The Evolution and Future of Earth’s Nitrogen Cycle

Donald E. Canfield,1* Alexander N. Glazer,2 Paul G. Falkowski3

Atmospheric reactions and slow geological processes controlled Earth’s earliest nitrogen cycle, and by ~2.7 billion years ago, a linked suite of microbial processes evolved to form the modern nitrogen cycle with robust natural feedbacks and controls. Over the past century, however, the development of new agricultural practices to satisfy a growing global demand for food has drastically disrupted the nitrogen cycle. This has led to extensive eutrophication of fresh waters and coastal zones as well as increased inventories of the potent greenhouse gas nitrous oxide (N₂O). Microbial processes will ultimately restore balance to the nitrogen balance, but the damage done by humans to the nitrogen economy of the planet will persist for decades, possibly centuries, if active intervention and careful management strategies are not initiated.

Nitrogen, the fifth most abundant element in our solar system, is essential for the synthesis of nucleic acids and proteins—the two most important polymers of life. Indeed, the nitrogen requirements for life are enormous; depending on the life form, for every 100 atoms of carbon incorporated into cells, between 2 and 20 atoms of nitrogen follow (1). The biogeochemistry of nitrogen is almost entirely dependent on reduction-oxidation (redox) reactions primarily mediated by microorganisms (2), and to a lesser extent on long-term recycling through the geosphere [e.g., (3)]. Despite the importance of nitrogen and its overwhelming abundance in the atmosphere, N₂ is virtually inert; hence, fixed nitrogen and its overwhelming abundance in the atmosphere on long-term recycling through the geoatmosphere, N₂ is virtually inert; hence, fixed nitrogen gas (N₂) to ammonium (Fig. 1). Microbial processes will ultimately restore balance to the nitrogen balance, but the damage done by humans to the nitrogen economy of the planet will persist for decades, possibly centuries, if active intervention and careful management strategies are not initiated.

What is the Metabolic Basis of the Modern Nitrogen Cycle?

An active biosphere ultimately requires incorporation of nitrogen into biological molecules through nitrogen fixation, a process where prokaryotes in the bacterial and archaeal domains reduce nitrogen gas (N₂) to ammonium (Fig. 1). Some eukaryotes (e.g., legumes and termites) also support nitrogen fixation, but only in symbiotic association with nitrogen-fixing prokaryotes. Although the reduction of N₂ is an exergonic reaction, the activation energy required to break its N≡N bond is formidable, and the reaction requires a catalyst to overcome the energy barrier. The heterodimeric enzyme complex, nitrogenase, serves this role by hydrolyzing ~16 adenosine triphosphate (ATP) molecules per molecule of N₂ fixed. Nitrogenase is an αβ₃ tetramer, in which each of the two α subunits catalyzes the ATP-dependent reduction of N₂ to NH₃. In its most common form, each α subunit contains a MoFe₇S₉ metal cluster (the MoFe cofactor) that donates electrons to N₂ (6), with the electrons coming from the respiration of organic carbon. The genes encoding the two nitrogenase subunits are highly conserved but are widely dispersed across many phyla of bacteria and archaea, which suggests that nitrogen fixation evolved once and subsequently spread by vertical inheritance and by horizontal gene transfer (5, 7–9, 41).

Although nitrogenase is widely distributed among prokaryotic lineages, most organisms cannot fix nitrogen but rather obtain their nitrogen directly as NH₄⁺ (or organic nitrogen) from the environment, or from the reduction of NO₃⁻ to NH₄⁺ through assimilatory nitrate reduction. Both prokaryotes and eukaryotes are able to mediate

1Institute of Biology and Nordic Center for Earth Evolution, University of Southern Denmark, Campusvej 55, Odense M, Denmark. 2Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA. 3Institute of Marine and Coastal Studies and Department of Earth and Planetary Sciences, Rutgers University, New Brunswick, NJ 08890, USA.

*To whom correspondence should be addressed. E-mail: dec@biology.sdu.dk

Fig. 1. The major biological nitrogen transformation pathways are linked by their associated enzymes (adapted from [63]). Genes encoding enzymes that conduct the important transformations include those for various nitrate reductases (nas, euk-nr, narG, napA), nitrite reductases (nir, nrf), nitric oxide reductase (norB), nitrous oxide reductase (nosZ), nitrogenase (nif), ammonium monoxygenase (amo), hydroxylamine oxidoreductase (hao), nitrite oxidoreductase (nxr), and hydrazine hydrolase (hh).

www.sciencemag.org
this process. Ammonium is returned to the environment when organisms die, and its fate (and the variety of subsequent forms of nitrogen) depends on whether the local environment contains oxygen (Fig. 1). In the presence of oxygen, NH₄⁺ is sequentially oxidized to NO₂⁻ by specific groups of bacteria and archaea. In this pathway, known as nitrification, organisms containing the enzyme ammonium monooxygenase first oxidize NH₄⁺ to hydroxylamine, which is subsequently oxidized to NO₂⁻ by hydroxylamine oxidoreductase, and finally the NO₂⁻ is oxidized to NO₃⁻ by nitrite oxidoreductase (Fig. 1). The electrons and protons derived during ammonium and nitrite oxidation are used by the microbes to fix inorganic carbon in the absence of light (i.e., chemolithotrophy) (10). The greenhouse gas N₂O is a byproduct in this process; indeed, nitrification from both marine and terrestrial environments is an important source of atmospheric N₂O (11, 12).

