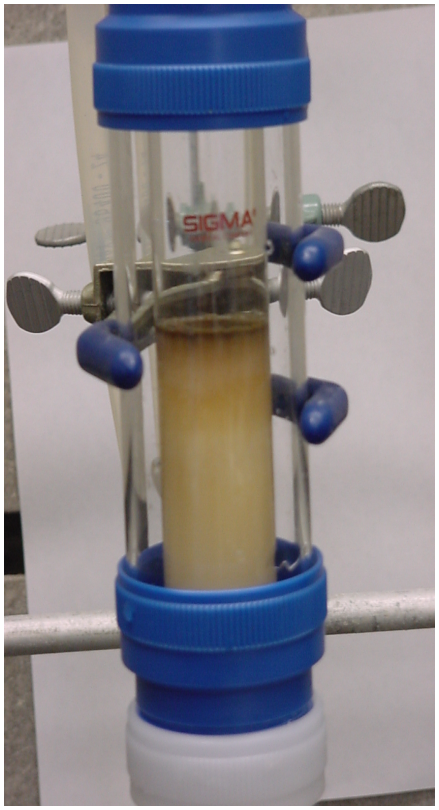
Isolation of DOM by adsorption onto hydrophobic resins



Seawater
(filtered; pH = 2)

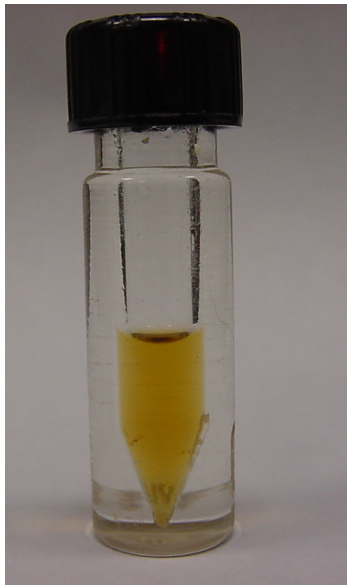
0.1N NaOH
or
1N NH_4OH/MeOH

XAD-8

XAD-4

(XAD = polystyrene)

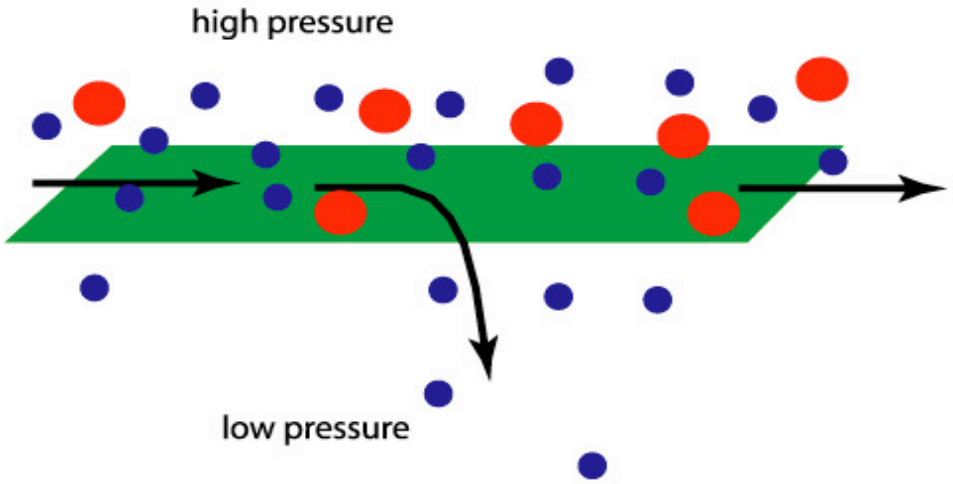
5-10% of DOC



sample

Isolation is chemically selective- based on affinity for resin.
XAD selects for hydrophobic organic matter with an old radiocarbon age.

Cross or tangential flow filtration, Ultra- or nanofiltration



Separation based on size

1 nm pore @ 1 kD

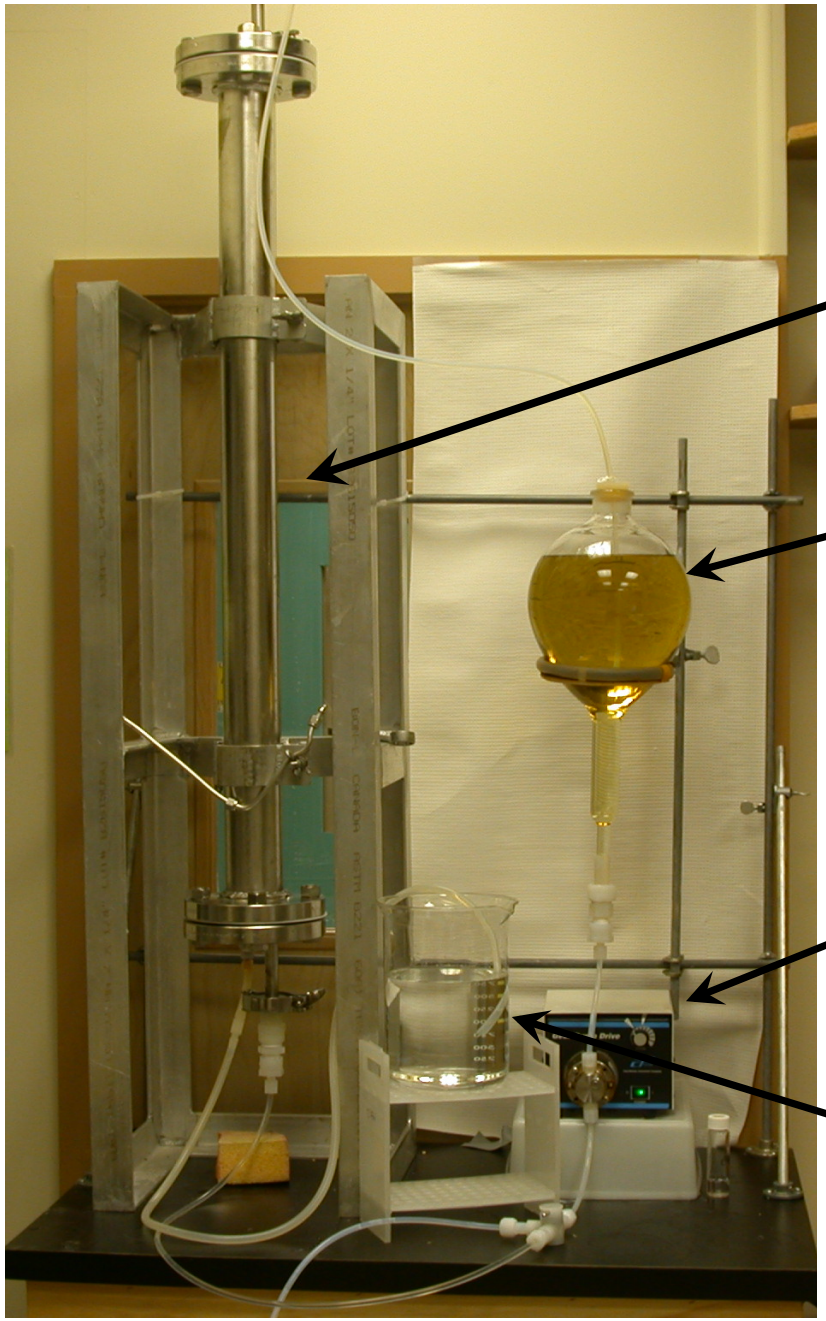
Selects for HMW fraction

about 30-35% TOC (now up to 80% using electrically assisted UF)

C/N = 15-16
C/P = 150 to > 500 (*f(z)*)

Membrane effects what is collected

DOC sampling



filter (1 nm)

sample concentrate
(> 1000 MW)

pump

filtrate (< 1000 MW)

Final product

30% of total DOC

C/N = 15 +/- 2

C/P = 150-500

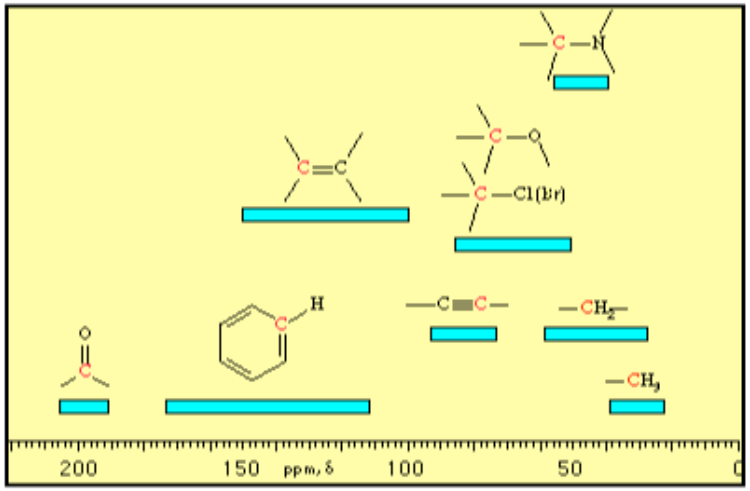
Nuclear Magnetic Resonance Spectroscopy (NMR)

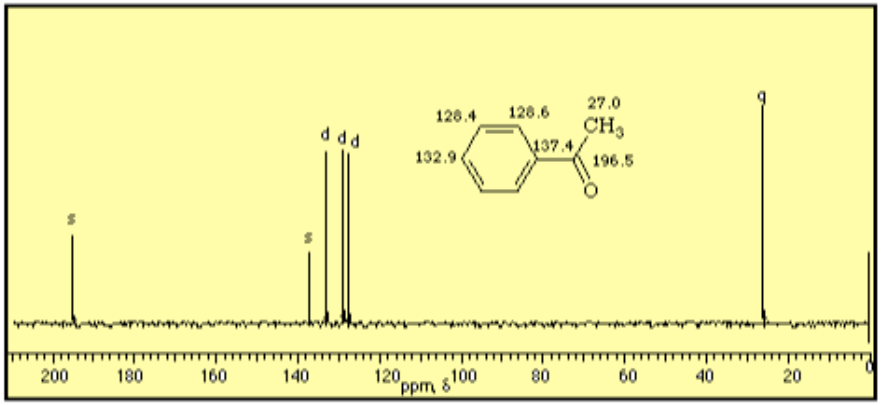
Can be tuned to different Nuclei of interest (C,N,P…).

Gives information on functional groups which, combined with a knowledge of biochemicals can be used to deduce composition and origin.

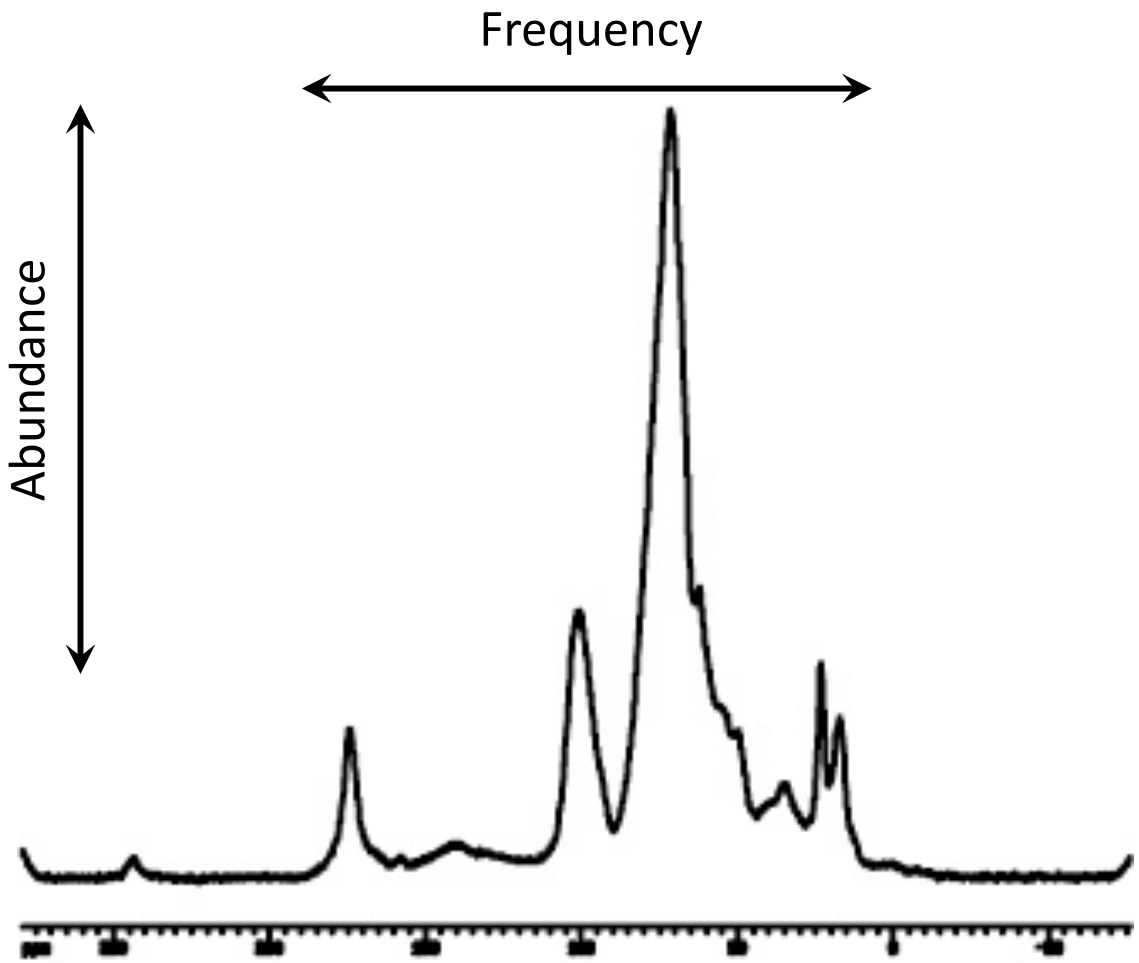
Internally quantitative.

