Global Implications of the Nitrogen Cycle

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Edited by

Trelita de Sousa

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Dedicated with love to my beautiful parents,
Colville and Maria Helena de Sousa

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FOREWORD

I was truly excited to learn that Dr. Trelita de Sousa had undertaken this task of bringing together a book on the nitrogen cycle. I have known Trelita since 2003 as her Professor when she was pursuing her Master's Degree in Microbiology at Goa University. As long as I can remember, she has been very passionate about the nitrogen cycle. Her profound interest led her to obtain a Ph.D. in Microbiology from Goa University for her contribution, "Response of denitrifying bacteria from coastal/estuarine ecosystems to hydrocarbons". She has also worked as an Erasmus Mundus Interweave Post-Doctoral Fellow at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal. Her dedicated research has transpired into worthy publications in reputed journals like Bioresource Technology and Chemosphere. After gathering worldwide experience over the years through her associations with the Universities of Goa (India), Porto (Portugal), and Washington (Seattle, U.S.A.), she continues to inspire and motivate young minds as an Assistant Professor at St. Xavier's College, Goa. This book is a small yet steady step that has the potential to make a great and lasting impact and is sure to leave a trail to follow.

Nitrogen makes up 78% of the Earth's atmosphere and is a crucial and essential component of life and its processes. Ironically, as much as it is the most abundant element in nature, it is often found in short supply and, therefore, poses as a limiting nutrient in most ecosystems. It is this very characteristic that enables its involvement with other cycles and administers its role in the regulation of marine productivity and the global climate. This book captures the intricate role of microorganisms in the various nitrogen transformations, the interaction of nitrogen with other biogeochemical cycles, and the impact of anthropogenic interference. Distinguished scientists and professors have offered their expert reviews on various aspects concerning the global nitrogen cycle thus, affording a comprehensive and contemporary overview of this increasingly significant area.

This book will, undoubtedly, be a valuable guide to every microbiologist, ecologist, marine environmentalist, and geologist, satiating the interest of graduates, post-graduates, Ph.D. students, researchers, and experienced professors and scientists alike. Above all, it is sure to provide a comprehensive representation of the nitrogen cycle and stimulate future work on its understanding, development, and application. The authors, editor, and publishers are to be applauded for an impressive effort and I wish them tremendous success and international acclaim. I hope that this is only the beginning of many more such undertakings!

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PREFACE

The nitrogen cycle is intimately associated with microbes making it the most intricate and fascinating among all the biogeochemical cycles. The dynamic cycle also inevitably exerts a significant influence on other nutrient cycles, in particular, carbon, sulphur, phosphorus, and iron, thereby occupying a central role in marine (both deep sea and coastal) and terrestrial biogeochemistry, biological productivity, and climate change.

Human interference has caused colossal perturbations to the global nitrogen cycle. Excessive anthropogenic activities amplified over several decades including agriculture, industrial nitrogen fixation, and the combustion of fossil fuels, have yielded an incredible amount of fixed nitrogen which gets exported to the ocean via rivers and atmospheric changes where its fate is still obscure. The erratic changes in the cycle favoured by prevailing oxygen fluxes in the ocean accelerate processes like nitrification, dissimilatory nitrate reduction, and denitrification and consequently, the emission of nitrous oxide, which is a powerful greenhouse gas. The massive acceleration of the global nitrogen cycle has induced critical impacts in the expansive oceanic habitats with ensuing influences on the primary productivity and carbon dynamics while contributing to eutrophication, smog formation, acid rain, global warming, and climate change.

This book is designed to improve our conceptual understanding of the highly interwoven nitrogen cycle and its ecological implications. The book is broadly categorised into three main sections. *Section I* (*The Microbial Transformation of Nitrogen*) renders an overview of the nitrogen cycle and formulates a critical review on each of its processes. *Section II (Implications of the Nitrogen Cycle on Primary Productivity and Biogeochemistry*) emphasises the importance of the nitrogen cycle in the environment and sheds light on the interaction of the nitrogen cycle with the other biogeochemical cycles. *Section III* (*Implications of Human Interference on the Nitrogen Cycle*) provides a detailed account of the human-induced acceleration of the nitrogen cycle and highlights the dynamic swings, ecological effects, and global repercussions.

