The Abdus Salam
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International Atomic Energy Agency

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Workshop and Conference on Biogeochemical Impacts of Climate and Land-Use Changes on Marine Ecosystems

2 - 10 November 2009

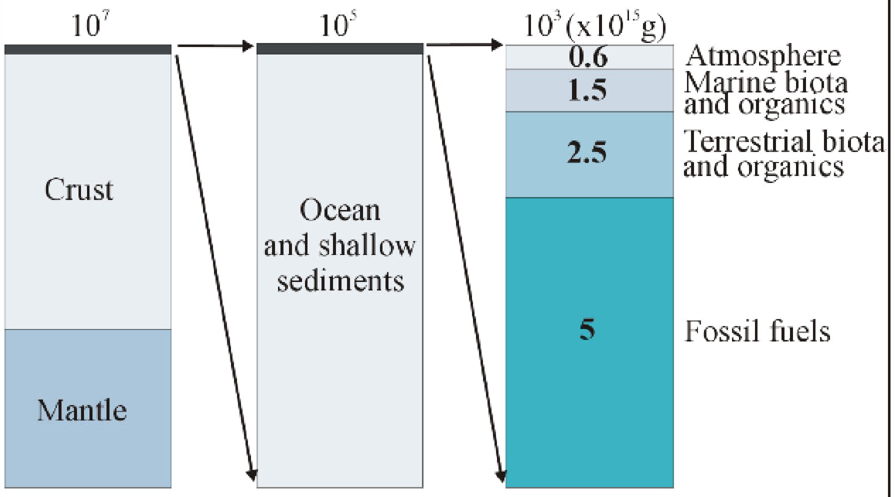
The Reduced Shakespeare Marine Nitrogen Cycle

D. Capone
USC
U.S.A.

The Reduced Shakespeare Marine Nitrogen Cycle

- N = 7, isotopes 13,14,15
- 99% of N on earth- N_2
- 6 ppb in crustal rocks
- Rutherford, 1772 (Priestly)
    - Phlogisticated air
- Nitre- greek for nitrate salt
- Azote (fr)

Carbon budget



10^7

10^5

10^3 ($x10^{15}$g)

Crust

Mantle

Ocean and shallow sediments

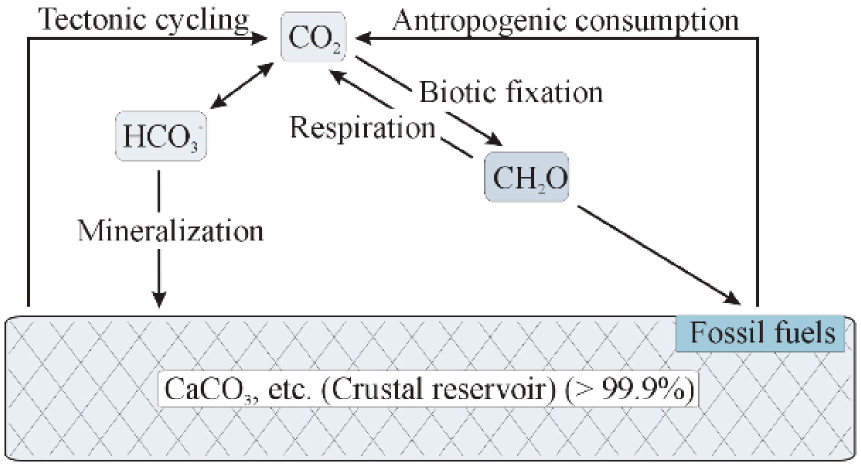
0.6 Atmosphere

1.5 Marine biota and organics

2.5 Terrestrial biota and organics

5 Fossil fuels

Carbon cycle



CO_2

Tectonic cycling

Antropogenic consumption

HCO_3^-

Biotic fixation

Respiration

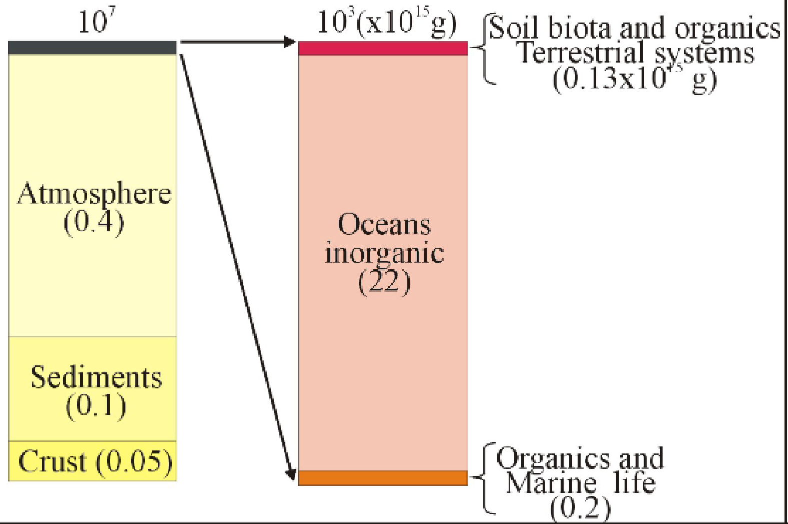
CH_2O

Mineralization

Fossil fuels

$CaCO_3$, etc. (Crustal reservoir) (> 99.9%)

Nitrogen budget



10^7

10^3($x10^{15}$g)

Soil biota and organics Terrestrial systems ($0.13x10^{15}$ g)

Atmosphere (0.4)

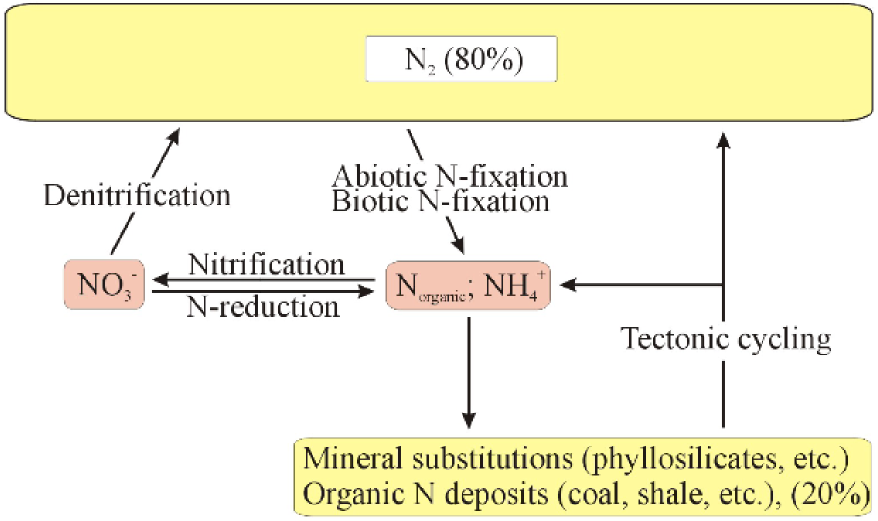
Sediments (0.1)

Crust (0.05)

Oceans inorganic (22)

Organics and Marine life (0.2)

Nitrogen cycle



N_2 (80%)

Denitrification

Abiotic N-fixation
Biotic N-fixation

NO_3^-

Nitrification

N-reduction

$N_{organic}$; NH_4^+

Tectonic cycling

Mineral substitutions (phyllosilicates, etc.)
Organic N deposits (coal, shale, etc.), (20%)

Elemental Composition of bacteria

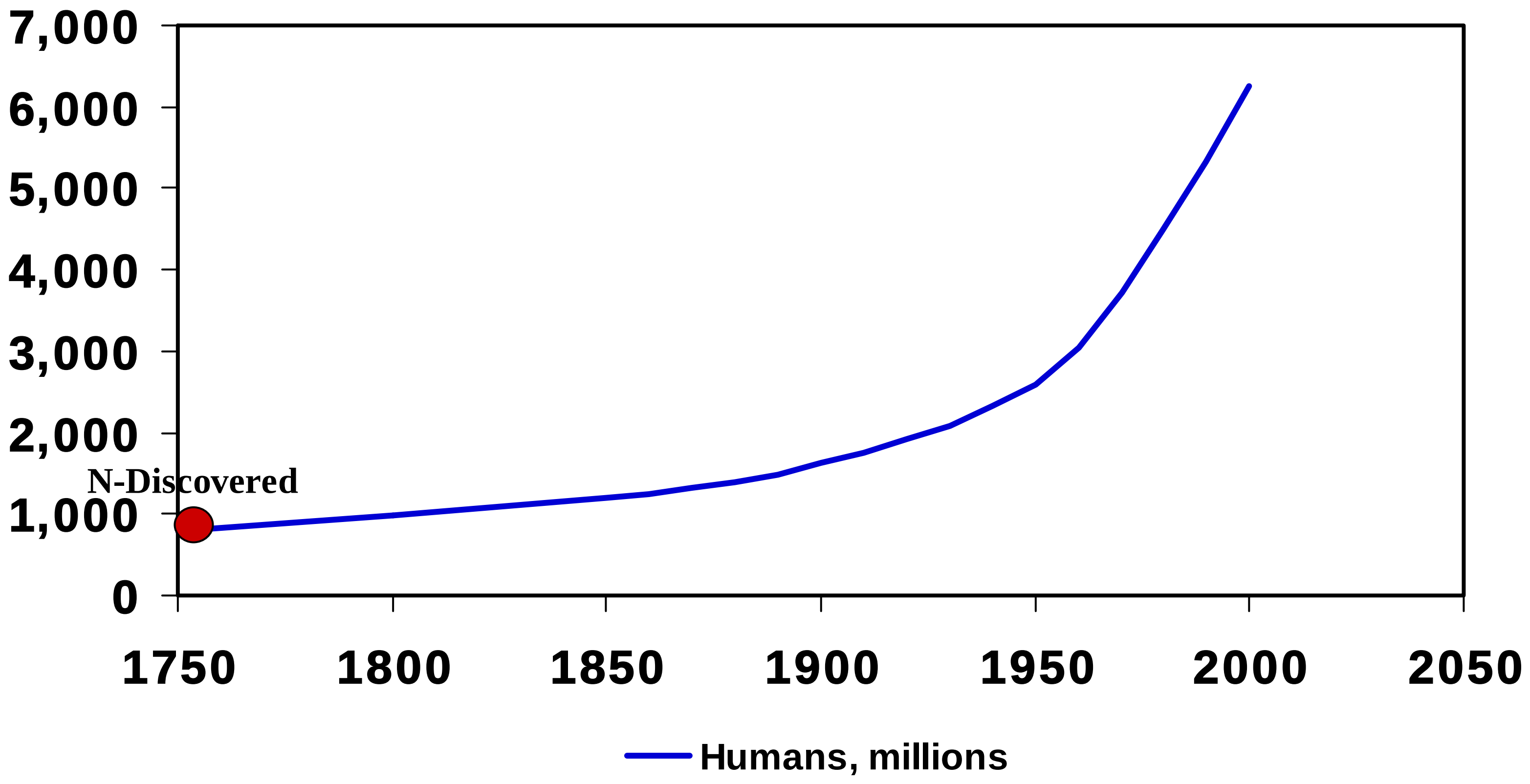
Element	% Dry Wt
C	55
O	20
N	10
H	8
P	3
S	1

Composition of bacteria

Monomer	Elements	Polymer	% Polymer
Amino acids	CHNOS	Proteins	52.4
Organic bases	CNOHP	Nucleic acids	19.9
Sugars	CHON	Polysaccharides	16.6
C-16 acid + P	CHOP	Phospholipids	9.4
		Total	97.3

The History of Nitrogen (from J. Galloway)

