Nitrogen Cycling in the Sea

The diagram illustrates the nitrogen cycle in the sea, showing the conversion between different nitrogen species such as ammonia ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), and nitrogen gas ($N_2$). The cycle involves processes like nitrogen fixation ($N_2$-Fixation), denitrification (Denitrification), mineralization (Mineralization), transport/mixing (Transport/Mixing), and sinking/mixing (Sinking/Mixing). The diagram highlights the movement of these nitrogen compounds between the atmosphere, illuminated ocean, and dark ocean layers.
Outline

• Nitrogen species in marine waters-distributions and concentrations
• New, regenerated, and export production
• The processes: Assimilation, $\text{N}_2$ fixation, Nitrification, and Denitrification / Anammox
Summary

• Microbes control global nitrogen budgets
• The oxidation-reduction potential of nitrogen facilitates the role of N-containing compounds as electron donors and acceptors.
• The principal transformations of N in the ocean include: assimilation, regeneration, nitrification, denitrification, and nitrogen fixation
• Total amount of fixed nitrogen depends on the balance between $N_2$ fixation and denitrification
• Ocean nitrogen budgets remain highly uncertain
Pools and pathways of nitrogen in the sea

Nitrogen as a nutrient (nitrogen assimilation):
- $\text{NO}_3^-$
- $\text{NO}_2^-$
- $\text{NH}_4^+$
- DON

$\text{N}_2$—only selected groups of prokaryotes use $\text{N}_2$

Nitrogen as an electron donor:
- $\text{NH}_4^+$: ammonium oxidation/annamox
- $\text{NO}_2^-$: nitrite oxidation
- DON: heterotrophic catabolism

Nitrogen as an electron acceptor:
- $\text{NO}_3^-$: denitrification
- $\text{NO}_2^-$: denitrification, annamox
- NO: denitrification
- $\text{N}_2\text{O}$: denitrification
Redox states and chemical forms of nitrogen

5 stable oxidation states

Oxidized N

Reduced N

Nitrate Assimilation

Assimilation
Nitrogen assimilation

• Nitrogen is an essential nutrient found in amino acids, protein, and nucleic acids.
• Nitrogen is assimilated by both autotrophs and heterotrophs.
• In large areas of the world’s ocean, nitrogen limits primary production.
• Nitrogen in organic matter is reduced; however, most “fixed” nitrogen in the ocean is oxidized (nitrate) and thus requires reductant for assimilation into biomass.
### Energy and reducing power required for assimilation of various N compounds

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme</th>
<th>Reaction</th>
<th>Electrons</th>
<th>ATP</th>
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</thead>
<tbody>
<tr>
<td>N₂</td>
<td>Nitrogenase</td>
<td>N₂ → NH₃</td>
<td>8</td>
<td>16</td>
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<tr>
<td>NO₃⁻</td>
<td>Nitrate reductase</td>
<td>NO₃⁻ → NO₂⁻</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>Nitrite reductase</td>
<td>NO₂⁻ → NH₃</td>
<td>6</td>
<td>0</td>
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<tr>
<td>NH₃</td>
<td>Glutamine synthetase Glutamate synthetase</td>
<td>NH₃ → Glutamate</td>
<td>2</td>
<td>1</td>
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</table>
The relationships between concentrations and planktonic uptake of reduced and oxidized N

Patterns of NO$_3^-$ and NH$_4^+$ disappearance due to preferential uptake of NH$_4^+$

Simultaneous rates of NO$_3^-$ and NH$_4^+$ uptake as a function of NH$_4^+$ concentration
Concentrations and distributions of ocean nitrogen

- $\text{NO}_3^-$: concentrations range nanomolar to micromolar
- $\text{NO}_2^-$: concentrations typically nanomolar
- $\text{N}_2\text{O}$: concentrations typically nanomolar
- $\text{N}_2$: biologically inert (mostly); concentrations $\sim 600 \ \mu\text{mol L}^{-1}$
- $\text{NH}_4^+$: rapidly consumed in the photic zone, concentrations typically nanomolar
- DON: concentrations typically 4-6 $\mu\text{mol L}^{-1}$
### Oceanic concentrations, inventories and turnover of nitrogen