I deeply appreciate the significant contributions of all the authors. I'm grateful to Dr. Saroj Bhosle not only for agreeing to pen down the foreword but also for her constant support. I wish to thank the entire team at Cambridge Scholars Publishing for helping me with this Herculean task! A special thanks to David Sleeman for offering this opportunity to me in the first place. This book would never have seen the light of day if it were not for the unconditional support and encouragement rendered by my dear family. A big thank you! Finally, I wish the readers an enriching experience!

SECTION I:

THE MICROBIAL TRANSFORMATION OF NITROGEN

CHAPTER 1

AN OVERVIEW OF THE MICROBIAL TRANSFORMATION OF NITROGEN

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Abstract

The cycling of nitrogen represents one of the most important nutrient cycles. In its elemental form, nitrogen makes up 78% of the atmospheric gases. The conversion of atmospheric nitrogen to a more usable form occurs via both physical and biological nitrogen fixation processes. Once nitrogen enters the biological system, it undergoes a series of oxidation and reduction reactions, under both aerobic and anaerobic conditions. These redox transformations are mediated by diverse groups of microorganisms and include nitrogen fixation, ammonification, nitrification, assimilatory nitrate reduction to ammonium, dissimilatory nitrate reduction to ammonium, denitrification, and anaerobic ammonium oxidation. This chapter provides an overview of these processes.

Introduction

Nitrogen is an essential nutrient for all living beings and is found in amino acids, proteins, nucleic acids, and chlorophyll (Francis et al. 2007, 19; Bernhard 2010, 1; Markov 2012, 347). Nitrogen constitutes the fourth most abundant element of the cellular biomass (Stein and Klotz 2016, R94). On average, it accounts for up to 6.25% of the dry cell biomass (Richardson et al. 2009, 389). The significance of nitrogen in cellular life is indisputable (Stein and Klotz 2016, R94). Nitrogen often limits growth in aquatic and terrestrial ecosystems and, in doing so, regulates biological productivity (Ward and Jensen 2014, 5; Roland et al. 2017, 1).

Nitrogen is found in a multitude of oxidation states. Compounds of nitrogen serve as electron acceptors and/or electron donors in many catabolic pathways (Simon and Klotz 2013, 114). The versatile redox chemistry of nitrogen is, in fact, the reason for its complex biogeochemistry (Ward and Jensen 2014, 5). The transformation to the different chemical forms of nitrogen through various biological, physical and geological processes constitutes the highly intricate nitrogen cycle (Thamdrup and Dalsgaard 2002, 1312; Markov 2012, 347). These redox reactions of the nitrogen cycle are mediated by different microorganisms via nitrogen fixation, ammonification, nitrification (including comammox), assimilatory nitrate reduction to ammonium (ANRA), dissimilatory nitrate reduction to ammonium (DNRA), denitrification, and anaerobic ammonium oxidation (anammox) (Fig. 1-1). Ammonification or the synthesis of ammonia (NH_3) refers to the process by which living organisms make NH_3 or ammonium ions (NH_4^+) (Markov 2012, 348). Ammonium can be produced via three microbial pathways: nitrogen fixation, ANRA, and DNRA. In nitrogen fixation (Fig. 1-1), dinitrogen (N_2) from the atmosphere (the main reservoir of molecular nitrogen on Earth) is reduced to NH_4^+ (Stein and Klotz 2016, R94). All life forms on earth depend on the process of nitrogen fixation for their nitrogen requirement (Markov 2012, 348). Ammonium, produced by nitrogen fixation, is either assimilated into biomass by autotrophs (Markov 2012, 348; Stein and Klotz 2016, R94) or is respired by aerobic or anaerobic NH_3-oxidising microbes (Stein and Klotz 2016, R94). The complete oxidation of NH_4^+ to nitrate (NO_3^-) is called nitrification (Scholz 2016), which occurs via two sequential steps, each mediated by a different set of microorganisms. Ammonium is first oxidised to nitrite (NO_2^-) via nitritation, which is then subsequently oxidised to NO_3^- via nitratation (Stein and Klotz 2016, R95). Ammonification and nitrification are the two primary microbial processes that control the availability of soil NH_4^+ in the environment (Wang et al. 2017a, 83). Nitrate may be assimilated in the form of NH_4^+ for biosynthesis through ANRA, lost as NH_3 by DNRA, or maybe reduced to N_2 via denitrification. Denitrification involves the sequential reduction of NO_3^- to N_2 via NO_2^-, nitric oxide (NO), nitrous oxide (N_2O), and finally N_2 (Stein and Klotz 2016, R95). Instead of oxygen, NO_3^- acts as an electron acceptor, thereby engineering an anaerobic mode of respiration of organic matter, reduced iron or sulphur species (De Sousa and Bhosle 2012; Stein and Klotz 2016, R95). Nitrogen may also be returned to the atmosphere by anammox (Fig. 1-1) (De Sousa and Bhosle 2012; Stein and Klotz 2016, R95). In anammox, NH_4^+ is anaerobically oxidised, coupled with NO_2^- reduction to produce N_2, with hydrazine (N_2H_4) as an intermediate via two different pathways, the NO pathway and the hydroxylamine (NH_2OH) pathway (Shalini and Joseph 2012).

