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Limitations on Limitation

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Key Points:
- The rate of marine primary productivity must have been at least 10 times smaller during the Proterozoic than it is today
- This rate is sufficiently small that the biosphere was unlikely to be nitrogen limited, even if molybdenum was efficiently scavenged
- Phosphorus is the most likely candidate for the limiting nutrient during the Proterozoic eon

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Abstract Phosphorus is believed to be the globally limiting nutrient in the modern ocean, but a number of nutrients have been invoked as limiting the Proterozoic biosphere. Mass balance calculations suggest that Proterozoic net primary productivity must have been 1 to 2 orders of magnitude less than today in order to maintain low oxygen levels despite increased burial efficiency in anoxic environments. The resulting demand for nutrients is so low that nitrogen, molybdenum, and iron could not have limited the rate of primary production following the evolution of extant nitrogenases. Phosphorus demand was approximately equal to the modern riverine flux, making phosphorus the most likely candidate for the limiting nutrient throughout the Proterozoic.

1. Introduction

Earth's oceanic biosphere produces about 4,000 Tmol of primary organic carbon each year through photosynthetic fixation of CO_2 (Field et al., 1998). Net primary production (NPP) of organic carbon is central to biogeochemical cycling on Earth's surface: it is the primary source of oxygen to the atmosphere, a major sink for CO_2, and the source of the organic material that supports heterotrophic life and drives redox cycling of sulfur and iron. Understanding variations in the rate of NPP over time can help us understand changes in Earth's surface environment.

Variations in NPP and oxygen production are of special interest for Proterozoic time, as proxy records record atmospheric oxygen levels at least 1 order of magnitude lower than today between the end of the Lomagundi carbon isotope excursion and the Neoproterozoic (Lyons et al., 2014; in this paper we use "Proterozoic" to refer to this period unless otherwise noted). The modern oxygen budget is balanced between its source, NPP, and a variety of sinks including reoxidation of organic carbon via oxic respiration. Under low-oxygen conditions, respiration proceeds more slowly (Katsev & Crowe, 2015), implying an imbalance in the Proterozoic O_2 budget that should have driven oxygen concentrations up toward modern values on geologically short time scales. A slower rate of NPP, sustained throughout the Proterozoic, was likely required to balance the sluggish consumption of O_2 (Laakso & Schrag, 2014) and stabilize an oxygen-poor atmosphere.

Why would net carbon fixation have been suppressed during the Proterozoic? Global NPP was probably dominated by the marine biosphere; a modern plant-dominated terrestrial biosphere did not arise until the middle Paleozoic (Kenrick et al., 2012). The modern rate of marine productivity is controlled by the supply of a limiting nutrient into well-lit surface waters; assuming this control also operated in the past, some nutrient must have been less available in the Proterozoic ocean than it is today. Mechanisms have been proposed for suppressing the supply of several different nutrients during the Proterozoic, including fixed nitrogen (Fennel et al., 2005), molybdenum (Reinhard et al., 2013), and phosphorus (Derry, 2015; Laakso & Schrag, 2014; Reinhard et al., 2017).

Tyrrell (1999) addressed the question of global nutrient limitation in the modern context, concluding that the globally limiting nutrient on long time scales is phosphorus. He argued that nitrogen can be locally or transiently limiting, as in parts of the modern ocean, but diazotrophic organisms can fix atmospheric N_2 into bioavailable form, presumably closing any nitrogen deficit given sufficient time.

In this paper, we build on Tyrrell's work in order to assess whether phosphate was also the limiting nutrient in a low-productivity Proterozoic ocean. This requires adapting his arguments to account for changes in ocean chemistry that may have limited the rate of biological nitrogen fixation. Several authors have argued that, unlike today, the Proterozoic diazotrophic community may not have been able to fix enough nitrogen to keep up with phosphorus input (Fennel et al., 2005; Reinhard et al., 2013). Such N limitation has been attributed to rapid loss of fixed nitrogen through denitrification in the Proterozoic (Fennel et al., 2005) and to a shortage of the metals necessary for the operation of nitrogenases, the nitrogen-fixing enzymes. The most widely distributed from of nitrogenase uses a molybdenum cofactor, and while Mo is the most abundant trace metal in the modern oxic ocean, it is efficiently scavenged into anoxic and euxinic sediments (Reinhard et al., 2013). If either of these arguments are correct, limitation by nitrogen could be the mechanism by which NPP was suppressed during the Proterozoic.

Understanding which nutrient was limiting at different points in Earth history is critical in determining the rate of net primary productivity, and thus the rate of oxygen production, over geologic time. We address this problem by calculating the rates of NPP that are consistent with balanced oxygen budgets at Proterozoic levels of atmospheric O_2. We then determine the resulting demand for phosphorus, fixed nitrogen, molybdenum, and iron and consider whether these demands could have been met in Proterozoic environments.

