DON bioavailability & approaches to study N uptake

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Department of Physical Sciences

Outline:

• DON utilization
  Allochthonous vs autochthonous

• Who is using what?

• Mechanisms of use
TDN - DIN = DON

TDN - (NO$_3^-$ + NO$_2^-$ + NH$_4^+$)
Based on Cresser 1977 Analyst
Allochthonous sources:

- Atmospheric deposition
- Rivers
- Terrestrial runoff
- Sewage effluent

REFRACTORY??

2 - 84% of N in atmospheric deposition is DON

Seitzinger and Sanders 1999 L&O

14 - 90% of N in rivers is DON

Seitzinger and Sanders 1997 MEPS
45 to 75% of the DON in rainwater was consumed

Atmospheric DON

Add DON

Riverine DON

Add DON

Incubate for 6 days

Seitzinger and Sanders 1999 L&O

Wiegner et al. 2006 AME
Transform Ion Cyclotron Resonance Mass Spectrometry

<table>
<thead>
<tr>
<th>Salinity</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>0</td>
<td>nd</td>
<td>nd</td>
<td>92</td>
<td>9</td>
<td>23</td>
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<td>10</td>
<td>80</td>
<td>99</td>
<td>100</td>
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<td>20</td>
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<td>20</td>
<td>59</td>
<td>76</td>
<td>0</td>
<td>2</td>
<td>14</td>
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<td>30</td>
<td>79</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>11</td>
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</table>

% EON that is labile

$N = 14.00674$

$C = 12.0107$
Making $^{15}$N-labeled humics

$^{15}$NH$_4^+$ → XAD resin → cut → $^{15}$N-labeled humics

3 months

spun in coastal seawater for 3 months in the dark

Use Killed Controls!

Humic uptake in culture

See et al. 2006 L&O
Autochthonous sources of DON

1 - Dust arrive Florida July 1, 1999

2 - Fe concentration increased in surface waters by 300%

3 - Trichodesmium counts increase 10x

4 - DON and NH$_4^+$ 300% increase in DON

5 - Karenia brevis blooms form in October

Bronk 2002 DOM book
Sipler et al. Submitted

Growth rate ($\mu$) = cellular division day
<table>
<thead>
<tr>
<th></th>
<th>Growth rate range (μ) divisions d⁻¹</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K. brevis in culture</strong></td>
<td>0.2-1.0 (μmax)</td>
<td>Loret et al., 2002</td>
</tr>
<tr>
<td><strong>K. brevis field population</strong></td>
<td>0.11-0.58</td>
<td>VanDolah et al., 2008</td>
</tr>
<tr>
<td><strong>K. brevis field population + Trichodesmium DON</strong></td>
<td><strong>0.95-1.16</strong></td>
<td>This Study</td>
</tr>
</tbody>
</table>

- masses detected in the K. brevis bloom water were bioavailable
- some bioavailable masses detected in the K. brevis bloom water were shared with the TCE concentrate
- 56% (94) of masses unique to the TCE treatment were bioavailable to the K. brevis community

Those masses >m/z 500 were more bioavailable than those <m/z 500
$^{15}$N-urea

**Drawbacks:**
- DON pool of unknown composition
- Few commercially available tracers
- $$$$$
### Coastal

<table>
<thead>
<tr>
<th>Location</th>
<th>NH$_4^+$</th>
<th>NO$_x$</th>
<th>Urea</th>
<th>AA</th>
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</thead>
<tbody>
<tr>
<td>Altamaha River, GA</td>
<td>56</td>
<td>27</td>
<td>9</td>
<td>8</td>
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<tr>
<td>Savannah River, GA</td>
<td>65</td>
<td>17</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Chesapeake Bay (Aug)</td>
<td>75</td>
<td>1</td>
<td>8</td>
<td>16</td>
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<tr>
<td>Orinoco River Plume</td>
<td>81</td>
<td>7</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Mississippi River plume</td>
<td>14</td>
<td>22</td>
<td>48</td>
<td>15</td>
</tr>
</tbody>
</table>

Bronk et al. In prep.

### Oceanic

<table>
<thead>
<tr>
<th>Location</th>
<th>NH$_4^+$</th>
<th>NO$_x$</th>
<th>Urea</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Atlantic Bight</td>
<td>53</td>
<td>15</td>
<td>25</td>
<td>5</td>
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<tr>
<td>Gulf of Mexico</td>
<td>66</td>
<td>6</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>Norway fjord</td>
<td>31</td>
<td>7</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>South Pacific</td>
<td>49</td>
<td>5</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td>ETN Pacific</td>
<td>50</td>
<td>9</td>
<td>22</td>
<td>18</td>
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</table>
DON Mean = 34 ± 18 %

Underestimate?
**Chesapeake Bay - August**

![Graph showing uptake rates in Chesapeake Bay - August](image)

**South Pacific**

![Graph showing DIN and DON uptake in South Pacific](image)

Bronk and Gilbert 1993 Mar Biol

Bronk & Campbell In prep.
Who is using the DON?

Bacteria → DON → Phytoplankton

$^{15}\text{NH}_4^+$

29-93% of bacteria retained on GF/F filters
Historical view.....

