Nitrogen Oxide Concentrations in Natural Waters on Early Earth

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Abstract A key challenge in origins-of-life studies is estimating the abundances of species relevant to the chemical pathways proposed to have contributed to the emergence of life on early Earth. Dissolved nitrogen oxide anions (NO$^-_X$), in particular nitrate (NO$^-_3$) and nitrite (NO$^-_2$), have been invoked in diverse origins-of-life chemistry, from the oligomerization of RNA to the emergence of protometabolism. Recent work has calculated the supply of NO$^-_X$ from the prebiotic atmosphere to the ocean and reported steady state [NO$^-_X$] to be high across all plausible parameter space. These findings rest on the assumption that NO$^-_X$ is stable in natural waters unless processed at a hydrothermal vent. Here, we show that NO$^-_X$ is unstable in the reducing environment of early Earth. Sinks due to ultraviolet photolysis and reactions with reduced iron (Fe$^{2+}$) suppress [NO$^-_X$] by several orders of magnitude relative to past predictions. For pH = 6.5–8 and T = 0–50 °C, we find that it is most probable that [NO$^-_X$] < 1 μM in the prebiotic ocean. On the other hand, prebiotic ponds with favorable drainage characteristics may have sustained [NO$^-_X$] ≥ 1 μM. As on modern Earth, most NO$^-_X$ on prebiotic Earth should have been present as NO$^+_X$ due to its much greater stability. These findings inform the kind of prebiotic chemistries that would have been possible on early Earth. We discuss the implications for proposed prebiotic chemistries and highlight the need for further studies of NO$^-_X$ kinetics to reduce the considerable uncertainties in predicting [NO$^-_X$] on early Earth.

1. Introduction

A key challenge for origin-of-life studies is determining the range of environmental conditions on early Earth under which life arose. Knowledge of these environmental conditions informs development of theories of the origin of life and enables assessment of the plausibility and probability of postulated prebiotic chemistries (e.g., Bada et al., 1994; Corliss et al., 1981; Ruiz-Mirazo et al., 2014; Todd et al., 2018; Urey, 1952; Xu et al., 2018). Consequently, extensive work has been done to place constraints on the prebiotic environment, including but not limited to the availability of liquid water, the redox state of the atmosphere, the ultraviolet (UV) irradiation environment, the pH and temperature of the early oceans, the physicochemical conditions at deep-sea hydrothermal vents, and the availability of sulfidic anions (Farquhar et al., 2000; Halev & Bachan, 2017; Krissansen-Totton et al., 2018; Mojzsis et al., 2001; Martin et al., 2008; Ranjan & Sasselov, 2017; Ranjan et al., 2018).

An important prebiotic environmental factor is the abundance of fixed nitrogen species in natural waters on early Earth. Dinitrogen’s high-energy triple bond renders it highly nonreactive, meaning that nitrogen generally must be “fixed” into its reduced or oxidized forms (e.g., NO$^-_2$, NO$^-_3$, and NH$^+_2$) to be useful to biology or prebiotic chemistry. Consequently, it is unsurprising that nitrogen fixation is thought to be ancient (Canfield et al., 2010; Fani et al., 2000; Stüeken et al., 2015; Zehr & Ward, 2002; Zerkle & Mikhail, 2017). Prebiotic chemists have been especially interested in nitrate (NO$^-_3$) and nitrite (NO$^-_2$), the oxidized anions of nitrogen (NO$^-_X$). These molecules are high-potential electron acceptors, have played a key role in microbial metabolisms since at least the Archaean (Canfield et al., 2010), and have been hypothesized to have been involved in the first metabolic pathways, for example, the oxidation of methane and hydrogenation of CO$_2$ at deep-sea vents (Ducluzeau et al., 2009; Nitschke & Russell, 2013; Shibuya et al., 2016). These molecules have also been invoked for the nonenzymatic synthesis and replication of oligonucleotides, in surficial (lake/pond) settings (Mariani et al., 2018). Crucially, NO$^-_X$ can be abiotically synthesized by the thermal decay of molecules like HNO, which are produced by high-energy events like lightning in a N$_2$-CO$_2$...
atmosphere (Ardaseva et al., 2017; Kasting & Walker, 1981; Mancinelli & McKay, 1988; Navarro-González et al., 1998), meaning these molecules may have been available for prebiotic chemistry on early Earth.

Motivated by the potential prebiotic relevance of NO$_3^-$ and NO$_2^-$, a number of studies have aimed to constrain their concentrations on early Earth. Mancinelli and McKay (1988) pointed out that atmospherically generated NO$_2^-$ would form NO$_3^-$ and NO$_3^-$ in the prebiotic ocean and accumulate and that solubility concerns would not limit the accumulation. However, Mancinelli and McKay (1988) did not quantify the concentrations to which NO$_3^-$ could accumulate. Wong et al. (2017) conducted atmospheric modeling, combining 3-D general circulation model estimates with 1-D photochemical models to estimate the supply of NO$_3^-$ to the oceans due to lightning. They identified the key variable controlling the NO$_3^-$ supply to be the CO$_2$ partial pressure (pCO$_2$). Under the assumption that the sole sink on NO$_3^-$ was destruction at high-temperature hydrothermal vents, Wong et al. (2017) computed [NO$_3^-$] in the bulk ocean to be $\geq 10\mu$M, and [NO$_3^-$] = 20 mM for pCO$_2 = 1$ bar. Laneuville et al. (2018) conducted a systems model of the prebiotic nitrogen cycle, including cometary delivery, impact synthesis, and lightning as sources of fixed nitrogen and destruction at hydrothermal vents as the sole sink of oceanic NO$_3^-$. They calculated [NO$_3^-$] $\approx 1\mu$M to 10 mM in the bulk prebiotic oceans, depending on a number of variables including atmospheric nitrogen fixation rate. These large ranges are due to the wide range of possible NO$_3^-$ supply. Both Wong et al. (2017) and Laneuville et al. (2018) suggest that the prebiotic oceans should have had [NO$_3^-$] $\geq 1\mu$M [NO$_3^-$], thought to be adequate for prebiotic chemistry. For comparison, on modern Earth, bioavailable fixed NO$_3^-$ achieves maximum concentrations of $\sim 40$ $\mu$M in the deep Pacific (Olsen et al., 2016; Zehr & Ward, 2002). NO$_3^-$ in the modern ocean is almost exclusively in the form of NO$_3^-$, except where NO$_3^-$ accumulates substantially within two depth horizons. The primary nitrite maximum, where nitrite concentrations reach hundreds of nanomolar to occasionally a few micromolar, is a global feature at the base of the photic zone, formed by leaking algal cells (Lomas & Lipschultz, 2006) and/or an imbalance in the ammonium and nitrite oxidation steps of nitrification (Santoro et al., 2013). The secondary nitrite maximum is a broad feature in the oxygen-deficient zones of the eastern tropical Pacific and Arabian Sea, where oxygen levels are reduced to < 10 nM (Revsbech et al., 2009). These secondary nitrite maxima can achieve several micromolars in concentration (e.g., Babbin et al., 2014) and are indicative of active denitrification regimes.

Overall, previous work has concluded that high [NO$_3^-$] (micromolar to millimolar) was present in the prebiotic ocean, under the assumption that the only sink of NO$_3^-$ in the prebiotic ocean was processing at hydrothermal vents and that atmospherically supplied NO$_3^-$ was otherwise stable in prebiotic waters. However, in the anoxic prebiotic environment, NO$_2^-$ and NO$_3^-$ are vulnerable to reduction to less soluble forms due to UV photochemistry and reactions with reductants (e.g., Fe$^{2+}$). These reduced species (NO, N$_2$O, and N$_2$) can then escape to the atmosphere, depleting the oceanic nitrogen pool (Carpenter & Nightingale, 2015).

In this paper, we explore the impact of these chemical sinks on the predicted concentrations of NO$_3^-$ in prebiotic waters. In section 2, we carry out a kinetic calculation, comparing the supply of NO$_3^-$ to natural waters to the sinks of NO$_3^-$ from reduction reactions and photochemistry to estimate the steady state concentration of NO$_3^-$ in oceans and ponds with $T = 0$–50 °C and pH = 6.5–8. In section 3, we examine the thermochemical stability of NO$_2^-$ and NO$_3^-$ in prebiotic conditions, finding results consilient with our kinetic calculations. In section 4, we discuss our calculations and explore their implications for prebiotic NO$_3^-$ levels, postulated prebiotic chemistries, and origin-of-life scenarios. We summarize our conclusions in section 5.

