Stable Isotope Notation

- Isotope data reported in standard ‘‰’ or ‘δ’ notation:

Nitrogen

\[
\delta^{15}N = \left[ \frac{\left( \frac{^{15}N/^{14}N \right)_{\text{sample}}}{\left( \frac{^{15}N/^{14}N \right)_{\text{AIR}}} - 1 \right] \times 1000
\]

Oxygen

\[
\delta^{18}O = \left[ \frac{\left( \frac{^{18}O/^{16}O \right)_{\text{sample}}}{\left( \frac{^{18}O/^{16}O \right)_{\text{VSMOW}}} - 1 \right] \times 1000
\]
The Nitrogen Cycle

N is present in many chemical forms, both organic and inorganic, in the atmosphere, biosphere, hydrosphere, and geosphere. It occurs in the gas, liquid (dissolved in water), and solid phases. N can be associated with organic species and with inorganic species. Important inorganic species include $\text{N}_2$, nitric acid ($\text{HNO}_3$), nitrate ($\text{NO}_3^-$), nitrite ($\text{NO}_2^-$), nitrous oxide ($\text{N}_2\text{O}$), nitric oxide (NO), N dioxide ($\text{NO}_2$), ammonium ($\text{NH}_4^+$), and ammonia ($\text{NH}_3$). Most organic N species are bio-molecules, such as proteins, peptides, enzymes, and genetic material (RNA and DNA). $\text{NO}_3^-$ and organic-N species exist in solution and as particulates.
The Nitrogen Cycle

Atmospheric N₂ 0‰. Dissolved N₂ 1‰. N fixation -2 to +2‰. Other -20‰ to +20‰ depending on processes involving fractionation and mixing.

Denitrification, assimilation, excretion prefer 14N, enriching residual nitrate.

*Figure 5* The δ¹⁵N distributions in ecosystems. See Figure 4 legend for explanation of symbols. Sources: 26, 27, 14, 48, 52, 60, 63, 71, 75, 76, 106, 108, 109.

(Peterson and Fry, 1987)
Terrestrial Nitrogen Cycle

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)

Terrestrial nitrogen cycle(s)
Aquatic Nitrogen Cycle

Table 4. Estimated Sources and Sinks in the Global Marine Nitrogen Budget Based on Codispoti and Christensen [1985]. This Study, and a Survey of the Recent Literature

<table>
<thead>
<tr>
<th>Process</th>
<th>Modified From Codispoti and Christensen [1985], Tg N yr⁻¹</th>
<th>This Study and Literature Survey, Tg N yr⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelagic N₂ fixation</td>
<td>25</td>
<td>110 ± 40²</td>
</tr>
<tr>
<td>Benthic N₂ fixation</td>
<td>25</td>
<td>15 ± 10²</td>
</tr>
<tr>
<td>River input (POC)</td>
<td>25</td>
<td>21 ± 10²</td>
</tr>
<tr>
<td>River input (PON)</td>
<td>25</td>
<td>24 ± 10²</td>
</tr>
<tr>
<td>Atmospheric deposition</td>
<td>24²</td>
<td>30 ± 5²</td>
</tr>
<tr>
<td>Total sources</td>
<td>74</td>
<td>181 ± 14²</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sources</th>
<th>This Study and Literature Survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelagic N₂ fixation</td>
<td>110 ± 40²</td>
</tr>
<tr>
<td>Benthic N₂ fixation</td>
<td>15 ± 10²</td>
</tr>
<tr>
<td>River input (POC)</td>
<td>21 ± 10²</td>
</tr>
<tr>
<td>River input (PON)</td>
<td>24 ± 10²</td>
</tr>
<tr>
<td>Atmospheric deposition</td>
<td>30 ± 5²</td>
</tr>
<tr>
<td>Total sources</td>
<td>181 ± 14²</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sinks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Benthic denitrification</td>
<td>88 ± 30²</td>
</tr>
<tr>
<td>Water column denitrification</td>
<td>88 ± 30²</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>24 ± 10²</td>
</tr>
<tr>
<td>N₂O loss</td>
<td>4 ± 2²</td>
</tr>
<tr>
<td>Total sinks</td>
<td>142</td>
</tr>
</tbody>
</table>

Oceanic N inventory

N₂ fixation

δ¹⁵N ~ 0 to -2‰

Avg. δ¹⁵N ~ 5‰

Water Column Denitrification

Sedimentary Denitrification ϵ_sed = 0

Typical Applications

• Identification of sources and cycling of N in aquatic and terrestrial systems (water, soil, air, organisms, petroleum, etc.)
• Food web dynamics
• Forensics
• Archeology
• Paleoclimate, paleoecology and evolution
Nitrogen Cycle