Carbon 13 Nuclear Magnetic Resonance Spectroscopy

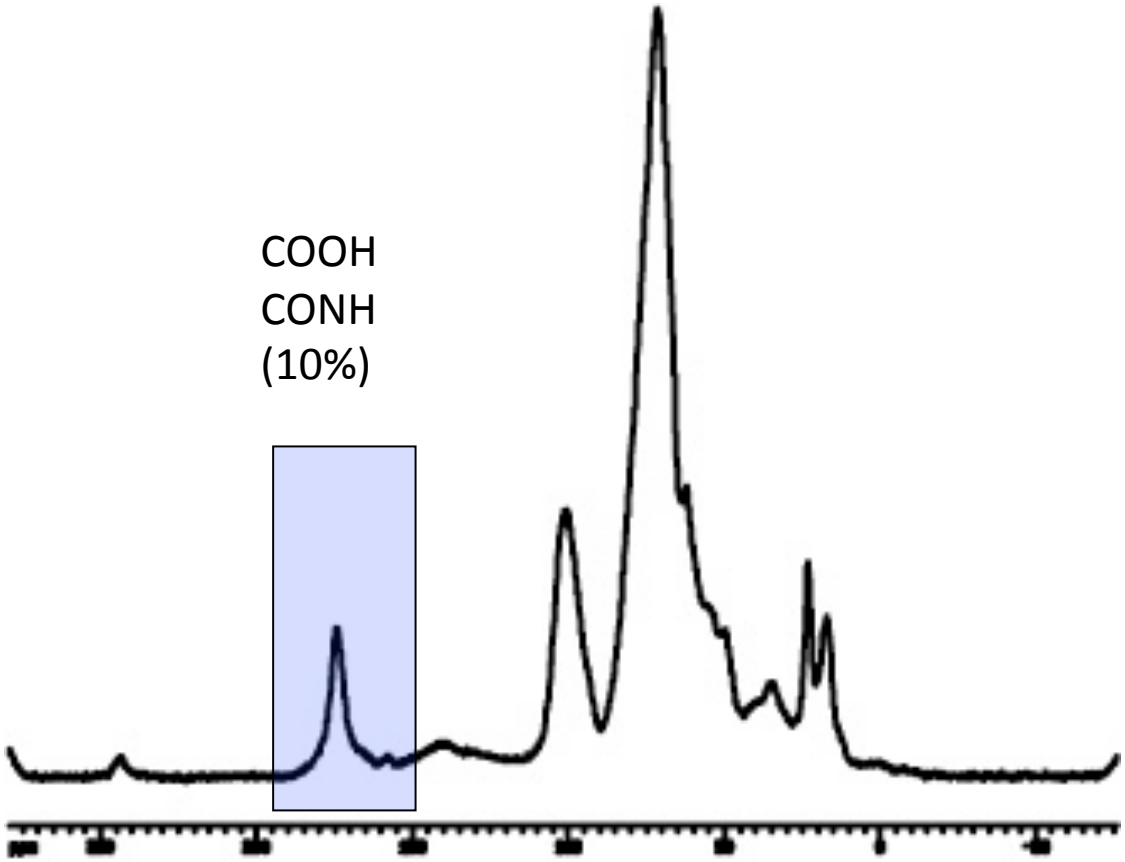




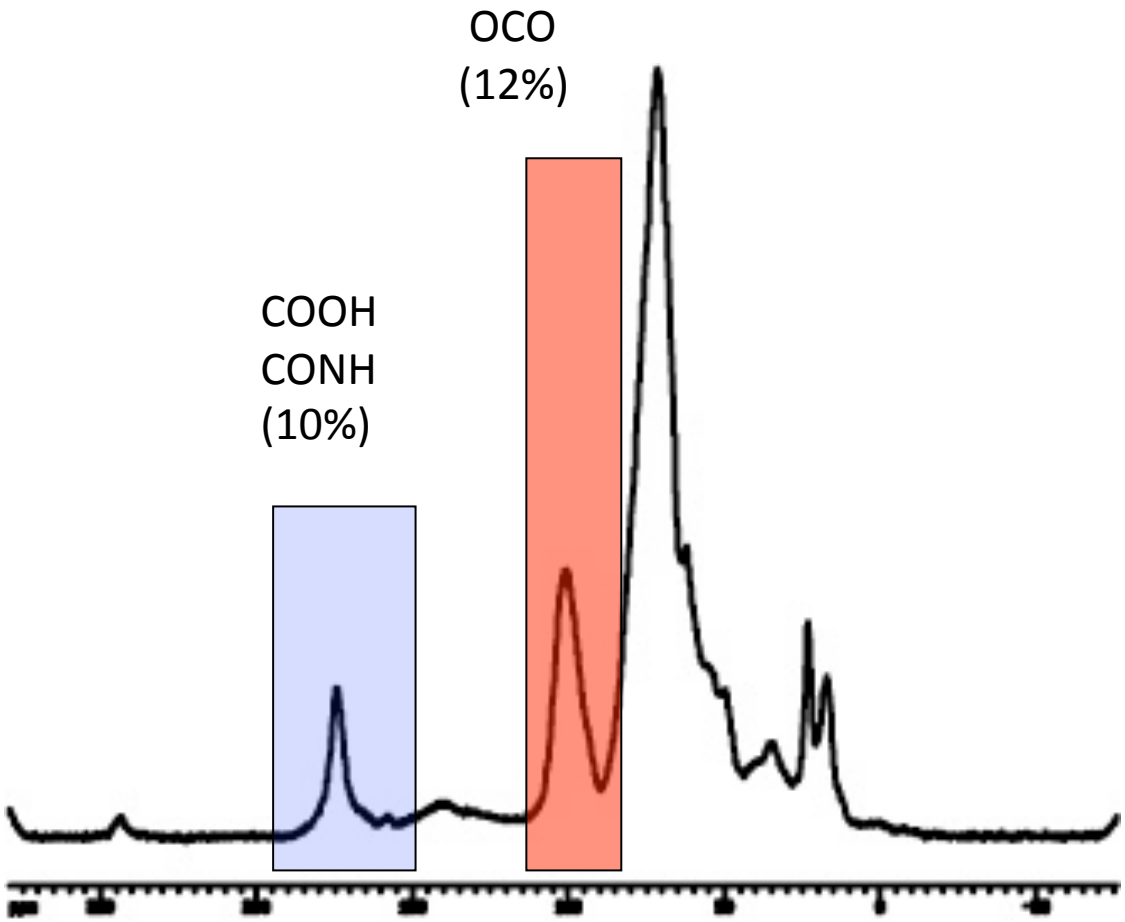
^{13}C Nuclear Magnetic Resonance Spectrum
of high molecular weight dissolved organic matter (C/N = 15)



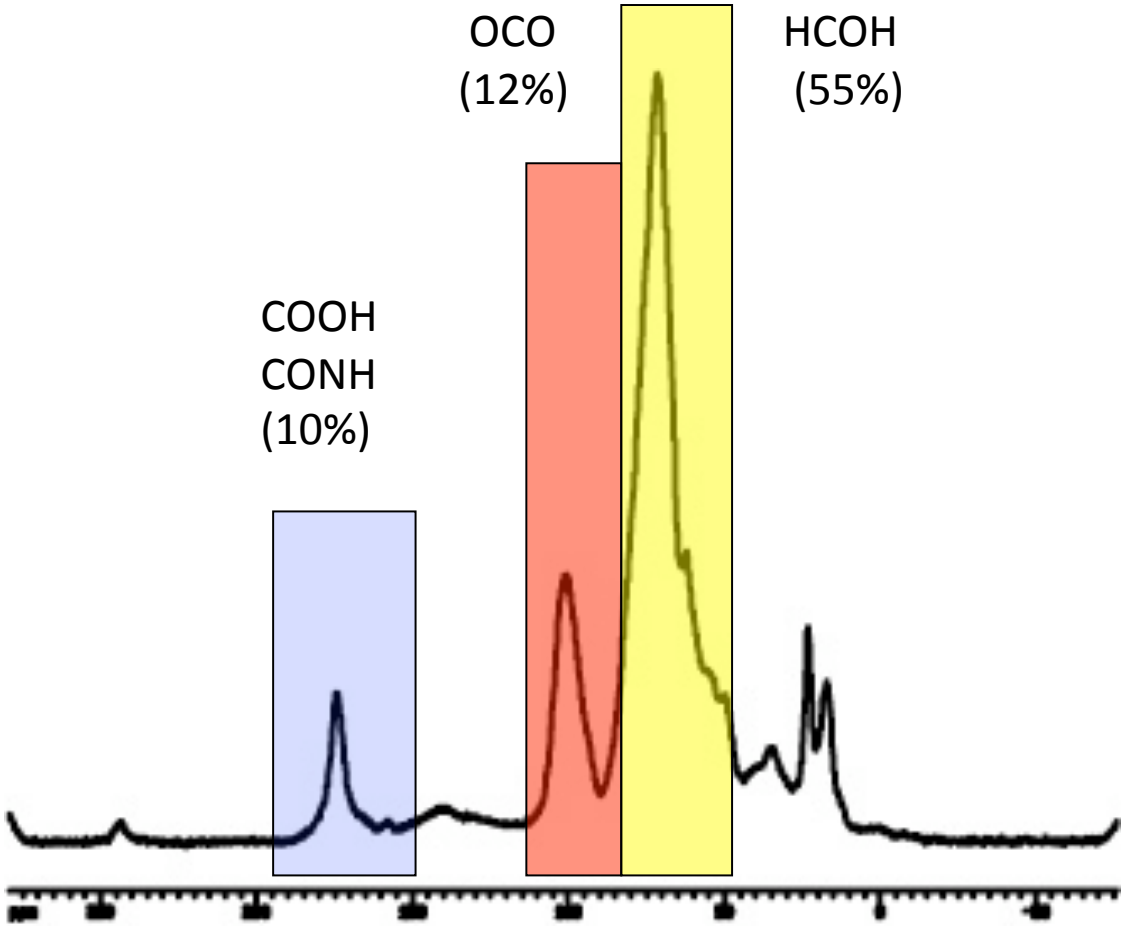
^{13}C Nuclear Magnetic Resonance Spectrum
of high molecular weight dissolved organic matter (C/N = 15)



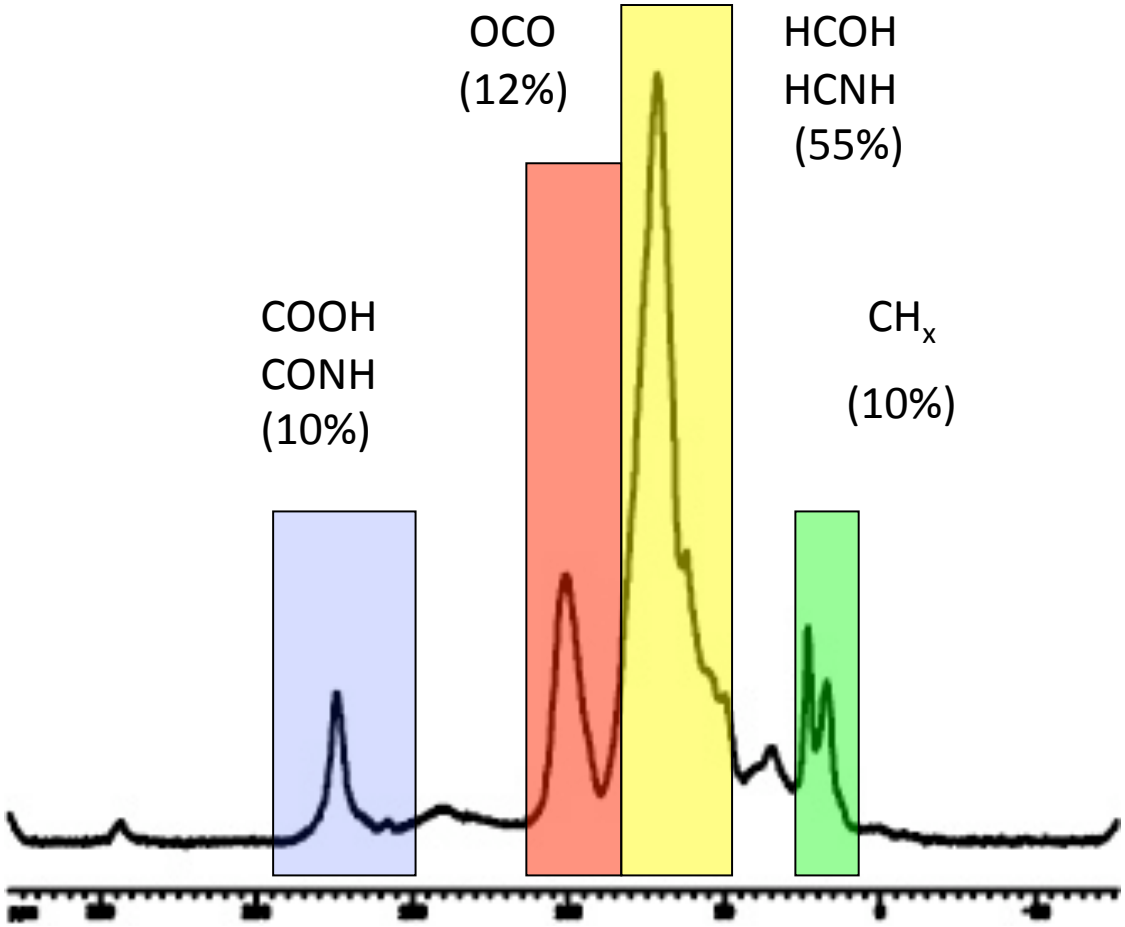
^{13}C Nuclear Magnetic Resonance Spectrum
of high molecular weight dissolved organic matter (C/N = 15)



^{13}C Nuclear Magnetic Resonance Spectrum
of high molecular weight dissolved organic matter (C/N = 15)



^{13}C Nuclear Magnetic Resonance Spectrum of high molecular weight dissolved organic matter (C/N = 15)



Proximate analysis of algal cells

Chlorophyceae (green algae)	**Protein**	**Carbohydrate**	**Lipid**	**Ash**
Tetraselmis maculata	72	21	7	(24)
Dunaliela salina	58	33	10	(8)
Chrysophyceae (golden brown algae)				
Monochrysis lutheri	53	34	13	(6)
Syracosphaera carterae	70	23	7	(37)
Bacillariophyceae (brown algae, diatoms)				
Chaetoceros sp.	68	13	16	(28)
Skeletonema costatum	58	33	10	(39)
Coscinodiscus sp.	74	16	10	(57)
Phaeodactylum tricornutum	49	36	14	(8)
Dynophyceae (dinoflagellates)				
Amphidinium carteri	35	38	23	(14)
Exuriella sp.	37	44	20	(8)
Average	**57**	**29**	**13**	

Most POM is protein, and this is probably a large fraction of reactive DOM
Dissolved "free" amino acids have been measured in seawater at 10's nM

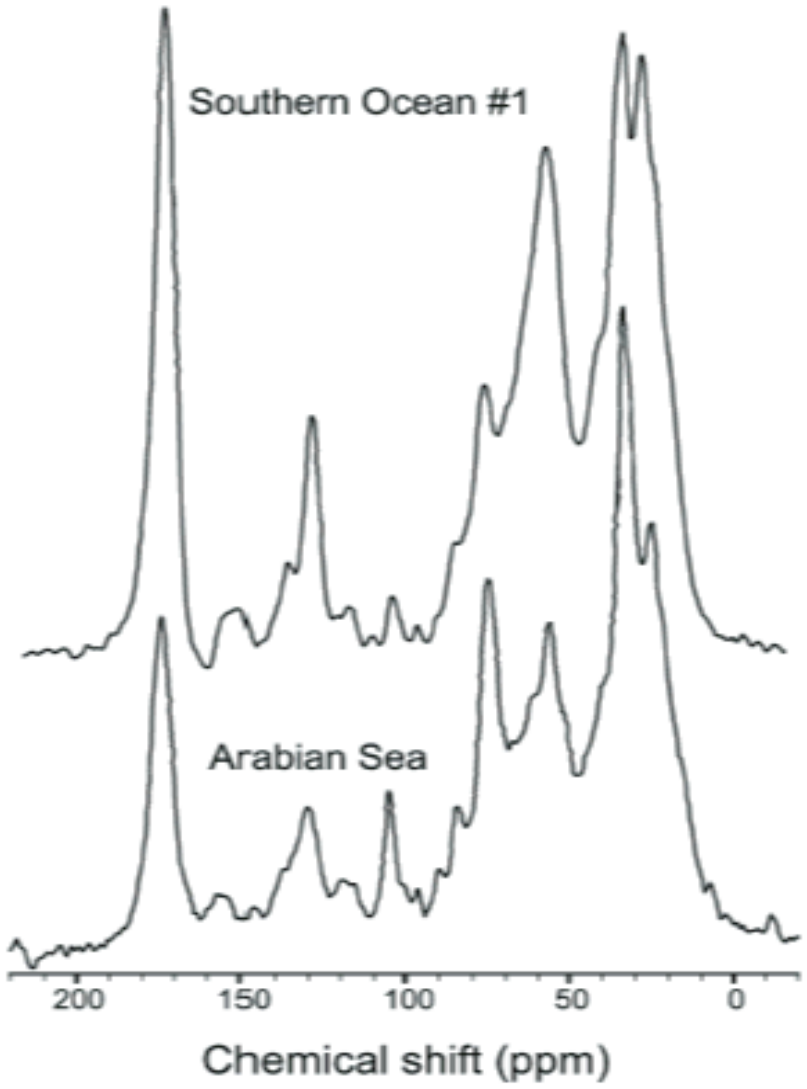
What are the biochemical ingredients of HMWDOM?

