Lecture 14: Nitrogen Cycle

Sources: Atlas and Bartha, Chapter 11
Brock Biology of Microorganisms, general reference
Maier et al., Environmental Microbiology, Chapters 14 and 15
NY Times article, “Too Much of a Good Thing Makes Benign Nitrogen a Triple Threat.”

1. Introduction (or Why are We Obsessed?)
   -- most commonly limiting nutrient (temperate bias)
   -- important plant nutrient
   -- critical for food production
   -- human manipulation is intense
   -- pollutant in water and air

2. Reservoirs

<table>
<thead>
<tr>
<th>Nitrogen reservoir</th>
<th>Metric tons nitrogen</th>
<th>Actively cycled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmosphere</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂</td>
<td>$3.9 \times 10^{15}$</td>
<td>No</td>
</tr>
<tr>
<td>Ocean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomass</td>
<td>$5.2 \times 10^{8}$</td>
<td>Yes</td>
</tr>
<tr>
<td>Dissolved and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>particulate organics</td>
<td>$3.0 \times 10^{11}$</td>
<td>Yes</td>
</tr>
<tr>
<td>Soluble salts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NO₃⁻, NO₂⁻, NH₄⁺)</td>
<td>$6.9 \times 10^{11}$</td>
<td>Yes</td>
</tr>
<tr>
<td>Dissolved N₂</td>
<td>$2.0 \times 10^{13}$</td>
<td>No</td>
</tr>
<tr>
<td>Land</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biota</td>
<td>$2.5 \times 10^{10}$</td>
<td>Yes</td>
</tr>
<tr>
<td>Organic matter</td>
<td>$1.1 \times 10^{11}$</td>
<td>Slow</td>
</tr>
<tr>
<td>Earth's crust*</td>
<td>$7.7 \times 10^{14}$</td>
<td>No</td>
</tr>
</tbody>
</table>

* This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Adapted from Dobrovolsky, 1994.)

Maier et al. 2000
-- the largest reservoirs are inert and the smallest ones cycle actively
-- dinitrogen (N$_2$) gas has been accumulating in the atmosphere since Earth formed
-- new N enters biosphere only through N fixation (microbial, lightning, or fertilizer production)
-- actively cycled N inputs through dry and wet deposition

*Figure 12.2* The global nitrogen cycle. Each flux is shown in units of $10^{12}$ g N/yr. Values are derived in the text.

Schlesinger 1997

3. N cycle reactions and related processes
-- 7 oxidation states of N
-- most transformations are biotic, most carried out by microorganisms
N fixation $\rightarrow$ $N_2 + 6e^- \rightarrow 2NH_3 \rightarrow$ Amino Acids $\rightarrow$ Proteins

-- large energy input required to break that N to N triple bond
-- free-living and mutualistic approaches to obtaining this energy
  • cyanobacteria pair photosynthesis and N$_2$ fixation
  • *Rhizobium* associates with plants, gets photosynthate in return for giving up some NH$_3$
  • *Frankia* associates with alder trees
  • more N$_2$ fixation in rhizosphere (*Azotobacter, Azospirillum, others*)
  • heterotrophy (*Azotobacter, others*)
-- typically occurs in environments with low or limiting NH$_3$ concentrations

-- N fixation done by enzyme complex, nitrogenase, encoded by *nif* genes
  • dinitrogenase reductase (Fe protein)
  • dinitrogenase (MoFe protein)
-- nitrogenase very sensitive to O$_2$
  • reduced oxygen tension (*Azospirillum*)
  • anaerobic heterotrophs (*Clostridium, Desulfovibrio, others*)
  • anoxygenic phototrophs (*Chromatium, Rhodospirillum, others*)
  • protective structures – root nodule for *Rhizobium*, heterocysts in cyanobacteria
  • nonheterocystous cyanobacteria – temporal separation between photosynthesis and N fixation; mats

**Haber-Bosch process** (industrial fertilizer production, i.e., human N fixation)

$$3CH_4 + 6H_2O \rightarrow 3CO_2 + 12H_2$$ (i.e., fossil fuel combustion)

$$4N_2 + 12H_2 \rightarrow 8NH_3$$ (under high temperature and pressure)
### Table 18.2: Representative Genera of Free-Living Nitrogen Fixers