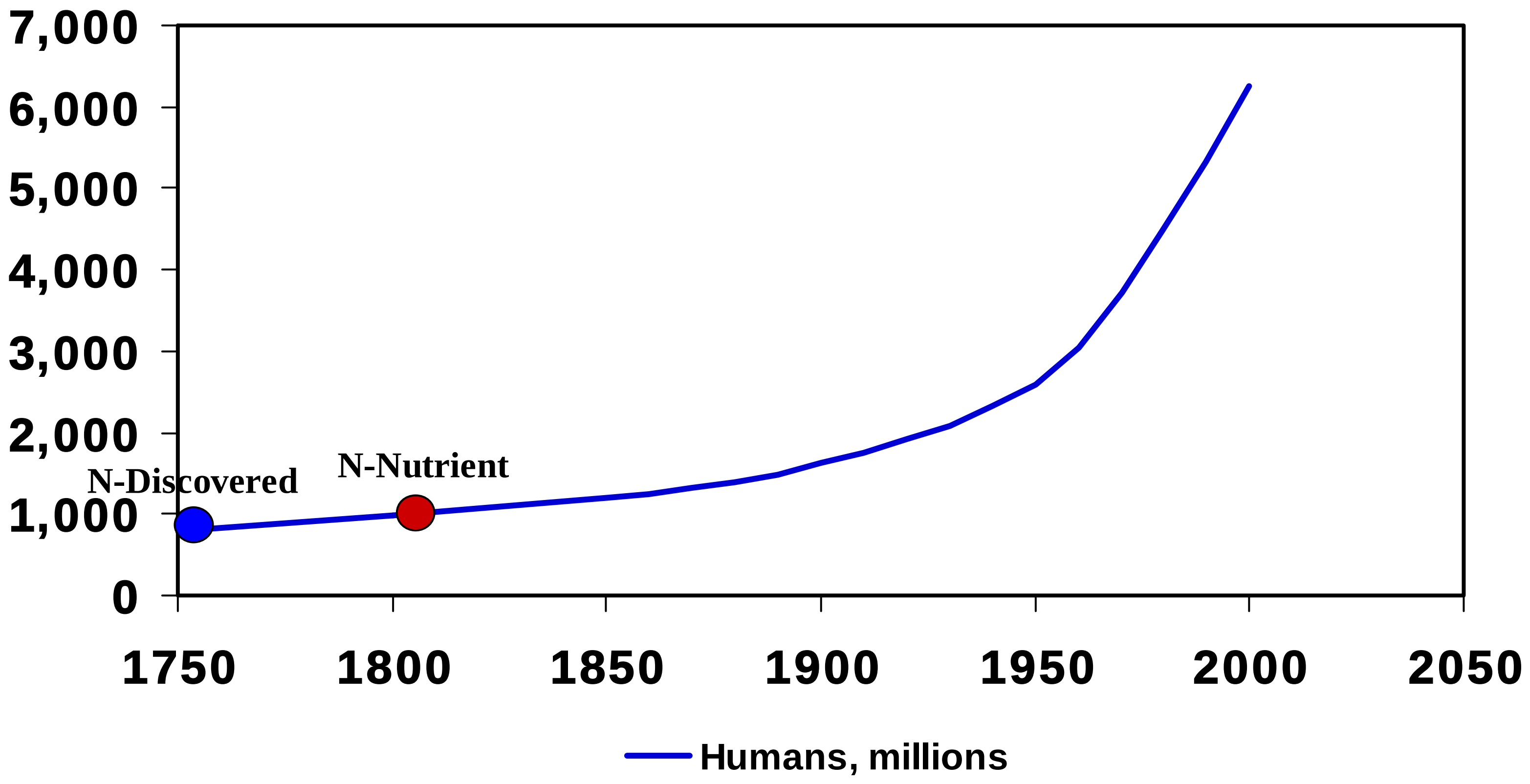
--Discovery—Rutherford/Priestly



Vaclav Smil, 2001. Enriching the Earth

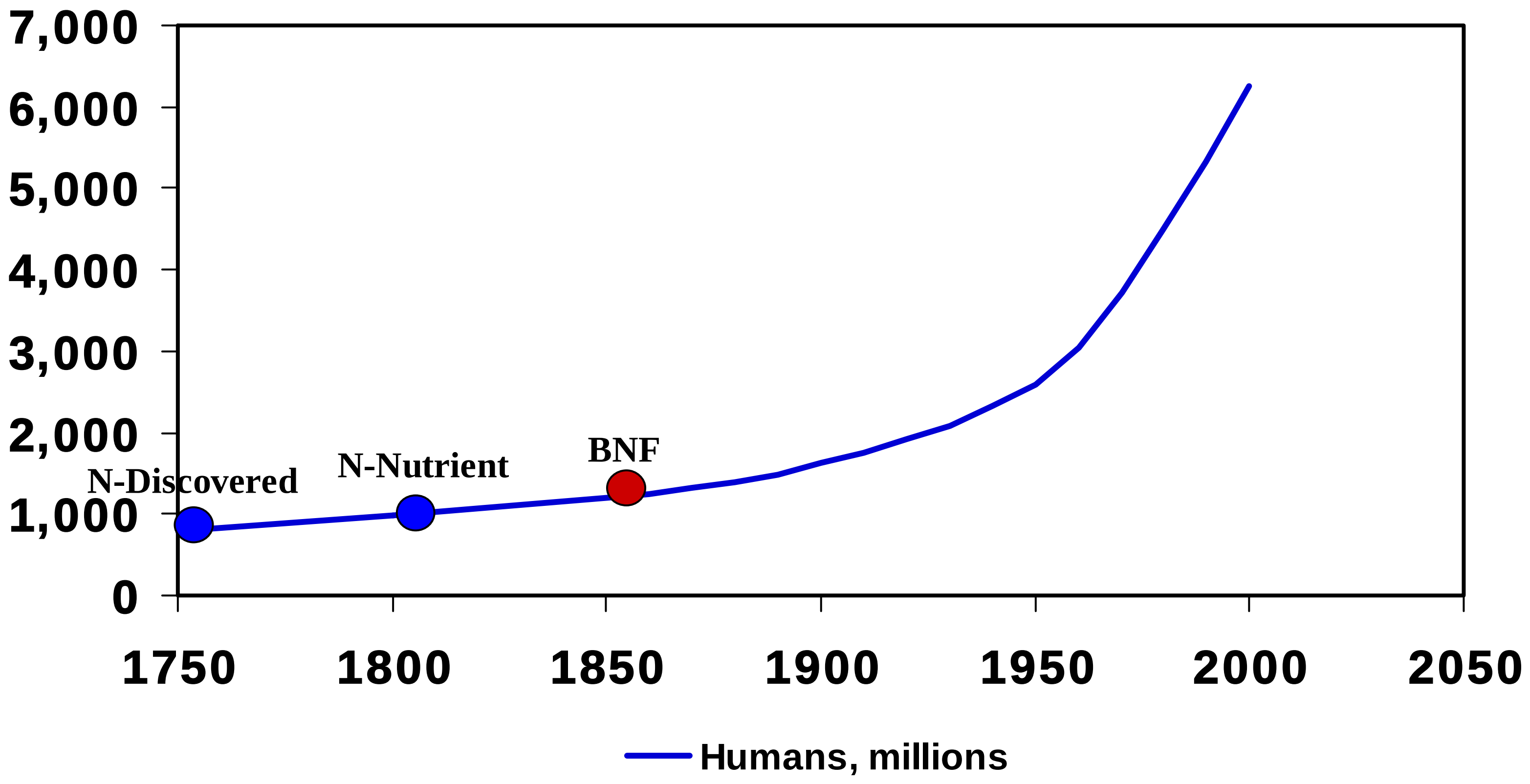
The History of Nitrogen

--N is a Nutrient--



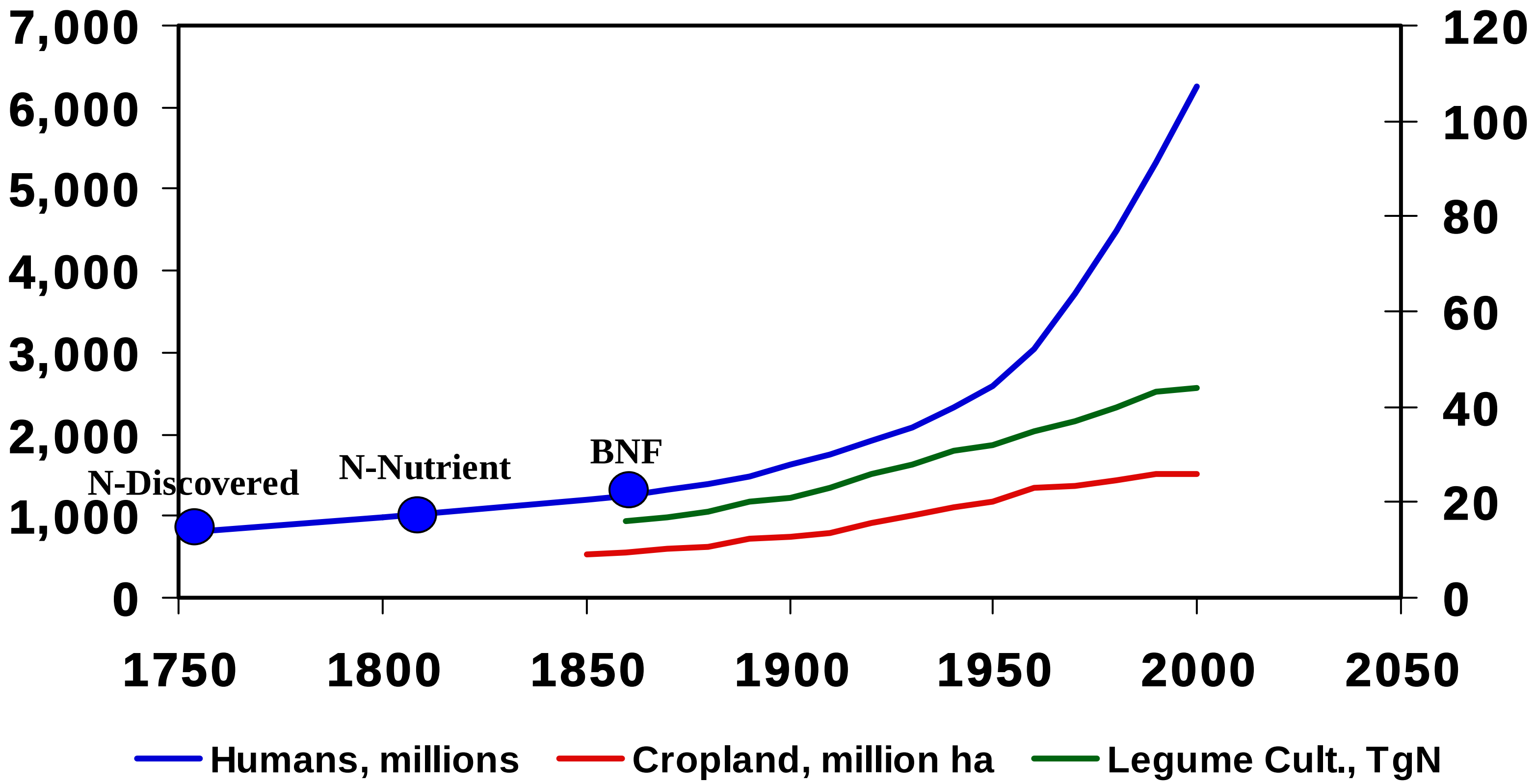
The History of Nitrogen

--Conversion to N_r--



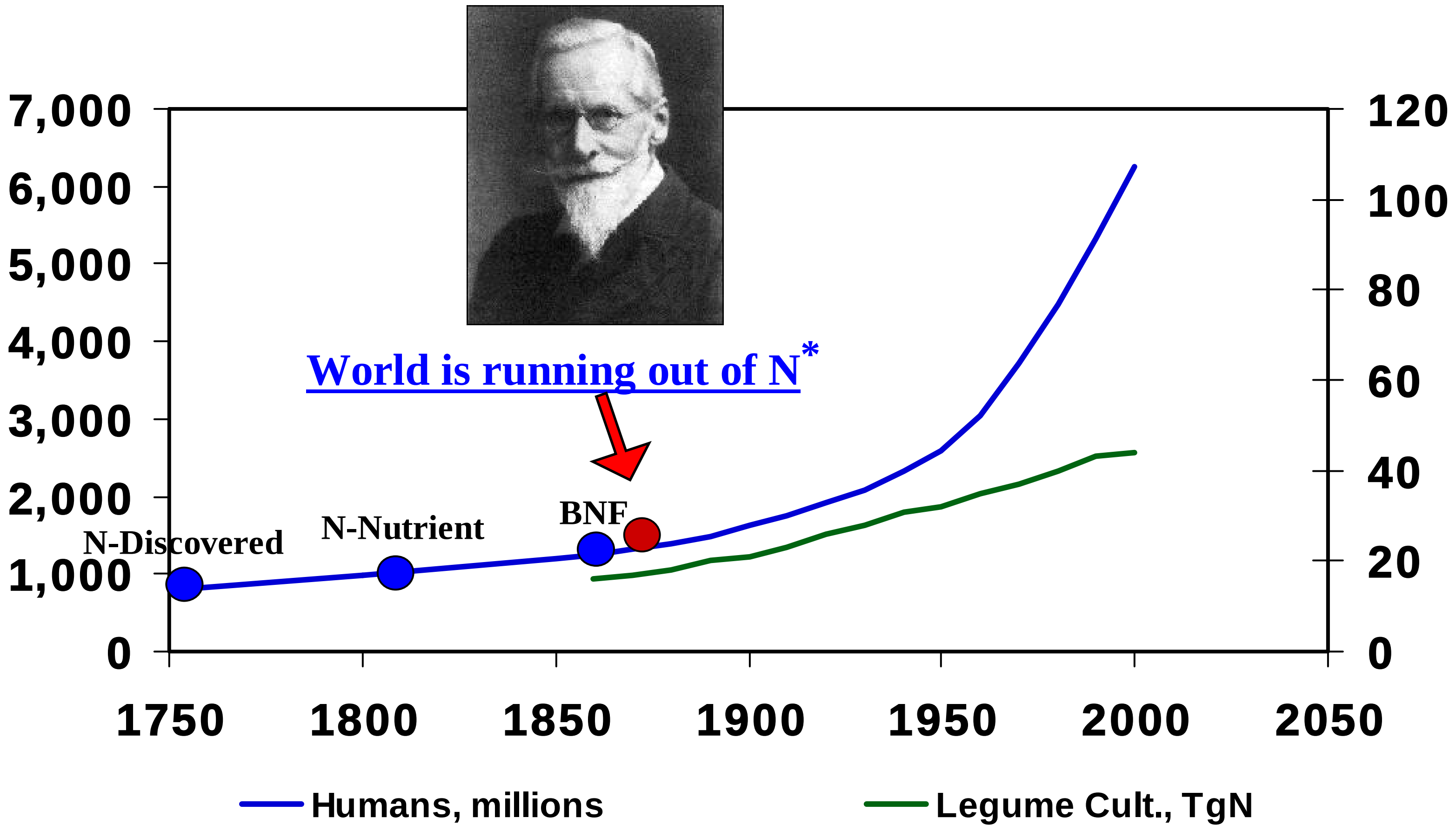
The History of Nitrogen

--Legumes--



The History of Nitrogen

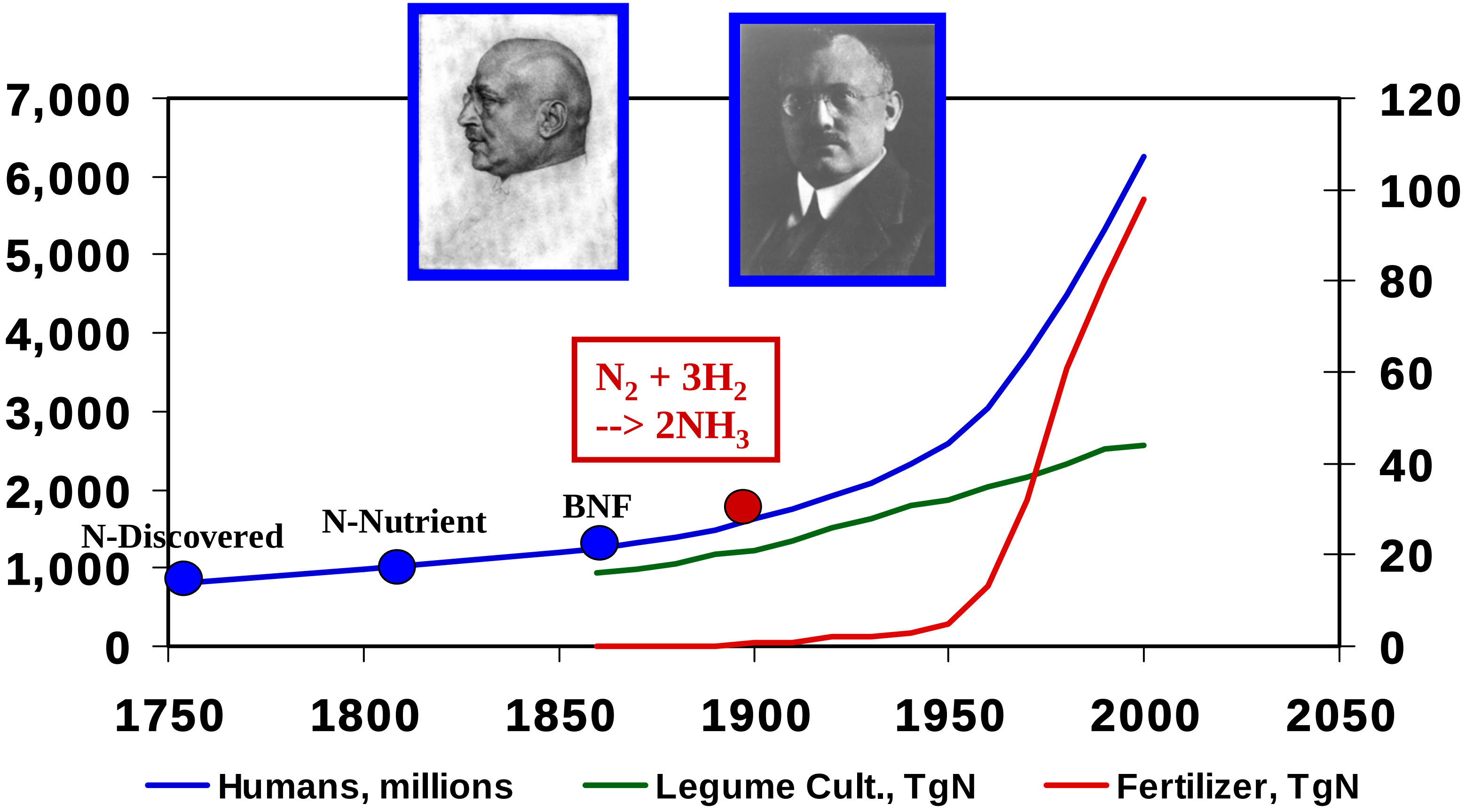
--N_r shortages--


World is running out of N*

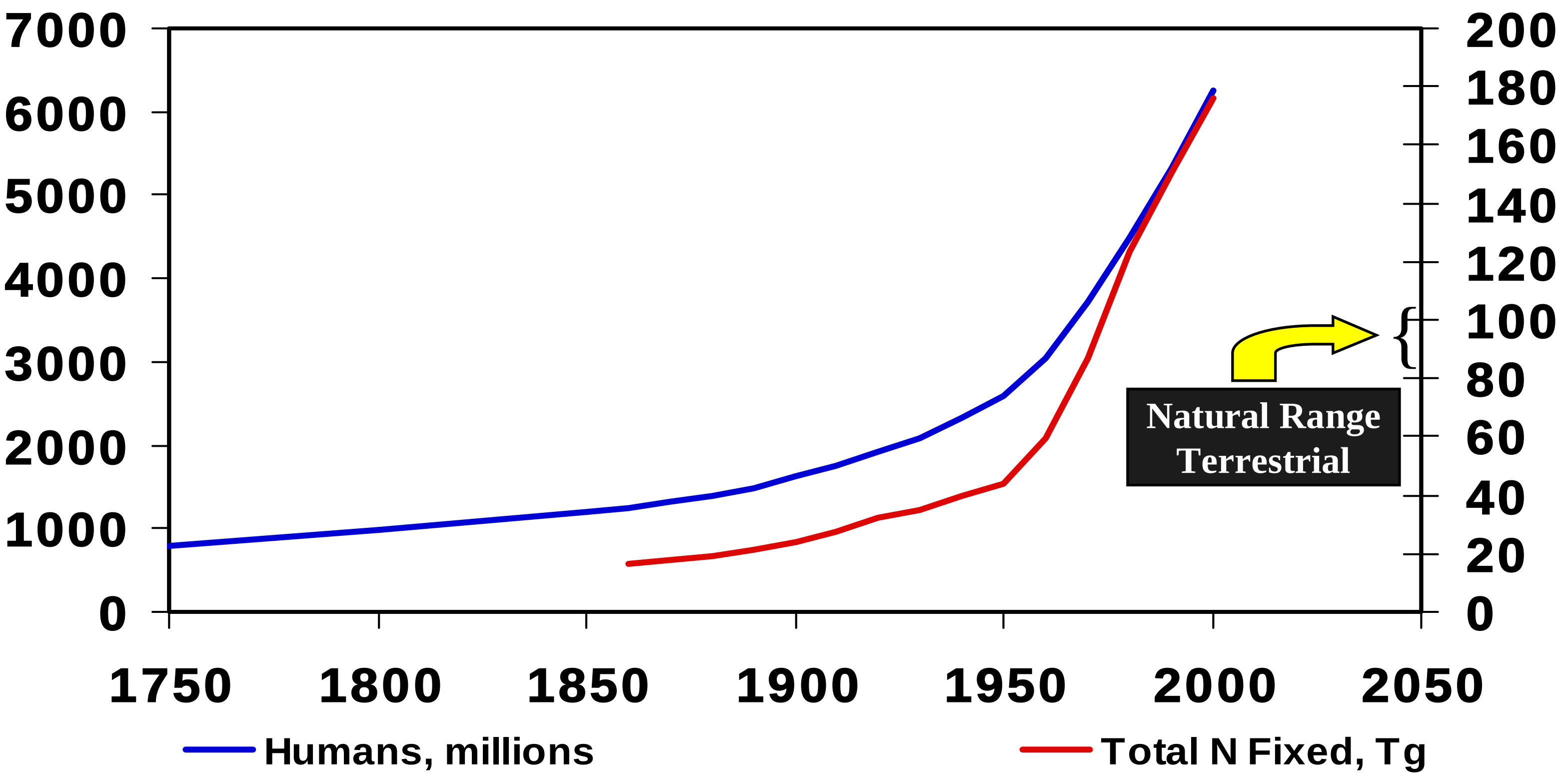
*1898, Sir William Crookes, president of the British Association for the Advancement of Science

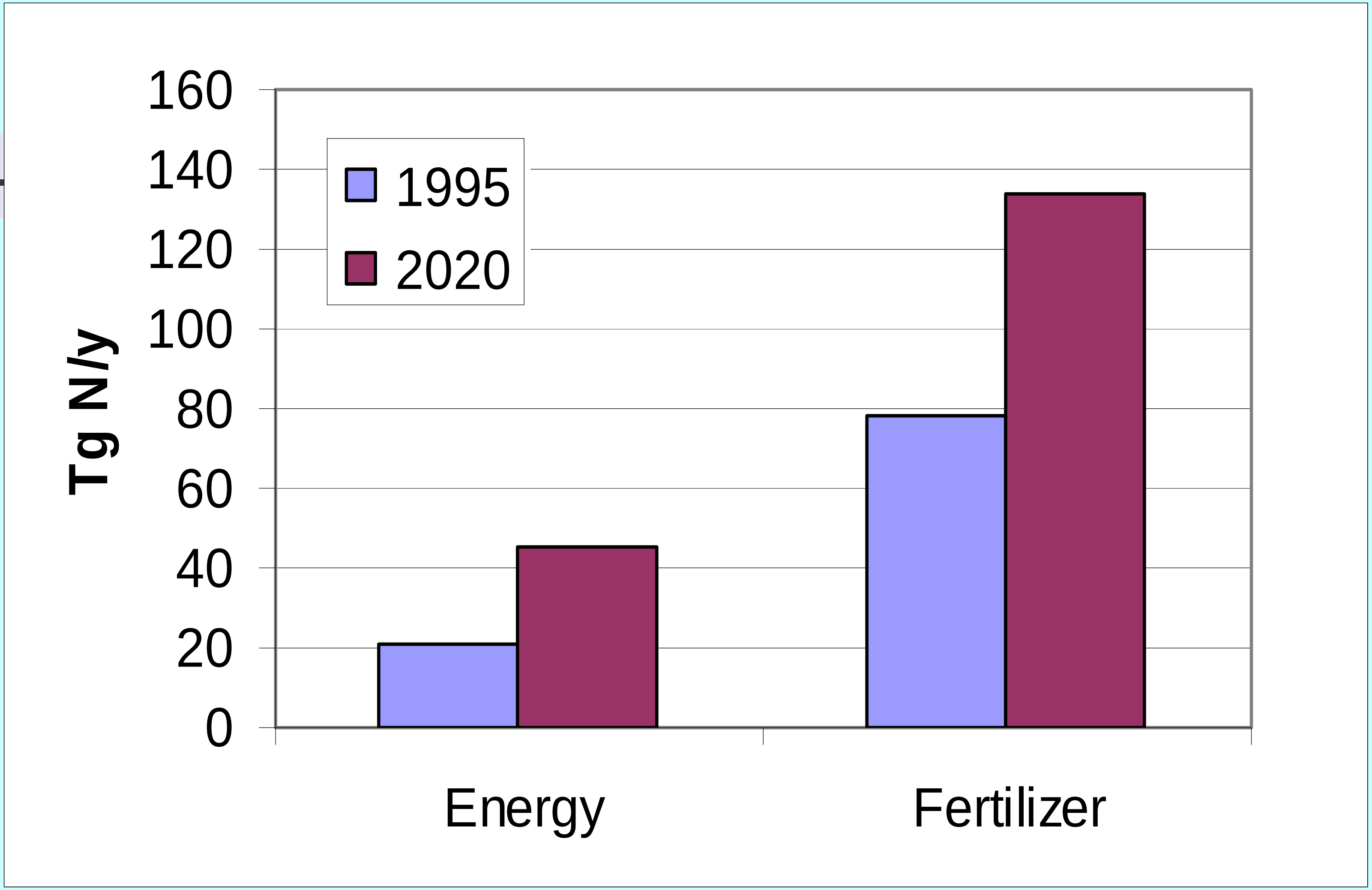
The History of Nitrogen

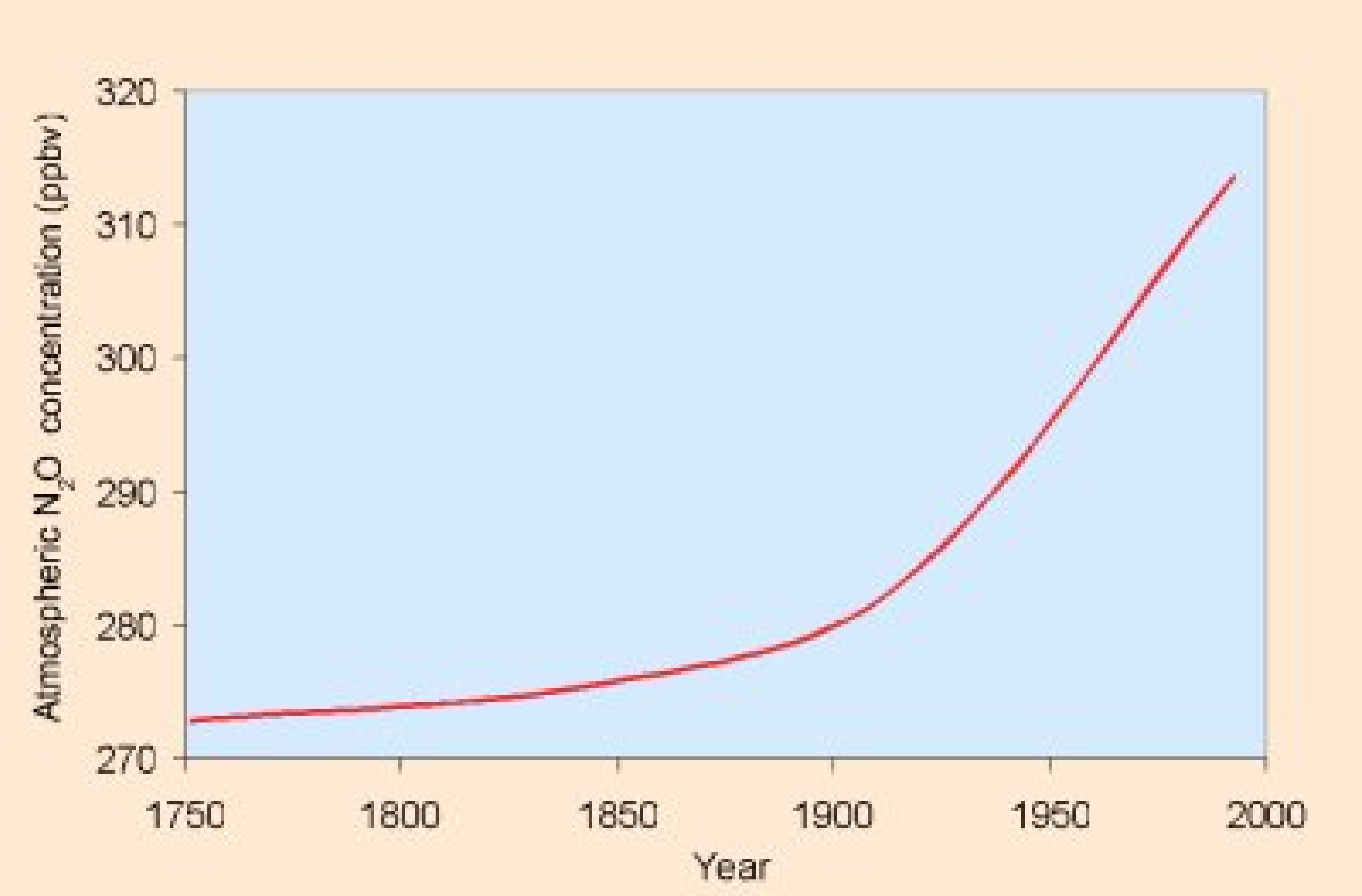
--Haber & Bosch!--



Major ongoing perturbation of N cycle




Tg N/y
160
140
120
100
80
60
40
20
0
1995
2020
Energy
Fertilizer


320
310
300
290
280
270
Atmospheric N_2O concentration (ppbv)
1750
1800
1850
1900
1950
2000
Year

MARINE N CYCLE (why do we care?)

