Chapter 1

ELECTRON DONOR LIMITATION OF THE BIOSPHERE BEFORE THE ORIGIN OF OXYGENIC PHOTOSYNTHESIS

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Abstract:

The evolution of oxygenic photosynthesis, and the resulting oxygenation of the atmosphere ~2.3 billion years ago, was the single largest shift in the size and structure of the biosphere since the origin of life. Oxygen-fueled metabolisms are highly energetic, and drove the evolution of complex life in Neoproterozoic and Phanerozoic time, and oxygenic photosynthesis itself came to dominate primary productivity on the surface of the Earth. Perhaps more revolutionary than the introduction of molecular oxygen, oxygenic photosynthesis introduced the ability for life to utilize water—an essentially infinite electron donor—for autotrophy. Before the evolution of oxygenic photosynthesis, the biosphere was supported by anaerobic metabolisms such as methanogenesis, acetogenesis, and anoxygenic photosynthesis. In addition to being less energetic than oxygen-based metabolisms, these early autotrophic processes depend on geologically supplied electron donors such as molecular hydrogen and ferrous iron as a source of electrons for reducing CO_2 to organic carbon. As a result, the geological fluxes of electron donors may have served as a fundamental limitation to the productivity of the early biosphere, rather than nutrients like phosphate or fixed nitrogen as today. Here, we integrate estimates for the geological fluxes of electron donor compounds, phosphate, and fixed nitrogen to determine the fundamental limits to pre-oxygen productivity. We demonstrate that even if iron

oxidation was occurring, it was a relatively minor fuel for productivity compared to molecular hydrogen. Furthermore, we show that fluxes of fixed nitrogen and phosphate were substantially in excess of rates of productivity allowed by the supply of electron donor compounds, even in the absence of biological nitrogen fixation. This suggests that until the evolution of oxygenic photosynthesis, the size and productivity of the biosphere were fundamentally constrained by the geological supply of electron donors, and that there may not have been evolutionary pressure to evolve biological nitrogen fixation until after the evolution of oxygenic photosynthesis.

Introduction:

While the antiquity of oxygenic photosynthesis by Cyanobacteria is a matter of significant debate (Johnson et al. 2013a, Johnson et al. 2013b, Crowe et al. 2013, Planavsky et al. 2014, Lyons et al. 2014, Fischer et al. 2016, Shih et al. 2016, Ward et al. 2016), molecular oxygen is constrained to extremely low concentrations earlier in Earth history (Farquhar et al. 2000, Johnson et al. 2013), but rapidly accumulated to significant concentrations at the GOE (Goldblatt et al. 2006, Bekker and Holland 2012, Ward et al. 2016, Luo et al. 2016).

Perhaps the most significant impact of oxygenic photosynthesis was not the introduction of O_2 into biogeochemical cycles, but rather the ability for life to make use of an essentially unlimited electron donor—water. The fixation of inorganic carbon into biomass fundamentally involves the reduction of CO_2 into more reduced forms via the transfer of electrons from an electron donor. Water as an electron donor for oxygenic photosynthesis is available in an essentially unlimited supply, and so rates of primary productivity today are constrained by other factors such as the availability of nutrients iron, fixed nitrogen, and phosphorous (Tyrrel 1999). Before the evolution of oxygenic photosynthesis—whenever this metabolism evolved, whether it was near the GOE or much deeper in Archean time—biology was restricted to more limited electron donor compounds, potentially including molecular hydrogen, ferrous iron, and reduced sulfur compounds (e.g. Kharecha et al. 2005, Canfield et al. 2006). These electron donors are geologically sourced and typically available at much lower abundances than water, raising the possibility that electron donors—rather than other nutrients like phosphate and fixed nitrogen—limited rates of primary productivity on the early Earth, before the evolution of oxygenic photosynthesis.

Here, we consider the contributions of electron donor compounds—particularly molecular hydrogen and ferrous iron—to fueling productivity on the pre-oxygen Earth, and the supply of these electron donors relative to nutrients like nitrogen and phosphorous. We conclude that nutrients, including phosphate and fixed nitrogen, were likely available in excess of the geological supply of electron donor compounds. This suggests that, to first order, the supply of electron donor compounds like H_2 set the tempo of early productivity, and that the resulting rate of productivity was at least 1000x lower than today. Furthermore, the efficiency with which these electron donors were consumed would be a major constraint to productivity, as consumption of H_2 via different metabolisms can affect the yield of organic carbon by an order of magnitude. We also conclude that at least at a global scale, abiotic nitrogen fixation pathways may have been sufficient to meet the nitrogen demands of the biosphere, and so there may not have been evolutionary pressure to evolve biological nitrogen fixation until the evolution of oxygenic photosynthesis. The relative abundance of nutrient fluxes relative to electron donors also suggests that there may have been an accumulation of nutrients in the pre-oxygen ocean, potentially providing a massive boost to productivity immediately following the evolution of oxygenic photosynthesis, leading to the rapid accumulation of O_2 at the GOE. The GOE therefore represents a singular break in the uniformitarian history of the scale of life on Earth, in which the tempo of productivity shifted geologically instantaneously form being set by slow geological rates of electron donor supply to being constrained by biological uptake and recycling of nutrients.

What limited productivity of the early biosphere?

