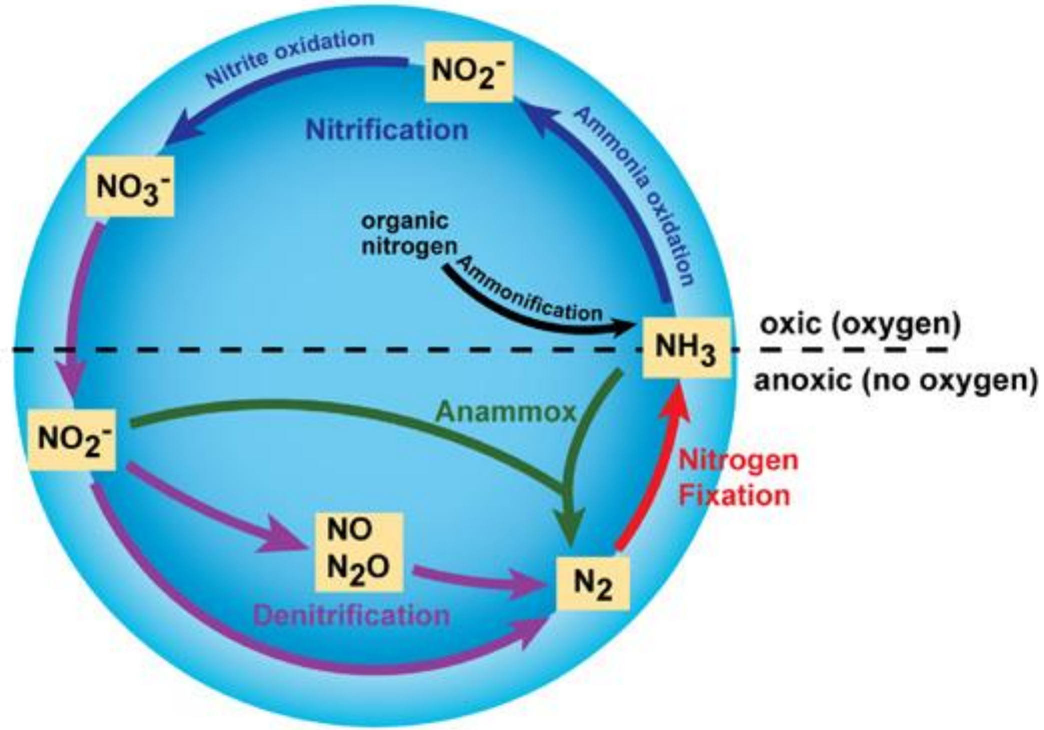
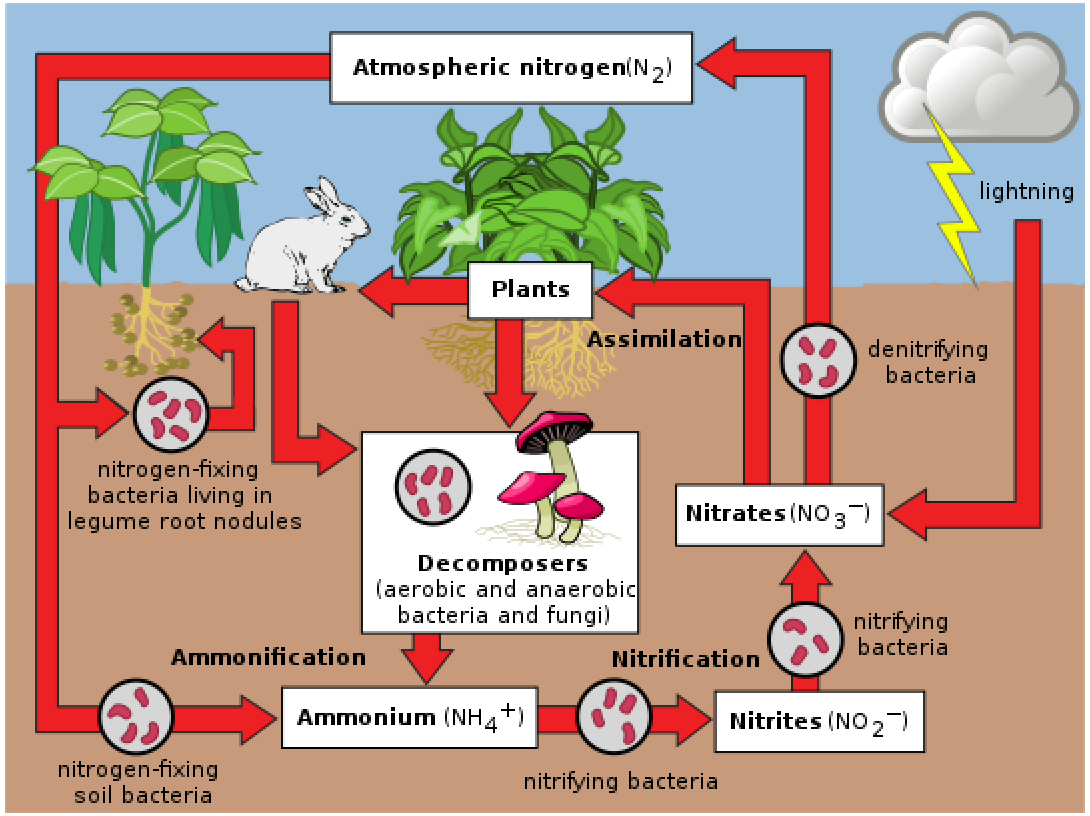
Nitrogen Cycle

