DON sources:
methods and processes

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Department of Physical Sciences

Outline:

• What is DON?
• Methods to study release
• Autochthonous sources
• Allochthonous sources
• DON as a mode of N delivery
## What is DON?

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<tr>
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<td>proteins</td>
<td>humic acids</td>
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<td>urea</td>
<td>DCAA</td>
<td>fulvic acids</td>
</tr>
<tr>
<td>nucleic acids</td>
<td>amino</td>
<td>porphorins</td>
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<td>methylamines</td>
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#### Methods for studying release:

1. **Bioassays**
2. **Radioactive tracers**
3. **Stable isotope tracers**
   a. Direct measures
   b. Isotope dilution
Bioassays & Radiotracers
\(^{3}H, \, ^{14}C, \, ^{32}P\)

- Absolute amounts
- Net rates

Stable isotope tracers
\(^{15}N, \, ^{13}C\)

- Ratios
  \(^{15}N/^{14}N\) or \(^{13}C/^{12}C\)

- Gross and net rates
- Uptake and regeneration simultaneously

\[\text{Rate} = \frac{\text{atom \% of target}}{\text{atom \% of source}} \times \frac{\text{Time}}{\text{[target]}}\]
\[ \text{Gross Uptake} = \frac{15\text{N in PN & DON}}{\text{Rate (atom \% } \text{NH}_4^+ \times \text{Time)}} \times [\text{PN}] \]

Bronk et al. 1994 Science

\[ \text{Gross Uptake} - \text{Net Uptake} = \text{DON Release} \]
Net Uptake = \frac{^{15}\text{N in PN}}{\text{Rate}} \times \text{atom }\% \text{ NH}_4^+ \times \text{Time} \times [\text{PN}]

\text{Isotope dilution}

\frac{^{15}\text{NH}_4^+}{^{14}\text{NH}_4^+}

Net Uptake = \frac{^{15}\text{N in PN}}{\text{Rate}} \times \text{atom }\% \text{ NH}_4^+ \times \text{Time} \times [\text{PN}]
\[ P_t = P_0 + (d - u)t \]

\[ \ln(R_t - R_a) = \ln(R_0 - R_a) - \frac{d}{(d - u)}[\ln \frac{P_t}{P_0}] \]

\( P_t \) and \( P_0 \) = ambient \( \text{NH}_4^+ \) conc at end and start of incubation
\( R_t \) and \( R_0 \) = atom % of the \( \text{NH}_4^+ \) pool at end and start of incubation.
\( u \) = absolute uptake rate
\( d \) = regeneration rate

Glibert et al. 1982 L&O

Autochthonous sources of DON

Direct release - Passive diffusion
Cell death and lysis (autolysis)
Bacterial exoenzyme release
Excretion of organic matter by phytoplankton: Do healthy cells do it?

Sharp 1977 L&O

Active release outflow model
Fogg 1966 OMBAR

Passive diffusion model
Fogg 1966 OMBAR

Reduction of viruses
Murray 1995 JPR

Bacteria as ectoparasites
Bjørnsen 1988 L&O

A bit of advice!

Bronk 1999 JPR
Autochthonous sources of DON

Zooplankton

Excretion
Sloppy feeding
Bactivory

DON (NH₄⁺)
Viral lysis

Viruses are unique in that they are “part” of the DOM pool (∼<2%).


Photochemical Ammonification

UV radiation

Humic or fulvic acids
Proteins
Large organic moieties

Phytoplankton

NH₄⁺
DPA
NO₂⁻

Bacteria

Bushaw et al. 1996 Nature
Field Methods

- $\text{NH}_4^+$
- $\text{NO}_2^-$/$\text{NO}_3^-$
- Urea
- DFAA
- DCAA
- Humic
- DON
- chl. $\alpha$

$^{15}\text{NH}_4^+$
$^{15}\text{NO}_3^-$

$\text{DON}$

Total Gross DIN Uptake and DON Release

- (ug-at N 1°a$^{-1}$)