- Primary limiting nutrient in most of ocean
- Major control on primary productivity and C export
- Control on climate? Paleo analysis
- Regional, basin scale and atmospheric perturbations

N Cycle Features

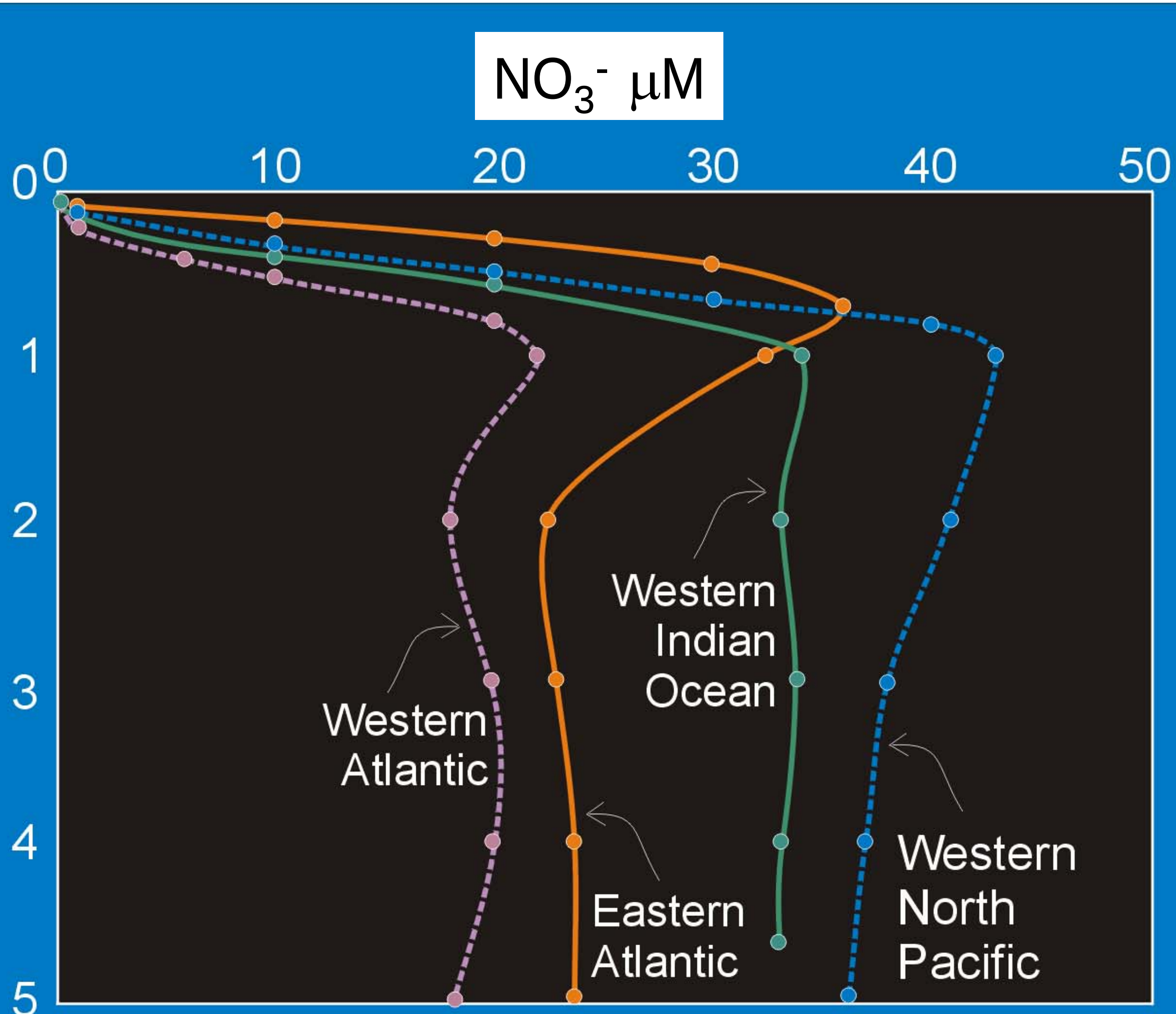
- Phase transitions
- Oxidation/ reduction reactions
  - Largely biological/ microbial
  - Trophic Structure
- Habitat/ Environment Specific Components
  - Photic/ aphotic (or shallow/ deep)
    - (e.g. NO_3^- uptake vs. nitrification)
  - Aerobic/ anaerobic (nitrification vs. denitrification)
  - Pelagic/ benthic
  - Coastal/ pelagic (quantitative aspects)

Marine N Pools

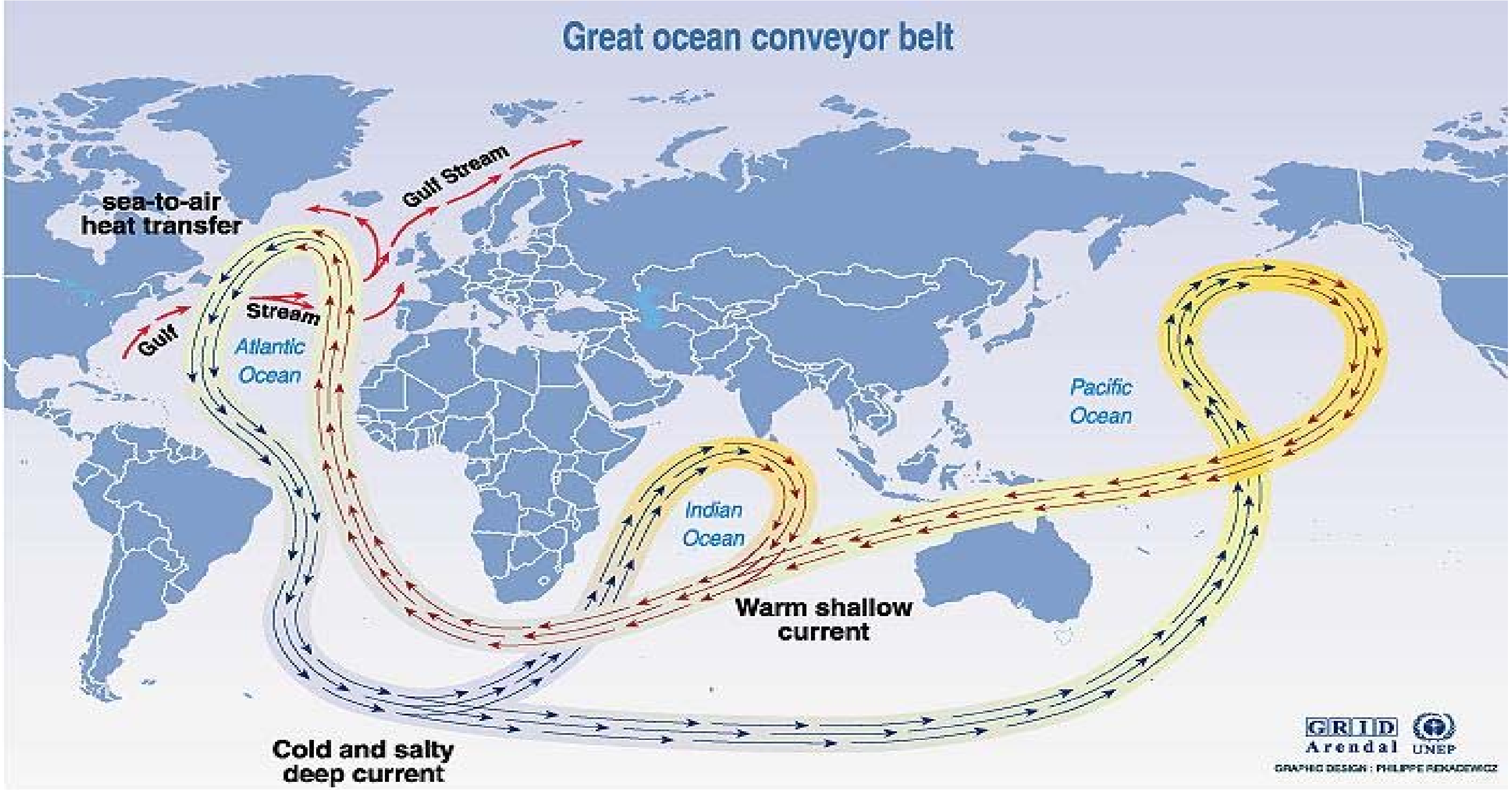
- N_2 dominates- but largely unavailable
- Large pools of NO_3^-, DON in ocean interior
- Pacificward enrichment- conveyor belt

Oceanic N Concentrations and Inventories

Species	Surface	Deep	Coastal µM	Estuarine	Reefs	TOTAL gN x10^{15}
N_2	800	1150	700 - 1100	700 - 1100	-	22,000
NO_3^-	0.2	35	0 - 30	0 - 350	0.1 - 2.7	570
NO_2^-	0.1	< 0.1	0 - 2	0 - 30	0.02 - 0.16	-
NH_4^+	< 0.5	3	0 - 25	0 - 600	0.02 - 1.7	7
DON	5	3	3 - 10	5 - 150	2- (70)	550
PON	0.4	< 0.1	0.1 - 2	1 - 100	-	3 - 24

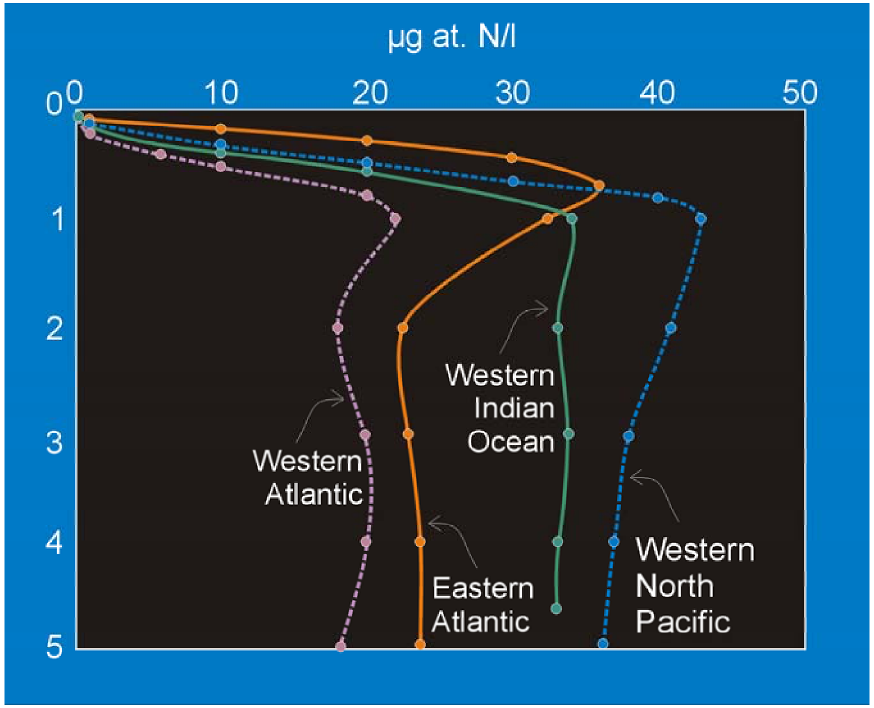

NO_3^- μM
0
10
20
30
40
50
0
1
2
3
4
5
Western Atlantic
Eastern Atlantic
Western Indian Ocean
Western North Pacific

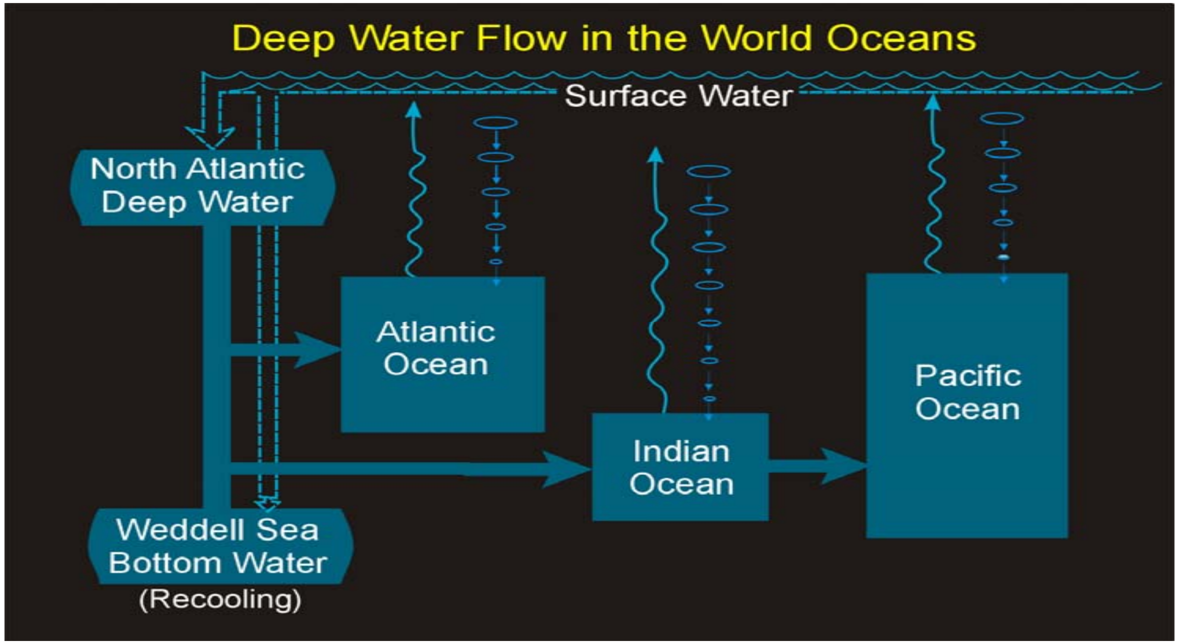
Conveyor Belt



Source: Broecker, 1991, in Climate change 1995, Impacts, adaptations and mitigation of climate change: scientific-technical analyses, contribution of working group 2 to the second assessment report of the intergovernmental panel on climate change, UNEP and WMO, Cambridge press university, 1996.

- Deep Water Circulation
    - Conveyor Belt
    - Global gradients of nutrient distributions
    - Function of input, regeneration

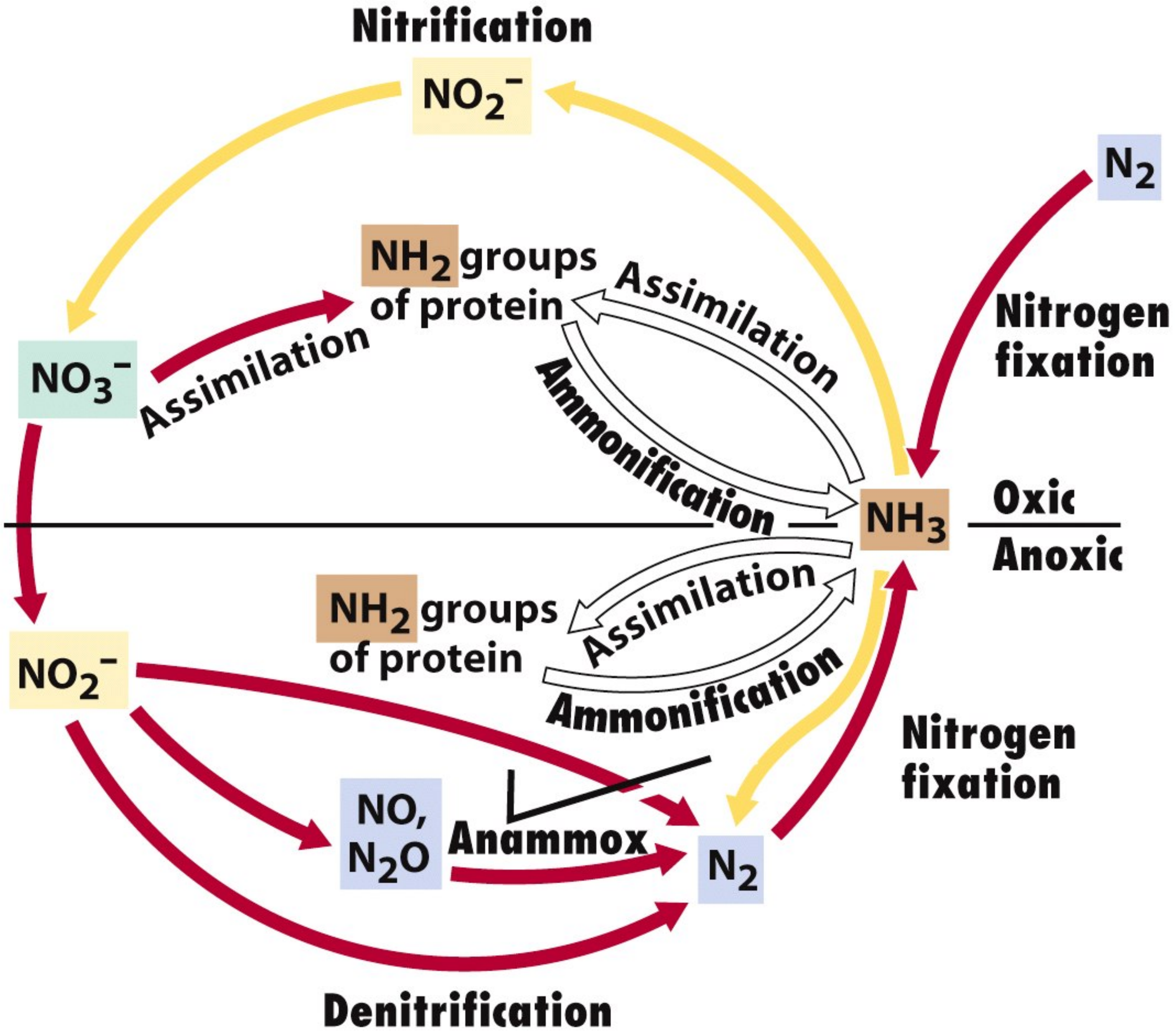




N Transformations

- Microbial oxidation/ reductions
- Habitat/ environment specific
- Trophic Structure specific

- Various Representations of the cycle



Nitrification

NO_2^-

N_2

NH_2 groups of protein

Assimilation

NO_3^-

Assimilation

Ammonification

Nitrogen fixation

NH_3

Oxic

Anoxic

NH_2 groups of protein

Assimilation

Ammonification

NO_2^-

Nitrogen fixation

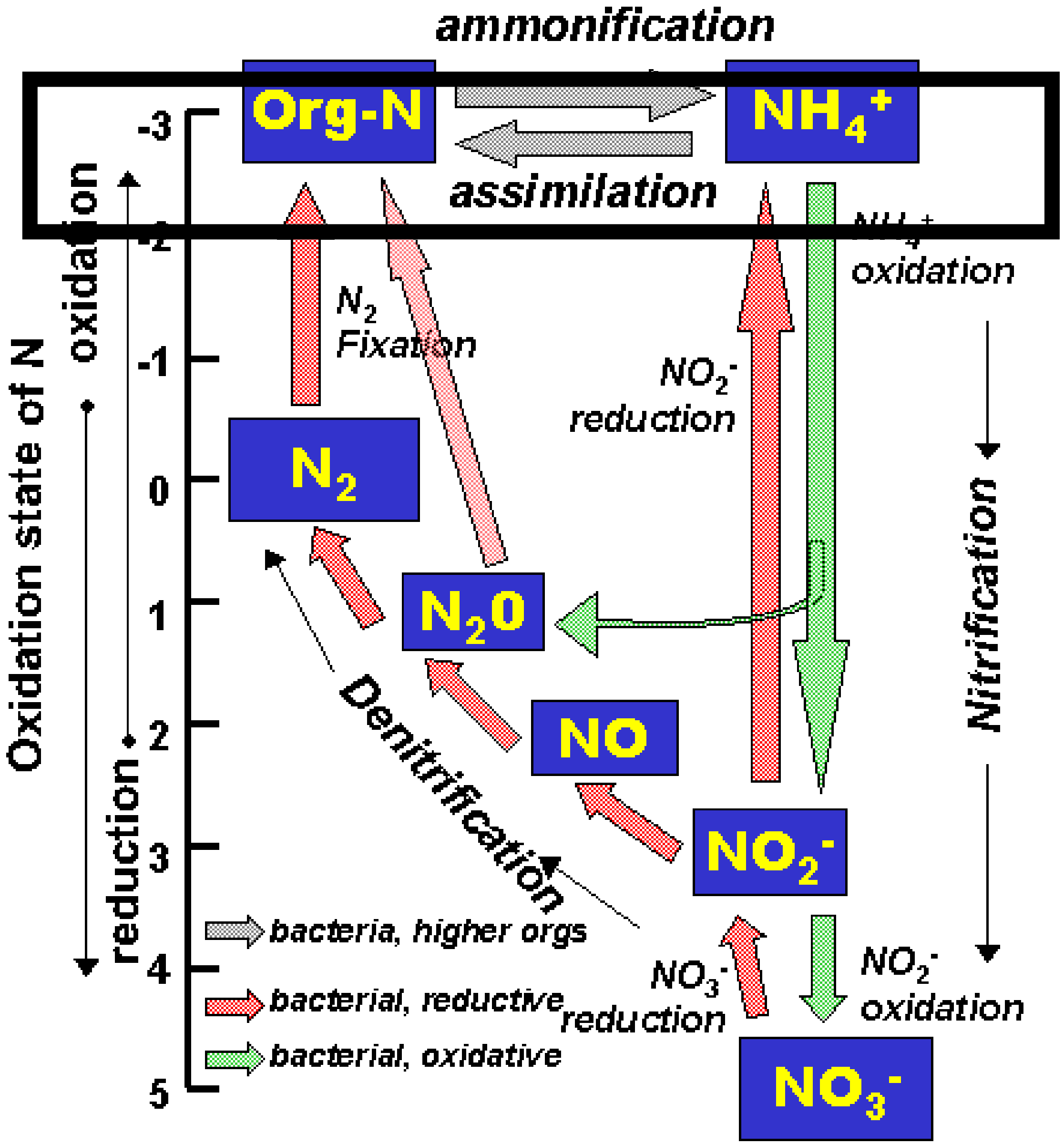
NO, N_2O

Anammox

N_2

Denitrification

Figure 19-28 part 2 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.


ammonification
Org-N
NH_4^+
assimilation
-3
-2
-1
0
1
2
3
4
5
Oxidation state of N
oxidation
reduction
N_2 Fixation
NH_4^+ oxidation
NO_2^- reduction
Nitrification
N_2
N_2O
NO
NO_2^-
Denitrification
NO_3^- reduction
NO_2^- oxidation
NO_3^-
bacteria, higher orgs
bacterial, reductive
bacterial, oxidative

