BIOLOGICAL TRANSFORMATIONS OF NITROGEN IN SOIL

- Animals
- Plant roots
- Plants
- Microorganisms
- Humus
- Animal residues
- Plant residues
- Nitrogen
- Nitrite
- Nitrate
- Ammonia
- Nitrous oxide

N.E.R. Campbell and H. Lees
Nitrogen Distribution in 10 Virgin Soils and Their Cultivated Counterparts

<table>
<thead>
<tr>
<th></th>
<th>Virgin Soil</th>
<th>Cultivated Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhydrolyzable (6N HCl)</td>
<td>25.4</td>
<td>24.0</td>
</tr>
<tr>
<td>Hydrolyzable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>74.6</td>
<td>76.0</td>
</tr>
<tr>
<td>Ammonium</td>
<td>22.2</td>
<td>24.7</td>
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<tr>
<td>Hexoseamine</td>
<td>4.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>26.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Unidentified</td>
<td>21.0</td>
<td>22.5</td>
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</table>

- Approximately 0.02 to 2.5% of soil is as Nitrogen.
- In a 2% OC soil (Ohio) the value would be about 0.2% Nitrogen.
- 1 to 3% of this N is mineralized each year.
NITROGEN MINERALIZATION

Conversion of organic nitrogen to the more mobile, inorganic state

AMMONIFICATION

Formation of ammonia from organic matter

NITRIFICATION

Oxidation of ammonium to produce nitrate

\[ N_i = \text{Organic matter mineralized} - (N_A + N_P + N_L + N_D) \]

- \( N_i \) = Inorganic Nitrogen
- \( N_A \) = Nitrogen Assimilated by microorganisms
- \( N_P \) = Nitrogen removed by Plants
- \( N_L \) = Nitrogen lost via Leaching
- \( N_D \) = Nitrogen lost via Denitrification
The disappearance of $N_1$ following the addition of a nitrogen-poor crop residue is called **immobilization**.

Immobilization is the result of microbial **assimilation** of inorganic nitrogen and is the converse of **mineralization**.

Ammonium is the most readily assimilated $N$ source for soil microorganisms.

$\text{NH}_4^+ > \text{NO}_3^- > \text{Organic N}$
Whether immobilization or mineralization of Nitrogen occurs depends on the C:N ratio. The C:N ratio where the two processes are in equilibrium is approximately 30 (1.5% N).

**C:N > 30**  
N is taken from mineral pool or degradation is slowed  
**Immobilization**

**C:N < 30**  
Mineral N is released  
**Mineralization**

**Nitrogen Factor**

The number of units of N immobilized for each 100 units of material being decomposed, i.e. the amount of N that must be added to avoid immobilization.

\[ N_{\text{imm}} = \frac{1}{100} \]
Changes in inorganic N in soil receiving various quantities of glucose
Changes in C:N ratio during the decomposition of plant residues
1. The lowering of the C:N ratio from an initially high value to a lower value occurs during decomposition.

2. The rate and extent of immobilization through microbial assimilation depends on the carbonaceous substances undergoing decay.

3. Whether N is immobilized or mineralized depends on the nature of the microbial population.

   (BACTERIA < FUNGI < ACTINOMYCETES)

4. Immobilization may be both harmful and beneficial.
   - Harmful if crop plants also are competing for N
   - Beneficial in serving as a store for N during times of low N requirements by plants
FACTORS INFLUENCING MINERALIZATION

1. Oxygen status
   - Ammonifying population includes both aerobes and anaerobes

2. Moisture Status
   - Wet / dry cycles

3. pH
   - greatest when the soil pH is near neutral

4. Temperature
   - Optimum is at 40 - 60°C, which is higher than most biological processes

5. C:N ratio
FATES OF NH₄⁺ PRODUCED

1. Taken up by plants

2. Taken up by microorganisms

3. Held onto the soil cation exchange sites

4. Is fixed within the clay lattice

5. Reacts with organic matter to form quinone - NH₂ complexes

6. Lost to the atmosphere through volatilization

7. Used as an energy source by chemoautotrophs
Mineralization of organic nitrogen and organic carbon are closely related to one another. The ratio of $\text{CO}_2$ - C to $N_i$ produced in an unamended soil is relatively constant, ranging from $7 \text{ to } 15 : 1$.