From our knowledge of cell biochemicals…
C/N….COOH:OCO:HCOH:CH_x…

HMWDOM	C/N = 15	CHx:HCOH:OCO:COOH =1:5.5:1.2:1
Proteins	C/N = 4,	CH_x(O):CON = 3:1
Carbohydrates	C maybe N	OCO:HCOH = 1:5
Lipids	C only	CH_x:COOH = 2-18 CH_x:COH = 2-30

unsolved mysteries #1…Carbohydrates are thought to be easily degraded by microbes, so why is the ocean filled with carbohydrate?

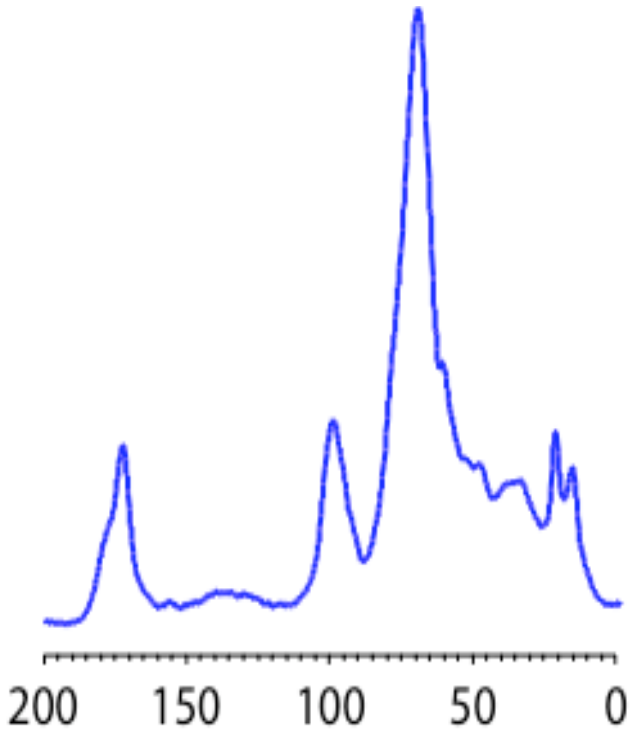
^{13}CNMR of plankton tows



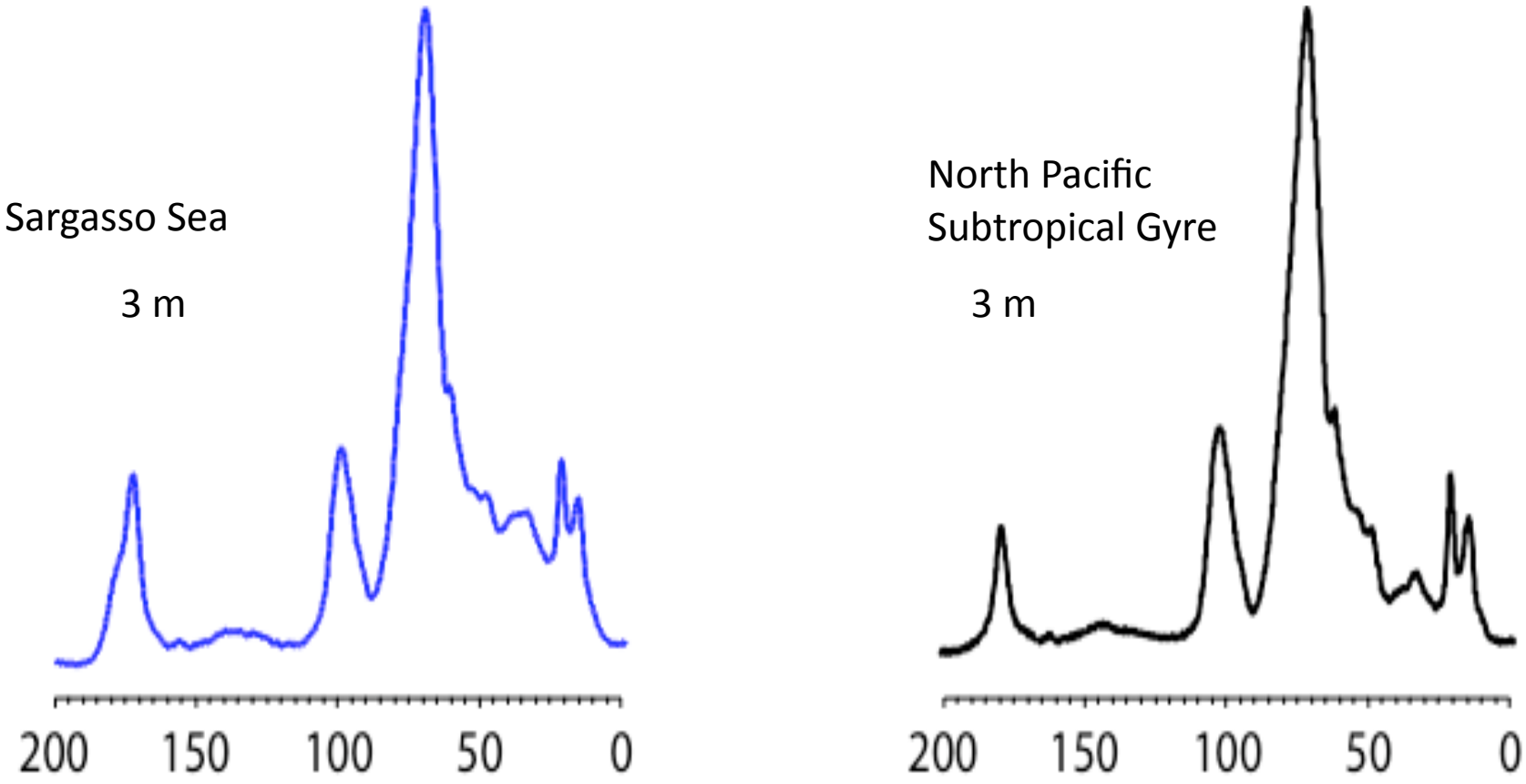
Hedges et al GCA 2001

^{13}CNMR of HMWDOM

Sargasso Sea
(surface)

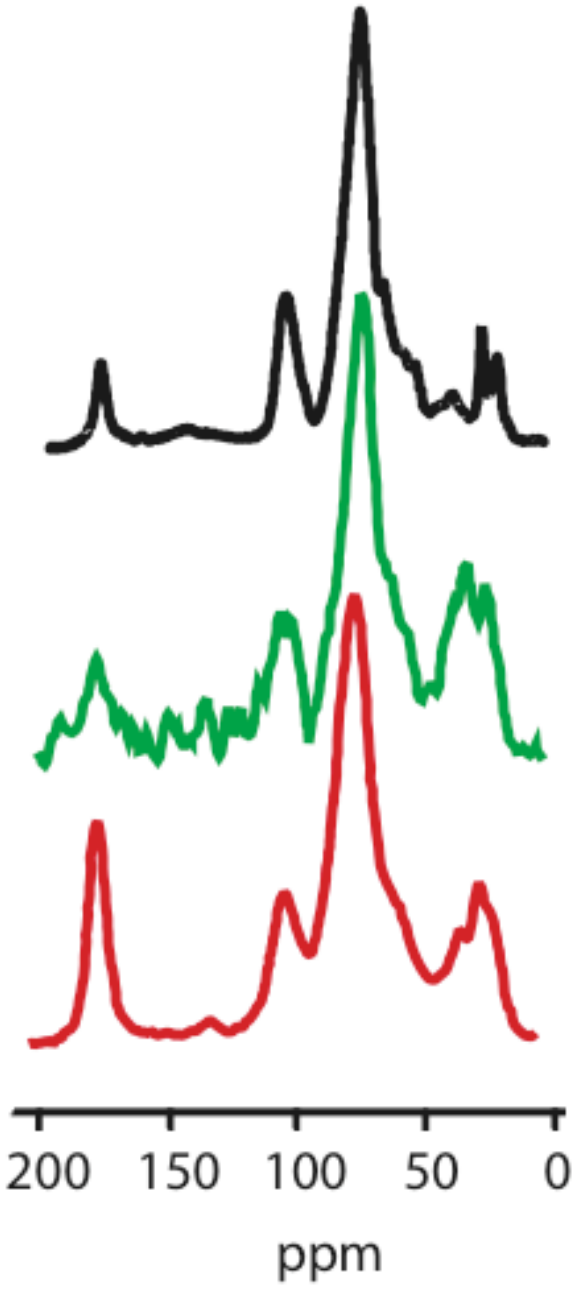


^{13}CNMR spectra of HMWDOM



unsolved mysteries #2…if the source of DOC is microorganisms and the dominant microbes change with location, why is the ocean filled with the same type of carbohydrate everywhere?

HMWDOM in different aquatic environments



NPSG

Andrews Creek

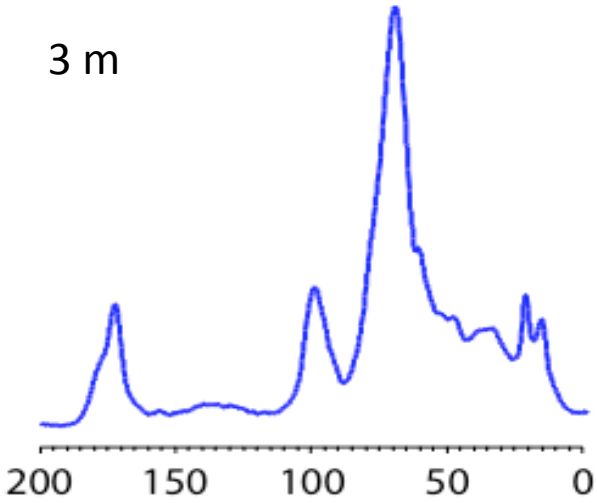
+3500 m in Rocky Mountains National Park

