$N_2$ fixation: Does physiology matter?

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Importance of $\text{N}_2$ fixation?

- Geochemical considerations – long-term inventories of nutrients & trace elements
- Ecological/oceanographic significance
  - New N; alleviates system-wide N limitation?
  - Elemental stoichiometry of ecosystems and basins
  - Community structure & export
- Implications for climate & C cycling
How do we estimate $N_2$ fixation in the ocean?

- Geochemical considerations – elemental budgets ($N^*$, $P^*$) & long-term averages
- Isotopic fractionation and mixing
- Direct measurements – acetylene reduction & $^{15}N_2$ uptake

- Are estimates affected by how the organisms actually work?
N₂ fixation – organisms & physiology

- Distributions
  - Tropical and subtropical distribution of *Trichodesmium*
  - Unicellular cyanobacteria more broadly distributed with latitude – IO?
  - α and γ-Proteobacteria
  - Diatom/diazotroph assemblages – IO?

- N₂ fixation rates and C fixation rates
- Stoichiometry of N₂ fixers
- N release and regeneration
- Limitation of N₂ fixation
Why is there N limitation anywhere? (or is there)

• What limits $N_2$ fixation?
  – Oxygen
  – Temperature
  – Light
  – Other elements (e.g., P and Fe)?
  – Excess N?
Potential from direct estimates

- *Trichodesmium* can fix N\(_2\) at high rates (up to 2.5 nmol N col\(^{-1}\) h\(^{-1}\)); can provide up to 50% of new production where they occur if abundant. IO distribution

- Unicells can fix >20 nmol N l\(^{-1}\) h\(^{-1}\) but generally up to 1 nmol N l\(^{-1}\) h\(^{-1}\) (Montoya et al. 2004, Falcon et al. 2004, Voss et al. 2004, Zehr et al. 2001, Mulholland et al. 2006), even in coastal waters (Mulholland et al. in prep); potentially ~10% of total new production in the oceans. IO?

- Diatom-diazotroph symbioses also fix significant amounts of new N and may be associated with coastal environments (Subramaniam et al. submitted). IO?

- Diazotrophic cyanobacteria & heterotrophic proteobacterial sequences found in deeper waters (Langlois et al. 2005). IO?
Trichodesmium always present but higher abundances Dec-Jan and April

- What limits $N_2$ fixation by *Trichodesmium*?
  - Temperature, light (photoautotroph), Fe or P, excess N?
Potential from direct estimates

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- Unicellular organisms can fix >20 nmol N l\(^{-1}\) h\(^{-1}\) but generally up to 1 nmol N l\(^{-1}\) h\(^{-1}\) (Montoya et al. 2004, Falcon et al. 2004, Voss et al. 2004, Zehr et al. 2001, Mulholland et al. 2006), even in coastal waters (Mulholland et al. in prep); potentially ~10% of total new production in the oceans. IO?

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Unicellular diazotrophs

- Small (< 10 μm) unicellular cyanobacteria and bacterioplankton (Zehr et al. 2001) fix N₂ (Falcon et al. 2004).
- Extended distribution so may be at least as important as colonial diazotrophs (Montoya et al. 2004).

- What limits N₂ fixation by unicellular cyanobacteria?
  - Light (photoautotroph), Fe or P, excess N?
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What limits $N_2$ fixation DDA?
- Light, Si, excess N?
Potential from direct estimates

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What limits $N_2$ fixation?

- Oxygen
- Temperature
- Light
- Other elements (e.g., P and Fe)?
- Excess N?
Outstanding questions for the IO

- **N₂ fixation** – where and who is it?
  - Limiting factors affect distributions
    - Physical – wind, water column stability
    - Biological – nutrient & trace element supply/availability
      - Ephemeral blooms and growth cycles
      - Rates - *Trichodesmium* and other N₂ fixers

- **Fate of newly fixed N₂**
  - Community interactions & trophic transfer
  - Export versus recycling

- **Long term controls and feedbacks**
  - Anthropogenic input of materials - rivers
  - Climatic forcing and feedbacks
Limitation Tendency: Fe (blue) N (red)

Upwelling of high N:Fe waters results in Fe limitation. Favorable for $N_2$ fixation? Bay of Bengal is always Fe replete.