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Euphotic zone (µmol L⁻¹)</th>
<th>Mean aphotic zone (µmol L⁻¹)</th>
<th>Oceanic inventory (Tg N)</th>
<th>Turnover rate (Tg N yr⁻¹)</th>
<th>Turnover time (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate NO₃⁻</td>
<td>7</td>
<td>31</td>
<td>5.8 x 10⁵</td>
<td>1570</td>
<td>370</td>
</tr>
<tr>
<td>Nitrite NO₂⁻</td>
<td>0.1</td>
<td>0.006</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium NH₄⁺</td>
<td>0.3</td>
<td>0.01</td>
<td>340</td>
<td>7000</td>
<td>0.05</td>
</tr>
<tr>
<td>Dissolved Organic Nitrogen DON</td>
<td>6</td>
<td>4</td>
<td>7.7 x 10⁴</td>
<td>3400</td>
<td>20</td>
</tr>
<tr>
<td>Particulate Nitrogen PN</td>
<td>0.4</td>
<td>0.01</td>
<td>400</td>
<td>8580</td>
<td>0.05</td>
</tr>
<tr>
<td>Nitrous Oxide N₂O</td>
<td>0.01</td>
<td>0.04</td>
<td>750</td>
<td>6</td>
<td>125</td>
</tr>
<tr>
<td>Dinitrogen gas N₂</td>
<td>450</td>
<td>575</td>
<td>1 x 10⁷</td>
<td>200</td>
<td>54,000</td>
</tr>
</tbody>
</table>
Distributions of nitrate in near surface waters

White contour lines are nitrate concentrations, colors are satellite ocean color derived Chl a
New and regenerated production

1) *new* production supported by external input of N (e.g. NO$_3^-$ and N$_2$),
2) *recycled or regenerated* production, sustained by *in situ* recycling of N.

-Assumes steady state:
Input of new N is balanced by export of N.
The f-ratio

\[ f = \frac{V_{NO_3^-}}{V_{NO_3^-} + \sum N_R} \]

F-ratio describes relative contribution of new production to total production.

\( N_R \) includes regenerated N uptake (historically thought to include urea and \( NH_4^+ \)).

In steady state N inputs are balanced by export/grazing loss.
Eppley and Peterson (1979) examined the contribution of nitrate and ammonium to total primary production (as determined by $^{14}$C-bicarbonate assimilation) to provide a quantitative evaluation of the amount of production able to support fisheries and sink to the deep sea.

New production (NO$_3^-$ based)

~5-50% of total production across ocean basins; lower in oligotrophic systems, greater in upwelling systems.
Redfield stoichiometry of production and remineralization

**Organic matter production:**

\[
106 \text{ CO}_2 + 16 \text{ HNO}_3 + \text{ H}_3\text{PO}_4 + 122 \text{ H}_2\text{O} \Rightarrow (\text{CH}_2\text{O})_{106} (\text{NH}_3)_{16} (\text{H}_3\text{PO}_4) + 138\text{O}_2 
\]

- Consumes CO\(_2\)
- Consumes nutrients
- Produces oxygen

This equation describes nitrate assimilation by photosynthesis.

**Aerobic remineralization of organic matter:**

\[
(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 138\text{O}_2 \Rightarrow 106\text{CO}_2 + 122\text{H}_2\text{O} + 16\text{HNO}_3 + \text{H}_3\text{PO}_4 
\]

- Consumes O\(_2\)
- Produces CO\(_2\)
- Produces nutrients
Station ALOHA nutrient profiles

DIC (µmol kg⁻¹)

NO₃⁻ + NO₂⁻ (µmol kg⁻¹)

PO₄³⁻ (µmol kg⁻¹)

NO₃⁻ + NO₂⁻ : PO₄³⁻
# Inorganic Nutrient Concentrations and Stoichiometry at Station ALOHA