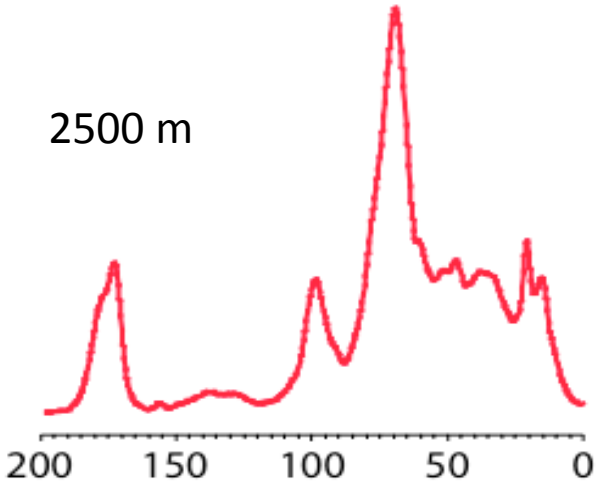
Great Salt Lake

Diane McKnight et al, BGC 1997; Jim Leenher, BGC 2004

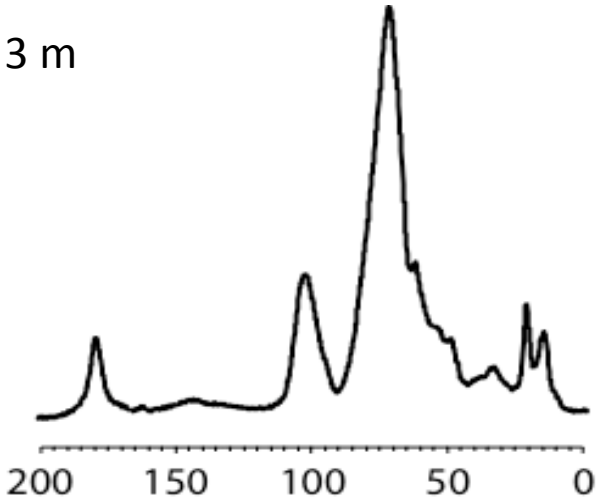
HMWDOM in the deep sea

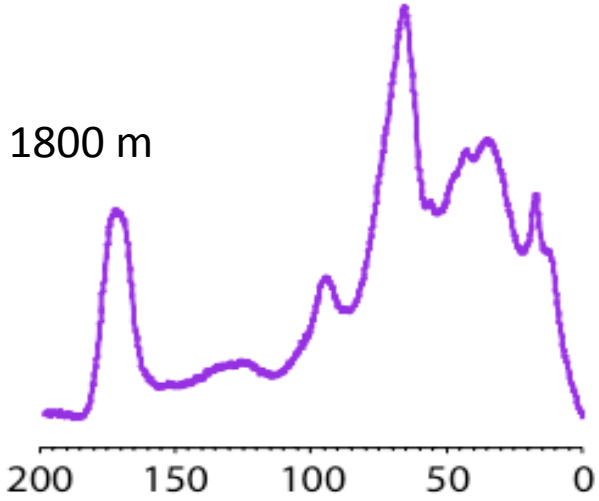
Sargasso Sea





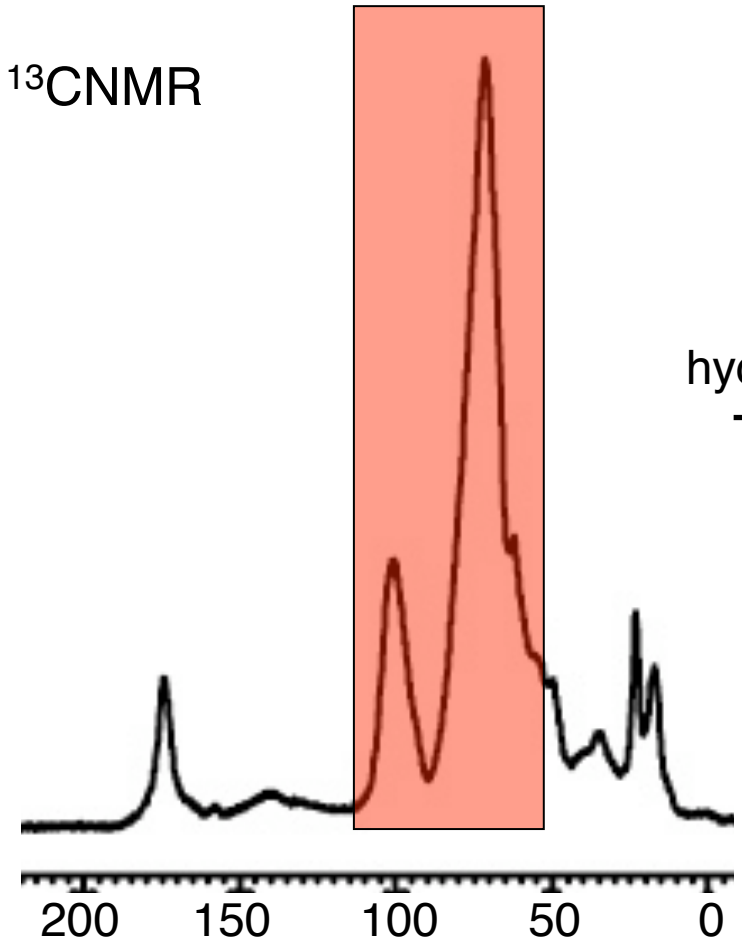
NPSG





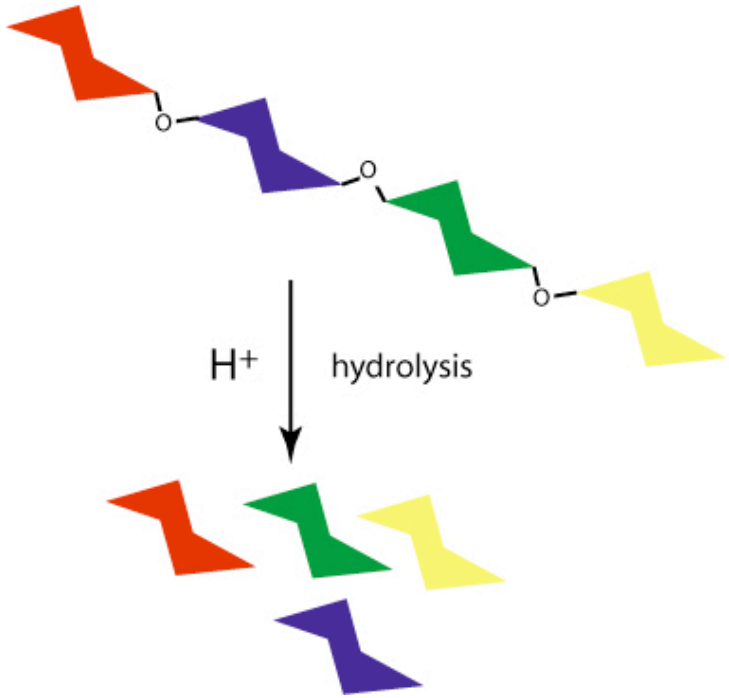
Spectral and chemical analyses of HMWDOC

Carbohydrate
70-90% of surface water HMWDOC

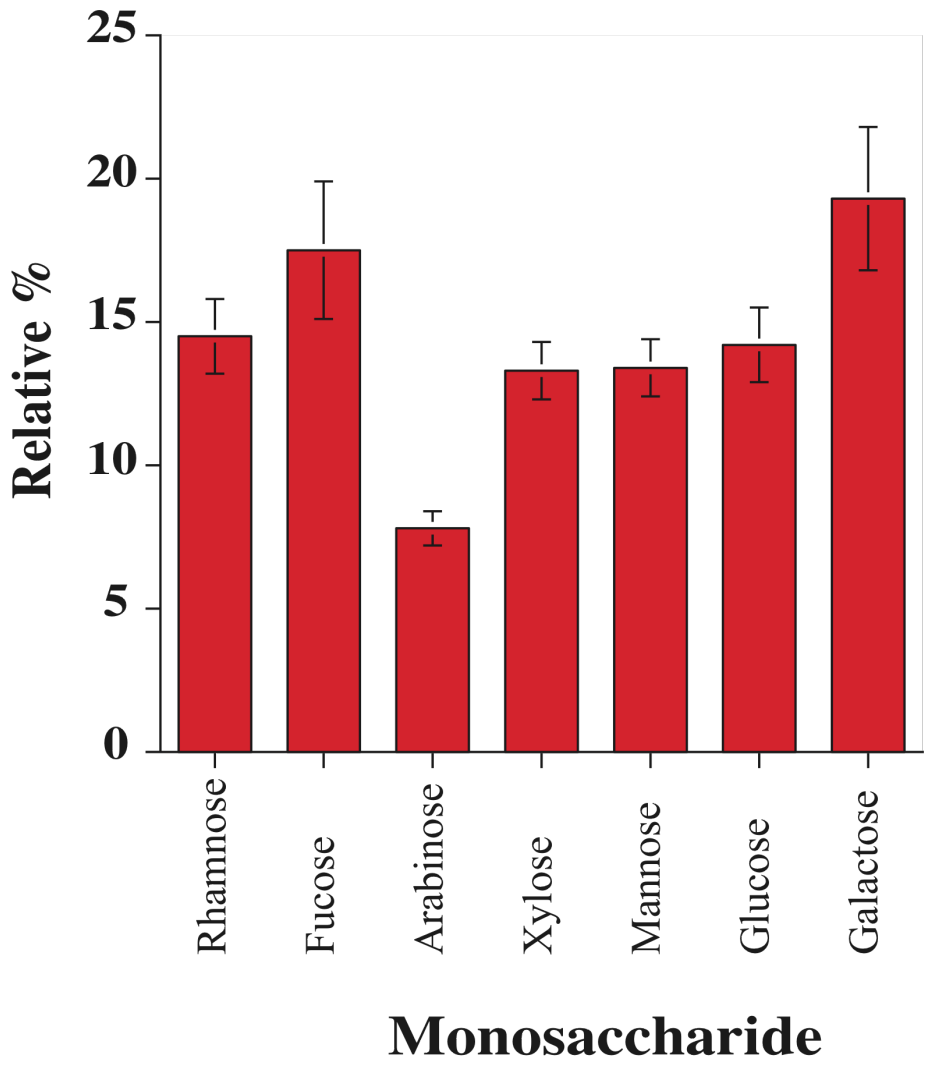


Acid hydrolysis followed by
Monosaccharide analyses
yields 7 major neutral sugars
that represent 10-20% of HMWDOC
in surface water

Acid
hydrolysis



Relative abundance of sugars in HMWDOC

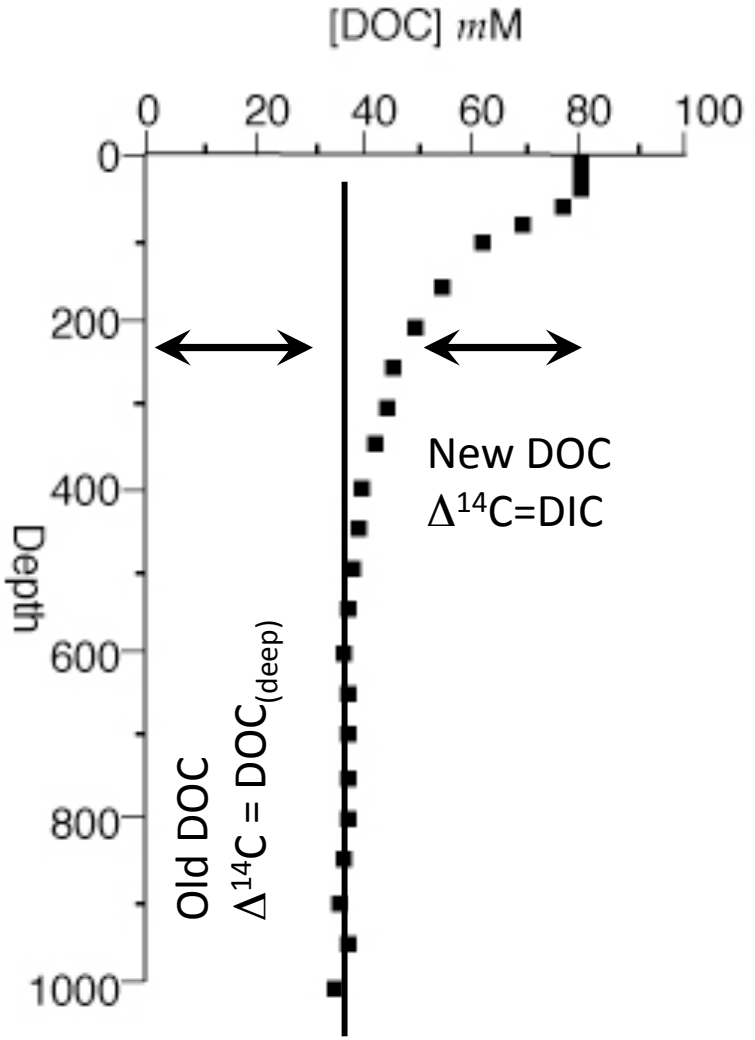


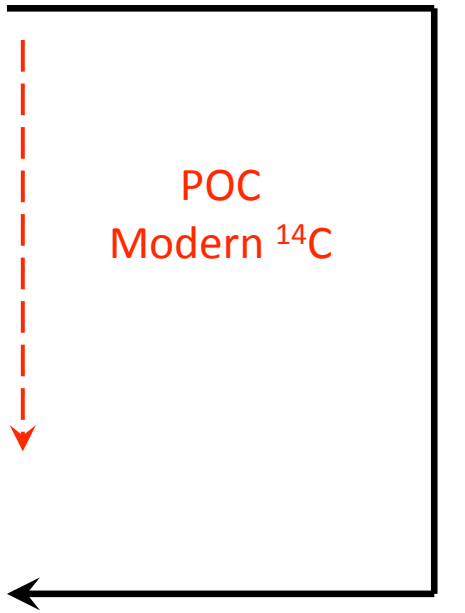
A good number of HMWDOM samples have been analyzed after hydrolysis for simple sugars or monosaccharides. In every case, hydrolysis yields seven major neutral sugars. Samples include HMWDOM from the North Pacific, North and South Atlantic, near shore, estuary, and open ocean water; surface and deep.

The mixture of neutral sugars found in HMW-DOM is not typical of polysaccharides, which tend to have a narrower range of sugars, and a few dominant sugars. HMWDOM has a large number of sugars in about equal amounts.

The small range in relative % composition suggests that these sugars are linked into a common macromolecular structure, and is not a mixture that can vary spatially and temporally.

Sequestration of DOC in the deep sea





Deep water formation
Older ^{14}C

Radiocarbon (Δ14C)analyses of HMWDOC carbohydrates in surface water

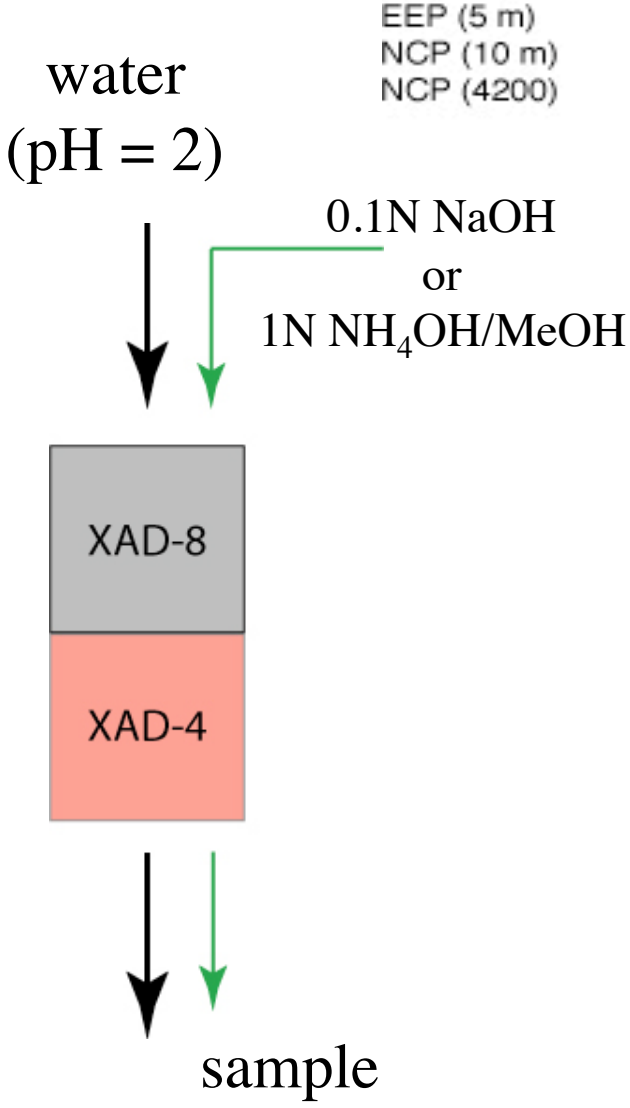
Sample	Hawaii	NPSG
DIC	72±7‰ (n=4)	89±7‰*
Glucose	47, 58	79
Galactose	67	103
Mannose	65	99
Xylose	52, 58	94
Arabinose	63	ND
Fucose	49, 52	69
Rhamnose	40, 57	57
Average sugar	56±6‰	89±13‰

