

# Global Implications of the Nitrogen Cycle

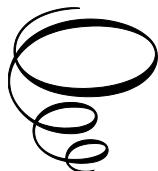


# Global Implications of the Nitrogen Cycle

Edited by

Trelita de Sousa

Cambridge  
Scholars  
Publishing



Global Implications of the Nitrogen Cycle

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This book first published 2020

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

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ISBN (10): 1-5275-5513-5

ISBN (13): 978-1-5275-5513-6



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



# CONTENTS

List of Illustrations .....	x
-----------------------------	---

List of Tables.....	xvi
---------------------	-----

Foreword .....	xix
----------------	-----

Preface .....	xxi
---------------	-----

## **Section I: The Microbial Transformation of Nitrogen**

Chapter 1 .....	2
-----------------	---

An Overview of the Microbial Transformation of Nitrogen

Valerie Gonsalves

Chapter 2 .....	37
-----------------	----

The Role of Plant-Associated Bacteria in the Soil Nitrogen Cycle  
and the Nitrogen Nutrition of Plants

Gauri A. Achari

Chapter 3 .....	71
-----------------	----

Nitrogen Fixation in Coastal Sand Dune Vegetation

Aureen L. Godinho Gomes

Chapter 4 .....	80
-----------------	----

The Role of Nitrogen-Fixing Bacteria in Nitrogen-Limiting Marine Waters

Milind Mohan Naik, Lakshangy Charya, Diksha Naik, Alisha Malik,  
and Meghanath S. Prabhu

Chapter 5 .....	100
-----------------	-----

Aerobic Ammonia-Oxidising Prokaryotes: A Perception of their Niche

Segregation across Estuarine Salinity Gradients

Hugo Ribeiro, João P. Santos, António G. G. Sousa, Paula Salgado,  
Maria Paola Tomasino, Mafalda S. Baptista, and Catarina Magalhães

Chapter 6 .....	136
The Ammonia-Oxidising Archaeal Community of Marine Sponges	
Sheryanne Velho-Pereira	
Chapter 7 .....	153
The Significance of Denitrification in the Nitrogen Cycle	
Genevieve L. Fernandes, Amara Begum Mulla, Larissa D. Menezes, and Samir R. Damare	
Chapter 8 .....	188
Anaerobic Ammonium Oxidation (Anammox)	
Rasika Gaokar Desai	
Chapter 9 .....	199
The Role of Eukaryotic Microbes in the Marine Nitrogen Cycle	
Varada S. Damare	
<b>Section II: Implications of the Nitrogen Cycle on Primary Productivity and Biogeochemistry</b>	
Chapter 10 .....	232
The Coupling of Nitrogen with the Carbon and Sulphur Cycles and the Ensuing Effect on the Global Climate	
Trelita de Sousa	
Chapter 11 .....	250
The Influence of Phosphate on the Altered Nitrogen Cycle	
Neha Prabhu	
Chapter 12 .....	265
The Implications of Iron in Nitrogen Cycling: A Review	
Teja Savoikar and Subhajit Basu	
Chapter 13 .....	278
The Global Implications of the Nitrogen Cycle: A Benthic Community Perspective	
Sanitha K. Sivadas, Afreen Hussain, and Rahul Nagesh	

**Section III: Implications of Human Interference on the Nitrogen Cycle**

Chapter 14 .....	324
The Changing Nitrogen Cycle: Consequences of Human Impedance	
Christina de Souza Veloso	
Chapter 15 .....	388
The Implications of the Altered Nitrogen Cycle on Coastal Ecosystems:	
Emphasis on Primary Producers	
Ravidas Krishna Naik, Priya M. D'Costa, R. K. Mishra, and Anil Kumar N.	
Chapter 16 .....	410
The Implications of Nitrous Oxide from Biogenic and Anthropogenic	
Sources on the Global Nitrogen Cycle	
Amrita Kharangate-Lad	
Chapter 17 .....	430
The Impact of Unbalanced Nitrogen Cycling on Human Health	
Flory Pereira and Vilas Desai	

