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THE GLOBAL NITROGEN CYCLE

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4.1 Introduction

The geobiology of nitrogen is dominated by large inert reservoirs and small biological fluxes. The largest reservoirs are nitrogen gas (in the atmosphere and dissolved in the ocean) and sedimentary nitrogen (sequestered in continental crust). Because most organisms cannot utilize gaseous nitrogen, we distinguish between fixed nitrogen compounds (which contain no N–N bonds) that are biologically available, and the dinitrogen gases (N_2 and N_2O) that are largely inaccessible to organisms. Fluxes into and out of the fixed nitrogen pools are biologically controlled, and microbes control the rates of transformations and the distribution of nitrogen among inorganic and organic pools.

Possibly more than any other biologically important element, the global nitrogen cycle has been perturbed by anthropogenic activities. The rate of industrial nitrogen fixation now approximately equals the natural rate, resulting in a two- to threefold increase in the total inventory of fixed N on the surface of the Earth through agricultural fertilizer applications (Galloway *et al.*, 2004). Nitrogen oxides enter the atmosphere via fossil fuel combustion and catalyse the atmospheric chemistry of ozone through pathways that were either nonexistent or insignificant prior to humans. Because of the relative biological inaccessibility of nitrogen, ecosystems have responded to the increased flux of fixed N with changes in the rates and fates of production, and in some cases, large changes in ecosystem chemistry and health.

The microbiology of nitrogen transformations has been intensely studied for over a century, but important new discoveries have been made in only the last decade.

These involve the discovery of previously suspected, but unknown processes, as well as the discovery of new and diverse microbes involved in many of the nitrogen cycle fluxes.

4.2 Geological nitrogen cycle

Nitrogen is an essential element for life on Earth and the fourth most abundant element (after C, O and H) in the biosphere (living plus dead organic matter) at about 0.3% by weight. Unlike the other major biologically important elements, nitrogen (N) is a minor constituent of the Earth's crust, averaging 50ppm in continental crust (Wedepohl, 1995). Fresh igneous rocks contain essentially no nitrogen (e.g. fresh volcanic lava), so nitrogen in rocks is ultimately derived from the atmosphere via biotic and abiotic nitrogen fixation. The process of N sequestration in rocks involves accumulation of organic matter in low energy marine environments, where it is slowly decomposed and the ammonium that is liberated partitions into clay minerals. In high energy environments, clays and organic matter do not accumulate and the coarser grained sands do not acquire ammonium (Boyd, 2001). Nitrogen is returned to the atmosphere during metamorphism. All of the ammonium in the Earth's crust has been derived ultimately from the atmosphere through nitrogen fixation (Boyd, 2001) and this constitutes trapping of about a quarter of the original atmospheric inventory.

Although primary rocks contain essentially no nitrogen, organic N is a relatively large reservoir in the global N inventory (biosphere plus PON and DON in the ocean and much of the N in soils and sediments;

Table 4.1 Major nitrogen reservoirs on Earth

Reservoir		Tg	Reference
Atmosphere	N ₂	3.7 × 10 ⁹	Sorai <i>et al.</i> , 2007
	N ₂ O	1.4 × 10 ³	Sorai <i>et al.</i> , 2007
Biosphere	Marine	3.0 × 10 ³	Lerman <i>et al.</i> , 2004
		5.0 × 10 ²	Sorai <i>et al.</i> , 2007
	Terrestrial	5.4 × 10 ³	Lerman <i>et al.</i> , 2004
		2.9 × 10 ⁴	Chameides and Perdue, 1997
Ocean	N ₂	7.7 × 10 ³	Sorai <i>et al.</i> , 2007
		1.46 × 10 ⁶	Calculated from Emerson <i>et al.</i> , 2002
	N ₂ O	0.34	Calculated from Emerson <i>et al.</i> , 2002
		6.0 × 10 ⁵	Mackenzie, 1998
	PON	9.0 × 10 ⁴	Sorai <i>et al.</i> , 2007
	DON	8.1 × 10 ⁵	Sorai <i>et al.</i> , 2007
	Geological	Continental crust	1.3 × 10 ⁹
Crustal rocks		6.4 × 10 ⁸	Boyd, 2001
Oceanic crust		8.9 × 10 ⁵	Li <i>et al.</i> , 2007
Coastal sediments		3.2 × 10 ⁴	Lerman <i>et al.</i> , 2004
Deep ocean sediments		2.0 × 10 ⁹	Chameides and Perdue, 1997
Soil		1.4 × 10 ⁵	Batjes, 1996
		2.2 × 10 ⁴	Lerman <i>et al.</i> , 2004

DON = dissolved organic nitrogen; PON = particulate organic nitrogen.

Table 4.1), and some of this material is preserved in sediments in the form of complex biomolecules that are studied as biomarkers of past microbial life. It has been estimated that fossil hopanoids (pentacyclic compounds similar to sterols that occur in bacterial membranes and are well preserved in sedimentary rocks) contain as much organic carbon as all living biota (~10⁶ Tg C; Ourisson *et al.*, 1987). Hopanoids vary widely in structure (Romer, 1993) and some contain nitrogen. These trace levels of nitrogen contribute to the geological reservoir of N in petroleum and other fossil organic deposits, and thus to the production of N oxides during fossil fuel combustion.

By far the largest reservoir of total nitrogen on Earth is the dinitrogen gas (N₂) in the atmosphere (Table 4.1). N₂ is also the major form of nitrogen in the ocean. The most abundant form of nitrogen in soils and marine sediments is organic nitrogen, produced by biological processes. Although organic N can also be degraded by biological processes, it tends to accumulate in soils and sediments because it becomes more and more resistant to biological attack as it ages. Thus the large organic N reservoirs on land and in the ocean have very long residence times and some of this material eventually transfers to the sedimentary and rock reservoirs. The molecular composition of dead organic matter is complex and largely unresolved, but much of the residual organic matter in soils and marine sediments

may be derived from decay-resistant residues of microbial cell walls.

Transformations and transfers among the reservoirs (Table 4.2) are predominantly controlled by biological processes, so it is interesting that the amount of nitrogen in the biomass itself is vanishingly small compared to the atmospheric and geologic reservoirs. The total amount of N in land and ocean biota is variously estimated at up to 10 × 10³ Tg, (10¹² g) a million-fold less than the atmospheric N₂ reservoir.