2. Model and Methods

We first calculate the rates of NPP that are consistent with a balanced oxygen budget for a given level of atmospheric oxygen, pO_2. We then determine the nutrient requirements of the marine biosphere and compare demand to expected rates of supply during the Proterozoic.

2.1. Net Primary Production Via the Oxygen Budget

The oxygen budget is a balance between oxygen production during photosynthesis and various oxidative sinks, including consumption of oxygen during the remineralization of organic carbon. It is useful to define the net source of O_2 as the difference between NPP and remineralization, which at steady state is equal to the rate of organic carbon burial in the sediments, B_o. This can expressed in terms of an organic burial efficiency, ε_o.

$$B_o = \varepsilon_o \cdot (\mathrm{NPP}) \tag{1}$$

Therefore, a balanced oxygen budget is given by

$$\varepsilon_o \cdot (\mathrm{NPP}) = g(pO_2) \tag{2}$$

where g is the rate of O_2 consumption by reductants other than organic carbon, which varies with oxygen concentration. As argued above, remineralization rates are slower in modern anoxic environments than their oxic equivalents (Katsev & Crowe, 2015; Van Mooy et al., 2002), leading to more efficient organic burial (larger values of ε_o). In the Proterozoic ocean this effect would have been exacerbated by the low concentrations of alternative electron acceptors, such as sulfate (Kah et al., 2004). The kinetics of many oxidative processes scale with oxygen concentrations (e.g., Daines et al., 2017), implying a smaller oxygen sink during this period (smaller value of g). Together, these effects imply that the Proterozoic oxygen budget could not have been balanced at modern levels of net primary productivity.

Steady state instead requires a smaller rate of NPP, such that its efficient burial does not lead to increase in the size of the oxygen source. Steady state is achieved when

$$\frac{\mathrm{NPP}'}{\mathrm{NPP}} = \frac{g'(O_2')}{g(O_2)} \frac{\varepsilon_o}{\varepsilon_o'} \tag{3}$$

where x denotes a modern value and x' its value during a low-oxygen point in Earth history. This is not a useful expression for NPP′, as the sink function g integrates a number of oxidative processes in many different environments, and its functional form may be complex (e.g., Goldblatt et al., 2006). Nevertheless, it should be a positive function of O_2. This gives a useful upper bound on Proterozoic rates of primary production that are consistent with low levels of atmospheric oxygen:

$$(\mathrm{NPP})' \leq \frac{\varepsilon_o}{\varepsilon_o'} (\mathrm{NPP}) \tag{4}$$

Recently, Kipp and Stüeken (2017) also argued that a lack of electron acceptors in the global ocean would have led to limited remineralization of organic-bound nutrients, depressing NPP and pO_2. This conclusion is in qualitative agreement with the approach and results discussed here. However, their study did not address whether the trade-offs between lower NPP and increased burial efficiency would allow for steady state oxygen budgets (as in equation (4)) and thus did not attempt to produce an internally consistent, quantitative estimate of steady state Proterozoic NPP.

Table 1
Carbon-to-Nutrient Ratios (r_U) Used for Estimates of Nutrient Demand

Nutrient	Phosphorus	Nitrogen	Molybdenum	Iron
r_U	75–195	5–9; 3–20	10^5–10^6	10^4–10^5

Note. The two different ranges given for nitrogen correspond to observed variations between average biomass in different ocean basins (Weber & Deutsch, 2010) and between individual organic particles (Geider & La Roche, 2002). Phosphorus ranges are from regional variations in particulate organic matter (Martiny et al., 2013). Molybdenum and iron ranges are based on samples of *Trichodesmium* and *Crocosphaera* (Nuester et al., 2012; Tuit et al., 2004).

2.2. Nutrient Demand

The global demand for a nutrient U is given by NPP/r_U, where NPP is measured in mol C yr^{-1}, and r_U is the average C:U ratio in the biomass of primary producers. However, a portion of NPP is remineralized within the photic zone, and these nutrients are likely to be reused. A more useful measure of demand is the rate at which nutrients are lost from the photic zone via export of organic matter to sediments or the deep ocean.

$$D'_U \le \frac{\varepsilon_o}{\varepsilon'_o}\frac{f'_E \mathrm{NPP}}{r_U} \tag{5}$$

where f_E is the fraction of NPP exported out of the photic zone. We assume a value of 40% for the Proterozoic ocean (see section 4). Note that, by definition, the total burial efficiency ε_o cannot be larger than this value.

r_U values for N and P are based on the Redfield ratio of 106C:16 N:1P, though we explore a range of C:N ratios as these are known to vary both between environment and organism. For Mo and Fe, r_U ranges are chosen based on C:U ratios in diazotrophs, which have higher quotas of these nutrients. This assumes the most extreme case for metal limitation, in which fixed nitrogen is available exclusively via biological nitrogen fixation, and thus, the entire community of primary producers is composed of diazotrophs actively fixing N_2. This gives an upper bound on demand, consistent with the preceding equation. These values are given in Table 1.