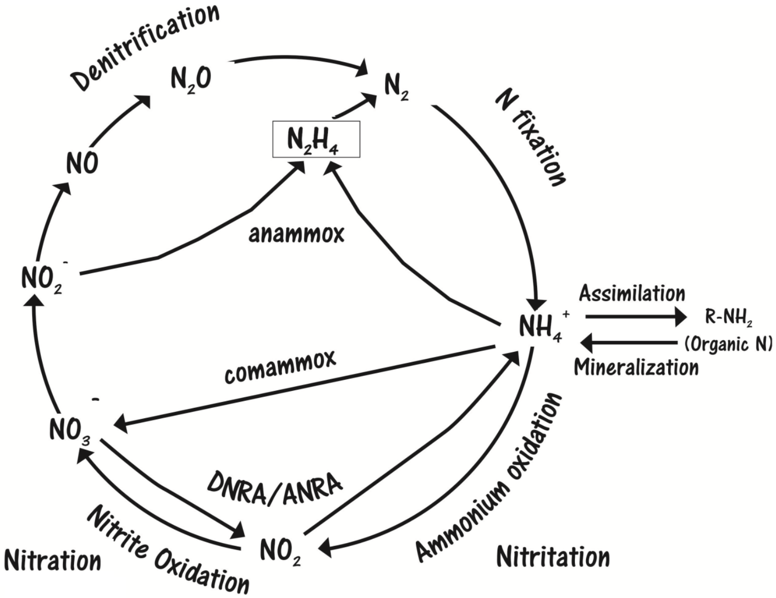

Figure 1-1: The Nitrogen Cycle: Ammonification may be accomplished either by the reduction of dinitrogen (nitrogen fixation) or by mineralisation. Nitrification is composed of the oxidation of ammonia to nitrite and the subsequent oxidation of nitrite to nitrate, or by the complete ammonium oxidation into nitrate (comammox). The reduction of nitrate to nitrite may be coupled with either the dissimilatory nitrate reduction to ammonium (DNRA) or the assimilatory nitrate reduction to ammonium (ANRA), denitrification, and anammox to release dinitrogen.

Thus, nitrogen from the atmosphere enters the biological system via nitrogen fixation and ammonification, where it undergoes a sequence of oxidation and reduction reactions under both aerobic (nitrification) and anaerobic (NO_3^- reduction) conditions and is finally replenished to the atmosphere via denitrification and anammox (Stein and Klotz 2016, R95). These processes influence and regulate the global inventory of fixed nitrogen, and consequently, the function of the ecosystem (Thamdrup and Dalsgaard 2002, 1312). This chapter presents an overview of each of these processes.

Nitrogen fixation

The atmosphere harbours the largest reservoir of nitrogen, where it exists mainly as inert, gaseous, triple bonded N_2, which makes up 78% of the total composition of the atmosphere (Francis et al. 2007, 19; Bernhard 2010, 1-2). Although N_2 is present freely and in large amounts in the atmosphere, in its molecular form, it is inaccessible to most organisms (Pidwirny 2006; Bernhard 2010, 1). Hence, it has to be fixed to a more usable form. Nitrogen fixation may occur by both biological and non-biological processes. The non-biological processes include lightning, combustion, and the man-made industrial Haber-Bosch process which has become the largest source of fixed nitrogen in the Earth's ecosystem.

Biological nitrogen fixation, discovered by the Dutch microbiologist, Martinus Beijerinck, is performed by several different prokaryotes including bacteria, actinomycetes, and certain anaerobic bacteria, collectively called diazotrophs. The reaction, catalysed by the enzyme nitrogenase, involves the conversion of atmospheric N_2 to NH_3 by the following reaction (Bernhard 2010, 3):

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$$

Nitrogenase is extremely sensitive to oxygen and functions only under anaerobic conditions even in aerobic microorganisms. The protection of the enzyme from oxygen is achieved by physical barriers (heterocysts in cyanobacteria), oxygen scavenging and oxygen-binding molecules (for example, leghaemoglobin in leguminous plants associated with *Rhizobium*), and high rates of metabolic activity. The NH_3 formed as the product of nitrogen reduction is immediately incorporated into organic matter as NH_4^+ ion to form amino acids, proteins, nucleic acids, and chlorophyll (Francis et al. 2007, 19; Bernhard 2010, 1; Markov 2012, 347).