In the modern ocean, it is the supply of nutrients such as phosphate and fixed nitrogen that limit primary productivity (Tyrrell 1999). However, this may not have been true on the early Earth, before the evolution of oxygenic photosynthesis. To determine the ultimate limiting factor on pre-oxygen productivity, we must consider the fluxes of nutrients to the Archean ocean, and the rates of productivity they can support (assuming an elemental composition of biomass approximating the Redfield Ratio of 106 C: 16 N: 1 P), compared against the number of electrons available for the fixation of organic carbon from a given flux of electron donor compounds (assuming an oxidation state of organic carbon similar to the modern, around -1, therefore requiring 5 electrons to fix one molecule of CO_2 to one molecule of organic carbon). We have assembled the best available estimates for the fluxes of essential nutrients to the pre-oxygen biosphere, focusing on phosphate, fixed nitrogen, and the electron donors molecular hydrogen and ferrous iron (Table 1). Though estimates of the availability and fluxes of these compounds are poorly constrained, in many cases relying on unknown conditions such as the composition of the early atmosphere, they are generally acceptable for an order of magnitude estimate—a level of accuracy acceptable for the results that follow.

Major electron donors

Previous estimates of Archean productivity have relied on steady state estimates of dissolved iron content of Archean seawater and atmospheric hydrogen concentrations with models of upwelling or diffusive flux into the photic zone (e.g. Kharecha et al. 2005, Canfield et al. 2006). These models assume steady state concentrations balanced by biological oxidation, recycling, and geological supply. Here, we are interested in the number of electrons delivered from the Earth to life, and the resulting amount of biomass (here considered as moles of organic carbon) that can be produced per year. We therefore use the flux of electron donors like ferrous iron and molecular hydrogen to the oceans/atmosphere, as estimated by geological processes like hydrothermal alteration of crust and volcanic outgassing, without assumption of particular steady state concentrations.

Production of oceanic crust and associated reduced iron was derived from Lécuyer and Ricard (1999). Based on Williams et al. (2012), it is assumed that mantle redox—and therefore the Fe^{2+}/Fe^{3+} ratio of new crustal material—has been constant since accretion. Hydrothermal fluxes were derived from Elderfield and Schultz (1996) for modern values as a reference. It has been argued that Archean hydrothermal fluxes may have been up to ten times higher than modern based on Des Marais (2000), although it is possible that Archean hydrothermal fluxes were lower than today (e.g., Korenaga 2006); as a result of the uncertainty of even the sign in this difference we use the modern hydrothermal flux.

Molecular hydrogen is supplied to the fluid Earth by a variety of sources, including volcanism and water-rock interactions in the oceans and continental subsurface. Fluxes of volcanic gases including H_2 have been estimated by Holland (2002) and the contribution of various pathways of continental and oceanic water-rock interactions to H_2 fluxes have been collected by Sherwood Lollar et al (2014) and summarized in Table 1.

The fluxes of ferrous iron and molecular hydrogen are estimated here to be of the same order (~1.5-6x10^{12} moles/year). If fluxes of both of these compounds are stoichiometrically oxidized to drive organic carbon fixation via anoxygenic photosynthesis, the contribution of hydrogen to productivity will be double that of iron as the oxidation of H_2 to H^+ provides two electrons to the one form Fe^{2+} to Fe^{3+}. Therefore even in the most productive scenario presented here (Scenario 1, Table 2), hydrogen will fuel several times more productivity than hydrogen.

Phosphate

Phosphate is necessary for all known life, and appears to be the primary limiting nutrient in the modern ocean over long timescales (Tyrrel 1999), and so it availability is crucial for constraining potential rates of primary productivity. However, it is challenging to constrain the availability of phosphate to the early biosphere, as the net abundance of marine phosphate is the result of the interplay of a wide range of geological, hydrological, biological, and other factors. We therefore consider a variety of influences on phosphate supply, recycling, and burial in order to make conservative estimates of the net availability of phosphate to the pre-oxygen biosphere.

The ultimate source of phosphate to the biosphere is chemical weathering of phosphate minerals in rocks. The extent of chemical weathering occurring on the Archean Earth—and therefore the delivery of phosphate to the oceans—is evident through the extensive production of carbonate minerals, which require a substantial flux of alkalinity (another product of chemical weathering) to the oceans (Higgins et al. 2009).

While chemical weathering of continental crust is the primary mechanism for oceanic phosphate delivery today, weathering and alteration of seafloor basalts—such as at hydrothermal vents—may have been significant on the early Earth. Basalt typically contains much higher phosphorous concentrations than average continental crust (up to ~8000ppm versus 700ppm, Taylor et al. 1995, Horton 2015), and so has the potential to be a major phosphate source to the ocean. Hydrothermal vents in the modern ocean are thought to be a net sink of phosphate (e.g. Elderfield and Schultz 1996), largely as a result of the sorption of phosphate to metal oxides that precipitate as metal-rich hydrothermal fluids interact with oxidized seawater (Wheat et al. 1996). Before the oxygenation of the oceans led to extensive water column metal oxidation, hydrothermal systems may have instead served as a major source of phosphate to the ocean.