Nitrogen Cycle

- The **nitrogen cycle** is the biogeochemical cycle by which nitrogen is converted into multiple chemical forms as it circulates among atmospheric, terrestrial, and marine ecosystems.
- The majority of Earth's atmosphere (78%) is atmospheric nitrogen, making it the largest source of nitrogen. However, atmospheric nitrogen has limited availability for biological use, leading to a scarcity of usable nitrogen in many types of ecosystems.
- Nitrogen is present in the environment in a wide variety of chemical forms including organic nitrogen, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2).
- Organic nitrogen may be in the form of a living organism, humus or in the intermediate products of organic matter decomposition.
- The processes in the nitrogen cycle is to transform nitrogen from one form to another.
- Many of those processes are carried out by microbes, either in their effort to harvest energy or to accumulate nitrogen in a form needed for their growth.
- The conversion of nitrogen can be carried out through both biological and physical processes.
- Important processes in the nitrogen cycle include fixation, ammonification, nitrification, and denitrification.

Figure 1: Major transformations in the nitrogen cycle





Nitrogen fixation

- For nitrogen to be available to make proteins, DNA, and other biologically important compounds, it must first be converted into a different chemical form.
- The process of converting N_2 into biologically available nitrogen is called nitrogen fixation.
- N_2 gas is a very stable compound due to the strength of the triple bond between the nitrogen atoms, and it requires a large amount of energy to break this bond.
- The whole process requires eight electrons and at least sixteen ATP molecules (Figure 2).
- As a result, only a select group of prokaryotes are able to carry out this energetically demanding process.
- Although most nitrogen fixation is carried out by prokaryotes, some nitrogen can be fixed abiotically by lightning or certain industrial processes, including the combustion of fossil fuels.

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

Biological Nitrogen Fixation

- Some nitrogen-fixing organisms are free-living while others are symbiotic nitrogen-fixers, which require a close association with a host to carry out the process.
- Most of the symbiotic associations are very specific and have complex mechanisms that help to maintain the symbiosis.
- For example, root exudates from legume plants (e.g., peas, clover, soybeans) serve as a signal to certain species of *Rhizobium*, which are nitrogen-fixing bacteria.
- This signal attracts the bacteria to the roots, and a very complex series of events then occurs to initiate uptake of the bacteria into the root and trigger the process of nitrogen fixation in nodules that form on the roots.
- Some of these bacteria are aerobic, others are anaerobic; some are phototrophic, others are chemotrophic (i.e., they use chemicals as their energy source instead of light) (Table 1).
- Although there is great physiological and phylogenetic diversity among the organisms that carry out nitrogen fixation, they all have a similar enzyme complex called nitrogenase that catalyzes the reduction of N_2 to NH_3 (ammonia), which can be used as a genetic marker to identify the potential for nitrogen fixation.
- One of the characteristics of nitrogenase is that the enzyme complex is very sensitive to oxygen and is deactivated in its presence. This presents an interesting dilemma for aerobic nitrogen-fixers and particularly for aerobic nitrogen-fixers that are also photosynthetic since they actually produce oxygen.
- Over time, nitrogen-fixers have evolved different ways to protect their nitrogenase from oxygen.
- For example, some cyanobacteria have structures called heterocysts that provide a low-oxygen environment for the enzyme and serves as the site where all the nitrogen fixation occurs in these organisms.
- Other photosynthetic nitrogen-fixers fix nitrogen only at night when their photosystems are dormant and are not producing oxygen.