2.3. Nutrient Supply

The goal of this study is to look for possible limiting nutrients, and so we will attempt to estimate *lower* bounds on nutrient supply. To this end, we assume that none of the nutrients remobilized during remineralization in the deep ocean or in the sediments is recirculated into the photic zone, presumably due to some highly efficient abiotic sink. Such mechanisms have been suggested for fixed nitrogen (denitrification; Fennel et al., 2005), molybdenum (removal with organic matter and sulfide phases; Reinhard et al., 2013), and phosphorus (vivianite precipitation; Derry, 2015). We recognize that it is unrealistic to assume that these sinks scavenged nutrients from the water column with perfect efficiency. However, this assumption provides a harsh lower bound on supply. If supply of a nutrient meets demand even in the absence of any organic recycling, that nutrient is very unlikely to have been limiting. The modern rates of input used to estimate the bioavailable supply are shown in Table 2. Note that some fraction of nutrient fluxes to the ocean may not be accessible to photosynthetic organisms as they are present in detrital or authigenic mineral phases. Only the bioavailable portion of supply is considered here.

Implicit in our approach is the assumption that the limiting nutrient is drawn down to growth-limiting concentrations by biological uptake itself. If this concentration is very low, mass balance then requires that net fixation of carbon is approximately equal to the rate of nutrient input, multiplied by the relevant carbon-to-nutrient ratio. This allows us to calculate NPP from global nutrient fluxes without specifying the concentration at which a particular nutrient becomes limiting for a particular organism. This approach will not be valid if there is a quantitatively significant alternative sink for a given nutrient in the photic zone that can

Table 2
Bioavailable Input of Nutrients to the Modern Ocean

Nutrient	Phosphorus	Fixed nitrogen	Molybdenum	Iron
Input Flux	60–300[a]	400[b]	3[c]	70[d]
Source	Apatite Weathering	Lightning	Oxidative Weathering	Weathering, Hydrothermal

Note. Input fluxes are given in Gmol yr^{-1}. Note that the rate of phosphorus input is highly uncertain, as the fraction of this supply that is bioavailable is poorly known.
[a]Benitez-Nelson (2000); [b]Galloway et al. (2004); [c]Reinhard et al. (2013); [d]Tagliabue et al. (2014).

out-compete biological uptake even at the low concentrations required to limit growth. This is discussed further in section 4.1.

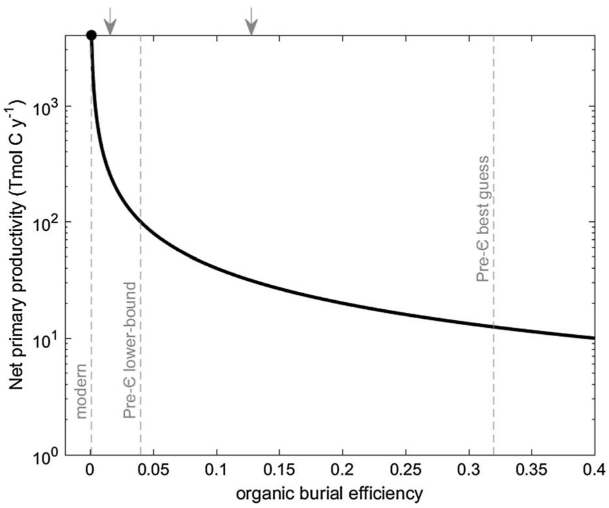

Figure 1. The maximum rate of net primary production consistent with a balanced oxygen budget. The arrows denote alternate estimates of the lower and upper bounds on Proterozoic burial efficiency, after allowing for changes in particle sinking rate (see section 4).

3. Results

Figure 1 plots NPP as a function of organic burial efficiency, $\varepsilon_o{}'$. This is the maximum productivity (NPP′) consistent with a balanced oxygen budget, following equation (4). Estimates of the organic burial efficiency in the low-oxygen environments of the Proterozoic are included for reference (see section 4). The plausible range of Proterozoic NPP is roughly between 10 and 100 Tmol C yr^{-1}, compared to a value of 4,000 Tmol C yr^{-1} in the ocean today.

Figures 2–4 show the maximum global nutrient demand consistent with a given efficiency of organic carbon burial. In each case, demand is normalized to the modern rate of supply. This "supply" is the input from weathering and hydrothermal activity and does not include nutrient recycling (see section 2). For a nutrient to be globally limiting, demand must *at least* exceed this lower bound on supply. Note that demand decreases at larger values of the burial efficiency, as this requires less primary production to balance the oxygen budget following equation (4). A plausible range of Proterozoic burial efficiencies is shown in each figure (see section 4).

Phosphorus demand is shown in Figure 2. The range in the ratio of demand to supply at a given burial efficiency is due to uncertainty in the fraction of modern riverine P fluxes that are available for biological uptake. Demand exceeds supply regardless of bioavailability for burial efficiencies as large as 5%, or ~50 times larger than modern. Above this value, demand continues to exceed supply if less than half of total P inputs are bioavailable.