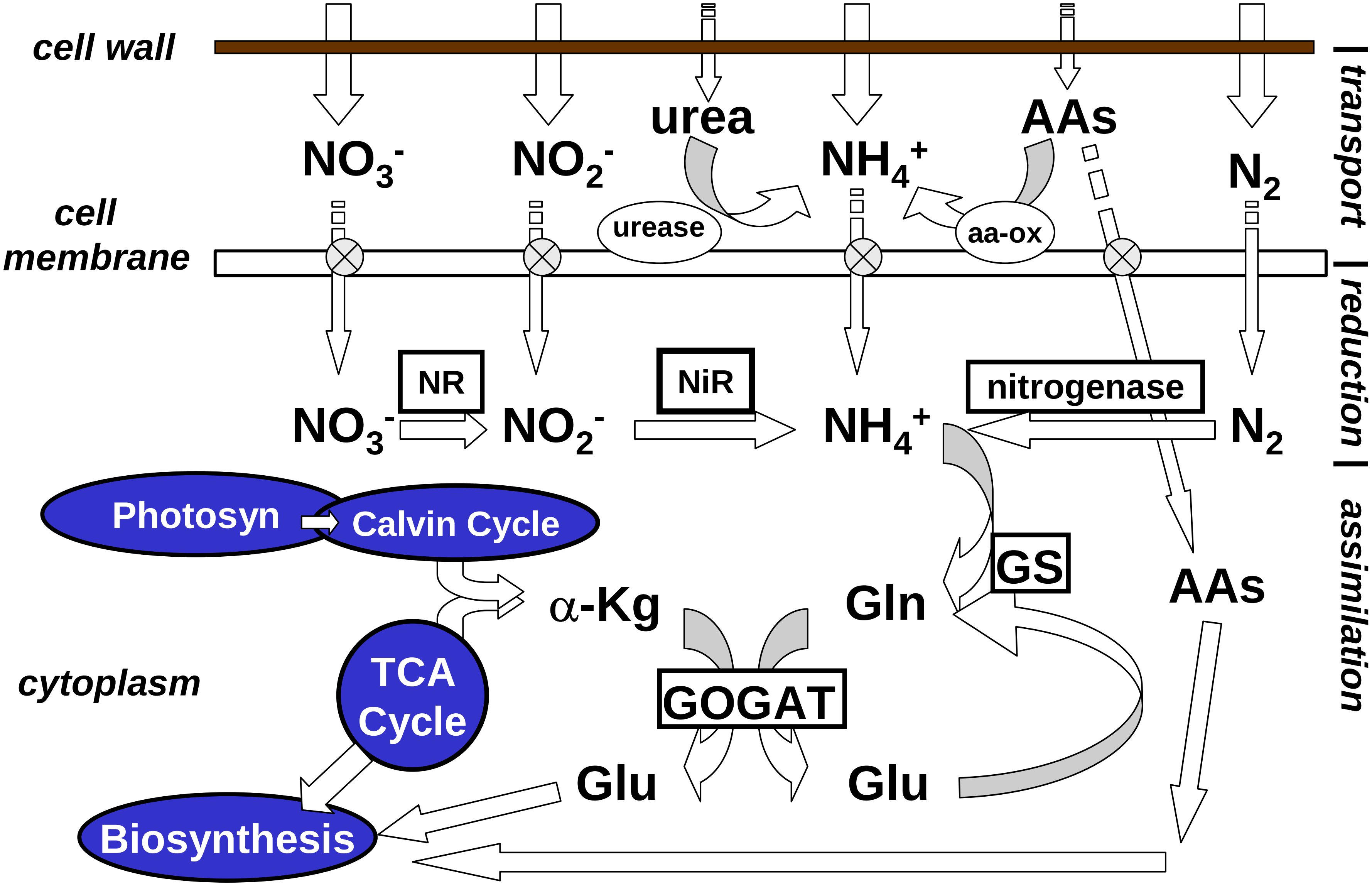
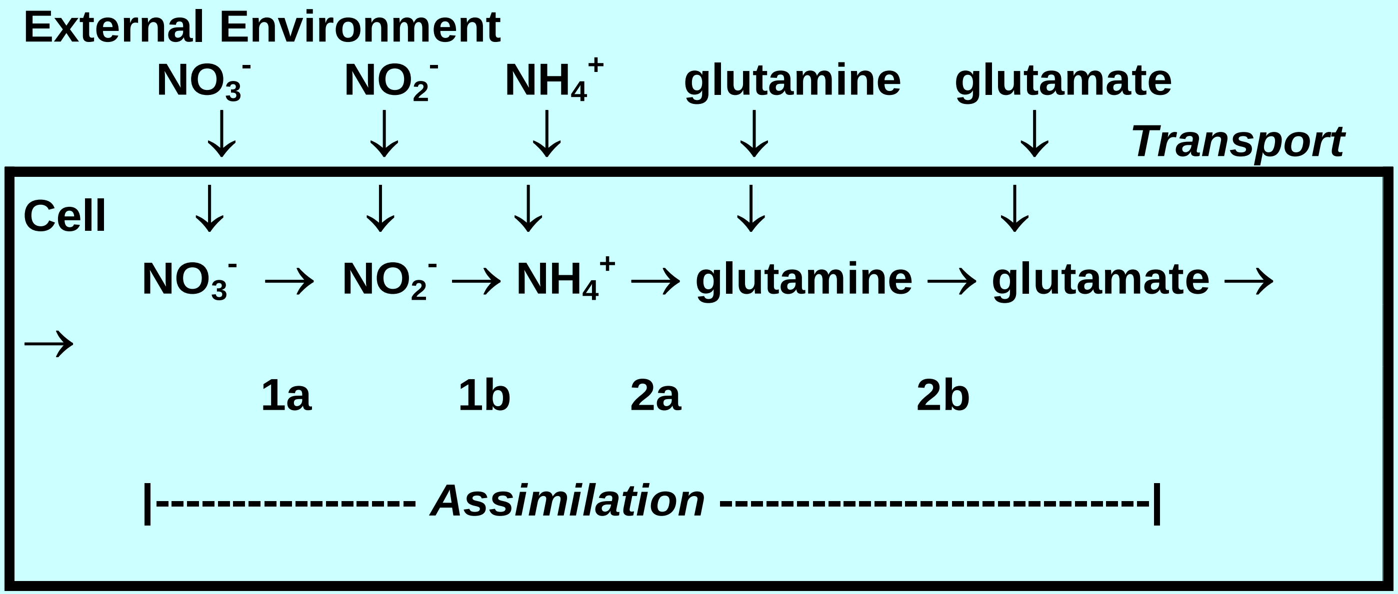


Figure 2. Capone

Uptake/ Assimilation of N

> Major pathways in Sea



External Environment

NO_3^- NO_2^- NH_4^+ glutamine glutamate

$\downarrow$ $\downarrow$ $\downarrow$ $\downarrow$ $\downarrow$ ***Transport***

Cell $\downarrow$ $\downarrow$ $\downarrow$ $\downarrow$ $\downarrow$

NO_3^- $\rightarrow$ NO_2^- $\rightarrow$ NH_4^+ $\rightarrow$ glutamine $\rightarrow$ glutamate $\rightarrow$

$\rightarrow$

1a 1b 2a 2b

|----------------- *Assimilation* ---------------------------|

NO_3^- Uptake: Algae, bacteria, fungi, plants

1a. Nitrate reductase (NaR)

1b. Nitrite reductase (NiR)

NH_4^+ Uptake: Algae, bacteria, fungi, plants

2a. Glutamine synthetase (GS)

2b. Glutamate synthase (GOGAT)

$NO_3^- + NAD(P)H + 6Fd_{(red)} + 9H^+$ -> $NH_4^+ + NAD(P)^+ + 6Fd_{(ox)}$

$G_o' = 289$ kJ/mole NH_4^+

Organic N: urea, amino acids

Nutrient limitation

- Liebig’s “Law” of the Minimum 1800s- agriculture
  - Limiting factor- substrate least available relative to growth needs
- What do we mean? (Howarth)

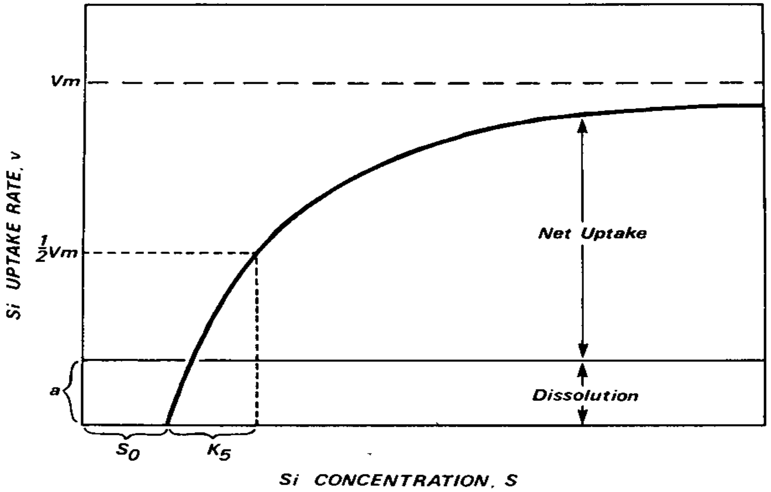
  Nutrient limitation of
  - biomass
  - growth of current populations
  - net primary production (autotrophs)
  - net ecosystem production
- Depends on perspective

For aquatic studies: Nutrient uptake model

- Michaelis- Menton enzyme uptake kinetics

$$v = V_{max} * \frac{[S]}{K_s + [S]}$$

Where: V_{max} = max velocity of uptake
Ks = nutrient concentration at ½ V_{max}



- Don’t always know [ambient]

$$v = V_{max} * \frac{[S_n + S_a]}{K_s + [S_n + S_a]}$$

Experimentally define uptake parameters- measure response of uptake of a specific nutrient over a concentration range (radio or stable tracers)

Compare within and among systems

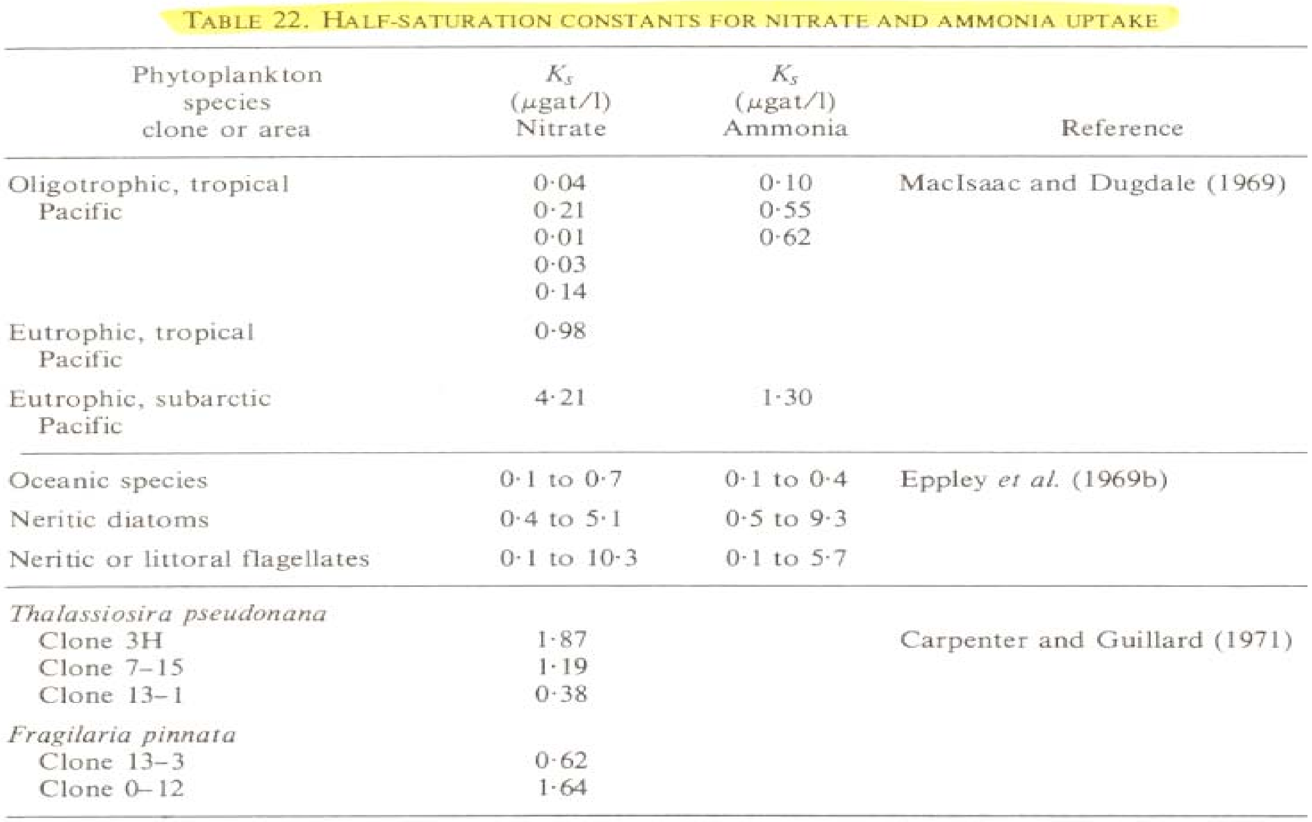


TABLE 22. HALF-SATURATION CONSTANTS FOR NITRATE AND AMMONIA UPTAKE

Phytoplankton species clone or area	K_s (μgat/l) Nitrate	K_s (μgat/l) Ammonia	Reference
Oligotrophic, tropical Pacific	0·04	0·10	MacIsaac and Dugdale (1969)
	0·21	0·55	
	0·01	0·62	
	0·03		
	0·14		
Eutrophic, tropical Pacific	0·98		
Eutrophic, subarctic Pacific	4·21	1·30	
Oceanic species	0·1 to 0·7	0·1 to 0·4	Eppley *et al.* (1969b)
Neritic diatoms	0·4 to 5·1	0·5 to 9·3	
Neritic or littoral flagellates	0·1 to 10·3	0·1 to 5·7	
Thalassiosira pseudonana			
Clone 3H	1·87		Carpenter and Guillard (1971)
Clone 7–15	1·19		
Clone 13–1	0·38		
Fragilaria pinnata			
Clone 13–3	0·62		
Clone 0–12	1·64		

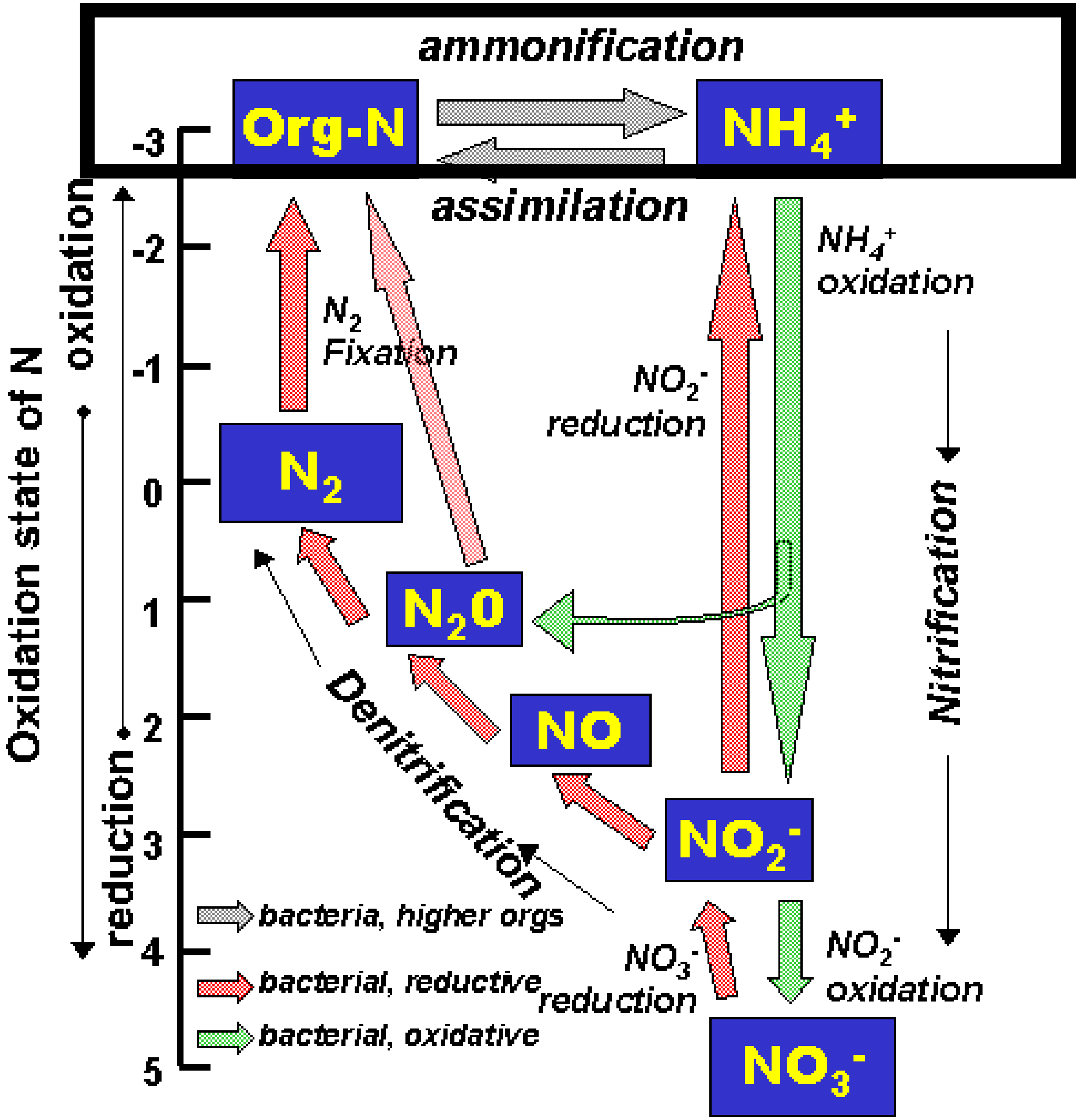
Example Ks for marine phytoplankton

Low Level Analysis

- Previous – to about 30 nM for NO_3, NH_4, PO_4
  - Greater than concentrations thru much of upper ocean
- Current methods to < 1 nM
  - Fluorometric NH_4
  - Long path length NO_3
  - NO_x Box (chemiluminescent)
  - MAGIC PO_4
- Will change our views on nutrient uptake in oligotrophic areas

Some Points

- Not all inorganic uptake is autotrophic
    - Heterotrophic bacterioplankton competition
    - Cell C:N vs substrate:
        - bacteria HAVE LOW C:N: typically 5-6
- Oceanic phytoplankton apparently not "nutrient stressed" based on C:N:P ratios
    - C:N:Ps of field populations reflective of C:N:P of cultures at high growth rate


ammonification
Org-N
NH4+
assimilation
-3
-2
-1
0
1
2
3
4
5
Oxidation state of N
oxidation
reduction
N2 Fixation
N2
N2O
NO
NO2-
NO3-
NH4+ oxidation
NO2- reduction
Nitrification
Denitrification
NO3- reduction
NO2- oxidation
bacteria, higher orgs
bacterial, reductive
bacterial, oxidative

N Regeneration

- Ammonification: DON/PON -> NH_4^+
  - hydrolytic deamination
- Amino Acid oxidation: aa -> NH_4^+
  - recent, cell surface enzymes
- Protein, peptide hydrolysis -> aa’s
- Redfield Model
  - Stoichiometry of regeneration
    - C:N demand vs. C:N of regeneration & availability

Redfield Ratios (Ecol Stoichiometry)

- Original Observation
  - N:P available dissolved in deep water very close to average plankton (phyto) tissues
    - C:N:P Deep water – dissolved = 1000:16:1
    - C:N:P Soft tissue = 106:16:1
  - Biology controls chemistry
    - Rain of organic material yields inorganic N:P after degradation
  - Considerable “plasticity” now recognized

- Stoichiometry of primary production & regeneration-
  - averaged over the oceans and over times scales of ocean circulation

$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138\ O_2$ ->

$106\ CO_2 + 122\ H_2O + 16\ NO_3 + PO_4 + 19\ H^+$

Redfield C:N:P: 106:16:1

N* Anomalies:

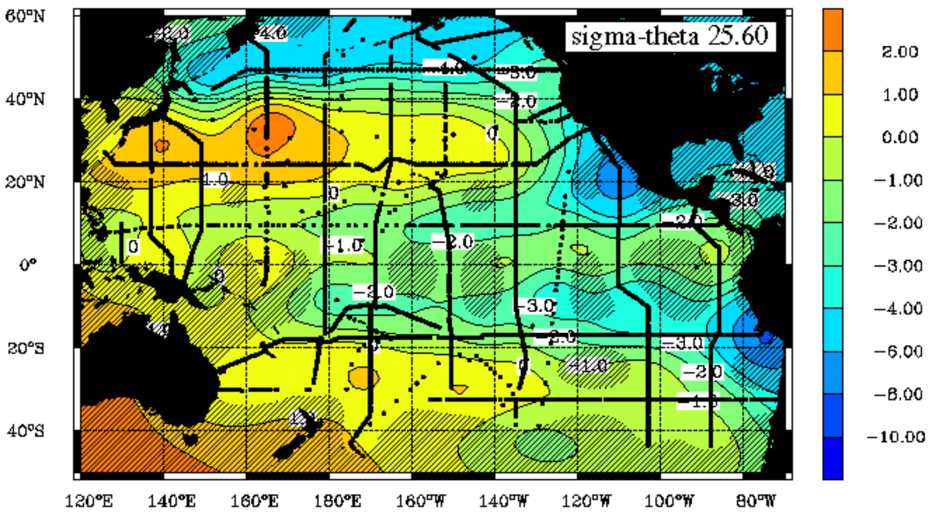
$N^* = N - 16P$

$+N^*$ = N regen in excess of Redfield

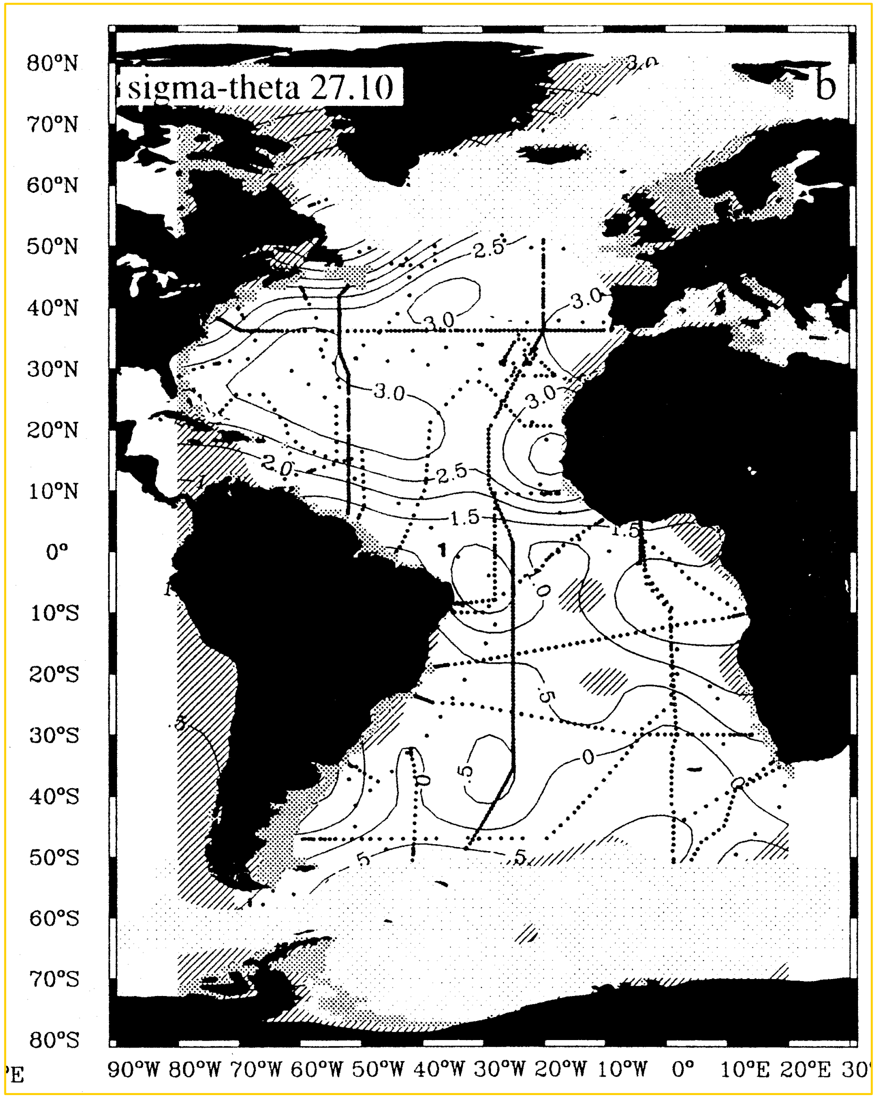
Diazotroph biomass

N:P > 16

N^* on isopycnal surface (μmol kg^{-1})