Monterey Bay

Bronk & Ward 1999 L&O
DOMINO
Dissolved Organic Matter IN the OceanS

DOC  →  DON

Measurements

NH₄⁺  NO₃⁻/NO₂⁻  DOC/TDN  Urea
Chl. a  VA  BA  PA  μZooA

< 150 μm

6 hr incubation

¹⁵NH₄⁺  ¹⁵NO₃⁻  ¹⁴HCO₃⁻

¹⁵NH₄⁺ Regen.  ¹⁵NO₃⁻  Grazing Rates
Ambient Conditions

$\text{NH}_4^+ \sim 9 \ \mu\text{M}$

$\text{NO}_3^- \sim 10 \ \mu\text{M}$

$\text{DOC} \sim 185 \ \mu\text{M}$

$\text{DON} \sim 10 \ \mu\text{M} \ (C:N \ 18)$

$\text{urea} \sim 0.6 \ \mu\text{M}$

Treatments

- Control
- 0 Virus
- 2X Virus

- + Grazers
- 0 Virus + Grazers
- 2X Virus + Grazers

*Acartia tonsa*
• Slight decrease in Chl. a when grazers are added.

• Grazers and viruses increased the rate of NH$_4^+$ regeneration.
  • Additive effect.
• Both grazers and viruses depressed primary production.
  • Additive effect.

• Grazers increased the % of primary production released as DOC.
  • Viruses tended to decrease it.
<table>
<thead>
<tr>
<th>Location</th>
<th>Compound Considered</th>
<th>Turnover Time</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oceanic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeastern Pacific</td>
<td>DON</td>
<td>0.91</td>
<td>years</td>
</tr>
<tr>
<td>Equatorial Atlantic (15N-25N)</td>
<td>DON</td>
<td>0.4 to 13.2c</td>
<td>years</td>
</tr>
<tr>
<td>Equatorial Atlantic (15S-15N)</td>
<td>DON</td>
<td>12.7 ± 26.1c</td>
<td>years</td>
</tr>
<tr>
<td>Equatorial Atlantic (35S-15S)</td>
<td>DON</td>
<td>2.1 to 300c</td>
<td>years</td>
</tr>
<tr>
<td>Caribbean Sea</td>
<td>DON</td>
<td>40.7 ± 10.4</td>
<td>days</td>
</tr>
<tr>
<td>Southern California Bight</td>
<td>DON</td>
<td>11 to 62</td>
<td>days</td>
</tr>
<tr>
<td>where?</td>
<td>HMW DON &gt;1kD</td>
<td>~238a</td>
<td>days</td>
</tr>
<tr>
<td>Northern Sargasso Sea</td>
<td>Protein</td>
<td>0.38 to 3.42</td>
<td>days</td>
</tr>
<tr>
<td>Northern Sargasso Sea</td>
<td>Modified proteind</td>
<td>9.04 to 32.71</td>
<td>days</td>
</tr>
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<td>9.04 to 32.71</td>
<td>days</td>
</tr>
<tr>
<td>Northern Sargasso Sea</td>
<td>DFAA</td>
<td>0.03 to 0.29</td>
<td>days</td>
</tr>
<tr>
<td>Central Arctic</td>
<td>DFAA</td>
<td>~2.72</td>
<td>days</td>
</tr>
</tbody>
</table>

Bronk 2002 Book chapter

semi-labile & refractory DON

Months

Years

Days

Hours

DFAA

urea

NA
Allochthonous sources of DON

Atm deposition

DON

Groundwater

Galloway et al. 2008 Science
Figure 2: Spatial patterns of total inorganic nitrogen deposition in 1860 (top) and early 1990 (bottom) in units of kg nitrogen per square kilometer per year.

