V. NITROGEN TRANSFORMATIONS IN SOIL

Required Readings:


Suggested Readings:


Nitrogen is one of the most abundant elements associated with this planet and N₂ is the major gas (79.1% by volume) in the earth's atmosphere. The significance of N arises from the fact that, after C, it is the element that most controls the biological activity in soil. The movement of N between the land, water, and atmosphere define the cycling of N on a global scale. However, the soil also contains an internal N cycle whereby N is converted from one form to another. This internal soil cycle interfaces with the global N cycle at several different points.

**Forms of N in Soil.** Mineral soils in the temperate regions of the world contain between 0.06 and 0.3% N. Approximately 90% of this N is in organic forms. Soil organic N can be fractionated by chemical procedures using hot mineral acids (or bases) to liberate the N from clay and organic colloids and bringing the N compounds into solution. Typically a soil is heated with 6 M HCl for 18 to 24 hours. The N is then separated into the fractions shown in Table 5.1.

<table>
<thead>
<tr>
<th>Nitrogen Fraction</th>
<th>Percent of Total Soil N</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Insoluble</td>
<td></td>
<td>19.2 – 34.3</td>
<td>24.0</td>
</tr>
<tr>
<td>Acid Soluble</td>
<td></td>
<td>65.7 – 80.8</td>
<td>76.0</td>
</tr>
<tr>
<td>Ammonium</td>
<td></td>
<td>18.7 – 29.0</td>
<td>24.7</td>
</tr>
<tr>
<td>Amino Acid</td>
<td></td>
<td>17.8 – 31.0</td>
<td>23.4</td>
</tr>
<tr>
<td>Amino Sugar</td>
<td></td>
<td>4.3 – 7.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
<td>19.5 – 28.9</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Specific N compounds that may be found in soils besides the amino acids and amino sugars occur only at low concentrations. These include the bases of nucleic acids, chlorophyll, phospholipids, a variety of amines, and vitamins. The 10% or less of the total N in soil in
inorganic forms exists primarily as \( \text{NH}_4^+ \) held within clay minerals. A second mineral form of N that is of major importance when discussing the soil N cycle is nitrate. Nitrate is a product of the oxidation of ammonium and, because of its mobility, is the most common N form taken up by plants.

Recently, studies have indicated that reflux of soil using the traditional 6 M HCl for 24 h and various detection systems grossly underestimate the amount of amino acid-N in soil. Hydrolysis and extraction with methanesulfonic acid (MSA) hydrolysis followed by amino acid quantification using ion chromatography with pulsed amperometric detection yielded recovery of N as amino acids equivalent to 85.6% of the total N content \((n = 22\) soils). Methanesulfonic acid has the chemical formula of similar to sulfuric acid but one of the \(-\text{O}^+\) groups has been replaced with \(-\text{CH}_3\).

The ammonium ion is the N compound that is most involved in the control of the N cycle in soil and some of the possible fates of \( \text{NH}_4^+ \) are summarized in the following list.

i. \( \text{NH}_4^+ \) can be taken up by plants and is a preferred N source in solution.

ii. \( \text{NH}_4^+ \) is the preferred source of N for microorganisms.

iii. \( \text{NH}_4^+ \) is held on the cation exchange sites of soil where it can be held in storage for later use.

iv. \( \text{NH}_4^+ \) is approximately the same size as the K+ ion. It can readily enter the interlayer of clays.

v. \( \text{NH}_4^+ \) reacts with organic matter to form quinone-NH\(_2\) complexes. These complexes are an intimate part of soil organic matter.
vi. The partial pressure of NH$_3$ is high. This can lead to losses of N from the soil.

vii. NH$_4^+$ can be used as an energy source by a special group of autotrophs in the nitrification process.

**Mineralization.** Conversion of organic to mineral forms of an element is called mineralization and occurs as a result of biological activity. For N, this involves several distinct steps with only the first step truly being mineralization. Ammonification is the process that converts organic N to NH$_4^+$ by heterotrophic microorganisms. The subsequent transformation of the NH$_4^+$ to NO$_3^-$ is called nitrification. Traditionally, nitrification has been attributed primarily to the combined activities of two groups of autotrophic bacteria, *Nitrosomonas*, which converts NH$_4^+$ to NO$_2^-$, and *Nitrobacter*, which converts NO$_2^-$ to NO$_3^-$. However, recent bacterial diversity studies have indicated this is an oversimplification and that there may be a relatively large and diverse group of both culturable and unculturable bacterial involved. Both anaerobic and aerobic microorganisms are involved in ammonification, whereas nitrifying bacteria are strict aerobes. Thus conditions that restrict the supply of O$_2$ permit NH$_4^+$ to accumulate.