### 2. Kinetic Steady State

In this section, we calculate abiotic loss of aqueous NO$_2^-$ and NO$_3^-$ due to reduction by UV light and Fe$^{2+}$ and compare to the NO$_3^-$ supply rate from processes such as lightning fixation and exogenous delivery, to estimate steady state NO$_3^-$ concentrations. We focus on these processes because they are dominant under the range of conditions available on early Earth. If abiotic NO$_3^-$ destruction is slow compared to abiotic NO$_3^-$ production, then it is possible for high levels of NO$_3^-$ to build up in natural waters. If destruction is fast compared to production, then NO$_3^-$ levels will be low.

#### 2.1. NO$_3^-$ Production

On modern Earth, lightning fixation is the largest nonbiological natural source of fixed nitrogen, via high-energy shocks which form free radicals and disrupt N$_2$ (Mancinelli & McKay, 1988). Wong et al. (2017) used photochemical models calibrated with general circulation model results to model the supply of NO$_3^-$ to the prebiotic surface due to lightning fixation. Most NO$_3^-$ reached the surface in the form of HNO, which
We find NO\textsuperscript{−} production to be strong functions of pN\textsubscript{2}/pCO\textsubscript{2} and pCO\textsubscript{2}. NO\textsubscript{X} production decreases as pN\textsubscript{2}/pCO\textsubscript{2} increases, because the probability of N atoms recombining to N\textsubscript{2} is higher (as opposed to reacting with CO\textsubscript{2}-derived oxygen to form NO\textsubscript{X}). We find NO\textsubscript{X} production increases with pCO\textsubscript{2} over the range pCO\textsubscript{2} = 0.1–10 bar. This contrasts to Wong et al. (2017), who report a maximum in NO\textsubscript{X} production at pCO\textsubscript{2} = 1 bar; this is because Wong et al. (2017) calculate lower lightning flash rates for high pCO\textsubscript{2} due to lack of moist convection in the warm troposphere they calculate for pCO\textsubscript{2} = 10 bar, while we fix the lightning flash rate. If we extrapolate the finding of Marty et al. (2013) that pCO\textsubscript{2} ≤ 0.7 bar and pN\textsubscript{2} ≥ 0.5 bar from 3–3.5 Ga to the prebiotic era (ca. 3.9 Ga) and assume lightning energies and flash densities similar to modern Earth, we find a tropospheric NO\textsubscript{X} production rate of φ\textsubscript{NO\textsubscript{X}} < 10\textsuperscript{9} cm\textsuperscript{−2}s\textsuperscript{−1}, in concordance with Wong et al. (2017). Biological fixation tends to decrease pN\textsubscript{2}, suggesting that pN\textsubscript{2} was not lower in the prebiotic era than in the Archaean (Johnson & Goldblatt, 2017). Krissansen-Totton, Olson, and Catling (2018) calculate that weathering restricted pCO\textsubscript{2} ≤ 1 bar at 4 Ga. We consequently retain 6.5 × 10\textsuperscript{8} cm\textsuperscript{−2}s\textsuperscript{−1} of Wong et al. (2017) as the upper bound on φ\textsubscript{NO\textsubscript{X}} but caution that if pCO\textsubscript{2} were higher or pN\textsubscript{2} lower than what we consider, φ\textsubscript{NO\textsubscript{X}} could have been up to an order of magnitude higher. For more details, see supporting information Text S4.

Comet delivery and impact fixation should also have supplied fixed nitrogen on prebiotic Earth; however, these mechanisms are thought to have supplied φ\textsubscript{NO\textsubscript{X}} < 2 × 10\textsuperscript{9} cm\textsuperscript{−2}s\textsuperscript{−1} and typically less, well within the range bracketed by lightning production (Laneuville et al., 2018). Airapetian et al. (2016) suggest that energetic protons from flares on the young Sun might also have powered nitrogen fixation and the supply of NO\textsubscript{X} to the surface; however, they do not quantify the magnitude of this supply. We consequently focus on the NO\textsubscript{X} production flux range defined by lightning production in the model of Wong et al. (2017) in our work (2.5 × 10\textsuperscript{9} – 6.5 × 10\textsuperscript{9} cm\textsuperscript{−2}s\textsuperscript{−1}).

### 2.2. NO\textsubscript{X} Destruction

We consider three processes in calculating NO\textsubscript{X} destruction in natural waters: processing at hydrothermal vents, UV photolysis, and reactions with Fe\textsuperscript{2+}. Past work has focused on processing at vents; in this work, we consider the effects of UV and Fe\textsuperscript{2+} as well. Supporting information Text S5 explores these processes in detail, along with other NO\textsubscript{X} loss processes we neglected in this work because they are dominated by the processes we consider here.

The presence of UV light on early Earth is attested to by the sulfur mass independent fractionation signal (Farquhar et al., 2001). The presence of Fe\textsuperscript{2+} is attested to by the presence of banded iron formations and other geological evidence (Cloud, 1973; Klein, 2005; Li et al., 2013; Walker & Brimblecombe, 1985). Recent estimates place [Fe\textsuperscript{2+}] = 30–600 μM in early Archean oceans, with higher [Fe\textsuperscript{2+}] in the aphytic deep oceans (Halevy & Bachan, 2017; Konhauser et al., 2017; Tosca et al., 2016; Zheng et al., 2018). In this work, we explore [Fe\textsuperscript{2+}] = 10–600 μM, bracketing this range. We do not consider reactions with other reductants,
such as Mn$^{2+}$, owing to paucity of constraints on the kinetics of these processes; consequently, we may underestimate NO$_X^-$ reduction rates in prebiotic natural waters.

The rates of NO$_X^-$ photolysis by UV and reduction by Fe$^{2+}$ are dependent on temperature and pH. In this work, we consider pH = 6.5–8 and T = 273–323 K, motivated by modeling work which predicts the early ocean to have been circumneutral (6.3 ≤ pH ≤ 7.2) and temperate (271 ≤ T ≤ 314 K; Krissansen-Totton et al., 2018; see also Halevy & Buchan, 2017). We consider the sensitivity of our conclusions to these assumptions in section 2.4.

### 2.2.1. NO$_X^-$ Destruction in Vents

The hot and acidic conditions at hydrothermal vents can destroy NO$_X^-$ (Brandes et al., 1998; Ray et al., 1917; Summers, 2005). It is debated how extreme conditions need to be to consume NO$_X^-$; under the assumptions that NO$_X^-$ is removed with unit efficiency at and only at black smoker-type vents ($T < 405^\circ$C, pH = 1–2; Martin et al., 2008), Wong et al. (2017) propose NO$_X^-$ destruction to be characterized by a first-order process with rate constant $k_{\text{vents}} = 8 \times 10^{-17}$ s$^{-1}$. Under the assumption that circulation through any hydrothermal vent would destroy 100% of NO$_X^-$, Laneuville et al. (2018) instead propose $k_{\text{vents}} = 1 \times 10^{-14}$ s$^{-1}$. We explore the range $k_{\text{vents}} = 8 \times 10^{-17}$ to $1 \times 10^{-14}$ s$^{-1}$ in this work.

### 2.2.2. NO$_X^-$ Destruction by UV

Irradiation by UV light in natural waters net photolyzes NO$_X^-$ to NO$_2^-$, and NO$_2^-$ to NO, which escapes the ocean to the atmosphere or is reduced to N$_2$O (Carpenter & Nightingale, 2015; Fanning, 2000; Mack & Bolton, 1999; Spokes & Liss, 1996; Stanton et al., 2018):

\[
\text{NO}_X^- + h\nu \rightarrow \text{NO}_2^- + \frac{1}{2}O_2
\]  

(1)

\[
\text{NO}_2^- + H_2O + h\nu \rightarrow \text{NO} + \text{OH} + \text{OH}^-
\]  

(2)

These processes are thought to be first order and have been measured both in the oceans and in lakes. In the modern surface ocean, these processes have median rate constants $k_{\text{NO}_2^-h\nu} = 2.3 \times 10^{-8}$ s$^{-1}$ and $k_{\text{NO}_2^-h\nu} = 1.2 \times 10^{-6}$ s$^{-1}$ for nitrate and nitrite, respectively (Mack & Bolton, 1999; Zafiriou & True, 1979a, 1979b). In pure water, NO$_2^-$ reacts with OH to reform NO$_X^-$; however, in the presence of OH scavengers like bicarbonate or Br$^-$, NO$_X^-$ is lost with 20–100% efficiency (Treinin & Hayon, 1970; Zafiriou, 1974; Zafiriou & True, 1979b; Zafiriou et al., 1984).