Since even the sign of difference in phosphate weathering between the Archean and modern Earth is unclear, we include as a first-order estimate of weathering-based phosphate delivery to the oceans from modern riverine input, around $7x10^{10}$ moles P/year (Tyrrel 1999) (Table 1). This estimate is approximate over a range of a fewfold, due to uncertainties in the lithology of the early crust and weathering rates, but is a reasonable starting estimate, particularly given that in the modern ocean most productivity is supported not by new delivery of phosphate, but by recycling. Biology has developed multiple strategies for maximizing uptake and recycling of this vital nutrient, and as a result the relationship between phosphate input and primary productivity in the modern ocean suggests that phosphorous is recycled on the order of 500 times between input to the ocean and burial (Tyrrel 1999). Whether this extent of efficient recycling occurred on the early Earth is an open question, but recycling almost certainly did occur. We therefore provide an additional estimate for phosphate delivery to the photic zone via upwelling of more nutrient-rich bottom waters in a manner analogous to the modern ocean, using estimates of deep-water phosphate concentrations (from phosphate concentrations in marine sedimentary rocks like banded iron formations) and estimates of rates of upwelling and diffusivity. Assuming deep-water phosphate concentrations constrained to about 10% of modern due to sorbtion of phosphate onto iron oxides (Bjerrum and Canfield 2002), along with a typical diffusivity of ~100 m/year, delivery of phosphate to the surface ocean would be on the order of 1.3×10^{12} moles P/year (Table 1). This is a conservative estimate, as the Archean iron cycle may have been dominated by ferrous silicates, not iron oxides (Box 1), and ferrous silicates are not expected to bind phosphate as efficiently as iron oxides.

Fixed nitrogen

In the modern ocean, fixed nitrogen is supplied primarily by biological nitrogen fixation using the nitrogenase enzyme (Canfield et al. 2010). The antiquity of nitrogenase is contested, with estimates ranging from its presence in the last universal common ancestor to a much later evolution, near the GOE (Weiss et al. 2016, Boyd et al. 2011). Before the evolution of nitrogenase, and potentially throughout the Archean, fixed nitrogen may have been provided primarily by abiotic nitrogen fixation processes (Navarro-Gonzalez et al. 2001). Abiotic nitrogen fixation occurs through a variety of processes, and the fluxes and products of these reactions dependent on atmospheric chemistry and other factors. These include the fixation of N_2 into reduced forms via high temperature and reducing conditions at hydrothermal vents (~10^{10} moles N per year, Brandes et al. 1998), fixation of N_2 to NO (which would reach the oceans as NO_3^-, Mancinelli and McKay 1988) by lightning (~10^{12} moles N per year, Navarro-Gonzalez et al. 1998) and the photochemical production of HCN (~$2x10^{12}$ moles N per year, Tian et al. 2011).

The primary mechanism of abiotic N fixation, and the N species produced, is expected to vary with the CH_4:CO_2 ratio of the early atmosphere. Under a high CO_2 atmosphere, lightning-based N fixation would produce large fluxes of NO, which would reach the oceans as NO_3^- on the order of 10^{12} moles N/year (Mancinelli and McKary 1988, Navarro-Gonzalez et al 1998, Navarro Gonzalez et al 2001). However, under the relatively high methane atmospheric conditions that are expected to have been present throughout the Archean (e.g. Kasting et al. 2001, Pavlov et al. 2001), lightning-based N fixation would have primarily produced HCN (Navarro Gonzalez et al. 2001), and photochemical HCN production may have been quite high (Tian et al. 2012), potentially totaling ~$3x10^{12}$ moles N/yr. Under either scenario, fluxes of fixed nitrogen would have been on the order of 10^{12} moles N per year, at least an order of magnitude in excess of the nitrogen demands of the electron donor-limited biosphere, even without recycling of organic nitrogen, which may be significant—particularly before O_2 was available to fuel nitrification and a complete nitrogen cycle (Zerkle and Mikhail 2017).

Other nutrients and electron donors

While sulfur isotope analysis of 3.45 Ga stromatolites indicates that microbial sulfate reduction and sulfur disproportionation were active at this time (Bontognali et al. 2012), it is expected that sulfur played only a minor role in the Archean ocean due to the rapid titration of sulfide to pyrite in an iron-rich water column (Canfield 2004). As a result, sulfate concentrations were very low in Archean seawater (Habicht et al. 2002, Crowe et al. 2014), and so the contribution of sulfate reduction to remineralization was likely minor, and recycling of sulfur was minimal. In sum it is therefore likely that sulfur metabolisms contributed only very small amounts of productivity to the Archean biosphere, orders of magnitude less than molecular hydrogen (Kharecha et al. 2005, Canfield et al. 2006). We therefore do not include a separate calculation for the expected productivity of sulfur-based metabolism; incorporation of a sulfur-based productivity model would shift our expected rate of productivity slightly, but because the availability of sulfur is much less than iron (Walker and Brimblecombe 1985), this shift would be even smaller than the difference between an anoxygenic phototrophic biosphere with and without iron oxidation (Scenario 1 and Scenario 2. Assuming a volcanic SO_2 outgassing flux of ~10^{11} moles/year (Ono et al. 2003), sulfur could fuel at most only about 4×10^{10} moles carbon fixed/year, less than 2% of our estimate for H_2-fueled productivity.

While it has been proposed previously that the supply of trace metal cofactors may have been a limitation to the productivity of early Cyanobacteria (e.g. Anbar and Knoll

2002, Saito et al. 2003), this has not been demonstrated for the anoxygenic organisms expected to drive primary productivity before the evolution of oxygenic photosynthesis. Consideration of individual trace metals shows that for metals important to methanogens and anoxygenic phototrophs (e.g. Fe, Ni) were relatively abundant early in Earth history relative to metals that are less essential for these organisms (e.g. Cu, Mo, Zn) (Williams and Rickaby 2012, Robbins et al. 2016). These organisms may even have inherited their suite of metal cofactors as a result of their evolution and diversification in the early ferruginous ocean as has been proposed for Cyanobacteria in a euxinic Paleoproterozoic ocean (Saito et al. 2003).