The great size of the atmospheric and geologic reservoirs suggests that abiotic processes should also be important in controlling the fluxes between and sizes of these reservoirs. Abiotic processes such as weathering may have changed the magnitude of fluxes in geological processes and the size of the N reservoir in the atmosphere and in rocks to a greater extent than could be accomplished by biological fluxes. A combination of biological and geological processes has caused major excursions in the atmospheric oxygen content over long stretches of Earth history (see Chapter 7). How has the concentration of N₂ in the atmosphere varied as compared to the historical variation in oxygen content of the atmosphere? The geological fluxes of nitrogen can be connected to those of carbon using a C/N ratio for representative materials (Table 4.3) (Berner, 2006). There is no apparent correlation between N/C and age of the shales or their organic content. Similarly, the N/C of coals does not

Flux		Tg yr ⁻¹	Reference
Inputs			
Fixation	Natural terrestrial	107	Galloway <i>et al.</i> , 2004 for early 1990s
	Natural oceanic	121	Galloway <i>et al.</i> , 2004 for early 1990s
		110	Capone <i>et al.</i> , 2001
		130–58	Deutsch <i>et al.</i> , 2007
	Leguminous crops	31.5	Cleveland <i>et al.</i> , 1999
	Chemical fertilizer	100	Galloway <i>et al.</i> , 2004 for early 1990s
	Fossil fuel combustion	24.5	Galloway <i>et al.</i> , 2004 for early 1990s
Lightning		21	Jickels, 2006
Volcanoes		5	Schumann and Huntreiser, 2007
Losses		0.04	Sano <i>et al.</i> , 2001
Denitrification	Natural terrestrial (land and rivers)	115	Galloway <i>et al.</i> , 2004 for early 1990s
		154	Lerman <i>et al.</i> , 2004
		123	Lerman <i>et al.</i> , 2004
	Natural oceanic	193	Galloway <i>et al.</i> , 2004 for early 1990s
		285	Middleburg <i>et al.</i> , 1996
		400	Codispoti, 2007
		2030	Archer <i>et al.</i> , 2002
Industrial combustion		7	Jickels, 2006
Biomass burning		41.6	Andrea and Merlet, 2001
Burial (ocean sedimentation)		25	Brandes and Devol, 2002

Table 4.2 Major fluxes in the global N cycle**Table 4.3** N content of geological reservoirs (calculated from Berner, 2006)

	% C	%N	N/C
Shales	0.013–23	0.005–0.7	0.011–0.083
Coal	57–86	1.0–2.1	0.015–0.024
Gases from volcanic/ metamorphic fumaroles	55–83.5	0.5–1.6	0.012–0.056

vary systematically with thermal grade. The overall range in N/C, a factor of 2–7, is relatively small and similar for all three reservoirs listed in Table 4.3.

With these observations and using geochemical models developed to study the carbon cycle (Berner, 2001, 2006), Berner (2006) computed that the total N₂ inventory in the atmosphere was nearly invariant over Phanerozoic time (the last 600 Mya). This is in clear contrast to the other major atmospheric component, oxygen, which has varied over the same time from as low as 10% to as high as 40% of the atmosphere (Berner *et al.*, 2003).

Before the colonization of the continents by land plants, most N fixation would have occurred in the oceans. If N-fixing microbes, such as cyanobacteria in mats, had been able to survive on land before the establishment of the ozone shield, then terrestrial N fix-

ation may have commenced long before the Ordovician/Silurian colonization of land by chlorophyte-type plants. This scenario would be compatible with Berner's contention (Berner, 2006) that the N₂ content of the atmosphere has not varied significantly over the entire Phanerozoic Eon. Biological N₂-fixation would have been established at close to its historical average level while photosynthesis gradually increased the oxygen content of the atmosphere, such that major changes in gas ratios would have occurred before the Phanerozoic. Similar scale changes in O₂/N₂ ratios have occurred during the Phanerozoic, but these have been due to changes in O₂, mirrored by large changes in CO₂ concentration.

4.3 Components of the global nitrogen cycle

The nitrogen cycle consists of fluxes (Table 4.2) between the reservoirs (Table 4.1). Because most organisms are not capable of capturing N₂ and "fixing" it into reduced nitrogen for biological processes, the distinction between N₂ (and N₂O) and all other forms of nitrogen is a meaningful one. The most important source of fixed N is biological nitrogen fixation (Table 4.2), which is the sole domain of a few restricted groups of Bacteria and Archaea. Production of fixed N also occurs via lightning and emissions from volcanoes (about 2.8 × 10⁹ mol yr⁻¹

including mid-ocean ridges, hot spots and subduction regions). These natural sources of fixed N are very small (Table 4.2), and are thought to be have been relatively invariant over Earth history (Sano *et al.*, 2001).

It is difficult to describe the preindustrial N cycle quantitatively. The most comprehensive and quantitative evaluation of historical, modern and future N cycling was performed by Galloway *et al.* (2004) who reconstructed the N cycle for 1860, compiled current data for 1993 and forecast future conditions for 2050. We can evaluate the early industrial N cycle by comparing the N budgets for 1860 and 1993. Human activities were already important factors in 1860. Taking the conditions of the late 1990's to represent 100% of the total anthropogenic change up to 1997, Vitousek *et al.* (1997) found that 25% of the total change in deforestation had occurred by ~1700, while 25% of the impact of industrial N fertilizer occurred by ~1975. Although only 16% of total atmospheric emissions were estimated to derive from human activities in 1860, changes in land use (deforestation, annual biomass burning) and concentrated food production had already made indelible marks on the global N cycle by that time.

Human-induced changes in the N cycle are easy to identify (Vitousek *et al.*, 1997):

1 N fertilizer: Organic fertilizers have been used since the beginning of agriculture, but they represent minor recycling of N, rather than net additions to the fixed N inventory. Preindustrial terrestrial biological N fixation (agriculture and natural) was estimated at 120 Tg N yr⁻¹, a value that had decreased about 15% by 1993 (Galloway *et al.*, 2004). Industrial N fixation via the Haber-Bosch process increased rapidly from essentially zero in the 1940s to about 100 Tg annually in 1995 and a further 20% to 121 Tg annually by 2005 (Galloway *et al.*, 2008). Most industrial fixed N (86%) is used to make fertilizer; at least half of global fertilizer applications are now in the developing countries of Asia.

2 Fossil fuel combustion continues to liberate N from the long-term geological reservoirs. Even though the N content of coal and oil is low and variable, the immense net transfer of old organic matter via combustion to the atmosphere now accounts for 24.5 Tg N yr⁻¹, or about a quarter of the total atmospheric emissions. Fossil fuels contributed ~0.3 Tg N yr⁻¹ in 1860 (Galloway *et al.*, 2004).

3 Nitrogen-fixing crops: Cultivation of naturally N-fixing crops has replaced mixed vegetation in natural systems, substantially increasing the total biological N fixation by 32–53 Tg N yr⁻¹ from agriculture (Galloway *et al.*, 2004). Thus total biological N fixation has increased, even though the natural rate has declined (see above).

4 N mobilization by various human activities liberates N from biological storage reservoirs:

deforestation/biomass burning, conversion of forest and savannahs to croplands, drainage of wetlands and peat burning, erosion, all increase biologically available N (Vitousek *et al.*, 1997). By 1993, human activities in total (industrial Haber Bosch process, biological N fixation in agriculture, and fossil fuel burning) had increased the production of fixed N to 156 Tg yr⁻¹ (Galloway *et al.*, 2004), more than doubling the natural rate of new nitrogen production.