Nitrite and nitrite photolysis rates, as measured by OH production, depend modestly on temperature. At $T = 0^\circ$C, photolysis rates are ≥ 0.5 times the rates at $T = 25^\circ$C, and at $T = 50^\circ$C, photolysis rates are ≤ 2 times the rates at $T = 25^\circ$C (Mack & Bolton, 1999; Zellner et al., 1990). The reaction rates measured by Zafiriou and True (1979a, 1979b) were measured at ambient temperature. Under the assumption that these ambient conditions corresponded to $T \approx 25^\circ$C, we explore $k_{\text{NO}_2^-h\nu} = 1.1 - 4.6 \times 10^{-8}$ s$^{-1}$ and $k_{\text{NO}_2^-h\nu} = 0.6 - 2.4 \times 10^{-6}$ s$^{-1}$, to account for the variation in photolysis rates with temperature for $T = 0$–50 $^\circ$C.

We calculate the global rate of nitrate/nitrite photolysis following a procedure motivated by that of Zafiriou and True (1979a, 1979b). We assume that nitrate and nitrite are lost at rates equal to half their surface photocatalytic losses down to their photic depths and that the loss rates are 0 below this threshold. The modern photic depth for nitrate and nitrite at the equator are 5 and 10 m, respectively; to average over latitude and obtain global mean photic depths, we scale these photic depths by two thirds (Cronin, 2014; Zafiriou & True, 1979a, 1979b). In the modern ocean, intense consumption by phototrophic microbes depletes surficial NO$_X^-$, meaning that NO$_X^-$ photolysis is only significant in upwelling areas where NO$_X^-$ is maintained at high concentrations due to supply from below. In the absence of biology, surface NO$_X^-$ would not be depleted, and NO$_X^-$ would be photolyzed from 100% of the surface area of the ocean. When calculating the loss of NO$_X^-$ in pond and lake environments, we assume the same photic depths and continue to take NO$_X^-$ to be lost from 100% of the surface area. We neglect possible enhancements in the conversion rate due to factors such as availability of shorter-wavelength UV radiation on early Earth and the anoxic nature of prebiotic natural waters, and we assume the lowest proposed efficiency for net loss of NO$_X^-$ to photolysis (i.e., 20%). Consequently, our estimates of NO$_X^-$ photolysis should be considered lower bounds.

### 2.2.3. Reduction of NO$_X^-$ by Fe$^{2+}$ to Nitrogenous Gas

Fe$^{2+}$ reduces NO$_2^-$ to yield nitrogenous gas (Buchwald et al., 2016):

\[
2\text{NO}_2^- + 4\text{Fe}^{2+} + 5H_2O \rightarrow N_2O(g) + 4\text{FeOOH} + 6H^+
\]  

(3)
Recent kinetic studies of these reactions are consistent with first-order kinetics with respect to both reactants and second-order kinetics overall, with rate constants that are dependent on pH (Buchwald et al., 2016; Grabb et al., 2017; Jones et al., 2015). From the data of Buchwald et al. (2016) and Grabb et al. (2017), we extract $k_{\text{NO}_2^-\text{Fe}^{2+}} = 3 \times 10^{-5}$ to $1 \times 10^{-2}$ M$^{-1}$s$^{-1}$ over pH = 6.5–8 and $T = 25^\circ$C (supporting information Text S5).

$k_{\text{NO}_2^-\text{Fe}^{2+}}$ depends on whether Fe$^{2+}$ or NO$_2^-$ is in excess, with reaction rates up to an order of magnitude lower if [NO$_2^-$] > [Fe$^{2+}$] compared to if [NO$_2^-$] < [Fe$^{2+}$] (Jones et al., 2015). To account for the potential dependence on relative NO$_2^-$ and Fe$^{2+}$ concentrations, we assign $k_{\text{NO}_2^-\text{Fe}^{2+}} ([\text{NO}_2^-] > [\text{Fe}^{2+}]) = 0.1$ $k_{\text{NO}_2^-\text{Fe}^{2+}} ([\text{NO}_2^-] < [\text{Fe}^{2+}])$. Further, The activation energy $E_A$ for NO$_2^-$ reduction by dissolved Fe$^{2+}$ has not been measured to our knowledge but is 18.4 kJ/mol for NO$_2^-$ reduction by mineralized Fe$^{2+}$ and is 70 kJ/mol for NO$_3^-$ reduction by dissolved Fe$^{2+}$ (Ottley et al., 1997; Samarkin et al., 2010). Mineralized Fe$^{2+}$ is a more effective reductant than dissolved Fe$^{2+}$, and NO$_2^-$ is more reactive than NO$_3^-$, suggesting $18.4 \leq E_A \leq 70$ kJ/mol. To ensure we do not underestimate the possible range of $k_{\text{NO}_2^-\text{Fe}^{2+}}$, we take $E_A = 70$ kJ/mol.

Combining these effects, in total we consider $k_{\text{NO}_2^-\text{Fe}^{2+}} = 2 \times 10^{-6}$ to $9 \times 10^{-2}$ M$^{-1}$s$^{-1}$ if [NO$_2^-$] < [Fe$^{2+}$] and $k_{\text{NO}_2^-\text{Fe}^{2+}} = 2 \times 10^{-7}$ to $9 \times 10^{-3}$ M$^{-1}$s$^{-1}$ if [NO$_2^-$] > [Fe$^{2+}$], corresponding to pH = 6.5–8 and $T = 0$–50 $^\circ$C.

### 2.2.4. Reduction of NO$_3^-$ by Fe$^{2+}$ to Nitrogenous Gas

Fe$^{2+}$ can reduce NO$_3^-$ to nitrogen gas, with proposed reactions (Postma, 1990; Samarkin et al., 2010):

$$2\text{NO}_3^- + 12\text{Fe}^{2+} + 11\text{H}_2\text{O} \rightarrow \text{N}_2\text{O}(g) + 4\text{Fe}_3\text{O}_4 + 22\text{H}^+ \quad (5)$$

$$2\text{NO}_3^- + 10\text{Fe}^{2+} + 14\text{H}_2\text{O} \rightarrow \text{N}_2(g) + 10\text{FeOOH} + 18\text{H}^+ \quad (6)$$

The kinetics of uncatalyzed reduction of NO$_3^-$ by Fe$^{2+}$ at room temperature are uncertain, because NO$_3^-$ reduction is very slow under these conditions and hence difficult to characterize in the laboratory. Ottley et al. (1997) reports the detection of uncatalyzed NO$_3^-$ reduction by Fe$^{2+}$ at room temperature over timescales of a week. However, Picardal (2012) report nondetections of NO$_3^-$ reduction by Fe$^{2+}$ in sterile incubations carried out under conditions and timescales similar to those of Ottley et al. (1997). As we are unable to favor one study above the other from available information, we consider a range of $k_{\text{NO}_3^-\text{Fe}^{2+}} = 0$–$9 \times 10^{-4}$ M$^{-1}$s$^{-1}$. The lower bound is derived from the reported nondetections of Picardal (2012). The upper bound is derived from the study of Ottley et al. (1997) and corresponds to pH = 8 and $T = 50^\circ$C, which should be the maximum rate possible over pH = 6.2–9 and $T = 0$–50 $^\circ$C (Petersen, 1979). We assume that the reduction of NO$_3^-$ by dissolved Fe$^{2+}$ is, like the reduction of NO$_2^-$, first-order with respect to both reactants, for a second-order reaction overall, motivated by the generally similar kinetics of NO$_2^-$ and NO$_3^-$ reduction by Fe(O), H$_2$, and mineralized Fe$^{2+}$ (Samarkin et al., 2010; Zhu &Getting, 2012). This range of $k_{\text{NO}_3^-\text{Fe}^{2+}}$ is very large; kinetic studies are required to constrain it.