from Deutsch et.al., manuscript submitted to Global Biogeochemical Cycles



Michaels et al. 1996
Gruber & Sarmiento 1997
Deutsch et al. 2001

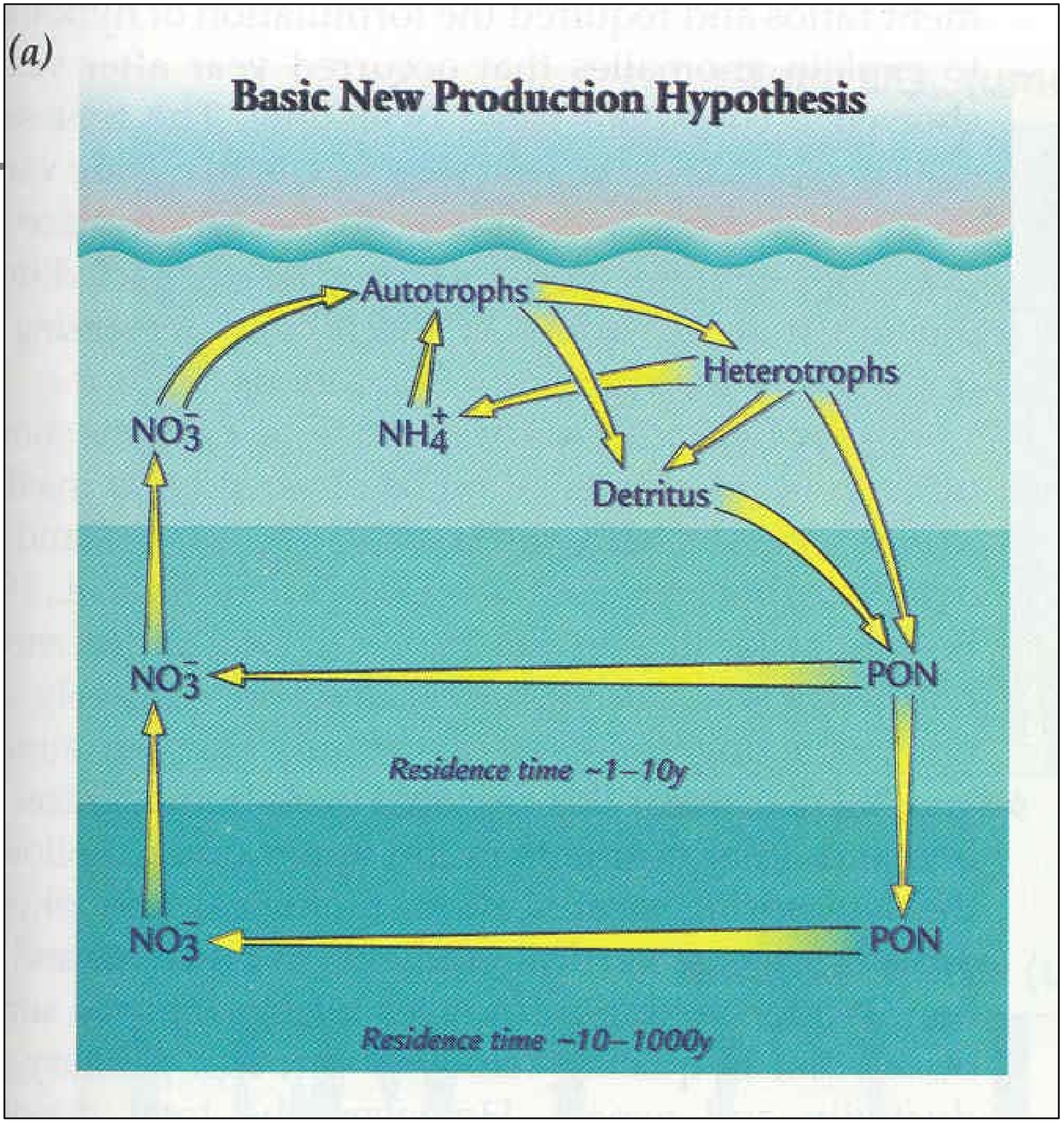
New Production Model

- Dugdale & Goering (1967)
- Eppley & Peterson (1979)
  - Recognized different forms of N relative to plankton uptake
    - recycled
    - new (from external to the system
  - Production based on “new” N set a limit on exportable production
  - Research largely focused on NO_3^-

Traditional Paradigm of Ocean N Cycle

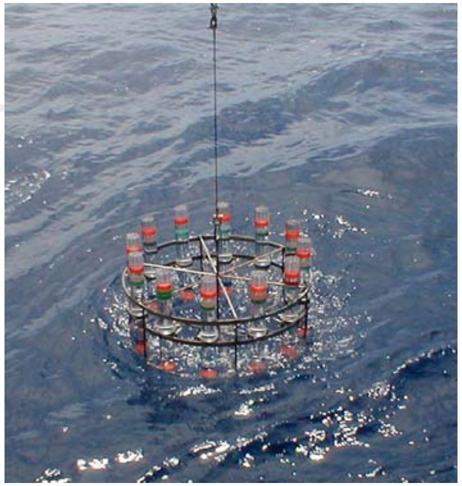
“Recycled” N supports much of net 1º production

“New” sources of nitrogen (e.g. deep nitrate) constrain export of organic N



APPROACHES/ New Production:

Constraints on organic export



- Integrated N Uptake: f ratio
- Gradient driven NO_3 flux
- Sediment traps: catch falling particles (export)
  - Floating (below euphotic zone)
  - Deep moored

- Upper water column DIC or O_2 dynamics integrated over time

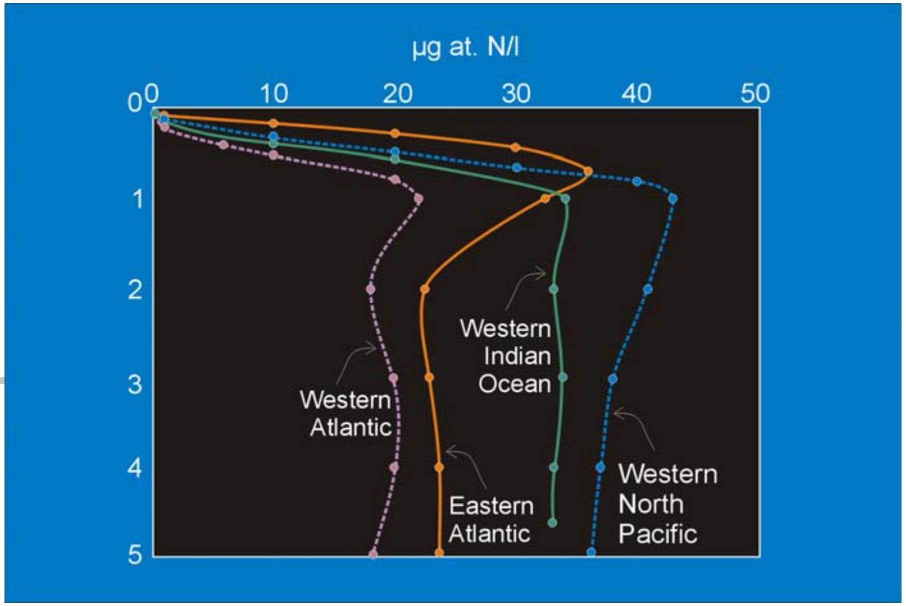
F Ratio

- Using - ^{15}N uptake

$$\text{f ratio} = \frac{\text{NEW Production}}{\text{TOTAL Production}} = \frac{V_{(NO3-)}}{V_{(NO3-)} + V_{(NH4+)}}$$

- Assumes no nitrification in euphotic zone (i.e. no NO_3^- prod – it all comes from below the euphotic zone)
- Assumes no other “new” sources other than NO_3^-,
- no other recycled sources other than NH_4^+ (Glibert; Capone)

Vertical NO_3 flux



- Estimate of vertical NO_3^- diffusion in highly stable, stratified waters
- Coefficient of diapycnal eddy diffusivity (K_z)- highly controversial – widely varying estimates
- Nitrate gradient - (umol N/(m3 m)

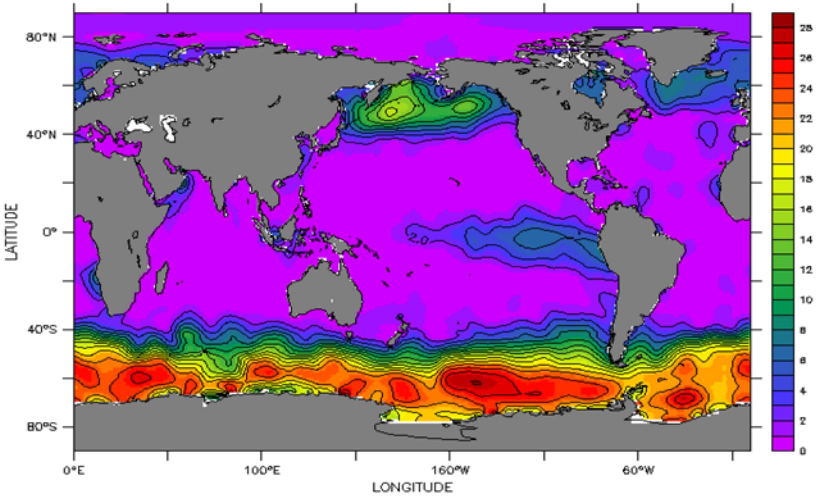
  (greater in Pacific)
- Estimates: 30 – 1500 umol N/ m^2 d

Table 1. Estimates of vertical NO_3^- flux into the euphotic zone

Location	NO_3^- gradient mmol/m^4	$K_z \times 10^5$ m^2/sec	N Flux µmol N/ m^2*d	Reference
Olig. E. Atlantic 28.5N, 23W,	0.045	3.7 (0.06-23)	139	Lewis et al. 1986
Sargasso Sea	0.02 - 0.03	[76]	1644	Jenkins 1988
Olig. E. Atlantic 26N 28W	0.03	1.1	27	Ledwell et al. 1993
Central Atlantic			380	Planas et al. 1999
Sub-trop N. Atlantic			274	Williams et al. 2001
trop. N. Pacific	0.15	0.5-5.1	180	Anderson (1978)
E. Pacific	n/a	8-40	-	Eppley et al. (1979)
E. trop. N. Pacif	n/a	-	380-1760	King & Devol (1979)
central N. Pacific	0.08	1-3.6	800	Platt et al. (1982)

% Recycled Production

Eppley & Peterson 1979



Nitrate (umol/l)

Biome	Depth of Euphotic zone (m)	Productivity g $C/m^{-2}y^{-1}$	Productivity X 10^{15} gCy^{-1}	% Recycled Production
Oligo	100	26	3.8	94%
Transitional	51	51	4.2	87%
Eq divergence, Subpolar	30-50	73	6.3	81%
Inshore	10-20	124	4.8	70%
Neritic (Shelf)	10-20	365	3.9	54%

Oceanic N demand- large flux

- 7200 Tg/ y – Net primary production
- 2500 Tg N/ y New or export
- 300 Tg N/ y for sequestered production
  - (i.e. that required for ocean biology to fix 2.0 Pg C)

Marine Nitrification

CONVENTIONAL UNDERSTANDING

- **2 steps, mediated by 2 distinct, specialized groups of strictly aerobic bacteria**
  [minor heterotrophic nitrification]

a. Ammonia oxidation: 6e$^-$ G_o' (KJ/mol)

$NH_4^+ + 1\ 1/2\ O_2 + H_2O \rightarrow NO_2^- + 2H_3O^+$ -273.9

Two step process- AMO and Hydroxyamine-OR

b. Nitrite oxidation 2e$^-$

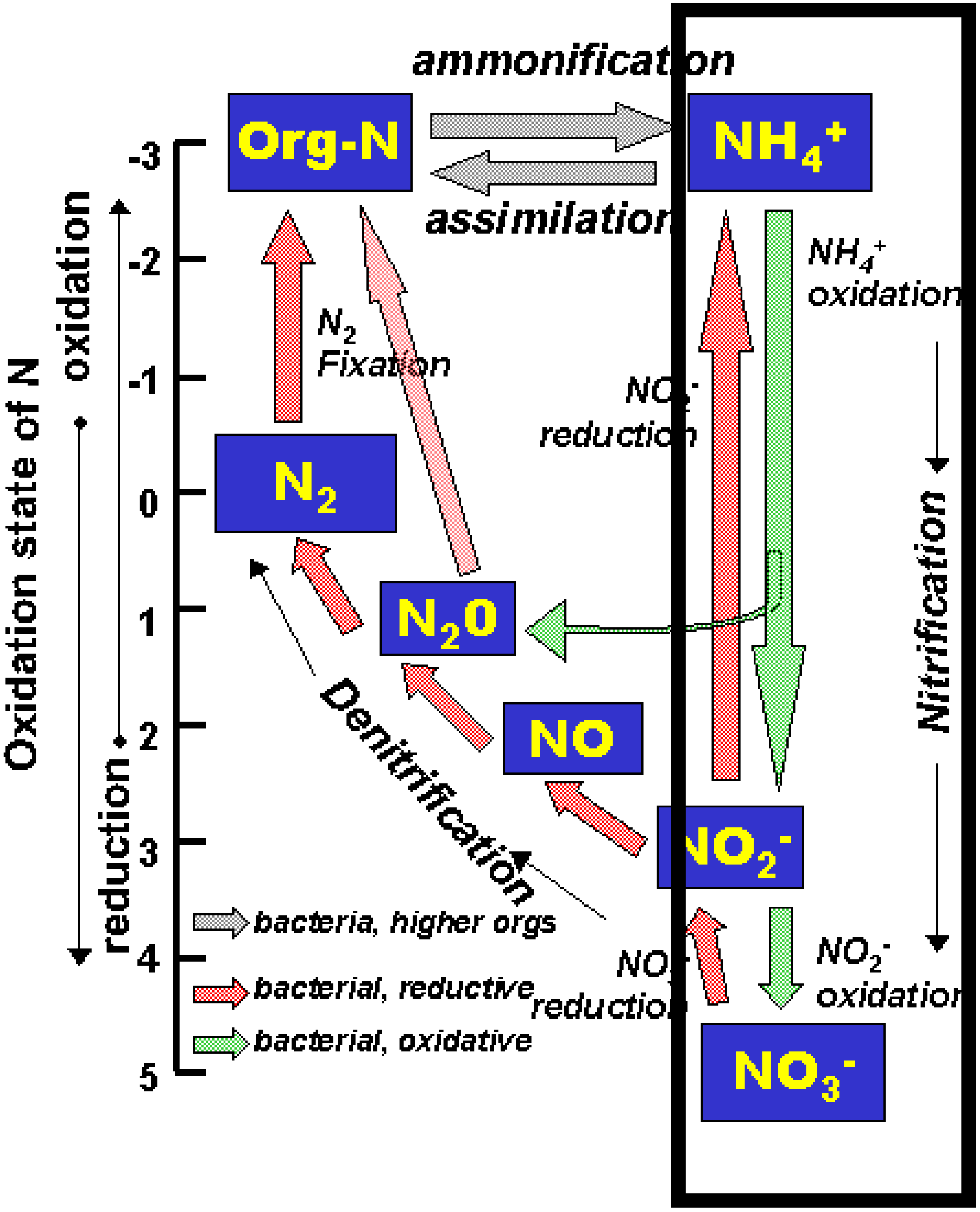
$NO_2^- + 1/2O_2 \rightarrow NO_3^-$ - 76.7

- **Physiology, Ecology**
  these oxidations coupled to
  - respiration for ATP formation
  - reductant (NAD(P)H_2) formation

for subsequent use in CO_2 reduction via CALVIN cycle

Autotrophs (chemoautotrophs)

1. **<u>Nitrosomonas</u> (Ammonium oxidizers)**
2. **<u>Nitrobacter</u> (Nitrite oxidizers)**

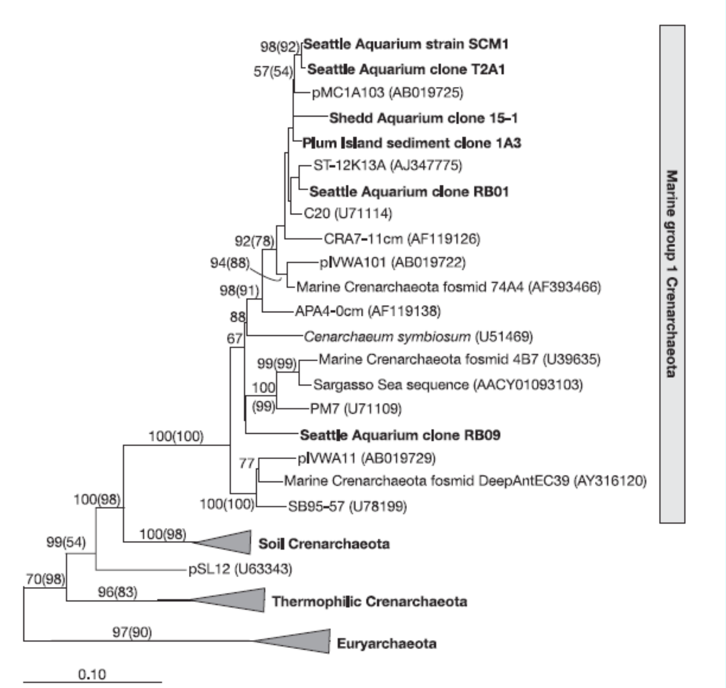


Recent Observations

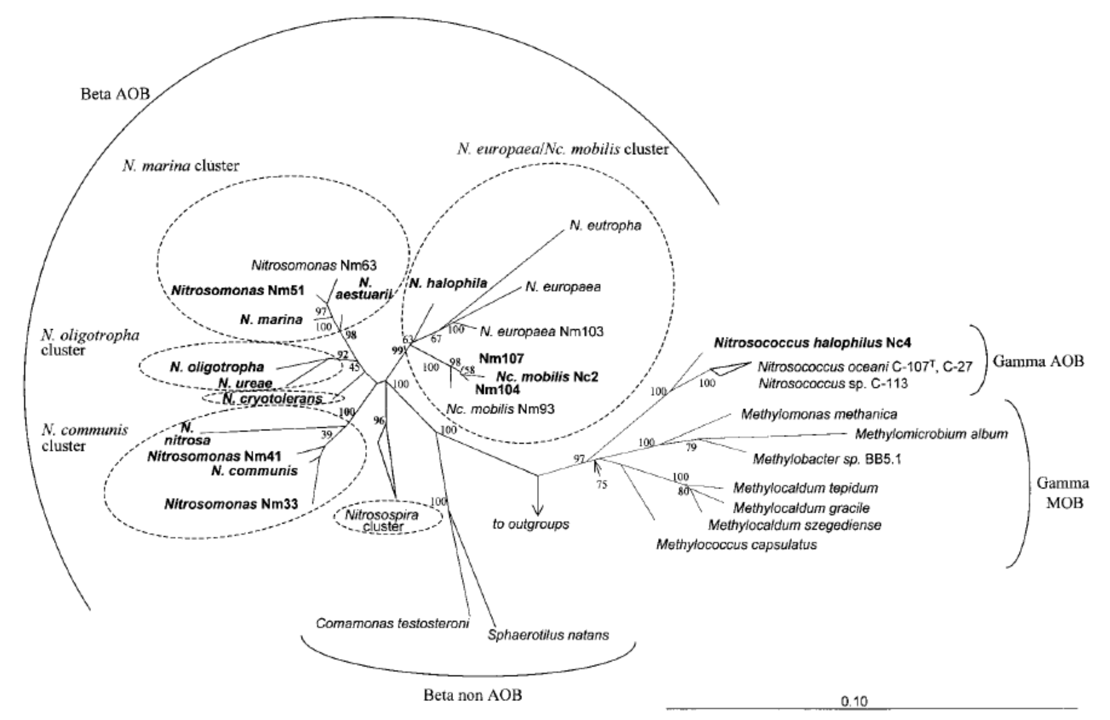
- Large populations of Archaea in the Sea
  - Fuhrman, deLong 1992
- Archaeal ammonium monoxygenase in Sargasso Sea metagenomics study
  - Venter et al. 2004
- Archael nitrifier isolated- (AOA)
  - Koennecke et al. 2005
- AOA are the predominant marine ammonium oxidizers
  - Wuchter et al. 2006
- AOA have competitive advantage w/ Ks
  - Martens-Habbena et a. 2009

