# A Mathematic Approach to Nitrogen Fixation Through Earth History

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**Abstract** Nitrogen is essential for life as we know it. According to phylogenetic studies, all organisms capable of fixing nitrogen are prokaryotes, both bacteria and archaea, suggesting that nitrogen fixation and ammonium assimilation were metabolic features of the Last Universal Common Ancestor of all organisms. At present time the amount of biologically fixed nitrogen is around $2 \times 10^{13}$ g/year (Falkowski 1997), an amount much larger than the corresponding to the nitrogen fixed abiotically (between $2.6 \times 10^{9}$ and $3 \times 10^{11}$ g/year) (Navarro-González et al. 2001). The current amount of nitrogen fixed is much higher than it was on Earth before the Cambrian explosion, where the symbiotic associations with *leguminous* plants, the major nitrogen fixer currently, did not exist and nitrogen was fixed only by free-living organisms as cyanobacteria. It has been suggested (Navarro-González et al. 2001) that abiotic sources of nitrogen fixation during Early Earth times could have an important role triggering a selection pressure favoring the evolution of nitrogenase and an increase in the nitrogen fixation rate. In this study we present briefly a method to analyze the amount of fixed nitrogen, both biotic and abiotic, through Earth's history.

## Introduction

Nitrogen is an essential component of all living organisms on Earth (Fig. 3.1). It is a basic component of aminoacids and nucleobases, and therefore of proteins and nucleic acids. In the human body, the quantity of nitrogen in our cells is about a 3%, i.e. around 1.860 Kg of the average mass. In prokariotic cells, present nowadays in a quantity of 4–6 $\times 10^{30}$ cells, i.e., around 85–130 $\times 10^{30}$ g of Nitrogen (Whitman

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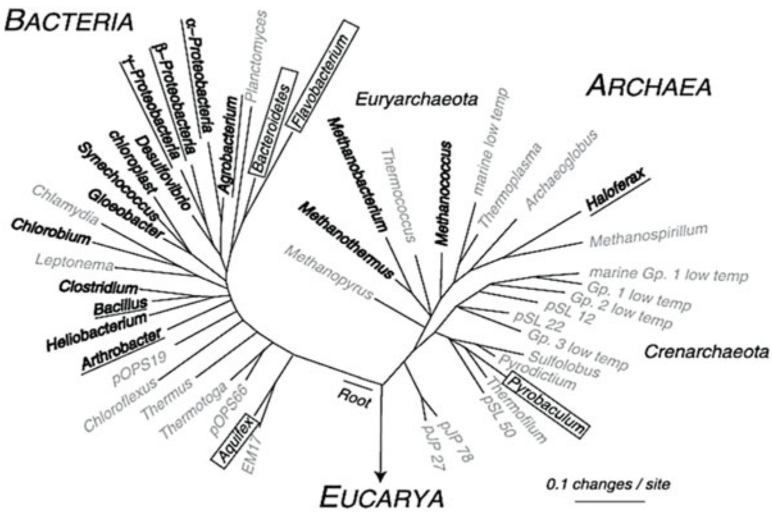


**Fig. 3.1** Phylogenetic tree of the three domains of life (*Bacteria*, *Archaea*, and *Eucarya*) (Adapted from Barns et al. (1996)). Members of the *Eucarya* are omitted for clarity. The genera listed are representative of major lineages of the two domains

et al. 1998), more than a half of the biomass. That this is an outstanding number can be easly seen when we compare it with the total amount of nitrogen content in the atmosphere ($1.3 \times 10^{16}$ g (Ehrlich 1996)).

Nitrogen is not a basic component of the Lithosphere, (although it can be found on clay minerals as $NH_4^+$ occuping the interlayer place of $K^+$), and then can be assumed that the source of the nitrogen incorporated to the biomass is the atmosphere. A relevant fact is that nitrogen in its molecular stable form, $N_2$, cannot be used generally as nutrient. This is because the strong triple bond between the N atoms of $N_2$ molecule makes it relatively inert. In fact, in order for plants and animals to be able to use nitrogen, $N_2$ gas must first be converted to more a chemically available form such as ammonium ($NH_4^+$), nitrate ($NO_3^-$), or organic nitrogen (e.g.urea – $(NH_2)_2CO$). The inert nature of $N_2$ means that biologically available nitrogen is often in short supply in natural ecosystems, limiting plant growth and biomass accumulation. This need to convert $N_2$ before beeing incorporated to organisms is an important difference with respect to other elements like Carbon (C) or Hydrogen (H).