### 2.2.5. Other Reactions of NO$_X^-$ with Fe$^{2+}$

Fe$^{2+}$ can also reduce NO$_2^-$ to NH$_3$, with proposed empirical reaction mechanism and rate law (Summers & Chang, 1993):

$$\text{NO}_2^- + 6\text{Fe}^{2+} + 7\text{H}^+ \rightarrow 6\text{Fe}^{3+} + 2\text{H}_2\text{O} + \text{NH}_3 \quad (7)$$

$$\frac{d[\text{NH}_3]}{dt} = k_7[\text{NO}_2^-][\text{Fe}^{2+}]^6(pH = 7.9) \quad (8)$$

This reaction should be a negligible sink on [NO$_2^-$] compared to Fe$^{2+}$ reduction of NO$_2^-$ to nitrogenous gas; to demonstrate this, we evaluate it for $k_7 = 4.2 \times 10^{-5}$ s$^{-1}$, corresponding to the maximum rate (pH = 7.6, $T = 40^\circ$C) measured by Summers and Chang (1993). UV photolysis and reduction by Fe$^{2+}$ dominate this process over the [NO$_2^-$] range we consider here (Figure 1).

Similarly, the anaerobic ammonium oxidation by NO$_2^-$ (anammox) to N$_2$ is negligible compared to other processes; to illustrate this, we compute its rate for [NH$_3$] = $6 \times 10^{-7}$ M and pH = 6.5, $T = 50^\circ$C, corresponding to conditions that maximize the reaction rate while conforming to the constraints (Kasting, 1982;
Figure 1. Kinetic loss rates for oceanic NO$_2^-$ (top) and NO$_3^-$ (bottom) for the processes summarized in Table 1, as a function of [NO$_2^-$] and [NO$_3^-$]. Also plotted are the extremal $\phi_{\text{NO}_X}$ calculated by Wong et al. (2017). The point at which the supply flux equals the destruction flux for a given process corresponds to the steady state concentration for that process; the largest destruction flux (leftmost curve at given $\phi_{\text{NO}_X}$) dominates the system. Note that NO$_3^-$ reduction by Fe$^{2+}$ cannot be used to set an upper limit on [NO$_3^-$] because we consider the possibility that $k_{\text{NO}_3^-\text{Fe}^{2+}} = 0$.

Krissansen-Totton, Arney, and Catling, 2018). We take the rate law from Nguyen et al. (2003) following Laneuville et al. (2018):

$$\text{NO}_2^- + \text{NH}_4^+ \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \quad (9)$$

$$\frac{d[N_2]}{dt} = A \exp\left(-E/RT\right)\text{[NH}_3\text{][HNO}_2\text{]}^2 \quad (10)$$

With $A = \exp(37.8) \text{ M}^{-2} \text{ s}^{-1} = 2.6 \times 10^{16} \text{ M}^{-2} \text{ s}^{-1}$ and $E = 65.7$ kJ/mol. At $T = 50^\circ\text{C}$, this corresponds to a rate constant of $k_9 = A \exp(-E/RT) = 6 \times 10^9 \text{ M}^{-2}\text{s}^{-1}$. UV photolysis and reduction by Fe$^{2+}$ dominate this process over the [NO$_2^-$] range we consider here (Figure 1).

We note that the results of Nguyen et al. (2003) were based on experiments with reactant concentrations $\geq 0.05\text{M}$. We assume this rate law to hold at lower concentrations as well; experimental studies are required to confirm this extrapolation. Our overall conclusions do not depend on these kinetics since these reactions are negligible compared to other processes.

2.3. Calculation of [NO$_X^-$] in Kinetic Steady State

We calculate the concentrations of NO$_2^-$ and NO$_3^-$ under the assumption of kinetic steady state, that is, that the loss rates of these molecules due to the destruction processes specified in section 2.2 and summarized in Table 1 equal their supply from the atmosphere (section 2.1).

To compare loss rates to the supply flux of NO$_X^-$ (cm$^{-2}$s$^{-1}$), we integrate over the water column, giving us a loss flux (cm$^{-2}$s$^{-1}$). We consider both ocean and pond environments, corresponding to different families of postulated prebiotic chemistries (e.g., Patel et al., 2015 vs. Shibuya et al., 2016). For the ocean, we
Table 1

Summary of NO\textsubscript{X} Loss Process Kinetics

<table>
<thead>
<tr>
<th>Process</th>
<th>Rate law</th>
<th>Rate constant</th>
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<tbody>
<tr>
<td>Hydrothermal vents</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_{\text{vents}}[NO\textsubscript{X}]]</td>
<td>(k_{\text{vents}} = 8 \times 10^{-17} - 1 \times 10^{-14} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(NO_2) UV photolysis</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_{\text{NO}_2, hv}[NO\textsubscript{X}]]</td>
<td>(k_{\text{NO}_2, hv} = 0.6 - 2.4 \times 10^{-6} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(NO_3) UV photolysis</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_{\text{NO}_3, hv}[NO\textsubscript{X}]]</td>
<td>(k_{\text{NO}_3, hv} = 1.1 - 4.6 \times 10^{-8} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(NO_2) reduction by Fe\textsuperscript{2+} to</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_{\text{NO}_2, Fe^{2+}}[Fe\textsuperscript{2+}][NO\textsubscript{X}]]</td>
<td>(k_{\text{NO}_2, Fe^{2+}} = 2 \times 10^{-6} - 9 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}, ([Fe\textsuperscript{2+}]&gt;[NO\textsubscript{2}]);)</td>
</tr>
<tr>
<td>(N_2, N_2O)</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_{\text{NO}_2, Fe^{2+}}[NO\textsubscript{X}][Fe\textsuperscript{2+}]]</td>
<td>(k_{\text{NO}_2, Fe^{2+}} = 0 - 9 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(NO_3) reduction by Fe\textsuperscript{2+} to</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_7[NO\textsubscript{3}][Fe\textsuperscript{2+}]\textsuperscript{1.8}]</td>
<td>(k_7 = 4.2 \times 10^{-5} \text{ M}^{-1.8} \text{ s}^{-1} (\text{Max.}))</td>
</tr>
<tr>
<td>(N_2, N_2O)</td>
<td>[\frac{d[NO\textsubscript{X}]}{dt} = k_7[NO\textsubscript{3}][Fe\textsuperscript{2+}]\textsuperscript{1.8}]</td>
<td>(k_7 = 4.2 \times 10^{-5} \text{ M}^{-1.8} \text{ s}^{-1} (\text{Max.}))</td>
</tr>
<tr>
<td>(NO_2) reduction by Fe\textsuperscript{2+} to</td>
<td>[\frac{d[NH_3]}{dt} = k_9[NH_3][HNO_2]\textsuperscript{2}]</td>
<td>(k_9 = 6 \times 10^{5} \text{ M}^{-2} \text{ s}^{-1} (T = 50\textdegree \text{C}))</td>
</tr>
</tbody>
</table>

Figure 2. Kinetic loss rates for pond NO\textsubscript{2} (top) and NO\textsubscript{3} (bottom) for the processes summarized in Table 1, as a function of [NO\textsubscript{2}] and [NO\textsubscript{3}]. Also plotted are the extremal \(\phi_{NO\textsubscript{2}}\) calculated by Wong et al. (2017), as well as these \(\phi_{NO\textsubscript{2}}\) scaled by 100 times to simulate a lake/pond with a large DR and fast drainage. The point at which the supply flux equals the destruction flux for a given process corresponds to the steady state concentration for that process; the largest destruction flux (leftmost curve at given \(\phi_{NO\textsubscript{2}}\)) dominates the system. Note that NO\textsubscript{3} reduction by Fe\textsuperscript{2+} cannot be used to set an upper limit on [NO\textsubscript{3}] because we consider the possibility that \(k_{\text{NO}_3, Fe^{2+}} = 0\). DR = drainage ratio.
Figure 3. Steady state concentrations of NO$_2^-$ and NO$_3^-$ as a function of atmospheric supply flux with UV photolysis and reduction by Fe$^{2+}$ to nitorgenous gas as the sinks, in the ocean and in a pond. The pond parameters ($d = 10$ cm, DR = 100) are favorable for NO$_X^-$ accumulation and hence should be considered an optimistic scenario. The horizontal dashed line demarcates micromolar concentrations, the putative boundary concentration for prebiotic relevance. The gray shaded area corresponds to the range of $\phi_{NO_X}$ calculated by Wong et al. (2017). DR = drainage ratio.

adopt a depth of $d_{\text{ocean}} = 3.8 \times 10^5$ cm, corresponding to the mean depth of the modern ocean (Rumble, 2017). Figure 1 presents the column-integrated destruction rates of oceanic NO$_2^-$ and NO$_3^-$ as functions of [NO$_2^-$] and [NO$_3^-$], as well as the range of plausible atmospheric supply rates from Wong et al. (2017). The point at which the supply flux equals the destruction flux for a given process corresponds to the steady state concentration for that process.