Techniques Used to Predict $N_i$ Production During a Growing Season

1. Determine the quantity of $N_i$ produced during a short laboratory incubation period.

2. Establishing what fraction of total organic nitrogen is correlated with nitrogen mineralization rate.

3. $\text{NO}_3^-$ - N (at time of planting) prior to side-dressing
DECOMPOSITION OF AMINO ACIDS
(removal of amino group)

1. Transamination (some directly enter TCA cycle)

2. Oxydative deamination

\[
R-C-COOH + NAD^+ \xrightarrow{H_2O} NADH (H^+) + R-C-COOH + NH_3
\]

3. Reductive deamination

\[
R-C-COOH + NADH (H^+) \rightarrow RCH_2COOH + NH_3
\]

4. Hydrolysis

\[
R-C-COOH + H_2O \rightarrow R-C-COOH + NH_3
\]

5. Desaturation

\[
R-C-CH_2-COOH \rightarrow R-CH=CHCOOH + NH_3
\]
DECOMPOSITION OF NUCLEIC ACIDS

Purine Base

Pyrimidine Base

(Base - Sugar - P)_N → Base - Sugar - P → Base - Sugar → Sugar + Purine or Pyrimidine Base
Pathway of Metabolism of Purines and Pyrimidines

**Purine bases**
- Adenine
- Hypoxanthine
- Guanine
- Xanthine
- Uric acid

**Pyrimidine bases**
- Cytosine
- Uracil

**Other compounds**
- Barbituric acid
- B-Ureidopropionic acid
- Urea
- Malonic acid
- B-Alanine
- Glyoxylic acid
- Allantoic acid
- Allantoin
- NH₃
- B-
NITRIFICATION

The biological formation (oxidation) of ammonium to produce nitrite and nitrate

\[ \text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \]

MICROORGANISMS INVOLVED

1. Obligate aerobes
2. Derive their carbon solely from CO\(_2\) or carbonates
3. Derive their energy from oxidation of NH\(_4^+\) or NO\(_2^-\)

NITROSOBACTER

\[ \text{NH}_4^+ \rightarrow \text{NO}_2^- \]

NITROSOBACTER

\[ \text{NO}_2^- \rightarrow \text{NO}_3^- \]
Postulated Steps in Nitrification

Nitrosomonas

\[ \Delta G \text{ Valance State} \]

-3 | +3.9 | -68.9 | +1 | +3

\( \text{NH}_4^+ \) ammonium

\[ \text{NH}_2 \text{ OH hydroxylamine} \]

\[ \text{(NOH) nitroxy radical} \]

\[ \text{N}_2 \text{O + H}_2 \text{O} \]

\( \text{NO}_2^- \) nitrite

\[ \text{H}_2 \text{O} \]

\[ \text{NO}_3^- \text{ hydrated nitrite} \]

\[ \text{H}_2 \text{O} \]

\[ \text{NO}_3^- \text{ nitrate} \]
Hydrazine (HN = NH) strongly inhibits NH₂OH but weakly inhibits NH₄⁺ → NO₂⁻.

Results have shown that NH₂OH can accumulate in small amounts in Nitrosomonas treated with hydrazine.

Oxidation of ammonium is blocked by copper enzyme poisons such as thiourea. However, NH₂OH → NO₂⁻ is not affected by thiourea.
The oxygen in the nitrate product is from $H_2O$, not molecular oxygen.

This is an example of a hydration (water addition) and dehydrogenation (hydrogen removal) reaction.

**Nitrosamine Formation**

$R\text{NH} + \text{NO}_2^- \rightarrow R\text{N} - \text{N} = \text{O} + \text{OH}^-$

Secondary Amine  Nitrosamine
\[
\text{NH}_2\text{OH} + \text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O}
\]
\[
\text{NOH} \rightarrow (\text{NOH}) \rightarrow \text{NO} \rightarrow \text{NO}_2^- \rightarrow 1/2 \text{N}_2\text{O} + 1/2 \text{H}_2\text{O}
\]

1. Incubation of hydroxylamine and Nitrosomonas causes production of NO and N\textsubscript{2}O.

2. N\textsubscript{2}O is not an intermediate in nitrification because it is not converted to NO\textsubscript{2}⁻.

3. Mechanisms are speculative. N\textsubscript{2}O may be produced from NO\textsubscript{2}⁻ still present in the Nitrosomonas.

