 4. Heterocysts Arrived Late in Cyanobacterial Evolution.

The schematic phylogeny of cyanobacteria summarises the molecular phylogenies published in references [86,87,91,107]. The morphological classifications of cyanobacteria (see [91,95]) are: Section V: filamentous branching, heterocyst-forming; Section IV: filamentous branching; Section III: filamentous; Section: I unicellular. Unicellular forms interleave in references [85–87,91,95,107] and are often not diazotrophic, symbolised by three unicellular lineages in the figure. Although the phylogenetic studies sample different lineages, employ different phylogenetic markers, and use different methods, they always identify Section V (heterocyst-forming) as a monophyletic derived group and as the sister to Section IV (also monophyletic). Diazocytes designate multifilament structures formed by some filamentous lineages such as *Trichodesmium* [55,90]. The most common form of nitrogenase protection in cyanobacteria is circadian expression (nitrogen fixation during the dark phase when there is no photosynthesis and respiration consumes oxygen); see [74,89]. The date of 2.4 billion years (left) indicates the time preceding the Great Oxidation Event as the approximate starting point of oxygenic photosynthetic cyanobacterial evolution. The arrow labelled 408 Ma signifies that the fossil record provides no evidence for heterocysts in rocks older than the Rhynie chert, 408 million years of age. Although akinetes extend further back in the fossil record (see text), at face value the data indicate that heterocysts did indeed arise in response to rising oxygen, as traditional reasoning on the topic has it [86,87,91,107], while this occurred under Phanerozoic rather than Proterozoic atmospheric O_2, and after the origin of land plants (see text; also Figure 1).

the excess cytoplasmic oxygen produced by photosynthesis was determined as 0.25 μM, three orders of magnitude lower than aqueous phase dissolved O_2 in equilibrium with today's atmospheric oxygen level. For *Gloeobacter*, the value was lower still, 0.025 μM [98]. The evolutionary pressure that selected for nitrogenase protection mechanisms stemmed from outside the cell (atmospheric oxygen), not from within (nascent photosynthetic oxygen).

A consequence of the late origin of nitrogenase-protective mechanisms in cyanobacteria (Figure 4) is that the N_2 fixing strategy of *Plectonema*, namely the lack of nitrogenase-protective mechanisms for O_2 levels exceeding 2%, is the ancestral state for the entire cyanobacterial group. In other words, 2% O_2 afforded sufficient generic nitrogenase protection for cyanobacteria prior to O_2 levels exceeding 2%, after which specific nitrogenase protective mechanisms (diurnal, **diazocytes**, heterocysts) originated in independent lineages.

The concept of limiting metal availability (Mo, V, or Fe) for nitrogenase activity [26,27] is an element of many proposals to account for low Proterozoic O_2. Our proposal differs from micronutrient limitation in a crucial aspect. Limiting the number of active nitrogenase enzymes in the environment by limiting metal availability only limits the rate at which cyanobacteria produce O_2, requiring other factors to impose limits upon the final O_2 partial pressure. Nitrogenase feedback inhibition by O_2 regulates the O_2 partial pressure directly, independent of the rate of photosynthesis, and generates a value for atmospheric O_2 partial pressure very close to the geochemical observation. Proterozoic primary production may then have been driven by a combination of restricted oxygenic photosynthesis coexisting with anoxygenic photosynthesis [18,99,100].

An O_2 Overshoot 2.3 Billion Years Ago?