For pond environments, a broad range of depths is possible. Larger depths correspond to lower [NO$_X^-$], since more column is available over which NO$_X^-$ is destroyed (or, equivalently, input NO$_X^-$ flux is distributed over a larger column). To obtain an upper limit on plausible [NO$_X^-$], we choose $d_{\text{pond}} = 10$ cm, corresponding approximately to the summer depths of Don Juan Pond in Antarctica, which hosts millimolar abiotic NO$_X^-$ (Marion, 1997; Samarkin et al., 2010; Torii et al., 1977). Figure 2 presents the column-integrated destruction rate of pond NO$_2^-$ and NO$_3^-$ as a function of [NO$_2^-$] and [NO$_3^-$], as well as the range of plausible atmospheric supply rates from Wong et al. (2017). Pond catchment areas can be much larger than their surface areas, meaning that ponds can concentrate atmospherically delivered NO$_X^-$ if the drainage timescale is short enough that the NO$_X^-$ does not decay en route. The catchment area/surface area ratio is often termed the drainage ratio (DR). A study of catchment areas in southern England indicates mean DR = 14 for lakes and DR = 500 for ponds (Davies et al., 2008). We therefore also present the supply fluxes scaled by a factor of 100, to simulate the potential concentrating effects of high DR.
Figures 1 and 2 show that UV photolysis and reduction by Fe$^{2+}$ to nitrogenous gas are the dominant sinks on NO$_X^-$ in natural waters; at a given [NO$_X^-$], the loss rates due to these processes are higher than the others, including processing at vents. We calculate [NO$_X^-$] and [NO$_3^-$] in the ocean as a function of $\mu_{\text{pNO}_2}$ including UV photolysis and reduction by Fe$^{3+}$ as sinks and exploring the full range of reaction rate coefficients identified in Table 1. We assume the NO$_X^-$ is supplied as 80% NO$_3^-$ and 20% NO$_2^-$, following the experimental work of Summers and Khare (2007); however, our results are not strongly sensitive to this assumption. We repeat this calculation for the case of a shallow pond with high drainage ratio and fast drainage ($d = 10$ cm, DR = 100), corresponding to a highly favorable scenario for NO$_X^-$ accumulation. Hence, this should be considered an approximate upper bound on plausible NO$_X^-$ concentration. From this calculation, the upper bound on oceanic NO$_X^-$ is $< 10 \mu$M and typically $< 1 \mu$M across most of parameter space. Ponds with favorable drainage characteristics can accumulate much more NO$_X^-$ (Figure 3).

2.4. Sensitivity to pH

We have focused on pH $= 6.5$–8, motivated by the findings of Halevy and Bachan (2017) and Krissansen-Totton, Arney, and Catling (2018) that the early ocean was circumneutral. However, this finding is a model prediction. Additionally, lakes/ponds can be buffered by local factors to an even wider range of pH; on modern Earth, lake pH ranges from pH $< 1$ to pH $> 11$ (Grant & Jones, 2000; Löhrl et al., 2005). Here, we consider the sensitivity of our results to our assumption of circumneutral pH.

As measured by OH production, NO$_3^-$ photolysis rates vary by $ \leq 2x$ from pH $= 4–11$, and nitrate photolysis rates by $\leq 3x$ from pH $= 2–14$, suggesting this process should be insensitive to pH (Daniels et al., 1968; Zafiriou & Bonneau, 1987; Zellner et al., 1990). However, for water with low concentrations of OH scavengers, nitrite photolysis should be reduced, since the OH can react with the photolysis products to reform the nitrite (Zafiriou, 1974; Zafiriou & True, 1979). Carbonate and bicarbonate are efficient OH scavengers. Consequently, achieving low OH scavenger concentrations requires pH $< 6$, such that dissolved inorganic carbon is present primarily as CO$_2$ as opposed to bicarbonate or carbonate at higher pH. At such pH, NO$_2^-$ is unstable. Hence, our choice of photolysis rate constants are valid from pH $= 4–11$.

$k_{\text{NO}_2^-,\text{Fe}^{2+}}$ increases with pH for pH $= 6–8.5$ (Moraghan & Buresh, 1977; Sørensen & Thorling, 1991). For pH $< 6$, nitrite protonates and self-decomposes (Rây et al., 1917; Park & Lee, 1988). Brown and Drury (1967) report fast reduction of NO$_2^-$ by Fe$^{3+}$ in alkaline solution (Fanning, 2000; though the experimental temperature is unclear). Thus, pH $\approx 6$ probably represents a minimum on thermal loss of NO$_2^-$. 

3. Thermochemical Equilibrium

In this section, we examine the stability of NO$_3^-$ and NO$_2^-$ in the anoxic early Earth environment under the assumption of thermal equilibrium. The purpose of this analysis is to test the implicit assumption of previous work that these molecules are stable in prebiotic waters absent processing at vents. Whether or not thermal equilibrium is achieved depends upon kinetic considerations. However, while our understanding of nitrogen kinetics in prebiotic natural waters may be incomplete (since we do not have an anoxic, prebiotic Earth-analog atmosphere-ocean system to study to confirm we have identified all relevant reactions), our equilibrium analysis depends only on known thermodynamic parameters and hence is robust.

We consider the general speciation of nitrogen in a reducing atmosphere-ocean system as present on early Earth, with H$_2$ as our reductant (pH$_2$ $\geq 1 \times 10^{-3}$ bar on early Earth; Kasting, 2014). We balance the nitrogen-converting redox half reactions with H$_2$ oxidation and take this to physically represent the amount of “reducing power” in the environment, even though the system could obtain its reducing power from other half reactions, for example, Fe(II) oxidation. pN$_2$ on early Earth is known to have been comparable to present-day levels ($0.5 < \text{pN}_2 < 1.1$ bar, Marty et al., 2013). We consider an initial atmosphere-ocean system with atmospheric pN$_2$ $= 1.1$ bar, oceanic [N$_2$] in equilibrium with the atmosphere (i.e., saturated in N$_2$), and no other initial carrier of N. We assume an N$_2$-dominated atmosphere and ocean volume equal to modern. Then, the total inventory of nitrogen atoms $N_X$ in the atmosphere/ocean system is

$$N_X = 2 \times ([N_2]V_{\text{ocean}} + \frac{pN_2}{\mu g}(4\pi R_{\text{Earth}}^2))$$

(11)
Table 2
Henry’s Law Constants (H) Under Standard Conditions and Zero Salinity for the Species Considered in This Analysis in Aqueous Solution

<table>
<thead>
<tr>
<th>Species</th>
<th>H (M/bar)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNO₃</td>
<td>2.6 × 10⁶</td>
<td>Chameides (1984)</td>
</tr>
<tr>
<td>HNO₂</td>
<td>5 × 10¹</td>
<td>Chameides (1984)</td>
</tr>
<tr>
<td>NO</td>
<td>1.9 × 10⁻³</td>
<td>Schwartz and White (1981)</td>
</tr>
<tr>
<td>N₂O</td>
<td>2.4 × 10⁻²</td>
<td>R. Sander (2015)</td>
</tr>
<tr>
<td>N₂</td>
<td>6.4 × 10⁻⁴</td>
<td>R. Sander (2015)</td>
</tr>
<tr>
<td>NH₃</td>
<td>6 × 10¹</td>
<td>Kavanaugh and Trussell (1980)</td>
</tr>
<tr>
<td>O₂ (g)</td>
<td>1.3 × 10⁻³</td>
<td>R. Sander (2015)</td>
</tr>
<tr>
<td>H₂ (g)</td>
<td>7.8 × 10⁻⁴</td>
<td>R. Sander (2015)</td>
</tr>
</tbody>
</table>

Here, \( V_{\text{ocean}} = 1.4 \times 10^{21} \text{ L} \) is the volume of the ocean (Pierazzo & Chyba, 1999), \( \mu = 28 \text{ g mol}^{-1} \) is the mean molecular mass of the N₂-dominated atmosphere, and \( g = 981 \text{ cm s}^{-2} \) is the acceleration due to gravity. From Henry’s Law, \([N_2] = H_{N_2} p_{N_2}\), where \( H_{N_2} = 6.4 \times 10^{-4} \text{ Mbar}^{-1} \) (Table 2). Then, \( N_N = 4 \times 10^{20} \text{ mol} \), comparable to present atmospheric N (Johnson & Goldblatt, 2015).