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>DIC (µmol L⁻¹)</th>
<th>NO₃⁻ + NO₂⁻ (µmol L⁻¹)</th>
<th>PO₄³⁻ (µmol L⁻¹)</th>
<th>DIC: NO₃⁻ (mol: mol)</th>
<th>DIC: PO₄³⁻ (mol: mol)</th>
<th>NO₃⁻: PO₄³⁻ (mol: mol)</th>
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<tbody>
<tr>
<td>5</td>
<td>1974</td>
<td>0.02</td>
<td>0.07</td>
<td>98700</td>
<td>28200</td>
<td>0.29</td>
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<tr>
<td>150</td>
<td>2027</td>
<td>1.1</td>
<td>0.15</td>
<td>1843</td>
<td>13513</td>
<td>7.3</td>
</tr>
<tr>
<td>300</td>
<td>2070</td>
<td>9.8</td>
<td>0.74</td>
<td>211</td>
<td>2797</td>
<td>13</td>
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<tr>
<td>750</td>
<td>2322</td>
<td>41</td>
<td>3.0</td>
<td>57</td>
<td>774</td>
<td>14</td>
</tr>
<tr>
<td>3000</td>
<td>2346</td>
<td>37.5</td>
<td>2.6</td>
<td>63</td>
<td>902</td>
<td>14</td>
</tr>
</tbody>
</table>

**Redfield**: 106C : 16 N : 1 P (mol:mol:mol)
For net carbon removal by biology, need to export material with greater C:N ratio than nutrient input C:N ratio

• A parcel of water brought to the surface from 150 m:
  \[ \Delta \text{DIC}:\Delta \text{NO}_3^- = (2027-1974) / (1.1) = 48 \]

• A parcel of water brought to the surface from 300 m:
  \[ \Delta \text{DIC}:\Delta \text{NO}_3^- = (2070-1974) / (9.8) = 9.7 \]

• A parcel of water brought to the surface from 750 m:
  \[ \Delta \text{DIC}:\Delta \text{NO}_3^- = (2322-1974) / (41) = 8.5 \]
Not all “new” nutrients are introduced to the euphotic zone from below…

- Atmospheric deposition (both dry and wet) can form an important source of nutrients.
- Advection: lateral input of nutrients
- N$_2$ fixation
• N₂ fixation is the primary mode of introducing “fixed” nitrogen to the biosphere.

• N₂ fixation converts N₂ to NH₃; process is exclusively mediated by prokaryotes

• Energy expensive to break triple bond in N₂

\[
N₂ + 8H^+ + 8e^- + 16 \text{ ATP} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{PO}_4^{3-}
\]
Table 1. Annual particulate nitrogen export fluxes at Station ALOHA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total PN flux (mmol N m(^{-2}) yr(^{-1}))</th>
<th>(\delta^{15}N)-F(_{PN})(^{*}) (% vs. air N(_2))</th>
<th>(\delta^{15}N)-supported fraction (%)</th>
<th>Contributions to flux (mmol N m(^{-2}) yr(^{-1}))</th>
<th>N(_2)</th>
<th>NO(_3^-)</th>
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<tr>
<td>1990</td>
<td>145</td>
<td>3.85</td>
<td>41</td>
<td>59</td>
<td>86</td>
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<td>1991</td>
<td>111</td>
<td>3.21</td>
<td>51</td>
<td>56</td>
<td>55</td>
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<tr>
<td>1992</td>
<td>80</td>
<td>3.58</td>
<td>45</td>
<td>36</td>
<td>44</td>
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<tr>
<td>1993</td>
<td>86</td>
<td>4.15</td>
<td>36</td>
<td>31</td>
<td>55</td>
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</tr>
<tr>
<td>1994</td>
<td>80</td>
<td>3.81</td>
<td>41</td>
<td>33</td>
<td>47</td>
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<tr>
<td>1995</td>
<td>81</td>
<td>3.76</td>
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<td>34</td>
<td>47</td>
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</tr>
<tr>
<td>1996</td>
<td>87</td>
<td>4.04</td>
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<td>33</td>
<td>54</td>
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<td>1997</td>
<td>113</td>
<td>3.29</td>
<td>49</td>
<td>56</td>
<td>57</td>
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</tr>
<tr>
<td>1998</td>
<td>116</td>
<td>3.21</td>
<td>51</td>
<td>59</td>
<td>57</td>
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<tr>
<td>1999</td>
<td>122</td>
<td>2.03</td>
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<td>84</td>
<td>38</td>
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<tr>
<td>2000</td>
<td>113</td>
<td>3.05</td>
<td>53</td>
<td>60</td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>

* Flux-weighted annual average.

