A possible nitrogen crisis for Archean life due to reduced nitrogen fixation by lightning

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Nitrogen is an essential element for life and is often the limiting nutrient for terrestrial ecosystems[1, 2]. The principal reservoir of nitrogen is molecular nitrogen in the atmosphere. However to be available for organisms nitrogen must be in the form of ammonia or nitrate, forms known as fixed nitrogen. Due to the strength of the triple bond in N_2, nitrogen fixation, while thermodynamically favored is kinetically restricted[3]. The development of biological nitrogen fixation must have occurred early in earth history when the biological demand exceeded the main abiotic source: production of nitric oxide by lightning in the early atmosphere composed of carbon dioxide and dinitrogen. Here we report an experimental study of the nitrogen fixation rate over the evolution of the pre-oxygenic atmosphere: from predominantly carbon dioxide to predominantly dinitrogen. Our results indicate that the production of nitric oxide drastically decreased from ~3.0×10^{11} g N yr^{-1} at the time of the origin of life when the CO_2 levels presumably

were high to ~2.6×10^9 g N yr^{-1} for the low CO_2 levels determined at 2.2 Gyr ago, just before the start of the rise of oxygen in the atmosphere. This reduction in NO production may have caused an ecological crisis that triggered the development of biological nitrogen fixation before a new increase in abiotic nitrogen fixation resulted from either the rise of atmospheric CH_4 or O_2 from biological activity.

Because biological nitrogen fixation is energetically expensive and does not occur if adequate supplies of fixed nitrogen are available, it has been generally thought that the development of metabolic pathways to fix nitrogen arose only in response to a crisis in the supply of fixed nitrogen on the early Earth. Cloud[4] and others[5, 6, 7] have suggested that this crisis occurred soon after the origin of life as the prebiotic source of organic material was depleted by the emerging life forms. In this scenario, the abiotic sources of fixed nitrogen were unable to sustain even simple microbial ecosystems. In contrast Mancinelli and McKay[8], noting that many modern microbially-dominated ecosystems are satisfied with abiotic inflows of fixed nitrogen and do not express nitrogen fixation, posited that nitrogen fixation arose late only when biological demand increased with the development of higher plants and exceeded the abiotic supply.

In both of these scenarios the key question is the rate of abiotic nitrogen fixation by lightning. Yung and McElroy[9] computed the lightning fixation rate on the early Earth for atmospheres containing N_2 and H_2O suggesting a source of ~10^{12} g N yr^{-1}. Kasting and Walker[10] and Kasting[11] considered more realistic atmospheres composed primarily of CO_2 and determined a production rate from thermodynamic equilibrium models of $\geq 10^{12}$ g N yr^{-1}, and noted that recycling by atmospheric photochemistry would return 25% of this to N_2, reducing the net source of fixed nitrogen[10]. The only previous

experimental determination of fixation in CO_2 dominated atmospheres is the work of Levine et al[12] who measured NO_x production in a simulated Venus atmosphere of 95.9% CO_2, 3.97% N_2 and traces of SO_2, Ar, and CO.

The thermodynamic equilibrium models cited above[9, 10] and used in all other previous studies of nitrogen fixation by lightning on the early Earth[8, 13] are only approximate because they compute the fixation rate by assuming that the chemical reactions proceed rapidly at high temperatures and then are quenched or "freeze out" at some specified temperature between 2000 and 3000 K. The equilibrium concentration at this freeze out temperature determines the final production. To accurately determine the nitrogen fixation rate by lightning in CO_2 atmospheres, we have experimentally determined the production of NO by simulated lightning as described in the methods.

Figure 1 shows the variation of the NO production rate with the carbon dioxide mixing ratio, χCO_2. For comparison the results obtained for the simulation of the present Venusian (Ref. *12*) and Terrestrial (Ref. *14*) lightning are presented. Our results indicate that the energy yield of production of nitric oxide increases from $\sim 1.9\times10^{15}$ molecule J^{-1} at $\chi CO_2 \sim 0.98$ to $\sim 1.3\times10^{16}$ molecule J^{-1} at $\chi CO_2 \sim 0.80$. This yield is insensitive down to about $\chi CO_2 \sim 0.50$. At lower mixing ratios the NO yield drastically decreases to $\sim 2.4\times10^{14}$ molecule J^{-1} at $\chi CO_2 \sim 0.20$ and then slowly diminishes to $\sim 1.1\times10^{14}$ molecule J^{-1} at $\chi CO_2 \sim 0.025$. Any further decrease in the atmospheric CO_2 content has little impact at this point since the water vapor present in the atmosphere contributes oxygen atoms for the production of NO, stabilizing it at about 7.0×10^{13}