We allow this N₂ to relax to equilibrium under a range of pH₂ and pH and calculate the speciation of nitrogen compounds at equilibrium. To do this, we consider the possible reactions between the nitrogen species by balancing the individual half reactions for interconverting nitrogen species with H₂ oxidation, and calculate cell potentials and logK for each reaction (see also supporting information Text S2). We identified the reactions of each species with H₂ with the largest logK; they are tabulated in Table 3. The Gibbs free energies of formation used in this study, \( \Delta G^{\circ}_{\text{rxn}} \), are compiled in Table 4.

We use these reactions to set up equations for concentrations at equilibrium using the definition of the equilibrium constant:

\[
K_1 = \frac{[N_2]}{[NO_3^-][H^+][H_2O]^3} 
\]

\[
K_2 = \frac{[N_2]}{[NO_2^-][H^+][H_2O]^3} 
\]

\[
K_3 = \frac{[NH_4^+]^2}{[NO][H^+][H_2O]^3} 
\]

\[
K_4 = \frac{[NH_4^+]^2}{[N_2O][H^+][H_2O]^3} 
\]

\[
K_5 = \frac{[NH_4^+]^2}{[N_2][H^+][H_2O]^3} 
\]
Table 4
Gibbs Free Energies of Formation Under Standard Conditions ($\Delta G^\circ_f$) for the Species Considered in This Work

<table>
<thead>
<tr>
<th>Species</th>
<th>$\Delta G^\circ_f$ (kJ/mol)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$ (aq)</td>
<td>−111.3</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>NO$_2^-$ (aq)</td>
<td>−32.2</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>NH$_4^+$ (aq)</td>
<td>−79.5</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>NO (g)</td>
<td>87.6</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>N$_2$O (g)</td>
<td>103.7</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>N$_2$ (g)</td>
<td>0</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>N$_2$ (aq)</td>
<td>18.8</td>
<td>Amend and Shock (2001)</td>
</tr>
<tr>
<td>O$_2$ (g)</td>
<td>0</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>O$_2$ (aq)</td>
<td>16.54</td>
<td>Amend and Shock (2001)</td>
</tr>
<tr>
<td>H$_2$ (g)</td>
<td>0</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>H$_2$ (aq)</td>
<td>17.72</td>
<td>Amend and Shock (2001)</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>−237.1</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>H$^+$</td>
<td>0</td>
<td>Rumble (2017)</td>
</tr>
</tbody>
</table>

NO$_3^-$, NO$_2^-$, and NH$_4^+$ undergo further acid/base equilibration, with partitioning governed by the reaction pKas (Table 5):

$$HA \rightarrow H^+ + A^- \quad (17)$$

$$K_a = \frac{[H^+][A^-]}{[HA]} = 10^{-pK_a} \quad (18)$$

where $A^-$ is the acid and HA its conjugate base.

Aqueous HNO$_3$, HNO$_2$, NO, N$_2$O, N$_2$, and NH$_3$ exist in equilibrium with their gaseous forms, with partitioning specified by Henry's Law (Table 5; Sander, 2015):

$$[X] = H_X pX \quad (19)$$

where $H_X$ is the Henry's Law constant for species $X$, and $pX$ its partial pressure.

Finally, we have the mass balance constraint that the sum of nitrogen atoms across all species equals the initial nitrogen inventory $N_N$:

$$N_N = ([NO_3^-] + [NO_2^-] + [NO] + 2[N_2O] + 2[N_2] + [NH_4^+] + [HNO_3] + [HNO_2] + [NH_3])V_{ocean} \quad (20)$$

$$+ \frac{pHNO_3 + pHNO_2 + pNO + 2pN_2O + 2pN_2 + pNH_3}{\mu g} (4\pi R_{Earth}^2) \quad (21)$$

Taken together, this system provides us with 15 equations, 15 unknowns ($[NO_3^-], [NO_2^-], [NO], [N_2O], [N_2], [NH_4^+], [HNO_3], [HNO_2], [NH_3],$ pHNO$_3$, pHNO$_2$, pNO, pN$_2$O, pN$_2$, pNH$_3$), and 3 prescribed conditions ([H$^+$], [H$_2$], $N_N$). We solve this system of equations for a range of pH ([H$^+$]) and reducing powers ([H$_2$]) for $N_N = 4 \times 10^{20}$ mol. We compute the fraction of atoms of N stored in each species (Figure 4).

Table 5
pKas for Relevant Dissociations of Acids/Bases in the Redox Network to Their Corresponding Conjugate Base/Acid

<table>
<thead>
<tr>
<th>Reaction</th>
<th>pKa</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNO$_3$ ↔ H$^+$ + NO$_3^-$</td>
<td>−1.38</td>
<td>Dean (1985)</td>
</tr>
<tr>
<td>HNO$_2$ ↔ H$^+$ + NO$_2^-$</td>
<td>3.25</td>
<td>Rumble (2017)</td>
</tr>
<tr>
<td>NH$_4^+$ ↔ H$^+$ + NH$_3$</td>
<td>9.25</td>
<td>Rumble (2017)</td>
</tr>
</tbody>
</table>
Figure 4 shows the speciation of nitrogen by redox state. The two oxidation states of nitrogen favored under plausible hydrogen concentrations are (−3) and (0), corresponding to NH₃/NH₄⁺ and N₂, respectively. N₂, which is favored at [H₂] ≤ 10⁶ M, will partition mostly into the atmosphere. The distribution of NH₃, NH₄ (aq), and NH₃ (g) depends on the pH of the aqueous solution. The concentration of NO₃ and NO₂ is subpicomolar over the scenarios considered here. These findings are insensitive to variations in pH, K, aK, and HX from T = 2–45 °C and variations in HX due to salinity from [NaCl] = 0–1 M; see supporting information Text S3 for details.

Overall, nitrate and nitrite are thermally unstable in the reducing conditions available on early Earth. If allowed to reach equilibrium, under most conditions NO₃ will relax to a gaseous species like N₂. This result is consistent with Van Cleemput and Baert (1978), who concluded that NO₃ in anoxic soils should decay, generally to N₂. The situation on reducing prebiotic Earth is very different from the situation on oxidizing modern Earth, where in equilibrium NO₃ is thermodynamically favored (Krissansen-Totton et al., 2016). Our thermochemical analysis confirms our kinetic analysis that previous work has overestimated prebiotic [NO₃⁻], increasing our confidence in this conclusion.

4. Discussion

4.1. [NO₃⁻] in the Prebiotic Oceans

Previous work has concluded that [NO₃⁻] in the prebiotic oceans was high, on the assumption that the dominant sink of NO₃ in the prebiotic ocean was processing at hydrothermal vents and that it was otherwise stable in the ocean (Laneuville et al., 2018; Wong et al., 2017). However, NO₃ is thermodynamically unstable in reducing environments. UV photolysis and reduction by Fe²⁺ are much stronger sinks than processing at vents and restrict NO₃ to orders of magnitude lower concentrations than previously suggested. Reduction by Fe²⁺ and UV photolysis suppresses NO₃ to submicromolar concentrations across most of the plausible parameter space. NO₃ at > 1μM requires ϕNO₃ > 1 × 10⁹ cm⁻² s⁻¹, higher than has been suggested in the literature. Achieving such high ϕNO₃ requires some combination of a high lightning flash rate, high pCO₂, and low pH/pCO₂. The required pCO₂ and pH are not favored by the available geochemical evidence, and we lack a robust prescription for global lightning flash rates, leading us to disfavor this possibility. Consequently, prebiotic oceanic NO₃ was likely submicromolar.