Dore et al. (2002)

At Station ALOHA, N\(_2\) fixation appears to contribute \(~30-84\%\) of new production—note that this is <5\% of total production.
Global estimate of $N_2$ fixation based on N-DIC drawdown in $NO_3$-depleted waters averages $0.8 \pm 0.3$ Pg C yr$^{-1}$.
$N_2$ fixation may also play an important role in controlling nutrient stoichiometry in the sea.

$$\text{TDN} = 14.57(\text{TDP}) + 1.5$$

Nearly identical slopes, but different intercepts.

$$\text{NO}_3^- = 14.62(\text{PO}_4^{3-}) - 1.08$$
Aerobic remineralization of organic matter:

\[(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 \Rightarrow 106CO_2 + 122H_2O + 16HNO_3 + H_3PO_4\]

- Consumes \(O_2\)
- Produces \(CO_2\)
- Produces nutrients

This reaction does not show the complex series of reactions required to transform organic nitrogen to nitrate (ammonification, ammonium oxidation, nitrite oxidation):

- \(DON \rightarrow NH_4^+\)
- \(NH_4^+ \rightarrow NO_2^-\)
- \(NO_2^- \rightarrow NO_3^-\)
Dissimilatory nitrogen transformations

NH$_2$OH $\rightarrow$ NO $\rightarrow$ N$_2$O

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

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

Anammox: \[ \text{NO}_2 + \text{NH}_4^+ \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \]
Nitrification

• Biological oxidation of NH$_3$ to NO$_3^-$ using oxygen as terminal electron acceptor.

• Two step process; ammonia oxidation followed by nitrite oxidation; both reactions yield energy.

• NO$_2^-$ serves as an important intermediate; incomplete nitrification also yields N$_2$O.
Redox states and chemical forms of nitrogen

Energy to be gained in oxidation
Degradation of organic N to ammonium occurs during heterotrophic metabolism.

Nitrification is a 2 step process that is mediated by different groups of microbes. The first step (termed ammonium oxidation) oxidizes $\text{NH}_4^+$ to $\text{NO}_2^-$, and the second step converts $\text{NO}_2^-$ to $\text{NO}_3^-$. 

\[ \text{NH}_2\text{OH} \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \]
Aerobic regeneration of nitrogen

Complete decomposition of organic matter

$$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 138\text{O}_2 \Rightarrow 106\text{CO}_2 + 122\text{H}_2\text{O} + 16\text{HNO}_3 + \text{H}_3\text{PO}_4$$

$$2\text{NH}_4^+ + 3\text{O}_2 \Rightarrow 2\text{NO}_2^- + 4\text{H}^+ + 2\text{H}_2\text{O}$$

$$2\text{NO}_2^- + \text{O}_2 \Rightarrow 2\text{NO}_3^-$$

These reactions yield energy (but not much…)

Most nitrifying microbes are chemoautotrophic (best studied are *Nitrosomonas* and *Nitrobacter*)
Recent isolation and cultivation of an abundant archaeal ammonium oxidizer

Figure 2 | Photomicrographs of SCM1. a, b, Fluorescence image of cells in identical fields of view stained with DAPI (a) and after hybridization with nucleotide polyprobes targeting SCM1 cells (b). Arrows indicate cells showing the characteristic peanut-like shape of marine Crenarchaeaota. Scale bars represent 1 μm. c, Transmission electron micrograph of negative-stained cells. Scale bar represents 0.1 μm. d, Scanning electron micrograph of Au/Pd-sputtered cells. Scale bar represents 0.1 μm.