While a source of inorganic carbon is necessary to support autotrophic carbon fixation, this is not expected to have been limiting to the early biosphere. The composition of the Archean atmosphere is poorly constrained, but it is generally thought that pCO_2 was significantly higher than today (e.g. Blättler et al. 2016), partially due to geological constraints such as the mineralogy of Archean paleosols (Rye and Holland 1995) and partially as a modeled solution for maintaining a clement climate under the faint young sun (Kasting 1987). CO_2, as dissolved inorganic carbon, should therefore have been well in excess of the demands of the early biosphere, both as a source of carbon for fixation of biomass as well as the terminal electron acceptor for cellular redox balance in metabolisms like methanogenesis and anoxygenic photosynthesis.

The electron donor-limited early biosphere

Our results suggest that electron donors, of which H_2 was likely most significant, were limiting to productivity before the evolution of oxygenic photosynthesis (Figure 1,

Table 2). Although iron oxidation has been considered a significant source of energy to the early biosphere, its relative contribution to rates of primary productivity is contested (e.g. Kharecha et al. 2005, Canfield et al. 2006), and may have been negligible (Box 1). Molecular hydrogen is thought to have been another major electron donor at this time, capable of fueling not only anoxygenic photosynthesis but also metabolisms like methanogenesis and acetogenesis. Based on our estimates of H_2 and Fe^{2+} fluxes to the pre-oxygen ocean, it appears that molecular hydrogen fluxes are somewhat higher than those of iron (Table 1), making hydrogen a more significant fuel for primary productivity than iron, even if photoferrotrophy oxidizes all of the ferrous iron sourced to the ocean. Global rates of primary productivity were likely no higher than 2.75×10^{12} moles C/yr, perhaps somewhat less if iron were not serving as an electron donor for photosynthesis. This value is more than 1000x lower than modern productivity, and much lower than previous estimates of Archean productivity (e.g. Kharecha et al. 2005, Canfield et al. 2006) (Figure 2). While local environments may have remained limited in other nutrients, on an averaged global scale it is the geological flux of electron donors that fundamentally limited the biosphere. This makes sense, as organisms can evolve to become more efficient with their use and recycling of nutrients, but electron donors are stoichiometrically consumed during the fixation of organic carbon.

Molecular hydrogen is an incredibly versatile and favorable electron donor for diverse metabolisms (hydrogenotrophy), including methanogenesis, acetogenesis, anoxygenic photosynthesis, and both aerobic and anaerobic lithotrophy. Of these, lithotrophy was likely not significant before the rise of oxygen, as it relies on respiratory electron acceptors such as oxygen or sulfate that were likely scarce or absent. Methanogenesis and acetogenesis are thought to be among the earliest metabolisms to have evolved (Battistuzzi et al. 2004), and so may have been responsible for driving productivity in the earliest stages of life on Earth. Sometime later, anoxygenic photosynthesis evolved. The evolution of anoxygenic photosynthesis may have led to a significant boost in primary productivity, as this metabolism can be more efficient at carbon fixation for a given flux of electron donor. Methanogenic productivity is limited not only by the flux of electron donor, but also limited by the necessity of consuming electron donor to supply cellular energy as well as to fix carbon—a limitation absent in anoxygenic phototrophs that can run cyclic electron flow to generate ATP (Madigan et al. 2012). Methanogens, as a result, must channel electrons into methane to conserve energy, and only fix on the order of 1 mole of organic carbon for every 10 moles of methane generated, dropping their expected productivity by an order of magnitude (e.g. Thauer et al. 2008). This leads to a drop in yield from ~2.4×10^{14} cells/mol H_2 for anoxygenic phototrophs to ~1.7×10^{13} cells/mol H_2 for methanogens (assuming average oxidation state of organic carbon of -1, ~10fg carbon per cell, and maximum yields for methanogens without cytochromes of 3g per mole of CH_4, Whitman et al. 1998, Thauer et al. 2008). As a result of this relative inefficiency of methanogenesis, primary productivity could have increased by about a factor of ten following the evolution and expansion of anoxygenic photosynthesis (from Scenario 2 to Scenario 3 in Table 2 and Figure 1). Determining the age of anoxygenic photosynthesis will therefore be important for plotting primary productivity through time.

Even under a hydrogen-fueled biosphere, iron is the ultimate source of most electrons from the solid Earth—water/rock interactions in hydrothermal vents, continental crust, and other environments result in the transfer of electrons from iron in rocks to molecular hydrogen, which is then released to the biosphere (Sherwood Lollar et al. 2014). Iron is the single largest reservoir of electrons in the Earth's crust (Walker and Brimblecombe 1985), yet is relatively inaccessible to biology, and so the extent to which life can be rock-powered is dependent on the extent of hydrothermal alteration and delivery to the oceans. Water is therefore a crucial intermediary in the transfer of electrons from rocks to life, particularly in the absence of photosynthesis.

Is a low-productivity Archean biosphere consistent with the carbon record?

An important question about the limited biological productivity predicted here is whether this rate of production is consistent with the abundance of organic carbon buried throughout the Archean. It has been suggested that organic carbon burial during the Archean was of similar order to today based on evidence from the organic carbon content of Archean sedimentary rocks and trends in the carbon isotope record (e.g. Lyons et al. 2014). These records, however, are consistent with the rates predicted here when differences in burial rates, and carbon isotope fractionation are taken into account.

