DON bioavailability & approaches to study N uptake

Deborah A. Bronk
Department of Physical Sciences

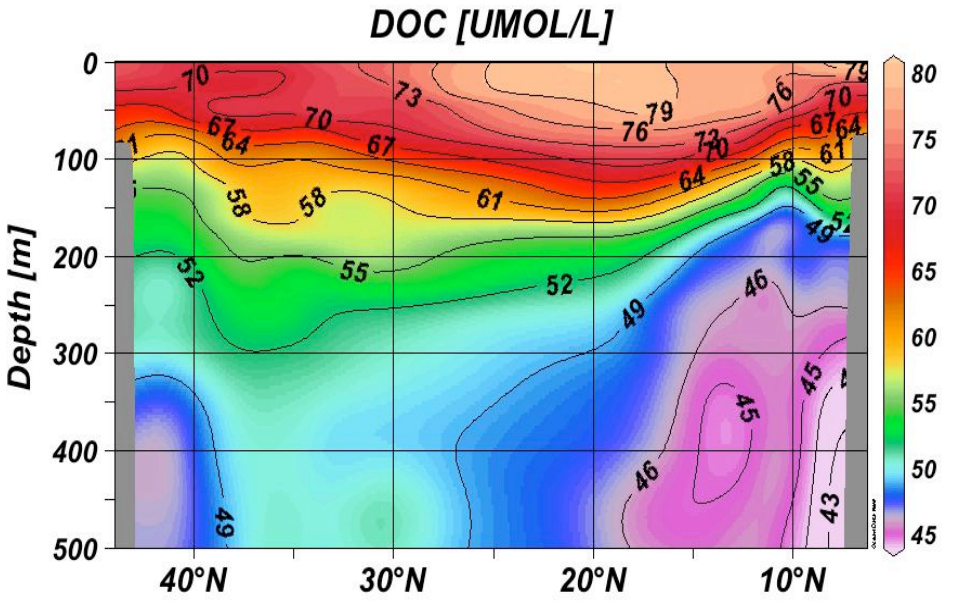
WILLIAM & MARY
VIMS
VIRGINIA INSTITUTE OF MARINE SCIENCE
SCHOOL OF MARINE SCIENCE

Outline:

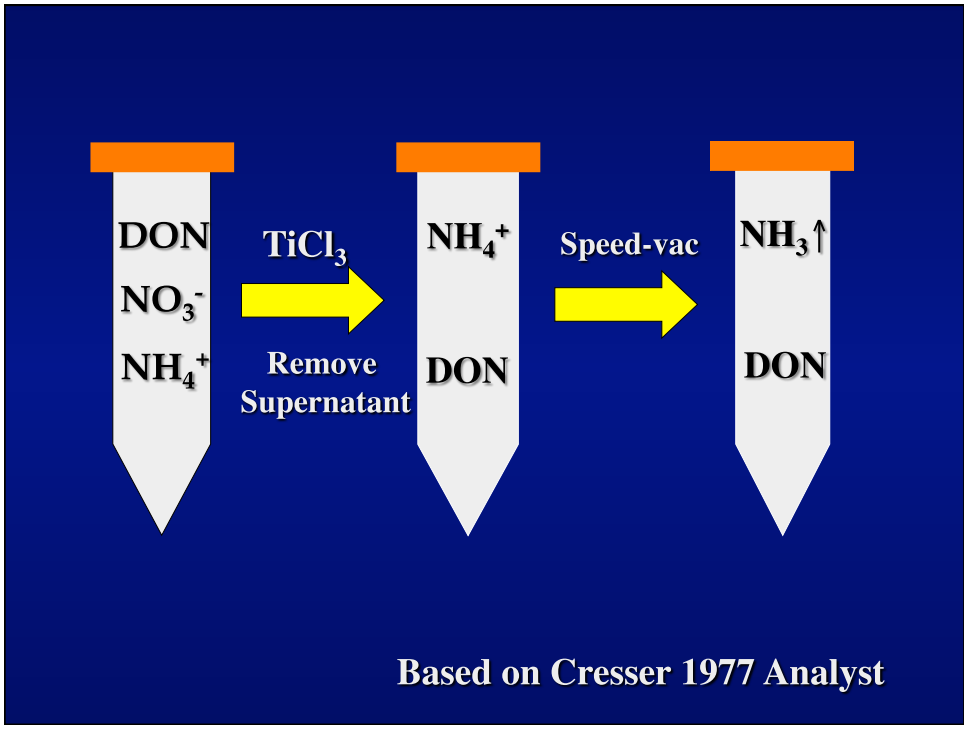
- DON utilization
  Allochthonous vs autochthonous
- Who is using what?
- Mechanisms of use

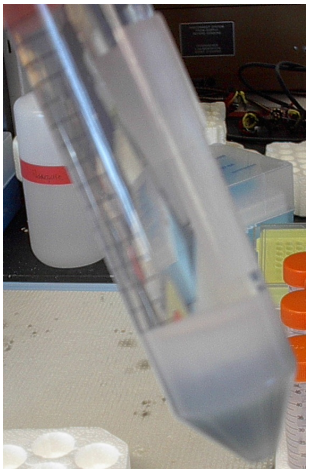
TDN - DIN = DON

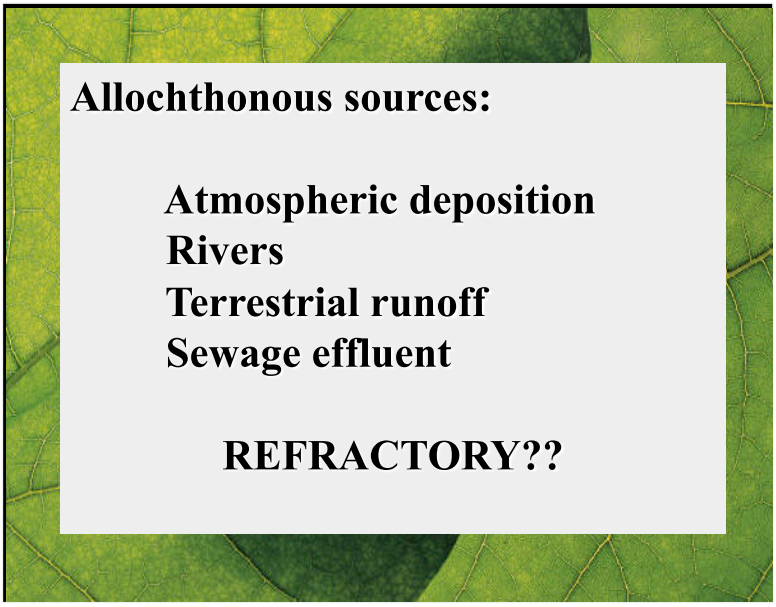
TDN - (NO_3^- + NO_2^- + NH_4^+)



From C. Carlson


DON
NO_3^-
NH_4^+
$TiCl_3$
Remove
Supernatant
NH_4^+
DON
Speed-vac
$NH_3\uparrow$
DON
Based on Cresser 1977 Analyst





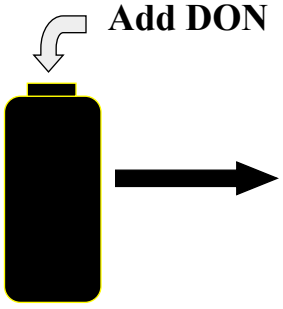
2 - 84% of N in atmospheric deposition is DON

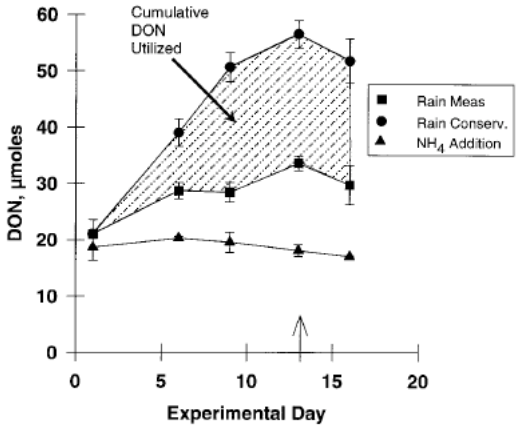
Seitzinger and Sanders 1999 L&O

14 - 90% of N in rivers is DON

Seitzinger and Sanders 1997 MEPS

Atmospheric DON

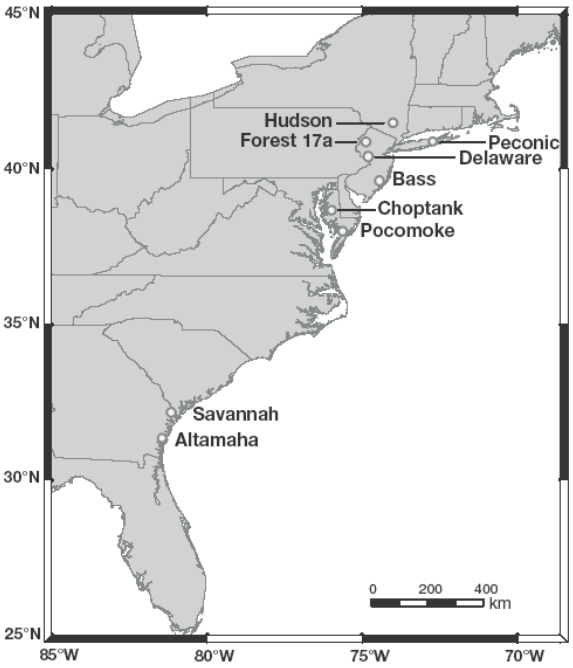




45 to 75% of the DON in rainwater was consumed

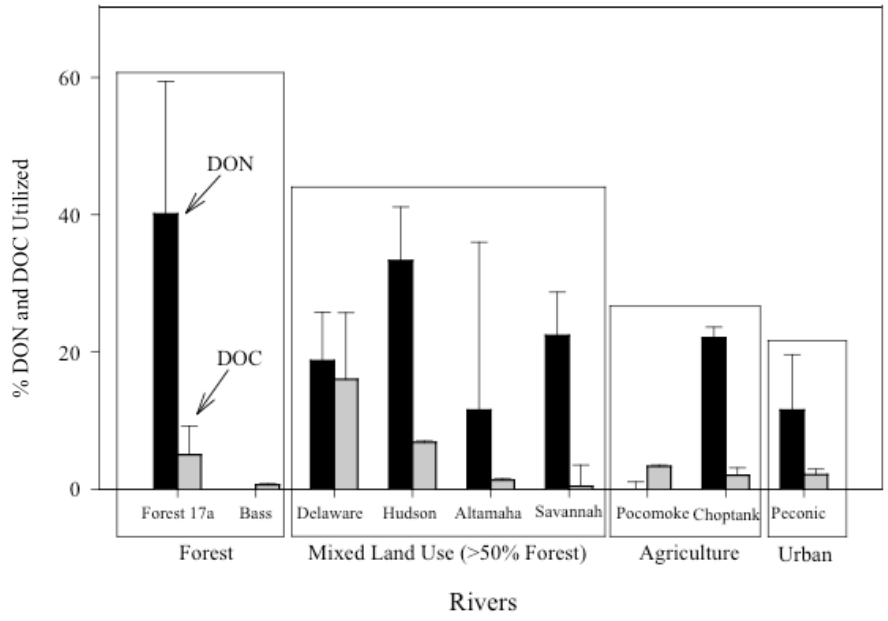
Seitzinger and Sanders 1999 L&O

Riverine DON



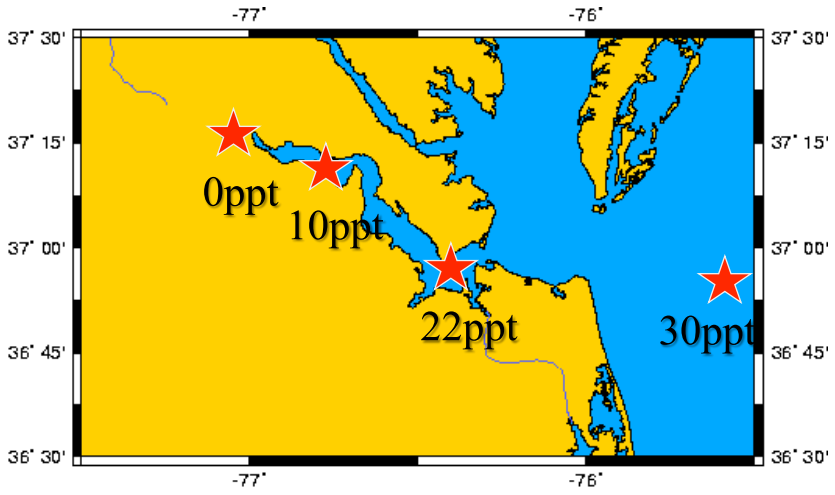
Incubate for 6 days

Wiegner et al. 2006 AME



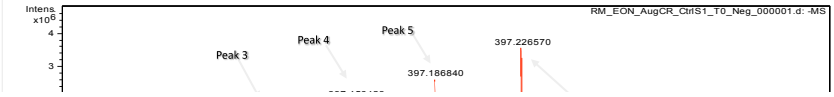
Wiegner et al. 2006 AME

Effluent Organic Nitrogen (EON)