**External Sources**

- **Nitrification**
  - $\text{NO}_3^-$ → $\text{NH}_4^+$

- **Assimilation**
  - $\text{NH}_4^+$ → Organic N

- **Remineralization**
  - Organic N → $\text{NH}_4^+$

- **Denitrification**
  - $\text{NO}_3^-$ → $\text{N}_2$ → $\text{N}_2\text{O}$

- **N fixation**
  - $\text{N}_2$ → Organic N

**Processes**

- **Nitrification**
- **Assimilation**
- **Remineralization**
- **Denitrification**
- **N fixation**
Nitrogen Cycle

- Assimilation
- Nitrification
- Remineralization
- Denitrification
- N fixation

- External Sources
- Organic
- NO₃⁻ → N₂O → N₂ → N fixation
- NH₄⁺ → Organic N
- δ¹⁸O
- H₂O
- O₂

Diagram showing the nitrogen cycle with key processes and chemical reactions.
Modified from Kendall, 1998
Nitrogen is a nutrient

- Vital to all living things
- Often limiting nutrient
  - added as fertilizer to increase crop yields
- Plays a central role in regulating ocean primary productivity – linked to global climate change
N is an environmental pollutant

- Byproduct of wastewater treatment, fossil fuel combustion, and agricultural fertilization
- Leads to ecosystem acidification, eutrophication (algal overgrowth) and hypoxia in aquatic ecosystems
- Impacts resident biota, impairs drinking water quality, and threatens human health
N Sources and Cycling

Through the use of stable isotopic tools, we can:

- **identify sources** of nitrogen to ecosystems
- **quantify** important ecosystem nitrogen **cycling** pathways
• Stable isotopes as indicators of nitrate sources and cycling

• Case Studies:
  Central California
  – San Francisco Bay
  – Monterey Bay
  – Elkhorn Slough
• Stable isotopes as indicators of nitrate sources and cycling

• Case Studies:
  Central California
    – San Francisco Bay
    – Monterey Bay
    – Elkhorn Slough
San Francisco Bay

• **Problem Statement:**
  – Urban Estuary: potentially high levels of NO$_3^-$ pollution

• **Study Objectives:**
  – Determine primary sources of NO$_3^-$
  – Evaluate controls on their distribution
  – Evaluate utility of $\delta^{18}$O as NO$_3^-$ tracer in an estuary?

• **Research Approach:**
  – Sample surface water at 48 sites with SF Bay
  – Measure nutrients and isotopic composition
San Francisco Bay

San Pablo Bay

Suisun Bay

Central Bay

South Bay

Lower South Bay
San Francisco Bay

$\delta^{15}$N indicates conservative mixing in northern bay and very little range in lower south bay

$\delta^{18}$O indicates something else going on...

Wankel et al., 2006. L&O
San Francisco Bay


Simple mixing should cause $\delta^{18}$O to increase but $\delta^{15}$N to decrease.

Fractionation via phytoplankton uptake causes $\delta^{15}$N and $\delta^{18}$O values to increase.

Patterns caused by combination of mixing and uptake.

Wankel et al., 2006. L&O
San Francisco Bay

- **Major Findings:**
  - Northern SF Bay
    - Sources: marine and riverine $\text{NO}_3^-$
    - $\delta^{18} \text{O}$ most useful characterization of $\text{NO}_3^-$ mixing
  - Southern SF Bay
    - Sources: marine and wastewater $\text{NO}_3^-$
    - Dual isotopes reveal combination of mixing and uptake
Outline

- Stable isotopes as indicators of nitrate sources and cycling

- Case Studies:
  - Central California
    - San Francisco Bay
    - Monterey Bay
    - Elkhorn Slough
Monterey Bay

• **Problem Statement:**
  – Ocean primary productivity → linked to global climate change → carbon sequestration →
  – N utilization – ‘new’ vs ‘regenerated’
“New Production”

‘f ratio:’

\[
\frac{\text{NO}_3 \text{ assimilation}}{\text{NO}_3 + \text{NH}_4 \text{ assimilation}}
\]

(Dugdale and Goering, 1967)
Coupled N and O isotopes

- \( \text{NO}_3^- \) drawdown in surface water by phytoplankton uptake
- Seasonal Coastal Upwelling

Wankel et al., GBC,
Coupled N and O isotopes

- Phytoplankton uptake fractionate residual NO$_3^-$ pool
- Causes $\delta^{15}$N and $\delta^{18}$O to increase in a predictable or coupled 1:1 ratio

Granger et al., 2004
Coupled N and O isotopes

Not 1:1

Increases in $\delta^{18}$O are higher than $\delta^{15}$N

Wankel et al., GBC,
Coupled N and O isotopes

• Not just assimilation?