DON in atmospheric deposition

<table>
<thead>
<tr>
<th>Source/Location</th>
<th>% org N</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker Branch, TN</td>
<td>34</td>
<td>Kelly and Meagher 1986</td>
</tr>
<tr>
<td>Coastal plain, FL</td>
<td>40–63</td>
<td>Riekkert 1983</td>
</tr>
<tr>
<td>Cascade Mnts., OR</td>
<td>46–72</td>
<td>Fredriksen 1976</td>
</tr>
<tr>
<td>Coastal plain, SC</td>
<td>49</td>
<td>Richter et al. 1983</td>
</tr>
<tr>
<td>Philadelphia, PA*</td>
<td>19–52</td>
<td>This study</td>
</tr>
<tr>
<td>Chesapeake Bay</td>
<td>57</td>
<td>Smullen et al. 1982</td>
</tr>
<tr>
<td>Rhode River, MD</td>
<td>18–44</td>
<td>Jordan et al. 1995</td>
</tr>
<tr>
<td>New Brunswick, NJ†</td>
<td>2–44</td>
<td>Seitzinger and Sanders unpbl. data</td>
</tr>
<tr>
<td>Narragansett, RI</td>
<td>19</td>
<td>Nixon et al. 1995</td>
</tr>
<tr>
<td>U.K.</td>
<td>21</td>
<td>Cornell et al. 1995</td>
</tr>
<tr>
<td>Czech Rep.</td>
<td>27</td>
<td>*</td>
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<tr>
<td>N. Carolina</td>
<td>21</td>
<td>*</td>
</tr>
<tr>
<td>Amazonia</td>
<td>22</td>
<td>*</td>
</tr>
<tr>
<td>Recife, Brazil</td>
<td>25</td>
<td>*</td>
</tr>
<tr>
<td>Bermuda</td>
<td>59</td>
<td>*</td>
</tr>
<tr>
<td>Tahiti</td>
<td>84</td>
<td>*</td>
</tr>
<tr>
<td>NE Atlantic</td>
<td>62</td>
<td>*</td>
</tr>
<tr>
<td>NE Atlantic</td>
<td>67</td>
<td>*</td>
</tr>
<tr>
<td>Cape Cod, MA</td>
<td>43</td>
<td>Valsiela et al. 1997</td>
</tr>
<tr>
<td>Lewes, DE</td>
<td>23</td>
<td>Scudlark et al. 1998</td>
</tr>
</tbody>
</table>

Seitzinger & Sanders 1999 L&O
Figure 3: Flux of reactive nitrogen from the landscape to coastal oceans in rivers for key contrasting regions of the world in the temperate zone, in units of kg nitrogen per square kilometer of watershed area per year.
The rise of urea

(a) Million metric tons N
(b) Urea as a % of Total N

Glibert et al. 2006 Biogeosciences
Allochthonous sources of DON

5-8 mg/L ——> 3 mg/L

EON

Effluent Organic Nitrogen

Mulholland et al. 2007 STAC

Nitrogen in Wastewater

Compounds poorly removed by treatment
Humics in source
Recalcitrant organics

Compounds formed during treatment

Dave Sedlak
Composition of EON

TABLE 1. Total Amino Acid Concentrations in the Secondary Treated Wastewater Effluents (Scully et al., 1988b; Confer et al., 1995; Grohmann et al., 1998; Pehlivanoglu and Seclik, in preparation)

<table>
<thead>
<tr>
<th></th>
<th>Wagott</th>
<th>Parkin</th>
<th>Elsässer</th>
<th>Hojzlar</th>
<th>Scully#</th>
<th>Scully</th>
<th>Confer</th>
<th>Pehlivanoglu</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg N/L</td>
<td>0.017</td>
<td>0.025</td>
<td>0.013</td>
<td>0.034</td>
<td>0.042</td>
<td>0.084</td>
<td>0.02</td>
<td>0.26</td>
</tr>
<tr>
<td>µM N</td>
<td>1.23</td>
<td>1.79</td>
<td>0.93</td>
<td>2.45</td>
<td>5–8</td>
<td>3–6</td>
<td>1.43</td>
<td>18.76</td>
</tr>
</tbody>
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Note: Wagott, Parkin, Elsässer, Hojzlar, and Scully data are obtained from Grohmann et al. (1998).

#Primary effluent.

Organics as a mode of inorganic N delivery!
Humic Extraction Method

Acidified Sample (pH < 2)

Humic Substances stick to the acidified resin

Humic fraction elutes from column with NaOH

Humics
C:N Ratio of Saturated Humics Before and After XAD Extraction

Samples Saturated at 4 μmol NH₄⁺^+ (mg humic-C)^-1

See & Bronk 2005 Mar Chem

See 2003 Dissertation
When humics hit ~15 % they dump NH$_4^+$

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