In the absence of oxygen, NO₃⁻ can be used by many microbes as a respiratory electron acceptor. Nitrate reduction is coupled to the anaerobic oxidation of organic carbon (Fig. 1), producing either NH₄⁺ in a process known as dissimilatory nitrate reduction to ammonium (DNRA) or, more commonly, N₂ gas during denitrification (Fig. 1). Denitrifiers include representatives of more than 60 genera of Bacteria and Archaea, as well as some eukaryotes (e.g., fungi, protozoa, and the planktonic Foraminifera and Gromiida) (13, 14). The process involves four metalloenzymes: dissimilatory nitrate reductase, nitric oxide reductase, nitric oxide reductase, and nitrous oxide reductases. N₂O is an obligate intermediate (Fig. 1), and some ultimately escapes to the atmosphere, making denitrification another important source of this greenhouse gas from both marine and terrestrial environments (15–17).

An alternative route from fixed nitrogen to N₂ is found among a group of bacteria known as the planctomycetes, where NH₄⁺ oxidation is coupled to NO₂⁻ reduction in a process called ammonium oxidation (anoxic ammonium oxidation), an exergonic reaction used for chemolithotrophic growth (18). This process dominates N₂ production in many marine environments, but, unlike classical denitrification, it does not lead to the production of N₂O (18). Together, denitrification and ammonox close the nitrogen cycle by returning N₂ gas back to the atmosphere (Fig. 1).

What Controlled Earth’s Earliest Nitrogen Cycle?
The form(s) of nitrogen delivered during planetary accretion, the rate of accretion, and the secondary atmosphere arising from volcanism controlled the prebiotic nitrogen cycle. Planetary accretion models generally assume that nitrogen was delivered to the protoplanet as solid (ice) NH₃, amino acids, and other simple organics. These reduced forms of nitrogen subsequently became oxidized via high-temperature reactions in the upper mantle with iron and other transition elements to form atmospheric N₂, which outgassed from volcanoes (19). Indeed, a sizable (but poorly constrained) proportion of the nitrogen on Earth is still associated with the mantle (Fig. 2). These abiotic processes are extremely slow, yielding an estimated turnover time for the nitrogen cycle of about 1 billion years (3).

Current models suggest that in the early Earth atmosphere, heat shock associated both with lightning (20, 21) and high-energy meteorite impacts (22) produced NO₃ which would have converted to NO₂⁻ and NO₃ through a series of subsequent photochemical and aqueous phase reactions (23, 24). The conversion of N₂ to NH₃ by these processes was probably extremely slow, with estimates ranging from ~2 x 10⁹ mol N year⁻¹ (assuming a low CO₂ atmosphere) to ~2 x 10¹⁰ mol N year⁻¹ (assuming a high-CO₂ atmosphere) (5.6 x 10³ moles N).

Fig. 2. The size of nitrogen reservoirs on Earth is highly variable. (44, 64–67).

These estimates are lower than modern rates of biological nitrogen fixation in the oceans by a factor of 50 to 5000 (25). In principle, NO₃⁻, NO₂⁻, and N₂ can be abiotically reduced to NH₄⁺ at high temperatures through interactions with metallic Fe, magnetite, or iron sulfide minerals (26, 27); however, no appreciable flux of NH₄⁺ is apparent in modern hydrothermal vent systems where such reactions should occur. Nitrite is also readily reduced to NH₄⁺ by Fe²⁺ at pH > 7.3 (28), which may have been of particular importance on the early Earth when the oceans were likely rich in Fe²⁺ (29). Because ultraviolet oxidation of atmospheric NH₃ (in equilibrium with NH₂⁻ in the oceans) would have formed N₂ (30), N₂ gas remained the dominant form of nitrogen in the atmosphere (Fig. 3). Ammonium and possibly some NO₃⁻ dominated ocean chemistry, whereas NO₂⁻ would have been a minor phase (Fig. 3).