Diazotrophs may be free-living (*Azotobacter, Clostridium, Rhodospirillum, Chlorobium,* and *Azospirillum*) or symbiotically associated with leguminous and certain non-leguminous plants (*Rhizobium, Bradyrhizobium, Frankia,* and *Anabaena*) (Pidwirny 2006; Bernhard 2010, 2-3; Stefanakis et al. 2014; Stein and Klotz 2016, R94). There also occurs several archaea like the methanogens (*Methanococcus thermolithotrophicus, Methanococcus maripaludis, Methanosarcina barkeri, Methanospirillum hungatei,* and *Methanobacterium bryantii*) that can also fix nitrogen (Leigh 2000, 125;

Isobe and Ohte 2014, 5). In the marine ecosystem, the fixed nitrogen is usually exhausted and so biological nitrogen fixation by photoautotrophs becomes the main process that controls nitrogen supply to all organisms (Isobe and Ohte 2014, 5). Both cyanobacterial and non-cyanobacterial nitrogen fixers contribute to the marine nitrogen budget (Isobe and Ohte 2014, 6). The principal diazotroph in the ocean is believed to be the filamentous non-heterocystous cyanobacteria of the genus *Trichodesmium*. Also, two oxygen-evolving unicellular diazotrophic cyanobacteria, UCYN groups A and B, were identified as the major nitrogen fixers in the oceans accounting for a large fraction of the nitrogen fixation in several marine ecosystems. Reports state that non-cyanobacteria nitrogen fixers, such as the *Alpha-, Beta-,* and Gamma-Proteobacteria also play a significant role in nitrogen cycling (Isobe and Ohte 2014, 6).

Ammonification

The NH_4^+ formed by nitrogen fixation is assimilated into the biomass as glutamine, which then serves as a nitrogen donor for the synthesis of other amino acids and heterocyclic nitrogen compounds (Richardson et al. 2009, 389). This organic nitrogen serves as a nutrient source for the various forms of life. If it is not utilised, the fixed organic nitrogen is returned to NH_3 by mineralisation via ammonification. This is accomplished by heterotrophic bacteria, which use the oxidation of organic carbon to CO_2 as an energy source but release the organic nitrogen as NH_4^+ since they cannot oxidise it to NO_2^- or NO_3^-.

Plants can use nitrogen in the form of NH_4^+ and/or NO_3^- ions (Pidwirny 2006). Animals acquire nitrogen by decomposition of living and dead organic matter (including plant and animal remains) by soil heterotrophic microbes via mineralisation, which converts the organic nitrogen to its inorganic form, NH_4^+ (ammonification) (Pidwirny 2006; Hopkinson and Giblin 2008; Bernhard 2010, 6; Wang et al. 2017a, 84). In mineralisation, nitrogen-containing organic polymers are depolymerised via proteolysis and aminisation to yield amide- or amine-R (R-NH_2). Some examples of such polymers are amino polysaccharides, nucleic acids, proteins, and chitin. In both terrestrial and aquatic environments, ubiquitous groups of microbes may be actively involved in the depolymerisation process. The end products (R-NH_2) of the microbial depolymerisation are deaminated and deamidated via the process of ammonification to yield NH_3 (Isobe and Ohte 2014, 10). Ammonium can be produced by the intracellular enzymes, namely deaminase and deamidase, by direct enzymatic cleavage of a free amino group, either amine- or amide-N (R-NH_2) (Isobe and Ohte 2014, 10; Stefanakis et al. 2014). Consequently, this process involves several consortia of microorganisms (Isobe and Ohte 2014, 10). Research states that ammonifying activity is widely distributed throughout the soil microbial community (Fuller 2005). Bright (1919, 318) demonstrated that the number of non-spore formers outnumbered the number of spore formers and that two non-spore formers, namely *Pseudomonas fluorescens* and *P. caudatus*, primarily contributed to the ammonification in manured soils.