Figure 3 | Near-stoichiometric conversion of ammonia to nitrite by SCM1. Growth of SCM1 in Synthetic Crenarchaeota Media containing ammonium chloride and bicarbonate as sole energy and carbon sources, respectively. DAPI-stained cells were directly counted on filters by fluorescence microscopy. Ammonium consumption and nitrite production were determined in triplicate as described previously.
Denitrification

The reduction of NO$_3^-$ and NO$_2^-$ to N$_2$ during heterotrophic respiration of organic matter. Denitrification implies N is lost from the ecosystem as a gaseous bi-product. Occurs predominately in anaerobic or suboxic environments.

\[ C_{106}H_{175}O_{42}N_{16}P + 104 \text{NO}_3^- \rightarrow 106\text{CO}_2 + 60\text{N}_2 + \text{H}_3\text{PO}_4 + 138\text{H}_2\text{O} \]

NO$_3^-$ and NO$_2^-$ serve as terminal electron acceptors during heterotrophic respiration.

NO$_2^-$ serves as an important intermediate, as do N$_2$O and NO.

Serves as the major sink for fixed nitrogen from aquatic environments.
NO$_2^-$ forms an important intermediate in many N-cycling reactions.
Anaerobic ammonium oxidation (anammox)

- $\text{NH}_4^+ + \text{NO}_2^- \rightarrow 2\text{N}_2 + 2\text{H}_2\text{O}$

- Anaerobic ammonium oxidation

- Major source of $\text{N}_2$ gas (along with denitrification)

- Anoxic sediments, marine water column, and sewage wastewater

- Mediated by *Planctomyces*
Oxygen concentrations along the 26.9 kg m\(^{-3}\) isopycnal surface (~500 m in the N. Pacific)

Chlorophyll distributions
High productivity in surface water due to upwelling of nutrients.

High organic matter flux depletes $O_2$ concentrations below the euphotic zone.

Fig. 1. The Benguela system. (a) Distribution of annual primary production (source: http://marine.rutgers.edu/opp/swf/Production/results/all2.swf.html). The white box indicates the extent of the Benguela upwelling system. (b) Vertical transect showing the lateral extension and fixed-inorganic-nitrogen deficit in the OMZ of Namibia at 23° south (see Nutrient Analyses in Methods). (c) Sites and nitrogen losses. The open circles represent sites used to construct the lateral transect in b. The blue bars represent the depth-integrated nitrogen loss (mmol m$^{-2}$ d$^{-1}$) through anammox determined from anaerobic $^{15}$N incubations of water collected from six or seven depths throughout the suboxic zone at sites M178, M182, M199, and M202. For sites M179 and M205 (red circles) anaerobic $^{15}$N incubations indicate anammox activity, but rates were not measured. The integrated nitrogen loss for site M166 (red circle) is not shown because anaerobic $^{15}$N incubations were performed for only one depth (107 m, Fig. 4). Anammox activity was undetectable at site M173 (green triangle), where oxygen concentrations in the bottom waters exceed 20 $\mu$M.
Coupling between $N_2$ fixation and denitrification
Global Nitrogen Budget

<table>
<thead>
<tr>
<th>Process</th>
<th>Nitrogen Flux (TgN yr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sources</strong></td>
<td></td>
</tr>
<tr>
<td>Pelagic $N_2$ fixation</td>
<td>120 ± 50</td>
</tr>
<tr>
<td>Benthic $N_2$ fixation</td>
<td>15 ± 10</td>
</tr>
<tr>
<td>River input (DON)</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>River input (PON)</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>Atmospheric deposition</td>
<td>50 ± 20</td>
</tr>
<tr>
<td><strong>Total Sources</strong></td>
<td>265 ± 55</td>
</tr>
<tr>
<td><strong>Sinks</strong></td>
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</tr>
<tr>
<td>Organic N export</td>
<td>1</td>
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<tr>
<td>Benthic denitrification</td>
<td>180 ± 50</td>
</tr>
<tr>
<td>Water column denitrification</td>
<td>65 ± 20</td>
</tr>
<tr>
<td>Sediment burial</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>$N_2O$ loss to atmosphere</td>
<td>4 ± 2</td>
</tr>
<tr>
<td><strong>Total Sinks</strong></td>
<td>275 ± 55</td>
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