Appendix A

The Nitrogen Cycle

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The nitrogen cycle is the biogeochemical cycle that describes the transformations of nitrogen and nitrogen-containing compounds in nature. Important reactions of the nitrogen cycle (Figure D1) include:

- **Nitrogen fixation** (1)- the microbial conversion of molecular nitrogen (N_2) to ammonia (NH_3). Ammonia exists in solution as the ammonium ion (NH_4^+). "Fixation" converts nitrogen gas to a salt that higher organisms can use. In other words, higher organisms are completely dependent on microorganisms for the nitrogen atoms in their proteins, nucleic acids, etc.
- **Nitrogen assimilation** (2)- ammonia can be incorporated into organic molecules ($R\text{-}NH_2$) such as nucleic acids.
- **Mineralization (Deamination)** (3)- conversely, organic molecules containing nitrogen are deaminated during decomposition of organic materials, producing ammonia.
- **Nitrification** (4)- soil bacteria collaborate to oxidize ammonia. The first oxidation product is the nitrite anion (NO_2-) produced by bacteria primarily of the genus *Nitrosomonas.* Nitrite is further oxidized by bacteria primarily of the genus *Nitrobacter,* producing the nitrate (NO_3^-) anion.
- **Denitrification (or nitrate reduction)** (5)- dissimilative nitrate reduction involves the microbial reduction of nitrate, producing nitrogen gas. Plants are able to do assimilative nitrate reduction (6), i.e. they use nitrate as a nitrogen source by reducing it and incorporating the nitrogen atoms into organic molecules.

Only microorganisms carry out nitrogen fixation, nitrification and denitrification.

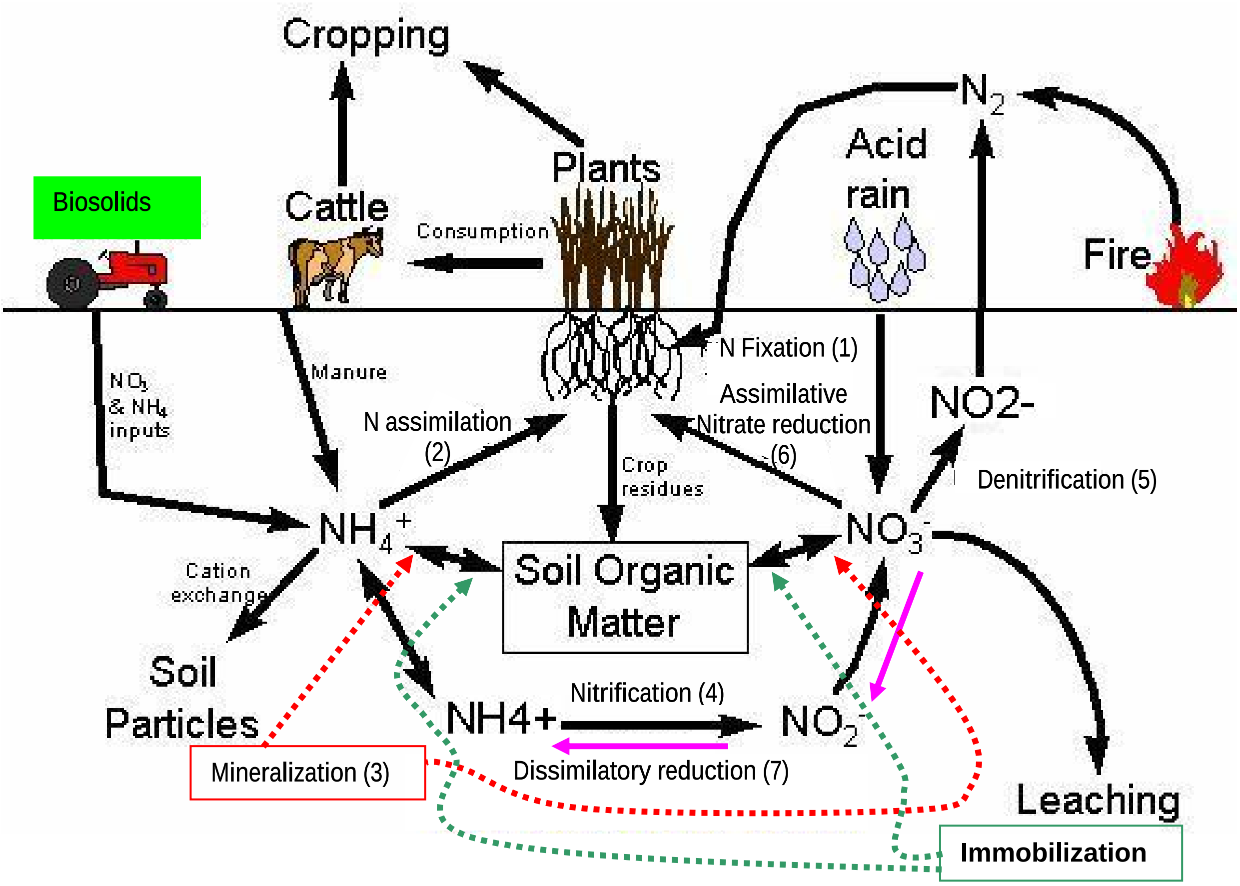

Figure D1. Nitrogen Cycle. Biosolids contribute nitrate and ammonia in fertilizing soil. (Cooper, 2007, Prescott et al, 1993)

Oxic vs. Anoxic?