Marine nitrifier phylogenies

Crenarchaeota



Eubacteria

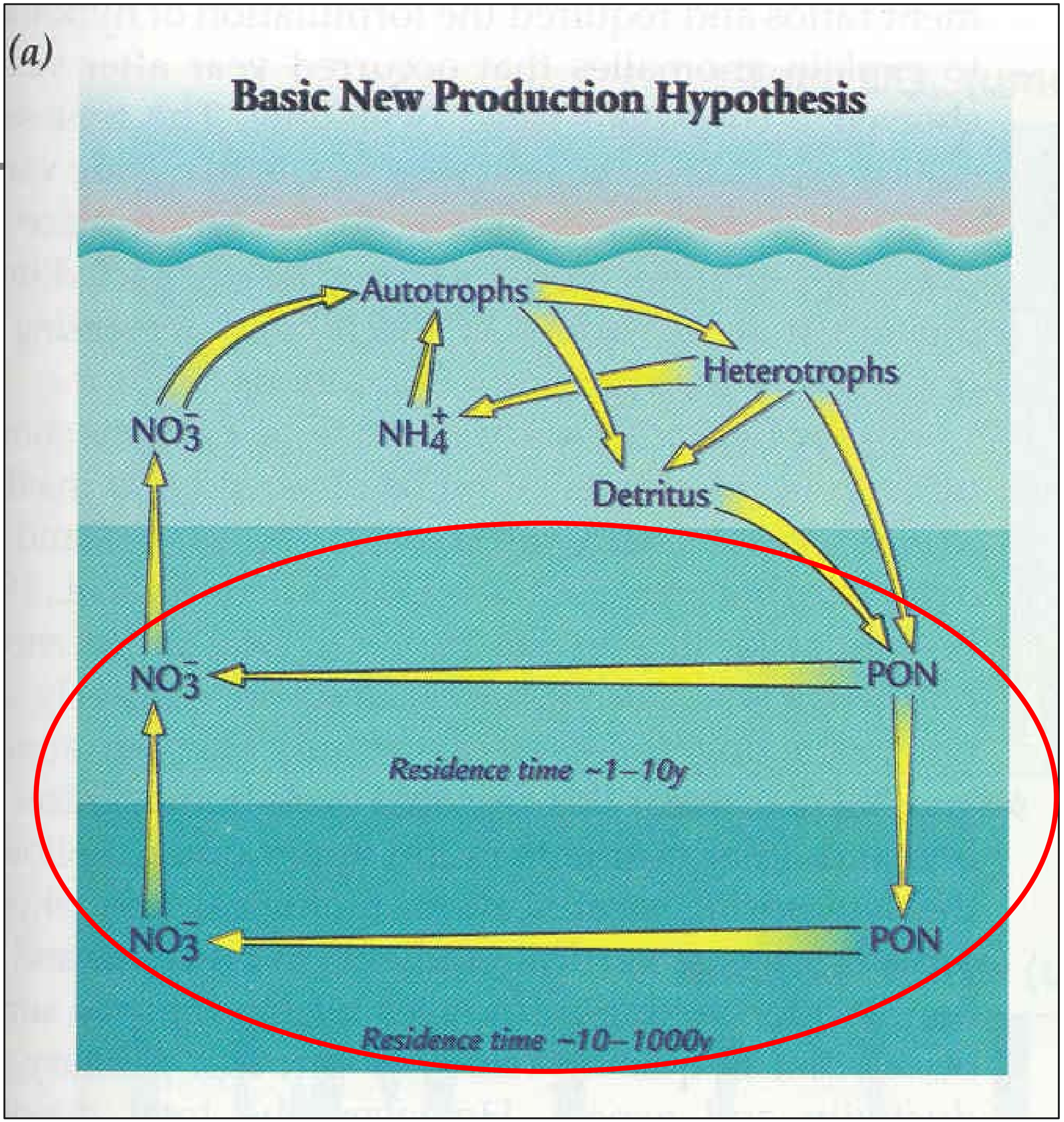


Nitrification Rates

Sample	mmol N/L*d	mg N/m^2day^1	gN * 10^{12}/y
Water Column			
Estuaries	15 - 2400		
Offshore	0.2 - 130		
Benthic			
Inshore	0 - 210	0 - 480	
Offshore		0.5 - 50	
TOTAL			2000

Oceanic nitrification should roughly balance new production based on NO_3^- :
Range: 500 (Eppley)- 2500 (Falkowski) Tg

Nitrification coupled to N regeneration accounts for nitrate production



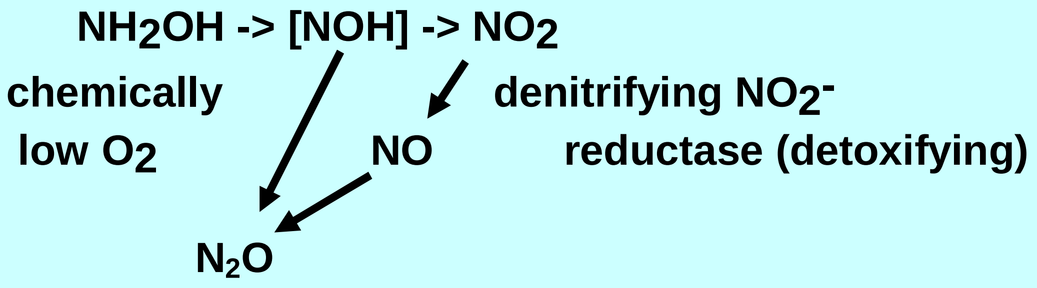
Nitrification (con't)

In general - activities proceed in concert
- nitrite maxima occasionally observed in various oxic environments
- evidence for differential inhibition of nitrifiers by light (Olson)
  - nitrite oxidizers more sensitive to light inhibition, low O_2

N_2O production by nitrifiers

minor byproduct with ecological importance

- potent greenhouse gas (300x CO_2)



NH_2OH -> [NOH] -> NO_2

chemically low O_2

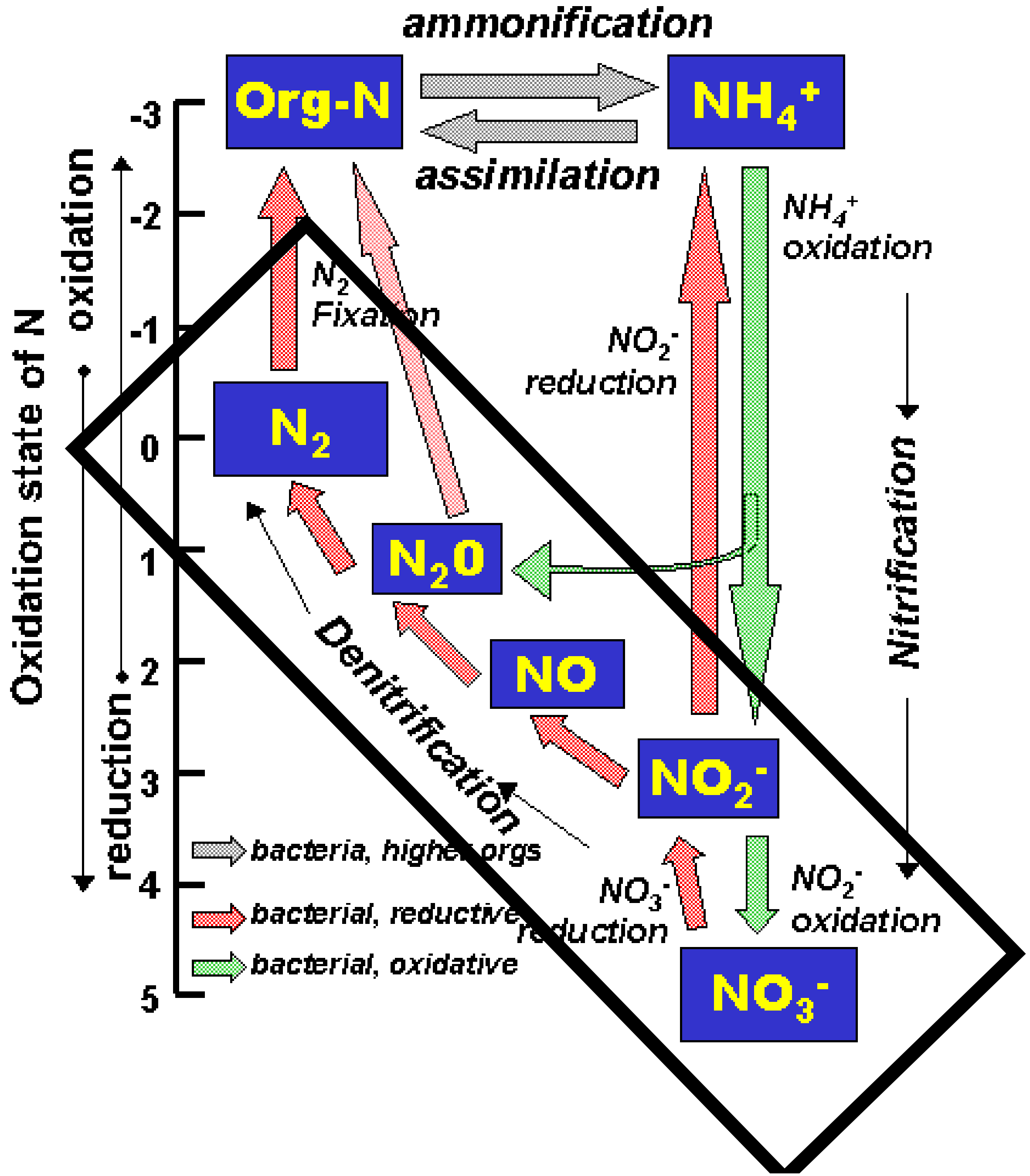
denitrifying NO_2^- reductase (detoxifying)

NO

N_2O

LOCATION: throughout Deep-Sea at low levels

At O_2/ NH_4^+ interfaces (water column, sediments)



ammonification

Org-N

NH_4^+

assimilation

Oxidation state of N

oxidation

reduction

-3

-2

-1

0

1

2

3

4

5

N_2 Fixation

NH_4^+ oxidation

NO_2^- reduction

Nitrification

N_2

N_2O

NO

NO_2^-

NO_3^-

Denitrification

NO_3^- reduction

NO_2^- oxidation

▨ bacteria, higher orgs

🟥 bacterial, reductive

🟩 bacterial, oxidative

Denitrification, Nitrate Reduction (respiratory, dissimilatory)

- Surface waters 5 mg O_2/L
- Denitrif. <0.2 mg O_2/L

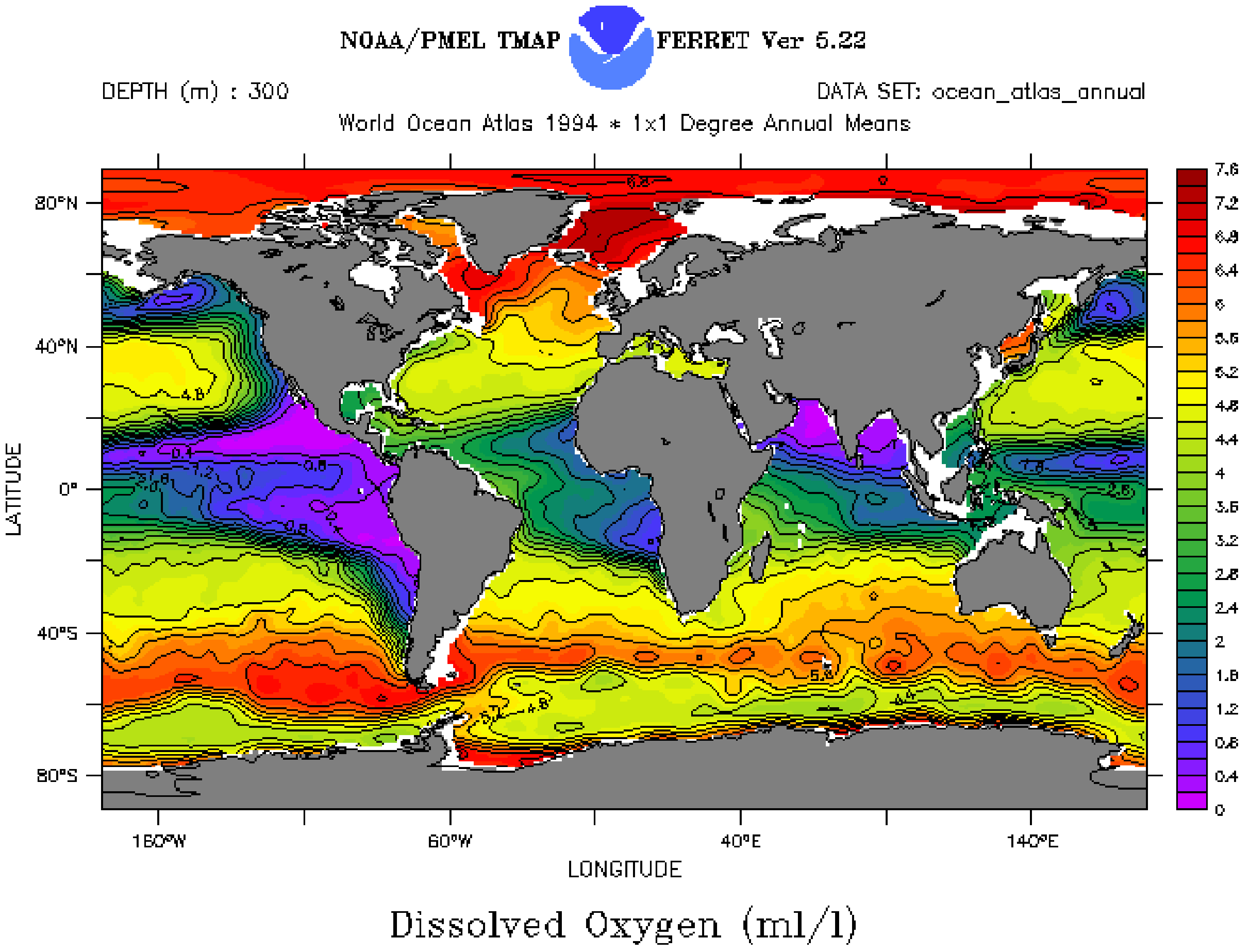
1. **Reactions** G_o'(KJ/mol)

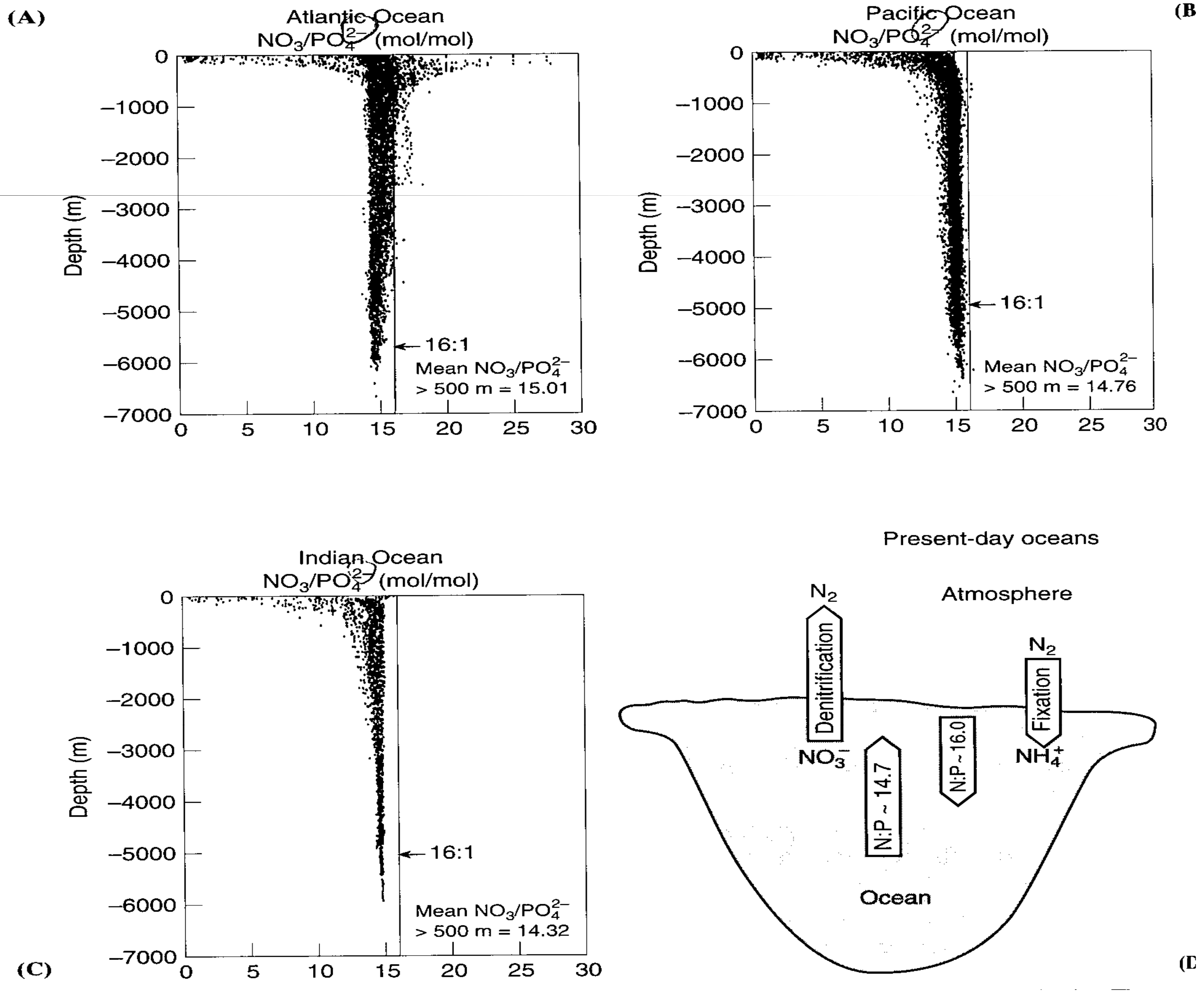
$2NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O$ ($10e^-$)	- 1121
($O_2 \rightarrow 1/2\ H_2O$)	(-1184) for comparison

2. **Enzymes**

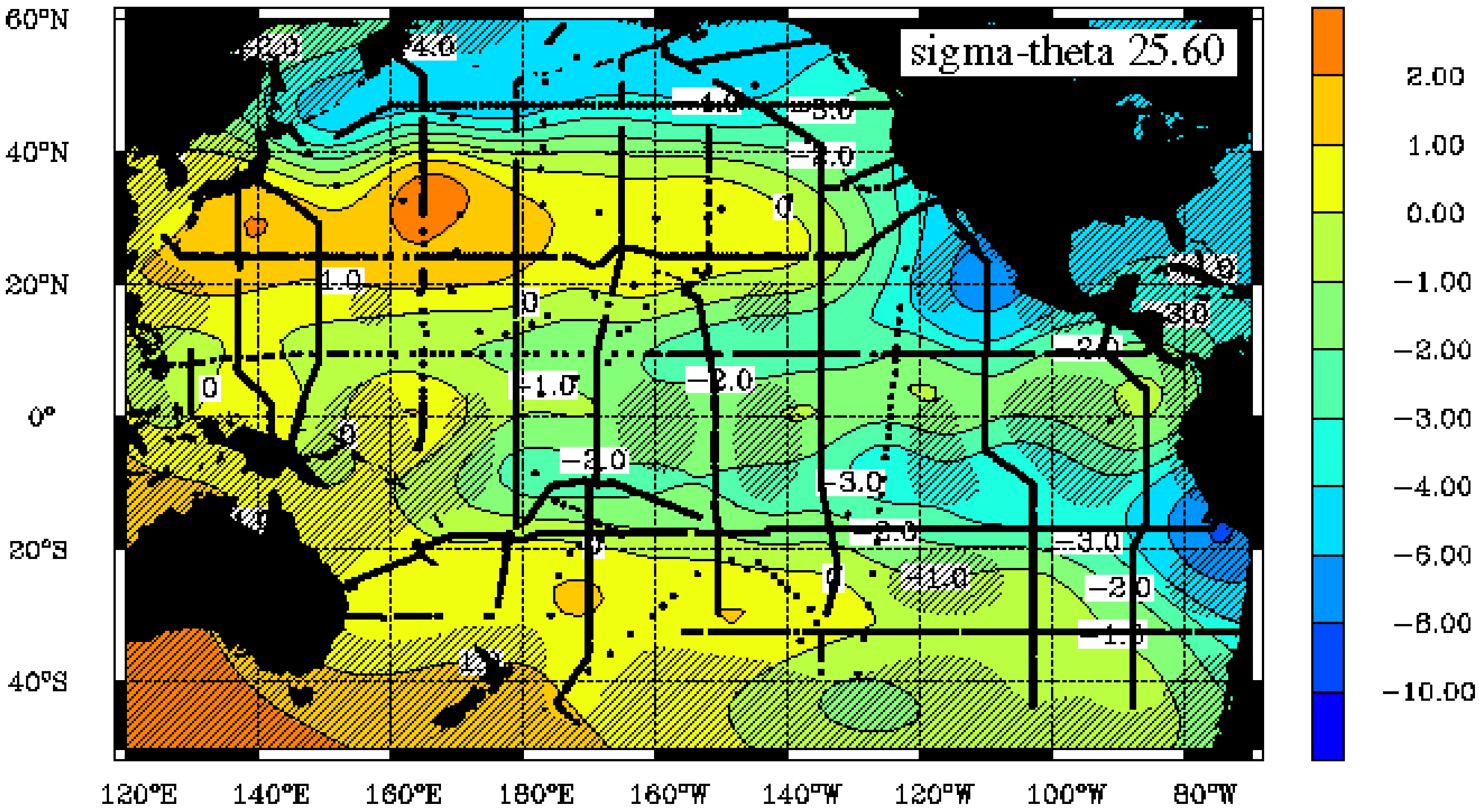
$$NO_3^- \xrightarrow{a} NO_2^- \xrightarrow{b} NO \xrightarrow{c} N_2O \xrightarrow{d} N_2$$

a. dissimilatory nitrate reductase
b. " nitrite "
c. nitric oxide reductase
d. nitrous oxide reductase


NOAA/PMEL TMAP FERRET Ver 5.22
DEPTH (m) : 300
DATA SET: ocean_atlas_annual
World Ocean Atlas 1994 * 1x1 Degree Annual Means
80°N
40°N
0°
40°S
80°S
LATITUDE
160°W
60°W
40°E
140°E
LONGITUDE
7.6
7.2
6.8
6.4
6
5.6
5.2
4.8
4.4
4
3.6
3.2
2.8
2.4
2
1.6
1.2
0.8
0.4
0
Dissolved Oxygen (ml/l)