…Biological Nitrogen Fixation

- Genes for nitrogenase are globally distributed and have been found in many aerobic habitats (e.g., oceans, lakes, soils) and also in habitats that may be anaerobic or microaerophilic (e.g., termite guts, sediments, hypersaline lakes, microbial mats, planktonic crustaceans.
- The broad distribution of nitrogen-fixing genes suggests that nitrogen-fixing organisms display a very broad range of environmental conditions, as might be expected for a process that is critical to the survival of all life on Earth.

Genus	Phylogenetic Affiliation	Lifestyle
Nostoc, Anabaena	Bacteria (Cyanobacteria)	free-living,aerobic, phototrophic
Pseudomonas, Azotobacter, Methylomonas	Bacteria	free-living, aerobic, chemoorganotrophic
Alcaligenes, Thiobacillus	Bacteria	free-living, aerobic, chemolithotrophic
Methanosarcina, Methanococcus	Archaea	free-living, anaerobic, chemolithotrophic
Chromatium, Chlorobium	Bacteria	free-living, anaerobic, phototrophic
Desulfovibrio, Clostridium	Bacteria	free-living, anaerobic, chemoorganotrophic
Rhizobium, Frankia	Bacteria	symbiotic, aerobic, chemoorganotrophic

Table 1: Representative prokaryotes known to carry out nitrogen fixation

Nitrification

- Nitrification is the process that converts ammonia to nitrite and then to nitrate and is another important step in the global nitrogen cycle.
- Most nitrification occurs aerobically and is carried out exclusively by prokaryotes. There are two distinct steps of nitrification that are carried out by distinct types of microorganisms.
- The first step is the oxidation of ammonia to nitrite, which is carried out by microbes known as ammonia-oxidizers (eg. *Nitrosomonas*).
- Aerobic ammonia oxidizers convert ammonia to nitrite via the intermediate hydroxylamine, a process that requires two different enzymes, ammonia monooxygenase and hydroxylamine oxidoreductase.

$$1)\ NH_3 + O_2 + 2\,e^- \longrightarrow NH_2OH + H_2O$$

$$2)\ NH_2OH + H_2O \longrightarrow NO_2^- + 5\,H^+ + 4\,e^-$$

- The process generates a very small amount of energy relative to many other types of metabolism; as a result, nitrosofiers are notoriously very slow growers.
- Additionally, aerobic ammonia oxidizers are also autotrophs, fixing carbon dioxide to produce organic carbon, much like photosynthetic organisms, but using ammonia as the energy source instead of light.
- Unlike nitrogen fixation that is carried out by many different kinds of microbes, ammonia oxidation is less broadly distributed among prokaryotes.
- Until recently, it was thought that all ammonia oxidation was carried out by only a few types of bacteria in the genera *Nitrosomonas*, *Nitrosospira*, and *Nitrosococcus*. However, in 2005 an archaeon was discovered that could also oxidize ammonia.

… Nitrification

- The second step in nitrification is the oxidation of nitrite (NO_2^-) to nitrate (NO_3^-).