How Did the First Organisms Influence the Nitrogen Cycle?
The earliest organisms on Earth likely gained energy from chemically reduced compounds delivered from Earth’s interior, of which H₂ was probably the most important (31). However, as a result of the limited H₂ supply and its escape to space, primary productivity was lower than contemporary rates by a factor of 1000 or more (Fig. 3) (32). Even so, heat shock processes may have kept pace with the demands of the emerging biosphere, especially if microorganisms evolved an early means (i.e., assimilatory nitrate reduction) to reduce any environmental NO₃⁻ to NH₄⁺ within the cell.

Early in biological evolution, anoxicogenic photosynthetic organisms evolved and harnessed the Sun’s energy to oxidize substrates such as H₂, H₂S, and Fe³⁺ (but apparently not NH₄⁺) and to use the reducing equivalents for carbon fixation. If fixed inorganic nitrogen was not limiting in the earliest prephotosynthetic biosphere, it almost cer-
from the stoichiometry of anoxicogenic photosynthesis with Fe$^{2+}$ (4 Fe$^{2+}$ per CH$_2$O fixed as organic carbon), and a C/N ratio of 6/1 in organic matter. On the basis of this stoichiometry, and given estimated Fe concentrations of 40 to 120 μM in the deep ocean (29), NH$_4^+$ levels would have been 27 to 80 μM.

**How Did the Nitrogen Cycle Respond to Changes in Earth’s Surface Chemistry?**

Both the evolutionary history of nitrogen metabolisms as well as their intensity depended on the evolution of Earth’s surface chemistry. Hence, although the anaerobic process of nitrogen fixation likely evolved early in biological evolution, the efficiency of this process and its importance in regulating the nitrogen inventory of the oceans depended on the evolution of ocean chemistry (9, 32). Thus, before ~2.5 billion years ago, the oceans were rich in dissolved Fe$^{2+}$ (29), which accumulated under very low atmospheric oxygen concentrations (33). Some nitrogen fixers contain paralogous genes that encode two alternative nitrogenases, where V or Fe replaces Mo. These alternative and less efficient forms are expressed when Mo is unavailable (34), and given the abundant availability of Fe on the early Earth and the lack of soluble Mo under conditions of low atmospheric oxygen, it is likely that the Fe form dominated under these conditions. Indeed, the more efficient Mo form may not have become widely distributed until some 500 to 600 million years ago, after oxygenation of the deep ocean led to an increase in soluble Mo concentrations (35). All known nitrogenases, however, are irreversibly inhibited by molecular oxygen. Hence, if Mo is unavailable (35, 38), nitrogen fixation would have been retarded [i.e., a negative feedback (9, 31)].

In such a situation, NH$_4^+$ would have been present in the deeper anoxic parts of the ocean, whereas NO$_3^-$ and NO$_2^-$ would have been concentrated at the oxic-anoxic transition as a result of nitrification just above and denitrification just below (Fig. 3). This situation is analogous to modern stratified basins such as the modern Black Sea, but some ventilation of the ocean interior may have accompanied the downwelling oxygen-enriched waters at high latitudes, as happens in the contemporary ocean.

While global rates of primary production increased markedly with the evolution of oxygenic photosynthesis (31) (Fig. 3), feedbacks among the nitrogen, carbon, and oxygen cycles potentially contributed to the retardation of Earth’s surface oxidation. Thus, massive loss of fixed nitrogen from the oceans through anammox and denitrification are predicted during periods of deep-water anoxia (9, 31), potentially limiting the availability of fixed inorganic nitrogen. With nitrogen limitation, the burial of organic carbon would have been reduced, and hence net oxygen accumulation would have been retarded [i.e., a negative feedback (9, 31)].
Stable isotopes provide some temporal constraints on the emergence of interactions between biogeochemical cycles. In the water column, both denitrification and annamox lead to an isotopic fractionation between the two stable isotopes of N; the lighter isotope, 14N, is preferentially converted to N2, leaving the pools of fixed inorganic nitrogen enriched in 15N. Indeed, isotopic analyses from late Archean shales (about 2.7 billion years ago) reveal organic matter with strong enrichments in 15N (47), suggesting that the nitrogen cycle had either an annamox or classical denitrification pathway. The expression of these pathways required active nitrate production through nitrification, which suggests that molecular O2 and the complete nitrogen cycle were present in the upper ocean for several hundred million years before the widespread oxygenation of the atmosphere (7).

How Did the Modern Nitrogen Cycle Evolve?

Oxygen rose to its modern levels over the last 550 million years [e.g., (42)], aided by the rise of terrestrial plants (43). With the oxygenation of the ocean interior, NO3− became the dominant nitrogen species, with minor concentrations of NH4+ and NO2− in the water column. On the modern Earth, rates of net primary production are nearly equally balanced between the land and sea at about 4 × 1015 mol year−1 each (44), and although current estimates are not well constrained, the natural, pre-industrial nitrogen cycles are also of a similar magnitude on land and in the sea (45) (Fig. 4).