Nitrogen demand is shown in Figure 3. Nitrogen demand exceeds the supply from lightning alone over the entire range of plausible burial efficiencies, assuming Redfield C:N ratios. However, lightning does provide between 10 and 50% of demand in this case. Fixation via lightning only exceeds demands for burial efficiencies of 20% or more and the lowest values of C:N observed in oceanic biomass.

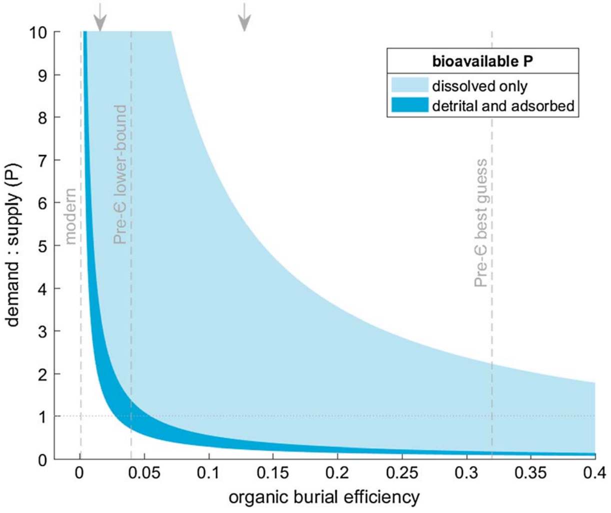

Figure 2. The maximum phosphorus demand consistent with a given efficiency of organic burial. Demand is normalized to the modern supply of P from weathering. Limitation can occur when the ratio of demand to supply exceeds 1. The range at a given burial efficiency results from assuming different C:P ratios in organic matter, and different proportions of the weathering supply that are bioavailable. Arrows are as in Figure 1.

Molybdenum and iron demand is shown in Figure 4. The modern supply of molybdenum exceeds demand for almost all plausible values of Proterozoic NPP; the only exception is for a combination of the lowest NPP values and the lowest C:Mo ratios in diazotrophs. The modern supply of dissolved iron greatly exceeds demand over the entire range of plausible burial efficiencies.

4. Discussion

The low-oxygen oceans of the Proterozoic eon would have buried organic carbon much more efficiently than they do today, leading to excess oxygen production and rapidly rising pO_2 unless the rate of organic carbon fixation was scaled back to compensate (Laakso & Schrag, 2014). Assuming that ~0.1% of modern oceanic NPP (4,000 Tmol C yr^{-1}; Field et al., 1998) is ultimately buried (Hedges & Keil, 1995), the residence time with respect to organic carbon burial for major oxidants (oxygen + sulfate) in the ocean and atmosphere today is about 10 million years. Given this time scale, an imbalance in oxygen fluxes cannot be consistent with the billions of years of low oxygen implied by the redox proxy record.

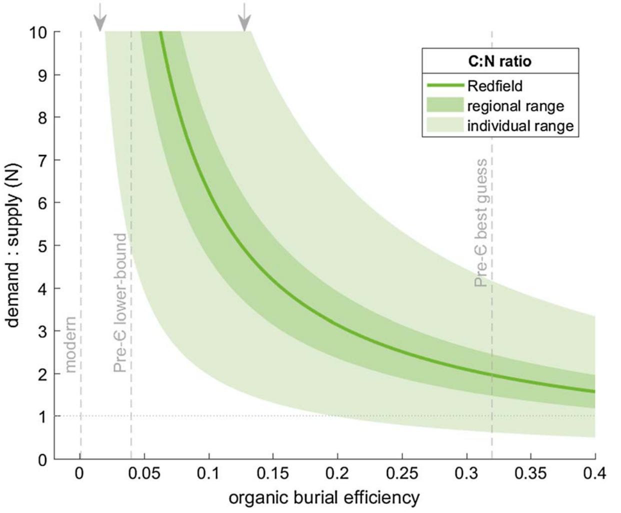

Figure 3. The maximum fixed nitrogen demand consistent with a given efficiency of organic burial. Demand is normalized to the modern supply of N from lightning. Limitation can occur when the ratio of demand to supply exceeds 1. The range at a given burial efficiency results from assuming different C:N ratios in organic matter. Arrows are as in Figure 1.

Lower rates of net primary productivity imply that nutrient demand was substantially lower throughout the Proterozoic. A quantitative estimate of demand requires an estimate of the burial efficiency during the Proterozoic, following equation (5). The modern burial efficiency, ε_o, is approximately 0.1%, with 1% of global NPP reaching the sediments and 10% of that material eventually buried at depth (Hedges & Keil, 1995). As organic matter sinks through the ocean, it is lost to remineralization following a power law with depth (Martin et al., 1987), and this attenuation is observed to be weaker in low-oxygen waters (Van Mooy et al., 2002). These empirical decay laws are not meant to be applied in the photic zone, where photosynthesis and rapid mixing give rise to more complex cycling. Nevertheless, one can recover the modern rates of export from the photic zone (20% of NPP) and to the deep ocean floor (1%) if Martin's law is assumed to apply from an "average" depth of production at 10 m. Applying the anoxic decay coefficient from Van Mooy et al. (2002), export from the photic zone is approximately 40% of NPP, and export to the seafloor is 10% of NPP.