$$NO_2^- + \frac{1}{2}O_2 \longrightarrow NO_3^-$$

- This step is carried out by a … as nitrite-oxidizing Bacteria.
- Some of the genera involved in nitrite oxidation include *Nitrospira*, *Nitrobacter*, *Nitrococcus*, and *Nitrospina*.
- Similar to ammonia oxidizers, the energy generated from the oxidation of nitrite to nitrate is very small, and thus growth yields are very low.
- In fact, ammonia- and nitrite-oxidizers must oxidize many molecules of ammonia or nitrite in order to fix a single molecule of CO_2.
- For complete nitrification, both ammonia oxidation and nitrite oxidation must occur.
- Ammonia-oxidizers and nitrite-oxidizers are ubiquitous in aerobic environments. They have been extensively studied in natural environments such as soils, estuaries, lakes, and open-ocean environments.
- However, ammonia- and nitrite-oxidizers also play a very important role in wastewater treatment facilities by removing potentially harmful levels of ammonium that could lead to the pollution of the receiving waters.
- Much research has focused on how to maintain stable populations of these important microbes in wastewater treatment plants.
- Additionally, ammonia- and nitrite-oxidizers help to maintain healthy aquaria by facilitating the removal of potentially toxic ammonium excreted in fish urine.

Anammox

- Traditionally, all nitrification was thought to be carried out under aerobic conditions, but recently a new type of ammonia oxidation occurring under anoxic conditions was discovered.
- Anammox (anaerobic ammonia oxidation) is carried out by prokaryotes belonging to the Planctomycetes phylum of Bacteria.
- The first described anammox bacterium was *Brocadia anammoxidans*.
- Anammox bacteria oxidize ammonia by using nitrite as the electron acceptor to produce gaseous nitrogen.

$$NH_4^+ + NO_2^- \longrightarrow N_2 + 2\,H_2O$$

- Anammox bacteria were first discovered in anoxic bioreactors of wasterwater treatment plants but have since been found in a variety of aquatic systems, including low-oxygen zones of the ocean, coastal and estuarine sediments, mangroves, and freshwater lakes.
- In some areas of the ocean, the anammox process is considered to be responsible for a significant loss of nitrogen. However, Ward *et al*. (2009) argue that denitrification rather than anammox is responsible for most nitrogen loss in other areas.
- Whether anammox or denitrification is responsible for most nitrogen loss in the ocean, it is clear that anammox represents an important process in the global nitrogen cycle.

Denitrification

- Denitrification is the process that converts nitrate to nitrogen gas, thus removing bioavailable nitrogen and returning it to the atmosphere.
- Dinitrogen gas (N_2) is the ultimate end product of denitrification, but other intermediate gaseous forms of nitrogen exist.

$$1)\ NO_3^- \longrightarrow NO_2^- \longrightarrow NO + N_2O \longrightarrow N_2$$

$$2)\ 2\ NO_3^- + 10\ e^- + 12\ H^+ \longrightarrow N_2 + 6\ H_2O$$

- Some of these gases, such as nitrous oxide (N_2O), are considered greenhouse gasses, reacting with ozone and contributing to air pollution.
- Unlike nitrification, denitrification is an anaerobic process, occurring mostly in soils and sediments and anoxic zones in lakes and oceans.
- Similar to nitrogen fixation, denitrification is carried out by a diverse group of prokaryotes.
- Some denitrifying bacteria include species in the genera *Bacillus*, *Paracoccus*, and *Pseudomonas*.
- Denitrifiers are chemoorganotrophs and thus must also be supplied with some form of organic carbon.
- Denitrification is important in that it removes fixed nitrogen (i.e., nitrate) from the ecosystem and returns it to the atmosphere in a biologically inert form (N_2).
- This is particularly important in agriculture where the loss of nitrates in fertilizer is detrimental and costly.
- However, denitrification in wastewater treatment plays a very beneficial role by removing unwanted nitrates from the wastewater effluent, thereby reducing the chances that the water discharged from the treatment plants will cause undesirable consequences (e.g., algal blooms).

Ammonification

- When an organism excretes waste or dies, the nitrogen in its tissues is in the form of organic nitrogen (e.g. amino acids, DNA).
- Various fungi and prokaryotes then decompose the tissue and release inorganic nitrogen back into the ecosystem as ammonia in the process known as ammonification.
- The ammonia then becomes available for uptake by plants and other microorganisms for growth.
- Enzymes involved are:
- GS: Glutamine synthetase
- GOGAT: Glutamate 2-oxoglutarate aminotransferase (Ferredoxin & NADH-dependent)
- GDH: Glutamate Dehydrogenase:
  - Minor Role in ammonium assimilation.
  - Important in amino acid catabolism.

Marine nitrogen cycle