Ammonification can occur under both aerobic and anaerobic conditions. However, the process occurs rapidly under aerobic conditions (Stefanakis et al. 2014). In the upper layers of soil, NH_3 present in organic matter is mineralised to NH_4^+ by decomposers, and taken up by plants and microorganisms for their growth and biosynthesis (Bernhard 2010, 6). The rate of ammonification inversely relates to the lignin content in the sediment (Hopkinson and Giblin 2008). Saltmarsh sediments and seagrass beds show higher rates of decomposition and ammonification than non-vegetated coastal marine sediments. The ammonification rates are higher near the surface and rhizosphere and decrease with depth with the decline in the concentration of organic nitrogen and oxygen (Hopkinson and Giblin 2008). The lowered rate of ammonification may be attributed to the reduced efficiency of heterotrophic decomposition under anaerobic conditions, and changes in soil physiology, such as pH and temperature (Scholz 2016). The optimum pH and temperature for ammonification are approximately 6.5-8.5 and 40-60°C, respectively (Stefanakis et al. 2014; Scholz 2016). In coral reef ecosystems, organic nitrogen regeneration may occur within the diverse symbiotic associations along with the various components of the reef (O'Neil 2008, 940). High bacterial activity and consequently, nutrient regeneration, is reported within the crevices of the reef structure. These organisms thus, aid in the various remineralisation processes, thereby contributing to the nitrogen concentration in the surrounding water column and consequently, the regeneration of nutrients in the marine system (O'Neil 2008, 953). Ammonia may be further oxidised by NH_3-oxidising microbes, via nitrification or anammox.

Nitrification

Ammonium ions may be taken up and used by plants to serve as a nitrogen source. However, in high amounts, they are extremely toxic to cells (Pidwirny 2006) and hence most of the NH_4^+ is converted to NO_3^-. This process of conversion of the most reduced form of nitrogen (NH_4^+) to the most oxidised form (NO_3^-) is called nitrification (Francis et al. 2007, 19; Bernhard 2010, 4; Casciotti and Buchwald 2012, 133; Stein and Klotz 2016, R94). Microbial nitrification plays a central role in the global nitrogen cycle (Gao et al. 2016, 1). The process of nitrification involves two separate sequential steps, mediated by two separate groups of organisms: the oxidation of NH_4^+ to NO_2^- (nitritation) and the subsequent oxidation of NO_2^- to NO_3^- (nitratation) (Markov 2012, 348; Gao et al. 2016, 1).

Nitritation

The oxidation of NH_4^+ to NO_2^- by nitritation is mediated by ammonia-oxidising bacteria (AOB), such as *Nitrosomonas, Nitrosococcus, Nitrosovibrio, Nitrosocystis,* and *Nitrosospira*; and ammonia-oxidising archaea (AOA), such as *Nitrosopumilus maritimus*. The reaction is as follows (Markov 2012, 348; Stein and Klotz 2016, R95):

$$NH_4^+ + O_2 \rightarrow NO_2^- + H_2O + H^+$$

Ammonium oxidation is the rate-limiting step of nitrification. Nitrite does not accumulate in the natural environment and serves as an intermediate or as a substrate in many nitrogen transforming processes (Isobe et al. 2011; Isobe et al. 2012, 61; Isobe and Ohte 2014, 6; Zhou et al. 2015, 1; Li et al. 2018, 1). In both AOB and AOA, NH_4^+ is first oxidised to NH_2OH by the enzyme ammonia monooxygenase (AMO) (McTavish et al. 1993, 2436; Vajrala et al. 2013, 1006; Li et al. 2018, 1). Subsequently, NH_2OH is oxidised to NO_2^- by the periplasmic enzyme, hydroxylamine oxidoreductase (HAO), in AOB (McTavish et al. 1993, 2436) and by a novel enzyme complex, in AOA (Vajrala et al. 2013, 1006). Ramos and Pajares (2018, 7) have reported that NH_2OH is first converted to NO which is then oxidised to NO_2^-. They demonstrated the production of NO, NO_2^-, and N_2 from NH_4^+ in marine environments under suboxic conditions.

For more than 100 years, NH_4^+ oxidation was thought to be performed solely by specialised bacteria of certain groups of chemolithoautotrophic Proteobacteria that grow exclusively on inorganic carbon and nitrogen (Schleper 2010, 1092; Hatzenpichler 2012, 7501; Isobe and Ohte 2014, 8; Zhou et al. 2015, 1). However, with the discovery and cultivation of AOA, and the recent discovery of homologs of *amo* genes in archaea, this notion has changed. It is now a well-known fact that both AOB and AOA are