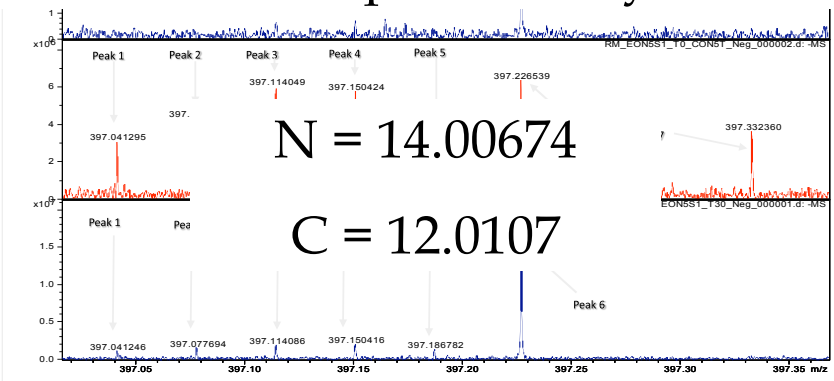


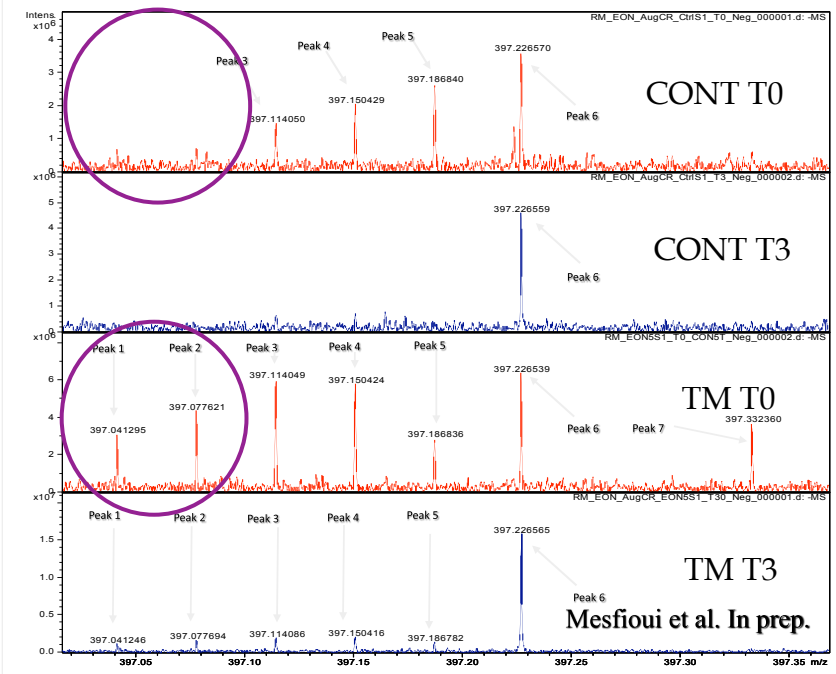
Salinity	% EON that is labile 1	2	3	4	5
0	nd	nd	92	9	23
10	80	99	100	5	20
20	59	76	0	2	14
30	79	26	0	0	11

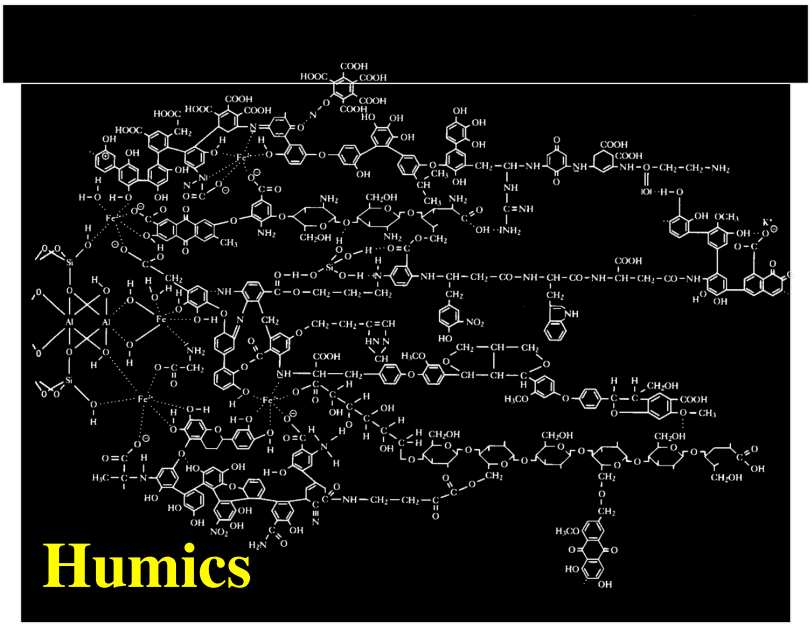
Bronk et al. Submitted



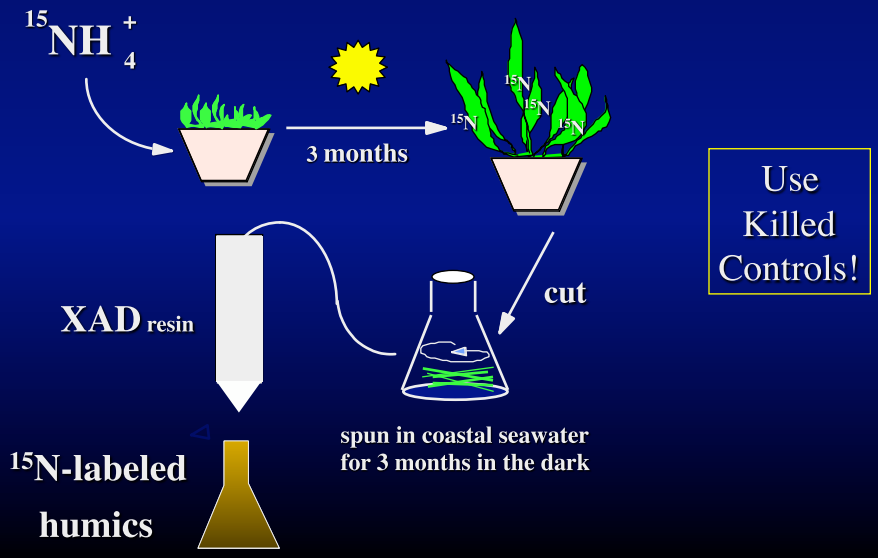
Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry




CONT T0
CONT T3
TM T0
TM T3
Peak 1
Peak 2
Peak 3
Peak 4
Peak 5
Peak 6
Peak 7
397.226570
397.186840
397.150429
397.114050
397.226559
397.226539
397.114049
397.150424
397.077621
397.041295
397.186836
397.332360
397.226565
397.041246
397.077694
397.114086
397.150416
397.186782
Mesfioui et al. In prep.

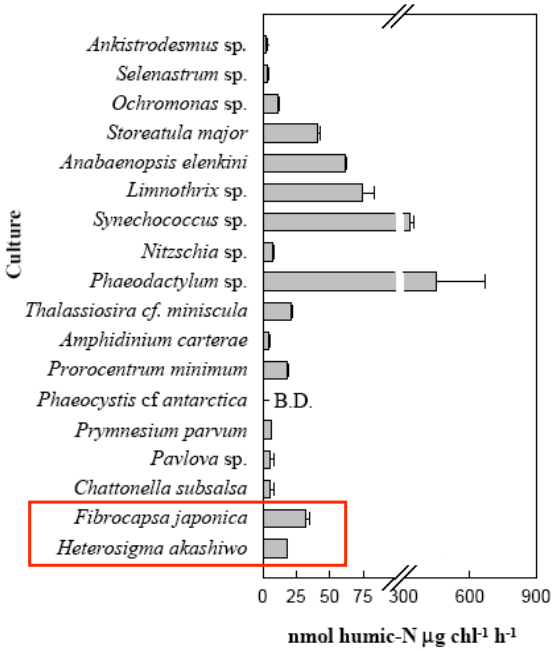

Humics

Making ^{15}N-labeled humics



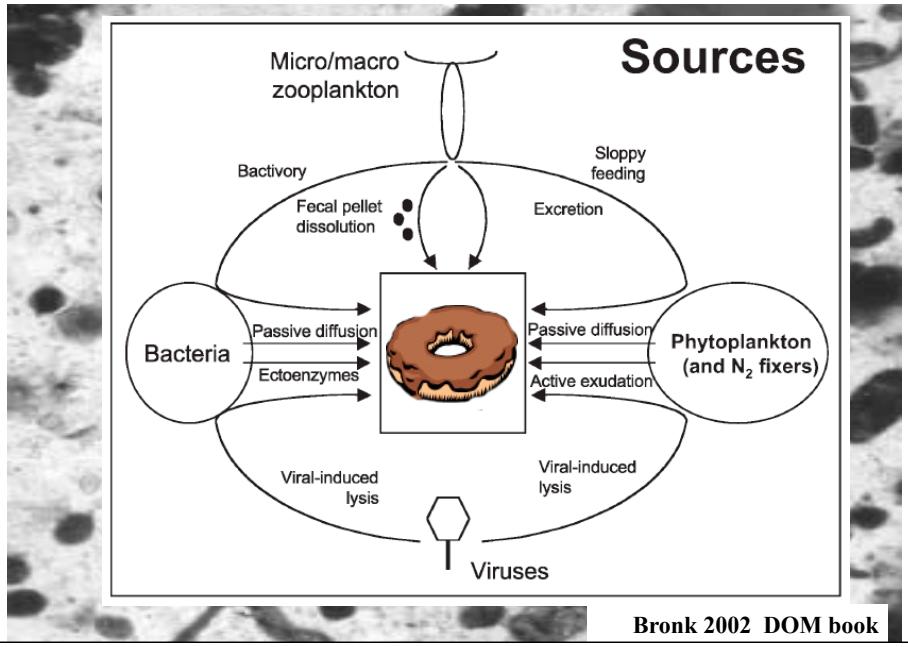
Use Killed Controls!