In the modern, well oxygenated ocean, organic carbon burial rates are on the order of 0.2% of NPP (Tyrrell 1999); while carbon burial rates on the early Earth are not well constrained (Ward et al. 2016), they were almost certainly much higher than today. Iron reduction is thought to be a major pathway for remineralization of organic carbon before the rise of oxygen (Konhauser et al. 2005, Posth et al. 2013), but if substantial iron oxidation were not occurring (Box 1), there would be no significant source of iron oxides to support this respiratory process. Remineralization would instead be left with only sulfate reduction (which would be insignificant in the open ocean due low sulfate concentrations, though may have been more significant in locally sulfate-rich environments, Ueno 2014), fermentation, methanogenesis, and acetogenesis—metabolisms that are less energetic and less productive than respiratory pathways. Based on modern analog systems such as Lake Matano in Indonesia, the proportion of organic carbon that escapes remineralization and is buried in ferruginous systems is likely on the order of 20-25% of gross primary productivity (Crowe et al. 2011). Even higher burial fractions of 40-50% are expected for anoxic water columns based on extrapolation to zero of oxygen exposure time (a common metric for estimating the burial efficiency of an aquatic system) (Hartnett et al. 1998). In the absence of anaerobic electron acceptors like iron oxides, the fraction of organic carbon that is buried may have been even higher in the Archean. Assuming a burial fraction of ~50% as predicted for anoxic water columns (Hartnett et al. 1998), and rates of primary productivity predicted in Table 2, burial rates may have been ~1.37×10^{12} moles C/yr, only a few fold lower than modern marine carbon burial despite several orders of magnitude lower productivity.

Furthermore, reburial of eroded sedimentary carbon is a major flux of carbon on the Earth today, and could account for a large proportion of organic carbon in Archean sedimentary rocks, as kerogen would be less easily degraded under an anoxic atmosphere. In modern environments, erosion of sedimentary organic carbon leads to delivery to the ocean by rivers of about 8×10^{12} moles C/year (Galy et al. 2015, Daines et al. 2017). This represents the fraction of eroded and resedimented fossil organic carbon that escapes oxygen; deriving the amount of fossil organic carbon that is initially eroded is challenging, as the fraction of eroded organic carbon that escapes oxidation is highly variable and depends on the maturity of the carbon and transport environment, but has been shown to vary within the range of about 10-80% (Bouchez et al. 2010, Hilton et al. 2014). Under an anoxic atmosphere, the amount of organic carbon reburied in sediments may have been much higher. However, even under the most conservative estimate based on modern environments, the resedimentation of eroded fossil organic carbon in the Archean was likely on the order of 10^{12} moles C/year, as much or even more than newly buried carbon.

Our understanding of the relative balances of carbon fixed and buried in Earth history are generally constrained by steady state models of the carbon cycle based on isotope mass balance of $\delta^{13}C$ in carbonates and organic carbon (e.g. Wickman 1956). These models are predicated on assumptions about steady state input and output of carbon through the fluid Earth, as well as the carbon isotope fractionations associated with carbon fixation. The value for fractionation of carbon isotopes during CO_2 fixation is generally assumed to be driven by RuBisCO (e.g. Berner 1991), the enzyme responsible for initial fixation of CO_2 in the Calvin cycle, used by all oxygenic phototrophs. Early in Earth history, however, this assumption may have been violated due to increased productivity from alternative carbon fixation pathways. Several alternative carbon fixation pathways, with fractionations both greater and lesser than that of the Calvin cycle, exist within bacteria and archaea (Hayes 2001). These alternative carbon fixation pathways are well established in niches focused around factors such as sensitivity to temperature and oxygen, growth rate, and yield (Berg 2011). Based on these factors, the Calvin cycle is thought to be relatively young, specialized to tolerate oxygen (Berg 2011). The Wood-Ljungdahl (reverse acetyl-CoA) pathway is thought to be much more ancient, perhaps present in the Last Universal Common Ancestor (Lane et al. 2010). This pathway is characterized by very large fractionations between CO_2 and organic carbon (Hayes 2001), of up to -68 ‰ (Blaser et al. 2013).

The Archean carbonate carbon isotope record typically records values of ~0 ‰, similar to today, but the organic carbon isotope record is typically depleted relative to today (e.g. Fischer et al. 2009). This likely records increased contributions from alternative carbon fixation pathways with higher characteristic fractionations, including the reverse acetyl-CoA pathway used by methanogens and acetogens (Slotznick and Fischer 2016). As a result, similar carbonate carbon isotope values can be reached as today with a much lower fraction of carbon buried as organic matter (albeit with a more depleted carbon isotope value). For instance, given a $\delta^{13}C$ of carbonates of 0 ‰, the interpreted fraction of carbon buried as organic carbon would be ~0.25 assuming a fractionation characteristic of the Calvin cycle, but as low as ~0.09 given fractionations achieved by some organisms utilizing the reductive acetyl-CoA pathway. Assuming an input of carbon to the Earth system of similar magnitude to today, this lower fraction of carbon buried as organic carbon is in line with the burial rates estimated above, making the Archean carbon isotope record consistent with our estimates of productivity before the evolution of oxygenic photosynthesis.

Implications of electron limitation and excess nutrients in the pre-oxygen biosphere

If fluxes of nutrients like phosphate and fixed nitrogen were in excess of the demands of biological productivity before the evolution of oxygenic photosynthesis, this suggests that these nutrients may have accumulated to substantial concentrations in the oceans. This large reservoir of nutrients would have been immediately available to the first oxygenic phototrophs, fueling their almost unchecked growth for an initial, brief, intense period. This would be consistent with a rapid initial rise in oxygen concentrations following the evolution of oxygenic Cyanobacteria, leading to both a rapid Great Oxygenation Event and potentially an oxygen overshoot before depletion of this initial nutrient pool and development of a new steady state as suggested by a range of modeling and geochemical studies (e.g. Bekker and Holland 2012, Canfield et al. 2013, Ward et al. 2016, Luo et al. 2016). This is furthermore consistent with indications that a rapid accumulation of atmospheric oxygen and drawdown of CO_2 may have destabilized a methane greenhouse and triggered the Paleoproterozoic Snowball Earth Event (Kopp et al. 2005).