<table>
<thead>
<tr>
<th>Status with respect to oxygen</th>
<th>Mode of energy generation</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobe</td>
<td>Heterotrophic</td>
<td><em>Azotobacter</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Beijerinckia</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Azotobacter</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td>Faculative anaerobe</td>
<td>Heterotrophic</td>
<td><em>Klebsiella</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus</em></td>
</tr>
<tr>
<td>Microaerophile</td>
<td>Heterotrophic</td>
<td><em>Xanthobacter</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Azospirillum</em></td>
</tr>
<tr>
<td>Strict anaerobe</td>
<td>Autotrophic</td>
<td><em>Thiobacillus</em></td>
</tr>
<tr>
<td></td>
<td>Heterotrophic</td>
<td><em>Clostridium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Desulfovibrio</em></td>
</tr>
<tr>
<td>Aerobe</td>
<td>Phototrophic (cyanobacteria)</td>
<td><em>Anabaena</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Nostoc</em></td>
</tr>
<tr>
<td>Faculative anaerobe</td>
<td>Phototrophic (bacteria)</td>
<td><em>Rhodospirillum</em></td>
</tr>
<tr>
<td>Strict anaerobe</td>
<td>Phototrophic (bacteria)</td>
<td><em>Chlorobium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Chromatium</em></td>
</tr>
</tbody>
</table>

### Table: Dinitrogen fixed (kg/ha/yr)

<table>
<thead>
<tr>
<th>Organism or system</th>
<th>Dinitrogen fixed (kg/ha/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free-living microorganisms</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria (&quot;blue-green algae&quot;)</td>
<td>25</td>
</tr>
<tr>
<td><em>Azotobacter</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Clostridium pasteurianum</em></td>
<td>0.1–0.5</td>
</tr>
<tr>
<td>Grass–bacteria associative symbioses</td>
<td>5–25</td>
</tr>
<tr>
<td>Plant–cyanobacterial associations</td>
<td></td>
</tr>
<tr>
<td><em>Gunnaria</em></td>
<td>12–21</td>
</tr>
<tr>
<td><em>Azolla</em></td>
<td>313</td>
</tr>
<tr>
<td>Lichens</td>
<td>39–84</td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
</tr>
<tr>
<td>Soybeans (Glycine max L. Merr.)</td>
<td>57–97</td>
</tr>
<tr>
<td>Cowpeas (Vigna, Lespedeza, Phaseolus, and others)</td>
<td>84</td>
</tr>
<tr>
<td>Clover (Trifolium hybridum L.)</td>
<td>104–160</td>
</tr>
<tr>
<td>Alfalfa (Medicago sativa L.)</td>
<td>128–300</td>
</tr>
<tr>
<td>Lupines (Lupinus sp.)</td>
<td>150–169</td>
</tr>
<tr>
<td>Nodulated nonlegumes</td>
<td></td>
</tr>
<tr>
<td><em>Alnus</em> (alders, e.g., red and black alders)</td>
<td>40–300</td>
</tr>
<tr>
<td>Hippophae (sea buckthorn)</td>
<td>2–179</td>
</tr>
<tr>
<td>Ceanothus (snow brush, New Jersey tea, California lilac)</td>
<td>60</td>
</tr>
<tr>
<td>Coriaria (&quot;tutu&quot; in New Zealand)</td>
<td>60–150</td>
</tr>
<tr>
<td>Casuarina (Australian pine)</td>
<td>58</td>
</tr>
</tbody>
</table>
Ammonification

Organic matter N $\rightarrow$ NH$_3$ (NH$_4^+$ in acid to neutral aqueous solution)

-- organic to inorganic, so decomposition
-- plant detritus C-C-C-C-NH$_2$, microbes eat up C for growth and energy
-- what happens to the N?
  • released into soil solution if microbe doesn’t need it (mineralization)
  • taken up by microbes if they need it to degrade more organic C (immobilization)
  • which process dominates depends on C:N of litter being decomposed

examples:
1. sawdust C:N = 225:1
   bacteria C:N = 6:1
   result: to get the C, the bacteria scavenges N from soil (bad for plants)
   IMMOBILIZATION

2. manure C:N = 15:1
   result: eventually C:N gets down to 6:1, still N around, released into soil
   MINERALIZATION