Using a compilation of modern sediments (Canfield, 1994), we find that an average modern preservation of 10% is best represented by oxic sediments with a sedimentation rate of roughly 0.01 g cm^{-2} yr^{-1}. Equivalent anoxic sediments have a burial efficiency of 40% (Katsev & Crowe, 2015), due to some combination of a direct oxygen effect (Hedges et al., 1999) and a lack of bioturbation (Jessen et al., 2017). The combination of both effects yields a low-oxygen burial efficiency (ε_o') of 4% or 40 times the modern value.

This should be a lower bound on ε_o'. The Proterozoic oceans was also depleted not only in oxygen but also in sulfate, the most abundant electron acceptor in today's ocean (Kah et al., 2004). Lake Matano, a ferruginous, sulfate-poor lake in Indonesia, buries 80–90% of the organic material exported out of the photic zone (Kuntz et al., 2015). Combining this with the photic zone export discussed above, the total burial efficiency may have been as high as 32% or $>$300 times the modern value. Given these estimates of burial efficiencies, Proterozoic NPP must have been 1 to 2 orders of magnitude smaller than it is today (Figure 1).

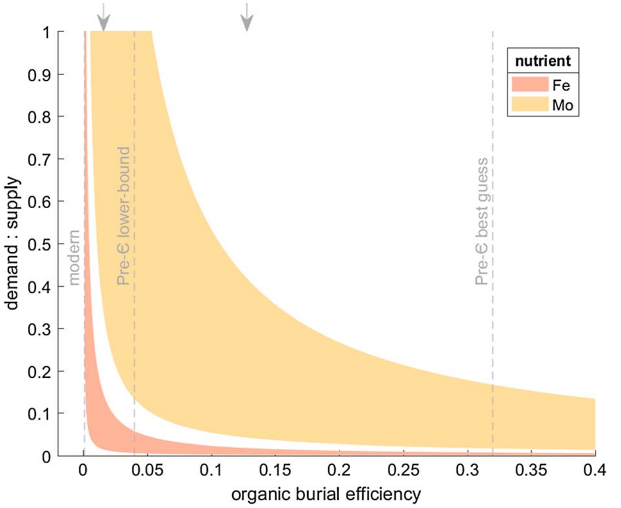

Figure 4. The maximum molybdenum and iron demands consistent with a given efficiency of organic burial. Demand is normalized to the modern supplies from weathering (Mo) and weathering, eolian input, and hydrothermal activity (Fe). Limitation can occur when the ratio of demand to supply exceeds 1. The range at a given burial efficiency results from assuming different C:Mo and C:Fe ratios in organic matter, based on modern diazotrophs. Arrows are as in Figure 1.

It is important to note that oxygen concentrations are not the only control on burial efficiency. Several authors have argued that biological innovations in the latest Proterozoic—such as larger body plans, fecal pellets, and biomineralization—increased the average size and density of organic particles (e.g., Butterfield, 2009; Logan et al., 1995; Rothman et al., 2003). These dense particles would sink more rapidly (Iverson & Ploug, 2010), increasing burial efficiency. Could this effect have countered the reduction in burial efficiency associated with rising oxygen levels during this period? Empirical export laws relate the flux of sinking organic matter to ocean depth z, usually following a power law ~z^{-b} (Martin et al., 1987). Assuming the degree of remineralization reflects oxidant exposure time, we can include the effects of sinking speed by allowing for changes in the time required to reach a particular depth:

$$\frac{f_{\mathrm{OM}}{}'(z)}{f_{\mathrm{OM}}(z)} = \frac{\left(z\frac{s}{s'}\right)^{-b'}}{z^{-b}} \tag{6}$$

where f_{OM} is the fraction of the export production remaining at a depth z; s is the sinking speed, and b is an empirical rate constant that depends on redox conditions (Van Mooy et al., 2002). x' continues to denote the value of x in the Proterozoic.

The terminal velocities of a small, nonbiomineralizing organism such as *Prochlorococcus* (0.5 mm diameter, 1.06 g/cm^3 density; Klaas & Archer, 2002) and a larger, biomineralizing organism such as *E. huxleyi* (5 mm diameter, 1.2 g/cm^3 density based on a cellular $CaCO_3$ content of 15 pg; Iglesias-Rodriguez et al., 2008) differ by roughly a factor of 10. Following equation (6), a tenfold decrease in sinking speed increases organic burial efficiency by a factor of 2.5, relative to the case where only oxygen effects are included. This calculation likely represents an upper bound on the global effects of ballasting, as cyanobacteria remain an important source of fixed carbon in today's ocean, and unballasted marine snow can aggregate in large particles with sinking rates that rival those of ballasted fecal pellets (Iverson & Ploug, 2010.)