Humic uptake in culture

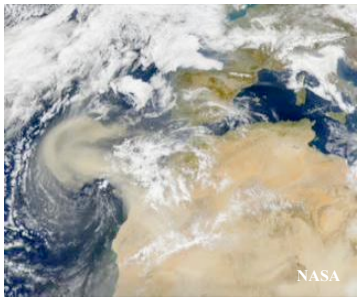


See et al. 2006 L&O

Autochthonous sources of DON



Bronk 2002 DOM book



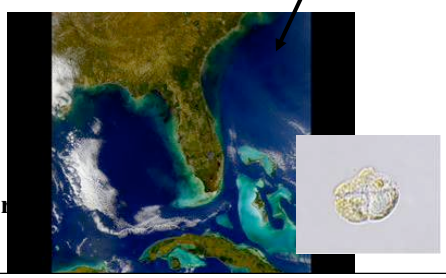
1 - Dust arrive Florida July 1, 1999

Fe

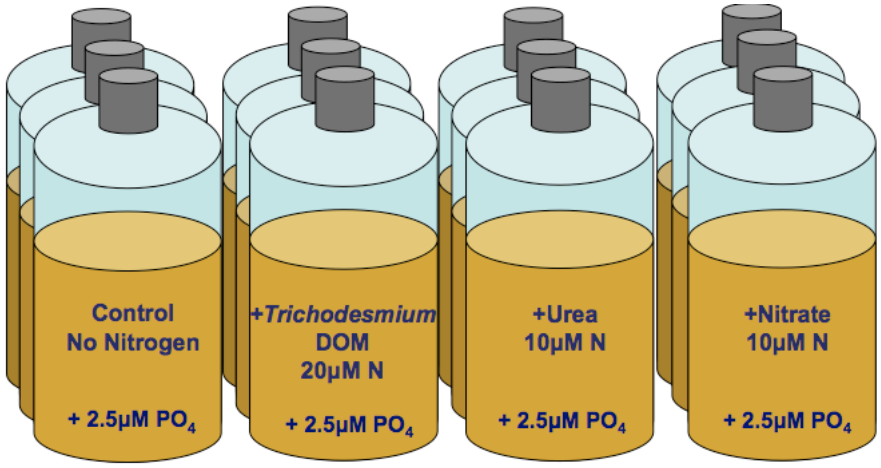
2 - Fe concentration increased in surface waters by 300%

3 - Trichodesmium counts increase 10x

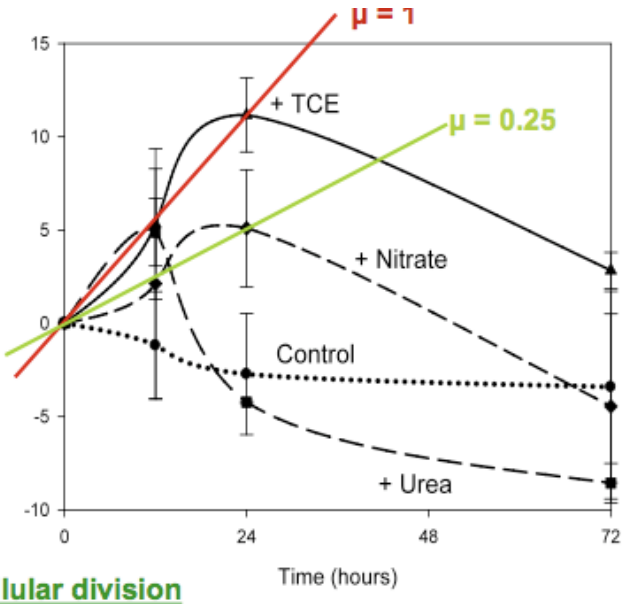
4 - DON and NH_4^+ 300 % increase in DON



5 - *Karenia brevis* blooms form in October

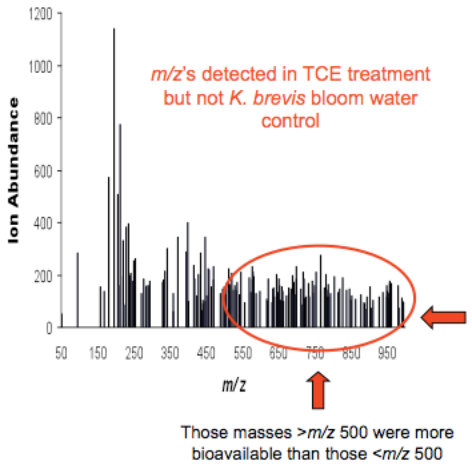


Sipler et al. Submitted

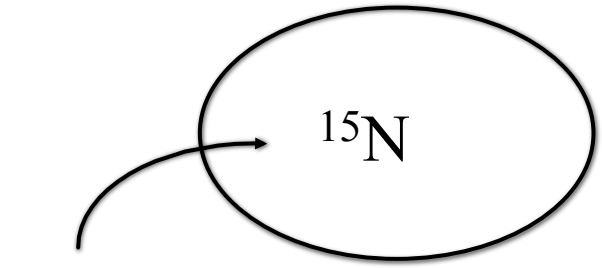


Growth rate (µ)= cellular division / day

	Growth rate range (μ) divisions d^{-1}	Reference
***K. brevis* in culture**	**0.2-1.0** (μmax)	Loret *et al.*, 2002
***K. brevis* field population**	**0.11-0.58**	VanDolah *et al.*, 2008
***K. brevis* field population + *Trichodesmium* DON**	**0.95-1.16**	This Study



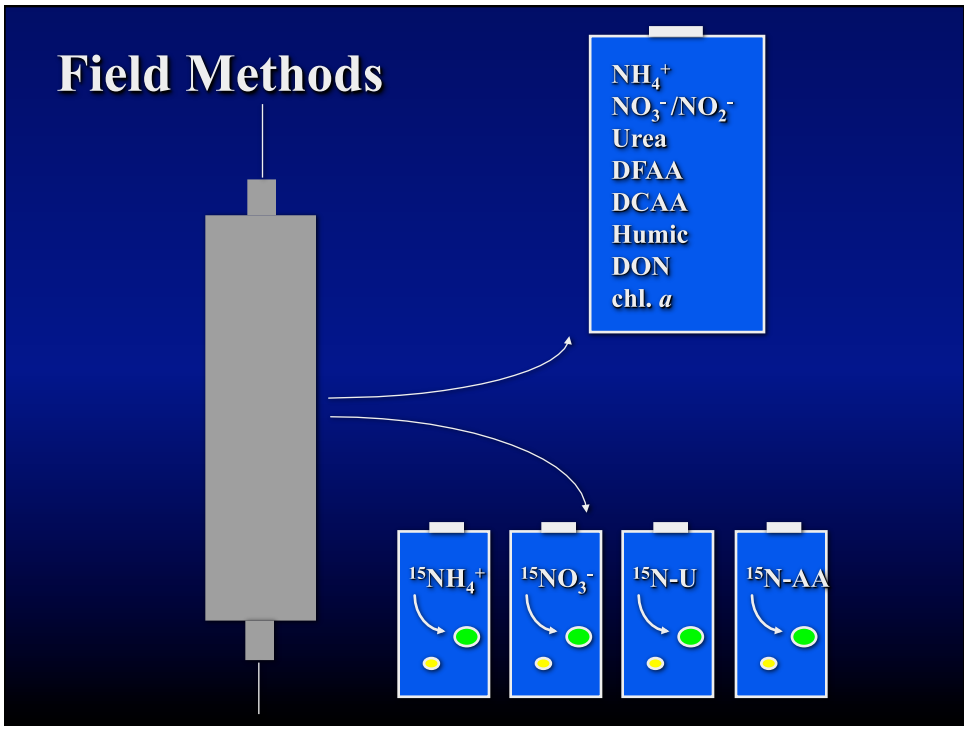
- masses detected in the *K. brevis* bloom water were bioavailable
- some bioavailable masses detected in the *K. brevis* bloom water were shared with the TCE concentrate
- 56% (94) of masses unique to the TCE treatment were bioavailable to the *K. brevis* community



^{15}N-urea

Drawbacks:

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



Coastal

	% Total Uptake			
	NH_4^+	NO_x	Urea	AA
Altamaha River, GA	56	27	9	8
Savannah River, GA	65	17	7	11
Chesapeake Bay (Aug)	75	1	8	16
Orinoco River Plume	81	7	11	1
Mississippi River plume	14	22	48	15

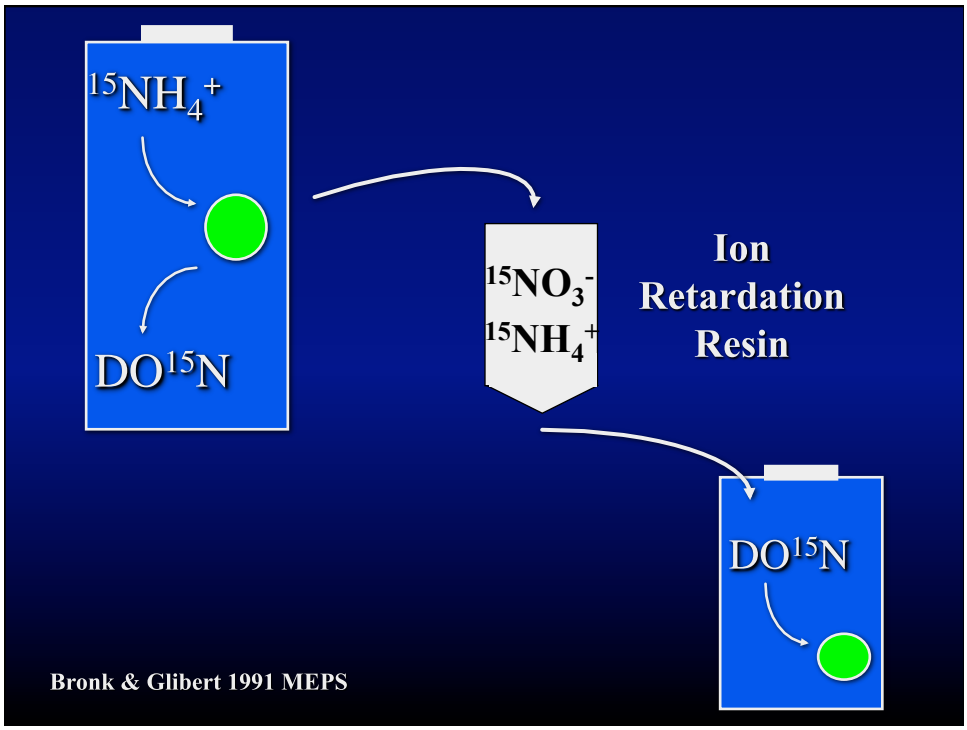
Bronk et al. In prep.