Need for Actual Observations

• **Stoichiometric considerations** – we may get it wrong
  - Ratios of rates
  - Ratios of particles and dissolved material

• **Absolute magnitude is unknown**
  - Net input versus export now but, specific processes may vary independently under future scenarios
Physiological Observations

- CO$_2$ fixation:N$_2$ fixation $\neq$ C:N
- CO$_2$ fixation:N$_2$ fixation generally higher than C:N ratio of *Trichodesmium* POM (5 – 6)
- Important when estimating N and C specific growth rates?
- Important in stoichiometric inferences estimating one from the other (e.g., Orcutt et al. 2001)
- Need actual measurements
**Literature**

<table>
<thead>
<tr>
<th>Location</th>
<th>C:N₂ fixation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>Gulf of Mexico</td>
<td>5.4 - 42.7</td>
<td>13.1</td>
</tr>
<tr>
<td>New Caledonia (lagoon)</td>
<td>9.2 - 77.7</td>
<td></td>
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<tr>
<td>North Atlantic (latitudinal gradient)</td>
<td>13.6 - 33.3</td>
<td>21.9</td>
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<tr>
<td>North Pacific</td>
<td>1.2 - 2.1</td>
<td>16</td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>1.5 - 87</td>
<td>128</td>
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<tr>
<td>BATS (puffs)</td>
<td>13 - 437</td>
<td>198</td>
</tr>
<tr>
<td>BATS (tufts)</td>
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<tr>
<td>N. Atlantic</td>
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<td>63</td>
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<tr>
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<td></td>
<td>49</td>
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<td>26</td>
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<tr>
<td><em>Trichodesmium</em> IMS101 (batch)</td>
<td>6.5 - 15.2</td>
<td>10</td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (semi-continuous)</td>
<td>6.5 - 25.2</td>
<td></td>
</tr>
<tr>
<td><em>Trichodesmium</em> IMS101 (continuous)</td>
<td>13.4 - 20.0</td>
<td></td>
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</table>

C:N uptake ratios > C:N of particulate material

Is C fixation too high or N₂ fixation too low?
C:N uptake

- Which is a better estimator of growth in nature?
- Net versus gross $N_2$ fixation
- $N:P$
- Fe scavenging strategies?
Evidence for substantial nitrogen release by *Trichodesmium*

- Accumulation of DIN and DON within blooms as they progress (Devassy et al. 1978 & 1979, Devassy 1987, Karl et al. 1997, Glibert & O’Neil 1999) and in cultures as they age (Mulholland et al. 1999, Mulholland and Capone 2001)
- Release ~50 % of recently fixed N\textsubscript{2} as LMW DON (Glibert and Bronk 1994) and amino acids (Capone et al. 1994)
- DON and NH\textsubscript{4}\textsuperscript{+} regeneration in cultures (Mulholland et al. 2001; 2004; Mulholland & Bernhardt 2005)
- What does this mean for overall isotopic signature of N pools and elemental stoichiometry on short timescales
- Trophic transfer – recycling in the euphotic zone (Mulholland et al. 2004)
N Regeneration Pathways

- Direct release of NH$_4^+$ or DON
- DOM decomposition via extracellular enzymes
- Grazing
- Viral cell lysis
- Bacterial remineralization
Ecological constraints on sinking flux

Trichodesmium OR Bacteria

Grazers

Virus

Other phyto

N\textsubscript{H}_4^+ \text{ & DOM}

Bacteria

Sinking
Pooled release rates from the Gulf of Mexico

**Percent release vs. Incubation time (h)**

- **Avg = 57%**

**Release (nmol N\(\text{col}^{-1}\text{h}^{-1}\)) vs. Incubation time (h)**

- **Avg = 0.63**
### Literature

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<tr>
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<td>3.3 - 36.8</td>
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<td>27 - 325</td>
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<td>Mulholland (unpub data)</td>
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<tr>
<td>North Atlantic (latitudinal gradient)</td>
<td>2.1 - 7.4</td>
<td>4.2</td>
<td>Mulholland et al. (in prep)</td>
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<td>North Pacific</td>
<td>3 - 10</td>
<td>1.9</td>
<td>Mague et al. (1977)</td>
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<td>Carpenter &amp; Price (1977)</td>
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<td>2.9</td>
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<td>Carpenter &amp; McCarthy (1975)</td>
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<td><em>Trichodesmium</em> IMS101 (cont.)</td>
<td>3.0 - 22.2</td>
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<td>Mulholland &amp; Bernhardt (2005)</td>
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Release artificially high in cultures but varies with growth conditions.
What controls \( \text{N}_2 \) fixation and release?

\( \text{N}_2 \) fixation from cultures

- Varies with growth rate – higher at higher growth rates
- Varies with P supply - higher when P is not limiting.
- Some indication of variability with temperature and CO\(_2\)
- Fe, oxygen, and light?
What about P and Fe?

- Fe limitation – may apply in some areas of IO
- P limitation
  - Rapid recycling of P (up to 100% of *Trichodesmium* P demand could be met through P regeneration in GOM)
  - P regeneration in *Trichodesmium* blooms of 2 - 15 nmol l\(^{-1}\) h\(^{-1}\) (compare with N regeneration)
  - High rates of alkaline phosphatase activity
  - Flexible N:P ratios?
- Excess N? Is it important (Langlois et al. 2005, Mulholland et al. in prep)
Correlations between $N_2$ fixers and other taxa.