(A)
Atlantic Ocean
NO_3/PO_4^{2-} (mol/mol)
Depth (m)
0
−1000
−2000
−3000
−4000
−5000
−6000
−7000
0 5 10 15 20 25 30
16:1
Mean NO_3/PO_4^{2-}
> 500 m = 15.01
(B
Pacific Ocean
NO_3/PO_4^{2-} (mol/mol)
Depth (m)
0
−1000
−2000
−3000
−4000
−5000
−6000
−7000
0 5 10 15 20 25 30
16:1
Mean NO_3/PO_4^{2-}
> 500 m = 14.76
(C)
Indian Ocean
NO_3/PO_4^{2-} (mol/mol)
Depth (m)
0
−1000
−2000
−3000
−4000
−5000
−6000
−7000
0 5 10 15 20 25 30
16:1
Mean NO_3/PO_4^{2-}
> 500 m = 14.32
Present-day oceans
N_2
Atmosphere
Denitrification
N_2
Fixation
NO_3^-
NH_4^+
N:P ~ 14.7
N:P ~ 16.0
Ocean
(D

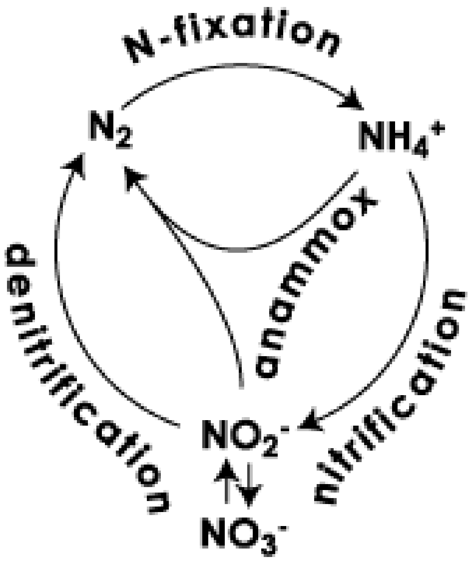
N^* on isopycnal surface (μmol kg^{-1})

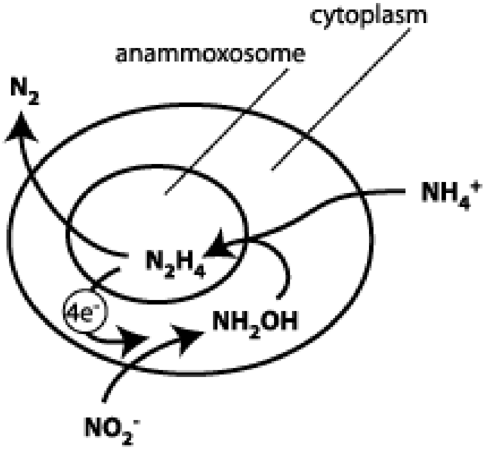


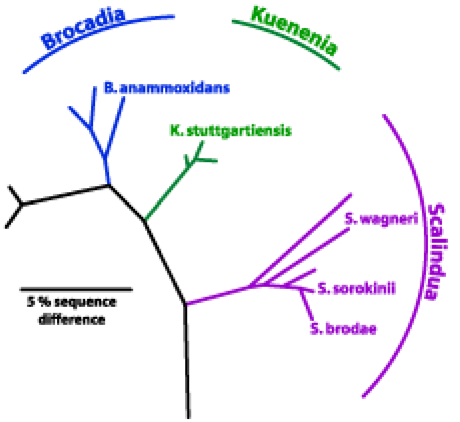
from Deutsch et.al., manuscript submitted to Global Biogeochemical Cycles

Anammox:
a new pathway

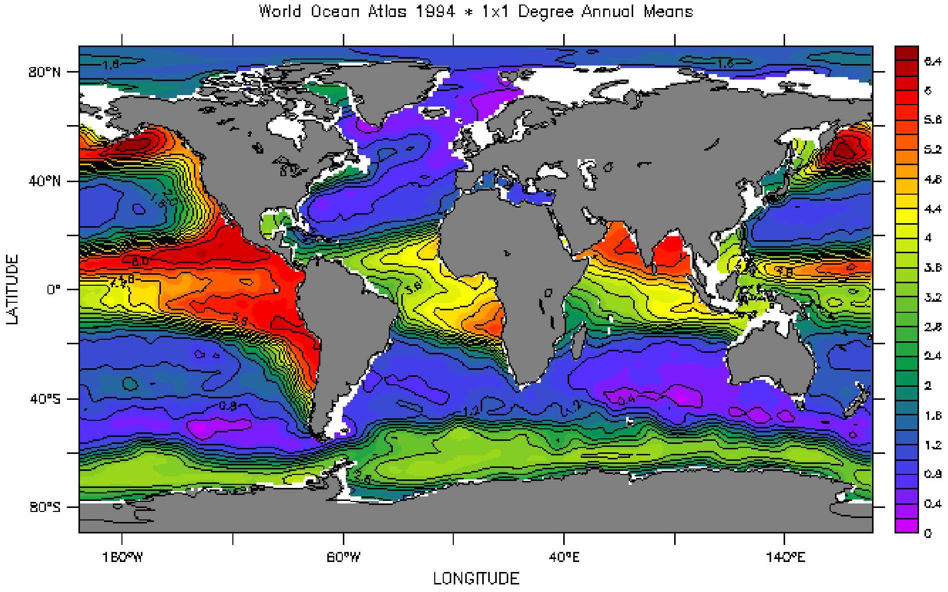
- First discovered: Sludge reactor 1995
  - Anaerobic oxidation of ammonium w/ nitrite
  - NO_2 + NH_4 -> N_2
  - Certain Planctomycete bacteria
    - autotrophs







Recent Observations



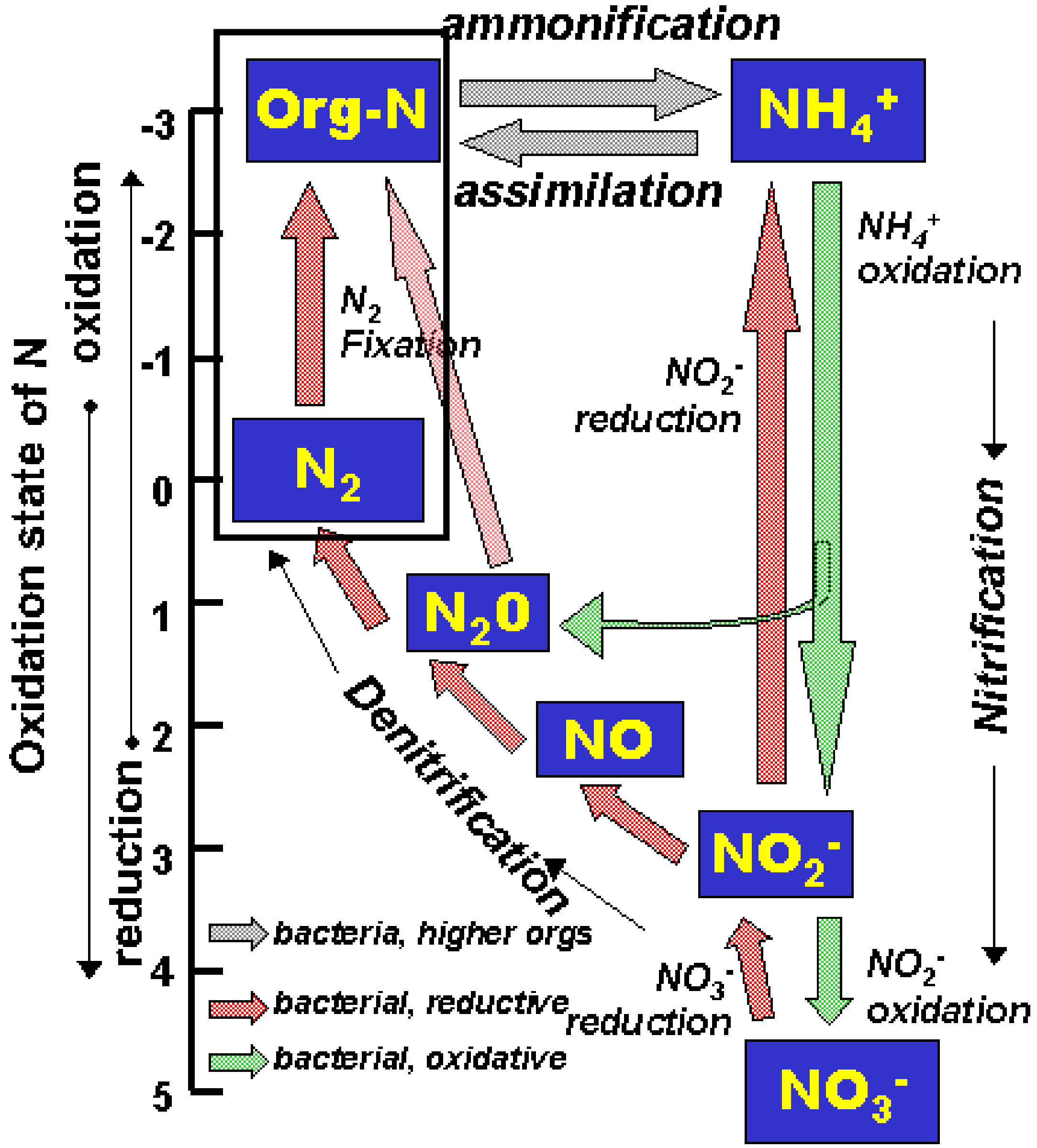
Apparent Oxygen Utilization (ml/l)

- Thamdrup & Dalsgaard 2002
  - Anammox in marine sediments
- Dalsgaard et al. Nature 2003
  - Golfo Dulce
- Kuypers et al. Nature- Black Sea
- Kuypers et al. PNAS 2005, Hamersley et al. 2008
  - Anammox in Benguela OMZ
  - Peru
  - No evidence for conventional denitrification
- Ward et al. 2009 – Denitrification in Arabian Sea
  - spatial differences among major OMZs?

Rates of Denitrification

Sample	mg $N*m^{-2}day^{-1}$	TgN /y
Water Column		
ETNP		10 - 230
ETSP		19 - 25
ET Atlantic		5
Indian Ocean		30
Total Pelagic		64 - 290
Benthic		
Estuaries	40	10 - 35
Pelagic sediments	0.01 - 6	
Shelf		50 - 75
Total Benthic		60 - 110
TOTAL		124 - 400

Completing the cycle

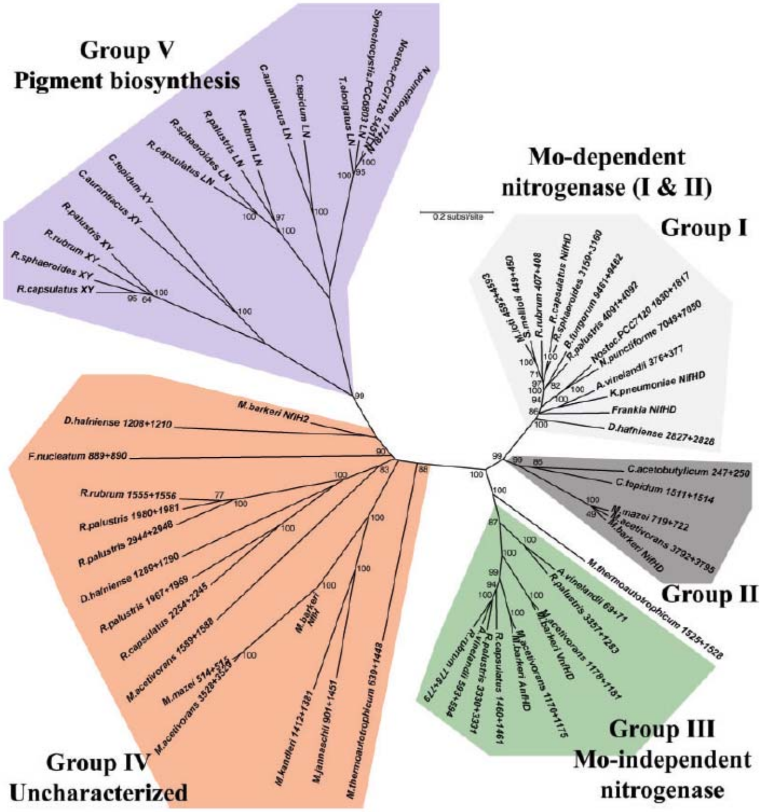


Nitrogen Fixation

- $N_2 \xrightarrow{\text{Nitrogenase}} 2\ NH_3 \longrightarrow$ amino and nucleic acids
- $C_2H_2 \longrightarrow C_2H_4$ (Acetylene reduction assay)
- Enzyme: Nitrogenase, (O_2 sensitive)
- Function: Biosynthetic, N Source
- $N_2 + 8H^+ \overset{8e^-}{\text{-->}} 2NH_3 + H_2$
- Energetically costly: $G_o{}' = + 340$ kJ/ mol
- ~ 15 ATP per mole of N_2

Nitrogen Fixation

- **Occurrence: physiologically and genetically diverse prokaryotes including Archaea**
- **Conventional N_2ase- Mo dependent**
- **Alternative nitrogenases (Fe, V, streptomyces)**
- **Ecological Function: Primary input of combined N into the Biosphere**
  - **New N: promotes uptake of atm CO_2 in the oligotrophic ocean**



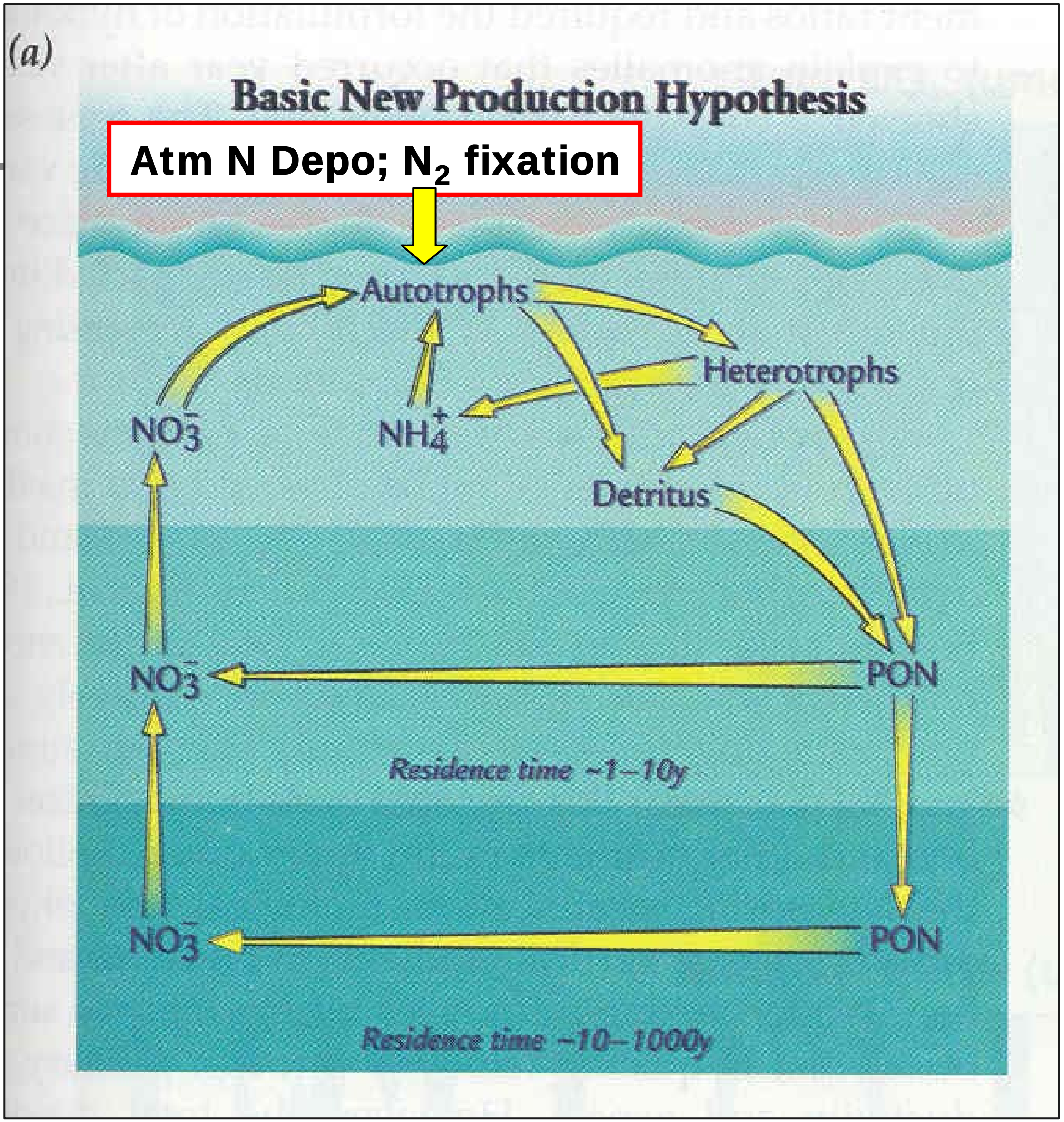
⌘*nif*H Phylogeny

(from Raymond et al. 2004)

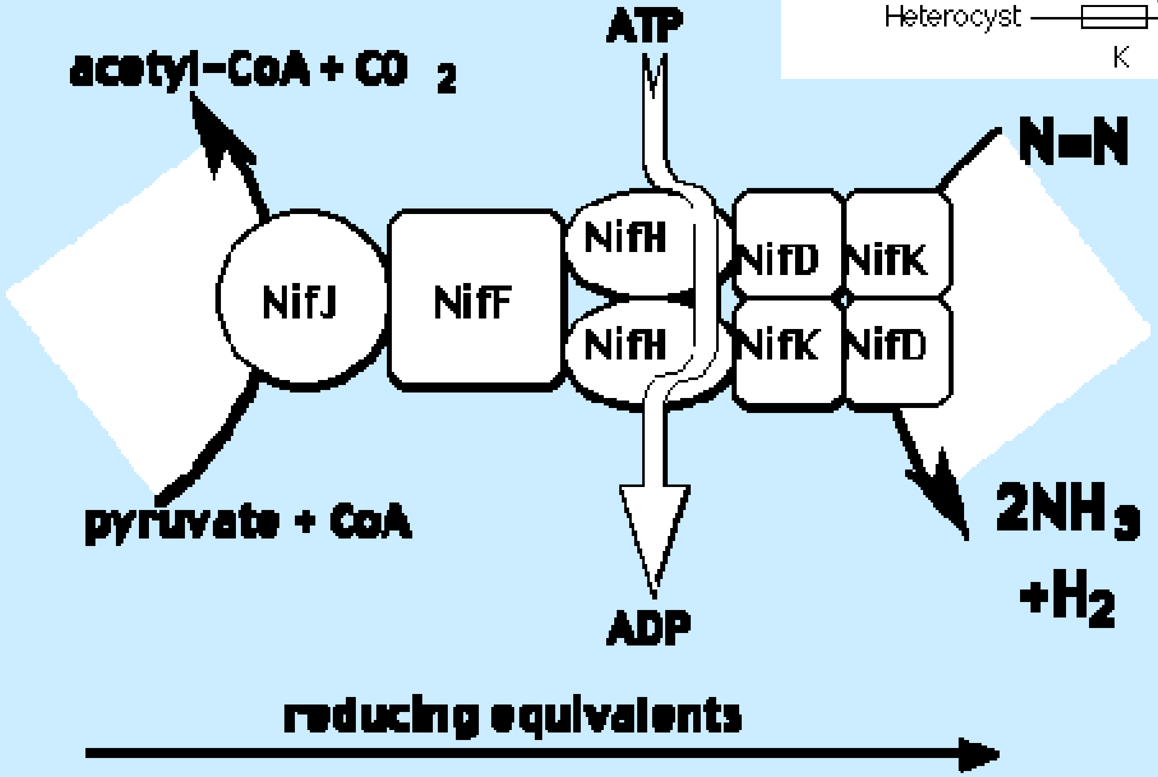
⌘*16sRNA similar*

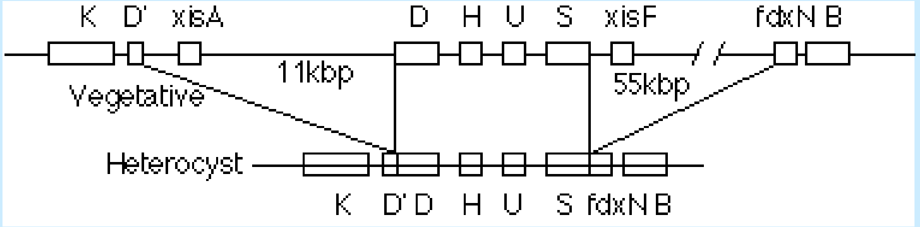
Traditional Paradigm of Ocean N Cycle

“Atmospheric Deposition and N_2 fixation also sources of “New” N.