Modern emissions of fixed N (NH₃, NO_x) to the atmosphere are dominated by anthropogenic sources, whereas in early industrial times, 1860, natural sources such as lightning and emissions from soil and vegetation dominated (Galloway *et al.*, 2004). The modern flux is dominated by reduced N (NH₃) coming from animals and agriculture and fossil fuel burning. Even in 1860, atmospheric emissions from food and energy production showed a clear fingerprint of human activity (Galloway *et al.*, 2004), and human activity increasingly controls the fixed N fluxes. Human inputs exceeded natural atmospheric emissions of oxidized (NO_x) and reduced N by about a factor of five in the early 1990s, and this dominance is expected to increase to a factor of 10 by 2050 (Galloway *et al.*, 2004).

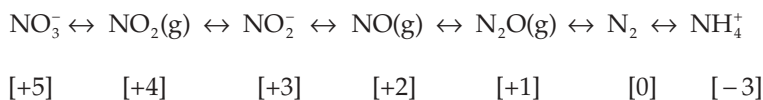
The oceans contribute a small net flux of reduced N (about 6% of total oxidized plus reduced inorganic N atmospheric emissions) to the atmosphere, but receive a quarter of the deposition. Deposition is predominantly in the form of oxidized nitrogen (nitrate or nitric acid) because the reduced forms tend to cycle rapidly and are deposited locally. The remainder is deposited on land, which leads to low-level N enrichment of the entire Earth, even the open ocean, which is far removed from the overwhelmingly terrestrial sources. Duce *et al.* (2008) concluded that anthropogenic N deposition in the open ocean could be responsible for ~3% of annual new primary production. The effects are much stronger on land and in estuaries and coastal oceans, where total N deposition is completely dominated by anthropogenic sources. The majority of these N additions cannot be directly accounted for and are assumed to have accumulated in soils, vegetation or groundwater, or been denitrified (Galloway *et al.*, 2008). Excess N loading from atmospheric deposition has been linked to eutrophication in temperate aquatic systems and to loss of biodiversity in temperate grasslands (Stevens *et al.*, 2004). Vitousek *et al.* (1997) concluded on the basis of an extensive literature review that human alterations of the N cycle had contributed substantially to the acidification of surface waters and accelerated the losses of diversity among terrestrial plants and animals, as well as contributing to long-term changes in estuarine and coastal ecosystems leading to fisheries declines.

Table 4.4 Oxidation states of biogeochemically important N compounds (adapted from Chamiendes and Perdue, 1997)

Compound	Name	N oxidation state
NH ₂ , NH ₄ ⁺	Ammonia, ammonium	-3
R-NH ₃	Amino acids, organic nitrogen polymers	-3
NH ₂ OH	Hydroxylamine	-1
N ₂	Dinitrogen gas	0
N ₂ O	Nitrous oxide	+1
NO	Nitric oxide	+2
HONO, NO ₂ ⁻	Nitrous acid, nitrite	+3
NO ₂	Nitrogen dioxide	+4
N ₂ O ₅ , HNO ₃ , NO ₃ ⁻	Dinitrogen pentoxide, nitric acid, nitrate	+5

4.4 Nitrogen redox chemistry

With an atomic number of 7 and an atomic weight of 14, nitrogen has five valence electrons and occurs in oxidation states ranging from -3 to +5. Nitrogen occurs in nature in six of its eight possible oxidation states (Table 4.4), and the odd oxidation states are most common, except for the ground state N₂ with an oxidation state of zero.



Nitrogen in the biosphere is usually in the -3 oxidation state as the amino group of biomolecules, and this form of nitrogen is most abundant in rocks and fossil organic matter. There are no N-containing primary minerals of any significance in the lithosphere, and, other than N₂ in the atmosphere and dissolved in natural waters, inorganic nitrogen is most abundant as nitrate in seawater and other aquatic systems. The most extreme oxidation states are those that are stable in the most oxidizing (e.g. ocean water) and most reducing (e.g. anoxic sediments) environments.

4.5 Biological reactions of the nitrogen cycle

Many organisms produce and obtain energy from the oxidation-reduction reactions that convert nitrogen among its stable states, and in so doing, control the distribution of nitrogen on Earth. These processes are described below, and they make up the biological nitrogen cycle, which is shown in Fig. 4.1. Most steps in the biological N cycle are uniquely the domain of microbes. The main role for macroorganisms is in the transfer of ammonium among various diverse organic nitrogen compounds, but the physiology of eukaryotes does very little to influence the nitrogen budget on either a local or

global basis. Some eukaryotes, such as humans, do have a huge effect on the N budget, however, as explained above, but this is not directly related to their metabolism or biochemical peculiarities.

4.5.1 Nitrogen fixation

N₂ is a relatively inert gas, due to the strength of the triple bond that joins the two atoms. The use of molecular nitrogen by organisms thus requires substantial energy expenditure, usually estimated at the cost of 16 ATP (and eight electrons) per molecule N₂ reduced. The only known biological mechanism of N fixation involves the enzyme nitrogenase, which is composed of two multi-subunit proteins. The most common form of Component I, which donates electrons to N₂, contains both iron and molybdenum at the active site. Alternative forms contain either iron and vanadium or iron alone. Component I proteins (nitrogenase, the protein that actually splits nitrogen) are coordinated with those of Component II (azoferredoxin, also known as dinitrogenase reductase, the protein that donates electrons to nitrogenase). Thus nitrogen fixation not only requires energy but also a high iron quota. The nitrogenase protein complex is

highly conserved, suggesting an ancient origin. Some degree of lateral gene transfer has no doubt occurred and accounts for the wide phylogenetic distribution of nitrogenase, but a high degree of coherence between the phylogenies inferred from 16S rRNA genes and nitrogenase genes supports early evolution from a common ancestor (Raymond *et al.*, 2004).

The first step in nitrogen fixation is the nitrogenase-catalysed reduction of N₂ to NH₃ within the cells of a restricted group of microbes.



The best known systems involve up to 20 proteins that must be coregulated, and the system is inducible, usually expressed only under nitrogen limitation. Recent reports of high nitrogen fixation rates in the presence of high external ammonium concentrations imply more complex regulation.

As for many transformations in the nitrogen cycle, study of nitrogen fixation has benefitted from the use of specific molecular assays to detect the genes that encode the key enzymes. Identification of nitrogen fixing microbes in the environment has focused on *nifH*, the gene that encodes azoferredoxin. Great diversity among

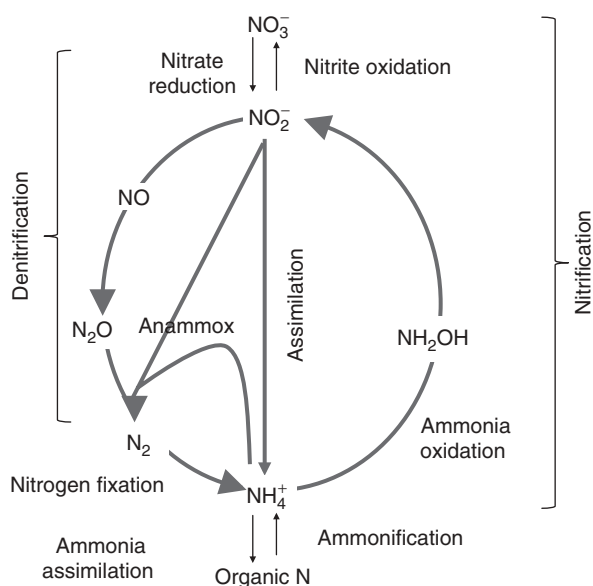


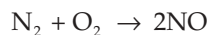
Figure 4.1 Diagram of the biological nitrogen cycle showing the main inorganic forms in which nitrogen occurs in natural and anthropogenically influenced environments.

nifH genes in a range of natural habitats has been reported, and the degree of diversity is not obviously correlated with the degree of nitrogen limitation or the observed rates of N fixation in the environment (Zehr *et al.*, 2003). Numerous and diverse nitrogenase genes have been detected in microbes living in environments where nitrogen is rarely if ever limiting, and where nitrogen fixation is rarely detected, suggesting (1) that organisms maintain the genetic capability for this energy-expensive process even when it is not used for long periods or (2) the presence of the genetic capability is not indicative of the actual process occurring in a particular place because microbes are motile and their distribution is determined by dispersal, depending on the physics of the system.