If nitrogen atoms incorporated in living organisms have their origin in Earth's atmosphere then the atmosphere must be different now from the one present before the origin of life. Nowadays Nitrogen is the main compound in the Earth's atmosphere with a 78.1%, and current models of the composition of Early Earth's atmosphere conclude that nitrogen abundance was more than 98% (Kasting 1993). In this paper we present a simple method to estimate the effects of life in the fixation and uptaken of nitrogen from Earth atmosphere through history.

## Nitrogen Fixation

Nitrogen fixation is the process wherein $N_2$ is converted to ammonium. It is essential because it is the only way that organisms attain nitrogen directly from the atmosphere. It is not a static process, because although there are abiotic methods (lightning) and biotic (nitrogen fixers) to fix nitrogen to the ground, there are several processes that give back nitrogen to the atmosphere.

We describe abiotic and biotic nitrogen fixation briefly:

– *Abiotic nitrogen Fixation*

High-energy natural events such as lightning, forest fires, and even hot lava flows can cause the fixation of smaller, but significant amounts of nitrogen. The high energy of these natural phenomena can break the triple bonds of $N_2$ molecules, thereby making individual N atoms available for chemical transformation.

In particular the enormous energy of lightning breaks nitrogen molecules and enables their atoms to combine with oxygen in the air forming nitrogen oxides. These oxides dissolve in rain, forming nitrates, that are carried to the surface. Lightning plays a minor part in the fixation of atmospheric nitrogen. Atmospheric nitrogen fixation probably contributes some 5–8% of the total nitrogen fixed. Nevertheless in the early stages of emergence of life its role could have been very important (Navarro-González et al. 2001).

In the abiotic fixation, $N_2$ would have been oxidized with $CO_2$ by lightning:

$$N_2 + 2CO_2 \; -> 2NO + 2CO$$

After this process, NO then gets converted to soluble nitrosyl hydride (HNO). An important point is that the fixed nitrogen depends on the abundance of $CO_2$. According to Navarro-González et al. (2001), for $CO_2$ mixing ratios from 0.04 to 0.5, the rate of fixation would be $2.6 \times 10^9$ to $3 \times 10^{11}$ gN year $^{-1}$(Catling and Kasting 2007), and references there in Hill et al. (1980).

In the Ocean, dissolved $N_2$ would have been converted into $NO_3^-$ and $NO_2^-$, according to Mancinelli and McKay (1998). However, the cycle of nitrogen also works in the Ocean, and ferrous iron $Fe^{2+}$ can reduce the dissolved forms to produce ammonia, $NH_3$, some of which would flux to the atmosphere, where it would be photolyzed to $N_2$ and $H_2$.

Another way that nitrogen could have been fixed abiotically was through HCN synthesis in atmospheres containing trace levels of $CH_4$.HCN is hydrolyzed in solution to form ammonium, $NH_4^+$.

– *Biological Nitrogen Fixation*

Between living beings the ability to fix nitrogen is found only in certain bacteria and archaea:

– Certain bacteria, for example those among the genus *Rhizobium*, are the only organisms that fix nitrogen through metabolic processes.