Current view.....
Flow Cytometric Sorting

Flow Cytometric Sorting

Phyto (+ chl)

Bact (- chl)

Traditional

GF/F
Phyto + some Bact

Stable Isotope probing

Individual Phyto groups

Individual Bact groups

\( {}^{15} \text{NH}_4 \)
Cross-System Comparison: Dissolved N

Bradley & Bronk Submitted

Overestimation of phyto uptake
Flow Cytometric Sorting

Phyto (+ chl)  Bact (- chl)

Phyto groups  Bact groups

Traditional

GF/F
Phyto + some Bact

Stable Isotope probing

Stable – Isotope Probing

Starting Population

Labeled Population

Extract DNA

PCR and Sequencing

Identity of the active members of the population

Modified from figure by Craig Phelps - Lee Kerkoff
Cesium Chloride (CsCl) Gradient

Modified from figure by Lee Kerkhoff

16S rRNA gene profiles of bacterial $^{15}$NO$_3^-$ uptake

<table>
<thead>
<tr>
<th></th>
<th>3m plume</th>
<th>Station 6-mid ORP site</th>
<th>14N-total community</th>
</tr>
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<tbody>
<tr>
<td><strong>$^{15}$N-active community</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>halocline</td>
<td></td>
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<tr>
<td>DCMAX</td>
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<td></td>
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</tbody>
</table>

Below euphotic

No amplification

Lee Kerkhoff et al. In prep.
Kerkhof et al. in prep.

**Orinoco River Plume site**

Synechococcus WH7803 $^{14}$N vs. $^{15}$N rbcL gene DNA as observed by qPCR

Wawrik and Bronk submitted
**Synechococcus** → \( \text{NH}_4^+ \) \( \text{DON} \) → **Diatoms** → \( \text{NO}_3^- \)
Diatom field control

West Florida Shelf 2008

Diatoms

Wawrik et al. submitted
<table>
<thead>
<tr>
<th>Substrate</th>
<th>% Syn</th>
<th>% Diatom</th>
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<tbody>
<tr>
<td>(\text{NH}_4^+)</td>
<td>78</td>
<td>35</td>
</tr>
<tr>
<td>(\text{NO}_3^-)</td>
<td>71</td>
<td>0-37</td>
</tr>
<tr>
<td>urea</td>
<td>53</td>
<td>0-43</td>
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<tr>
<td>AA</td>
<td>34</td>
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</tr>
<tr>
<td>glut acid</td>
<td>46</td>
<td>15</td>
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</table>

Wawrik & Bronk Submitted

Phytoplankton mechanisms to access organic N:

- Organic oxidases
- Peptide hydrolysis
- Pinocytosis
- Phagocytosis
- Photochemical processes
- Salinity release
N
N
N
Farming nitrogen from “refractory” compounds!
Humic Uptake Mechanisms?

- = $^{13}\text{C}$
- = $^{15}\text{N}$

Direct Uptake (Pinocytosis)

Enzymatic Cleavage (Amino Acid Oxidation)

Photoproduction of labile N

- UV radiation
- Humic or fulvic acids
- Proteins
- Large organic moieties

$\text{NH}_4^+$
$\text{DPA}$
$\text{NO}_2^-$

Based on Bushaw et al. 1996 Nature
### Eastern Tropical North Pacific

<table>
<thead>
<tr>
<th>Date (m)</th>
<th>Depth (m)</th>
<th>% NH$_4^+$ Uptake</th>
<th>DPA</th>
<th>NO$_2^-$</th>
<th>% NH$_4^+$ Regen.</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>NH$_4^+$</td>
<td>DPA</td>
<td>NO$_2^-$</td>
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</tr>
<tr>
<td>10</td>
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<td>0.0</td>
<td>0.0</td>
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<td>65.3</td>
<td>3.0</td>
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<tr>
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<td>64.6</td>
<td>70.3</td>
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<td>168.8</td>
<td>11.4</td>
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<td>19.9</td>
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</table>

Mean production:

- NH$_4^+$: 23.3
- DPA: 28.3
- NO$_2^-$: 62.1
- NH$_4^+$ Regen.: 4.4

Standard deviation:

- NH$_4^+$: 27.2
- DPA: 30.3
- NO$_2^-$: 61.8
- NH$_4^+$ Regen.: 3.6
**NH₄⁺ photoproduction from *Tricho DON***

![NH₄⁺ Concentrations](image)

**PO₄⁻ photoproduction from *Tricho DON***

![PO₄⁻ Concentrations](image)
$\text{NH}_4^+$ Conc (µmol L$^{-1}$) vs. Salinity (ppt)

KW

TM

Bronk et al. Submitted
• A significant fraction of both autothonomous and allochthonous DON is labile on time scales of days.
• Both bacteria AND phytoplankton use DON.

Big Questions:
Who is using what and how do they do it?
Acknowledgments:
Marta Sanderson and Quinn Roberts
Paul Bradley, Lynn Killberg, and Jason See

EON work: Margie Mulholland, Nancy Love, Liz Canuel

SIP work: Lee Kerkhof and Boris Wawrik