1. Anoxic reactions
   a. Nitrogen fixation, even though some organisms that do this live in aerobic environments.
   b. Denitrification
2. Oxic
   a. Nitrification

Nitrate is the product of nitrification and the substrate of denitrification. If these two processes share a common metabolite, how can nitrification be oxic while denitrification is anoxic? Nitrate must move from the oxic environments, where it is produced, to the anoxic environments where it is utilized. For instance, as water seeps into soils, it carries nitrate produced at the aerobic surface to anaerobic layers of the soil where the nitrate it is reduced.

Nitrogen fixation (1)

Nitrogenase is the diagnostic enzyme for nitrogen-fixing organisms; it consists of two proteins

(i) Dinitrogenase - This component reduces nitrogen (N_2) to ammonia (NH_3). It contains an iron-molybdenum cofactor (abbreviated FeMoCo) that accepts electrons from dinitrogenase reductase.

(ii) Dinitrogenase reductase - This component transfers electrons to dinitrogenase. It contains an iron atom that is involved in the redox chemistry. A noteworthy limitation: dinitrogenase reductase is irreversibly inactivated by oxygen. As a result, nitrogen fixation cannot be done in the presence of oxygen.

Nitrogenase catalyses the following reaction:

$$N_2 + 8\ e^- + 8\ H^+ + ATP \dashrightarrow 2\ NH_3 + H_2 + ADP + Pi$$

Nitrogen fixers - Despite the extreme oxygen-sensitivity of nitrogenase, some microbes that live in aerobic environments can fix nitrogen. Organisms that fix nitrogen include:

(i) *Azotobacter* spp. - soil bacteria; occur in oxic environments (fix ~ 0.26 lbs N2 per agricultural acre per year);

(ii) *Klebsiella* spp. - soil bacteria, can live in the presence or absence of oxygen;

(iii) Cyanobacteria (e.g. *Anabeana* spp., *Nostoc*) - water bacteria; occur in oxic environments (fix ~ 22 lbs N_2 per agricultural acre per year);

(iv) Rhizobia spp. - plant symbionts; responsible for significant fixation (fix ~ 220 lbs N_2 per agricultural acre per year).

Nitrogen assimilation (2)

Uptake of mineral nitrogen by plants

Mineralisation (3)

Conversion of organic nitrogen to mineral nitrogen

Nitrification (4)

Nitrification is defined as a biological oxidation of ammonium to nitrite and nitrate, or more generally as the biological transformation of reduced forms of nitrogen to oxidized forms. In many soils nitrification is a key process in nitrogen transformations as it converts the exchangeable cation, NH_4^+, to the mobile anions NO_2^- and NO_3^-, which rapidly undergo other transformations (denitrification to N_2 and N_2O, assimilatory and disimilatory nitrate reduction to ammonia, nitrate respiration, or are easily leached from the soil, Figure 1. High rates of nitrification thus usually lead to high losses of nitrogen from soil. Moreover, as the oxidation of nitrogen compounds during nitrification produces hydrogen ions (H+), nitrification results in acidification of the soil.

Nitrification involves the collaborative, energy-generating oxidation of ammonia (NH_3). Organisms that perform these reactions are strict aerobes because oxygen is the electron acceptor during the oxidation of ammonia.

1. Nitrosofiers such as *Nitrosomonas* spp. oxidize ammonia to nitrite (NO_2-) in two steps.

a. The first step generates the intermediate hydroxylamine (NH2OH):

NH_3 + O2 + 2 H+ + 2e- -----> NH_2OH + H_2O + energy

The enzyme ammonia monooxygenase catalyzes this reaction. Note the similarity to methane monooxygenase, the first enzyme used by the methanotrophs in the aerobic oxidation of methane (CH4), generating methanol (CH3OH).

b. The second step produces nitrite from hydroxylamine:

NH_2OH + H2O -----> NO_2- + 2 H+ + 2e- + energy

2. Nitrifiers such as Nitrobacter spp. oxidize nitrite to nitrate (NO_3-):

NO_2- + H_2O -----> NO_3- + 2 H+ + 2 e- + energy

This reaction produces nitric acid (NO_3- + H+).