Most environmentally important nitrogen fixers are identified as (1) endosymbionts with leguminous plants (including crops and trees), (2) cyanobacteria, especially filamentous types in the ocean, microbial mats and lakes, (3) anaerobes, including methanogens, sulfate reducers and fermenters, and (4) free living soil bacteria such as *Azotobacter* (Zehr *et al.*, 2003). Thus, nitrogen fixers are distinguished mainly by metabolic attributes not associated with nitrogen fixation, and they all have close relatives with similar metabolic characteristics that are unable to fix nitrogen. The ecological advantage of nitrogen fixation enables growth under conditions that would be prohibitive for non-fixers, but comes at the cost of a high demand for reducing power.

The triple bond of N_2 can also be broken by abiotic processes, including lightning and combustion of fossil

fuels and forest fires. In this case, the resulting fixed N is in the form of NO gas.



Using very high temperatures and pressures, the industrial fixation of N_2 to NH_3 by the Haber–Bosch process has doubled the global rate of atmospheric N_2 fixation since 1950, a major factor in the historical changes to the global N cycle (see above).

4.5.2 Ammonium assimilation

Ammonium is assimilated into organic matter by microbes during both autotrophic and heterotrophic growth. Several enzymes are involved in the process, and they all result in the assimilation of ammonium into amino acids, which are then incorporated into proteins. Proteins are the basis of enzymes, which do the catalytic work of biological processes, and they are the main reservoir of N in living biomass. N is also an important component of the nucleotides that comprise DNA and RNA.

Although it is dangerous to make wide generalizations, it is probably safe to say that the vast majority of, if not all, microbes are capable of ammonium assimilation. For many organisms, it is the preferred (or only) inorganic nitrogen source, probably because ammonium is at the same oxidation state as amino acids, and thus little metabolic energy is required for its incorporation.

4.5.3 Ammonification or mineralization

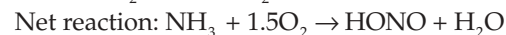
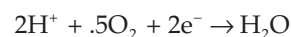
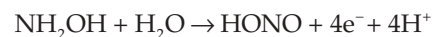
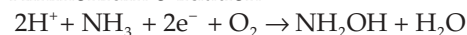
The nitrogen in biomass is returned to the ecosystem by degradation of the macromolecules assembled from proteins and nucleotides. This is referred to as mineralization because it returns the organic N to the inorganic, or mineral, form. The essential step is deamination, removal of the amino group from proteins and amino acids, which results in the release of ammonium. This process is excretion, which occurs during recycling of macromolecules during growth and metabolism of organisms. Ammonium is also returned to the environment when organic matter is degraded by heterotrophic microbes. Because it is in the reduced form required for incorporation into biomass with minimal energy expenditure, ammonium rarely accumulates in the environment, but is rapidly recycled into biomass. An exception occurs in anoxic sediments, where ammonium accumulates to high levels, due to the absence of oxidants needed to support microbial utilization of the ammonium.

4.5.4 Nitrification

For a few specialized microbes, ammonium serves as a source of reducing power and not simply a source of

nitrogen for building biomass. Nitrifying bacteria oxidize ammonium to nitrite, and then to nitrate. The two steps are performed by two different groups of organisms; no single organism is known to catalyse the complete conversion of ammonium to nitrate. All of the bacteria that perform nitrification are primarily autotrophic, utilizing the reducing power of ammonium or nitrite to fix CO₂ by the Calvin cycle. While slow and inefficient growth results, the process has an important effect on the N cycle, by catalyzing the net conversion of ammonium to nitrate. Recently, some Archaea have been shown to be able to oxidize ammonium. The pathway of archaeal ammonium oxidation is still unknown, but it appears to be quite different from the well known pathway in bacteria. Archaeal ammonia oxidizers are also autotrophic, fixing CO₂ by the 3-hydroxypropionate pathway, but they may also be mixotrophic, relying on organic carbon assimilation to an unknown degree. The reactions of bacterial nitrification are shown below.

Ammonium oxidation



Nitrite oxidation



In oxic environments, nitrate is the stable form, and it accumulations up to tens or hundreds of micromolar concentrations in seawater and lakes. Although not shown in the reactions above, ammonia-oxidizing bacteria also produce nitrous oxide via two pathways. One is a reductive pathway that is analogous to the nitrite reduction of denitrifiers (see next section) and the other is a less well characterized pathway thought to involve decomposition of hydroxylamine. The proportion of ammonium that is released as N₂O vs. NO₂⁻ is negatively correlated with oxygen concentration at high cell densities in culture. It has proven very difficult, however, to determine what controls N₂O production in nitrifiers and the correlation with oxygen concentration may not apply to naturally occurring population densities. N₂O production has been detected in cultivated Archaeal ammonia (Santoro et al., 2011). Archaeal ammonia oxidizers may thus play an important role in producing N₂O in aquatic and terrestrial environments.

The key enzyme in ammonium oxidation by both Bacteria and Archaea is the first enzyme in the process, ammonia monooxygenase, encoded by the *amoABC* genes. *amoA* has been widely used as a genetic marker for the organisms responsible for the process (Ward and

O'Mullan, 2005). As is usual for molecular studies, a broader diversity of *amoA* genes has been discovered than was known from cultivated microbes. The ammonia-oxidizing Archaea were first identified from their genes, and only later cultivated (Konneke et al., 2005). They appear to be very abundant in both aquatic and terrestrial environments, and in some environments, much more abundant than their better known bacterial counterparts (Wuchter et al., 2006, Mincer et al., 2007). Cultivated aerobic nitrifiers have relatively long generation times, on the order of a day, which is consistent with the low energy yield of ammonium and nitrite oxidation, and the energetic costs of CO₂ fixation.

