What is the largest reservoir of nitrogen on the planet?
Nitrogen: The Basics

Nitrogen is (almost always) a limiting nutrient.

All plants (marine, aquatic, and terrestrial), phytoplankton, and bacteria can use NH\textsubscript{4} (ammonia) as a nitrogen source.

Most plants and some bacteria can use NO\textsubscript{3} (nitrate) as a nitrogen source.

Only a few cyanobacteria and microbes can use N\textsubscript{2} as a nitrogen source.
Redox is Important: Controls Speciation in Compounds

<table>
<thead>
<tr>
<th></th>
<th>Reduced</th>
<th></th>
<th></th>
<th></th>
<th>Oxidized</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-3</td>
<td>-2</td>
<td>-1</td>
<td>0</td>
<td>+1</td>
<td>+2</td>
<td>+3</td>
<td>+4</td>
<td>+5</td>
</tr>
<tr>
<td>Protein</td>
<td>R-NH₂</td>
<td>NH₃/NH₄⁺</td>
<td></td>
<td></td>
<td>NO₂⁻</td>
<td>NO₃⁻</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-NH₂</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NH₃/NH₄⁺</td>
<td></td>
<td></td>
<td></td>
<td>assimilation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| ammonia nitrogen fixation | | | | | | | | | nitration
| N₂(g) | N₂O(g) | NO(g) | NO₂⁻ | NO₃⁻ | | | | | |
| | | | | | | | | | |
| N₂(g) | | | | | | | | | demitrification

Diagrams:
- Assimilation
- Nitrification
- Demitrification
- Nitrogen fixation
What Controls $\delta^{15}$N Variation in Soils?

Amundson et al. 2003
What Controls $\delta^{15}N$ Variation in Plants?

Australia Plants

Count

$\delta^{15}N$
Major Themes

Chemical transformations are key to isotopic fractionation.

Although there are exceptions, the conversion of organic to inorganic material is most often the primary interest.

Separate internal cycling from additions and losses.
Chalkboard
Nitrification

NO$_3^-$

Soil Organic Matter

Labile

Recalcitrant

The Living

Assimilation

Mineralization

NH$_4^+$

Nitrification

NO$_3^-$
## Soil Processes: Observed $\Delta^{15}\text{N}$

<table>
<thead>
<tr>
<th>Process</th>
<th>Fractionation (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{N}_2$ fixation</td>
<td>$-2$ to $2$</td>
<td>(1)</td>
</tr>
<tr>
<td>Assimilation</td>
<td>$-1$ to $1.6$</td>
<td>(2)</td>
</tr>
<tr>
<td><strong>Large</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrification</td>
<td>$12$ to $35$</td>
<td>(3), (1)</td>
</tr>
<tr>
<td>Denitrification</td>
<td>$0$ to $33$, $26$</td>
<td>(1), (4)</td>
</tr>
<tr>
<td>Ammonia volatilization</td>
<td>$20$ to $27$</td>
<td>(1)</td>
</tr>
<tr>
<td><strong>Small</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineralization</td>
<td>$-1$ to $1$</td>
<td>(2)</td>
</tr>
<tr>
<td>Ion exchange</td>
<td>$-1$ to $-8$</td>
<td>(5)</td>
</tr>
<tr>
<td>Enzymatic hydrolysis</td>
<td>$10$ to $24$</td>
<td>(6)</td>
</tr>
<tr>
<td>$\text{N}$ transfer, ECM fungi to plant host</td>
<td>$8$ to $10$</td>
<td>(6)</td>
</tr>
<tr>
<td>$\text{N}$ transfer, AM fungi to plant host</td>
<td>$0$ to $3.5^a$</td>
<td>(7)</td>
</tr>
</tbody>
</table>

Positive values indicate that the reactant is enriched in $^{15}\text{N}$ (e.g., $\text{NH}_4^+$ in nitrification) and the product is depleted in $^{15}\text{N}$ (e.g., $\text{NO}_3^-$ in nitrification). Sources: (1) Högberg (1997), (2) Kendall (1998), (3) Shearer and Kohl (1986), (4) Pörlt et al. (2007), (5) Hübner (1986), (6) Hobbie and Colpaert (2003), (7) Handley et al. (1999b)

*ECM* ectomycorrhizal, *AM* arbuscular mycorrhizal

*a* Based on maximum difference between nonmycorrhizal and mycorrhizal plants
Major Transformations

The Living

Observe $\Delta^{15}N$

$^{14}\text{NH}_3$

Mineralization (-1‰ to +1‰)

Assimilation

Labile

Recalcitrant

Soil Organic Matter

$\text{NH}_4^+$

Nitrification

$\text{NO}_3^-$
The Living

Soil Organic Matter

Labile

Recalcitrant

NH₄⁺

Observed Δ¹⁵N

¹⁴NH₃

Mineralization (-1‰ to +1‰)