molecule J^{-1}. For experiments conducted at $\chi CO_2 \sim 0.80$, it was found that the NO production yield is not pressure dependent. The efficient production of NO in CO_2 dominated mixtures can be explained by the thermal dissociation of carbon dioxide leading to the formation of atomic oxygen according to reaction 1:

$$CO_2 \rightarrow O + CO \quad (1)$$

Most of the O produced in reaction 1 combines to form O_2. However at temperatures above 1500°C, a small fraction of the O results in the formation of NO *via* the following reaction chain:

$$O + N_2 \rightarrow NO + N \quad (2)$$

$$N + CO_2 \rightarrow NO + CO \quad (3)$$

In competition are the following reactions which destroy NO:

$$NO + N \rightarrow N_2 + O \quad (4)$$

$$NO + O \rightarrow N + O_2 \quad (5)$$

At low CO_2 mixing ratios, water vapor from the atmosphere contributes to the formation of atomic oxygen *via* the following set of reactions:

$$H_2O \rightarrow H + OH \quad (6)$$

$$OH + OH \rightarrow H_2O + O \quad (7)$$

The drastic reduction in the NO production in N_2 dominated atmospheres ($\chi CO_2 \leq 0.20$) is attributed to the efficient third-body conversion of atomic nitrogen into dinitrogen[15, 16] (reaction 8), transforming reaction 3 into a negligible channel.

$$2N + N_2 \rightarrow 2N_2 \quad (8)$$

In contemporaneous lightning where oxygen would be a dominant species, reaction 1 and 5 would be replaced by the following set of equations, respectively:

$$O_2 \rightarrow O + O \quad (9)$$

$$N + O_2 \rightarrow NO + O \quad (10)$$

The formation of atomic oxygen in reaction 10 enhances the production of NO *via* reactions 2 and 10. The ratio of NO to CO in the product gases varies as expected based on this reaction scheme. In our experiments the CO to NO ratio is over 200 for CO_2 mixing ratios near unity and then drops rapidly to values between 30-60 for CO_2 mixing ratios between 0.8 and 0.2 (figure 2). Below CO_2 mixing ratios of 0.2 the CO to NO ratio climbs again to high values, reaching 180 for $\chi CO_2 = 0.025$. The shape of this curve for $\chi CO_2 > 0.2$ is as expected if the freeze out temperature is constant. However, the increase in CO/NO at low χCO_2 is not predicted by a constant freeze out model. Freezout temperature variations of about 1000 K, and different freeze out temperatures for NO and CO would be required to explain this difference. The increasing importance of reaction 8 at low χCO_2 in our proposed chemical scheme can satisfactorily account for this increase in the CO/NO ratio.

It is unclear how the lightning flashing rate would have varied on the early Earth; however, assuming that it remained constant at 1×10^{18} J yr^{-1} over time[17], our results (Figure 1) imply that the annual production rate of NO was of the order of ~3.0×10^{11} g N whenever CO_2 was the dominant atmospheric gas. This is likely to have been the case during the Hadean, when chemical evolution and the origin of life probably occurred. However, paleosol measurements[18] indicate that at 2.2 Gyr ago CO_2 levels had dropped to < 0.04 bar. As seen from Figure 1, this drop in CO_2 caused lightning production of NO to rapidly decrease to ~2.6×10^{9} g N yr^{-1} until large increase in abiotic nitrogen fixation resulting either from the rise of atmospheric oxygen or production of HCN in a CH_4 rich atmosphere[19]. We suggest that a crisis in the supply of fixed nitrogen occurred as a result of this factor of a 100 decrease in NO production prompting the development of biological nitrogen fixation.

Abiotic production might have increased before the rise of O_2 due to HCN formation from CH_4 by methanogenic bacteria. Models of the early greenhouse effect suggest that biogenic methane levels increased as the CO_2 levels declined and became the dominant greenhouse gas[20,21] with a mixing ratio of ~10^{-3}. Photochemical models by Zahnle[19] suggest that HCN formation occurs at a significant rate when the CH_4 mixing ratio exceeds 10^{-5} for a 100 times the present atmospheric levels of CO_2 (see Fig. 1). Climate models[20] suggest that this occurred only when the CO_2 mixing ratios fell to below 0.1. Thus the nitrogen crisis would have occurred when the CO_2 mixing ratio was $0.1 \geq \chi CO_2 \leq 0.2$. The atmospheric CO_2 removal rate for the Precambrian is unknown; however, assuming that the CO_2 levels dropped linearly from the Hadean to early Proterozoic, when the oldest CO_2 paleosol record exist[18], this crisis could have

lasted in the order of 10^8 yrs even though it was confined to a narrow window in the atmospheric CO_2 concentration. Even if this figure were over estimated by several orders of magnitude, a shortage of reactive nitrogen to the Archean Biosphere of more than 10^3 yrs would have had dramatic ecological consequences.