  • magic C:N ~ 25:1
  • perspective – oak litter C:N = 80:1; maple litter C:N = 50:1
  -- immobilization is assimilation by cells into amino acids (organic N)
  -- balance of immobilization and mineralization is net mineralization
  -- lots of guys do this

-- fate of NH$_4^+$
  • plant uptake
  • microbial uptake
  • volatilization
  • bound to soil colloids or humus
  • bound to clay
  • nitrification

\[
\begin{align*}
\text{Nitrification:} & \quad \text{Nitrosomers} \quad \text{Nitrifiers} \\
& \quad \text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-
\end{align*}
\]

1 = ammonia monooxygenase
2 = hydroxylamine oxidoreductase
3 = nitrite oxidoreductase

-- nitrifiers are aerobic chemolithoautotrophs (use energy from oxidation of ammonia to fix carbon for assimilation)
-- two step process (NH$_4^+$ $\rightarrow$ NO$_2^-$ and NO$_2^-$ $\rightarrow$ NO$_3^-$)
• first step by nitrosofiers (*Nitrosomonas*)
• second step by nitrifiers (*Nitrobacter*)
• steps are closely coupled
• both energy-yielding processes, but not much (35 moles NH₃ for one mole CO₂, 100 moles NO₂⁻ for one mole CO₂)

-- “pack of lies”
• aerobic
• limited number of genera
• limited substrate range
• autotrophic

-- nitrifiers are sensitive to environmental stress; population sizes typically low
-- marine and soil environments

-- fate of NO₃⁻
• plant uptake
• leaching, runoff
• reduction to NH₄⁺ (assimilatory and dissimilatory pathways)
• reduction to dinitrogen gas (N₂)

Assimilatory Nitrate Reduction → NO₃⁻ → NH₃ → Amino Acids

-- done by lots of guys with NO₃⁻ and NO₂⁻ reductases
-- preferential uptake of NH₄⁺ rather than NO₃⁻ for assimilation
-- inhibited by NH₄⁺ but not by O₂

Dissimilatory NO₃⁻ Reduction
-- redox reaction; NO₃⁻ converted to variety of reduced products, while organic matter is oxidized
-- uses NO₃⁻ as terminal e⁻ acceptor
-- two pathways (DNRA and denitrification)

Dissimilatory Nitrate Reduction to Ammonia (DNRA)

\[ \text{NO}_3^- + 4\text{H}_2 + 2\text{H}^+ \rightarrow \text{NH}_4^+ + 3\text{H}_2\text{O} \]

-- multistep process
• \[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NH}_4^+ \]
• 1st step energy-producing step
• 2nd step regenerates reducing equivalents used to oxidize C substrates
-- environmentally-limited process (consumes lots of reducing equivalents)
• carbon-rich environments
• few e⁻ acceptors
• sewage sludge
• stagnant water
• some sediments (high organic matter)
• rumen
  -- inhibited by O₂, not NH₄⁺ (opposite of assimilatory nitrate reduction)
  -- many are facultative anaerobes, heterotrophic, fermentative (Escherichia, Vibrio, Enterobacter, others)

Denitrification  \[ \text{NO}_3^- + 5\text{H}_2 + 2\text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O} \]