Wong et al. (2017) point out that Fitzsimmons et al. (2014) have detected dissolved iron thousands of kilometers from hydrothermal sources and suggest that the survival of this iron for such large distances on NO₃-rich modern Earth means that Fe³⁺ oxidation by NO₃ is ineffcient. However, Fitzsimmons et al. (2014) also point out that only 0.02–1% of hydrothermal Fe survives transport over these distances in the dissolved phase, meaning that the vast majority of hydrothermal Fe is oxidized. Moreover, the Fe that does avoid oxidation is thought to do so by forming colloids and/or by complexing with organic ligands (Fitzsimmons et al., 2014; Hawkes et al., 2013; Sander & Koschinsky, 2011; Taglabue, 2014). In other words, hydrothermal Fe appears to survive long-distance transport because it is protected by complexing, mineralization, and colloidation, not because its reactions with oxidants are inefficient. Additionally, mineralized Fe²⁺ is typically a more effective reductant than dissolved Fe³⁺ (Dhakal, 2013; Hansen et al., 1996; Sørensen & Thorling, 1991). We consequently argue that it is not possible to dismiss reduction by Fe²⁺ as a sink on NO₃, especially in light of evidence that Fe³⁺ levels were high on early Earth.

4.2. [NO₂⁻] in Prebiotic Ponds

As in the oceans, reduction by Fe²⁺ and photochemical loss are major sinks of NO₂⁻. However, since ponds are much shallower than oceans, the impact of thermal reactions is muted, and UV photolysis is proportionately more important.

[NO₂⁻] could have been above oceanic in shallow ponds (d ≤ 3 m) with large DR and short transit times. Shallow ponds permit higher NO₂⁻ buildup because NO₂⁻ destruction processes have a shorter column over which to operate. Large drainage ratios permit ponds to collect NO₂⁻ rainout from a larger area. Short
transit times minimize the probability that the NO$_X$ will decay en route due to encounters with reductants in the soil. For a pond with $d = 10$ cm, DR = 100, and fast drainage, [NO$_X$] can build up to micromolar concentrations for $\phi_{NO_X} \geq 4 \times 10^7$ cm$^{-2}$ s$^{-1}$ and at lower $\phi_{NO_X}$ if the pond is cold and acidic. For $\phi_{NO_X} = 6.5 \times 10^6$ cm$^{-2}$ s$^{-1}$, [NO$_X$] can build up to near-millimolar concentrations in such a lake.

We consider whether such a pond is plausible. A study of water bodies in southern England indicated that the ratio between the total catchment area and total surface area for lakes and ponds was 14 and 500, respectively, and a study of boreal lakes in Sweden found DR as high as 1,000, indicating DR = 100 to be plausible (Davies et al., 2008; Sobek et al., 2003). Assuming neutral, room-temperature groundwaters with [Fe$^{2+}$] $\leq 10^{-4}$ M, the lifetime of NO$_X$ is $\geq 400$ days, implying transit times $\leq 400$ days are required to ensure negligible decay of NO$_X$ during transport. Catchment transit times $\leq 400$ days exist, particularly for smaller catchments, but are not universal, indicating that only a subset of ponds will meet this criterion (Brosig et al., 2008; Broxton et al., 2009; McGuire & McDonnell, 2006; Rodhe et al., 1996). Further, if present, mineralized Fe$^{2+}$ in the ground may more efficiently reduce NO$_X$ (Dhakal, 2013). These challenges will be avoided in terrain where rain is immediately lost to the pond as surface runoff; this is especially likely to occur in catchments in bare, rocky terrain (Li et al., 2011). Ponds with high concentrations of NO$_X$ do exist on modern Earth, as predicted from our modeling; for example is Don Juan Pond, which is thought to be abiotic and which features [NO$_X$] = 6 mM (Samarkin et al., 2010). In summary, ponds with high [NO$_X$] should have existed on early Earth but were probably not typical; hence, pond prebiotic chemistries which require high NO$_X$ must specify such a pond as part of their scenario.

### 4.3. Implications for Prebiotic Chemistry

Oceanic NO$_X$ could only have achieved prebiotically relevant levels if atmospheric supply rates were very high. Achieving [NO$_X$] $\geq 1$ mM requires $\phi_{NO_X} \geq 1 \times 10^9$ cm$^{-2}$ s$^{-1}$, which requires some combination of high flash rates, high pCO$_2$, and low pN$_2$/pCO$_2$. These conditions are not at present favored in the literature (Marty et al., 2013; Krissansen-Totton et al., 2018; Wong et al., 2017). Consequently, oceanic NO$_X$-dependent origin-of-life scenarios (e.g., those that invoke NO$_X$ as electron acceptors at deep-sea hydrothermal vents; Ducluzeau et al., 2009; Nitschke & Russell, 2013; Shibuya et al., 2016) must invoke either extreme planetary parameters or local circumstances which can concentrate [NO$_X$] levels beyond the oceanic mean.

NO$_X$ could have achieved above-oceanic concentrations in favorable pond environments, that is, ponds with large DR and short catchment transit times. Low temperatures and acidic pH would also favor NO$_X$ buildup, especially as NO$_X^-$. [NO$_X$] could be even higher at polar latitudes where photolysis rates are suppressed by low UV surface radiances due to larger solar zenith angles (Ranjan & Sasselov, 2017). Such a pond would be able to sustain [NO$_X$] $\geq 1$ mM across most of the range of $\phi_{NO_X}$ calculated by Wong et al. (2017). Such ponds are plausible but not typical and hence must be explicitly invoked when considering NO$_X$-dependent pond prebiotic chemistries. Their nonuniversality must also be considered when estimating the plausibility of NO$_X$-dependent prebiotic chemistries.

NO$_X^-$ is orders of magnitude more stable than NO$_2^-$. This suggests that in both lake and oceanic environments, prebiotic NO$_X$ should have existed primarily as NO$_X^-$, as in natural waters on modern Earth and in experimental studies of abiotic nitrogen fixation (Summers & Khare, 2007). Consequently, NO$_2^-$-utilizing prebiotic chemistries are more plausible than NO$_X^-$-dependent prebiotic chemistries, and prebiotic chemists should consider using NO$_2^-$ instead of NO$_X^-$ in their studies.

In this work, we have focused on concentrations of NO$_2^-$ and NO$_X^-$, under the broad category of NO$_X^-$. We have ignored more complex derivatives of these compounds. For example, Mariani et al. (2018) point out that under UV irradiation, NO$_X^-$, Fe, and HCN combine to yield nitroprusside, a compound in which NO$_X^-$ is protected from reduction by Fe$^{2+}$ and which is stable in the dark on a timescale of $\geq 5$ months. However, nitroprusside is unstable to irradiation by the visible light which accompanies UV irradiation, though this may be due to the more rapid degradation of nitric oxide (NO) following photodissociation of nitroprusside in the oxygenic atmosphere of modern Earth (Schulz et al., 2010; Vesey & Batistoni, 1977; Van Loenen & Hof’s-Kemper, 1979; Wolfe & Swinehart, 1975). Measurements of the kinetics of nitroprusside formation and destruction under prebiotically relevant conditions are required to determine the range of plausible steady state concentrations of nitroprusside in prebiotic natural waters.

We note in passing that the prospects for abiotic NO$_X$ buildup may be enhanced on planets orbiting M-dwarfs, due to their much lower surface UV irradiation and consequently much slower NO$_X$ photolysis.
Consequently, NO\textsubscript{X} dependent prebiotic chemistries may proceed especially well on such worlds relative to early Earth.

### 4.4. Validity of Simplifying Assumptions

In this work, we have approximated the activity of ionic species (e.g., NO\textsubscript{X}, H\textsuperscript{+}) by their concentrations, neglecting the effects of ion-ion and ion-water interactions on their reactivity. For the species relevant to this work, the activity coefficient \( \gamma \leq 0.26 \) for solutions with ionic strengths \( I \leq 1 \) (supporting information Text S1). For context, the ionic strength of the modern oceans is \( I = 0.7 \), and studies of fluid inclusions in quartz suggests that Archean ocean salinity was \( \leq \) modern (Marty et al., 2018; Rumble, 2017). Our order-of-magnitude conclusions are insensitive to such variations, motivating this simplifying assumption.