The key reactions that form HCN in a CH_4-rich atmosphere are:

$$^3CH_2 + N \rightarrow HCN + H \quad (11)$$

$$CH_3 + N \rightarrow HCN + H_2 \quad (12)$$

but their rate constants and products have not yet been studied experimentally. In fact, these reactions were considered to be similar to another reaction[22]:

$$CH + N \rightarrow HCN \quad (13)$$

The rate of this reaction has been measured and is fast but the products have not yet been identified. Current kinetic databases[16] suggest that reaction 11 does not produce HCN but rather CH + NH based on bond energy-bond order theory. If NH radical is produced instead, this could lead to the production of ammonia. Therefore, the question of abiotic nitrogen fixation in CH_4-rich atmospheres is unresolved and further work is needed. If no net production of fixed nitrogen took place in CH_4-rich atmospheres, then abiotic nitrogen fixation increased only with the rise of atmospheric O_2 at about 2.2 Gyr ago.

There are other indications that nitrogen fixation arose early in the history of life. These include: (i) Evidence of the phylogenetic antiquity of the genes for biological nitrogen fixation[23]; (ii) Nitrogen 15/14 isotopic ratios[24] consistent with fixation before 3.5 Gyr ago; (iii) The oxygen sensitivity of the nitrogen fixation process[1] which implies an origin predating the rise of atmospheric oxygen[25] about 2 Gyr ago; (iv) The presence of heterocysts formed by nitrogen fixing cyanobacteria in fossils[26] of 1.5 – 1.3 Gyr age; and (v) The antiquity of cyanobacteria as indicated by hopanoids found in 2.7 Gyr sediments[27], by morphological evidence for cyanobacteria at 3.5 Gyr ago showing both microfossils similar to modern cyanobacteria as well as stromatolites[28] , and by possible evidence for cyanobacteria at 3.8 Gyr ago in the carbon isotopes[29] showing a characteristic enrichment in ^{12}C.

Our results suggest that the development of biological nitrogen fixation arose in response to changes in atmospheric composition that resulted in a reduction in the production of abiotically fixed nitrogen. The biochemistry of the nitrogen fixing process, in particular its sensitivity to oxygen, may reflect the timing of nitrogen crisis and illustrates the co-evolution of the metabolic pathways in life and the environment of the early Earth.

Methods

Lightning in the laboratory was simulated by a plasma generated with a Q-switched Nd-YAG laser. The temperature of this plasma[30] has been determined to be about 20,000 K at 1 μs after initiation. The laser delivers a beam of 1.06 μm photons with an energy of up to 600 mJ per pulse in 5-7 ns at 10 Hz. A beam with 300 mJ per pulse was focused

inside a closed 1-liter Pyrex flask with a plano-convex optical glass lens which is expected to have a focal aberration of ~10 μm. The power deposited into the system was determined calorimetrically. Our power meter was calibrated with the heating rate produced by passing a known electric current through a resistor. The power deposited was found to depend on the chemical composition of the gas mixture used, varying from 1.5 W for 100% CO_2 to 1.9 W for 50% CO_2 in N_2 to 2.3 W for 100% N_2. The energy dissipated per unit length in this simulated lightning was estimated to be ~10^4 J m^{-1}, in close agreement with that from natural lightning. Most experiments were conducted at 1 bar total pressure. In another series of experiments, the pressure was varied (0.2 to 1.0 bar). In some additional experiments water vapor was introduced into the reactors to achieve a 100% relative humidity. The main nitrogen-containing lightning product was determined to be nitric oxide by infrared and mass spectroscopy. The NO production rate was derived from the slope of a linear fit to the number of molecules *vs* energy deposited.

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Figure 1. Variation of the nitric oxide yield as a function of the CO_2 mixing ratio in simulated lightning in primitive atmospheres composed of CO_2-N_2 at 1 bar under anhydrous (○) and hydrated (●) conditions. All samples were irradiated at 20°C from 5 to 30 minutes. Immediately after irradiation, an aliquot (2 ml) was introduced with an automatic gas sampling loop into a gas chromatograph interfaced in parallel with a FTIR-detector and a quadrupole mass spectrometer. Two capillary chromatographic columns were used for the separation of lightning products: PoraPlot Q (25 m × 0.32 mm I.D.) and Alumina/KCl (50 m × 0.32 mm I.D.). For comparison the results obtained for the simulation of the present Venusian (Δ) and Terrestrial (□) lightning are presented. Also shown is the possible production of HCN based on the Zahnle mechanism[19] for methane mixing ratios (dotted line) needed to offset the

reduced CO_2 and keep surface temperature constant at 288.15 K (present level) based on Kasting *et al*[21]. Time proceeds nonlinearly from left to right.