Oceanic

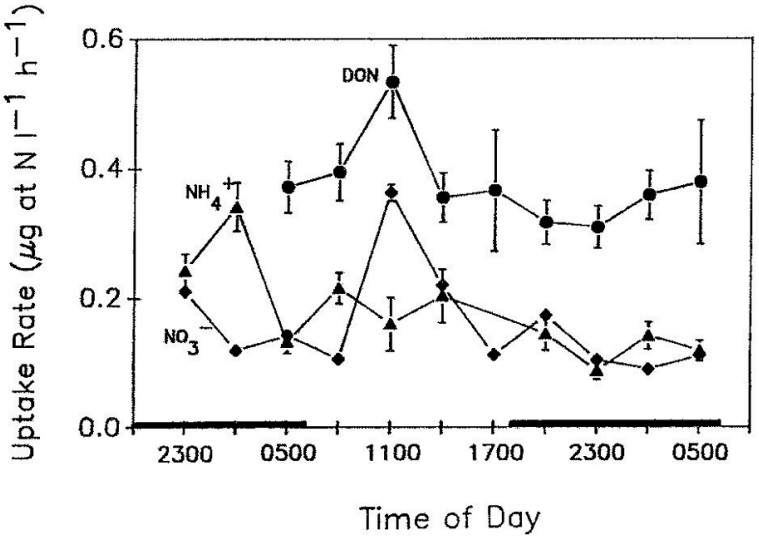
	% Total Uptake			
	NH_4^+	NO_x	Urea	AA
South Atlantic Bight	53	15	25	5
Gulf of Mexico	66	6	21	7
Norway fjord	31	7	60	2
South Pacific	49	5	39	7
ETN Pacific	50	9	22	18

DON Mean = 34 ± 18 %

Underestimate?