*data from Ellen Druffel

Enrichment of $\Delta^{14}C$ in deep sea HMWDOM carbohydrate

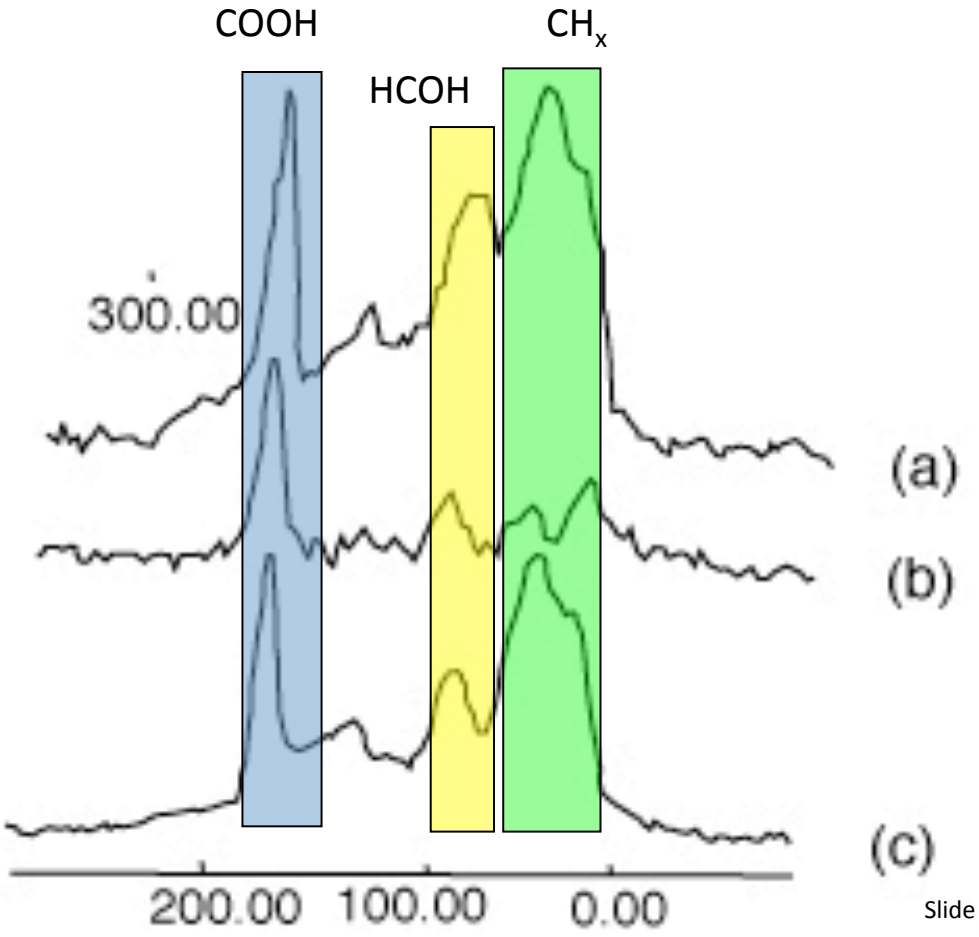
	DIC$\Delta^{14}C$(‰)	HMWDOC$\Delta^{14}C$	sugars $\Delta^{14}C$
Surface	89±7	46	84±17
600m	-155±7	-259	-122±18
3600m	-240±7	-381	-145±23

Hydrophobic (XAD collected) DOM in North Pacific Ocean

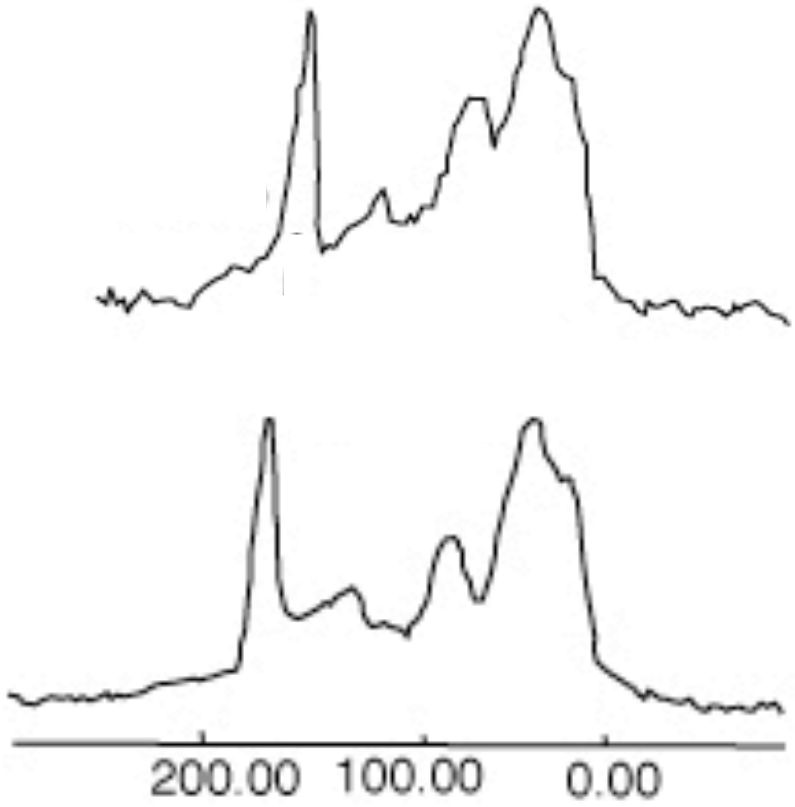


Pacific Ocean samples

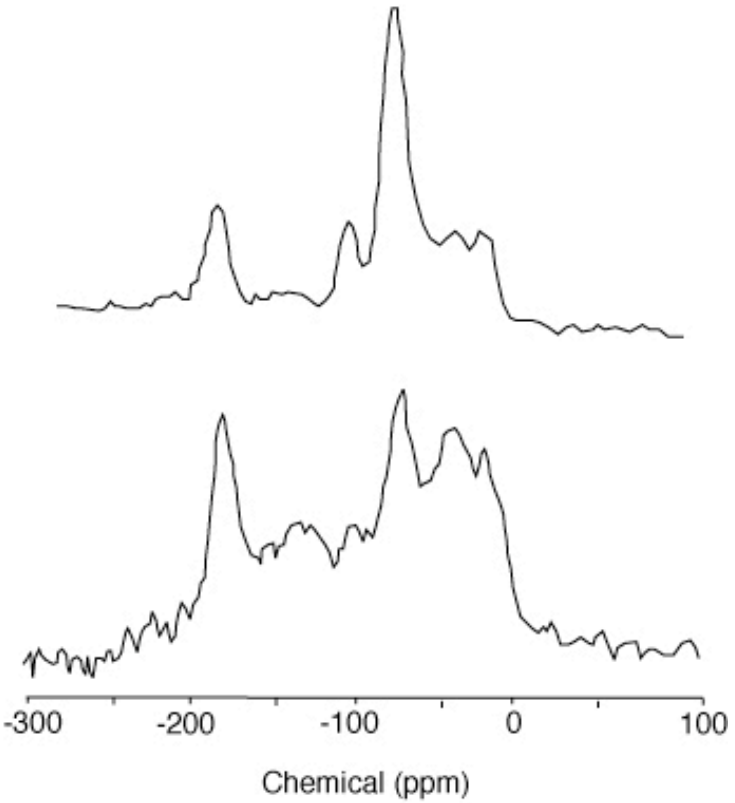
EEP (5 m)	51	36	1.6	45	18	18	15	5	-22
NCP (10 m)	48	46	1.2	44	20	20	16	ND	-22
NCP (4200)	50	43	1.2	46	17	19	15	3	-22



A comparison of ^{13}CNMR spectra of XAD and ultrafiltration collected DOM in surface (top) and 4200 m (bottom) Pacific seawater



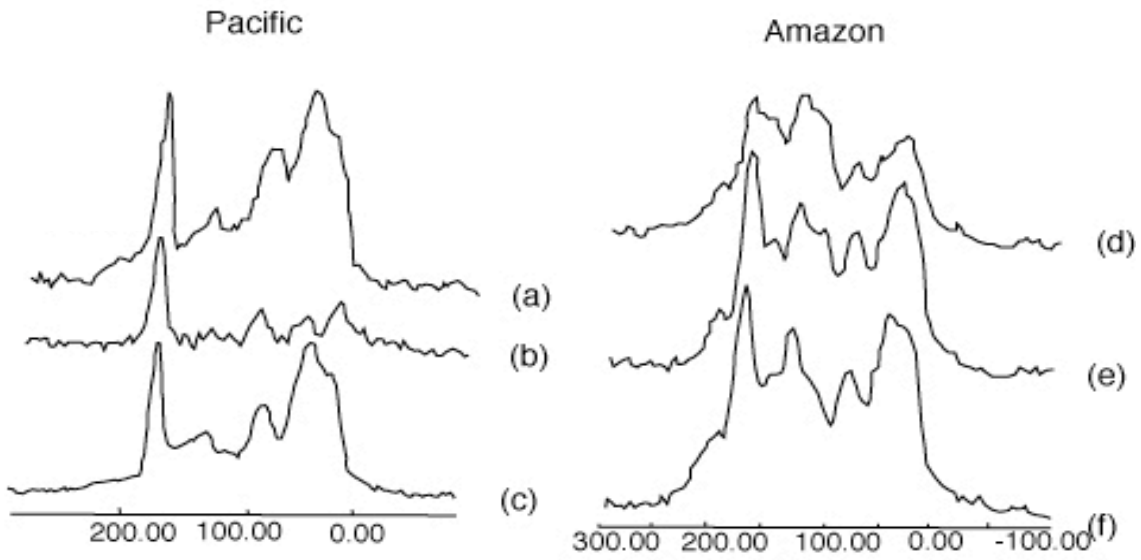
XAD DOM



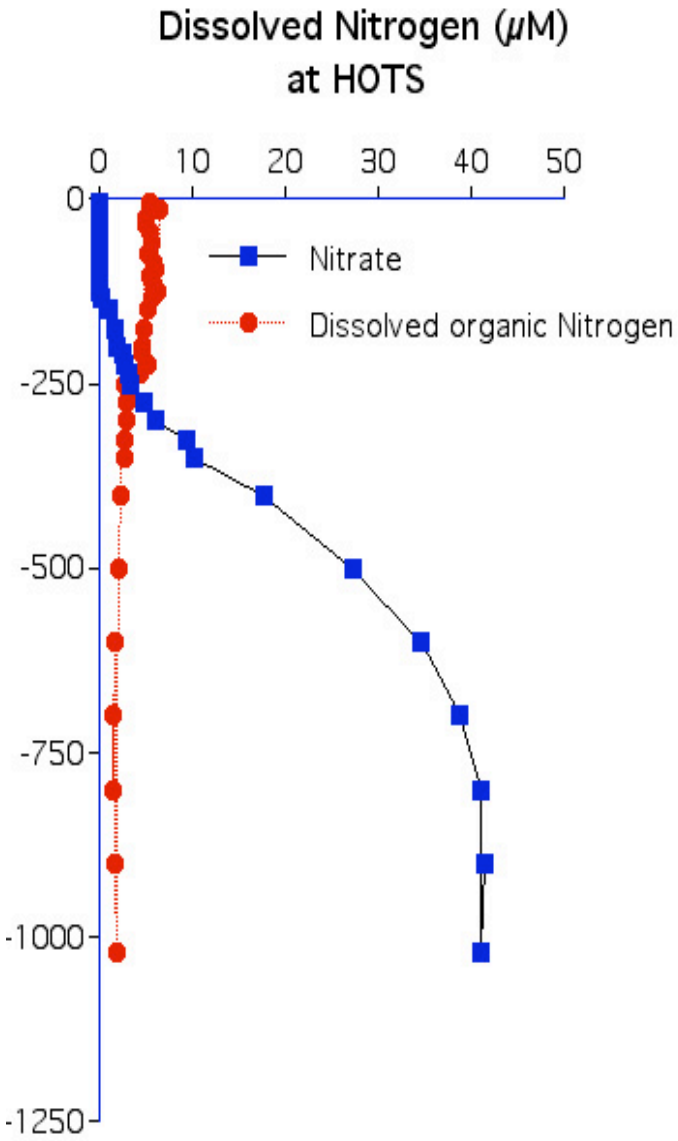
HMWDOM

The composition of humic substances in the Amazon River and North Pacific Ocean

Sample	%OC	C/N	H/C	CHx	HCOH	C=C	COO	HC=O	C-13
				Amazon River samples					
Rio Negro FA	52	85	0.95	32	15	33	16	4	-29.0
Rio Negro HA	52	58	0.79	27	9	41	18	6	-29.2
main branch FA	51	60	1.00	32	13	31	17	7	-29.0
				Pacific Ocean samples					
EEP (5 m)	51	36	1.6	45	18	18	15	5	-22
NCP (10 m)	48	46	1.2	44	20	20	16	ND	-22
NCP (4200)	50	43	1.2	46	17	19	15	3	-22