If nitrogen were not limiting to productivity before the rise of oxygen, there may not have been strong evolutionary pressure to evolve biological nitrogen fixation. Nitrogen fixation is a costly process, especially for the energy- and electron-limited organisms characteristic of the pre-oxygen biosphere, and so there would be a clear advantage to making use of abiotically fixed nitrogen rather than investing in fixing nitrogen via nitrogenase. While there may have still been locally nitrogen-limited environments conducive to the early evolution of nitrogenase, it is conceivable that the evolution of this enzyme (or at least emergence of the crown group forms that we see today), were relatively late, postdating the evolution of anoxygenic photosynthesis, and potentially as late as the GOE. This is consistent with some phylogenetic and molecular clock analyses of the evolution of the nitrogenase family (e.g. Boyd et al. 2011) but will require further evidence to confidently assess.

Alternatively, nitrogenase may have evolved much earlier but for a different function and was only later coopted for fixation of N_2. Under high-methane conditions, the product of atmospheric (lightning and photochemical) nitrogen fixation would primarily be HCN (Navarro-Gonzalez et al. 2001, Tian et al. 2012). Following rainout to the ocean, HCN could hydrolyze to form NH_4OH (Zahnle 1986), but can also be taken up directly by biology via nitrogenase (Dekas et al. 2009). Large fluxes of HCN to the oceans may therefore have triggered the evolution of nitrogenase first as a way to detoxify and take up HCN, and was only later coopted to N_2 fixation, a scenario that has been hypothesized previously (e.g. Silver and Postgate 1973, Raymond 2005).

In the modern ocean, phosphate availability is often incredibly limited, leading to diverse strategies by phytoplankton to reduce their phosphate requirements such as substitution of membrane phospholipids with alternatives such as sulfonolipids (e.g. Van Mooy et al. 2006), sometimes pushing C:P ratios away from the Redfield value of 106:1 to values on the order of 500:1 (e.g. Bertilsson et al. 2003). However, these adaptations appear to be relatively recent, and phospholipids appear to be ancestral. If phosphate were much more abundant on the early Earth, this would explain the widespread use of phosphate head groups in membrane lipids, as they evolved when phosphate was available in great excess relative to productivity, and adaptations to low phosphate only evolved after the evolution of oxygenic photosynthesis and drawdown of marine phosphate concentrations.

Our predictions of excess nutrient fluxes before the GOE have as corollaries testable hypotheses that can be verified with careful analysis of the rock record. One such testable hypothesis is that if phosphate fluxes were in excess of biological demand before the evolution of oxygenic photosynthesis, then the phosphorous cycle should have been dominated by abiotic processes, with the largest sinks for marine phosphorous being in the form of the precipitation and burial of phosphate minerals. If our model is correct, primary phosphate minerals—such as apatite—should have precipitated in the Archean ocean, potentially preserved in the rock record.

A major implication of this result is that without use of water or a similarly abundant electron donor, biology is fundamentally constrained in the mass and flux of biomass that can be fixed by the number of electrons supplied from geological processes. This was likely true on the early Earth, and would also be true of any biosphere on ice-covered worlds like Europa and Enceladus, where the use of water as an electron donor is impossible due to the lack of light penetration to the ocean. As a result, the size and productivity of any biosphere on these worlds would be limited by the flux of electron donor compounds from water/rock interactions at the base of their subsurface oceans. Furthermore, since anoxygenic photosynthesis would also be impossible in these environments, the efficiency and yield from consumption of these electron donors may be low, potentially more in line with Scenario 3 above than Scenarios 1 or 2. This would be true whether oxygen is supplied to the ocean from radiolysis of ice on the surface or not;

aerobic lithotrophic metabolisms typically have low efficiency per electron donor, of a similar order to methanogenesis (e.g. 40 Fe^{2+} oxidized to Fe^{3+} per 1 C fixed when using O_2, compared to 4-6 Fe^{2+} oxidized to Fe^{3+} per 1 C fixed when using light, Ehrenreich and Widdel 1994, Neubauer et al. 2002). This has substantial implications for the potential abundance, productivity, and detectability on icy moons, and should be kept in mind when planning life detection missions.

Table 1: Phosphate, fixed nitrogen, and electron donor fluxes to the pre-oxygen biosphere.

Flux	**Nutrient flux (10^{12}moles/yr)**	**Citation**	**Notes**
Phosphate (rivers)	0.0722	Tyrrel 1999	New nutrient
Phosphate (upwelling)	1.3	10% average modern marine deep water P concentrations per Bjerrum and Canfield 2002, textbook diffusivity for circulation rate	Includes recycling, high estimate using modern deep water phosphate concentration
Fixed N (photochemistry)	2	Tian et al. 2011	Production of HCN in relatively high CH_4 atmosphere
Fixed N (hydrothermal)	0.01	Brandes et al 1998	
Fixed N (lightning)	0.714	Navarro-Gonzalez et al. 1998, Navarro-Gonzalex et al. 2001	NO from high CO_2 atmosphere, or HCN from high CH_4
H_2 (volcanic outgassing)	4.7	Holland 2002	
H_2 (water-rock interactions)	1.36	Sherwood Lollar et al 2014	Sum of continental and oceanic fluxes
Fe^{2+} (hydrothermal)	0.19	Elderfield and Schultz 1996	
Fe^{2+} (production of new crust)	1.4	Lecuyer and Ricard 1999	

Table 2:

Productivity scenarios referenced in Figure 1. 1) Best estimate for electron donor-limited anoxygenic photosynthetic productivity. 2) Methanogen-dominated productivity. 3) Anoxygenic photosynthetic productivity without photosynthetic iron oxidation. 4) Modern marine oxygenic productivity.