## LIST OF ILLUSTRATIONS

**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.

**Figure 1-2:** The anaerobic ammonium oxidation process via the nitric oxide pathway and the hydroxylamine pathway

**Figure 2-1:** A schematic representation of the biological nitrogen cycle in agricultural soils. The plant-beneficial processes include nitrogen fixation, mineralisation, nitrification, and DNRA which provide  $\text{NH}_4^+$  or  $\text{NO}_3^-$  which are readily taken up by the plants. Denitrification and anammox cause N losses and the release of GHG. Immobilisation of N compounds due to the uptake by microbes can result in competition and temporary N losses. Efficient regulation of these interlinked pathways is crucial for enhancing the N synchrony in plants and curbing N loss and the release of GHG.

**Figure 3-1:** The transformation of nitrogen in the soil  
[Adapted and modified from Brady and Weil (2000) and Sylvia et al. (2005)]

**Figure 5-1:** A description of the overall nitrification pathway

**Figure 6-1:** A vertical cross-section of a sponge

**Figure 7-1:** The denitrification pathway depicted with their soluble and gaseous products. The genes participating in each step are shown near their respective functions.

**Figure 8-1:** The possible mechanisms of anammox observed in bacteria wherein the reduction of nitrite and the oxidation of hydrazine; a) occur at different sites of the same enzyme; or b) are catalysed by different enzymes connected via an electron transport chain (modified from Jetten et al. (1998, 285) and Ni and Zhang (2013, 4).

**Figure 8-2:** The biological nitrogen removal systems employed for wastewater treatment; a) The ANAMMOX Reactor; b) The SHARON Reactor; c) The SHARON-ANAMMOX Reactor; d) The CANON Reactor (modified from Paredes et al. (2007, 19) and Bagchi et al. (2012, 1369).

**Figure 9-1:** The tree of life for the protists as modified from Adl et al. (2012). The thick lines show the supergroups and the groups within. The thin lines are the *incertae sedis* groups as reported by Adl et al. (2012). The tree only shows phylogenetic placement irrespective of the time of evolution. Hence the length of the lines may be ignored.

**Figure 9-2:** A microphotograph of the diatom *Chaetoceros* sp. as viewed under a bright field microscope with a 10X magnification. (Picture courtesy: Dr. Ravidas Naik)

**Figure 9-3:** A microphotograph of a thraustochytrid isolate grown on an agar medium (Modified Vishniac medium). The photograph shows spherical cells of 5-15  $\mu\text{m}$  size and with biflagellate zoospores (marked by a black arrow). The zoospores are ellipsoidal to oval in shape depending on the plane in which they were captured during their flagella-aided motion. The thraustochytrid cells produce rhizoid-like structures called ectoplasmic net elements (marked by a white arrow) which are extensions of the plasma membrane. These structures aid in the attachment to the substratum and the secretion of extracellular enzymes and are a characteristic feature of Labyrinthulomycetes.

**Figure 9-4:** A microphotograph depicting the aboral side view and the top view of the dinoflagellate *Noctiluca* sp. as observed under a bright-field microscope with a 10X magnification. (Picture courtesy: Dr. Ravidas Naik)

**Figure 9-5:** Scanning electron microphotographs of foraminifera (a) *Bulimina marginata* and (b) *Brizalina striata*. (Picture courtesy: Dr. Rajani Panchang)

**Figure 10-1:** The Global Carbon Cycle. Carbon transforms to both organic and inorganic forms, as it flows through its various reservoirs. The flow estimates are expressed as gigatonnes of carbon per year. One gigatonne of carbon is equivalent to one billion metric tonnes, equal to  $10^{15}$  g.

**Figure 10-2:** The interactions between the nitrogen and carbon cycles. The anthropogenic-induced factors are indicated in red.