Ammonification involves all forms of organic N including proteins, nucleic acids and other types of N compounds in soil. The process of ammonification involving proteins can be represented as follows:

\[
\text{proteins} \rightarrow \text{peptides} \rightarrow \text{amino acids} \rightarrow \text{ammonia (ammonium)}
\]

The degradation of proteins to form peptides and then individual amino acids involve a wide diversity of enzymes called proteases and/or peptidases. The important of the protease reaction is
that it represents the rate-limiting step in the N mineralization process of proteins in soils. The proteases catalyze the hydrolysis of peptide bonds in proteins. They are generally characterized by their substrate affinity and the catalytic rate of the reaction and four major families of proteases have been defined; (1) serine proteases, (2) cysteine proteases, (3) aspartate proteases and (4) metallo proteases. This classification does not relate to the substrate specificity of the proteases themselves. Instead the name of the protease family refers to an amino acid (e.g. aspartate) or a metal as co-enzyme at the active site of the enzyme.

Once the protein has been broken down into individual amino acids, the N can be released as ammonia via oxidative deamination, reductive deamination, hydrolysis or desaturation. Several papers have been published on the enzymes involved in hydrolysis in soils of amino acids such as asparagine, aspartic acid, and glutamine. The $K_m$ values for these hydrolysis reactions often exceed the concentrations measured in soil extracts. This would, on the surface, suggest such reactions are not really relevant in natural systems. However, this overlooks the fact that microenvironments where proteins are deposited in soil, may release amino acids to create concentrations much greater than those measured in soil extracts.

Nucleic acids undergo a similar set of reactions in which the nucleic acid is first broken down to form the individual bases that are then further metabolized to eventually release ammonia.

$$\text{nucleic acid} \rightarrow \text{base-sugar-P} \rightarrow \text{base-sugar} \rightarrow \text{base} \rightarrow \text{ammonia (ammonium)}$$

The first step in this case involves a large number of restriction enzymes and is followed by other steps that eventually yield either a purine (adenine or guanine) or pyrimidine (thymine, cytosine,
or uracil) structure. With purine bases as the starting point, the end product is one molecule of glyoxylic acid (CHOCOOH) and two molecules of urea (NH₂CONH₂). For the pyrimidine bases, the end products are either urea and malonic acid or ammonia, carbon dioxide and beta-alanine. The urea is then converted to ammonia by urease as follows:

\[
\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \rightarrow 2 \text{NH}_3 + \text{CO}_2
\]

The other major class of N compounds in soils, the amino sugars, also are readily deaminated to yield ammonia plus the sugar. This can be illustrated using glucosamine. Glucosamine is a glucose molecule in which one of the -OH groups has been replaced with –NH₂.

\[
\text{glucose-NH}_2 + \text{H}_2\text{O} \rightarrow \text{glucose} + \text{NH}_3
\]

As you may have guessed by now, these deamination reactions that occur in soil are catalyzed by an entire family of enzymes which show specificity towards the sugar portion of the amino sugar molecule.

**Nitrogen Immobilization.** This is an assimilatory process whereby nitrate or ammonium is taken up by a cell and used to build cellular material. Ammonium is often the preferred N source as the cell does not need to expend energy to reduce the ammonium-N prior to its being incorporated into cellular compounds. If nitrate is assimilated, it must first be reduced in a process that is distinct from that of nitrate reduction that characterizes denitrification (see notes later in this section). Assimilatory nitrate reduction is oxygen insensitive but sensitive to reduced N compounds that often inhibit the process.
The net change in the total amount of inorganic N in soil is a result of immobilization and mineralization reactions. Whether immobilization or mineralization is the predominant reaction depends on the growth requirements of the microbial population for N and the N content of the soil organic matter. The critical concentration of N in organic matter resulting in immobilization and mineralization being approximately equal is often expressed in terms of C:N ratios. In natural materials with approximately 40% C, levels of N greater than 1.2 to 1.8% or a C:N ratio less than 20 to 30:1 results in net mineralization.

Nitrogen Fixation [http://helios.bto.ed.ac.uk/bto/microbes/xanthan.htm#rhizobium]. There is an abundant supply of N in the earth's atmosphere - nearly 79% in the form of N\textsubscript{2} gas. However, N\textsubscript{2} is unavailable for use by most organisms because there is a triple bond between the two N atoms, making the molecule almost inert. In order for N to be used for growth it must be "fixed" (combined) in the form of ammonium (NH\textsubscript{4}) or nitrate (NO\textsubscript{3}) ions. The weathering of rocks releases these ions so slowly that it has a negligible effect on the availability of fixed N. So, N is often the limiting factor for growth and biomass production in all environments where there is suitable climate and availability of water to support life.