Nitrification

NO₃⁻
Fractionation During NH$_4$ Uptake

Intermediate $\Delta^{15}$N
Enzyme Kinetic Fractionation

Intermediate to High $\Delta^{15}$N
Equilibrium & Kinetic Isotope Effect
$\text{NH}_4 \leftrightarrow \text{NH}_3 + H^+$

Relatively Low $\Delta^{15}$N
$\delta^{15}N_{\text{plant}} \approx \delta^{15}N_{\text{source}}$

Fogel and Cifuentes 1993
Fractionation During NH$_4^+$ Uptake

- Nitrogen Limited
- Enzyme Limited
- Diffusion Limited

Extracellular NH$_4^+$ (uM) vs. Fractionation ($\Delta^{15}$N)

Fogel and Cifuentes 1993
Enzymes Associated with NH₄ Uptake

**GDH pathway**
- glutamate dehydrogenase

- \( \text{NH}_4^+ \)
- GDH
- Glutamate
- 2-oxoglutarate
- NADPH
- NADP

**GS-GOGAT pathway**
- glutamine synthetase-glutamate synthase

- \( \text{NH}_4^+ \)
- ATP → ADP + Pi
- Glutamate
- Glutamine
- 2-oxoglutarate
- NADP
- NADPH

- **One net glutamate**
- **No ATP** consumed
- **Low** affinity for \( \text{NH}_4^+ \) \( (K_M = 1.0 \text{ mM}) \)

- **One net glutamate**
- **One ATP** consumed per \( \text{NH}_4^+ \)
- **High** affinity for \( \text{NH}_4^+ \) \( (K_M = 0.1 \text{ mM}) \)

\[ \Delta^{15}N = 0-20\% \]

Van Heeswijk et al. 2013
Where Does This Occur?

Cytoplasm

\[ \text{NH}_4^+ \rightarrow \text{Glutamine} \rightarrow \text{Glutamate} \]

Chloroplast

\[ \text{Glutamine} \rightarrow \text{Glutamate} \rightarrow \text{Fd/NADH-GOGAT} \]

\[ \text{Glutamate} \rightarrow \text{Chlorophyl} \rightarrow \text{Light} \]

\[ \text{Chlorophyl} \rightarrow \text{PSI & PSII} \rightarrow \text{ATP/NADPH} \rightarrow \text{Calvin cycle} \rightarrow \text{Starch} \rightarrow \text{Sugar} \]

Peroxisome

\[ \text{2-oxoglutarate} \rightarrow \text{GGAT} \rightarrow \text{Glutamate} \]

2-oxoglutarate

Mitochondrion

\[ \text{NH}_4^+ \rightarrow \text{Glutamate} \rightarrow \text{GDH} \rightarrow \text{2-oxoglutarate} \]

\[ \text{NH}_4^+ \rightarrow \text{GS1} \rightarrow \text{Glutamine} \rightarrow \text{GS2} \rightarrow \text{2-oxoglutarate} \]

\[ \text{NH}_4^+ \rightarrow \text{NH}_4^+ \rightarrow \text{NH}_4^+ \rightarrow \text{NH}_4^+ \]

\[ \text{NH}_4^+ \rightarrow \text{Amt1.1} \rightarrow \text{NH}_4^+ \rightarrow \text{roots and transport to the shoot} \]

Ranathunge et al. 2014
Mean: +18‰ (±12‰ to ±35‰)

Nitrification - Observed $\Delta^{15}N$

Mineralization: -1‰ to +1‰

Soil Organic Matter

Labile

Recalcitrant

The Living

Assimilation: (-1‰ to +1‰)

15$^\text{NH}_4^+$

Nitrification Mean: +18‰ (+12‰ to +35‰)

14$^\text{NH}_3$

14$^\text{NO}$

14$^\text{N}_2O$
Nitrification

\[ 2\text{NH}_4 + 3\text{O}_2 \rightarrow 2\text{NO}_2 + 4\text{H} + 2\text{H}_2\text{O} \]
\((\text{Nitrosomonas, Comammox})\)

\[ 2\text{NO}_2 + \text{O}_2 \rightarrow 2\text{NO}_3 \]
\((\text{Nitrobacter, Nitrospira, Comammox})\)

OR

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

Nitrifying ammonia-oxidizing bacteria (AOB) and archaea (AOA)
Nitrifying organisms are chemoautotrophs (use CO\(_2\))
Nitrification takes place only in the presence of oxygen (aerobic)
Important in agricultural systems (converts NH\(_3\) to NO\(_3\))
Increases soil leaching because NO\(_3\) is more soluble in water
Major Transformations