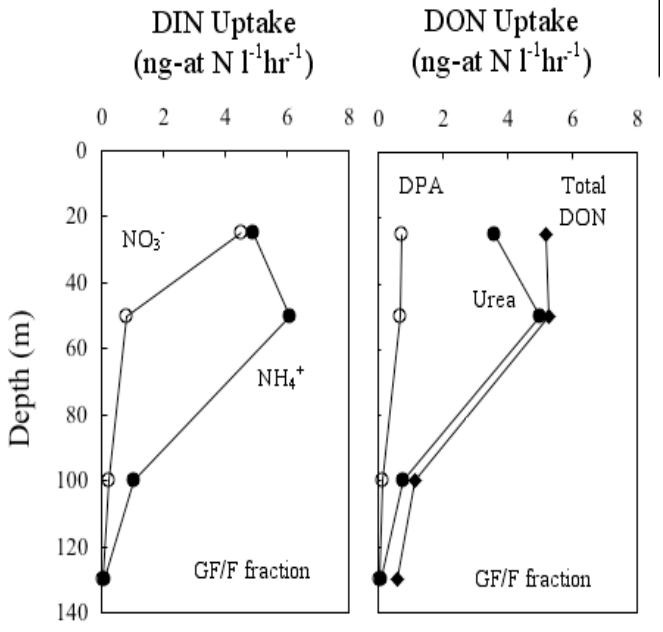


Chesapeake Bay - August

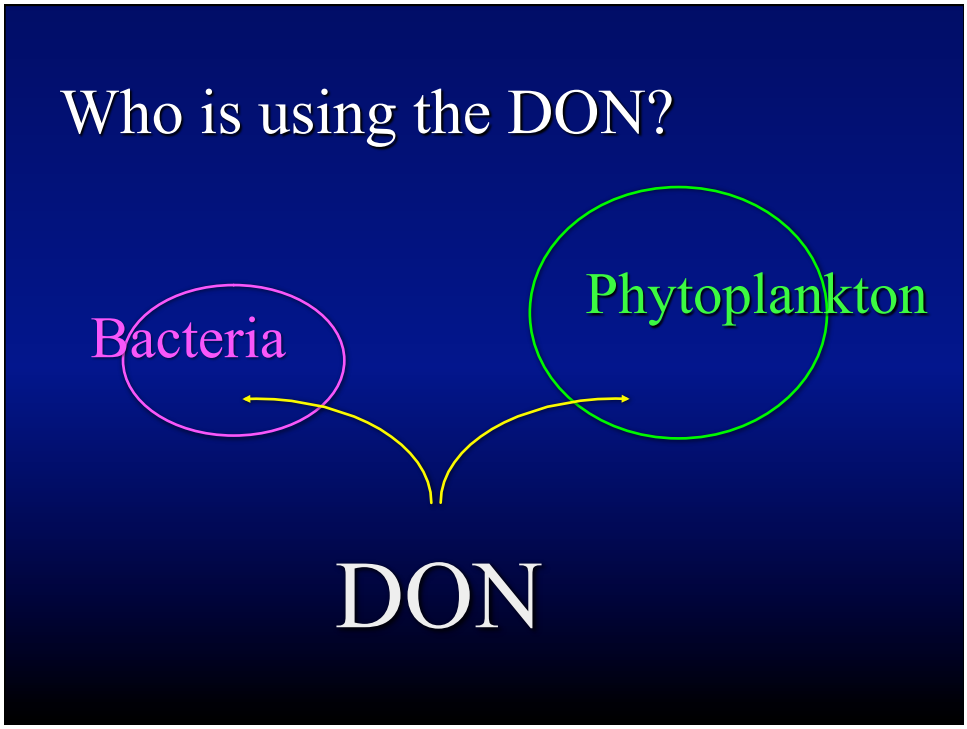


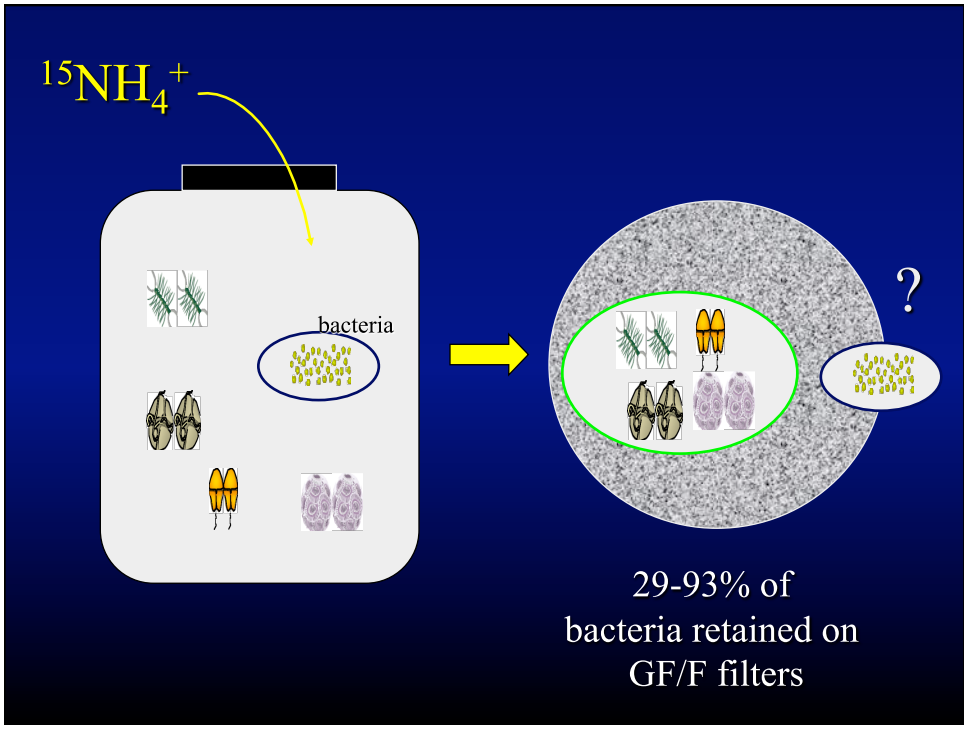
Bronk and Glibert 1993 Mar Biol

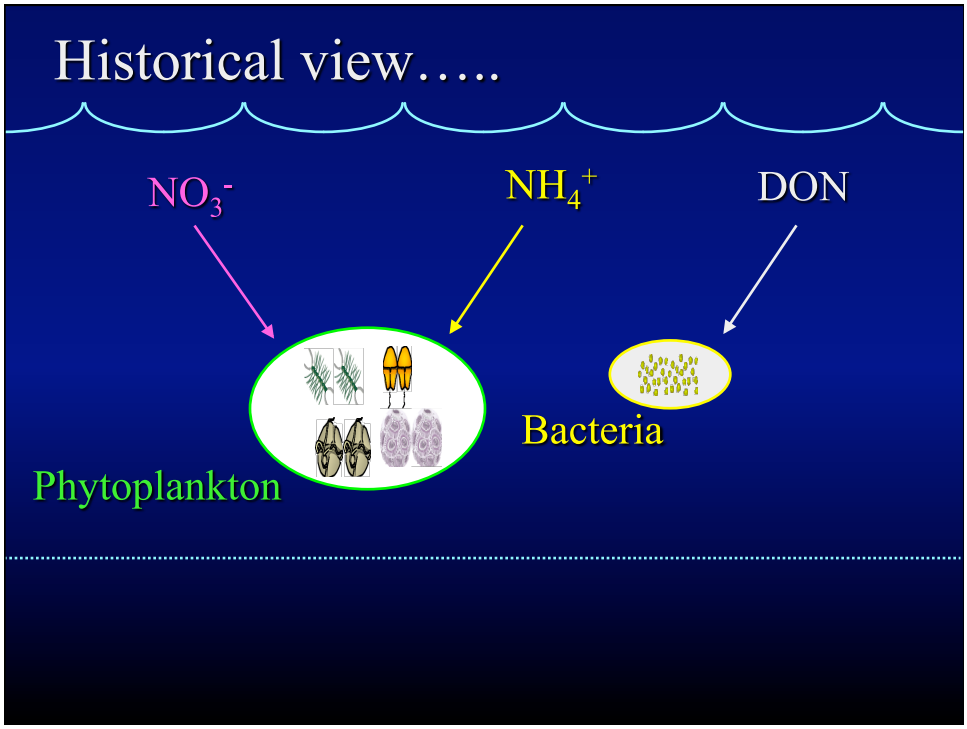
South Pacific

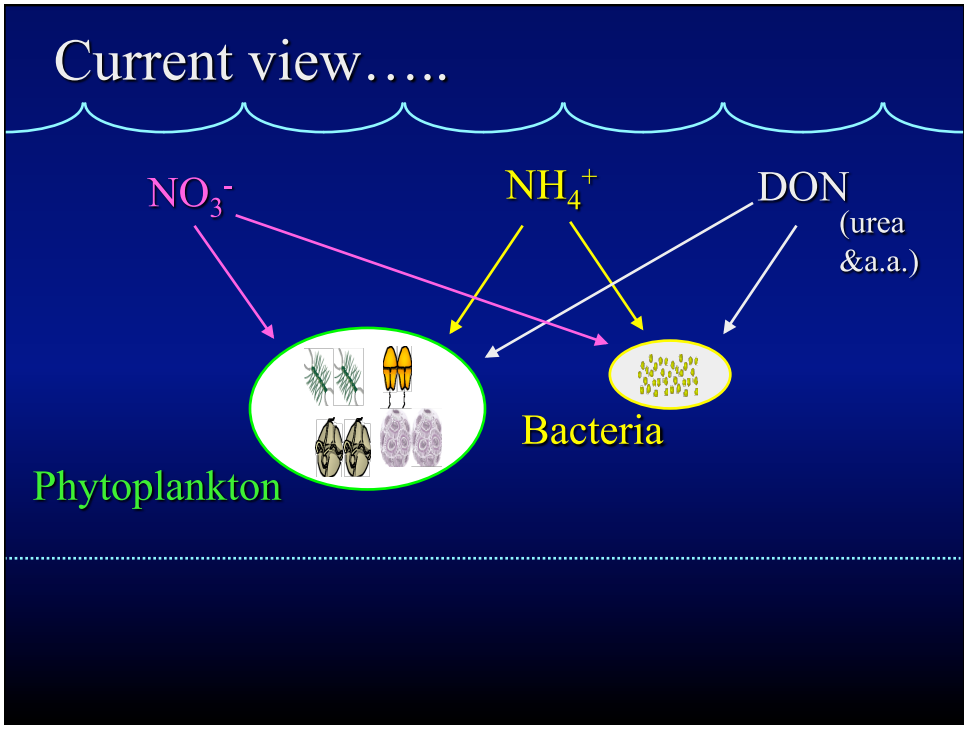


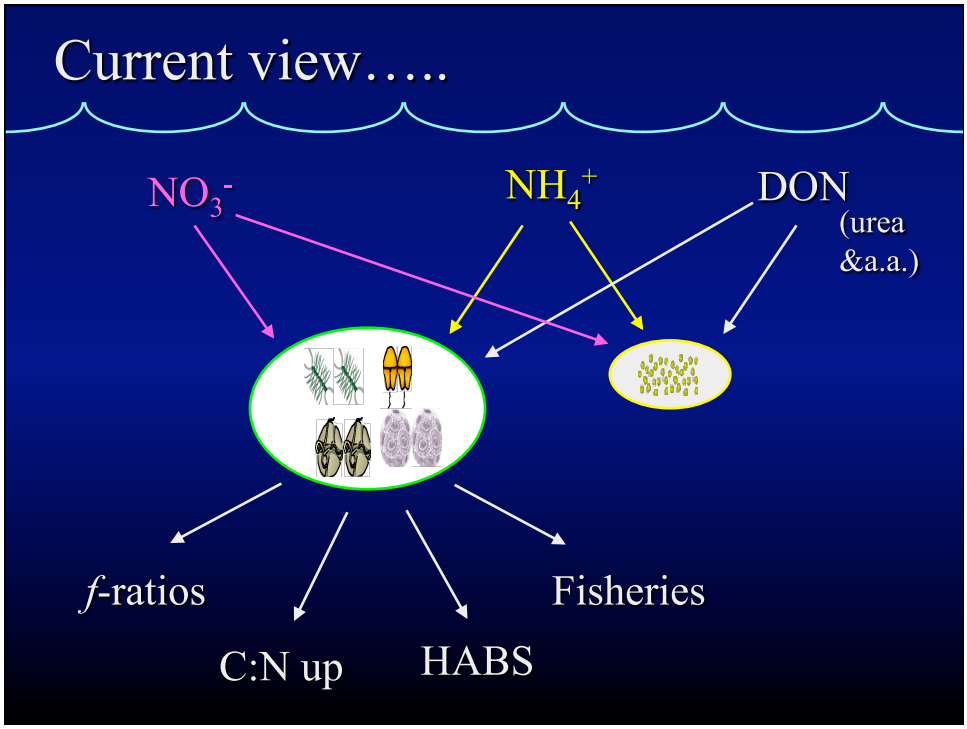
Bronk & Campbell In prep.

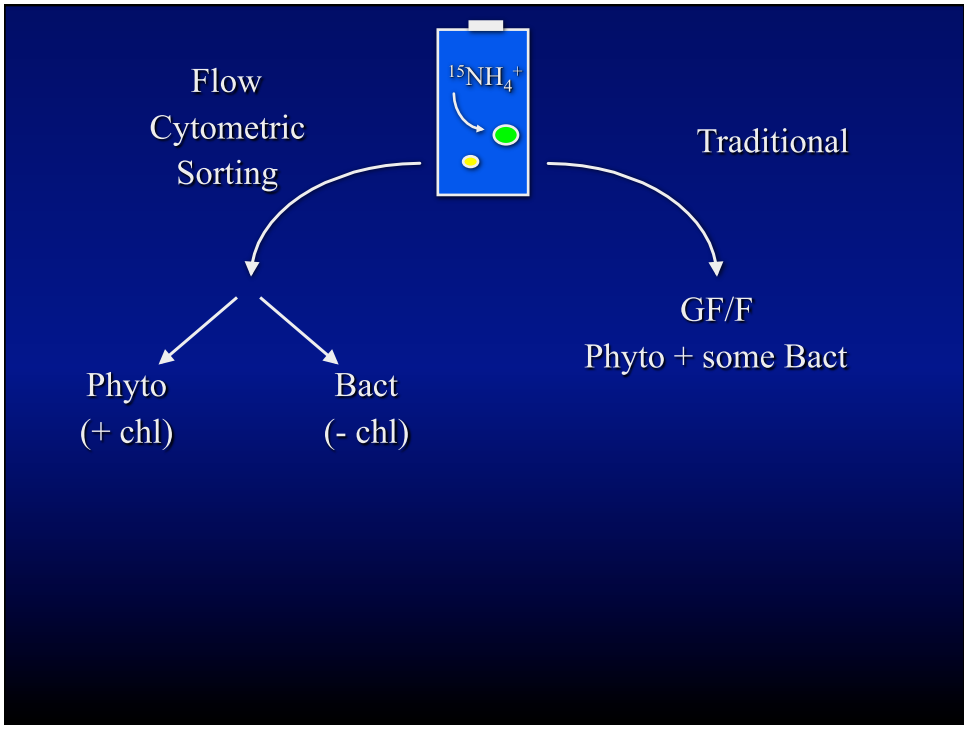

Who is using the DON?
Bacteria
Phytoplankton
DON


$^{15}NH_4^+$
bacteria
?
29-93% of
bacteria retained on
GF/F filters

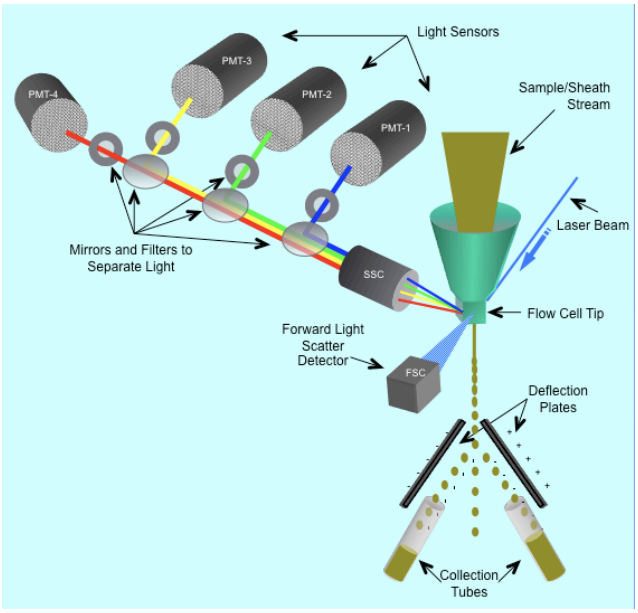

Historical view…..
NO_3^-
NH_4^+
DON
Bacteria
Phytoplankton

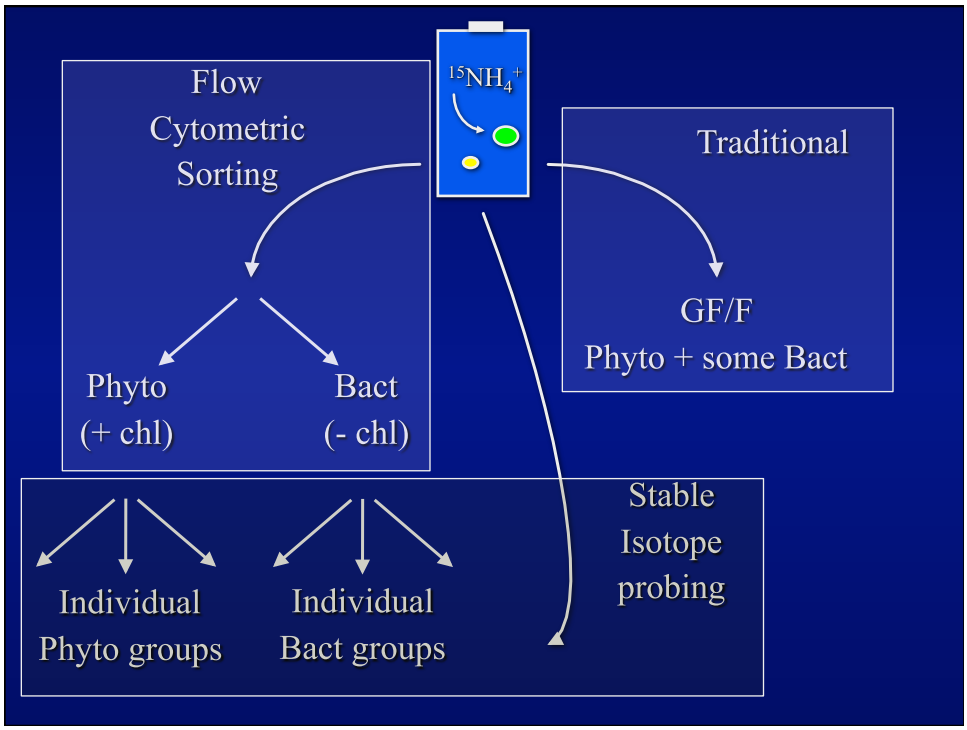

Current view…..
NO_3^-
NH_4^+
DON
(urea
&a.a.)
Bacteria
Phytoplankton


Current view…..
NO_3^-
NH_4^+
DON
(urea
&a.a.)
f-ratios
C:N up
HABS
Fisheries

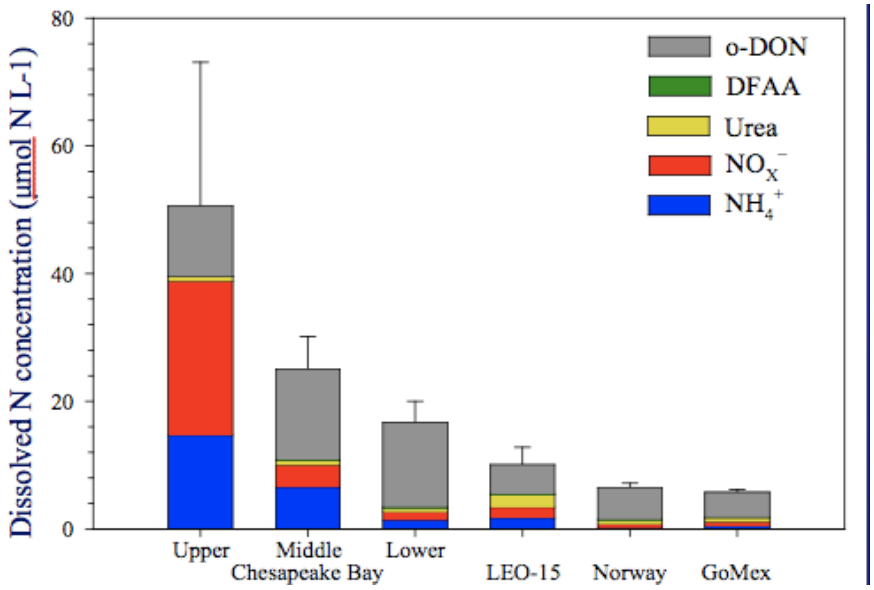

Flow
Cytometric
Sorting
$^{15}NH_4^+$
Traditional
Phyto
(+ chl)
Bact
(- chl)
GF/F
Phyto + some Bact