• Ward 2005 – nitrification rates in Monterey Bay euphotic zone

Wankel et al., GBC,
Coupled N and O isotopes

Surface Ocean – Steady-State Box Model

Org N

NO$_3^-$ Assimilation

ε$_p$

NH$_4^+$ assim.

Remineralization

Nitrification

Export

Exchange (Upwelling/Downwelling)

Wankel et al., GBC,
Coupled N and O isotopes

\[
\delta^{15}N_{NO3} = \delta^{15}N_{deep} + \varepsilon_p \times f_n
\]

where:

- \(f_n = [\text{Export/In}] = '\text{drawdown}'\)
- \(f_a = (A_{NH4}/\text{Remin})\)
  - fraction of Remin directly reassimilated as \(NH_4^+\)
- \(f_w = (NTR/A_{NO3})\)
  - fraction of total \(NO_3^-\) assim supported by NTR

Wankel et al., GBC,
Coupled N and O isotopes

- Steady-State Fluxes
  Oxygen Mass Balance

For NO$_3^-$:

\[
\delta^{18}O_{surf} = \delta^{18}O_{deep} + \varepsilon_p \times f_o
\]

Where $f_o$ is equal fraction of gross production supported by NO$_3^-$

Wankel et al., GBC,
Coupled N and O isotopes

Deviation [Δ(15,18)] is proportional to amount of productivity supported by nitrification

Wankel et al., GBC,
Coupled N and O isotopes

- Monterey Bay
  - On average ~30% of surface water NO$_3^-$ has been regenerated in the euphotic zone

The concept of the “f-ratio” (Dugdale and Goering, 1967)

$$\text{NO}_3^- \text{ uptake/ (NH}_4^+ + \text{NO}_3^- \text{ uptake)} = \text{‘new’ production}$$

may be misleading…

…should be…

$$\text{NO}_3^- \text{ uptake/ (NH}_4^+ + \text{NO}_3^- \text{ uptake} - \text{NTR)} = \text{‘new’ production}$$

Wankel et al., GBC,
Outline

• Stable isotopes as indicators of nitrate sources and cycling

• Case Studies:
  Central California
  - San Francisco Bay
  - Monterey Bay
  - Elkhorn Slough
Elkhorn Slough, CA

- Army Corps of Engineers opened mouth in 1947
  - Tidally muted $\Rightarrow$ tidally flushed
- Dramatic erosional loss of marsh habitat
- Ecologically important – Pacific Migratory Flyway
- Watershed largely dominated by agriculture
- Very high $\text{NO}_3^-$ in some areas of the watershed
Problem Statement:
- Agricultural estuary: potentially high levels of NO$_3^-$ pollution from watershed

Study Objectives:
- Determine extent to which watershed sources impact Elkhorn Slough marshes

Research Approach:
- Periodically sample major watershed inputs
- Sample along main channel transect and channel cross section over full tidal cycle
  - Evaluate mixing of various sources
Sampling Sites

Monterey Bay

Watershed Sites

‘OSR’
Carneros Creek

Channel Sites

‘Old Salinas River’
Elkhorn Slough Summary

- **Wet season:**
  - OSR ~66%
  - Mont Bay ~29%
  - Marsh ~5%

- **Dry Season:**
  - OSR ~34%
  - Mont Bay ~42%
  - Marsh ~25%

- Dual isotope approach allowed determination of mixing of three ‘sources’ – influencing the composition of nitrate in the main channel

- Third source, however, was the result of simultaneous uptake and production of $\text{NO}_3^-$
The importance of $N_2$-fixation in oligotrophic waters: 

*Trichodesmium* abundance and $\delta^{15}N$ of zooplankton

- $\delta^{15}N$ values lowest with highest abundance of *Trichodesmium*,
  - $\delta^{15}N$ -1 to -2‰

- $\delta^{15}N$ values highest in areas with low abundance of *Trichodesmium*
  - $\delta^{15}N$ waters ~0‰