-- multistep process
  • \( \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \)
  • 1ˢᵗ step by nitrate reductase, 2ⁿᵈ step by nitrite reductase, etc.
  • enzymes are increasingly sensitive to O₂, \( \text{N}_2\text{O} \) reductase also sensitive to low pH
-- energy-yielding process (anaerobic respiration), lots of energy
  • energy used to drive oxidation of organic matter
-- lower carbon environments, rich in e- acceptors
  • controlled by presence of O₂, \( \text{NO}_3^- \), and organic matter
  • often coupled with nitrification
-- inhibited by O₂, not NH₄⁺ (like DNRA, opposite of assimilatory \( \text{NO}_3^- \) reduction)
-- many are heterotrophic but respiratory, but some are \( \text{N}_2 \) fixers, autotrophs, fermenters
-- fate of products (NO, \( \text{N}_2\text{O} \), \( \text{N}_2 \))
  • gaseous loss to atmosphere
  • output of N from ecosystem
  • reduces soil fertility
  • reduces \( \text{NO}_3^- \) leaching to surface and groundwater
<table>
<thead>
<tr>
<th>Genus</th>
<th>Interesting characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organotrophs</strong></td>
<td></td>
</tr>
<tr>
<td><em>Alcaligenes</em></td>
<td>Common soil bacterium</td>
</tr>
<tr>
<td><em>Agrobacterium</em></td>
<td>Some species are plant pathogens</td>
</tr>
<tr>
<td><em>Aquaspirillum</em></td>
<td>Some are magnetotactic, oligotrophic</td>
</tr>
<tr>
<td><em>Azospirillum</em></td>
<td>Associative N₂ fixer, fermentative</td>
</tr>
<tr>
<td><em>Bacillus</em></td>
<td>Spore former, fermentative, some species thermophilic</td>
</tr>
<tr>
<td><em>Blastobacter</em></td>
<td>Budding bacterium, phylogenetically related to <em>Rhizobium</em></td>
</tr>
<tr>
<td><em>Bradyrhizobium</em></td>
<td>Symbiotic N₂ fixer with legumes</td>
</tr>
<tr>
<td><em>Brachymella</em></td>
<td>Animal pathogen</td>
</tr>
<tr>
<td><em>Chromobacterium</em></td>
<td>Purple pigmentation</td>
</tr>
<tr>
<td><em>Cytophaga</em></td>
<td>Gliding bacterium; cellulose degrader</td>
</tr>
<tr>
<td><em>Flavobacterium</em></td>
<td>Common soil bacterium</td>
</tr>
<tr>
<td><em>Flexibacter</em></td>
<td>Gliding bacterium</td>
</tr>
<tr>
<td><em>Halobacterium</em></td>
<td>Halophilic</td>
</tr>
<tr>
<td><em>Hyphomicrobium</em></td>
<td>Grows on one-C substrates, oligotrophic</td>
</tr>
<tr>
<td><em>Kingeria</em></td>
<td>Animal pathogen</td>
</tr>
<tr>
<td><em>Neisseria</em></td>
<td>Animal pathogen</td>
</tr>
<tr>
<td><em>Paracoccus</em></td>
<td>Halophilic, also lithotrophic</td>
</tr>
<tr>
<td><em>Propionibacterium</em></td>
<td>Fermentative</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>Commonly isolated from soil, very diverse genus</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>Symbiotic N₂ fixer with legumes</td>
</tr>
<tr>
<td><em>Wolinella</em></td>
<td>Animal pathogen</td>
</tr>
<tr>
<td><strong>Phototrophs</strong></td>
<td></td>
</tr>
<tr>
<td><em>Rhodopseudomonas</em></td>
<td>Anaerobic, sulfate reducer</td>
</tr>
<tr>
<td><strong>Lithotrophs</strong></td>
<td>Uses H₂, also heterotrophic, common soil isolate</td>
</tr>
<tr>
<td><em>Alcaligenes</em></td>
<td></td>
</tr>
<tr>
<td><em>Bradyrhizobium</em></td>
<td>Uses H₂, also heterotrophic, symbiotic N₂ fixer with legumes</td>
</tr>
<tr>
<td><em>Nitrosomonas</em></td>
<td>NH₃ oxidizer</td>
</tr>
<tr>
<td><em>Paracoccus</em></td>
<td>Uses H₂, also heterotrophic, halophilic</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>Uses H₂, also heterotrophic, common soil isolate</td>
</tr>
<tr>
<td><em>Thiobacillus</em></td>
<td>S-oxidizer</td>
</tr>
<tr>
<td><em>Thiomicrospira</em></td>
<td>S-oxidizer</td>
</tr>
<tr>
<td><em>Thiosphaera</em></td>
<td>S-oxidizer, heterotrophic nitrifier, aerobic denitrification</td>
</tr>
</tbody>
</table>

From Myrold, 1998.

