DON sinks

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• DON sinks
  Autochthonous vs allochthonous
• Mechanisms of use DON
• Who is using what?

DON constitutes a large percentage of the dissolved N in many systems.

Traditional Dogma
DON is refractory!
Methods for studying release:

1. Bioassays
2. Radioactive tracers
3. Stable isotope tracers
   a. Direct measures
   b. Isotope dilution

Allochthonous sources:
- Rivers
- Terrestrial runoff
- Agricultural
- Urban
- Forested
- Combined sewage overflows
- Sewage effluent
- Atmospheric deposition

Seitzinger and Sanders 1999 L&O

2 - 84% of N in atmospheric deposition is DON

14 - 90% of N in rivers is DON

Seitzinger and Sanders 1997 MEPS

Atmospheric DON

45 to 75% of the DON was consumed

Seitzinger and Sanders 1999 L&O
Up to 60% of the DON was consumed in 6 days

Incubated for ~2, 4, and 7 days - monitored nutrient and biomass parameters.
Response varied with salinity

\[ + \text{EON} = \text{net consumption} \]

Control - production and consumption

52%  27%

Making \(^{15}\text{N}\)-labeled humics

\[^{15}\text{N}\text{H}_4 \rightarrow \text{XAD resin} \rightarrow \text{cut} \rightarrow \text{spin in coastal seawater for 3 months in the dark} \]

Use Killed Controls!

Humic uptake in culture

See et al. 2006 L&O
Humic Uptake Rates

*Thalassiosira cf. miniscula*

![Graph showing humic uptake rates over time](image)

**Autochthonous sources of DON**

*Sources*

- Micro/macro zooplankton
- Bacteria
- Phytoplankton (and N₂ fixers)
- Viruses

**Field Methods**

- NH₄⁺
- NO₃⁻/NO₂⁻
- Urea
- DFAA
- DCAA
- Humic DON
- chl. a

**15N-urea**

- DON pool of unknown composition
- Few commercially available tracers
- $$$$

**Drawbacks:**

Berman & Bronk 2003 MEPS
Uptake characterization

Altamaha

\[ \text{NO}_3^- \]
\[ \text{NH}_4^+ \] humic
\[ \text{DPA} \]

Savannah

\[ \text{NO}_3^- \]
\[ \text{NH}_4^+ \] humic
\[ \text{DPA} \]

South Atlantic Bight

\[ \text{NO}_3^- \]
\[ \text{NH}_4^+ \] urea
\[ \text{DPA} \]

ETNP

\[ \text{NO}_3^- \]
\[ \text{NH}_4^+ \] Urea
\[ \text{DPA} \]

Berman & Bronk 2003 MEPS

Mississippi River Plume - July 2005

\begin{align*} 
\text{NO}_3^- & \rightarrow \text{NH}_4^+ \\
\text{NH}_4^+ & \rightarrow \text{DPA} \\
\text{DPA} & \rightarrow \text{NH}_4^+ \\
\text{NH}_4^+ & \rightarrow \text{humic} \\
\text{humic} & \rightarrow \text{DPA} \\
\end{align*}

Outside Plume

\begin{align*} 
\text{CONC (µM)} & \rightarrow \text{DEPT (m)} \\
\end{align*}

Inside Plume

\begin{align*} 
\text{CONC (µM)} & \rightarrow \text{DEPT (m)} \\
\end{align*}

Outside Plume

\begin{align*} 
\text{CONC (µM)} & \rightarrow \text{DEPT (m)} \\
\end{align*}

Inside Plume

\begin{align*} 
\text{CONC (µM)} & \rightarrow \text{DEPT (m)} \\
\end{align*}
Outside Plume

Inside Plume

Orinoco River Plume - Oct 2006

The rise of urea

Glibert et al. 2006 Biogeosciences
f-ratio = \frac{\text{New production}}{\text{New + Regenerated Production}}

f-ratio = \frac{\text{NO}_3^- \text{ uptake}}{\text{NH}_4^+ + \text{NO}_3^- + \text{U} + \text{DPA} \text{ uptake}}

Is the urea really regenerated?
Is the NO$_3^-$ really new?

Bronk & Glibert 1993 Mar Biol - Chesapeake Bay - August

South Pacific

Bronk & Campbell In prep.
Phytoplankton mechanisms to access organic N:

- Organic oxidases
- Peptide hydrolysis
- Pinocytosis
- Phagocytosis
- Photochemical processes
- Adsorption - Desorption

Farming nitrogen from "refractory" compounds!

Cell Surface Enzymes

- $^{14}$C
- NH$_4^+$
- $^{14}$C-Keto acid
- H$_2$O$_2$
- O$_2$
- $^{14}$C Amine

Palenik et al. 1988

Humic Uptake Mechanisms?

- $^{13}$C
- $^{15}$N

Direct Uptake
(Pinocytosis)

Enzymatic Cleavage
(Amino Acid Oxidation)
Photoproduction of labile N

Based on Bushaw et al. 1996 Nature

ETNP Buoy

Who cares?

In estuararies and lakes...

- Phytoplankton $\rightarrow$ $O_2$
- Bacteria take up $O_2$ $\rightarrow$ $CO_2$
- Phytoplankton $\rightarrow$ higher trophic levels
- Phytoplankton can $\rightarrow$ HABs

Who uses what?

Bacteria

Phytoplankton

N

• UV radiation
  • Humic or fulvic acids
  • Proteins
  • Large organic moieties
  • $NH_4^+$
  • DPA
  • $NO_2^-$

• Phytoplankton

• Bacteria

• $NH_4^+$
• DPA

$ETNP$ Buoy

Concentration ($\mu$M)

$NH_4^+$

$DPA$
In the ocean

\[ \text{NO}_3^- \quad \text{NH}_4^+ \quad \text{DON} \]

Phytoplankton

\[ \text{sink!} \]

\[ \text{Bacteria don’t!} \]

\[ \begin{align*}
\text{GF/F filters retain} & \quad 25-75\% \text{ of all bacterial cells} \\
\text{Lipschutz (1995)} & \quad -^{15}\text{N} \\
\text{Zubkov et al. (2004)} & \quad -^{35}\text{S}
\end{align*} \]

Phytoplankton vs. Bacteria N Uptake

Flow Cytometric Sorting

GF/F filters retain 50-65\% of all bacterial cells
Flow Cytometric Sorting

Chesapeake Bay
July 2004

Chesapeake Bay - AA uptake

Absolute Uptake (µmol N L⁻¹ hr⁻¹)

Bradley & Bronk In prep

Stable – Isotope Probing

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

DON
• A significant fraction of both autothonomous and allochthonous DON is labile on time scales of days.

• Both bacteria AND phytoplankton use DON.

Big Question:  Who is using what?

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