Nitrogen cycle

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The collective term given to the natural biological and chemical processes through which inorganic and organic nitrogen are interconverted. It includes the processes of ammonification, ammonia assimilation, nitrification, nitrate assimilation, nitrogen fixation, and denitrification.

Nitrogen exists in nature in several inorganic compounds, namely N_2, N_2O, NH_3, NO_2^-, and NO_3^-, and in several organic compounds such as amino acids, nucleotides, amino sugars, and vitamins. In the biosphere, biological and chemical reactions continually occur in which these nitrogenous compounds are converted from one form to another. These interconversions are of great importance in maintaining soil fertility and in preventing pollution of soil and water.

Nitrogen reserves exist in five major sinks: the primary rocks, the sedimentary rocks, the deep-sea sediment, the atmosphere, and the soil-water pool. Although primary rocks contain as much as 97.8% of the Earth's total (2.1×10^{17} tons or 1.9×10^{17} metric tons), their N_2 contributes little to the nitrogen cycle. Of the remaining nitrogen, 2% is in the atmosphere as N_2, and about 0.2% is in sedimentary rocks. The biosphere, consisting of the soil-water pool, contains only a small portion of the Earth's total nitrogen (2.6×10^{13} tons or 2.4×10^{13} metric tons), and even here the predominant species (2.4×10^{13} tons or 2.2×10^{13} metric tons) is N_2 dissolved in the sea. In spite of this, it is in this soil-water pool that the major reactions of the nitrogen cycle occur.

An outline showing the general interconversions of nitrogenous compounds in the soil-water pool is presented in **Fig. 1**. The reactions are much more complex than in the outline, and biological agents have evolved intricate ways to manipulate these nitrogenous compounds for their own use. There are three primary reasons why organisms metabolize nitrogen compounds: (1) to use them as a nitrogen source, which means first converting them to NH_3, (2) to use certain nitrogen compounds as an energy source such as in the oxidation of NH_3 to NO_2^- and of NO_2^- to NO_3^-, and (3) to use certain nitrogen compounds (NO_3^-) as terminal electron acceptors under conditions where oxygen is either absent or in limited supply. The reactions and products involved in these three metabolically different pathways collectively make up the nitrogen cycle and are discussed below.