Nitrosomonas and *Nitrobacter* have long been identified as the typical, and presumably most important, genera of ammonia-oxidizing and nitrite-oxidizing bacteria, respectively. These are well characterized from laboratory studies as capable of true chemolithoautotrophy – obtaining all reducing power from either ammonium or nitrite, and all carbon from CO₂. Genetic investigations, however, have shown that the cultivated types of ammonia oxidizers are not representative of those in the environment. Among the ammonia-oxidizing bacteria, the most prevalent in the ocean is a *Nitrosospira* type (Bano and Hollibaugh, 2000; O'Mullan and Ward, 2005) that is not present in culture collections and has proven resistant to cultivation. This lack of cultivation might be because its lifestyle and metabolic requirements differ from the cultured forms and researchers have been unable to determine their requirements.

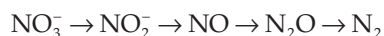
In addition to *Nitrobacter*, a more recently discovered and cultivated genus known as *Nitrospira* is also abundant in the environment. A relative of this genus appears to be the most abundant nitrite-oxidizing bacterium in the ocean (Mincer et al., 2007)

The genomes of several cultivated nitrifying bacteria, both ammonia and nitrite oxidizers, have been completely sequenced. As expected, the complete pathways that allow chemolithoautotrophic growth on either ammonium or nitrite and CO₂ are represented in the genomes. Although they also possess a complete tricarboxylic acid cycle, which is usually involved in degradation of carbon substrates for heterotrophic growth, the nitrifiers appear to favour an autotrophic lifestyle due to a dearth of transporters for organic carbon molecules. Even the nitrite oxidizers that were suspected of mixotrophic growth are not capable of growth on 6-carbon compounds and appear to have very limited heterotrophic capabilities. The heterotrophic capabilities of the ammonia oxidizing Archaea are uncertain at present.

4.5.5 Denitrification

The nitrate formed by nitrification, or resulting from the oxidation of N in the atmosphere, can be utilized by a

wide range of bacteria as a respiratory substrate. Most denitrifying organisms are bacteria, and most are facultative aerobes. They respire oxygen when oxygen is present, but when oxygen is depleted, denitrifiers switch to an anaerobic respiration in which NO_3^- is the initial electron acceptor. Complete denitrifiers reduce nitrate sequentially to nitrite, then to nitric oxide, nitrous oxide and finally to dinitrogen gas:



Each intermediate is used as a respiratory substrate, but growth is best on nitrate. The strong bonds connecting two N atoms are established at the N_2O step; N_2O is a linear molecule in which the nitrogen atoms are linked by a double bond. Dinitrogen gas is the final product, thus completing the N cycle and returning N_2 gas to the atmosphere and removing it from biological availability. Denitrification (and anammox, see below) is thus the budgetary balance for nitrogen fixation. The balance between nitrogen fixation and denitrification determines the total inventory of fixed N on Earth. Whether the nitrogen budget is in balance or not is the subject of much current debate (see below).

N_2O is an obligate intermediate in the complete denitrification pathway, and it is assumed that incomplete denitrification is responsible for the accumulations of N_2O that occur in anoxic environments. Because the enzymatic pathway by which N_2O is produced in denitrifiers is homologous with the reductive pathway in bacterial nitrifiers, however, it is difficult to determine which organisms are responsible for N_2O production in stratified environments.

Most denitrifiers are heterotrophs and thus utilize organic carbon for energy and to build biomass. The complete degradation of organic matter by denitrification results in the net production of ammonium (ammonification from nitrogen-bearing organic materials). A few kinds of denitrifying bacteria couple nitrate respiration to the oxidation of an inorganic substrate and use the reducing power from that substrate to fix CO_2 , thus achieving a lithoautotrophic lifestyle. Reduced sulfur is usually the lithotrophic substrate, and these organisms are well suited for life at the anoxic interface of calm water and sediments.

The diversity and distribution of denitrifying bacteria have been investigated from a genetic approach, focusing on genes in the nitrate reduction pathway. Three genes have been widely applied for this purpose. Two of them encode the reduction of nitrite to nitric oxide; this is the only step in the pathway for which two different enzymes are known. The genes that encode these enzymes are known as *nir*, and genetic investigations suggest that there are thousands of different versions, as

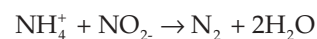
proxies for thousands of different kinds of bacteria capable of denitrification, in the environment. The third signature gene is *nosZ*, which encodes the nitrous oxide reduction enzyme. It is also diverse and widely distributed, although not all denitrifiers are capable of reducing N_2O .

Bacteria capable of denitrification are very common in aquatic and terrestrial environments, especially as many of them grow well by aerobic respiration. In oxygen depleted environments, such as found in hemipelagic marine sediments, and the three major oxygen minimum zones of the world ocean, denitrifiers can be a significant if not dominant portion of the total microbial community.

A related metabolism is that of dissimilatory nitrate reduction to ammonium (DNRA) in which the first step is nitrate respiration as in denitrifiers. The nitrite is then reduced directly to ammonium, without the intermediates of denitrification. Organisms that carry out DNRA perform the process as a respiratory mechanism, and can couple it to heterotrophic or autotrophic growth based on sulfur oxidation.

4.5.6 Anammox

Anaerobic ammonium oxidation (anammox), in which ammonium is oxidized microbially using nitrite as an oxidant, is a thermodynamically favourable reaction, but until 1995, organisms responsible for it had never been detected or identified.



Biogeochemical distributions in sediments and across oxic–anoxic interfaces in some aquatic environments had suggested that such a process was occurring (Richards, 1965; Bender *et al.*, 1977). Organisms capable of the process were first identified in wastewater treatment systems (Mulder *et al.*, 1995; van de Graaf *et al.*, 1995), and have now been found in marine, terrestrial and freshwater environments as well. The anammox organisms are all members of a previously obscure phylum of bacteria known as the Planctomycetes. Typical of Planctomycetes, the anammox organisms possess unique intracellular membrane bound compartments, and in the case of anammox, these appear to be linked to their unique metabolism. The oxidation of ammonium with nitrite involves hydrazine as an intermediate, an explosive known more commonly as rocket fuel. The oxidation reactions take place inside the anammoxosome (van Niftrik *et al.*, 2004), a membrane-bound compartment. The membrane lipids of the anammox cell contain novel lipids known as ladderanes, from their ladder like structure. These have now been detected in ancient sediments.

Four different genera of anaerobic ammonia-oxidizing Planctomycetes are known, but it appears that only one of them is common in natural environments. Those in culture grow very slowly, with minimum generation times of several days to a few weeks. This is much slower than most other bacteria under optimal conditions, and it remains to be determined whether anammox bacteria in nature grow at a similarly low rate.

Anammox bacteria from wastewater systems live in apparently obligate consortia with other organisms, usually denitrifiers or aerobic ammonia-oxidizing bacteria (Sliekers *et al.*, 2002, Third *et al.*, 2001). Wastewater influent contains very high ammonium levels and trace levels of oxygen. The ammonia-oxidizing bacteria consume the oxygen in the process of oxidizing some of the ammonium to nitrite. The anammox bacteria combine the nitrite with ammonium to produce N_2 gas at the very low levels of oxygen maintained by the ammonia-oxidizing bacteria. Thus, anammox performs the same role in the nitrogen budget as denitrification under conditions usually associated with denitrification.