**Figure 10-3:** The cycling of sulphur through the terrestrial, ocean, and atmospheric systems

**Figure 10-4:** The coupling of nitrogen and sulphur pathways

**Figure 11-1:** The nitrogen cycle in the rhizosphere

**Figure 11-2:** The phosphorus cycle in soil

**Figure 11-3:** The effect of phosphorus deficiency on plant uptake (grey box), nitrate and ammonia uptake (blue box), and nitrogen fixation (green box)

**Figure 12-1:** The role of iron in nitrogen fixation in leguminous plants

**Figure 13-1:** A schematic diagram showing the various processes of the nitrogen cycle. Nitrogen fixed by the primary producer is exported to the benthic system. The organic matter is converted to  $\text{NH}_4^+$  (ammonification) which, in turn, is oxidised to nitrite ( $\text{NO}_2^-$ ) and then nitrate ( $\text{NO}_3^-$ ) (nitrification) which is further reduced to nitrogen gas ( $\text{N}_2$ ) (denitrification). Under anaerobic conditions, ammonium reacts with  $\text{NO}_2^-$  to produce nitrogen gas (anammox). PON: particulate organic nitrogen, DON: dissolved organic nitrogen,  $\text{O}_2$ : oxygen,  $\text{NH}_4^+$ : ammonium, NO: nitric oxide, and  $\text{N}_2\text{O}$ : nitrous oxide.

**Figure 13-2:** The changes in the nitrogen cycle due to bioturbation by benthic organisms: A) sediment system with benthic fauna and B) sediment system without benthic fauna. The bioturbation by the benthic fauna introduces oxygen deep into their burrows, oxidising the sediment system with benthic fauna. The oxidised layer is depicted by a light-brown region (A). In the sediment without the benthic fauna (B) the oxic layer is reduced.

**Figure 13-3:** The nitrogen cycle at important locations in the coral reefs. Nitrogen cycling in the coral reef sediments is similar to that observed in the benthic sediments (Fig. 1) and includes nitrogen fixation, ammonification, nitrification, and denitrification. Sponges (A) take up dissolved nitrogen from the surrounding seawater; the prokaryotes symbiotically associated with the sponges transform the nitrogen (B); symbiotic algae efficiently utilise the inorganic nutrients (DIN) released by the corals and also facilitate the uptake of nitrogen in the form of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  from the seawater (C). The diazotrophic assemblages function as nitrogen fixers. Also, corals actively take up particles and zooplankton as additional nutrients.

**Figure 14-1:** The modes of creation of reactive nitrogen (Erisman et al. 2015).

**Figure 14-2:** The sources of human-induced alterations to the global N cycle (Vitousek et al. 1997a).

**Figure 14-3:** The reactive N cycle (Erisman et al. 2015)

**Figure 14-4:** The fate of fertiliser N produced by the Haber-Bosch process from the factory to the mouth for (a) a vegetarian diet, and (b) a carnivorous diet (Galloway and Cowling 2002).

**Figure 14-5:** The correlation of the global progression from 1900 to 2012 between human population and the creation of total anthropogenic  $\text{N}_r$  over the 20<sup>th</sup> century (Erisman et al. 2015)

**Figure 14-6:** The period in which the symptoms of eutrophication and hypoxia/anoxia began in the developed countries of the world and the more recent unfolding of these events in developing countries (Rabalais et al. 2014).

**Figure 14-7:** A global distribution of dead zones associated with eutrophication (Diaz and Rosenberg 2008).

**Figure 14-8:** A conceptual diagram depicting the alteration of an ecosystem's energy flow by hypoxia. The green portion of the diagram is an indication of the range of energy that is transferred to predators at the higher level from the benthos under conditions of normoxia, generally 25–75% of macrobenthic carbon. With the onset of mild or periodic hypoxia,

surges of benthic energy to predators may be observed. Such surges are short-lived and do not always occur. As oxygen declines, predation at the higher level decreases but may continue at the benthic level, and the degree of the energy transfer from the benthos to microorganisms increases rapidly (orange). Under conditions of persistent hypoxia, some amount of energy is still processed by tolerant benthos. Microorganisms transform all benthic energy as H<sub>2</sub>S and anoxia develop (red) (Diaz and Rosenberg 2008).