- Some live in a symbiotic relationship with plants of the legume family (e.g., soybeans, alfalfa). Symbiotic nitrogen fixation occurs in plants that harbor nitrogen-fixing bacteria within their tissues. Each of these is able to survive independently (soil nitrates must then be available to the legume), but life together is clearly beneficial to both. In this relationship, nitrogen fixing bacteria inhabit legume root nodules and receive carbohydrates and a favorable environment from their host plant in exchange for some of the nitrogen they fix. Only together can nitrogen fixation take place.
- Some establish symbiotic relationships with plants other than legumes (e.g., alders).
- Some establish symbiotic relationships with animals, e.g., termites and "shipworms" (wood-eating bivalves).
- Some nitrogen-fixing bacteria live free in the soil.
- Nitrogen-fixing cyanobacteria are essential to maintaining the fertility of semi-aquatic environments like rice paddies. In aquatic environments, blue-green algae (really a bacteria called cyanobacteria) is an important free-living nitrogen fixer.
- Within the last century, human activities have become as important a source of fixed nitrogen equivalent to all natural sources combined. Burning fossil fuels, using synthetic nitrogen fertilizers, and cultivation of legumes all fix nitrogen. Through these activities, humans have more than doubled the amount of fixed nitrogen that is pumped into the biosphere every year

In order to model the amount of nitrogen taken from the atmosphere and biologically reduced we need to know the population of bacteria at each time since life appeared on Earth.

## Nitrogen Cycle

The nitrogen cycle is the set of biogeochemical processes by which nitrogen undergoes chemical reactions, changes form, and moves through difference reservoirs on earth, including living organisms. The nitrogen cycle has changed through Earth's history (Fig. 3.2). For example it is believed that during the Archean there was no-denitrification, which means that all the nitrogen fixed remained in that way. After that period of time, denitrification is a fact and nitrogen cycle started, coupled to other biochemical cycles such as Carbon cycle and Phosphate cycle.

In this work, we assume a rate of denitrification about 11% (Do-Hee Kim et al. 1997). This value is similar to the denitrification rate at present day, knowing that, although is very probable that during that time the denitrification rate was smaller, we are trying to estimate the minimum value of the nitrogen fixed. Also, the organic nitrogen formed in the atmosphere is photodissociated with a rate of 25% (Kasting and Walker 1981).

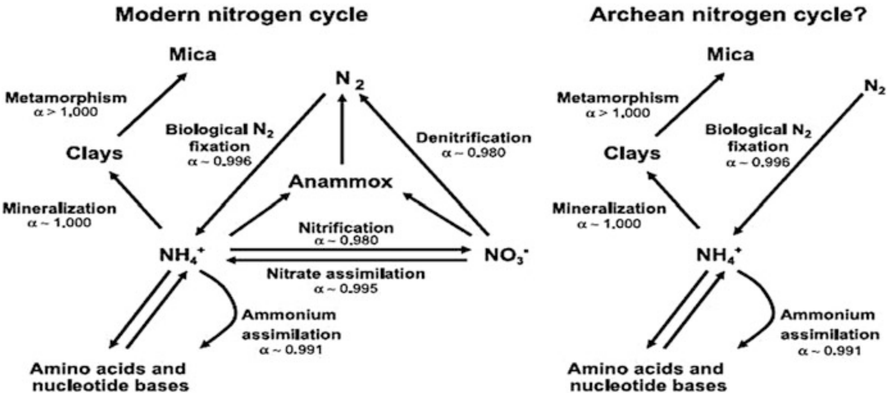


**Fig. 3.2** *Left*: A simplified modern nitrogen cycle with known fraction effects for the nitrogenous reaction product. *Right*: The proposed nitrogen cycle operating during the Archean (From Papineau et al. (2005))

## The Population of Bacteria

It is usually attributed to Malthus (1798) the merit to propose the first model for population growth based on a geometrical equation. Its mathematical expression is an exponential dependent of one parameter, $\mu$, called the growth factor:

$$\mathbf{P} = \mathbf{P}_0 \cdot \mathbf{e}^{\mu t}$$

Other more sophisticated models of growth have been proposed in the past, as for example the logistical model proposed by Verhust (1838), dependent on three-parameters: $k$ (maximum of the population), $\mu$ (the growth constant), and $\tau$ (the instant in which the population is k/2):

$$\mathrm{P} = \mathrm{k}/[1 + \mathrm{e} - \mu(\mathrm{t} - \tau)]$$

More detailed formulations of the population growth have been done increasing the number of parameters of the equation. Independently of the model, the fundamental parameter that determine the population at one instant of time will be the growth constant, $\mu$.