When it became clear that the anammox process occurs in nature, it was also recognized that previous estimates of denitrification rates had probably also included anammox. In many ways, it probably does not matter whether denitrifiers or anammox bacteria are responsible for the loss of fixed nitrogen. But because the two groups of organisms have such different lifestyles and metabolic constraints, it might make a difference to environmental regulation and response to changing environmental conditions. Both groups perform their N_2 production under very low or zero oxygen conditions, but denitrifiers usually have an alternative metabolism and can live quite well as facultative aerobes; not so for anammox bacteria, which appear to live only anaerobically (Kartal *et al.*, 2008). Denitrifiers are versatile heterotrophs, capable of utilizing a wide variety of organic carbon compounds; anammox bacteria are obligate autotrophs. Thus denitrification has an important role in the carbon cycle, which is the degradation of organic matter under oxygen poor conditions. In contrast, the slow, energy-limited growth of anammox bacteria is likely to have a negligible effect on the carbon cycle directly. Denitrifiers produce and consume N_2O , while it does not appear that N_2O is involved in the nitrogen transformations of anammox. All of these differences suggest that denitrification and anammox respond differently to environmental conditions such as supply and concentration of oxygen, organic carbon, etc. and leave a different imprint on the chemistry of their environment.

Despite the complications of a consortium, the genome of an anammox bacterium has been published (Strous *et al.*, 2006). The pathways by which the anammox bacteria make a living were not immediately obvious, even from the complete genome. The

organisms grow as obligate autotrophs, fixing CO_2 , although they are capable of metabolizing a few simple organic acids. Unlike the aerobic nitrifiers who utilize the Calvin cycle for CO_2 fixation, anammox uses the acetyl CoA pathway, although the genes in this pathway were not highly expressed during growth. Similarly, the enzymes responsible for the oxidation of hydrazine are still unclear.

Because the key enzymes and genes involved in the nitrogen metabolism of anammox have only recently been discovered, molecular detection of anammox organisms in the environment has relied on their 16S rRNA genes, depending on the assumption that only the few Planctomycetes genera, which can be defined by their 16S rRNA genes, are involved. On the basis of 16S rRNA genes, only the genus *Scalindua* appears to be abundant in the environment (Schmid *et al.*, 2007). Ladderane lipids are also used as a unique biomarker for anammox, but these cannot distinguish between the genera.

4.5.7 Linked reactions in the biological nitrogen cycle

From this discussion of the biological N cycle, it is clear that several of the steps are tightly coupled and directly dependent upon each other. Some of these interactions are interesting because they lead to counterintuitive distributions of N compounds and concentrations that seem to contrast with the magnitude of the fluxes. We explore some of the specific coupled reactions below (Fig. 4.2).

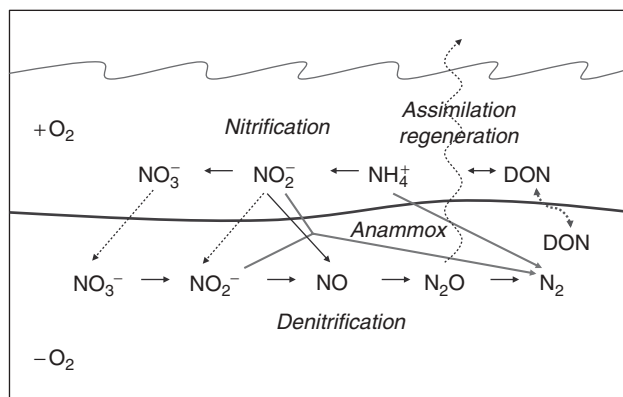


Figure 4.2 Transformations in the nitrogen cycle emphasizing the linkages between processes that occur in aquatic environments. Denitrification and anammox both occur in anoxic conditions, but are linked ultimately to nitrification (favoured under oxic conditions) for supply of substrates. Ammonium, the initial substrate for nitrification, is regenerated by mineralization of organic matter, but is also assimilated as the preferred N source by many organisms. Solid arrows signify microbial transformations while dotted arrows imply diffusion.

4.5.7.1 Coupled nitrification/denitrification

The oxidation of ammonium to nitrite and nitrate produces substrates that can be used for respiration by conventional denitrifying bacteria as well as by anammox bacteria. In order to be coupled, however, the organisms must be situated in a gradient such that oxygen supply is sufficient to allow aerobic nitrification but not high enough to inhibit the anaerobic processes of denitrification and anammox. Stable gradients that allow this coupling occur at sediment–water interfaces in the coastal ocean and lakes, in the stratified water column of oxygen minimum zones in the oceans, in stratified basins and lakes, and in the interstices of soils. The result of coupling these processes across diffusional gradients is the net consumption of ammonium and the net production of N_2 gas. While this represents a net removal of fixed nitrogen, this is sometimes desirable in estuarine and coastal environments where excess N loading can lead to increased primary production and eutrophication. In N limited systems, as N input increases, organic production increases, leading to increased oxygen demand for its decomposition. Under aerobic conditions, decomposition produces the ammonium that drives the coupled nitrification/denitrification cycle. If organic loading leads to an oxygen demand in excess of supply by ventilation or mixing, the nitrification part of the couple will be inhibited, and the ammonium will tend to accumulate in the system. Fixed N will then be maintained in the system instead of being removed as N_2 via denitrification or anammox. Under anoxic conditions, it should be possible for anammox to oxidize this ammonium anaerobically, but many anoxic systems accumulate high levels of ammonium, suggesting that anammox is limited by the absence of an oxidized N compound.

4.5.7.2 Denitrification/anammox

While denitrification and anammox have the same net effect on the N cycle (i.e., the loss of fixed nitrogen through N_2 production), their relative contributions to this flux are poorly known and probably quite variable among ecosystems. Denitrification depends on substrates produced by aerobic nitrification, while anammox depends on substrates that, under anoxic conditions, could be supplied by denitrification. Ammonium is produced by ammonification of organic compounds during anaerobic growth of denitrifiers, and the nitrite required for anammox is an intermediate in denitrifier respiration. Anammox apparently must be coupled to nitrification, dissimilatory nitrate reduction to ammonium or denitrification for supply of its N substrates, and the natural lifestyle of anammox organisms is thus not surprisingly as members of consortia.

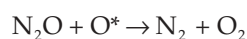
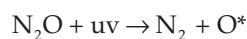
4.5.7.3 Ammonium assimilation/regeneration

Ammonium has already been identified as a key substrate and product in the coupled N cycle reactions described above. In both aerobic and anaerobic environments, ammonium cycles rapidly between biomass and the inorganic pool without being involved in redox chemistry. As the most favourable N compound for assimilation into biomass, as well as the first product of organic nitrogen degradation, ammonium is usually transferred rapidly by assimilation and regeneration. Thus its concentration in the environment is usually very low under oxic conditions, despite its involvement in very large fluxes. The rates of ammonium oxidation and assimilation may therefore be more correlated with ammonium flux than with actual concentrations.