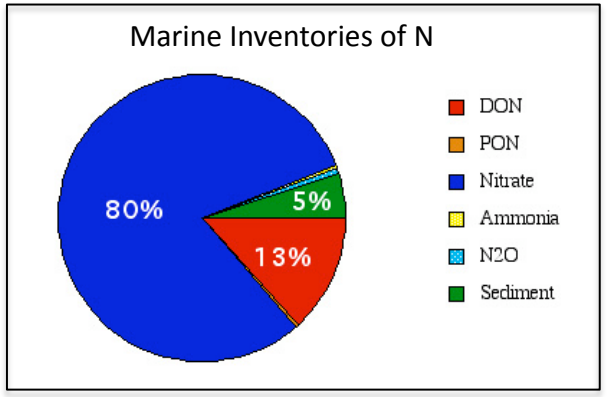


Dissolved Organic Nitrogen

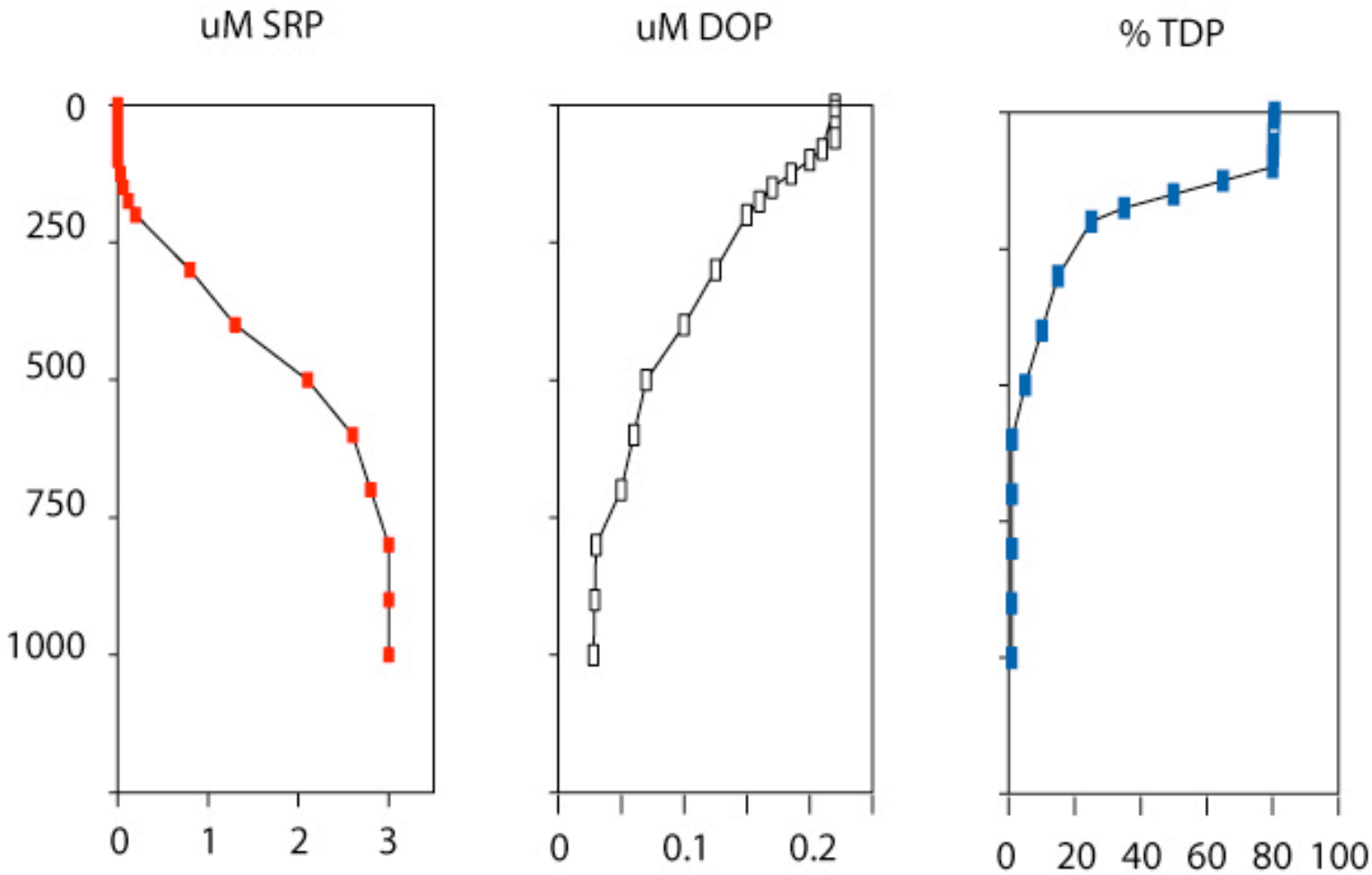


Total Dissolved N (TDN) = DON+DIN

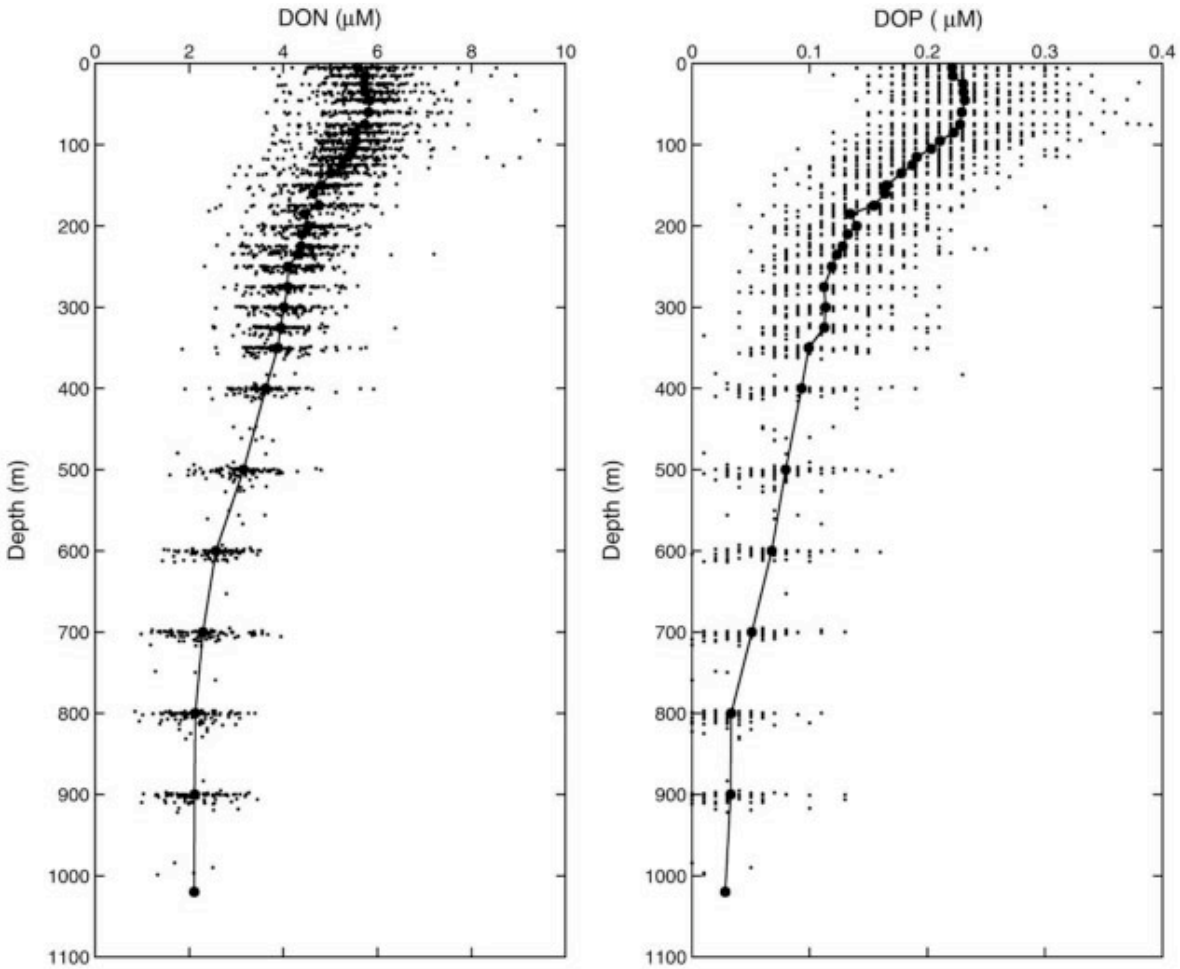
(where DIN = ammonia + nitrate + nitrite)



Soluble reactive phosphorus (SRP), dissolved organic phosphorus (DOP) and DOP as % total dissolved phosphorus (DOP/DOP+SRP) at Station ALOHA



Temporal variation in DON and DOP at station ALOHA



DIN vs DIP, and TDN vs TDP at station ALOHA

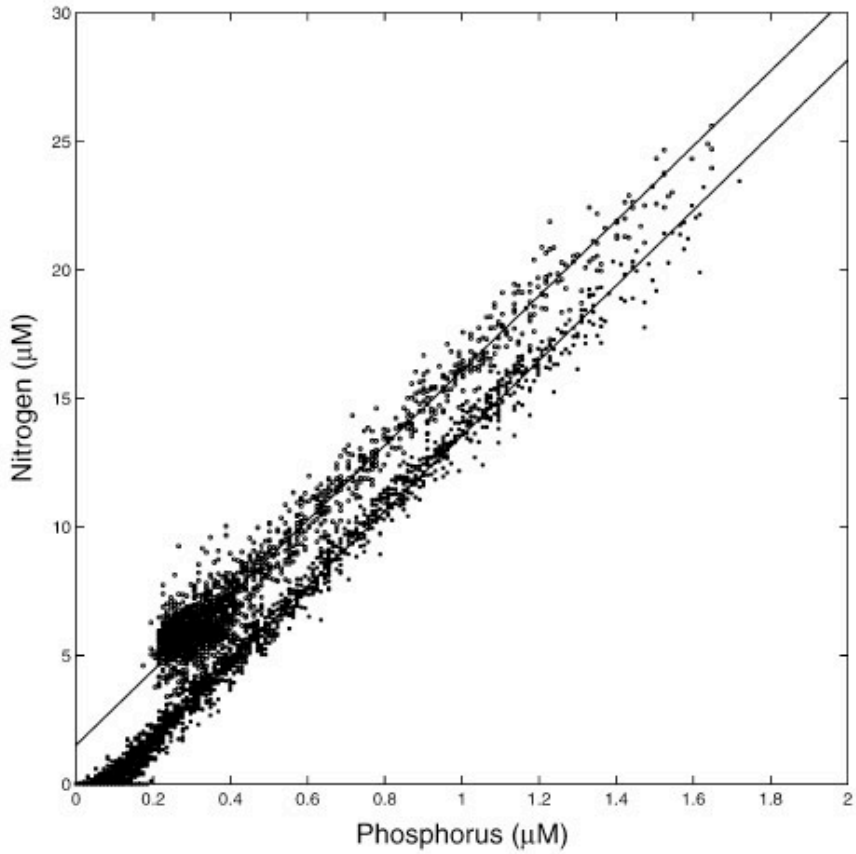
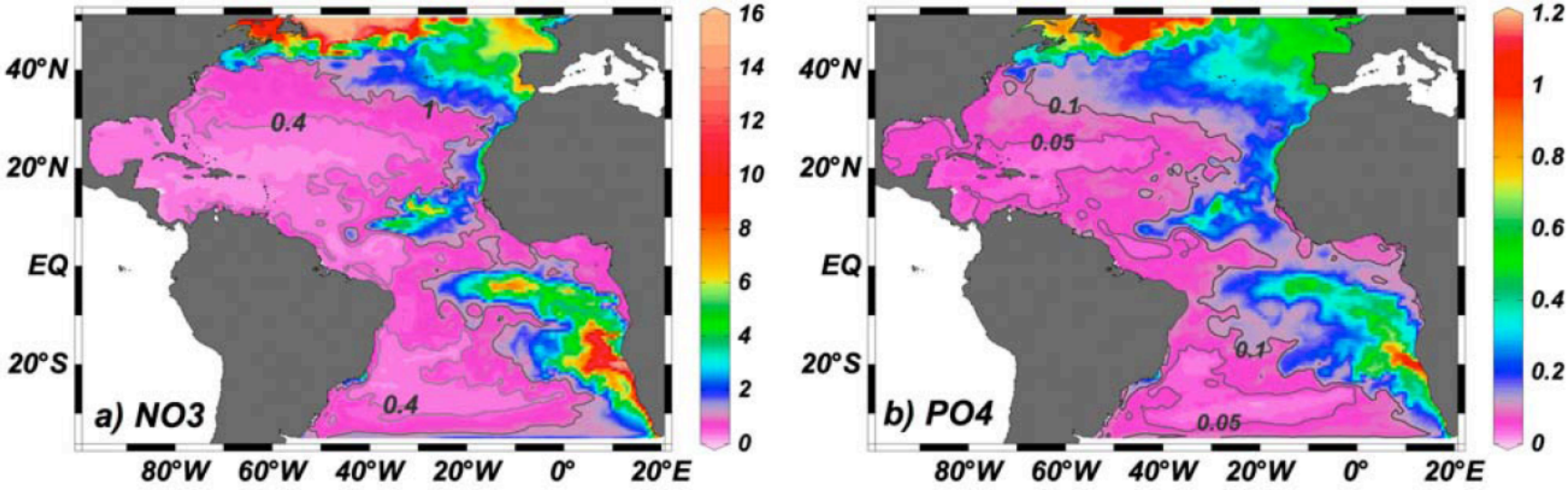
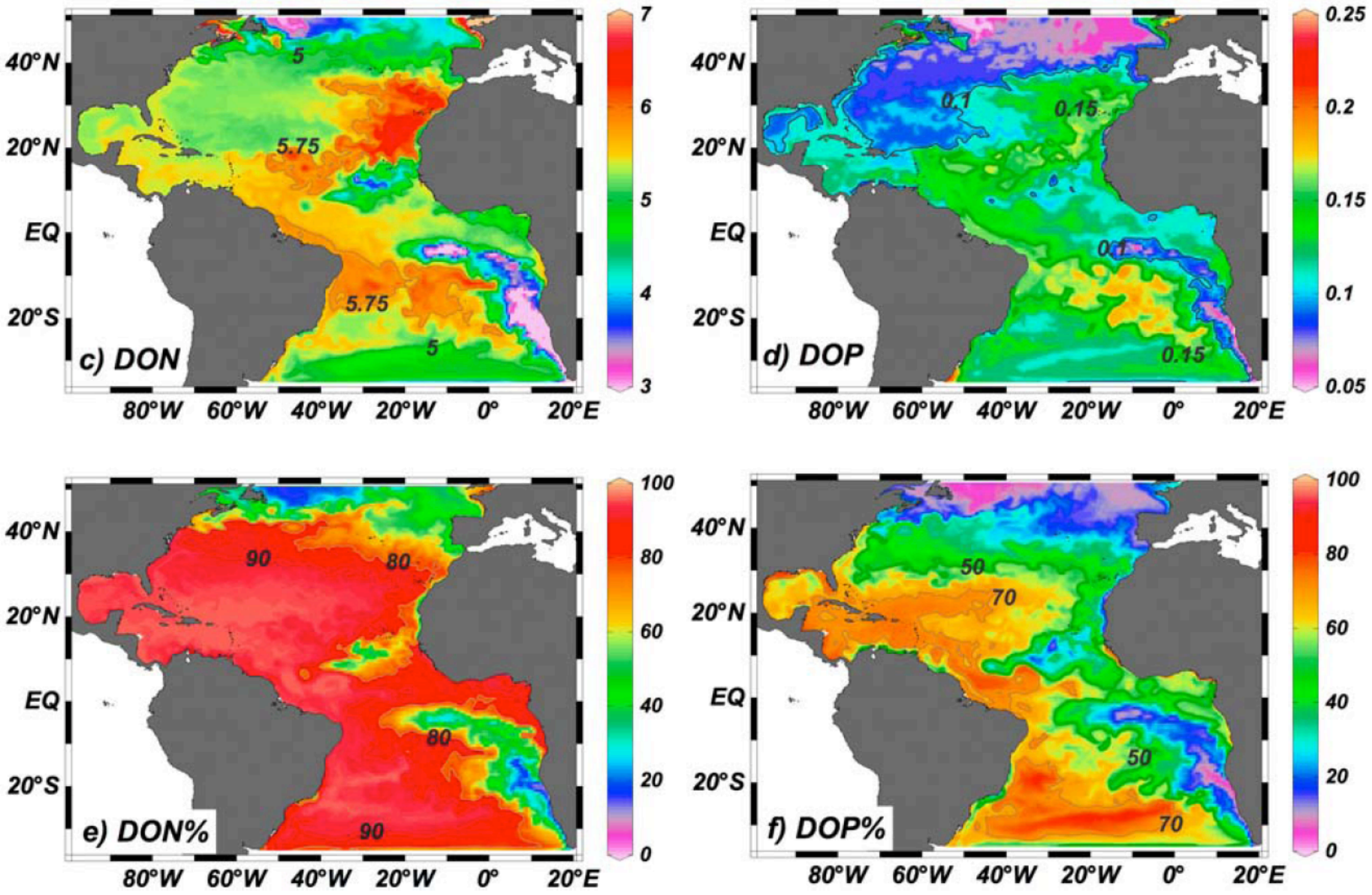


Fig. 10. Nitrogen versus phosphorus correlations for samples collected in the upper 0–400 m of the water column at Sta. ALOHA during the period Oct. 1988 to Dec. 1997. The bottom line is for nitrate plus nitrite (N + N) versus soluble reactive phosphorus (SRP) concentrations and the top line is for total dissolved nitrogen (TDN) versus total dissolved phosphorus (TDP). Model II linear regression analyses: N + N (µM) = 14.62 [14.58 to 14.66] SRP (µM) − 1.08 [− 1.10 to − 1.06], $r = 0.996$, $n = 3299$ and TDN (µM) = 14.57 [14.45 to 14.69] TDP (µM) + 1.50 [1.44 to 1.56], $r = 0.981$, $n = 2060$. Values in brackets indicate the 95% confidence intervals of the respective slope and intercept values.