Flow Cytometric Sorting

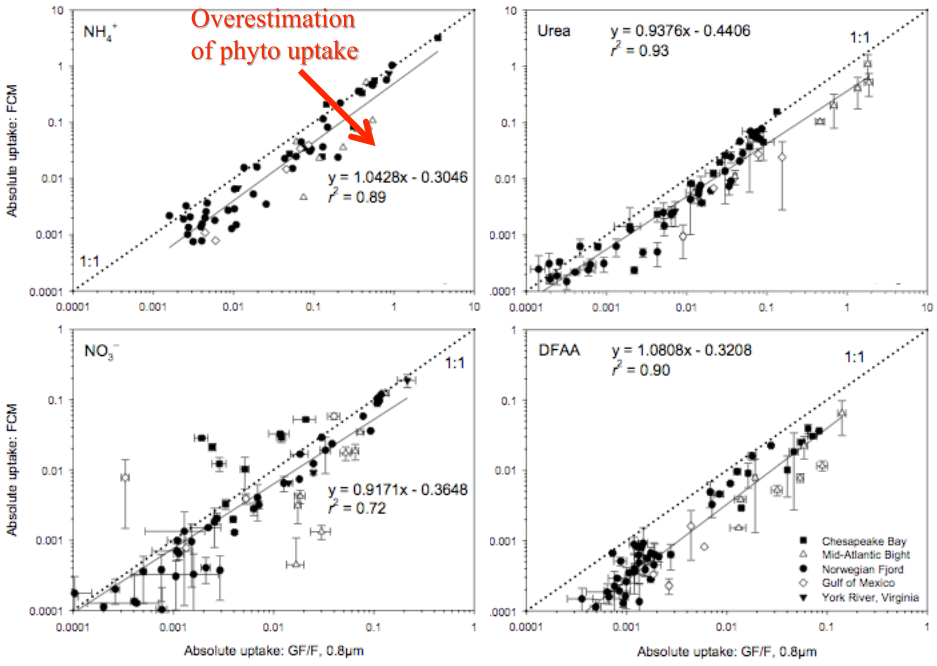


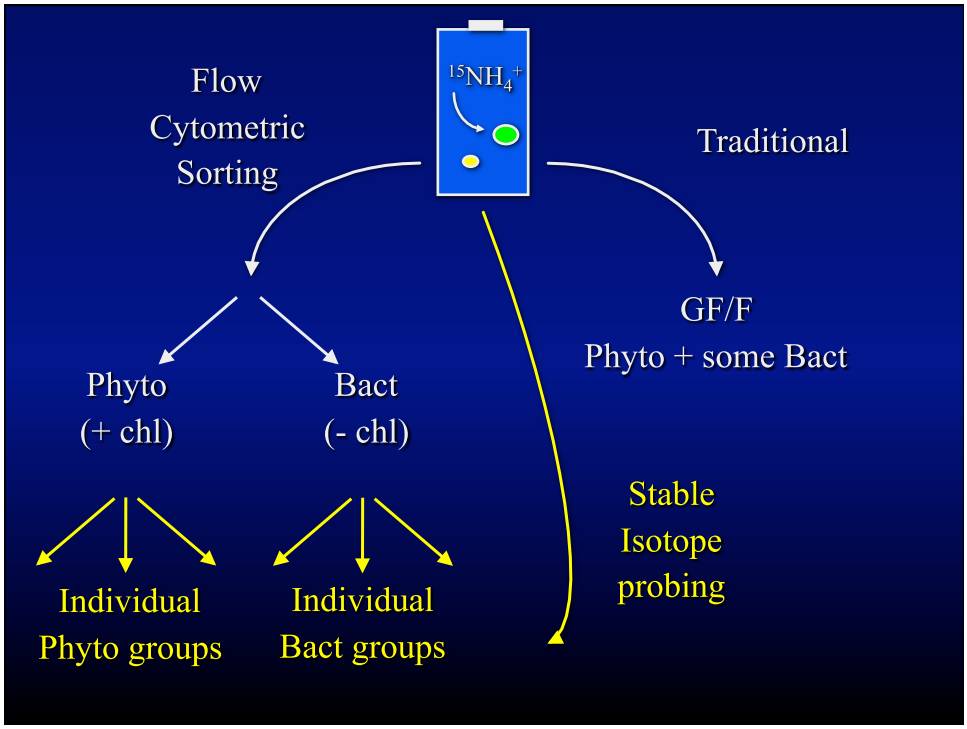


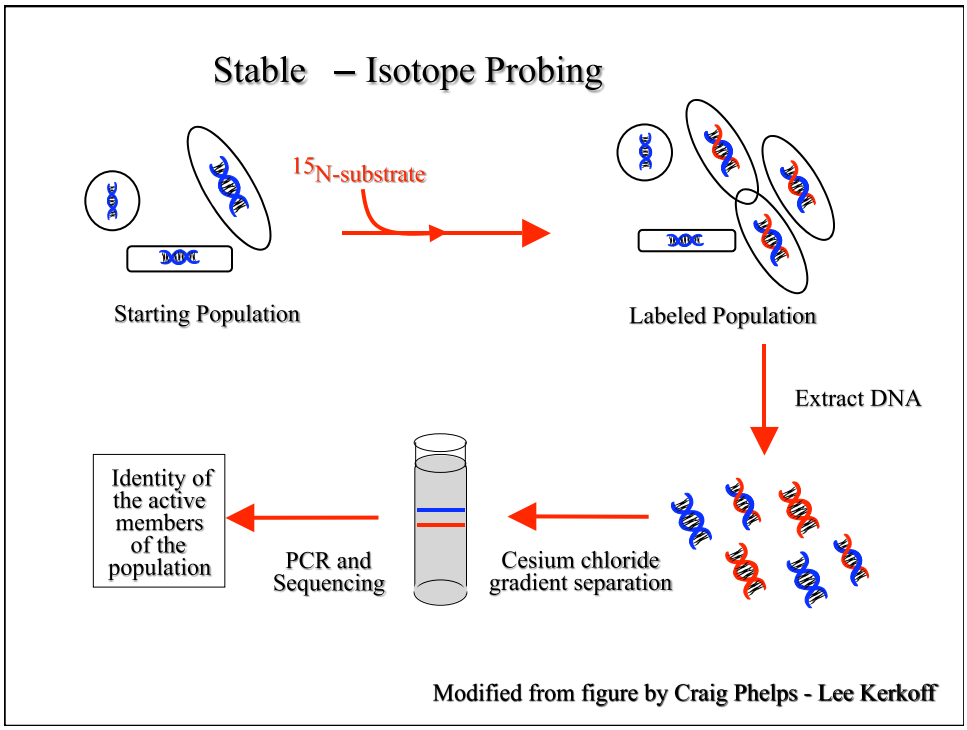
Cross-System Comparison: Dissolved N



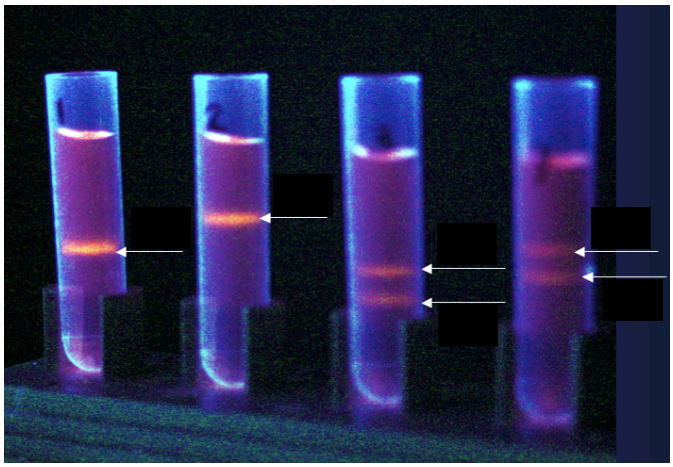
Bradley & Bronk Submitted




Flow
Cytometric
Sorting
$^{15}NH_4^+$
Traditional
GF/F
Phyto + some Bact
Phyto
(+ chl)
Bact
(- chl)
Stable
Isotope
probing
Individual
Phyto groups
Individual
Bact groups

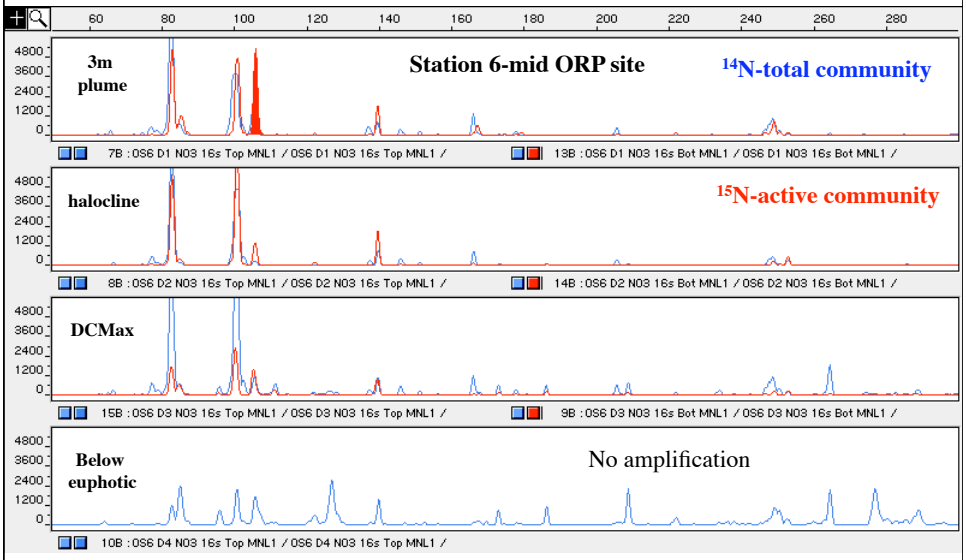

Stable – Isotope Probing
^{15}N-substrate
Starting Population
Labeled Population
Extract DNA
Identity of the active members of the population
PCR and Sequencing
Cesium chloride gradient separation
Modified from figure by Craig Phelps - Lee Kerkoff

Cesium Chloride (CsCl) Gradient

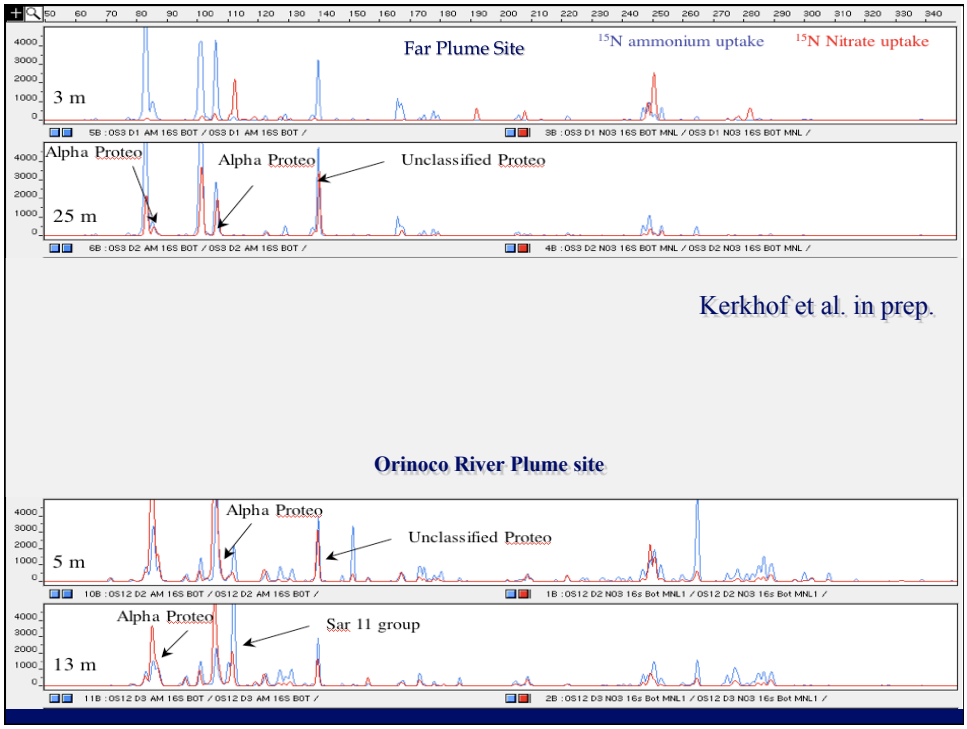


Modified from figure by Lee Kerkhoff

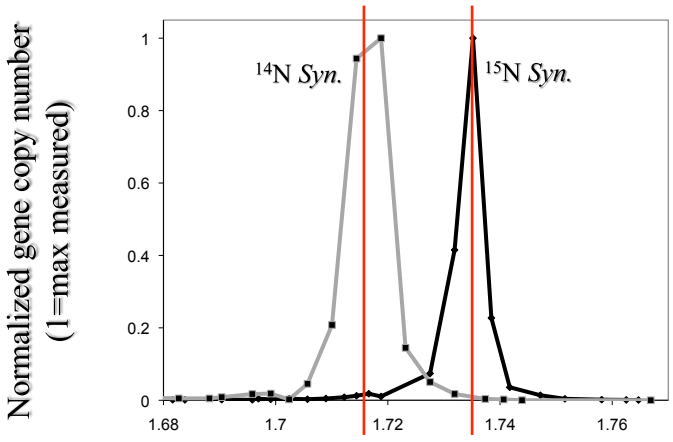
16S rRNA gene profiles of bacterial $^{15}NO_3^-$ uptake