If Proterozoic burial efficiencies were lower than expected due to the prevalence of small, low dense cells, higher rates of NPP would have been possible without violating the oxygen budget. The upper bound of this effect, discussed above, is shown in Figure 1 (arrows). However, the oxygen effect still dominates, and burial efficiencies remain well above their modern values. This suggests that Proterozoic NPP must have been 1 to 2 orders of magnitude smaller than in the modern ocean. If productivity was maintained at such low rates for billions of years, the supply of the limiting nutrient must have been much smaller than it is today—but which nutrient was limiting?

4.1. Nitrogen Limitation

Today, N_2 is converted to biologically useful forms primarily by nitrogen-fixing organisms, with a minor contribution from lightning strikes. The biosphere of the Proterozoic was sufficiently small (Figure 1) that modern lightning strikes would have provided a large fraction of total nitrogen demand, as defined in equation (5). Given a typical C:N ratio of 6.6, modern lightning-based fixation would have met between 5 and 50% of total demand (Figure 2). If average C:N ratios were at the high end of the modern range, lightning alone may have met 100% of demand.

The nitrogen demand calculated in Figure 2 is a maximum, as we have assumed that 100% of the fixed nitrogen exported from the photic zone is lost to subsequent denitrification in ferruginous deep waters. This is an extreme version of the argument made by Fennel et al. (2005), who point out that cycles of nitrification and denitrification would have become a net sink for fixed N in a sufficiently oxygen-poor water column. Some fraction of the organic nitrogen, nitrate, or ammonium was likely recycled into the biosphere (e.g., Michiels et al., 2017), and this would further reduce demand, making lightning an even more important source of fixed N.

These results imply a greatly reduced demand for biological nitrogen fixation. Nevertheless, much of the parameter space that describes the Proterozoic requires at least a small contribution from biological N fixation in order to meet demand (Figure 2). The nitrogen yield of lightning strikes may also have been lower in Proterozoic atmospheres (Navarro-Gonzalez et al., 2001). If lightning is insufficient to meet demand, nitrogen may have become transiently limiting. Diazotrophs gain a competitive advantage over other photosynthetic organisms in such an environment, and their population expands until the total community is again limited by some other nutrient (Tyrrell, 1999). This simple feedback should always prevent N limitation given enough time. Even in the extreme case, where 100% of the fixed nitrogen (outside living biomass) is immediately denitrified, diazotrophs will simply compose the entire photosynthetic community.

It is important to note that, while diazotrophs may have slower cells-specific growth rates than other photosynthetic microorganisms (Tyrrell, 1999), this does not imply that a diazotroph-dominated *community* will have a lower rate of NPP, as was argued by Fennel et al. (2005). If cell-specific growth is slow, nutrients will accumulate in the photic zone, allowing the total population to expand until the limiting nutrient is again consumed completely.

More precisely, the cell population n will be at steady state when its cell-specific growth rate balances its cell-specific mortality rate, m:

$$\mu \frac{\mathrm{U}}{\mathrm{U} + K_{\mathrm{M}}} = m \tag{7}$$

where μ is the maximum cell-specific growth rate, U is the concentration of the limiting nutrient, and the growth rate is controlled by the rate of nutrient uptake following Michaelis-Menten kinetics with Michaelis constant K_M. The population will expand or contract until nutrient concentrations satisfy the steady state given above:

$$U^* = \frac{mK_M}{\mu - m} \tag{8}$$

This will occur when nutrient fluxes into and out of the photic zone are at steady state:

$$\begin{aligned} \text{organic uptake} &= \text{nutrient input} \\ \mu\frac{U^*}{U^* + K_M}n = mn &= \text{nutrient input} \end{aligned} \tag{9}$$

Thus, the population size will simply adjust until all nutrients are consumed. This may not be true if there is a kinetically rapid inorganic sink for nutrients in the photic zone itself—slower-growing cells could be outcompeted for nutrients by this process, requiring an additional term in the nutrient balance. However, the existence of such a sink is unlikely if the limiting nutrient is either phosphorus or molybdenum. In both cases, inorganic scavenging is a minor sink from modern oxic waters (Benitez-Nelson, 2000; Emerson & Huested, 1991), and the photic zone was at least weakly oxidized even in the Proterozoic (e.g., Poulton et al., 2010).

4.2. Molybdenum-Mediated Nitrogen Limitation

Tyrrell's argument holds that long-term nitrogen limitation is not possible due to expansion of diazotroph communities in the event of transient N limitation. This argument assumes that the diazotrophic community will always expand until some other nutrient becomes limiting. These organisms have unusually high needs for molybdenum, which is a cofactor in the most widely distributed form of nitrogenase. Due to efficient abiotic scavenging from sulfidic water masses, molybdenum has been suggested as limiting nitrogen-fixation rates during periods of low oxygen (e.g., Anbar & Knoll, 2002; Reinhard et al., 2013; Scott et al., 2008).