Chlorate (Dinitrophenol) inhibits the formation of nitrate from nitrite by Nitrobacter. This can be used to study the reaction facilitated by Nitrosomonas.

\[
\text{NH}_4^+ \rightarrow \text{NO}_2^- \xrightarrow{\text{chlorate blocks reaction}} \text{NO}_3^-
\]

The formation of nitrite from ammonium can thus be measured. This reaction is important because it is the rate limiting step.
The end product of fertilizer N is nitrate whether the fertilizer is added as urea, anhydrous ammonia, or ammonium nitrate.
CONTROL OF NITRIFICATION

1. Control of nitrification inhibitors
2. Use of nitrogen compounds that release N slowly because of limited solubility in water or slow rate of microbial decomposition
3. Use of nitrogen compounds coated with material that will delay the release of N

DESIRABLE CHARACTERISTICS OF NITRIFICATION INHIBITORS

1. Specific in blocking $\text{NH}_4^+ \rightarrow \text{NO}_3^-$
2. Able to move with fertilizer through soil
3. Non-toxic to plants
4. Non-toxic to plant root micro-environment
5. Not easily bio- or chemical degradable
**NITRIFICATION INHIBITORS**

**POTENTIAL ADVANTAGES**

1. Reduced gaseous loss of N
2. Reduced loss of NO$_3^-$ by leaching and runoff
3. Reduced risk of NO$_3^-$ toxicity

**DISADVANTAGES**

1. Increased gaseous loss of N (NH$_4^+$ Volatilization)
2. Increased fixation or immobilization of NH$_4^+$
3. Some plants do not use NH$_4^+$, but primarily NO$_3^-$
MOST EFFECTIVE INHIBITORS OF NITRIFICATION

1. N - SERVE

2 - Cloro - 6 - (Trichloromethyl) - Pyridine

2. AM

2 - Amino - 4 - Cloro - 6 - Methyl - Pyrimidine

3. ATC

4 - Amino - 1, 2, 4 - Triazole

4. CS$_2$

(or Na$_2$CS$_3$) Sodium Trithiocarbamate

\[2\text{Na}_2\text{CS}_3 + 2\text{H}_2\text{O} + 5\text{CO}_2 \rightarrow 4\text{NaHCO}_3 + 3\text{CS}_2\]

5. DWELL
DENITRIFICATION

The reduction of nitrate to nitrite and then to gaseous nitrogen products which are lost from the aquatic or soil system. It is a dissimilatory reduction reaction.

ASSIMILATORY NITRATE REDUCTION

\[
\text{NO}_3^- \rightarrow \text{NH}_4^+ 
\]

The ammonium is used for cell growth. This reaction is oxygen insensitive and is inhibited by reduced N compounds.

DISSIMILATORY NITRATE REDUCTION

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

This reaction provides energy for growth, but the nitrogen is not used directly for synthesis of microbial products. This reaction is very oxygen sensitive and ammonium insensitive.
1. Represents a major loss pathway of nitrogen from soils (30%)

2. Nitrous oxide, a product of denitrification, may lead to destruction of the ozone layer

3. Importance in commercial wastewater treatment plants to reduce nitrate levels

4. A key intermediate, nitrite, readily reacts with secondary amines to produce carcinogenic nitrosamines

\[
\text{R} \text{NH} + \text{NO}_2^- \rightarrow \text{R} \text{N} - \text{NO} + \text{OH}^-
\]

5. N cycling on global basis

**Note!**
A key feature distinguishing denitrification from assimilatory nitrate reduction is the formation of a nitrogen - nitrogen bond in denitrification.
ASSIMILATORY REACTION

\[ 2\text{NO}_3^- \rightarrow 2\text{NO}_2^- \rightarrow 2\text{NH}_2\text{OH} \rightarrow 2\text{NH}_4^+ \]

DENITRIFICATION

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

Normal Sequence of Events

<table>
<thead>
<tr>
<th>Time</th>
<th>mg of Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3^-$</td>
</tr>
<tr>
<td></td>
<td>NO$_2^-$</td>
</tr>
<tr>
<td></td>
<td>N$_2$O</td>
</tr>
<tr>
<td></td>
<td>N$_2$</td>
</tr>
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# METHODS OF DETERMINING DENITRIFICATION RATES

1. **Nonrecovery of total N or $^{15}$N nitrogen**
   - A. Sensitivity of detection losses are low
   - B. Short term studies not possible
   - C. Analytical errors are important

2. **Disappearance of denitrification intermediates**
   - A. $\text{NO}_3^-$ or $\text{NO}_2^-$ disappearance
     (works only if nongaseous N losses are minimal)
   - B. $\text{N}_2\text{O}$ disappearance
     - Nondisturbing to soil system and specific to one step of denitrification pathway
     - Difficult to calculate data (on a soil basis)

3. **Production of $\text{N}_2\text{O}$ and N**
   - A. Sensitive and very precise
   - B. Background of N$_2$ is high, making a natural system difficult to use