4.6 Atmospheric nitrogen chemistry

The nitrogen cycle in aquatic and terrestrial environments is dominated by the biological processes described above. The nitrogen cycle of the atmosphere, however, is dominated by abiotic interactions, that are nevertheless closely coupled to biological processes in the water and on land. The inorganic nitrogen gases NO , NO_2 and N_2O are all produced as intermediates or side reactions in nitrification and denitrification, and ammonia volatilization adds another reactive N molecule to the atmosphere. For an excellent discussion and quantitative treatment of the effect of biological and human processes on atmospheric nitrogen chemistry, see Chamiedes and Perdue (1997).

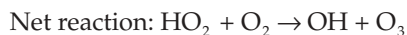
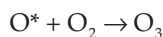
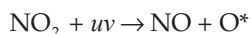
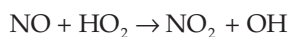
Nitrous oxide is the most stable of the nitrogen gases other than N_2 , and it is relatively unreactive in the troposphere. It is emitted to the atmosphere at the Earth's surface through biological and abiotic processes (fossil fuel burning) and is destroyed only after being transported to the stratosphere, where it is destroyed by photochemical reactions. In the troposphere, most N_2O is converted to N_2 , either by direct photolysis by UV light, or by reaction with singlet oxygen, an interaction that can also yield NO :



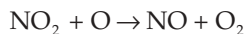
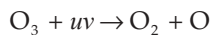
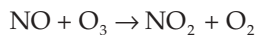
The abundance of N_2O in the stratosphere is of interest for two reasons. First, N_2O itself is a powerful greenhouse gas with a life time of about 150 years, comparable to that of CO_2 in the atmosphere, but with a radiative forcing equivalent to ~200-fold that of CO_2 on a per molecule basis. The concentration of CO_2 is about 1000-fold higher than that of N_2O , but the total radiative forcing due to N_2O is almost 20% of that attributed to CO_2 (IPCC, 2007).

The concentration of N_2O in the atmosphere has increased approximately 16%, from about 275 parts per billion by volume (ppbv) before the industrial revolution to about 320 ppbv today. Some of this increase is due to the oxidative liberation of trace N in fossil fuels during energy generation. Some N_2O production is also due to agricultural practices, and results ultimately from increased fertilizer additions to partially water-logged soils, where coupled nitrification/denitrification under low oxygen conditions results in the net release of N_2O .

The second reason that even trace levels of N_2O in the atmosphere are significant is that NO produced by interaction with oxygen atoms exerts a controlling influence over the concentration of ozone in the stratosphere. In the troposphere and lower stratosphere, where ozone concentrations are relatively low, NO contributes to ozone production by interaction with hydroperoxy radicals:



NO also causes the catalytic destruction of ozone in the stratosphere.



Atmospheric chemists tend to consider all the nitrogen gases that do not include N-N bonds as NO_y , which includes NO_x (NO and NO_2) and NO_z (NO_3 , N_2O_3 , HONO, HNO_3 , RNO_3 , etc). NO_y gases are very reactive and are involved in a complex suite of interactions that include the oxidation of volatile organic carbon compounds and CO, and the generation of ozone and hydrogen peroxide. NO_y compounds are involved in the production of photochemical smog in the troposphere.

4.7 Summary and areas for future research

The global nitrogen cycle includes important geological and biological components, in which relatively small biological fluxes control the availability of nitrogen between large reservoirs. The versatile redox chemistry of nitrogen means that it occurs in a wide range of compounds, whose concentrations are mostly controlled by microbial transformations.

As mentioned in the discussion of individual processes in the biological N cycle above (and described

in more detail in Chapter 13), nature is provided with an astounding diversity of microbes that are involved in every step of the N cycle. Does each variety of, for example, denitrifier or nitrogen fixer occupy a unique niche, the dimensions of which we are not yet capable of identifying? Does this immense diversity provide resilience for ecosystem function, such that overall rates of denitrification or nitrogen fixation are not seriously perturbed when the environment changes, although the suite of organisms performing the reactions changes to those more suitable for the new environment? Among the diverse organisms with the potential for each of the N cycle reactions, it often appears that just a few are dominant in a particular environment. Are these key players important in similar ecosystems around the world? Are the key players interchangeable such that dominance results from chance interactions between the diverse assemblage and some key environmental factor that may change at any time?

The inventory of fixed nitrogen on Earth is controlled by the balance between nitrogen fixation and denitrification/anammox. There is no *a priori* reason to think that the two processes should always be equivalent, but the ramifications of life on Earth of a long-term imbalance would be substantial. Already one of the least abundant elements in the lithosphere, in contrast to its relatively high requirement for life, further depletion of nitrogen by excess denitrification would constrain production on a global basis. As the macronutrient most limiting for life, any sustained additions through excess nitrogen fixation would likely have large effects on the composition and growth rates of natural communities. This could lead to increased CO_2 consumption and to limitation by other, more trace level, elements (Chapter 6).

Nitrous oxide is produced and consumed in both nitrification and denitrification, and although the pathways are fairly well known in cultivated organisms, it is still not clear to what degree the two processes are involved in N_2O production in nature. In the ocean, for example, highest N_2O concentrations are found in oxygen-limited environments. These are the same locations where coupled nitrification/denitrification occurs, so it is not obvious whether both or one of the processes is responsible for net N_2O production. If excess N loading to the coastal or even open ocean were to lead to increased production of organic matter, which subsequently resulted in increased oxygen demand and the expansion of anoxic environments, would this lead to an increase in N_2O release to the atmosphere (Duce *et al.*, 2008)?

Through net production of fixed nitrogen and net fertilization of natural and managed ecosystems, humans have already perturbed the natural nitrogen cycle. Even beyond changes to the biological N cycle, these changes have the potential to influence atmospheric chemistry

far into the future as well. Although the NO_y compounds are highly reactive and short lived, N_2O has very long residence time in the atmosphere. Because it is the source of some of the NO_y compounds via its photochemistry, the long-term increase in N_2O already documented has probably already affected the concentration of trace NO_y compounds in the atmosphere, and thus their reaction rates. Global warming aside, what will be the long-term effects of the anthropogenic nitrogen perturbations on the atmosphere and the quality of the environment?