**Figure 14-9:** The progressive changes in fish and invertebrate fauna with the decrease in oxygen concentration from 2 mg/L to anoxia (Rabalais et al. 2002).

**Figure 14-10:** The amplified effects of N<sub>r</sub> induced by the nitrogen cascade (Erisman et al. 2015).

**Figure 16-1:** The emission of the greenhouse gas (GHG) nitrous oxide during nitrification, dissimilatory nitrate reduction to ammonium (DNRA), and denitrification by microorganisms in the nitrogen cycle in nature (Giles et al. 2012, 2).

**Figure 16-2:** The eutrophication of a water body due to the incessant application of nitrogenous fertilizers.

**Figure 16-3:** The formation of acid rain, acid snow, and dry deposition from nitrous oxide and sulphur dioxide emissions from anthropogenic sources and their effect on environmental ecosystems.

**Figure 16-4:** The formation of photochemical smog due to ozone, nitric acid, and the volatile organic compounds (VOCs)

**Figure 16-5:** The photolytic breakdown of nitrous oxide in the atmosphere to form free oxygen radicals and its reaction with it to yield nitric oxide which catalyses the breakdown of ozone.

**Figure 16-6:** Ozone production in the troposphere in a nitric oxide rich environment

**Figure 17-1:** Trends and future projections in the global emissions of NH<sub>3</sub> (Based on data from Lamarque et al. (2010), Masui et al. (2011), and Sutton et al. (2013)).

**Figure 17-2:** Trends in the global emissions of N<sub>2</sub>O levels: Current and historic atmospheric N<sub>2</sub>O

**Figure 17-3:** The nitrate levels at river mouths (UNEP 2006)

**Figure 17-4:** Trends and future projections in the global emissions of NO<sub>2</sub> (Based on data from Lamarque et al. (2010), Masui et al. (2011), and Sutton et al. (2013)).

**Figure 17-5:** The effects of an unbalanced nitrogen cycle on human health

## LIST OF TABLES

**Table 2-1:** The important N cycling routes in agricultural soil and the measures to conserve the plant-available N

**Table 4-1:** The effect of oil spills on the different marine biota

**Table 4-2:** The major oil spills of the world

**Table 5-1:** A summary of the metabolic activity, taxonomic relatedness, and general colonisation of the ammonia and nitrite-oxidising prokaryotes (Koops et al. 2006; Daims et al. 2016; Alves et al. 2018)

**Table 5-2:** The archaeal *amoA* gene and the betaproteobacterial *amoA* gene abundances at different salinity ranges (Low, Intermediate, and High) obtained for various estuarine systems. The most abundant group (AOA or  $\beta$ -AOB), or similar ( $\text{AOA} \approx \beta\text{-AOB}$ ) is displayed in each range of salinity (Mosier and Francis 2008; Santoro et al. 2008; Magalhães et al. 2009; Moin et al. 2009; Abell et al. 2010; Bernhard et al. 2010; Wankel et al. 2011; Zheng et al. 2014; Li et al. 2015; Veettil et al. 2015; Zhang et al. 2015; Monteiro et al. 2017; Li et al. 2018).

**Table 5-3:** The dominant ammonia-oxidising archaea (AOA) and bacteria (AOB) in various estuarine ecosystems (Freitag et al. 2006; Magalhães et al. 2007; Mosier and Francis 2008; Sahan and Muyzer 2008; Moin et al. 2009; Bernhard et al. 2010; Zheng et al. 2014; Li et al. 2015; Monteiro et al. 2017; Li et al. 2018)

**Table 9-1:** The various pathways of the nitrogen cycle observed in different eukaryotic groups

**Table 9-2:** The various pathways of the nitrogen cycle proposed in different eukaryotic groups

**Table 14-1:** The global impact of the nitrogen cascade (Compton et al. 2011)

**Table 14-2:** The global terrestrial contributions to the biological N fixation in the year 2000 (Fowler et al. 2015)

**Table 14-3:** The impacts of the factors that affect the marine N fixation (Fowler et al. 2015)

**Table 14-4:** The global N fertiliser consumption, ammonia production, and urea production in the year 2000 (Fixen and West 2002)