Observed $\Delta^{15}N$

The Living

Soil Organic Matter

Labile

Recalcitrant

$^{14}\text{NH}_3$

$^{14}\text{NO}$

$^{14}\text{N}_2\text{O}$

$^{14}\text{N}_2$

Denitrification
Mean: +25‰
(0‰ to +33‰)

Mineralization
(-1‰ to +1‰)

Nitrification
Mean: +18‰
(+12‰ to +35‰)

Assimilation
(-1‰ to +1‰)

$\text{NH}_4^+$

$\text{NO}_3^-$
Denitrification

\[
\text{NO}_3^- + 12\text{H}^+ + 10\text{e}^- = \text{N}_2 + 6\text{H}_2\text{O}
\]

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

Denitrifying bacteria (nitrate reductase enzyme).
Takes place only in the absence of oxygen (anaerobic).
Deep soils, stagnant waters, ocean depths.
Lowers soil fertility by converting useful NO\(_3^-\) into useless N\(_2\).
Additions

- Biological N₂ Fixation
- Atmospheric Deposition
- Industrial N₂ Fixation

The Living

Soil Organic Matter

Labile

Recalcitrant

NH₄⁺

NO₃⁻

Denitrification
Mean: +25‰
(0‰ to +33‰)

Mineralization
(-1‰ to +1‰)

Nitrification
Mean: +18‰
(+12‰ to +35‰)

Assimilation
(-1‰ to +1‰)
Nitrogen Fixation: Nitrogenase

Fe Protein and Mo-Fe Protein
Reduced Ferredoxin (electron supply)

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

1) \text{N}_2
2) \text{HN}=\text{NH}
3) \text{HN}=\text{NH}
4) \text{H}_2\text{N}–\text{NH}_2
5) \text{H}_2\text{N}–\text{NH}_2
6) 2\text{NH}_3

Nitrogenase Enzyme Complex
Discrimination During N$_2$ Fixation: 0‰ to -2‰

Nitrogen Fixing Microbes

\[
\begin{array}{ccc}
\delta^{15}N_{\text{substrate}} & \delta^{15}N_{\text{algal}} & \Delta^{15}N_{\text{algal-N$_2$}} \\
(\%o) & (\%o) & (\%o) \\
-0.53 & -2.85 & -2.36 \\
-0.44 & -2.93 & -2.44 \\
-0.49 & -2.74 & -2.25 \\
-0.49 & -2.84 & -2.35 \\
\end{array}
\]
Nitrogen fixation is not perfect:
- Fixed nitrogen (NH$_3$) can be altered before plants assimilate it.
- Within plant fractionation (enzymatic or diffusion).
- Plants take up multiple sources (N$_2$/NH$_4$/NO$_3$)
The Living

Additions
- Biological N\(_2\) Fixation
- Atmospheric Deposition
- Industrial N\(_2\) Fixation

Labile

Recalcitrant

\(\text{SOIL ORGANIC MATTER}\)

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

\(\text{NO}_3^-\)

Denitrification Mean: +25‰ (0‰ to +33‰)

Mineralization Mean: +18‰ (+12‰ to +35‰)

Assimilation (-1‰ to +1‰)

Nitrification Mean: +18‰ (+12‰ to +35‰)
Productive Humboldt Current (Eastern Boundary Current). Seabirds breed on offshore rocks absent of predators. Seabird poo (guano): ammonium oxalate, urate, phosphates: high $\delta^{15}$N. A rare but important commodity that created conflicts among countries.
Industrial $N_2$ Fixation

- Fritz Haber (1886–1934)
- 1918 Nobel Prize (Chemistry)
  - “Father of Chemical Warfare”
- Anthropogenic generation of fertilizers
  - >50% of current fertilizer production.
- Industrially completing the same reaction as biological $N_2$ fixation.
  - $2N + 3H_2 = 2NH_3$
- Fractionation close to zero but can vary by the process.
  Becomes progressively positive with processing.
### Power Plant NO₂ Pollution

<table>
<thead>
<tr>
<th>CAT175</th>
<th>CTH110</th>
<th>DCP114</th>
<th>HWF187</th>
<th>KSF112</th>
<th>LYK123</th>
<th>OXF122</th>
<th>PSU106</th>
</tr>
</thead>
</table>

- **Power plant NO₂ emissions**
- **Natural NO₂ emissions** (Lightning and biogenic soil emissions)

**NO₂ δ¹⁵N**

![Graph showing Power Plant NO₂ Pollution](image_url)
Atmospheric Deposition

Altieri et al. 2013
What Controls $\delta^{15}$N Variation in Plants

- Precipitation
- Nitrogen Content in Soils
- Source of Inorganic Nitrogen
- Nitrogen Fixation
- Microbes in Soils/Sediments