Maier et al. 2000
Anammox (anoxic ammonia oxidation)  \(5\text{NH}_4^+ + 3\text{NO}_3^- \rightarrow 4\text{N}_2 + 9\text{H}_2\text{O} + 2\text{H}^+\)

1. \(\text{NH}_3 + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}\) (Energy metabolism)
2. \(\text{CO}_2 + 2\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{Biomass} + \text{NO}_3^-\) (Anabolic)

-- \(\text{NO}_2^-\) is e\(^-\) acceptor (reaction 1) and e\(^-\) donor (reaction 2)
-- anoxic conditions
-- chemolithoautotrophic
-- theorized to happen (depending on e\(^-\) acceptor) used based on thermodynamics, but no one had ever isolated the bug who did it despite much effort
-- originally described in 1995 in wastewater purification system
-- also found in some marine sediments
-- planctomycetes *Candidatus Brocadia* anammoxidans
  - reproduce by budding
  - anammoxosome takes up 30-60% of cell volume
  - dense, impermeable membrane perhaps contains toxic intermediates, hydrazine (\(\text{N}_2\text{H}_4\)) and hydroxylamine (\(\text{NH}_2\text{OH}\))
Abiotic processes
-- ammonia volatilization; NH$_3$ loss to atmosphere
-- deposition; wet and dry; NO$_3^-$, NH$_4^+$, dissolved organic nitrogen (DON)
-- lightning; N$_2$ fixation; momentary conditions of high pressure and temperature allow N$_2$ and O$_2$ to combine
-- pyrodenitrification; biomass burning, N$_2$ loss to atmosphere
-- weathering
4. **N balance on ecosystem and global scales**
   -- ecosystem example; upland, non-legume, temperate forest, 70 years old

**Pools (kg N/ha)**
- Soil organic matter: 7,000
- Plants: 500
- Inorganic pool: 30

**Fluxes (kg N/ha/yr)**

**Inputs**
- N₂ fixation: 1
- Deposition: 10

**Internal Cycling**
- Litterfall: 70
- Plant uptake: 80
- Net mineralization: 70
- Nitrification: ~30

**Outputs**
- Denitrification: 1
- Leaching: 1
Net Balance
Inputs = 11
Outputs = 2

NET RETENTION

-- similar in most temperate, terrestrial ecosystems
  • naturally low and high fertility systems have different retention rates and potential
  • can predict losses as a result of disturbance (like clearcutting)

-- where does N go? probably accumulating in soil organic matter, but then why are
  systems still N limited?
  • are we screwing up outputs?
  • is it unavailable?

-- NET LOSS from some systems like alder forests or at some times of year (dormant
  season)

-- net loss from human-altered systems
  • humans have doubled amount of new N added to systems (Vitousek et al.)
  • agriculture – harvesting, fertilizers, higher quality litter
  • increasing N deposition from fossil fuel combustion
  • N saturation – N provided in excess of plant and microbial requirements

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Figure 3. A conceptual model combining elements of the models of Aber et al. (1989) and Stoddard (1994). This model identifies and characterizes the four stages of N saturation along gradients of N deposition and forest health.
Figure 2. Hypothetical response of temperate forest ecosystems to chronic, elevated nitrogen addition. Abbreviations at the top refer to study sites in New England (HFHW = Harvard Forest hardwood stand; HFP = Harvard Forest pine stand; BBW = Bear Brook Watershed; Ascutney = Mt. Ascutney, Vermont; Transect = 161 spruce-fir stands along a N deposition gradient in New York and New England). Site begin in different stages of N saturation due to N inputs or land use history and progress along the stages due to community species composition. Source: Aber et al. 1998

-- too much nitrogen → vegetation changes
- species composition changes
- exotic invasions
- weedy species
- loss of species adapted to low nutrient conditions
- forest decline from nutrient imbalances and soil acidification
-- NO$_3^-$ loss to surface and groundwaters
- eutrophication
- freshwater acidification
- blue baby syndrome
-- 

- N₂O, NO loss to atmosphere
  - increased concentration of greenhouse gas N₂O (long residence time, 200 times more effective at trapping heat than CO₂)
  - N₂O and NO are involved in protective stratospheric ozone depletion

\[
\begin{align*}
N₂O + hv & \rightarrow N₂ + O \\
N₂O + O & \rightarrow 2NO \\
NO + O₃ & \rightarrow NO₂ + O₂ \\
NO₂ + O & \rightarrow NO + O₂
\end{align*}
\]

- atmospheric concentration of N₂O increasing at 0.3% year
- NO is a precursor of acid/N deposition and tropospheric ozone production – 80% of NO emissions are human-caused

-- fixes
  - more efficient fertilizer use
  - wetland restoration
  - riparian buffer strips
  - more efficient fossil fuel combustion
  - alternative energies