**Table 14-5:** The estimates for anthropogenic nitrous oxide emissions (Davidson 2009)

**Table 14-6:** The impacts of livestock production on the N cycle (Steinfeld and Wassenaar 2007)

**Table 14-7:** Point and non-point sources of anthropogenic N entering aquatic ecosystems (Carpenter et al. 1998; Carmago and Alonso 2006)

**Table 14-8:** The adverse effects of anthropogenic acidification on freshwater plants and animals (Carmago and Alonso 2006)

**Table 14-9:** The ecological and toxicological effects of anthropogenic eutrophication in water bodies (Carpenter et al. 1998; Carmago and Alonso 2006; Diaz and Rosenberg 2008; Compton et al. 2011)

**Table 14-10:** The relationship between nitrogen related ambient air pollutants and human health (Peel et al. 2013)

**Table 14-11:** The fluctuations in the concentrations of greenhouse gases due to human activities (Ebi et al. 2003)

**Table 14-12:** The impacts of reactive N ( $N_r$ ) on climate change (Pinder et al. 2012)

**Table 14-13:** The favourable and damaging effects of reactive N ( $N_r$ ) (Galloway and Cowling 2002)

**Table 15-1:** The strategies adopted by phytoplankton for acquiring dissolved organic compounds based on Bronk et al. (2007) and Burkholder, Glibert, and Skelton (2008)

**Table 17-1:** The global nitrogen fluxes from 2000-2010 (Sutton et al. 2013, 24)

## 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!



Dr. (Mrs.) Saroj Bhosle  
Ex Prof and Dean  
Department of Microbiology  
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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**

### **VALERIE GONSALVES**

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#### **Abstract**

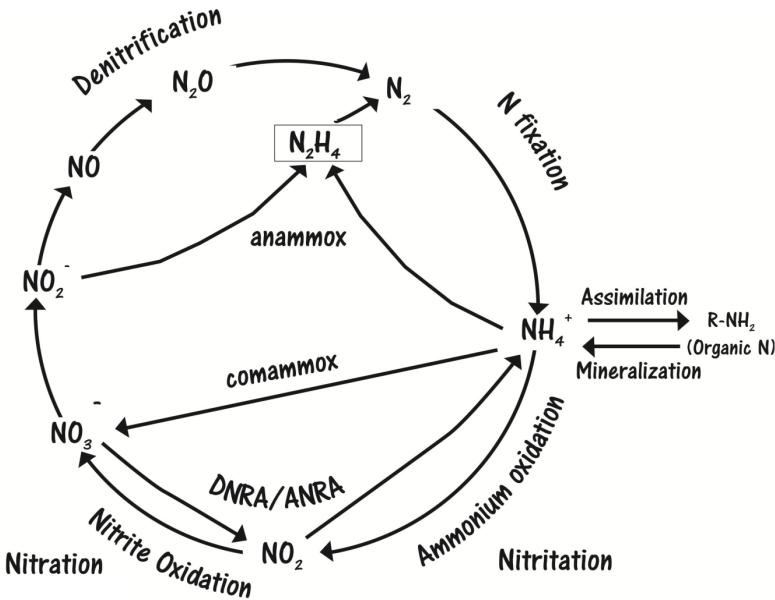
The cycling of nitrogen represents one of the most important nutrient cycles. In its elemental form, nitrogen makes up 78% of the atmospheric gases. The conversion of atmospheric nitrogen to a more usable form occurs via both physical and biological nitrogen fixation processes. Once nitrogen enters the 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 ( $\text{NH}_3$ ) refers to the process by which living organisms make  $\text{NH}_3$  or ammonium ions ( $\text{NH}_4^+$ ) (Markov 2012, 348). Ammonium can be produced via three microbial pathways: nitrogen fixation, ANRA, and DNRA. In nitrogen fixation (Fig. 1-1), dinitrogen ( $\text{N}_2$ ) from the atmosphere (the main reservoir of molecular nitrogen on Earth) is reduced to  $\text{NH}_4^+$  (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  $\text{NH}_3$ -oxidising microbes (Stein and Klotz 2016, R94). The complete oxidation of  $\text{NH}_4^+$  to nitrate ( $\text{NO}_3^-$ ) 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 ( $\text{NO}_2^-$ ) via nitritation, which is then subsequently oxidised to  $\text{NO}_3^-$  via nitratation (Stein and Klotz 2016, R95). Ammonification and nitrification are the two primary microbial processes that control the availability of soil  $\text{NH}_4^+$  in the environment (Wang et al. 2017a, 83). Nitrate may be assimilated in the form of  $\text{NH}_4^+$  for biosynthesis through ANRA, lost as  $\text{NH}_3$  by DNRA, or maybe reduced to  $\text{N}_2$  via denitrification. Denitrification involves the sequential reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  via  $\text{NO}_2^-$ , nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ), and finally  $\text{N}_2$  (Stein and Klotz 2016, R95). Instead of oxygen,  $\text{NO}_3^-$  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,  $\text{NH}_4^+$  is anaerobically oxidised, coupled with  $\text{NO}_2^-$  reduction to produce  $\text{N}_2$ , with hydrazine ( $\text{N}_2\text{H}_4$ ) as an