Most nitrification is carried out by chemolithoautotrophic bacteria belonging to the family *Nitrobacteraceae*. The family consists of two main groups, the ammonia-oxidizing or nitrosobacteria and the nitrite-oxidizing or nitrobacteria. Nitrifying bacteria are obligate aerobes and gain energy from the oxidation of reduced nitrogen compounds to fix CO_2 to organic carbon. Although nitrite and nitrate are main nitrification products, there is an increasing evidence that also some gaseous nitrogen species, namely NO and N_2O, are produced as by products during the autotrophic nitrification.

In addition to chemolithoautotrophic nitrifying bacteria, many other (heterotrophic) soil bacteria and fungi have an ability to oxidize reduced nitrogen compounds, both mineral (NH_4^+) and organic in the process called heterotrophic nitrification - heterotrophy is related to use of organic compounds as a source of carbon for biomass synthesis. Heterotrophic nitrifiers apparently do not obtain energy from the process and thus its physiological importance is not clear. Nevertheless, heterotrophic nitrification can prevail in (micro)sites with unfavourable conditions for autotrophic nitrifiers in, for example, acidic forest soils. As with autotrophic nitrification, nitrogen gases may also be produced during heterotrophic nitrification, although the significance of both autotrophic and heterotrophic sources of NO and N_2O has not been clearly demonstrated. It has been recently found that substantial amounts of NO and N_2O can be produced by autotrophic nitrifiers under conditions with lowered pO(2). At high pO(2) the organisms use molecular oxygen to oxidise NH; during nitrification, which depletes the O_2 in their microenvironment.

Denitrification (5)

Denitrification is reduction of nitrate (NO_3^-) to nitrogen gas or to organic nitrogen compounds. Two types of denitrification may be distinguished - dissimilative and assimilative.

1. Dissimilative denitrification (5)

The dissimilative pathway returns nitrogen atoms to the atmosphere by reducing nitrate to nitrogen gas. Several intermediates are involved:

$$NO_3^- \text{ -----> } NO_2^- \text{ -----> NO -----> } N_2O \text{ -----> } N_2 \text{ gas}$$

Major denitrifiers:
Denitrification is done only by microorganisms

(i) Usually facultative anaerobes

(ii) Predominantly:

- *Pseudomonas spp.* (e.g., *Pseudomonas denitrificans*)
- *Bacillus spp.*

In order to maintain catabolism, nitrifying bacteria may switch to using either nitrate or nitrite as an electron acceptor in respiration reactions, saving any remaining O_2 for activation of NH_4^+ by ammonium monooxygenase enzyme. This in fact means that they carry out (dissimilative) denitrification reactions which were found to be an important source of nitrogen gases. The process is called nitrifier denitrification and is believed to contribute substantially to NO and N_2O production in many soils.

3. Assimilative denitrification (6) do you want this bold in keeping with others? Do want 1. and 2. lined up?

4.

The assimilative pathway introduces nitrogen atoms into biological molecules by reducing nitrate to ammonia (NH_3). Ammonia is the precursor to organic amino groups ($R\text{-}NH_2$). Several intermediates are involved:

NO_3^- ----->NO_2^- -----> NH_2OH -----> NH_3 -----> $R\text{-}NH_2$

Assimilative denitrification is performed by plants, fungi and various prokaryotes.

Assimilative nitrate reduction (6)

Uptake of nitrate by plants

Dissimilatory reduction (7)

In dissimilatory reduction nitrate is transformed to ammonia. This activity is performed by a variety of bacteria, including *Geobacter metallireducens*, *Desulfovibrio* spp., and *Clostridium*.

Organic Matter

Organic matter in soil is required for N-mineralization activity. This organic matter includes proteinaceous components (proteins, peptides and amino acids, about 40%), amino sugars (about 5-6%), heterocyclic N compounds (including purines and pyrimidines –the building blocks of nucleic acids, about 35%), NH_3 (about 19%, approximately a ¼ of the NH_3 is fixed NH_4^+). Thus proteinaceous materials and heterocyclics appear to be the major components for N components in organic matter in soil (Schulten and Schnitzer, 1998).

Regulation of Nitrification

Nitrification is controlled by many environmental variables, but the principal regulatory factors are ammonium as the substrate and partial pressure of molecular oxygen. The latter is a result of balance between soil air and soil moisture content, which is controlled both directly and indirectly by many environmental conditions.

The only practical way for controlling the rate of nitrification in the field is thus the use of specific chemical compounds known as nitrification inhibitors, or to manage soil N to prevent substantial NH_4^+accumulation at times when plant demand is small. Several techniques for estimation of nitrification rates in soil were developed, of which short-term nitrifying enzyme assay is a promising tool for the indication of recent capacity of the soil to nitrify.

In sewage treatment and bioremediation dissimilative denitrification is desirable. Dissimilative denitrifiers aid in converting organic nitrogen to clean nitrogen gas that escapes to the atmosphere. This method allows for very clean disposal of nitrogenous pollutants.

References

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