---

**Fig. 5.** (A) *Trichodesmium* abundance and (B) $\delta^{15}N$ of zooplankton during leg 2 of cruise SJ9603.
N Isotopes - $N$ Utilization

• Phytoplankton discriminate against $^{15}\text{N}$ ($\varepsilon \sim 5 \, \text{‰}$). Residual pool heavier
  – Little affect when nitrate scarce
  – $\downarrow \text{Utilization} = \downarrow \delta^{15}\text{N}$ (sedimentary marine organic matter)

• Reduction of $\text{NO}_3^-$ by denitrifying bacteria
  – strongly fractionates product (prefer light N)
  – $\text{N}_2$ lost to atmosphere $\Rightarrow$ residual $\text{NO}_3^-$ $\uparrow \delta^{15}\text{N}$
Nitrate Uptake by Phytoplankton

\[ \delta_{15}^{\text{NO}_3,\text{surf}} = \delta_{15}^{\text{NO}_3,\text{deep}} - \varepsilon \ln(t) \]

\[ \delta_{15}^{\text{N-PN}} = \delta_{15}^{\text{NO}_3,\text{deep}} + \varepsilon \frac{t \ln(t)}{1-t} \]
Farrell et al., 1995
Determination of the relative importance of nitrate sources to a groundwater system. Two sources fertilizer and manure. Both are undergoing denitrification. Mixing model suggests ~60% of the nitrate is contributed by the fertilizer.
Amundson et al., 2003
Forensic Geology

Stable isotopes can be used to identify the geo-location of heroin (and morphine) and cocaine.

Ehleringer et al. (1999)
Nitrogen Isotopes in Food Webs

N in the Body: Kinetic Fractionation, Open System
Enrichment in $^{15}\text{N}$ leads to $\sim 3$ per mil disparity between every trophic level.
Why does $\downarrow$ Water availability $\uparrow \delta^{15}N$ in Animal Tissue?

Two General Theories:

1. Diet/plant $\delta^{15}N$ increases in arid habitats
   - $\uparrow$ aridity = larger relative $^{14}N$-rich gas loss (soil denitrification)

2. Metabolic enrichment theories
   - $\uparrow$ urine excreted is isotopically heavy (rich in $\delta^{15}N$) (Ambrose & DeNiro 1986, Sealy 1987)
   - $\downarrow$ protein diets in arid regions promote urea recycling for N
(Murphy & Bowman, 2006)
Results

- Grass foliage $\delta^{15}\text{N}$ (‰)
- Bone collagen $\delta^{15}\text{N}$ (‰)

Water availability index
Summary: Roo Study

• Near identical negative pattern of $\delta^{15}$N in grass and kangaroo bone collagen with water availability.

• Plant $\delta^{15}$N is main cause, with no change in metabolism.
  – Model which best explained data incorporated only plant $\delta^{15}$N values, and NOT metabolism.

• Support for historic trophic ecology and past climate change data that rely on direct relationship between herbivores and plants which is not confounded by animal metabolism.
Tracing Atmospheric Nitrate

Diagram showing the chemical processes involving atmospheric nitrate, including reactions involving O₃, NO, NO₂, O₂, OH, H₂O, NO₃⁻, and particulate NO₂. A graph illustrates the variation in δ¹⁸O(NO₃) and δ¹⁵N(NO₃) over time from July 03 to December 04.
Nitrate deposition in 33 monitoring sites in the midwestern and northeastern U.S. is dominated by inputs of NOx from power plants rather than vehicles.

Elliot et al., 2007
Hanalei Bay Kauai

δ¹⁵N (%oo)

Princeville Hotel
Hanalei River
Far West
Waikoko Bridge
Offshore
Taro Fertilizer
Cesspool Sample
Hanalei Bay Kauai

N+N concentration (µmol L⁻¹) inferred for fresh SGD component

Distance to nearest golf course (km)
Nitrogen Balance: Starvation

Kinetic Fractionation, Closed System

- Generalization: Starvation increases $\delta^{15}N$ of tissue.

Diet $\rightarrow$ Body (enriched in $^{15}N$) $\rightarrow$ Hair, milk, feces...

-6 per mil

Urea (depleted in $^{15}N$)

Body Mass Lost

Isotope ratio

Urea

$6\%\%$

Body

$\delta^{15}N$
Future Applications

- Diagnose eating disorders, disease, nutritional stress
- Studies of nitrogen balance associated with anorexia, bulimia, exercise, disease, weight loss, burns