4. **Inhibition of $\text{N}_2\text{O}$ reduction by acetylene**
   $$\text{N}_2\text{O} \xrightarrow{\text{acetylene}} \text{N}_2$$
   Denitrification is measured by the accumulation of $\text{N}_2\text{O}$ in the presence of $\text{C}_2\text{H}_2$

5. **Isolation and enumeration of denitrifiers**
FACTORS WHICH CONTROL DENITRIFICATION IN SOIL

1. Oxygen and redox potential
2. Water content
3. Concentration of NO$_3^-$ or NO$_2^-$
   - First order to zero order reaction rates occur at low concentrations of NO$_3^-$ or NO$_2^-$
   - $K_m = 4 - 7$ uM
   - NO$_3^-$ inhibits NO$_2^-$ $\rightarrow$ N$_2$ so that early intermediates accumulate
   - NO$_3^-$ inhibitions cause greater N$_2$O production relative to N$_2$
4. Organic C
5. pH (optimum is 7.0 - 8.0)
   - At low pH, more N$_2$O is produced relative to N$_2$
   - NO$_2^-$ can accumulate at low pH
6. Temperature
   - Optimum occurs between 60 - 75°C. Whether chemical processes are of great importance in this temperature range is still not known.
DECOMPOSITION OF UREA

\[
\begin{align*}
\text{Urea} & \xrightarrow{\text{Urease}} \text{Ammonium Carbamate} \\
H_2N - C - NH_2 & \quad H_2NCONH_2 \quad 2NH_3 + CO_2 \\
\text{H}_2\text{N} - C - \text{NH}_2 + \text{CO}_2 & \quad \text{Allophanic Acid} \\
& \rightarrow \text{H}_2\text{NCNHCOOH} \quad 2\text{NH}_3 + \text{CO}_2
\end{align*}
\]
USE OF UREA AS A \( \text{N} \) SOURCE IN SOILS

Advantages

1. High N content (45-46% N)
2. Low cost of manufacture, transport, storage, and distribution
3. High solubility in water
4. Ease in handling (nonexplosive, noncaking)
5. Suitable for dry blends or in solution blends
6. Can be applied as a foliar spray
7. Used in production of compound fertilizers (Urea phosphates)

Disadvantages

- Rapid hydrolysis to ammonium carbamate
1. Raises pH and causes volatilization losses of N
2. Nitrite toxicity
3. Seedling germination can be inhibited
FACTORs PROMOTING NH$_3$ VOLATILIZATION LOSSES

1. pH values > 6
2. Low moisture content
3. High temperatures
4. Low CEC of soils
5. Addition of highly nitrogenous fertilizer N sources without incorporation
APPROACHES USED TO DECREASE VOLATILIZATION LOSSES FROM UREA

1. Urease inhibitors
   - Hg, Ag are the most effective inorganic inhibitors
   - Catechol, hydroquinone - most effective organic inhibitors
   - Phosphorodiamides,
     \[
     \text{NBPT} \rightarrow \text{N (n-buty)} \text{ thiophosphoric triamide}
     \]
     (oxon form is active form)

2. Slow release urea fertilizers
   - Formation of water insoluble urea compounds (urea-forms)
   - Coat urea granule with an inert or water resistant material (sulfur-coated urea)
   - Conversion to acidic derivative (urea phosphate)

3. Addition of divalent cations with application of urea
(NH₄)₂X + CaCO₃ ⇌ (NH₄)₂CO₃H₂O + CaX↓  
where X = SO₄²⁻ or HPO₄³⁻  

(NH₄)₂CO₃·H₂O → 2NH₄OH + CO₂↑  
NH₄⁺ + OH⁻ ⇌ NH₄OH  
NH₄OH ⇌ NH₃↑ + H₂O  

at alkaline pH the reaction goes to the right

2NH₄X + CaCO₃ ⇌ (NH₄)₂CO₃·H₂O + CaX₂  
X = Cl⁻, NO₃⁻, H₂PO₄⁻

CO(NH₂)₂ + 3H₂O → (NH₄)₂CO₃·H₂O

(NH₄)₂CO₃·H₂O + 2H⁺ → 2NH₄⁺ + CO₂↑ + 2H₂O

(NH₄)₂CO₃·H₂O + CaX₂ → CaCO₃↓ + 2NH₄X

soluble salt of divalent cations

CaCO₃ precipitation and pH depression (exchange of Ca for H⁺ on exchange sites) as mechanisms of ammonia volatilization reduction!

[see equations 6 and 7]