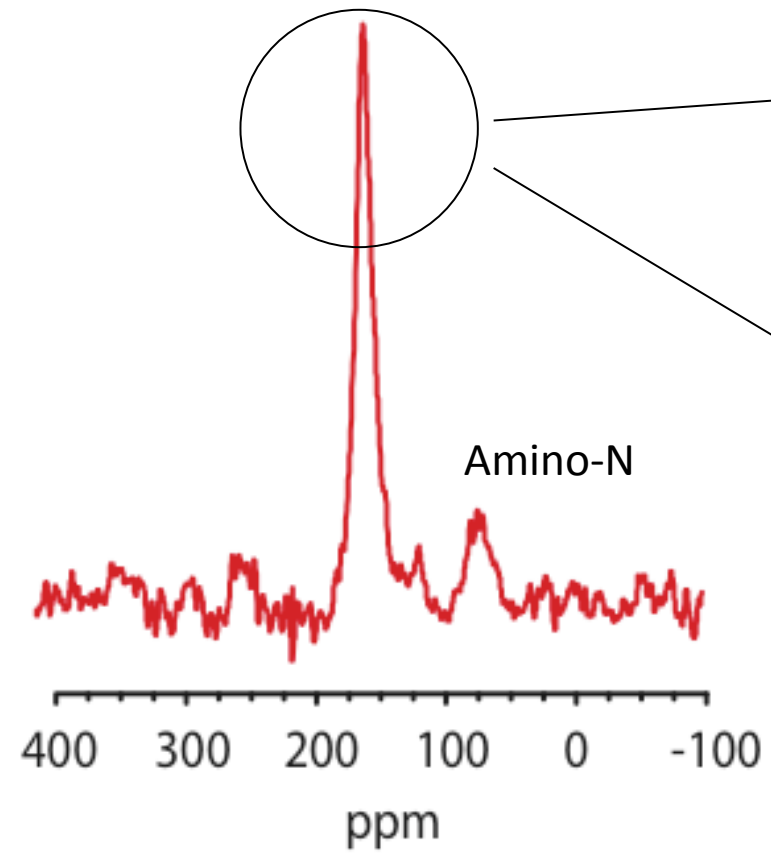

40°N
20°N
EQ
20°S
80°W
60°W
40°W
20°W
0°
20°E
a) NO3
0.4
1
0.4
16
14
12
10
8
6
4
2
0
b) PO4
0.1
0.05
0.1
0.05
1.2
1
0.8
0.6
0.4
0.2
0



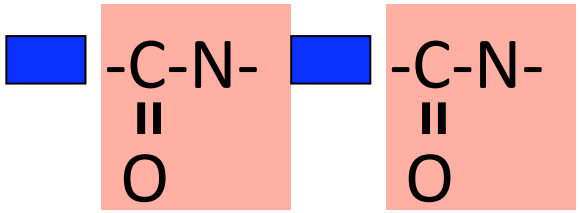
Torre-Valdes et al. GBC 2009

What else is in HMWDOM ?

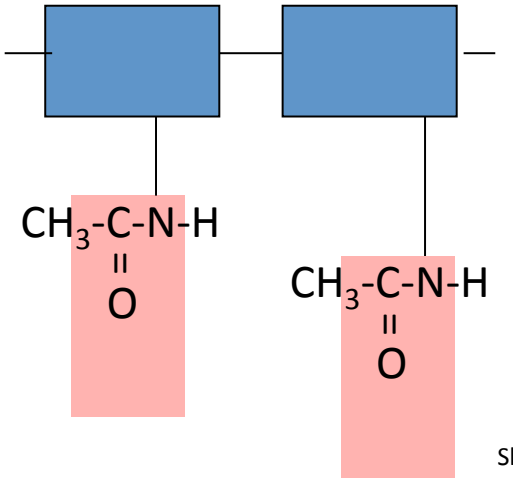
^{15}N NMR



proteins

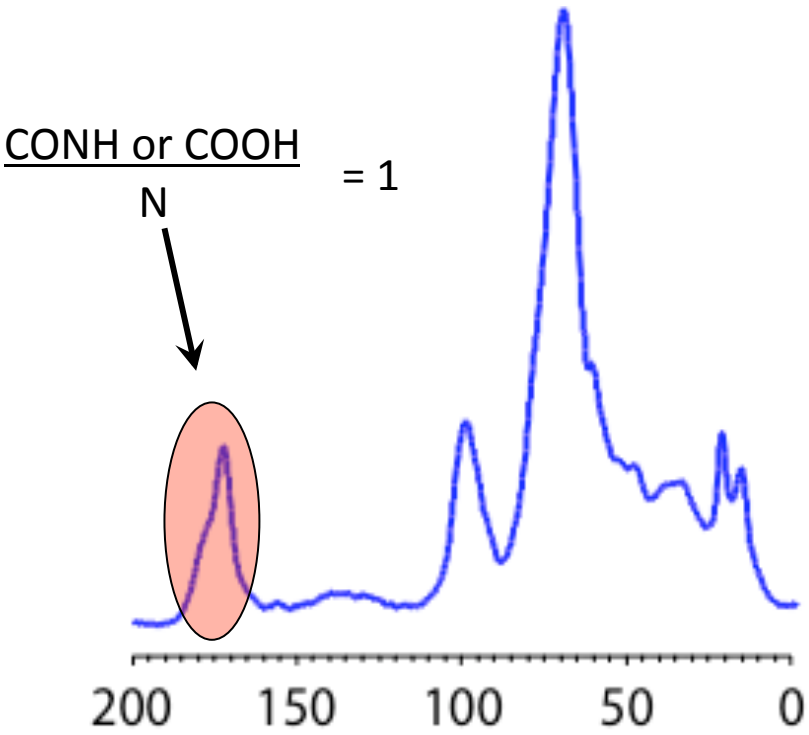


amino sugars

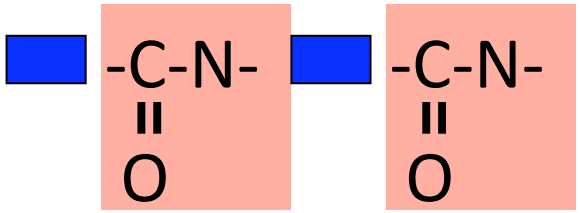


McCarthy et al, 1997; Aluwihare et al. 2005

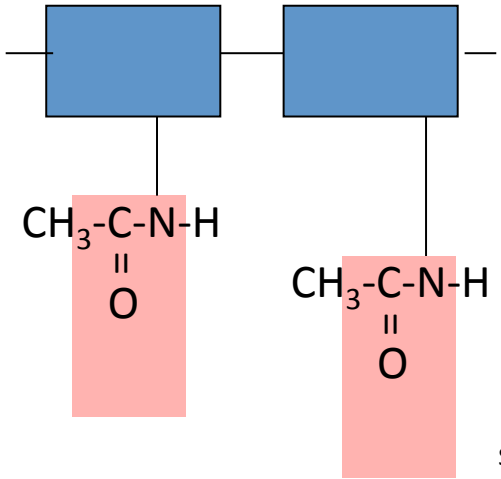
C/N = 15, if all N is amide, then CON should be about 7% of the total C, which it is….



proteins

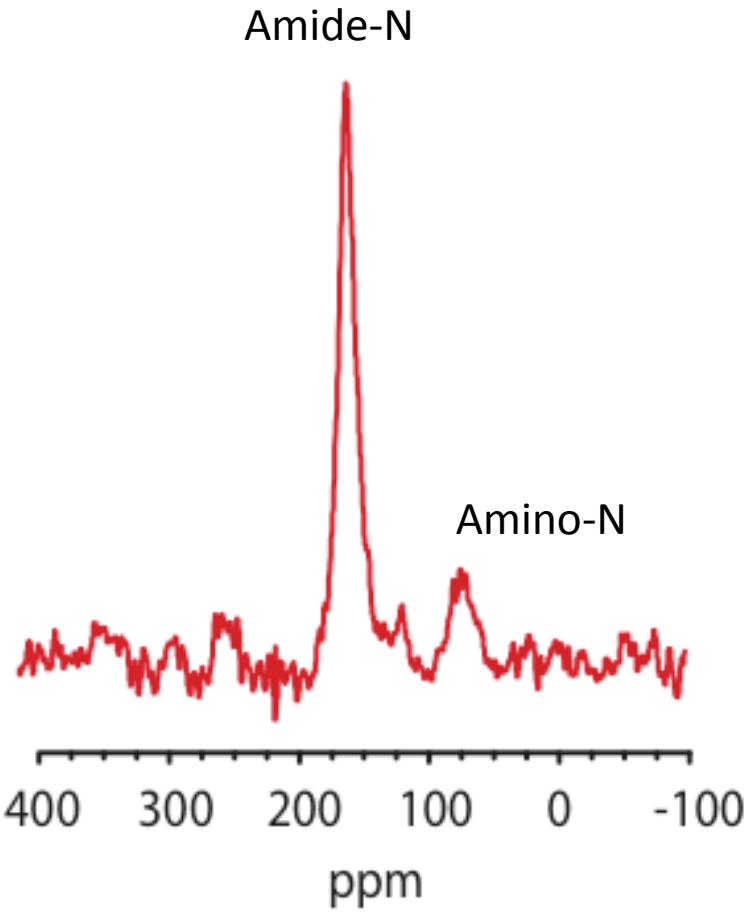


amino sugars

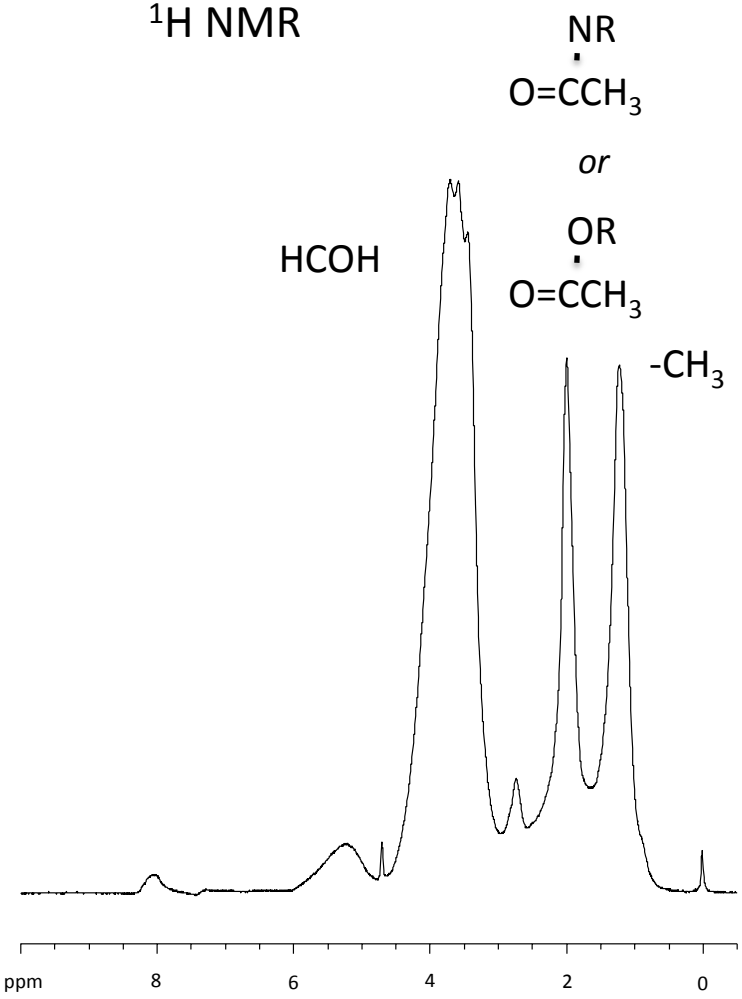


What else is in HMWDOM ?