Nitrogen compounds as nutrients

The synthesis of organic nitrogen compounds from inorganic nitrogen and carbon compounds begins with NH_3 incorporation. One major reaction, catalyzed by glutamic acid dehydrogenase, involves 2-ketoglutarate, NADH
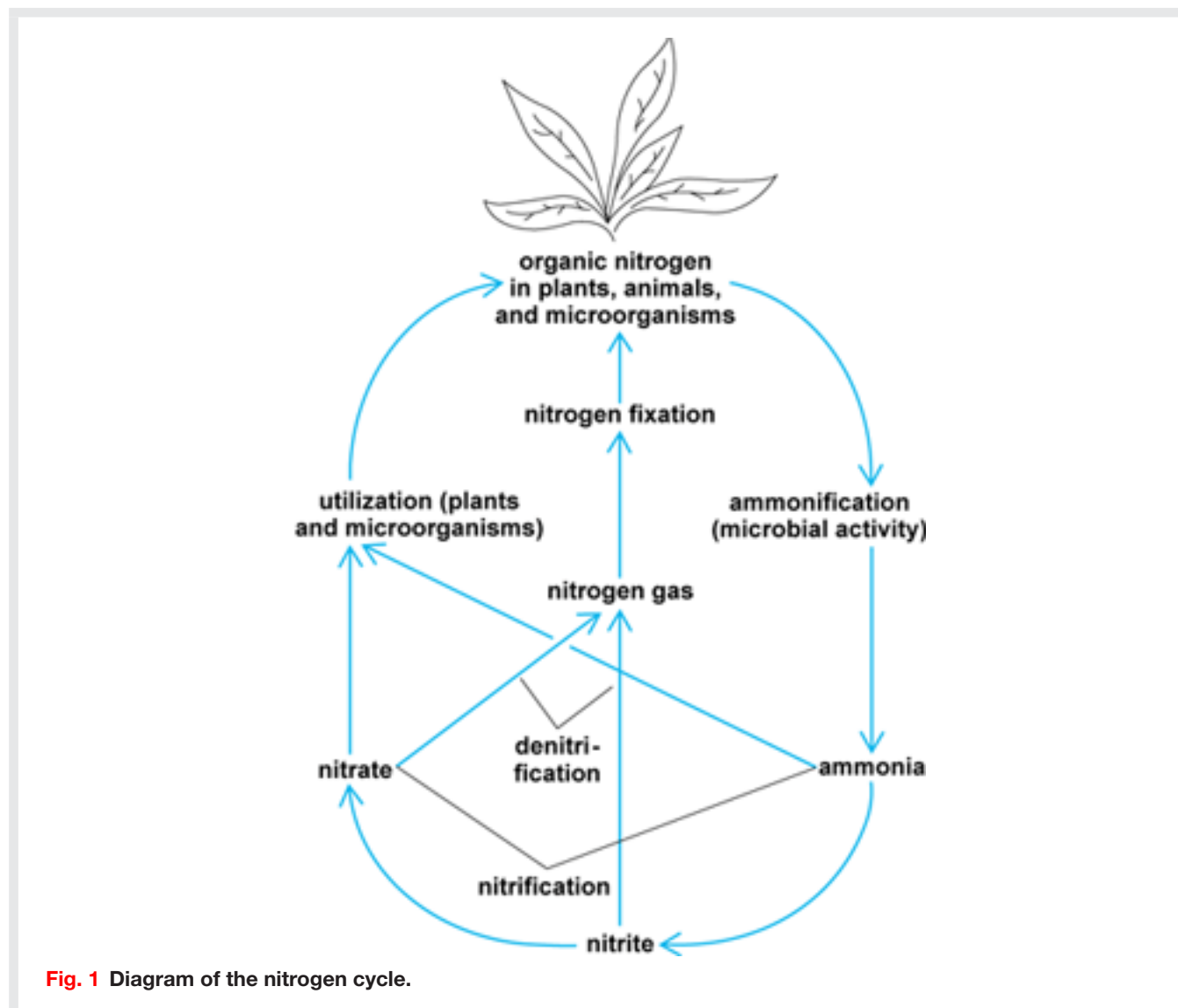
Fig. 1 Diagram of the nitrogen cycle.