References

- Andrea MO, Merlet P (2001) Emission of trace gases and aerosols from biomass burning. *Global Biogeochemical Cycles* **15**, 955–966.
- Archer DA, Morford JL, Emerson SR (2002) A model of suboxic sedimentary diagenesis suitable for automatic tuning and gridded global domains. *Global Biogeochemical Cycles* **16**, doi:10.1029/2000GB001288.
- Bano N, Hollibaugh JT (2000) Diversity and distribution of DNA sequences with affinity to ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in the Arctic Ocean. *Applied and Environmental Microbiology* **66**, 1960–1969.
- Batjes NH (1996) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* **47**, 151–163.
- Bender ML, Fanning KA, Froelich PN, Heath GR, Maynard V (1977) Interstitial nitrate profiles and oxidation of sedimentary organic matter in the eastern equatorial Atlantic. *Science* **195**, 605–609.
- Berner RA, Beerling DJ, Dudley R, Robinson MG, Wildman, RA Jr, (2003) Phanerozoic atmospheric oxygen. *Annual Review of Earth and Planetary Science* **31**, 105–134.
- Berner RA (2001) Modeling atmospheric O_2 over Phanerozoic time. *Geochimica et Cosmochimica Acta* **65**, 685–694.
- Berner RA (2006) Geological nitrogen cycle and atmospheric N_2 over Phanerozoic time. *Geology* **34**, 413–415
- Boyd SR (2001) Nitrogen in future biosphere studies. *Chemical Geology* **176**, 1–30.
- Brandes JA, Devol AH (2002) A global marine fixed-nitrogen isotopic budget: implications for Holocene nitrogen cycling. *Global Biogeochemical Cycles* **4**, 1120–1134.
- Capone DG (2001) Marine nitrogen fixation: what's the fuss? *Current Opinion in Microbiology* **4**, 241–348.
- Chamiedes WL, Perdue EM (1997) *Biogeochemical Cycles: A computer-interactive study of earth system science and global change*. Oxford University Press, Oxford
- Cleveland CC, Townsend AR, Schimel DS, et al. (1999) Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemical Cycles* **13**, 623–645.
- Codispoti LA (2007) An oceanic fixed nitrogen sink exceeding 400 Tg N a^{-1} vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences* **4**, 233–253.
- Duce RA, et al (2008) Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* **320**, 893–897.
- Deusch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP (2007) Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* **445**, 163–167.
- Emerson S, Stump C, Johnson B, Karl DM (2002) In situ determination of oxygen and nitrogen dynamics in the upper ocean. *Deep-Sea Research I* **49**, 941–952.
- Galloway JN, Dentener FJ, Capone DB, et al. (2004) Nitrogen cycles: past, present and future. *Biogeochemistry* **70**, 153–226.
- Galloway, JN, Townsend AR, Erisman JW, et al. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**, 889–892.
- IPCC (2007) *Climate Change 2007: Synthesis Report*. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland, 104 pp.
- Jickels T (2006) The role of air-sea exchange in the marine nitrogen cycle. *Biogeosciences* **3**, 271–280.
- Kartal G, Keltjens KT, Jetten MSM (2008) The metabolism of anammox. In: *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd, Chichester. doi: 10.1002/9780470015902.a0021315.
- Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**, 543–546.
- Lerman A, Mackenzie FT, Ver LM (2004) Coupling of the perturbed C-N-P cycles in industrial time. *Aquatic Geochemistry* **10**, 3–32.
- Li L, Bebout GE, Idleman BD (2007) Nitrogen concentration $\delta^{15}\text{N}$ of altered oceanic crust obtained on ODP Legs 129 and 185: Insights into alteration-related nitrogen enrichment and the nitrogen subduction budget. *Geochimica et Cosmochimica Acta* **71**, 2344–2360.
- Mackenzie FT (1998) *Our changing planet: An introduction to Earth system science and environmental change*, 2nd edn. Prentice-Hall, Upper Saddle River, NJ.
- Middleburg JJ, Soetaert K, Herman PMJ, Heip CHR (1996) Denitrification in marine sediments: A model study. *Global Biogeochemical Cycles* **10**, 661–673.
- Mincer TJ, Church MJ, Taylor LT, Preston C, Karl DM, Delong EF (2007) Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the north Pacific subtropical gyre. *Environmental Microbiology* **9**, 1162–1175.
- Mulder, A, van de Graaf AA, Robertson LA, Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiology Ecology* **16**, 177–184.
- O'Mullan GD, Ward, BB (2005) Relationship of temporal and spatial variabilities of ammonia-oxidizing bacteria to nitrification rates in Monterey Bay, CA. *Applied and Environmental Microbiology* **71**, 697–705.
- Ourisson G, Rohmer M, Poralla K (1987) Prokaryotic hopanoids and other polyterpenoid sterol surrogates. *Annual Reviews of Microbiology* **41**, 301–333.
- Raymond J, Siefert JL, Staples CR, Blankship, R E (2004) The natural history of nitrogen fixation. *Molecular Biology and Evolution* **21**, 541–554.
- Richards FA (1965) Anoxic basins and fjords. In: *Chemical Oceanography* (Eds. Riley JP and Skirrow G). Academic Press, London, pp. 611–645.
- Romer M (1993) The biosynthesis of triterpenoids of the hopane series in the Eubacteria: a mine of new enzyme reactions. *Pure and Applied Chemistry* **65**, 1293–1298.

- Sano Y, Takahata N, Nishio Y, Fischer TP, Williams SN (2001) Volcanic flux of nitrogen from the Earth. *Chemical Geology* **171**, 263–271.
- Santoro AE, Buchwald C, McIlvin MR, Casciotti KL (2011) Isotopic signature of N₂O produced by marine ammonia-oxidizing Archaea. *Science* **333**, 1282–1285.
- Schmid MC, Risgaard-Petersen N, *et al.* (2007) Anaerobic ammonium-oxidizing bacteria in marine environments: widespread occurrence but low diversity. *Environmental Microbiology* **9**, 1476–1484.
- Schumann U, Huntreiser H (2007) The global lightning-induced nitrogen oxides source. *Atmospheric Chemistry and Physics* **7**, 3823–2907.
- Sliekers AL, Derwort N, Gomez JLC, Strous M, Kuenen JG, Jetten MSM (2002) Completely autotrophic nitrogen removal over nitrite in one single reactor, *Water Research* **36**, 2475–2482.
- Sorai M, Yoshida N, Ishikawa M (2007) Biogeochemical simulation of nitrous oxide cycle based on the major nitrogen processes. *Journal of Geophysical Research* **112**, doi: 10.1029/2005JG000109.
- Stevens CJ, Dise, NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science* **303**(5665), 1876–1879.
- Strous M, Pelletier E, Mangenot S, *et al.* (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature* **440**, 790–794.
- Third KA, Sliekers AO, Kuenen JG, Jetten MSM (2001) The CANON system (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: Interaction and competition between three groups of bacteria. *Systematic and Applied Microbiology* **24**, 588–596.
- van de Graaf AA, deBruijn P, Mulder, A, *et al.* (1995) Anaerobic ammonium oxidation is a biologically mediated process. *Applied and Environmental Microbiology* **61**, 1246–1251.
- van Niftrik LA, Fuerst JA, Sinninghe Damste JS, Kuenen, JG, Jetten MSM, Strous M (2004) The anammoxosome: an intracytoplasmic compartment in anammox bacteria. *FEMS Microbiological Letters* **233**, 7–13.
- Vitusek PM, Howarth RW, Likens GE, *et al.* (1997) Human alteration of the global nitrogen cycle: causes and consequences *Issues in Ecology* **1**, 1–17.
- Ward BB, O'Mullan GD (2005) Community level analysis: Genetic and biogeochemical approaches to investigate community composition and function in aerobic ammonia oxidation. In: *Methods in Enzymology* **397**, 395–413.
- Wedepohl KH (1995) The composition of the continental crust. *Geochimica et Cosmochimica Acta* **59**, 1217–1232.
- Wuchter C, Abbas B, Coolen MJL, *et al.* (2006) Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences, USA* **103**, 12317–12322.
- Zehr JP, Jenkins BD, Short SN, Steward GF (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environmental Microbiology* **5**, 539–554.