A functional gene approach to studying nitrogen cycling in the sea

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March 20, 2007
Overview

★ Climate change, carbon cycling, and ocean biology
★ Distributions of nutrients and marine production
★ The ocean’s nitrogen cycle
★ Case Study: Nitrogen fixation from a functional gene perspective
CO$_2$ concentrations in the atmosphere-past and present
The Ocean’s response to increasing CO$_2$: Temporal variability in surface ocean CO$_2$ and pH
In the open ocean, biology controls bioelemental cycling
The Influence of Biology on Carbon Cycling

![Diagram showing the influence of biology on carbon cycling.](image)

- **Total inorganic carbon** ($\mu$mol C kg$^{-1}$)
- **Depth (m)**
- **Total inorganic carbon** ($\mu$mol C kg$^{-1}$)

The diagram illustrates the processes involved in carbon cycling, including phytoplankton carbon uptake, zooplankton respiration, excretion, physical mixing, aggregate formation, sinking particles, decomposition, bacteria, zooplankton migration, carbon flux, and respiration.
What controls the distribution of marine biological production?

Ocean primary production

Mike Behrenfeld, 
http://marine.rutgers.edu/opp/swf/Production

http://iridl.ldeo.columbia.edu/SOURCES/.NOAA/.NODC/.WOA01/
Depth profiles of light, temperature, nutrients, and chlorophyll in the open ocean
Satellite-derived estimates of global chlorophyll distributions
Ecosystem controls on marine production

• **Physical**: turbulence, light, temperature
• **Chemical**: nutrient availability, trace elements
• **Biological**: community composition, food web structure, N₂-fixation
• **Climate and Human Influences**: ENSO, PDO-NAO, land use, population, deserts-dust
### OLIGOTROPHIC GYRES OF THE WORLD OCEAN

<table>
<thead>
<tr>
<th>Basin</th>
<th>Area (x 10^3 km^2)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific</td>
<td>90,105</td>
<td>24.6</td>
</tr>
<tr>
<td>Atlantic</td>
<td>30,624</td>
<td>8.3</td>
</tr>
<tr>
<td>Indian</td>
<td>19,599</td>
<td>5.3</td>
</tr>
<tr>
<td>Other</td>
<td>8,000</td>
<td>2.2</td>
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<tr>
<td>TOTAL</td>
<td>148,329</td>
<td>40.4</td>
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</table>
The Hawaii Ocean Time-series (HOT)

- Established in 1988 as part of the U.S. JGOFS program.
- Monthly measurements of ocean physics, biology, and chemistry at Station ALOHA
- Primary objectives: characterize time-dependent dynamics in carbon, nitrogen, and phosphorus inventories and fluxes.
Highly stratified ocean ecosystems
= high light, very low nutrient concentrations
= low chlorophyll
Vertical profile of light, nutrients, and primary production

- \([\text{NO}_3^- + \text{NO}_2^-]\) ~ 1-10 nM
- \([\text{PO}_4^{3-}]\) ~ 10-100 nM
- Annual rates of net primary production >15 mol C m\(^{-2}\)
Global Models and Predictions

- Ocean circulation models coupled with biology
- Increased temperature will impact both CO$_2$ the oceans ability to absorb atmospheric CO$_2$
In the low nutrient upper ocean very small, but very diverse plankton populations comprise the majority of biomass and production.
The microbial soup at Station ALOHA

Photosynthetic unicellular bacteria
(*Prochlorococcus, Synechococcus*)

Colonial cyanobacteria bacteria
(*Trichodesmium*)

Non-photosynthetic Bacteria and Archaea
New production in the ocean

2 forms of primary 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.

-Over short time scales, input of new N supports higher trophic levels and supports carbon export.
Nitrogen serves as both an essential nutrient, and because of its redox potential also serves as an energy source and electron acceptor.
N$_2$ fixation contributes a major fraction (~30-84%) of new production in the open ocean.