(reduced nicotinamide adenine dinucleotide) and ammonia, and the product is glutamic acid. There are two ways in which organisms obtain ammonia. One is to use nitrogen already in a form easily metabolized to ammonia. Thus, nonviable plant, animal, and microbial residues in soil are enzymatically decomposed by a series of hydrolytic and other reactions to yield biosynthetic monomers such as amino acids and other small-molecular-weight nitrogenous compounds. These amino acids, purines, and pyrimidines are decomposed further to produce NH_3 which is then used by plants and bacteria for biosynthesis, or these biosynthetic monomers can be used directly by some microorganisms. The decomposition process is called ammonification. Not all organic nitrogen is ammonified easily, and resistant nitrogenous residues constitute humus, a complex component of great importance to soil structure and water-holding capacity.
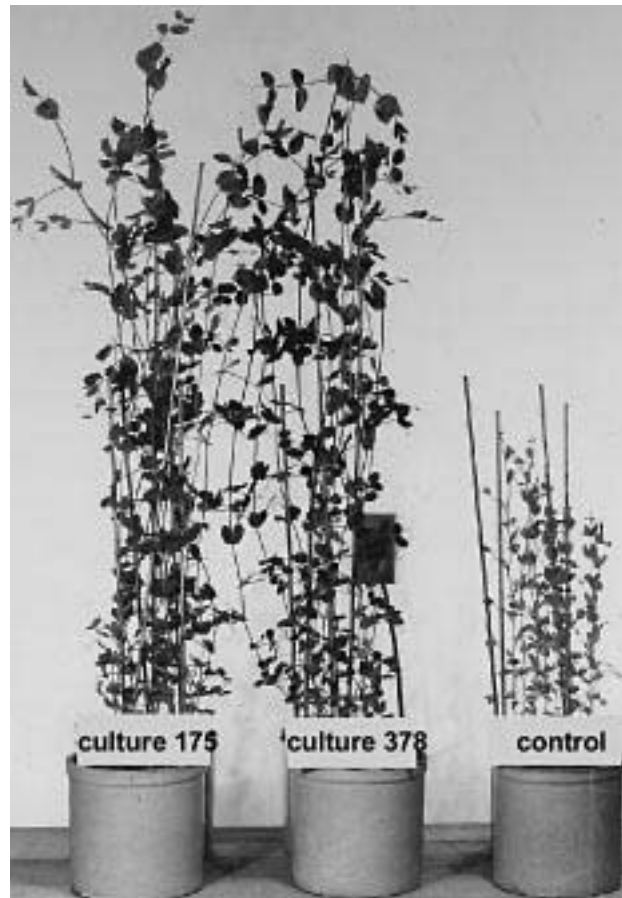
Fig. 2 Effect of inoculation with two strains (cultures 175 and 378) of the nitrogen-fixing *Rhizobium leguminosarum* on the development of peas.

The second way in which inorganic nitrogen is made available to biological agents is by nitrogen fixation (this term is maintained even though N_2 is now called dinitrogen), a process in which N_2 is reduced to NH_3. Since the vast majority of nitrogen is in the form of N_2, nitrogen fixation obviously is essential to life. The N_2-fixing process is confined to prokaryotes (certain photosynthetic and nonphotosynthetic bacteria). The major nitrogen fixers (called diazotrophs) are members of the genus *Rhizobium*, bacteria that are found in root nodules of leguminous plants (**Fig. 2**), and of the cyanobacteria (originally called blue-green algae). Even though rhizobia have recently been cultured so that they can fix nitrogen in the absence of the plant, the conditions needed for this fixation, such as a very low O_2 concentration supplied by oxyleg-hemoglobin and the necessary carbon and energy sources supplied by the plant, are ideal in the nodule and are not easily met in the laboratory or elsewhere in nature. There are many "free-living" diazotrophs, and even though they appear to contribute little to soil nitrogen, most knowledge of the biochemistry of nitrogen fixation comes from studies of three of them, *Clostridium pasteurianum, Azotobacter vinelandii*, and *Klebsiella pneumoniae*.

The nitrogen-fixing system of all these organisms is made up of two protein components. One, called the Fe protein, is a dimer of molecular weight about 60,000 daltons, and it contains an $Fe_4S_4^*$ center (similar to that of ferredoxin) that is involved in accepting and transferring the electrons needed for N_2 reduction. It is the Fe protein that initially binds the magnesium adenosine triphosphate (MgATP) needed for N_2 reduction. The second component, called the MoFe protein, is a tetramer of about 220,000 daltons and contains four $Fe_4S_4^*$ centers and two $Fe_8Mo_1S_6$ centers, with the latter centers probably being the sites where N_2 is reduced to NH_3. For N_2 reduction the MoFe protein accepts electrons from the Fe protein, and to facilitate the transfer, ATP is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate. The N_2 fixation process consumes as much as a third of the cell's energy supply, so for obvious reasons the N_2-fixing system is not synthesized if a usable nitrogen compound other than N_2 is available, that is, nitrogenase synthesis is repressed in the presence of NH_3.