The bioavailable form of molybdenum, dissolved $MoO_4{}^{2-}$, is released to rivers during oxidative weathering (Emerson & Huested, 1991). Unlike phosphorus, the modern supply of molybdenum from weathering exceeds Proterozoic demand across nearly the entire range of parameter values (Figure 3). Exposed molybdenum-bearing minerals have likely weathered to completion since 2.4 billion years ago (Reinhard et al., 2013), when rising oxygen levels led to complete oxidation of continental sulfide minerals, one of the primary host minerals for molybdenum. It is possible that an increase in the weatherability associated with the evolution of land plants in the Paleozoic could have accelerated overall inputs, though any acceleration would have been partially suppressed by a decrease in the rate of silicate dissolution driven by silicate weathering "thermostat" (Lenton et al., 2016). It is therefore unlikely molybdenum could have limited NPP to sufficiently small values during the Proterozoic eon. The Proterozoic ocean as a whole was molybdenum poor due to efficient scavenging from its anoxic subsurface waters (Reinhard et al., 2013). However, since riverine input to the photic zone is more than sufficient to meet the needs of the small Proterozoic biosphere for most plausible values of NPP, deep-water Mo depletion needs not imply global limitation of nitrogen-fixing communities.

4.3. Iron-Mediated Nitrogen Limitation

There is a small range of burial efficiencies for which molybdenum may have been limiting, particularly if the effects of ballasting are significant (Figure 4). Some authors have argued that, if molybdenum had somehow become limiting, diazotrophs would simply have utilized an alternative nitrogenase that does not require Mo (Zhang et al., 2014). Alternative nitrogenases may utilize iron or vanadium as a substitute cofactor (e.g., Boyd et al., 2011). We focus on the possibility of iron limitation, as it should have been abundant in the ferruginous oceans of the Proterozoic (Poulton & Canfield, 2011).

The modern source of dissolved, bioavailable iron to the oceans greatly exceeds the demands of a diazotroph-dominated Proterozoic ocean (Figure 3). This modern source consists of the dissolved component of ferric iron in rivers, iron transported to the ocean by dust, and the portion of hydrothermal iron that is transported to the photic zone. At 70 Gmol Fe yr^{-1} (Tagliabue et al., 2014), this represents a small fraction of the teramoles of iron mobilized by weathering alone; under oxic conditions, the majority of iron precipitates as nonsoluble ferric particulates (Poulton & Raiswell, 2002). In the more reducing environment of the Proterozoic, the dissolved flux was likely much higher. The deep ocean was ferruginous (Poulton & Canfield, 2011), and iron-rich water masses would have upwelled into the photic zone. The cell-specific growth rates of such a community may be low (e.g., Zerkle et al., 2006), but productivity is ultimately controlled by total nutrient supplies (see section 4.1), which far exceed demand in the case of iron.

The supplies of molybdenum and iron are individually large enough to meet the needs of Proterozoic primary producers, even if denitrification was extremely efficient and abiotic sources of fixed nitrogen were much smaller than they are today. This suggests that long-term nitrogen limitation did not occur. A similar conclusion was reached by Michiels et al. (2017) based on evidence for slower rates of denitrification to N_2, but we stress that nitrogen limitation would not have occurred even in the presence of very rapid denitrification, because Mo and Fe would have been sufficiently abundant to support a large Proterozoic community of diazotrophic organisms.

Was this also true during the Archean? Demand for all nutrients would have been even lower, as organic burial efficiencies would been even larger in an environment where even surface waters had very low oxygen levels, leading to efficient export. On the other hand, the bioavailability of molybdenum may have been lower during the Archean. Under sufficiently low-oxygen conditions, physically weathered molybdenum is present as reduced particulates that are not biologically accessible (Emerson & Huested, 1991), and the low molybdenum content of Archean sediments is consistent with inefficient mobilization (Lyons et al., 2014). It is possible that the diazotrophic community, if limited by molybdenum, simply switched to use of an iron cofactor. Phylogenetic analysis of nitrogenase-bearing organisms shows that the molybdenum-free nitrogenases evolved after the Mo-dependent form (Boyd et al. 2011), but an inefficient, iron-based precursor may have predated all modern forms of nitrogenase (Boyd et al., 2011). Organisms with such an enzyme could have supplied sufficient nitrogen to the Archean biosphere (see section 4.1), unless their iron quotas were many orders of magnitude higher than in modern diazotrophs (Figure 3). Further insight into the path and timing of nitrogenase development will be required to resolve Archean nitrogen cycling in detail.