The population growth depends mainly on temperature and nutrients (Ratkowsky et al. 1982; Savage et al. 2004). During the glaciations periods for instance, the growth constant was near zero due to the fact that most known bacteria need a range of temperature very limited, and if it goes below 0°C, it cannot grow properly. Also, the availability of nutrients is a very big limitation for the population of bacteria, nitrogen and carbon being the main compounds affecting the growth constant (Demoling and Figueroa 2007).

In this work, the influence of these parameters is not taken into account directly but it is actually included in the formulation, as we have taken stromatolites as a reference, and the natural conditions at any time are having influence in the number of cells and therefore in the growth constant we obtain from the fit.

## Stromatolites as a Test for the Model

The arrow of time goes in one unique direction and it is not possible to have direct proof of the validity of a model estimating the evolution. A source of information from the past that can help us to test our model is the fossile record, particulary stromatolites. Stromatolites are the fossiles that bacteria and archea left behind after their dead. This fossilized microbial mat changes their abundance with time as showed in Table 3.1. Although the different opinions about the appearance of metazoa and the decline of stromatolites are well explained, there seems to exist in fact a relation, due to the change of sediments by metazoa and the possible fossilization of microbial mats associated to it (Awramik 1984; Walter and Heys 1985).

Although stromatolites are not only composed of cyanobacteria but other organisms such as Planctomycetes, Proteobacteria or Archea, and their abundance is only about 5% of the total number of cells (Papineau et al. 2005), it can be used as a marker for estimating the cyanobacteria growth factor. We understand that this is a good assumption, even when the % of cyanobacteria is different from one fossil to another.

Taking into account the amount of stromatolites, we can fit the data to an exponential function since 3,500 Myr until approximately 0.600 Ma (Fig. 3.3), the time in which the Cambrian explosion modified the fossil record. The parameters of this fit are:

$$\mu = 2.1319 + / - 0.06374(2.99\,\%)$$

and the data are plotted in Fig. 3.4.

**Table 3.1** Abundance of stromatolites classified as function of geological epoch (Adapted from Walter and Heys (1985))

| Boundary | Interval | Duration (Ga) | Abundance |
|---|---|---|---|
| 0.5 | Cambrian | 0.07 | 86 |
| 0.57 | Vendian | 0.11 | 140 |
| 0.68 | Late Riphean | 0.38 | 357 |
| 1.05 | Middle Riphean | 0.3 | 274 |
| 1.35 | Early Riphean | 0.3 | 170 |
| 1.65 | Late Proterozoic | 0.55 | 153 |
| 2.2 | Early Proterozoic | 0.3 | 8 |
| 2.5 | Archean | | |

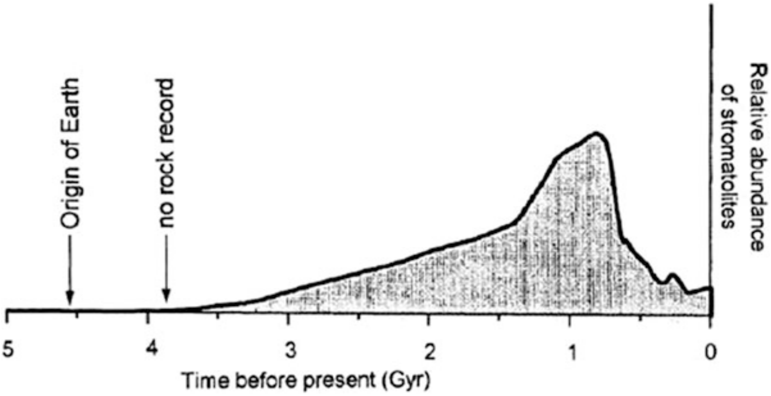


**Fig. 3.3** Relative abundance of stromatolites (Adapted from "The Ecology of Cyanobacteria; Their diveristy in time and space", Whitton and Potts (2002))