^{15}N NMR

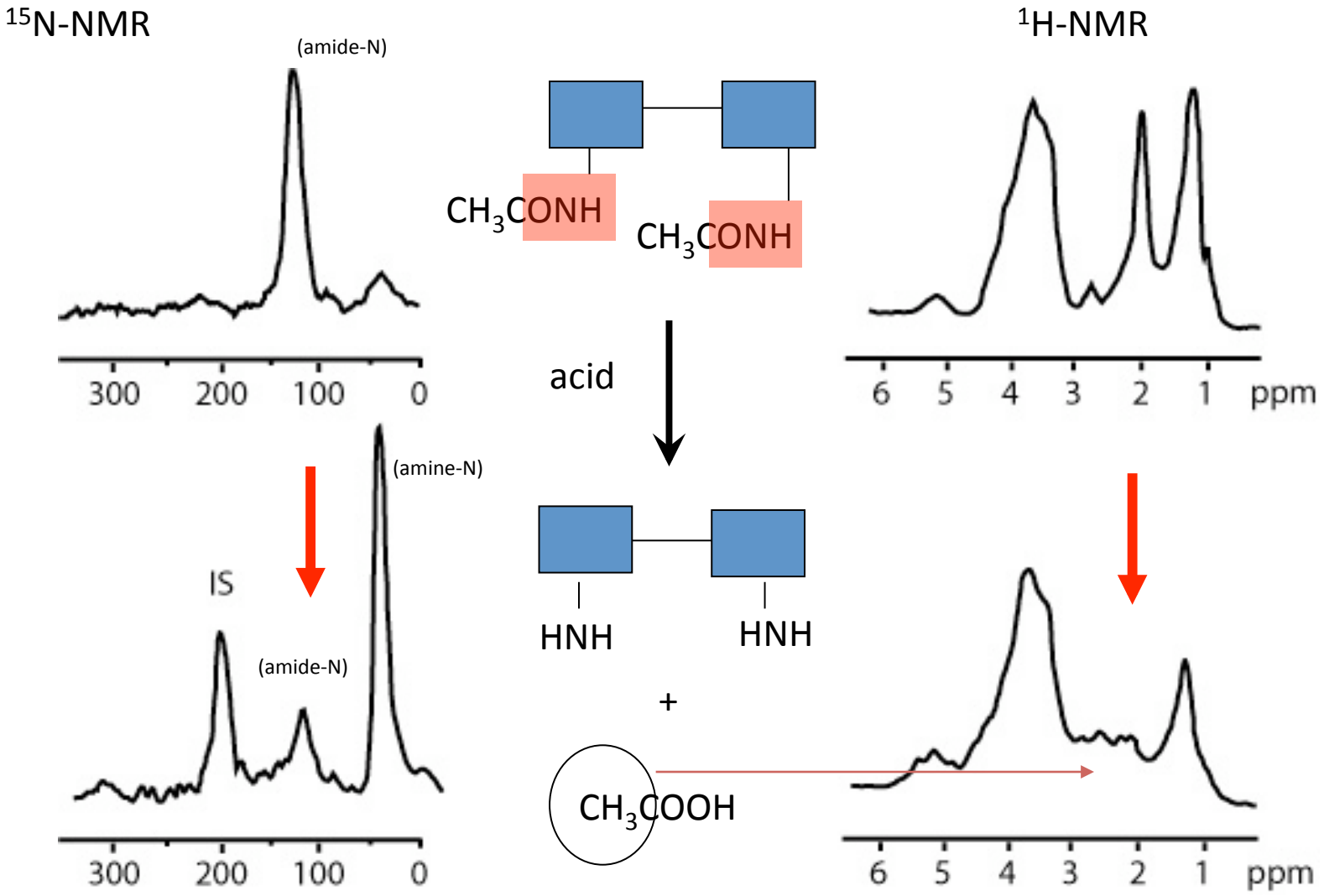


^{1}H NMR



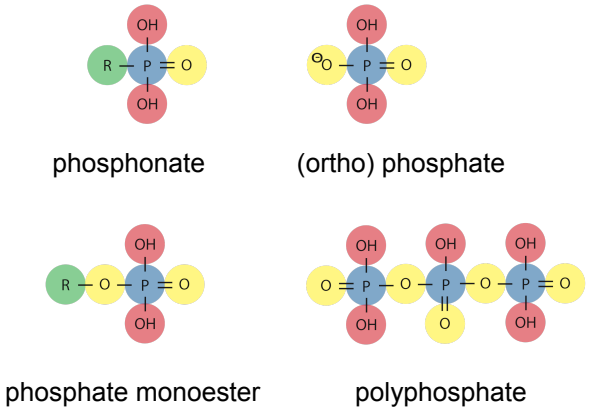
McCarthy et al, 1997; Aluwihare et al. 2005

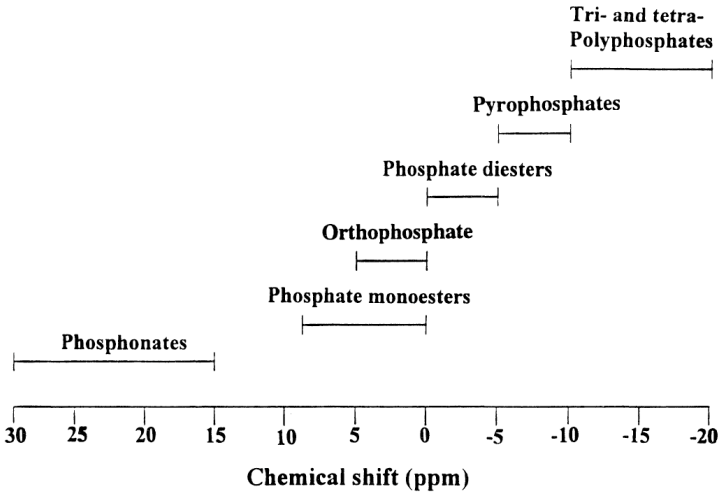
Is a large fraction of HMWDOC and HMWDON from amino sugars?



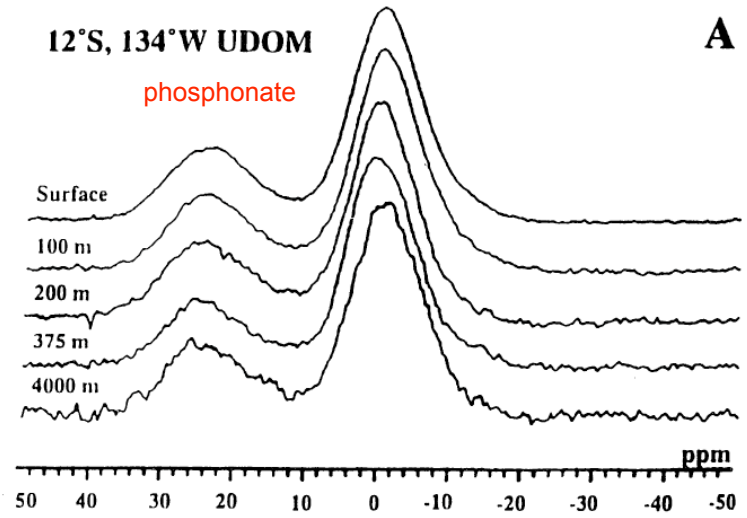
Composition of HMWDOP

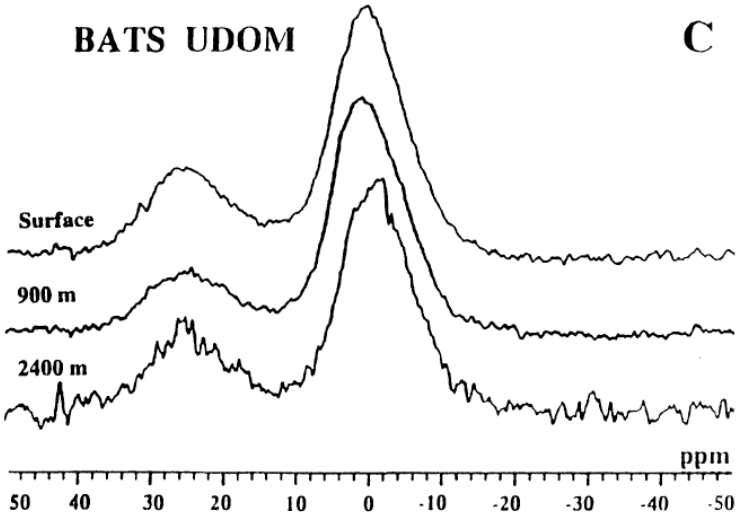
Organic phosphorus nomenclature





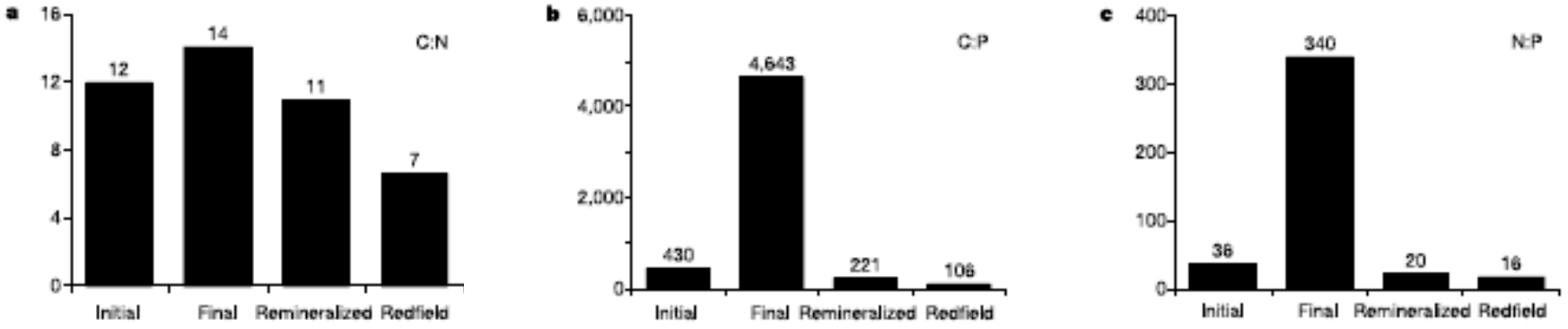
phosphate mono- & di-esters

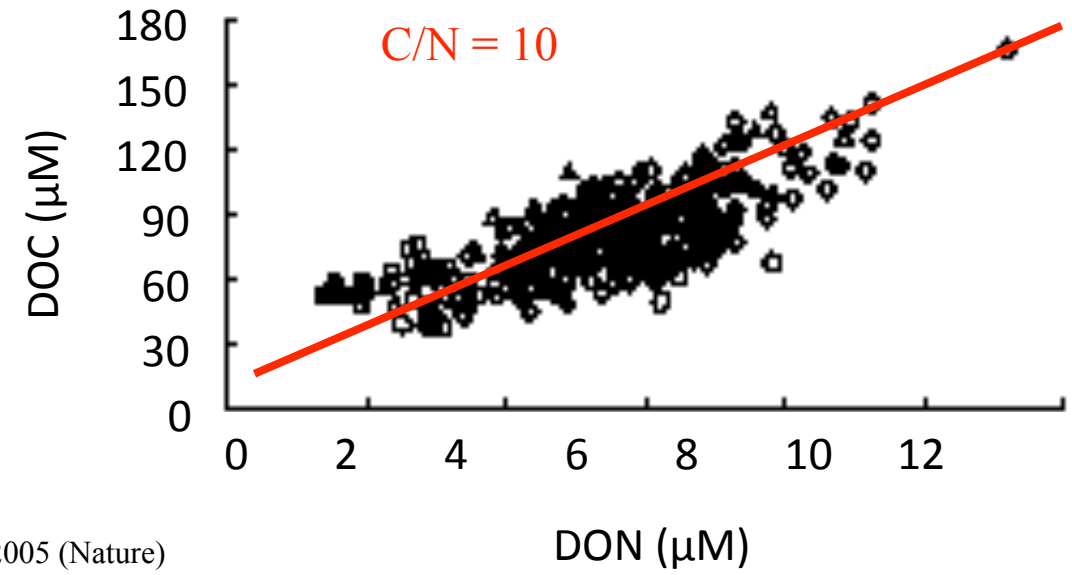




Kolowith et al., L&O 2001

What is the degradation stoichiometry of recalcitrant DOC ?

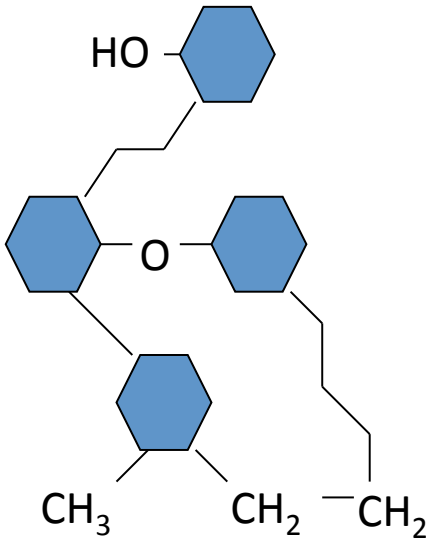




Hopkinson and Vallino 2005 (Nature)

Composition, reactivity, flux and distribution of DOM

Non-reactive DOM



Aliphatic or "Humic" substances

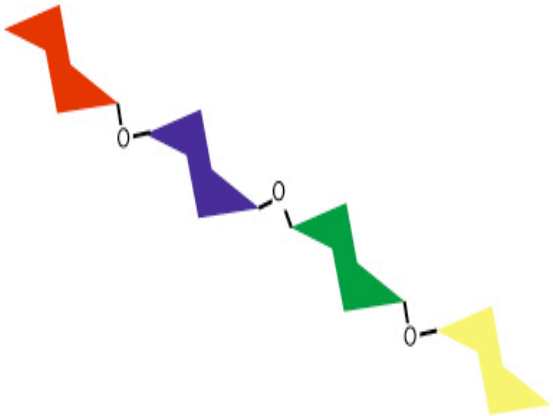
Concentration 40 µM

Inventory = 640 GT C

$\Delta^{14}C$= -400 to -600‰

Annual flux = 0.1 GTC

Semi-reactive DOM



Biopolymers

(polysaccharides, proteins)*

Concentration 0-40 µM

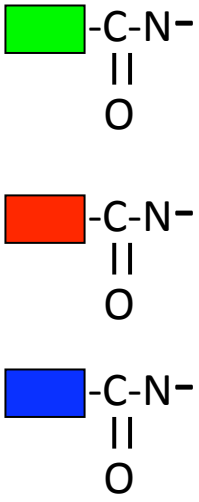
Inventory = 10-20 GT C

$\Delta^{14}C$= modern (DIC)

Annual flux = 10's GT C?

* = 80% of cell C, N

Very reactive DOM



Simple biomolecules

(amino acids, sugars)*

Concentration 1-2 µM

Inventory = 0.1-0.3 GT C

$\Delta^{14}C$= modern (DIC)

Annual flux = 10's GT C?

* = 10-20% of cell C, N

Summary

DOM is isolated from seawater using either adsorption onto hydrophophic resin or ultrafiltration (molecular filtration). Adsorptive techniques selects DOM that has a high affinity for the adsorption media, while ultrafiltration relies on the hydrodynamic diameter of the organic matter.

Adsorptive techniques select for DOM that is depleted in radiocarbon, while ultrafiltration selects for "HMWDOM" that is enriched in radiocarbon.

NMR analysis of HMWDOM shows it to have a remarkably uniform distribution of functional groups that is conserved across ocean basins. Carbohydrates are the dominant biochemical class present in HMWDOM. Chemical hydrolysis Only recovers 10-20% of carbohydrate as neutral sugars, characterized by seven major neutral sugars (rhamnose, fucose, arabinose, mannose, glucose, xylose, galactose). These data suggest that the HMWDOM is a biopolymer with a specific composition.

The old, hydrophobic fraction of DOM is relatively more enriched in aliphatic carbon and highly oxidized, with a high COOH/CHx ratio.

Dissolved organic nitrogen (DON) and phosphorus (DOP) have high concentrations in surface water, and lower concentrations at depth.

HMWDON occurs primarily as amide-N, linked through N-acyl amino sugars, while HMWDOP occurs primarily phosphate esters and some phosphonate.