Scenario	**Description**	**Primary Productivity (10^{12}moles C/yr)**	**Required P flux (10^{12}moles/yr)**	**Required N flux (10^{12}moles/yr)**
1	Electron donor limited anoxygenic photosynthesis (H_2 and Fe^{2+})	2.75	0.0259	0.415
2	Electron donor limited anoxygenic photosynthesis without photoferrotrophy (H_2)	2.43	.0229	0.367
3	Electron donor limited methanogenesis (H_2)	0.243	0.00229	0.0367
4	Modern marine productivity (oxygenic photosynthesis)	4000	37.7	604

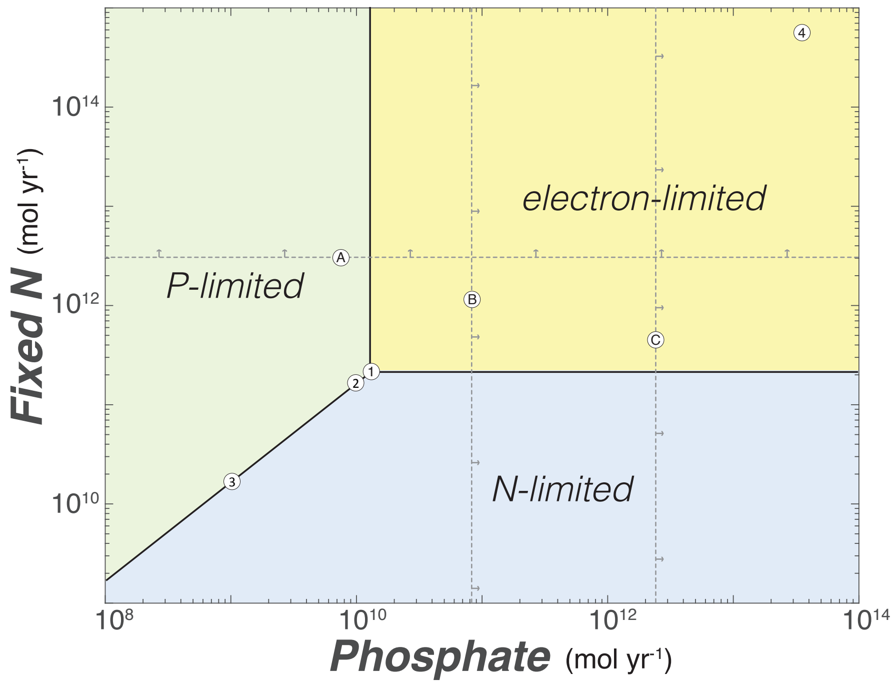

Figure 1:

Phosphate and fixed nitrogen fluxes to the biosphere, and resulting limitation on productivity. Electron donor, N, and P limitation fields assuming productivity in Scenario 1. Scenarios 2 and 3 shift the electron limited field down and to the left. Predicted nitrogen and phosphate fluxes (A-C) are well in excess of the demand of the electron limited rates of productivity predicted here.

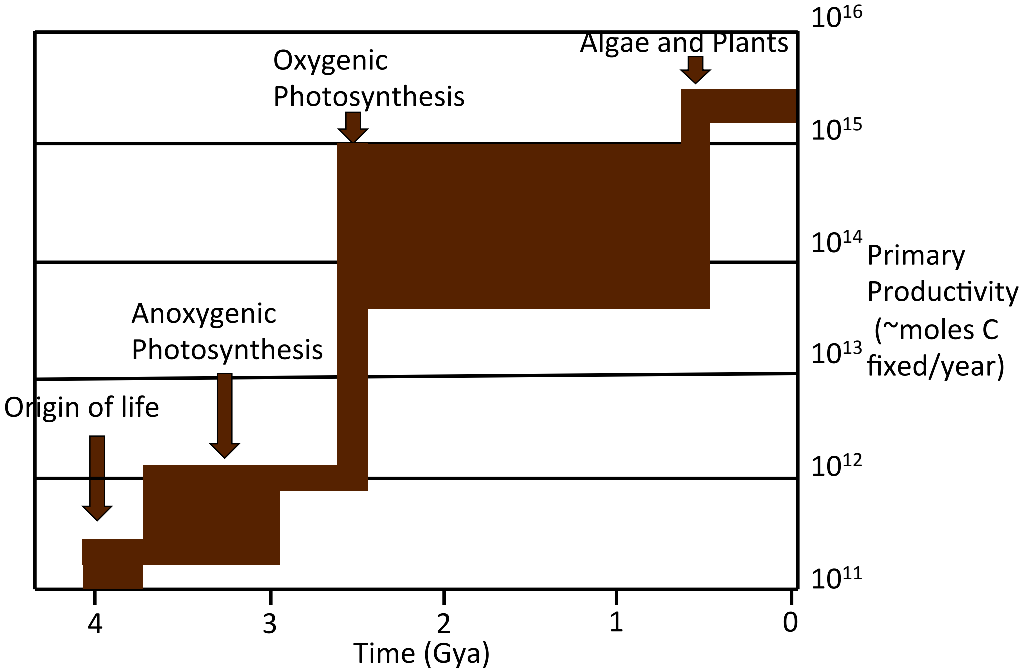

Figure 2:

Productivity of the biosphere (in terms of moles organic carbon fixed per year, on a log scale) through time (in billions of years). To first order, the productivity of life on Earth has increased through time, beginning near 0 at the origin of life to massive productivity today. The Great Oxygenation Event was associated with the largest boost to productivity since the origin of life, as a result of water splitting by oxygenic photosynthesis freeing life from electron donor limitation. An earlier, lesser jump to rates of productivity was likely associated with the evolution of anoxygenic photosynthesis, as a result of the conservation of life energy increasing the efficiency with which life could use electron donors to fix organic carbon.