Nitrogen compounds as energy source

Many microorganisms can use organic nitrogen compounds as energy sources, but in most cases the nitrogen of the compound is first removed and excreted as NH_3, and then the reduced carbon compound remaining is catabolized to yield both energy and organic carbon intermediates. A relatively few microorganisms (including some fungi) are able to aerobically convert the excreted NH_3 to NO_2^-, and NO_2^- to NO_3^-, and couple these oxidations to the production of ATP and the creation of a membrane potential needed for biosynthetic reactions. Thus, NH_3 added to aerobic soils is rapidly converted by such organisms to NO_3^-. Two chemoautotrophs (organisms that grow using an inorganic compound as an energy source and CO_2 as a carbon source) are primarily responsible for NO_3^- production. First *Nitrosomonas* converts the NH_3 to NO_2^-, and then the relatively toxic NO_2^- is rapidly oxidized to NO_3^- by *Nitrobacter*. Plants and microorganisms readily use this NO_3^- as a nitrogen source by first reducing it to NH_3. The overall process of oxidation of NH_3 to NO_3^- is called nitrification. The process whereby NO_3^- is reduced to NH_3 is called nitrate assimilation.

Nitrogen compounds as electron acceptors

When NO_3^- accumulates in soils in which metabolizable carbon compounds are available and when such soils become anaerobic because growth of aerobic organisms exhausts the O_2, certain organisms such as *Pseudomonas, Micrococcus, Achromobacter*, and *Bacillus* use the NO_3^- either as a normal electron acceptor or as an electron acceptor in place of O_2. The electron acceptor is needed by such cells to allow electrons from cellular oxidations to flow through the array of electron carries in the cell membrane. This electron flow is needed to facilitate proton (H^+) transfer across the membrane, which in turn creates a membrane potential and a pH gradient. The potential energy of the pH gradient is used in conjunction with the cells adenosine triphosphatase (ATPase) to allow ATP to be synthesized. The NO_3^- is the terminal electron acceptor in the electron flow and becomes reduced to NO_2^-, and the NO_2^- in turn is further reduced to N_2 (some N_2O may also be produced). The N_2 (and some N_2O) is released into the atmosphere. This process, called denitrification or

nitrate respiration, is responsible for ridding many bodies of water and soil of excess fixed nitrogen that could lead to pollution by overproduction and decay of algae and other bacteria.

N_2 cycle in the oceans

Most information on the N_2 cycle comes from studies of the soil-water system. Much less is known about the nitrogen cycle in seas, even though it is estimated that as much as 20% of the N_2 fixed on Earth occurs in the ocean. This N_2 seems to be primarily fixed by cyanobacteria, although in localized areas some contribution by other photosynthetic and nonphotosynthetic bacteria also occurs. One calculation estimates that a single bloom of the cyanobacterium *Trichodesmium* could fix as much as 1100 tons (100 metric tons) of N_2 per day. The other processes associated with the nitrogen cycle, ammonification, nitrification, and denitrification, although obviously present in oceans, have been studied even less. Heterotrophic organisms were not shown to be responsible for nitrification in seas, and therefore chemoautotrophic marine nitrifiers, such as *Nitrospira* and *Nitrococcus*, appear to be the major nitrifiers in oceans. The denitrification step of the ocean's nitrogen cycle may account for a third of the Earth's total denitrification, but there is relatively little information on this. One suspects that most of this denitrification takes place in the anaerobic conditions of marine mud.

Summary

The major microbial reactions discussed above are the three major contributors to the N_2 cycle. First N_2 is fixed (reduced) to ammonia, and the ammonia is used by N_2-fixing microorganisms and by plants harboring N_2-fixing microorganisms. Then these plants and bacteria, and animals and plants living off these plants and bacteria, die and lyse, and the nitrogen of their nitrogenous compounds is converted to ammonia by ammonification. The released ammonia is converted rapidly by nitrifying bacteria to NO_3^-, or it is used directly by microorganisms or plants. The NO_3^- produced by nitrification is used by plants and by bacteria as a nitrogen source; or if anaerobic conditions are created, the NO_3^- is denitrified (reduced) to N_2 and N_2O. This completes the nitrogen cycle.

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