- First observed in the Indian Ocean (Devassy)
- Australia (Revelante & Gilmartin, Furnas, Burford et al., O’Neil et al.)
- A variety of species live in association with colonies (Sellner)
- Gulf of Mexico (Walsh & Steidinger, Lenes et al.)
N$_2$ fixation and other taxa

- Unpublished research
- Anecdotal information
- Historical monitoring data
- Correlation between timing and magnitude of organisms
Weekly mean *K. brevis* surface abundance
(within 9 km of Cedar Key)

From Walsh & Steidinger (2001)
For Gulf of Mexico
Not including unicells!

- Acetylene reduction
  ~0.3 – 2.6 nmol N col\(^{-1}\) h\(^{-1}\)

- \(^{15}\)N\(_2\) uptake
  0.05 – 2.2 nmol N col\(^{-1}\) h\(^{-1}\)

- \(^{15}\)N release
  0.1-1.8 nmol N col\(^{-1}\) h\(^{-1}\)
  As \(\text{NH}_4^+\) (0.1-1.1 nmol N col\(^{-1}\) h\(^{-1}\))

- *Trichodesmium* abundance
  - Cruises 0.6-20 col l\(^{-1}\)
  - Summer *Trichodesmium* 20 col l\(^{-1}\)
  - Background 0.75 col l\(^{-1}\)
  - Bloom >1000 col l\(^{-1}\)

- Water column N uptake
  - ~20 - 100 nmol N l\(^{-1}\) h\(^{-1}\)
  - Up to 1000 during bloom
Direct links: Trophic transfer of regenerated N

Bottles with *Trichodesmium* sealed in dialysis bags.

$^{15}$N$_2$ gas is fixed by *Trichodesmium* within the bags and some portion is released (as DON or NH$_4^+$), which is then available for re-incorporation by cells outside of dialysis bags.
Results from direct measurements

• Up to 43% of recently fixed N moved into co-occurring plankton in 4-6 h incubations

• Uptake rates of released N are on the order of observed ambient uptake rates in the Gulf of Mexico (or even total N)
The fate of new N in tropical systems

- Non-Redfield and variable stoichiometry of CO$_2$:N$_2$ fixation rates – can one be reliably inferred from the other? (Orcutt et al. 2001)
- Fuels regenerated and heterotrophic processes
- DOM production versus particle flux
- Rapid and non-Redfield regeneration of N and P in the euphotic zone
- What does that mean for C?
N cycling in colonies

\[ \text{NHNH}_4^{++} \quad \text{NHNH}_4^{++} \quad \text{NHNH}_4^{++} \quad \text{NHNH}_4^{++} \]

\[ \text{GluGlu} \quad \text{GlnGln} \quad \text{GlnGln} \quad \text{AAO/PHAAO/PH} \]

\[ \text{N}_2 \quad \text{N}_2 \quad \text{N}_2 \quad \text{N}_2 \]

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

\[ \text{N}_2 \quad \text{Gln} \quad \text{Gln} \quad \text{Gln} \quad \text{Gln} \]

\[ \text{AAO/PH} \quad \text{NH}_4^+ + \text{keto acid} \]

\[ \text{N}_2 \quad \text{N}_2 \quad \text{N}_2 \quad \text{N}_2 \]

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

\[ \text{N}_2 \quad \text{Glu} \quad \text{Glu} \quad \text{Glu} \quad \text{Glu} \]

\[ \text{N cycling in colonies} \]
What about the future (& past)

- Do rates of N$_2$ fixation vary over time?
- Do associated processes (CO$_2$ fixation, elemental stoichiometry, regeneration) vary over time?
- Does the system change in a balanced way?
For both isolates (IMS101 & GBR), raising pCO$_2$ to year 2100 levels dramatically increased N$_2$ fixation rates, regardless of either temperature or limitation by P (Hutchins et al., in review).
1) **Biomass-normalized N₂ fixation rates** increased as pCO₂ increased.

2) **Biomass-normalized CO₂ fixation rates** also increased by >50% between 375 and 1500 ppm CO₂.

3) **Cellular molar N:P ratios** increased from near-Redfield values of 17 at present day CO₂ concentration to 21-23 at 750-1500 ppm.

4) *Trichodesmium* cannot survive long at a low pCO₂ (150 ppm)
Implications

- Future anthropogenic scenarios
- No $N_2$ fixation by *Trichodesmium* at glacial maximum
- Increase in $N_2$ fixation during interglacial (as for denitrification are they coupled?)
- Other $N_2$ fixers?
Indian Ocean

A. Classical gyres

B. Fe limited but high N
   Omani coast

C. N₂ fixation/denitrification
   OMZ
Conclusions & future directions

• Still need measurements to get magnitude of $N_2$ fixation in Indian Ocean
  – Coupling or other relationship with denitrification?
  – Changes over time in competing processes

• Physiology (all diazotrophs)
  – Relative rates of N and C uptake
  – Elemental cycling under range of environmental conditions
  – Controls on $N_2$ fixation

• Ecology & Oceanography: Fate of the recently fixed $N_2$
  – Timescales of export and regenerated production signals
  – Other diazotrophs
  – Role of bacteria & trophic linkages