Box 1: Did iron fuel the early biosphere?

Banded iron formations (BIFs) are among the most characteristic lithotypes in the Precambrian sedimentary record. These finely laminated, iron-rich (>15% Fe by weight) deposits are commonly thought to provide a record of biological activity on the early Earth (e.g. Harder 1919, Konhauser et al. 2002, Kappler et al. 2005). It is generally hypothesized that BIFs formed as a result of transport and concentration of ferrous iron (as $Fe^{2+}_{(aq)}$) in seawater under anoxic and sulfur-poor conditions, followed by oxidation and precipitation of iron as ferric iron phases (Holland 1973, Drever 1974, Holland 1984). The oxidation of iron in BIF deposition is commonly hypothesized to have been driven by biology, either indirectly by O_2 sourced from oxygenic photosynthesis by Cyanobacteria (e.g. Cloud 1973), or directly by aerobic iron-oxidizing bacteria (e.g. Chan et al. 2016) or anaerobic iron-oxidizing phototrophic bacteria (photoferrotrophs) (e.g. Widdel et al. 1993, Kappler et al. 2005). However, the complex depositional and alteration histories of ancient rocks have made the original composition of BIFs difficult to interpret, and as a result other hypotheses for pre-GOE BIF formation that do not rely on biological iron oxidation are viable. Demonstrating that early BIFs were not deposited via photoferrotrophy—and therefore not contributing to primary productivity—may have significant implications for our understanding of the Archean biosphere.

It is commonly thought that Archean BIFs were deposited as primary ferric iron oxides, and then reduced to ferrous minerals as a result of a combination of microbial iron respiration and diagenesis (Konhauser et al. 2005, Fischer and Knoll 2009), and it is under this depositional model that biological iron oxidation mechanisms are invoked. However, a growing body of evidence suggests that Archean BIFs were deposited primarily as ferrous silicates (e.g. greenalite, Rasmussen et al. 2013, Rasmussen et al. 2015, Rasmussen et al. 2016; Rasmussen et al. 2017), a process that does not require microbial iron oxidation. In fact, if BIFs were being precipitated as ferrous minerals, this would not only be an absence of evidence for microbial iron oxidation, but would in fact be evidence of the absence of this process.

If Archean BIFs were precipitated as ferrous silicates, this suggests that photoferrotrophs were not oxidizing most of the iron in the Archean ocean. This raises an intriguing problem: if ferrous iron was abundant, and photoferrotrophy was a viable metabolism, why was it not occurring? Solutions to this problem could take one of a few forms. The first is that photoferrotrophy had not yet evolved. This seems an unlikely situation—photoferrotrophy seems to have been an ecologically viable niche in the Archean ocean, and is biochemically feasible given its presence in modern organisms (e.g. Widdel et al. 1993). For it not to have evolved by Archean time, as late as *ca* 2.5 Gya, when iron formations appear to have been deposited as ferrous silicates (e.g. Rasmussen et al. 2015, Rasmussen et al. 2016), runs counter to assumptions about the rapid evolution of microbes to exploit any available, thermodynamically viable redox couple (e.g. Broda 1977). It is especially curious given that photoferrotrophy appears to have evolved independently at least twice, in both the Chlorobi and the Proteobacteria (Ilbert and Bonnefoy 2013, Posth et al. 2013), implying that this metabolism is not as evolutionarily difficult as other metabolisms such as oxygenic photosynthesis that have evolved only once (Falkowski 2011). A more compelling explanation is that photoferrotrophy was not a viable niche. This could be the case if, perhaps, ferrous iron were precipitated out of solution before reaching the photic zone. The flux of hydrothermal iron into an ocean with dissolved silica may have removed iron from the water column as amorphous ferrous silicate phases. In the modern ocean, dissolved silica concentrations are kept low due to the precipitation of silica biominerals, primarily by eukaryotic algae; in the Archean ocean, before the evolution of these oganisms, abiotic silica precipitation would have dominated, resulting in a more silica-saturated ocean (Stefurak et al. 2014). In an ocean where silica concentrations were close to saturation with respect to cristobalite or amorphous silica (Rasmussen et al. 2015), this process would titrate iron from the Archaean ocean, restricting the flux of ferrous iron to the photic zone, and limiting primary productivity by photoferrotrophy. Under this scenario, iron and light would not have substantially cooccurred in the Archean ocean, and photoferrotrophy would not have been a viable niche. This solution can be tested by investigating depth transects of BIF occurrences. Archean BIF are typically restricted to deep-water environments (Klein 2005), which is consistent with precipitation below the photic zone. In formations where depth transects can be traced between deep and shallow water facies, iron minerals are typically found only in deeper water facies (e.g. Tice and Lowe 2004). This could be consistent with dissolved iron not reaching the photic zone.

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