A curious feature of the modern terrestrial nitrogen cycle is that denitrification and nitrogen fixation are largely balanced. A sizable fraction of the nitrogen transfer from the land to the sea results from a combined loss of anthropogenic nitrogen inputs through rivers and atmospheric transport of gaseous phases (e.g., NOx, NH3, and N2O) from the continents to the oceans (45, 46). There is apparently also a large annual storage of nitrogen on the continents (46). In the oceans, rates of denitrification appear to be higher than those of nitrogen fixation and terrestrial input, suggesting an imbalance in the system (45, 46) (Fig. 4). Overall, denitrification in the oceans is governed by oxygen supply (47). In coastal sediments, and in regions of low oxygen in the water column (such as in the eastern tropical Pacific, southwest Africa, and the Arabian Sea), denitrification is extensive. In contrast, N2 fixation is primarily found in the tropical and subtropical regions of the Northern Hemisphere where continents deliver a biologically available dust source of iron (48). Assuming an average molar ratio for nitrogen and phosphate of 16/1 in organic matter (i.e., the Redfield ratio), there is a NO3− deficit of ~2 μmol/liter in the contemporary ocean, presumably reflecting a slight imbalance between N2 fixation and denitrification (2). Because nitrogenase has a high absolute Fe requirement, and because Fe availability is variable from place to place in the oceans, nitrogen deficits may have been the norm in the global ocean, with nitrogen fixation lagging to fill the demands of the biosphere as necessitated by fixed nitrogen loss through denitrification.

Has Human Activity Created an Imbalance?

In the 20th century, humans began to have an enormous impact on the global nitrogen cycle by developing industrial processes to reduce N2 to NH4+, by implementing new agricultural practices that boost crop yields, and by burning fossil fuels (49). Agriculture alone contributes about 4.09 × 1012 mol N year−1 to the global N cycle (25). For these crops, the nitrogen use efficiency is typically below 40%, meaning that most applied fertilizer either washes out of the root zone or is lost to the atmosphere by denitrification before it is assimilated into biomass. Given the rising costs of synthetic fertilizer production, this overuse is not only economically expensive, but also initiates a cascade of large-scale environmental impacts (52). Worldwide, nearly 90% of nitrogen fertilizer is NH4+, where nitrifying bacteria can convert it to highly mobile NO3−, which in turn can leach into rivers, lakes, and aquifers. This results in nitrogen loss and leads to eutrophication of coastal waters, creating huge hypoxic zones around the world (53).

Under anoxic conditions (e.g., as found in wet soils), denitrification forms mainly N2 but also forms N2O, a fraction of which is lost to the atmosphere and increasingly contributes to the rise in atmospheric N2O concentrations (54). As a greenhouse gas, N2O has 300 times (per mol) the warming potential of CO2, and it also reacts with and destroys ozone in the stratosphere (55). Because nitrification also produces N2O as an intermediate, agricultural systems represent huge sources of N2O to the atmosphere, accounting for about one-quarter of global N2O emissions (56).

What Will the Future Nitrogen Cycle Look Like?

Humans may have produced the largest impact on the nitrogen cycle since the major pathways of the modern cycle originated some 2.5 billion...
years ago. Natural feedbacks driven by microorganisms will likely produce a new steady state over time scales of many decades (i.e., excess nitrogen added from human sources will no longer accumulate, but will be removed at rates equivalent to rates of addition). However, because of the projected increase in human population through at least 2050, there will be demand for a concomitant increase in fixed nitrogen for crops to feed this population. One potential consequence of increased fixed nitrogen use will be increased fluxes of riverine nitrogen to coastal zones (57), leading in turn to enhanced biological productivity, increased coastal anoxia, detrimental impacts on water quality, and increased fluxes of \( \text{N}_2 \text{O} \) to the atmosphere.

Several new approaches and a much wider use of more sustainable time-honored practices, however, can decrease nitrogen use substantially. These include (i) systematic crop rotation [e.g., legume cropping in maize-based systems supplying the nitrogen otherwise provided by synthetic fertilizers (58)], (ii) optimizing the timing and amounts of fertilizer applied to increase the efficiency of their use by crops (59), (iii) breeding or developing genetically engineered varieties for improved nitrogen use efficiency (60), (iv) improving the ability of economically important varieties of wheat, barley, and rye to produce nitrification inhibitors through traditional breeding techniques (60, 61), and (v) further developing cereals and other crops with endosymbiotic nitrogen-fixing bacteria to supply their nitrogen needs [e.g., (62)]. Market forces may drive these improvements because the rising economic and environmental costs of nitrogen fertilizers will accelerate a demand for increased nitrogen use efficiency in agriculture (17). Thus, humans can do something about managing the nitrogen cycle, and microbial processes will ensure that a new balance in the cycle will be reached. However, even with management, the future cycle will likely be different from the one that preceded the Industrial Revolution.

References and Notes