An isotopic anomaly called the **Lomagundi excursion** needs to be addressed. At 2.3 to 2.2 Ga ago, the isotopic record first reported from the Lomagundi formation in Zimbabwe indicates burial of heavy (^{13}C enriched) carbon [4]. This ^{13}C increase is sometimes interpreted as indicating the presence of large amounts of O_2 on a global scale [4,12]. If that interpretation is correct, its least explicable aspect is that following the Lomagundi excursion, oxygen levels drop once again [4]. Yet they do not drop to precyanobacterial levels, rather they drop to oxygen levels very near 2% O_2 [v/v], the oxygen partial pressure that nitrogenase feedback inhibition generates in laboratory cultures [32,55]. If the Lomagundi excursion is taken as a valid proxy for high global O_2 levels, the following situation at the GOE ensues. O_2 is a strong oxidant. Its contribution to metabolic evolution was not just new metabolic pathways [101], but more complete oxidation of existing organic substrates [102]. O_2 mobilised organic nitrogen and carbon sequestered in biomass, thereby overwhelming pre-existing redox buffers [103]. By liberating sequestered nitrogen (and carbon as CO_2) that was previously inaccessible to anaerobes, the onset of O_2 accumulation at the GOE initiated a positive growth feedback loop for aerobic autotrophs that were not reductant limited: cyanobacteria. When anaerobically deposited nitrogen reserves had been liberated, nitrogenase feedback inhibition set in, driving O_2 levels down to Proterozoic levels and keeping them low for over a billion years thereafter. The modern, oxygen-dependent nitrogen cycle may have been initiated, only to become restricted as O_2 levels fell [104]. Our proposal does not at all hinge upon the Lomagundi excursion, yet if it is interpreted as evidence for transiently high global O_2 levels, for high rates of CO_2 liberation, or both, our proposal could account for its emergence (nitrogenase-independent N availability during the excursion) and, perhaps more importantly, for the subsequent return to low O_2.

If our proposal for the Lomagundi excursion is correct, then it recorded, globally and over millions of years, a spike in O_2 concentration that inhibited nitrogenase and during which previously available fixed nitrogen became depleted. Exactly this pattern is reported in a modern microbial mat during the natural diel cycle [105]. In these observations, and following the onset of illumination, increased oxygen inhibits both nitrogenase activity and *nif* gene transcription [105]. The initial effect of illumination, however, is a transient increase in the rate of nitrogen fixation to give a maximum value by means of increased ATP and reductant from photosynthetic electron transport. After a lag of a few hours, O_2 concentration again becomes inhibitory to nitrogen fixation [105].

When and why did feedback inhibition at nitrogenase cease to keep O_2 low? At the origin of land plants, the nature of biomass changed and O_2 production by upright terrestrial plants became physically separated from N_2 fixation in aquatic environments and soil. Deposition by land plants of nitrogen-free cellulose [6,7], billions of tonnes of it, became a massive sink for CO_2 without exerting similar effects on nitrogen availability, thus allowing O_2 to increase through the standard mechanism of carbon burial, bypassing control by aquatic nitrogenase feedback.

Concluding Remarks and Future Perspectives

By oxidising water to free, molecular oxygen [3], the first cyanobacteria [33,91,106–108] introduced a strong electron acceptor into the atmosphere, revolutionizing Earth's chemistry, geology, and biology [35,96,97]. We suggest that oxygen inhibition of any ecosystem's cornerstone enzyme activity, nitrogenase, created an initial bottleneck for oxygenic primary production that is sufficient to account for low oxygen levels throughout the Proterozoic, or the boring (2) billion. Nitrogenase feedback inhibition could directly account for Proterozoic low oxygen stasis. Oxygen-inhibition of nitrogenase would have driven down transiently higher O_2 levels ensuing from previously accumulated fixed nitrogen and would have ceased at the origin of land plants. With its testable predictions and attendant questions (see Outstanding Questions), our model (Figure 3) requires light, CO_2, and N_2 in the photic zone, and accommodates local and global variation in geochemical conditions while remaining robust to their effects. The factor limiting Proterozoic O_2 accumulation was not geochemical. It was biological, and the attribute of a single enzyme, nitrogenase, contained within and synthesised by living cells.

Outstanding Questions

Some cyanobacteria can use H_2S facultatively as their electron donor for anoxygenic photosynthesis. Which protein complexes are involved and how do these cyanobacteria switch between electron donors?

Catalytic metal clusters of nitrogenase and oxygenic photosystem II arose only once in 4 billion years. They transformed our planet and have never been superseded. Where did these singularities in evolution come from and why did they arise only once?

Before the origin of water splitting, reductant was limiting for primary production. What was the succession of rate-limiting steps in global primary production?

Recalling that nutrients like iron can limit the rate of O_2 accumulation but not its final value, what biological factor or factors, other than nitrogenase inhibition, could have kept O_2 low for 2 billion years?

Mechanisms of nitrogenase protection are different in different cyanobacterial lineages (heterocysts, diazocytes, diurnal expression), and some cyanobacteria lack nitrogenase protection altogether. Which selection pressures other than late oxygenation could have elicited such diverse evolutionary responses?

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