Lee Kerkhoff et al. In prep.



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



Wawrik and Bronk submitted

NH_4^+
DON

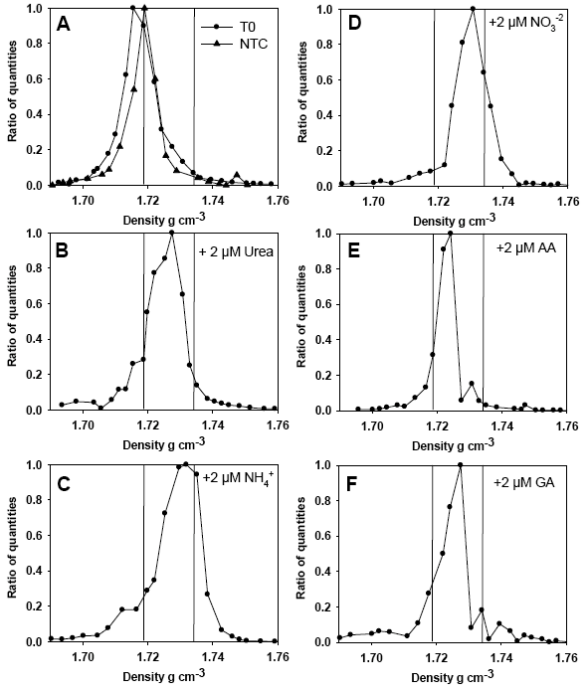
NO_3^-

Synechococcus

Diatoms

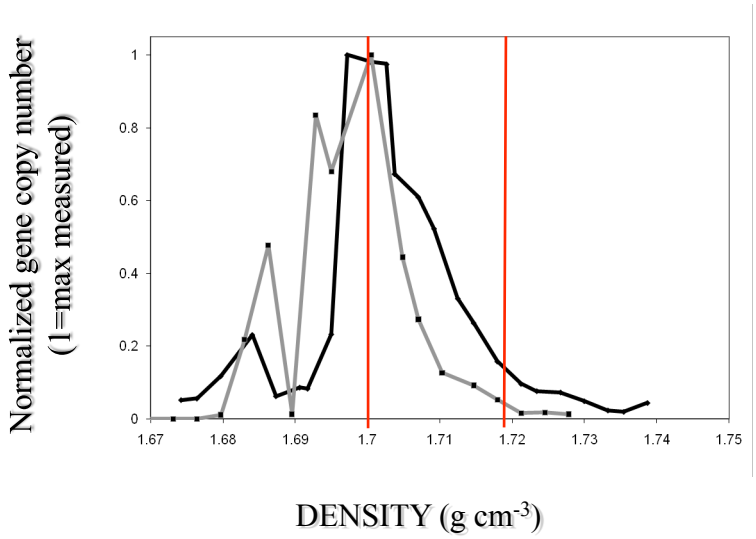
West Florida Shelf 2008

***Synechococcus* sp.**



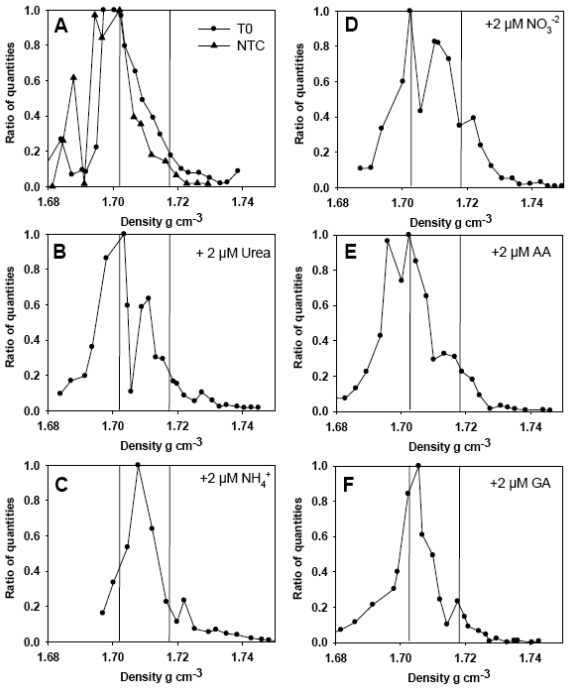
Wawrik et al. submitted

Diatom field control



West Florida Shelf 2008

Diatoms



Wawrik et al. submitted

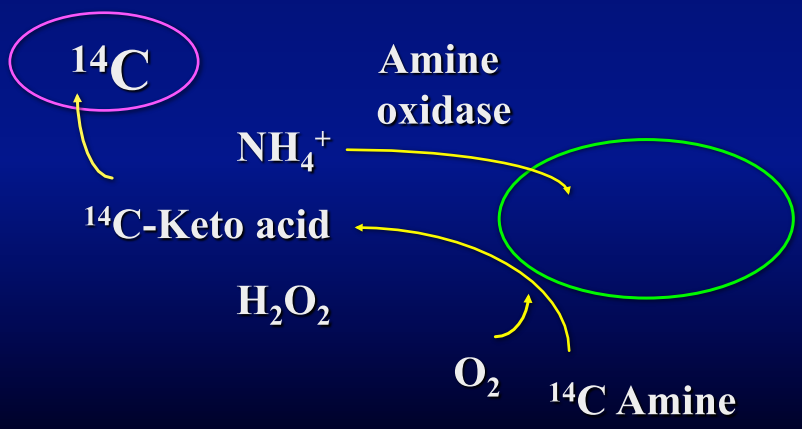
Substrate	% label incorporation *Syn*	Diatom
NH_4^+	78	35
NO_3^-	71	0-37
urea	53	0-43
AA	34	2
glut acid	46	15

Wawrik & Bronk Submitted

Phytoplankton mechanisms to access organic N:

Organic oxidases
Peptide hydrolysis
Pinocytosis
Phagocytosis
Photochemical processes
Salinity release

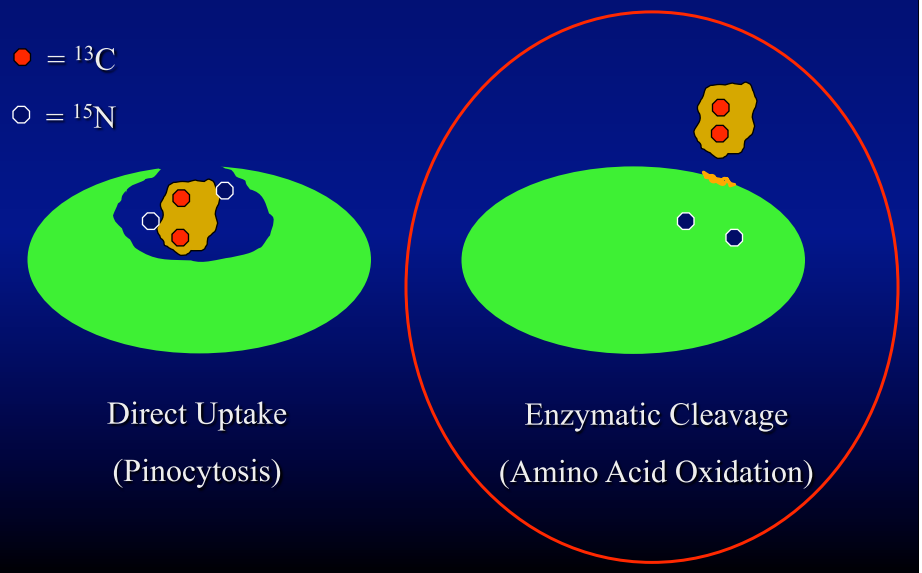
Cell Surface Enzymes



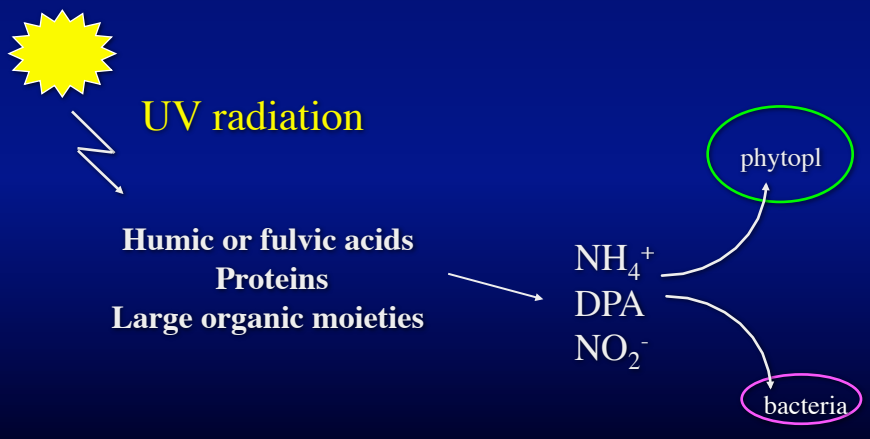
Palenik et al. 1988

Farming nitrogen from "refractory" compounds!