Figure 2. Ratio of NO to CO produced in laser discharge as a function of CO_2 concentration (○). Also shown are the predictions (line) of the freeze out models for freeze out temperatures between 2000 and 3000 K, the extreme range of freeze out models.

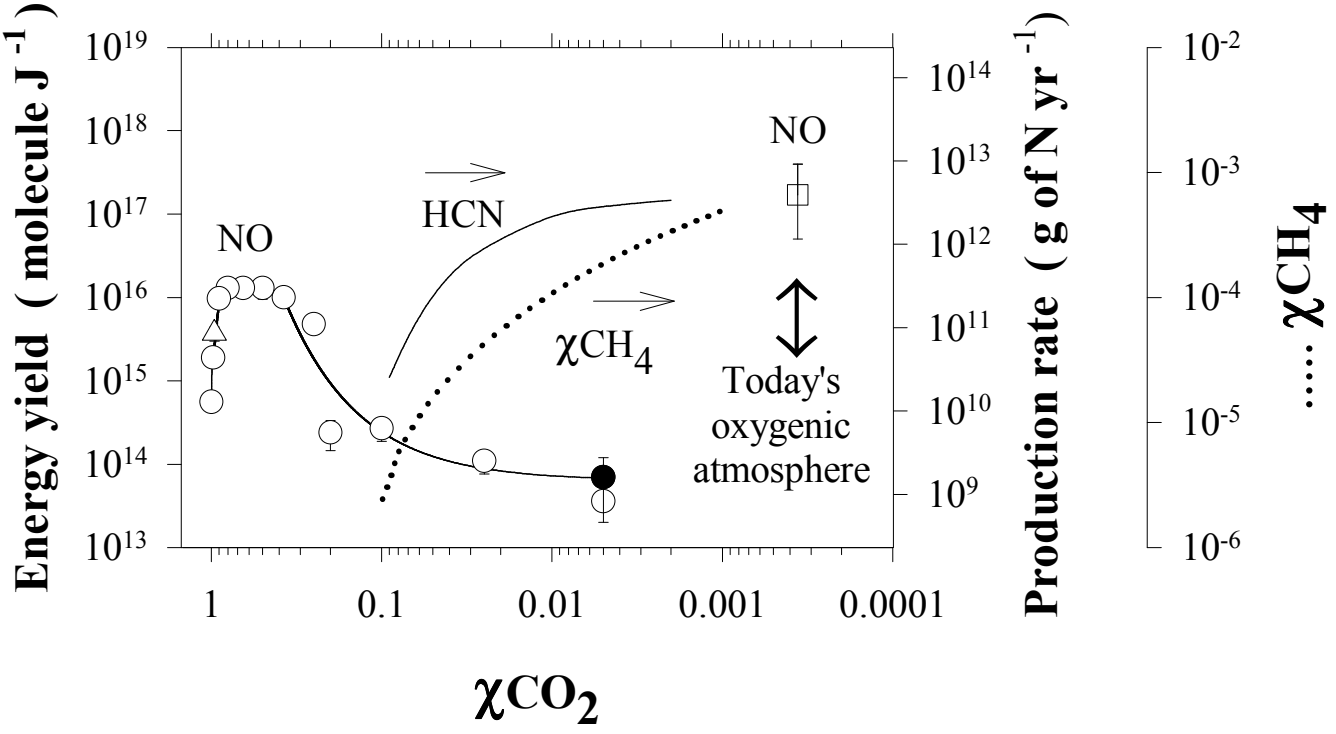

Figure 1
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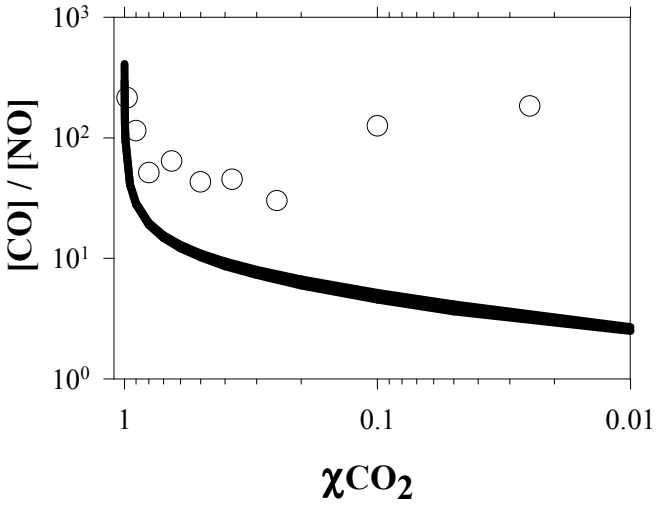

Figure 2
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