4.4. Phosphorus Limitation

Returning to the Proterozoic, we now consider the possibility of phosphorus limitation. At the lower end of its estimated range, the modern riverine supply of bioavailable phosphorus to the oceans is too slow to support productivity at Proterozoic levels (Figure 2), making phosphorus a plausible candidate for the limiting nutrient over Earth history. Bioavailability today is controlled by sorption processes that may be reversible under certain conditions (Colman & Holland, 2000; Froelich, 1988). Adsorption onto poorly crystalline iron phases can be more efficient in weakly oxidizing conditions (Mayer & Jarrell, 2000), potentially reducing riverine phosphate concentrations and making P limitation more probable (Figure 2; Laakso & Schrag, 2014).

However, rivers supply only a few percent of the phosphorus required to support modern productivity. The majority of P reaches the photic zone through upwelling of phosphate that has been remineralized in the deep ocean. Suppressing NPP to 100 Tmol C or less requires a much smaller upwelling source, such that the total supply does not much exceed modern weathering inputs (Figure 2). This is partially achieved through the increase in burial efficiency, which leads to less recycling of organic P into the water column. Others have argued that remineralized phosphate is abiotically scavenged from deep waters, either due to precipitation of ferrous-phosphate phases in a ferruginous ocean (Derry, 2015) or an efficient iron trap formed at the redox-cline (Reinhard et al., 2017). Another possibility is that phosphate precipitated rapidly from sedimentary pore waters as authigenic apatite, which is believed to be the dominant abiotic sink today (Ruttenberg & Berner, 1993). However, P is *less* likely to be retained in anoxic sediments (Anderson et al., 2001), making authigenic apatite a poor candidate for scavenging during the Proterozoic.

In order to ensure that phosphorus limitation is consistent with a balanced P budget, we can consider a simple global mass balance for phosphorus. Balancing sources against sinks

$$(\text{riverine input}) = (\text{organic burial}) + (\text{inorganic sinks})\ (r_P W_P)' = \varepsilon_o'\left(\text{NPP}'\right) + S' \tag{10}$$

where S is an abiotic sink for phosphate. Applying the relationship between modern and Proterozoic NPP [4] and assuming a similar mass balance applies today, the condition for phosphorus limitation becomes

$$(r_P W_P)' - S' \leq (r_P W_P) - S \tag{11}$$

Equation (7) suggests that at least one of the two abiotic sinks mentioned above, riverine or marine, most likely played a role in the P cycle in order to ensure phosphorus limitation during the Proterozoic. The observed rise in the phosphorus content of siliciclastic sediments (Reinhard et al., 2017) and the apparent transition to an algal-dominated productive community (Brocks et al., 2017) have both been attributed to a rise in seawater phosphate concentrations during the Cryogenian. Both observations are consistent with an increase in oxygen, remineralization of phosphate, and ultimately P-limited productivity, during the Neoproterozoic.

The mass balance calculations presented here agree with a number of recent studies arguing for global phosphorus limitation in the Proterozoic eon, due to limited P regeneration from oxidant-poor waters (Kipp & Stüeken, 2017), P scavenging by iron (Derry, 2015; Laakso & Schrag, 2014; Reinhard et al., 2017), or efficient recycling of bioavailable nitrogen Michiels et al., 2017). Our results complement this earlier work by demonstrating that, under low-oxygen conditions, balanced budgets for carbon and oxygen demand sufficiently low NPP that long-term global nitrogen limitation is not plausible even under the most conservative assumptions.

5. Conclusions

Oxygen mass balance calculations suggest that the global rate of NPP must have been less than ~100 Tmol C yr^{-1} during the Proterozoic, just a few percent of modern NPP. The nutrient demand of this biosphere is sufficiently small that fixed nitrogen is very unlikely to have been limiting. Modern rates of fixation by lightning could have met a substantial portion of demand. The remainder would have been supplied by biological nitrogen fixation, at sufficiently low rates that riverine supplies of iron and molybdenum would have exceeded demand. Limitation by nitrogen directly, or by molybdenum, may have occurred before the evolution of the relevant nitrogenases, the timing of which is still poorly known.

These results leave phosphorus as the most likely candidate for the limiting nutrient of the Proterozoic. Apatite weathering produces dissolved phosphate at approximately the rate required to limit NPP to the necessary value of $<$100 Tmol C yr^{-1}. However, productivity would exceed this bound if a substantial fraction of organic phosphorus were regenerated to the photic zone; for example, 99% of organic P is regenerated in the modern ocean, leading to production that exceeds the rate of river phosphorus input by a factor of 100. Several mechanisms have been proposed for scavenging inorganic phosphorus from ocean or river waters (Derry, 2015; Laakso & Schrag, 2014; Reinhard et al., 2017). Additional laboratory and field work exploring the efficiency of these mechanisms under various conditions may shed light on which may have operated in Proterozoic environments.

We have considered several nutrients in this paper, but it is possible that other nutrients were limiting in ancient environments. Limitation is often discussed in terms of nutrient availability, as this has almost certainly fluctuated over time for many nutrients (e.g., Anbar & Knoll, 2002). However, demand also varies over time, and Phanerozoic rates of oxygenic photosynthesis and nutrient consumption are likely to represent maxima in Earth history.

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