intermediate via two different pathways, the NO pathway and the hydroxylamine ( $\text{NH}_2\text{OH}$ ) pathway (Shalini and Joseph 2012).



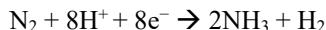
**Figure 1-1:** The Nitrogen Cycle: Ammonification may be accomplished either by the reduction of dinitrogen (nitrogen fixation) or by mineralisation. Nitrification is composed of the oxidation of ammonia to nitrite and the subsequent oxidation of nitrite to nitrate, or by the complete ammonium oxidation 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 ( $\text{NO}_3^-$  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<sub>2</sub>, which makes up 78% of the total composition of the atmosphere (Francis et al. 2007, 19; Bernhard 2010, 1-2). Although N<sub>2</sub> 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<sub>2</sub> to NH<sub>3</sub> by the following reaction (Bernhard 2010, 3):



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<sub>3</sub> formed as the product of nitrogen reduction is immediately incorporated into organic matter as NH<sub>4</sub><sup>+</sup> 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 thermolithothrophicus*, *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  $\text{NH}_4^+$  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  $\text{NH}_3$  by mineralisation via ammonification. This is accomplished by heterotrophic bacteria, which use the oxidation of organic carbon to  $\text{CO}_2$  as an energy source but release the organic nitrogen as  $\text{NH}_4^+$  since they cannot oxidise it to  $\text{NO}_2^-$  or  $\text{NO}_3^-$ .

Plants can use nitrogen in the form of  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  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,  $\text{NH}_4^+$  (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 ( $\text{R}-\text{NH}_2$ ). 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 ( $\text{R}-\text{NH}_2$ ) of the microbial depolymerisation are deaminated and deamidated via the process of ammonification to yield  $\text{NH}_3$  (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 ( $\text{NH}_4^+$ ) to the most oxidised form ( $\text{NO}_3^-$ ) 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  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (nitritation) and the subsequent oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (nitratation) (Markov 2012, 348; Gao et al. 2016, 1).

### **Nitritation**

The oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  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):



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,  $\text{NH}_4^+$  is first oxidised to  $\text{NH}_2\text{OH}$  by the enzyme ammonia monooxygenase (AMO) (McTavish et al. 1993, 2436; Vajrala et al. 2013, 1006; Li et al. 2018, 1). Subsequently,  $\text{NH}_2\text{OH}$  is oxidised to  $\text{NO}_2^-$  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  $\text{NH}_2\text{OH}$  is first converted to NO which is then oxidised to  $\text{NO}_2^-$ . They demonstrated the production of NO,  $\text{NO}_2^-$ , and  $\text{N}_2$  from  $\text{NH}_4^+$  in marine environments under suboxic conditions.

For more than 100 years,  $\text{NH}_4^+$  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