A relatively small amount of ammonia is also produced by lightning or produced industrially by the Haber-Bosch process, using an iron-based catalyst, very high pressures and fairly high temperature. But the major conversion of N\textsubscript{2} into ammonia, and thence into proteins, is achieved by microorganisms in the process called N\textsubscript{2} fixation (or dinitrogen fixation). Nitrogen fixing bacteria are either free-living or form symbiotic associations with plants or other organisms (e.g. termites, protozoa).
Biological N₂ fixation can be represented by the following equation, in which two moles of ammonia are produced from one mole of N gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions):

\[
\text{N}_2 + 8\text{H}^+ + 8e^- + 16\text{ATP} = 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{Pi}
\]

This reaction is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed nitrogenase. This enzyme consists of two proteins - an iron protein and a molybdenum-iron protein, as shown below.

The reactions occur while N₂ is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N₂, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to H₂N-NH₂, and this in turn is reduced. Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation.

The nitrogenase enzyme complex is highly sensitive to oxygen. It is inactivated when exposed to oxygen, because this reacts with the iron component of the proteins. Although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species such as cyanobacteria (which generate oxygen during photosynthesis) and the free-living aerobic bacteria of soils, such as *Azotobacter* and *Beijerinckia*. These organisms have various methods to overcome the problem. For example, *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very
low level of oxygen in their cells. *Azotobacter* species also produce copious amounts of extracellular polysaccharide (as do *Rhizobium* species in culture). By maintaining water within the polysaccharide slime layer, these bacteria can limit the diffusion rate of oxygen to the cells. In the symbiotic N$_2$-fixing organisms such as *Rhizobium*, the root nodules can contain oxygen-scavenging molecules such as leghaemoglobin, which shows as a pink color when the active N$_2$-fixing nodules of legume roots are cut open. Leghaemoglobin may regulate the supply of oxygen to the nodule tissues in the same way as haemoglobin regulates the supply of oxygen to mammalian tissues. Some of the cyanobactia have yet another mechanism for protecting nitrogenase: N$_2$ fixation occurs in special cells (heterocysts) which possess only photosystem I (used to generate ATP by light-mediated reactions) whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water to supply H$_2$ for synthesis of organic compounds).

**Nitrification.** This seemingly simple process of transforming ammonium to nitrate actually involves a complex series of reactions that can be summarized in chemist shorthand as:

\[
\text{NH}_3 + \text{O}_2 \rightarrow \text{NO}_2^- + 3\text{H}^+ + 2\text{e}^- \\
\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 2\text{H}^+ + 2\text{e}^-
\]

These balanced chemical equations tell us that ammonium is combined with oxygen to produce bacterial cell mass, nitrite (NO$_2^-$) and acid and energy in the form of electrons. Then the nitrite is combined with oxygen that comes from water to form nitrate, acid and more energy. The energy is used to convert carbonic acid to bacterial cell mass.
Several important points that come out of these equations is that nitrification is really a series of steps that start with ammonium and end with nitrate. There are other intermediates that are not shown in the simplified reactions above. It is also an acidifying process, producing a gradual build up of nitric acid. The process, itself, does not remove any N from the system but merely changes it from one form to another.

Denitrification. This is the process that completes the N cycle and converts nitrate back to N gas. Like the immobilization or assimilation process of nitrate, this is also a reduction process, but in this case the N is not used directly for cellular synthesis. Instead the process provides energy for growth. The reaction is very oxygen sensitive as nitrate will generally only serve as the electron acceptor if oxygen is limiting and not available. It is ammonium insensitive.

The reduction of nitrate to N gas occurs via two electron transfer steps (see below) and the key reaction that commits nitrate to the denitrification step is the formation of an N-N bond.

\[
2 \text{NO}_3^- \rightarrow 2 \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]

There are several good methods to study denitrification in soil, but the most commonly used method is to use acetylene to block the last step in the process from N\(_2\)\(_2\)O to N\(_2\). N\(_2\)O can be sensitively quantified using a gas chromatograph.

The major environmental factors that control denitrification in soil are (1) a low redox status brought about by excess water limiting oxygen content in the soil, (2) readily available organic carbon to serve as a source of electrons, and (3) optimum pH (near pH 7.0) and temperature.
Gains and Losses of N from Soil. From a global perspective, the N that enters the soil via natural processes is derived from biological N$_2$ fixation and atmospheric deposition of combined N forms (i.e. NH$_3$, NH$_4^+$, and NO$_3^-$). An additional input to agricultural soils is fertilizer. Data concerning global N fluxes indicates that biological N$_2$ fixation returns approximately $175 \times 10^9$ kg to the earth's surface, of which nodulated legumes grown for agricultural purposes contribute about 68%. The remainder of the N$_2$ fixed is a result of nodulated nonlegumes, blue-green algae, free-living microorganisms, and plant-algal associations.