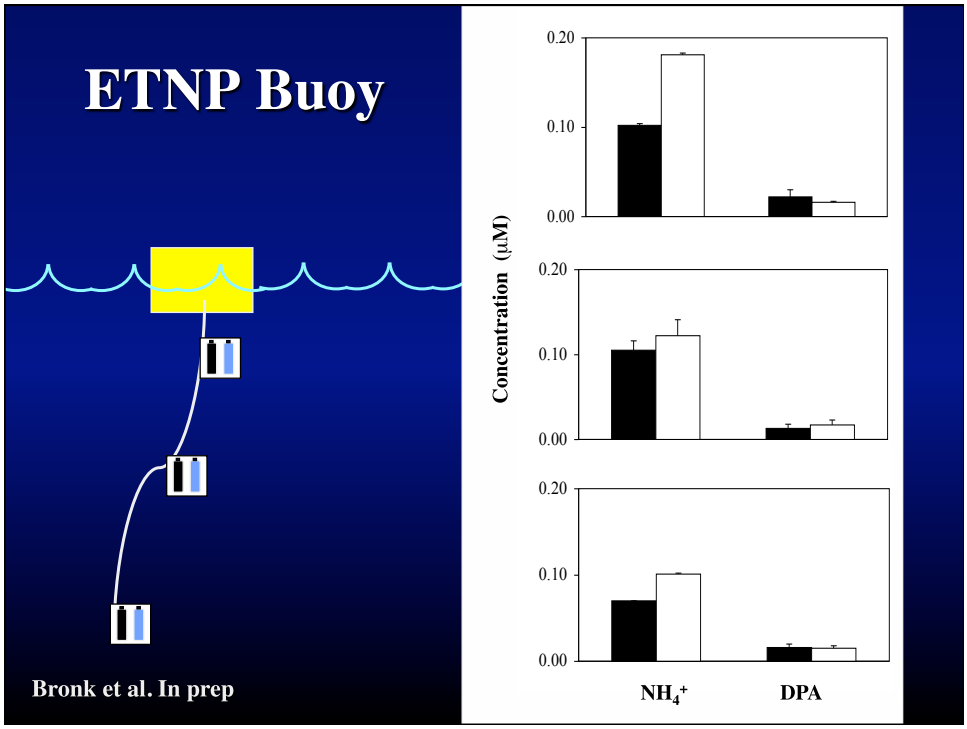
Humic Uptake Mechanisms?



Photoproduction of labile N



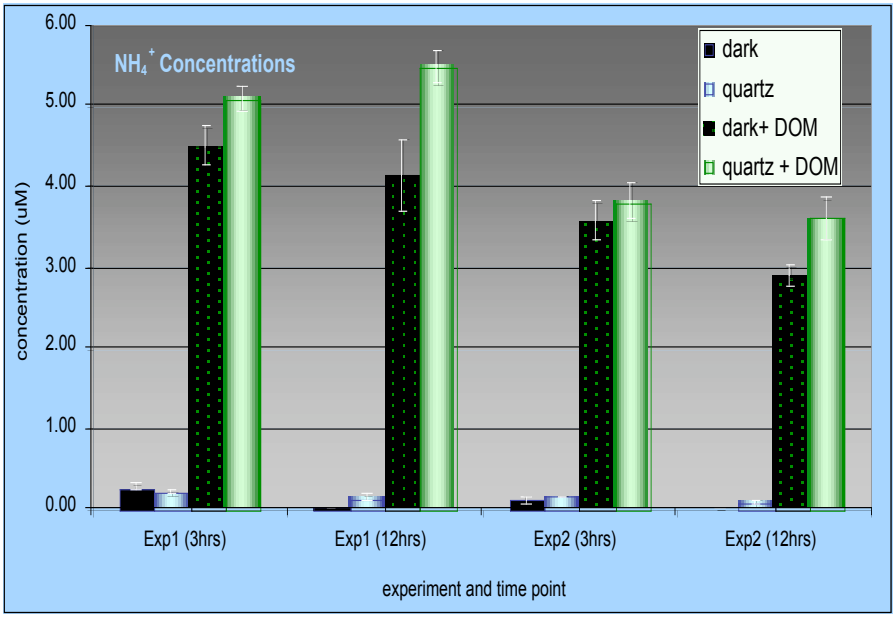
Based on Bushaw et al. 1996 Nature



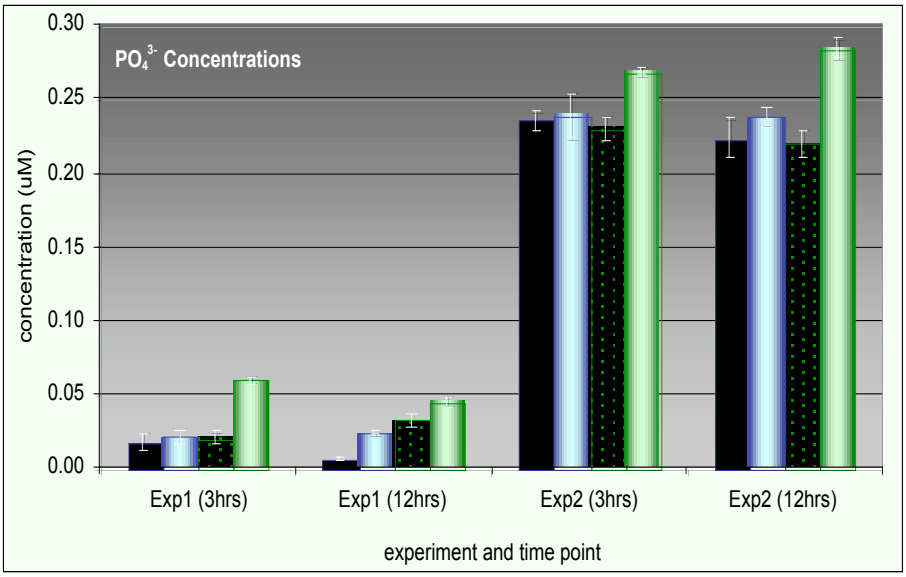
Date	Depth (m)	% Uptake NH_4^+	% Uptake DPA	NO_2^-	% NH_4^+ Regen.
Eastern Tropical North Pacific					
	10	0.0	70.5		0.0
	50	17.0	29.6		5.7
	600	12.2	0.0		4.1
	1200	25.6	12.2		8.6
	100	4.2	0.0	65.3	3.0
	1200	5.6	0.0	92.7	4.1
	80	0.0	43.4	14.8	0.0
	1200	64.6	70.3	99.9	8.1
	100	17.3		117.2	2.3
	400	86.0		168.8	11.4
	1	50.7		0.0	6.7
	6	0.0		0.0	0.0
	13	19.9		0.0	2.6
Mean production		**23.3**	**28.3**	**62.1**	**4.4**
std		**27.2**	**30.3**	**61.8**	**3.6**

Bronk et al. In prep

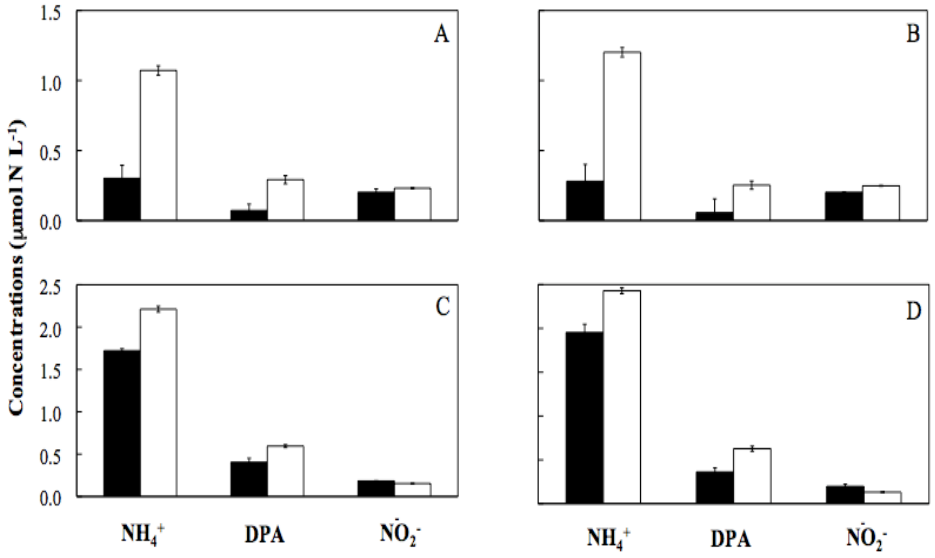
NH_4^+ photoproduction from *Tricho* DON



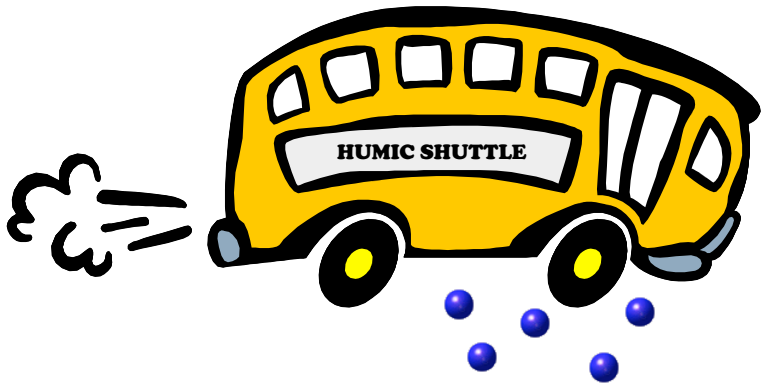
PO_4^- photoproduction from *Tricho* DON

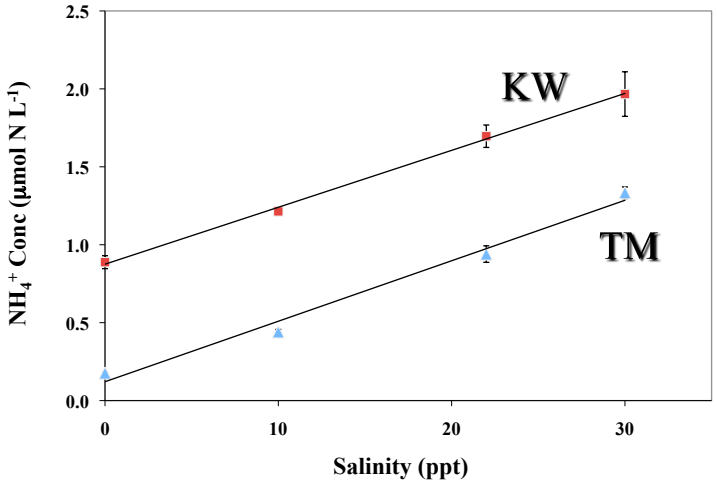


Effluent Organic Nitrogen



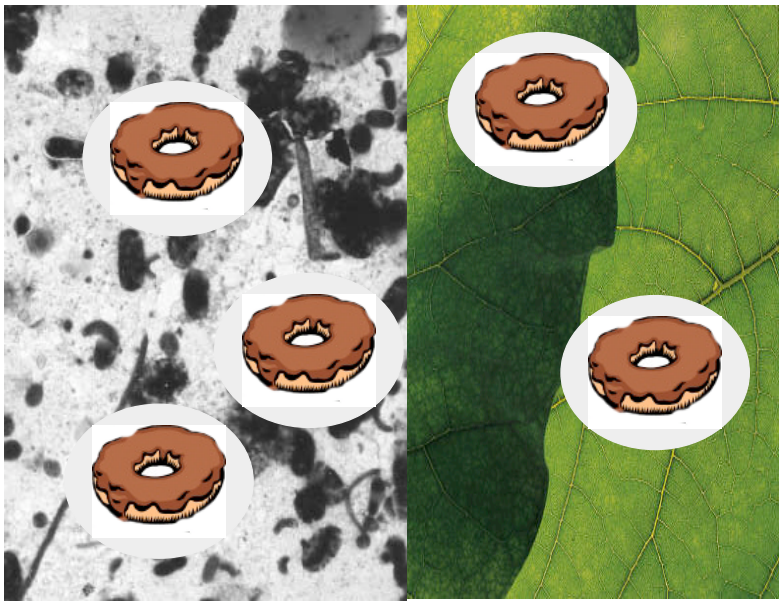
Bronk et al. Submitted





Bronk et al. Submitted

DON



- A significant fraction of both autothonous 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