Van Cleemput and Baert (1983) suggest that the kinetics of NO\textsubscript{X} reduction by Fe\textsuperscript{2+} are second order with respect to nitrite concentrations at acidic pH. We repeated our analysis assuming second-order dependence on \([\text{NO}_\text{X}]\); our conclusions were unchanged, indicating our analysis is insensitive to this possibility.

Our photolysis calculations assume photolysis rate constants equal to the modern value. While shortwave surface UV irradiation (200 – 300 nm) on anoxic early Earth was much higher than on modern Earth, surface UV irradiation over the full UV range (200 – 400 nm) was 20% lower on prebiotic Earth compared to modern Earth, suggesting we may slightly overestimate the photolysis rate (Ranjan & Sasselov, 2017). However, (1) our conclusions are robust to variations in photolysis rate of a few tens of percent, and (2) the magnitude of NO\textsubscript{X} photolysis is sensitive to the action spectrum of NO\textsubscript{X} photolysis; if shorter wavelengths are much more effective at photolyzing NO\textsubscript{X}, then our methods may underestimate photolysis rates (Cockell, 2000; Claire et al., 2012; Ranjan & Sasselov, 2016). Further measurements of the action spectrum of NO\textsubscript{X} photolysis are required to rule on this possibility.

Our photolysis calculations assume all NO\textsubscript{X} entering the ocean goes to NO\textsubscript{1} and neglects reactions of NO\textsubscript{X} with other reductants which may have been abundant on early Earth, such as H\textsubscript{2}, CH\textsubscript{4}, or Mn\textsuperscript{2+} (Fischer et al., 2016; Tian et al., 2005; Zahnle et al., 2018). Consequently, our estimates should be considered upper bounds on prebiotic \([\text{NO}_\text{X}]\).

This box model approach we have taken averages over the entire natural water body under consideration. This approach permits us to place bounds on the mean concentrations of NO\textsubscript{X} in prebiotic natural waters with minimal assumptions and is in line with past work (e.g., Laneuville et al., 2018; Wong et al., 2017). This approach is a good approximation to well-mixed shallow lakes and ponds. However, the oceans are not necessarily well mixed; \([\text{NO}_\text{X}]\) may be a function of depth. Resolved 1-D models are required to probe this effect; mean oceanic concentration should be similar, but NO\textsubscript{X} concentrations should be higher at the surface where it is supplied and lower at depth. In summary, our approach suffices for NO\textsubscript{X} estimates in ponds, and for estimates of the mean NO\textsubscript{X} concentration in the ocean, but resolving the heterogeneity of the ocean requires higher-dimensional models.

### 4.5. Importance of Better Kinetic Constraints

Measurements of NO\textsubscript{X} kinetics under conditions relevant to the early Earth are scarce. While our calculations are motivated by and consistent with available data, improved measurements of these kinetics can decrease the uncertainty in these calculations and improve the confidence of these results. In particular, (1) the literature contains contradictory reports as to whether uncatalyzed NO\textsubscript{X} reduction by Fe\textsuperscript{2+} is significant at room temperature (Ottley et al., 1997; Picardal, 2012). Experimental studies are required to resolve the dichotomy between these studies; if this process is indeed significant, as Ottley et al. (1997) report, then oceanic NO\textsubscript{X} concentrations would be suppressed to concentrations lower than we model here. (2) The activation energy for reduction of NO\textsubscript{X} by Fe\textsuperscript{2+} is not known; knowledge of this quantity would enable tighter constraints on \( k_{\text{NO}_\text{X};\text{Fe}^2+} \). (3) The rate constants for Fe\textsuperscript{2+} reduction used in this work are generally derived from measurements made at large [Fe\textsuperscript{2+}] than thought to have been available on early Earth. Determination of these rate constants at prebiotically plausible [Fe\textsuperscript{2+}] (10–600 \( \mu \)M) under early Earth conditions (e.g., anoxia) could confirm the applicability of these rate constants at lower [Fe\textsuperscript{2+}]. The extension of studies like Stanton et al. (2018) for NO kinematics to NO\textsubscript{X} kinematics could improve the precision and potentially accuracy of this work. (4) Measuring the rate constant of NO\textsubscript{X} photolysis in simulated prebiotic natural waters, under irradiation by a source simulating the prebiotic UV environment in both magnitude and spectral shape, could directly verify our extrapolation from modern photolysis rates, and in particular could confirm whether the shorter-wavelength UV radiation available on early Earth would affect overall reaction rates.
5. Conclusions

Constraining the abundance of trace chemical species on early Earth is relevant to understanding the plausibility and guiding the development of proposed prebiotic chemistries. In this work, we have used box model kinetic calculations to constrain the plausible range of $\text{NO}_2^-$ and $\text{NO}_3^-$ concentrations in oceans and ponds on prebiotic Earth.

Prebiotic oceanic $\text{NO}_2^-$ was likely much lower than calculated in previous work (Laneuville et al., 2018; Wong et al., 2017) due to UV photolysis and reactions with Fe$^{2+}$. Oceanic $\text{NO}_2^-$ could only have built up to $\geq 1 \mu$M in an extremal realm of parameter space, in particular if the $\text{NO}_2^-$ supply flux was much higher than currently favored in the literature. Consequently, origins-of-life scenarios which require elevated $\text{NO}_2^-$ in the ocean must invoke either an extremal planetary conditions or specialized local conditions which concentrate $\text{NO}_2^-$ above the oceanic mean. $\text{NO}_3^-$ was not an inevitable part of the prebiotic milieu, and the prebiotic plausibility of oceanic origin-of-life scenarios can be improved by utilizing alternative feedstocks, for example, an alternative electron donor for protometabolism (Duchelleau et al., 2009).

Prebiotic $\text{NO}_2^-$ could have built up above oceanic levels in shallow ponds with large, fast-draining catchment areas. Such environments should have been extant but likely uncommon. In these environments, $\text{NO}_2^-$ could have built up to prebiotically relevant levels ($\geq 1 \mu$M) over a much broader range of planetary parameters than in the ocean and in particular over most (but not all) of the proposed range of $\text{NO}_3^-$ supply flux. Consequently, $\text{NO}_2^-$ dependent prebiotic chemistries which can function in shallow ponds (e.g., Mariani et al., 2018) are prebiotically plausible, with the caveat that they impose requirements on the environment. Near-millimolar $\text{NO}_2^-$ concentrations are possible if the $\text{NO}_2^-$ supply flux was at the upper end of what has been proposed in the literature ($\phi_{\text{NO}_2^-} \geq 6.5 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1}$) and if the pond were cool and mildly acidic. This finding is in line with past work which suggests shallow lakes/ponds to be especially compelling venues for origin-of-life chemistry (Deamer & Damer, 2012; Mulikdjanian et al., 2012; Patel et al., 2015; Ranjan et al., 2018). We emphasize that our estimates are upper bounds; if a significant fraction of input $\text{NO}_2^-$ failed to go to $\text{NO}_3^-$ or reactions with other reductants present on early Earth were significant compared to the processes considered here, $[\text{NO}_2^-]$ would have been proportionately lower.

For both oceanic and pond environments, $\text{NO}_2^-$ dependent prebiotic chemistries that can function at lower $[\text{NO}_2^-]$ are more prebiotically plausible. Similarly, prebiotic chemistries that utilize $\text{NO}_3^-$ are more plausible than those which utilize $\text{NO}_2^-$, since most $\text{NO}_2^-$ should be present as $\text{NO}_3^-$ due to its greater stability.

Our analysis could be most improved by better characterization of $\text{NO}_2^-$ reaction kinetics under prebiotically relevant conditions, especially its reduction by Fe$^{2+}$ and Mn$^{2+}$ and its reduction by UV in prebiotic natural waters (e.g., the extension of studies like Stanton et al., 2018, to $\text{NO}_2^-$). Studies with higher-dimensional models could also help determine if there exist areas in the ocean which should have concentrated $\text{NO}_2^-$ above the oceanic mean, perhaps to prebiotically relevant levels.

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