Losses of N occur because of the great mobility of NO$_3^-$ and the high vapor pressure of NH$_3$. In addition, biological reactions involving N compounds result in net losses from the soil. Five major pathways of N loss from soil are possible. These include bacterial denitrification, chemodenitrification, NH$_3$ volatilization, leaching losses, and erosion losses. Denitrification has already been discussed. Ammonia volatilization is a major loss mechanism when soils have pH values above 6 and especially above 7, there is low moisture content, high temperatures, low cation exchange capacity and addition of highly nitrogenous fertilizers or proteinaceous materials without incorporation.

The world's most commonly used N fertilizer is urea and urease in soil will hydrolyze urea as follows to form carbon dioxide and ammonia.

$$H_2N(C=O)NH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

Because of the potential for N loss by volatilization following urea fertilizer applications, especially granular urea on no-tillage fields, efforts have been made to develop effective
inhibitors of the urease enzyme, thus improving overall N use efficiency from urea.

**Impact of N on Health and Environment.** Nitrogen, while being a major essential element for life, also has the potential to adversely affect our health and the environment. There exists a conflict, however, between the increased use of fertilizer N for greater worldwide production of food and fiber and the need for controlling levels of NO$_3^-$ in supplies of our drinking water. Potential health and environmental problems associated with N are listed in Table 5.2.

The soil N cycle impacts the problems primarily through the NO$_3^-$ ion. Natural biological reactions and agricultural practices that lead to an increase in NO$_3^-$ concentrations in the soil will

<table>
<thead>
<tr>
<th>Problem</th>
<th>Causative Agent</th>
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<tbody>
<tr>
<td>Human Health:</td>
<td></td>
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<tr>
<td>Methemoglobinemia</td>
<td>Excess NO$_3^-$ and NO$_2^-$ in water and food</td>
</tr>
<tr>
<td>Cancer</td>
<td>Nitrosomines from NO$_2^-$ and secondary amines</td>
</tr>
<tr>
<td>Respiratory Illness</td>
<td>Peroxy and alkyl nitrates, NO$_3^-$ aerosols, NO$_2^-$, and HNO$_3$ vapors in urban areas</td>
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<tr>
<td>Animal Health:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excess NO$_3^-$ in feed and water</td>
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<tr>
<td>Plant Growth:</td>
<td></td>
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<tr>
<td>Stunted growth</td>
<td>High levels of NO$_3^-$ in soil</td>
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<tr>
<td>Excessive growth</td>
<td>Excess available N</td>
</tr>
<tr>
<td>Environmental Quality:</td>
<td></td>
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<tr>
<td>Eutrophication</td>
<td>Excess available N in surface waters</td>
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<tr>
<td>Stratospheric ozone depletion</td>
<td>N$_2$O from denitrification</td>
</tr>
<tr>
<td>Materials and ecosystem damage</td>
<td>HNO$_3$ in rainfall</td>
</tr>
</tbody>
</table>
indirectly have potential to affect our health and environment. Both point and nonpoint sources of \( \text{NO}_3^- \) result from our modern industrial and agricultural practices. Large localized animal production facilities are examples of point sources of \( \text{NO}_3^- \) and the widespread use of fertilizer N is an example of a practice that results in nonpoint sources of \( \text{NO}_3^- \).

**Use of \( ^{15}\text{N} \) in Soil N Studies.** Our understanding of the behavior of N in soils has been greatly aided by the use of the stable isotope \( ^{15}\text{N} \). Unlike the other major nutrient elements, N does not exist in a radioactive form that can be easily used for research purposes. However, \( ^{15}\text{N} \) can be utilized as a tracer because it is naturally present only in very low amounts (0.366% of the total N) in nature. \( ^{15}\text{N} \)-labelled materials are analyzed with a mass spectrometer and are used in tracer experiments in the same manner as radioactive labels are used. Several assumptions are made when using \( ^{15}\text{N} \) as a tracer. One, the isotopic composition of N in the natural state is constant and two, living systems are unable to distinguish one isotope from another. In reality, slight variations occur in the isotopic composition of N that can be ignored if sufficient \( ^{15}\text{N} \) label is added to make this natural variation small compared to the variation imposed by the addition of the label. Natural variations in \( ^{15}\text{N} \) content may ultimately prove useful in evaluating the N cycle in soil as we obtain more data on the relative abundance of this isotope in the various soil N fractions.