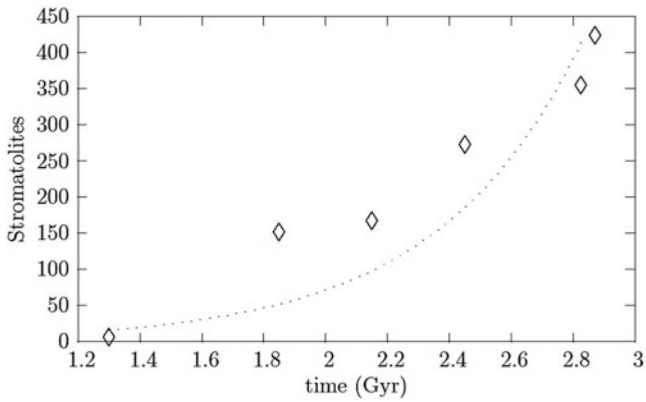


**Fig. 3.4** Exponential fit for stromatolites in time

## Summary and Conclusions

We have developed a very simple model to estimate the effect of nitrogen fixation and uptaken from life in the abundance of atmospheric nitrogen through Earth's history.

Although the first cyanobacteria fossiles found in stromatolites are dated back from 3.5 Gyr ago there are evidences for the existence of life on Earth before 3.8 Gry ago based on Carbon isotopes studies (Mojzsis et al. 1996), although this is still debated. Assuming that only one living cell was present at that time, we have modelled the evolution of a population of cells based on a realistic value for the growth constant from stromatolites and estimated the amount of cells during the evolution of Earth till the Cambrian Explosion. This calculation provides a

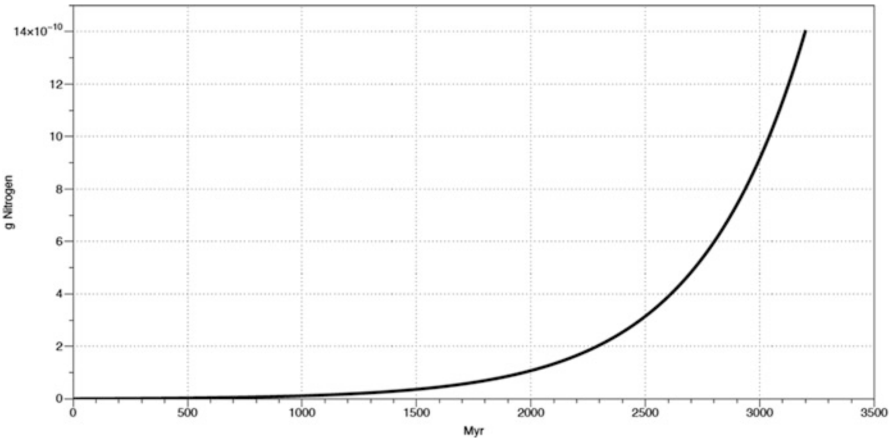


**Fig. 3.5** Biological nitrogen fixed since the origin of life until the Cambrian explosion

minimum value for the number of cells present at each instant of time and, with that value, it is possible to estimate the biological fixation during the Precambrian epoch. Therefore, this is not a very precise model for the estimation of biological fixation, but it is useful to understand the relative importance of lighting and abiotical fixation with respect to the biological fixation.

From the data showed in Table 3.1 and Fig. 3.5 we conclude that the nitrogen biological fixed at the time of the Cambrian explosion was about $1.4 \times 10^{-9}$ g

Considering that 3% of a cell is nitrogen, and an average cell weight is about $10^{-12}$ g, the amount on nitrogen present in a cell is $3 \times 10^{-14}$ g. By the time of the cambrian explosion, our model provides a value for the number of cells around 900,000 cells, which means $2.7 \times 10^{-8}$ g of nitrogen needed to maintain the population.

This estimated number of cells needs more biological nitrogen than the amount they can fix, so this result indicates that an additional source of abiotic nitrogen fixation was needed during the Precambrian to substain this amount of living systems.

This additional source could be, for example lightning, as proposed by Navarro-González et al. (2001). According to these authors, lightning could be a source of nitrogen fixation of about $10^{12}$ g N year$^{-1}$, which means that can be fixed abiotically in a year more than bioticaly in all the Precambrian.

Nevertheless, today the biological fixation is many times bigger than the abiotical one, and this is not because the number of cells increased, but because of the associations between different organisms. A symbiotic association of leguminous can fix nitrogen thousand times more efficiently than a free-living organism.

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