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>N$_2$-supported fraction (%)</th>
<th>Contributions to flux (mmol N m$^{-2}$ yr$^{-1}$)</th>
</tr>
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<tbody>
<tr>
<td>1990</td>
<td>145</td>
<td>3.85</td>
<td>41</td>
<td>59</td>
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<tr>
<td>1991</td>
<td>111</td>
<td>3.21</td>
<td>51</td>
<td>56</td>
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<td>1992</td>
<td>80</td>
<td>3.58</td>
<td>45</td>
<td>36</td>
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<td>1993</td>
<td>86</td>
<td>4.15</td>
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<td>31</td>
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<tr>
<td>1994</td>
<td>80</td>
<td>3.81</td>
<td>41</td>
<td>33</td>
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<tr>
<td>1995</td>
<td>81</td>
<td>3.76</td>
<td>42</td>
<td>34</td>
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<td>1996</td>
<td>87</td>
<td>4.04</td>
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<td>1997</td>
<td>113</td>
<td>3.29</td>
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<td>56</td>
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<tr>
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<tr>
<td>1999</td>
<td>122</td>
<td>2.03</td>
<td>69</td>
<td>84</td>
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<tr>
<td>2000</td>
<td>113</td>
<td>3.05</td>
<td>53</td>
<td>60</td>
</tr>
</tbody>
</table>

* Flux-weighted annual average.
Nitrogen sources supporting plankton growth

Eukaryotes: NO₃⁻, NO₂⁻, NH₄⁺, DON, N₂ (*)

Prochlorococcus: NO₂⁻, NH₄⁺

Diazotrophs: NO₃⁻, NH₄⁺, N₂

Heterotrophs: NO₃⁻, NO₂⁻, NH₄⁺, DON, N₂

Not all nutrients are created equal...
Genes and processes in the nitrogen cycle

Nitrate assimilation
- Nitrate (NO₃) to Nitrite (NO₂)
  - Genes: narB, nirK, nirS

Ammonia oxidation
- Nitrite (NO₂) to Ammonia (NH₄⁺)
  - Genes: hao, nosZ, aax

Ammonium assimilation
- Ammonia (NH₄⁺) to Glutamine (glnA)
  - Genes: amoA

Denitrification
- Ammonia (NH₄⁺) to N₂ (nitrogen gas)
  - Genes: nirA, nirB, nirK, nirS, nirH

N₂ fixation
- N₂ gas to Ammonia (NH₄⁺)
  - Genes: nifH, glnA

Identifying genes encoding enzymes that catalyze specific biochemical reactions.
Selected sources of nitrogen supporting plankton growth in the upper ocean

- Nitrate / nitrite reductase (nar, nir)
- Nitrogenase (nif)
- Glutamine synthetase (gln)
- Urease (ure)
- NO$_3^-$
- NO$_2^-$
- NH$_4^+$
- DON
Fe protein is inactivated upon oxidation; cyanobacteria must isolate nitrogenase (spatially or temporally) from O$_2$ evolved during photosynthesis.

Nitrogenase
From genes to proteins

Transcription of *nifH* DNA:

5’-TATTAGGCCAATCCGC-3’

3’-ATAATCCGGTTAGGC-5’

Translation of *nifH* mRNA:

5’-UAUUAGGCCCAUUGGGCCG-3’

*nif operon*

*nifH gene*

*nifH mRNA transcript*

Nitrogenase
Phylogenetic relationships among nifH genes
1. What are the predominant N$_2$-fixing bacteria in the open ocean?
Sample and extract plankton DNA and mRNA.

Amplify *nifH* genes by PCR and reverse transcriptase (RT) PCR using degenerate primers, clone and sequence amplified products.
Diazotrophic bacteria at Station ALOHA

- Richelia
- Heterocystous cyanobacteria
- Unicellular cyanobacteria (Group A and B)
- Filamentous cyanobacteria (Trichodesmium)
- Proteobacteria
- Anaerobes

Size ranges:
- 100 µm
- 2-10 µm
- 10-2000 µm
- >0.2 µm
Questions

1. How abundant are \textit{nifH}-containing microorganisms?

2. What processes regulate \textit{nifH} expression and N$_2$ fixation?
An introduction to the quantitative polymerase chain reaction (QPCR)

Environmental DNA or reverse transcribed mRNA

**Denature**
- 94°C

**Anneal**
- 64°C

**Extend**

Fluorescence is directly proportional to the abundance of the initial target
i.e., $C_n = C_0 \times 2^n$

Where $n=$PCR cycles
Vertical distributions of various nifH phylotypes at Station ALOHA (Dec. 2002)
Temporal patterns of nifH expression at Station ALOHA (Dec. 2002)

**Group A unicellular**

**Group B unicellular**

**Trichodesmium spp.**

**Heterocystous cyanobacteria**

Dec. 13, 2002

Dec. 14, 2002

Dec. 15, 2002