NITROGENASE





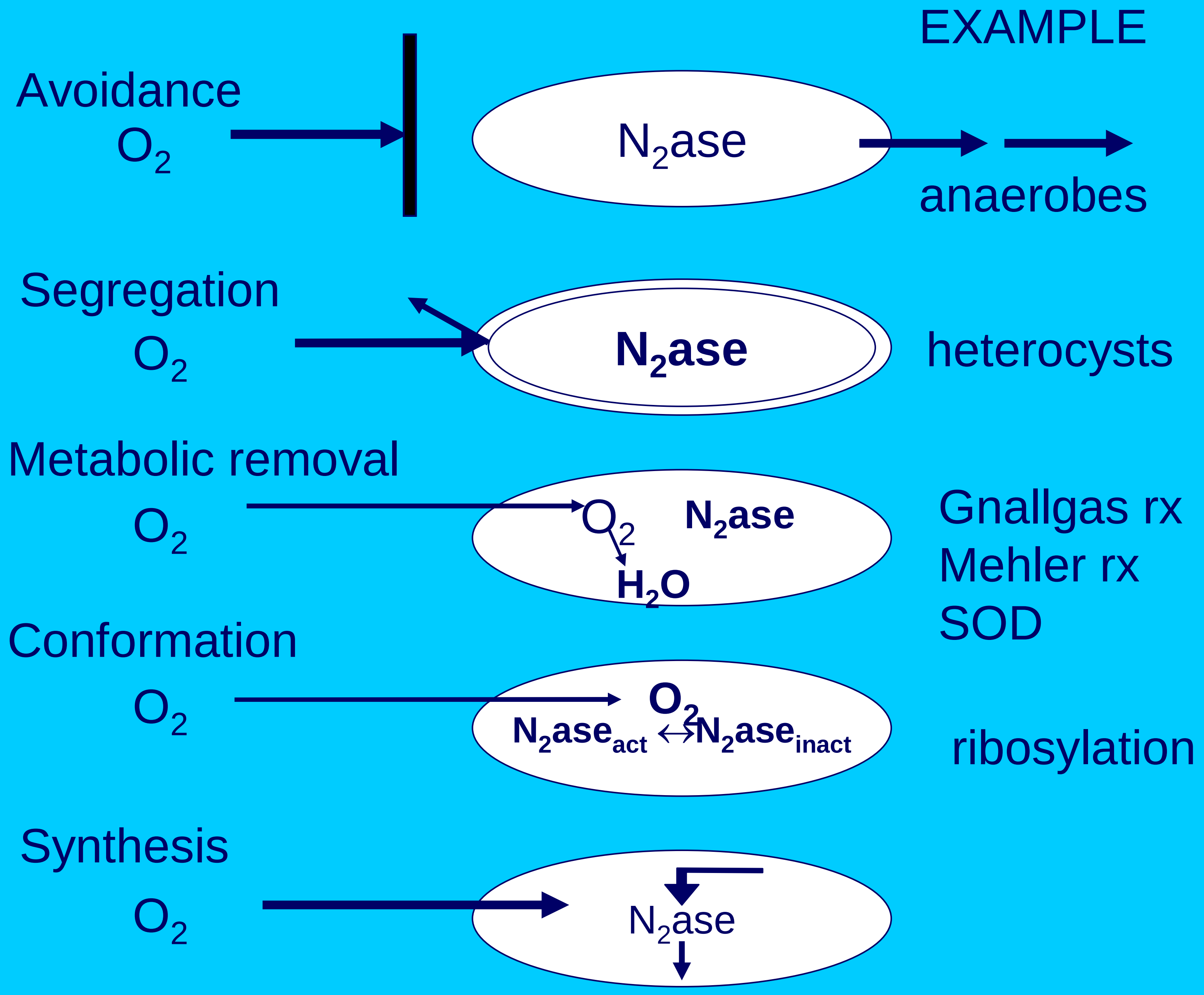
- Four component enzyme complex: Mo-Fe dimer (nitrogenase) & Fe-S dimer (dinitrogenase reductase)
  - Highly O_2 sensitive
  - Highly conserved gene cluster with structural (nifHDK) and regulatory operons: protein function highly conserved

Oxygen Issues

- All nitrogenases - O_2 sensitive
  - O_2 directly inhibits activity of both components
  - coarse genetic control by O_2 in many diazotrophs

- Oxygen-forming photoautotrophs-
  - Incompatable processes

- Strategies:



EXAMPLE

Avoidance

O_2

N_2ase

anaerobes

Segregation

O_2

N_2ase

heterocysts

Metabolic removal

O_2

O_2 **N_2ase**

H_2O

Gnallgas rx

Mehler rx

SOD

Conformation

O_2

O_2

$N_2ase_{act} \leftrightarrow N_2ase_{inact}$

ribosylation

Synthesis

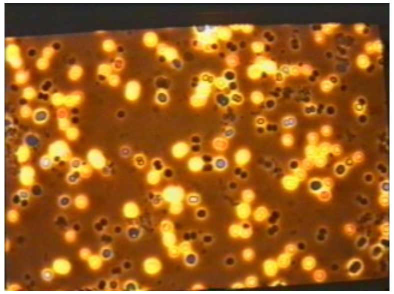
O_2

N_2ase

Cyanobacteria

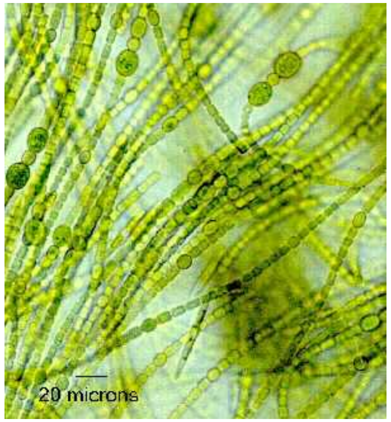
- Photosynthetic prokaryotes
  - Fossils back to about 3.2 BYA
- Invented oxygenic photosynthesis
  - Oxygenation of atmosphere- 2.2 BYA
  - Progenitors of chloroplasts
- Many fix atmospheric nitrogen
- Role in atmospheric CO_2 regulation

Cyanobacterial N_2 Fix Strategies

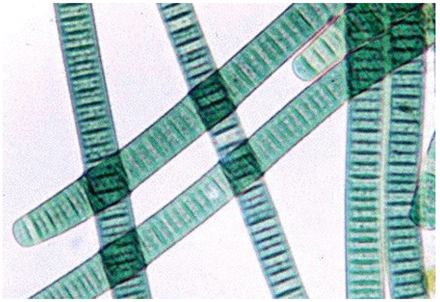


⌘ **Coccoid**

- Coccoids, Filamentous forms
  - Temporal segregation
    - Photosynthesize in light/ daytime
    - Fix nitrogen in dark/ nighttime
- Heterocystous forms
  - Compartmentalize nitrogenase
    - Can simultaneously fix C and N

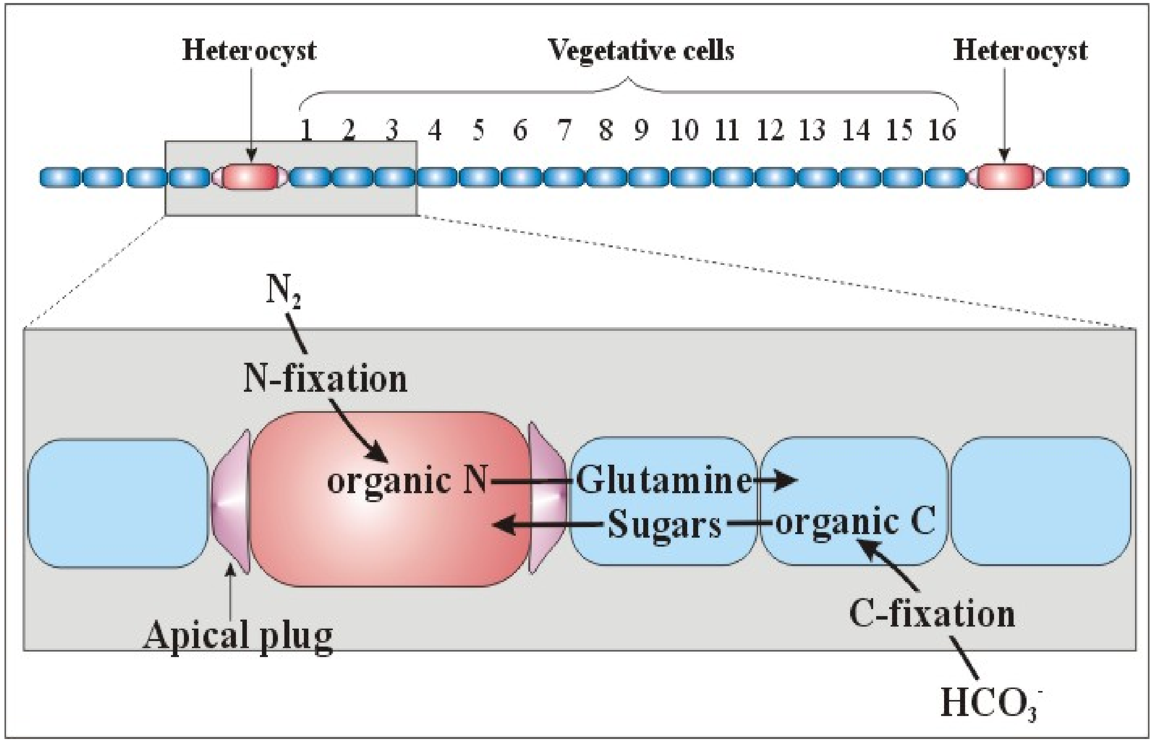


⌘ **Heterocystous**



⌘ **Filamentous**

Heterocysts



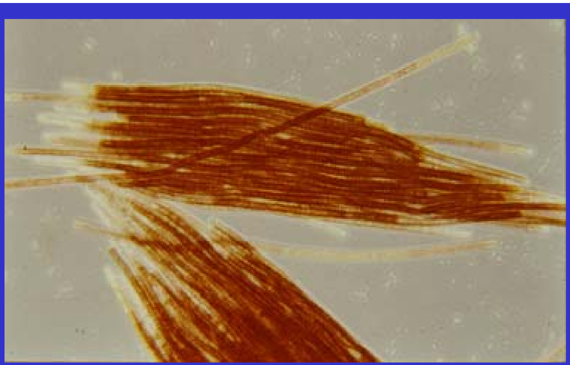
Basic structure of an *Anabaena* filament. N_2 fixation occurs in heterocysts, while vegetative cells fix C coupled to oxygenic photosynthesis.

The exchange of N and C between cells is represented.

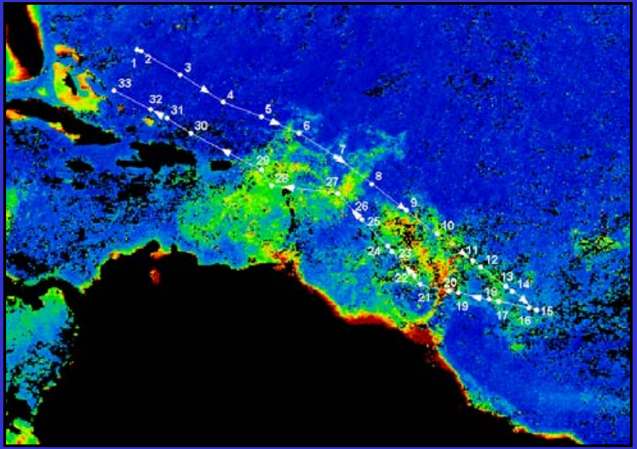
- No photosystem II in heterocyst

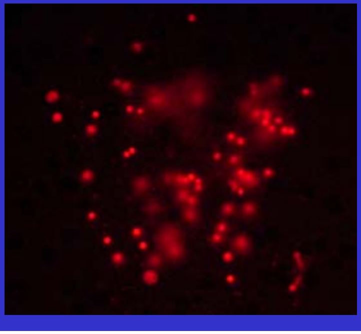
Marine Diazotrophs

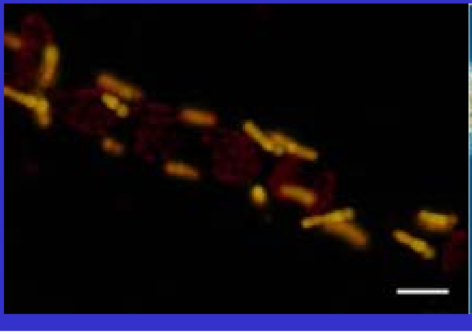
Functional groups of diazos

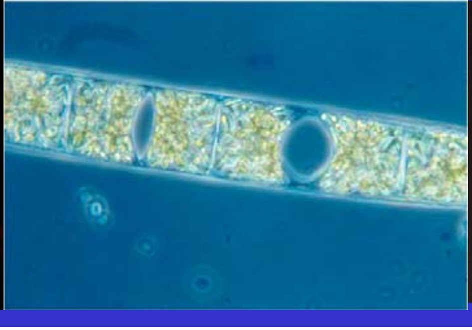


- **Filamentous: *Trichodesmium* and relatives**
- **Diazotrophic diatom associations (DDAs) *(e.g. Richelia / Hemiaulus)***
- **Coccoid cyanobacteria**
- **Bacterioplankton- α & γ proteobacteria**
- **Copepod gut flora**
- **Deep sea / vents Archaea**

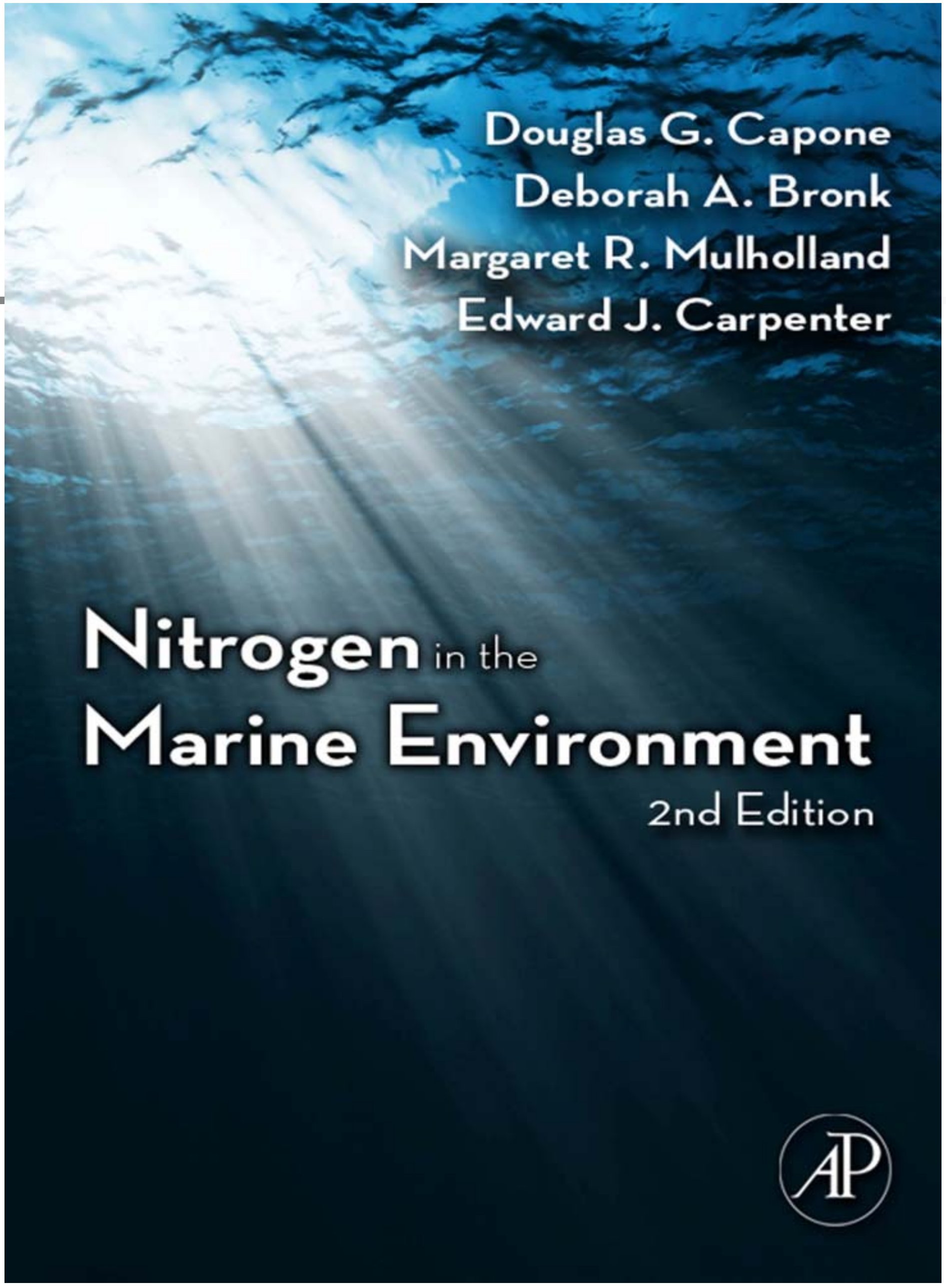








Commercial moment: Now at your local newsstand!



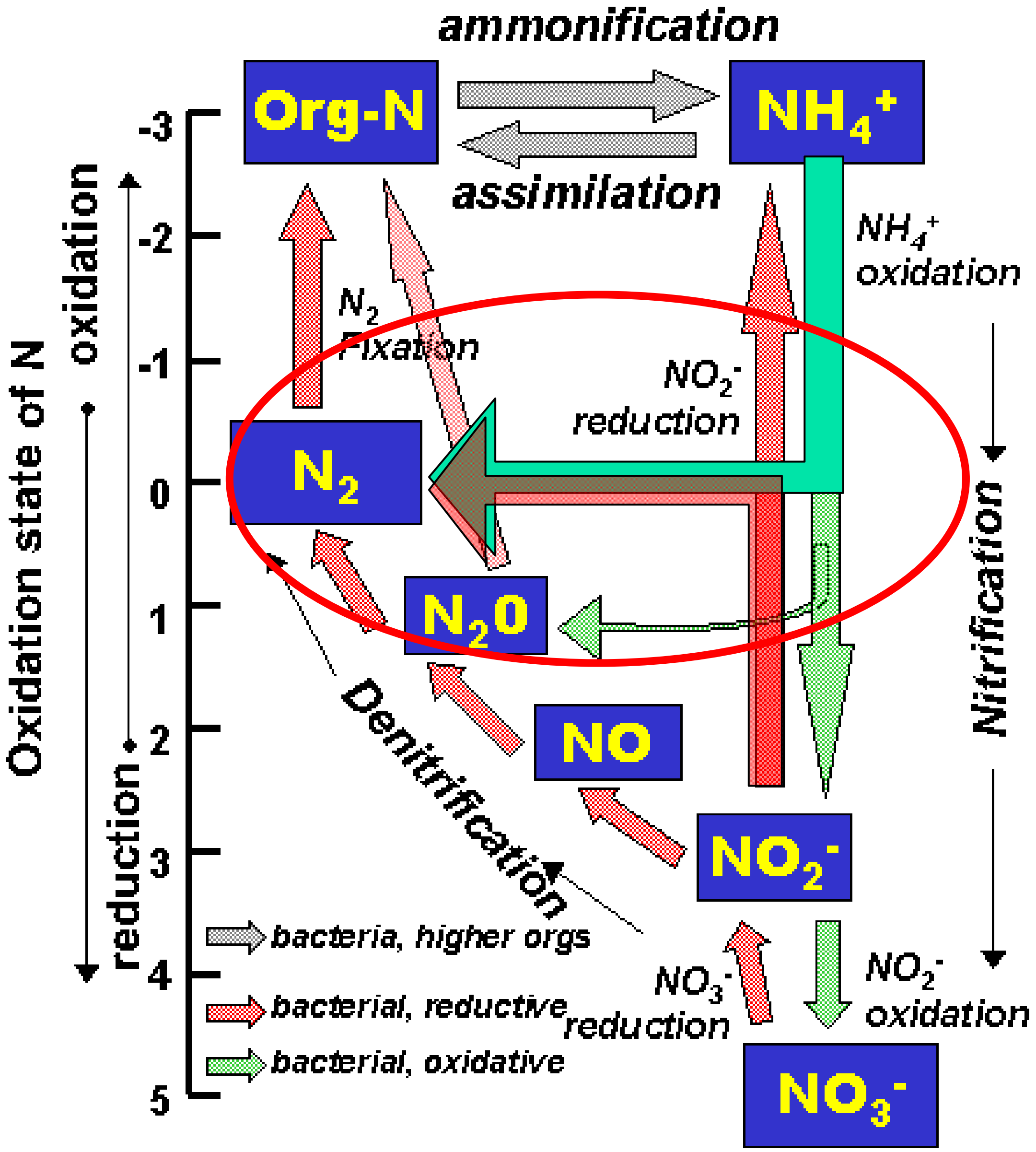
Douglas G. Capone
Deborah A. Bronk
Margaret R. Mulholland
Edward J. Carpenter

Nitrogen in the
Marine Environment
2nd Edition

Simple Stoichiometric Model

	C	N	P
Prod	106	16	1
Prod	6.6	1	
Regen		14.7	1

	C Fix 10^{15}g	C 10^{12} mol	N 10^{12} mol	P 10^{12} mol	N Tg N
Net	47.5	3958	597	37.3	7197
Export (33%)	16	1333	201	12.6	2424
Sequest	2				303
Regen		1333	185	12.6	
Difference			16		229
Current Water Column Denitrification					64-290



ammonification

assimilation

Org-N

NH_4^+

N_2

N_2O

NO

NO_2^-

NO_3^-

N_2 Fixation

NO_2^- reduction

NH_4^+ oxidation

Nitrification

Denitrification

NO_3^- reduction

NO_2^- oxidation

Oxidation state of N

oxidation

reduction

-3

-2

-1

0

1

2

3

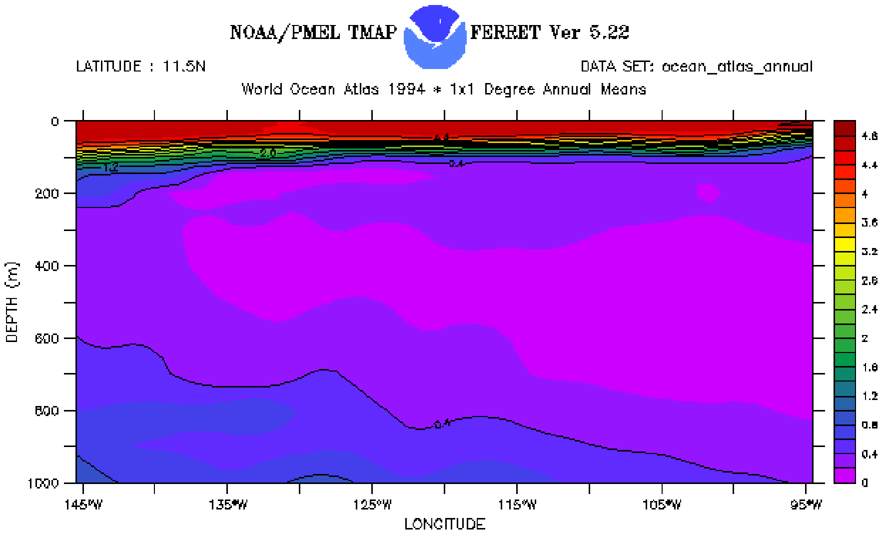
4

5

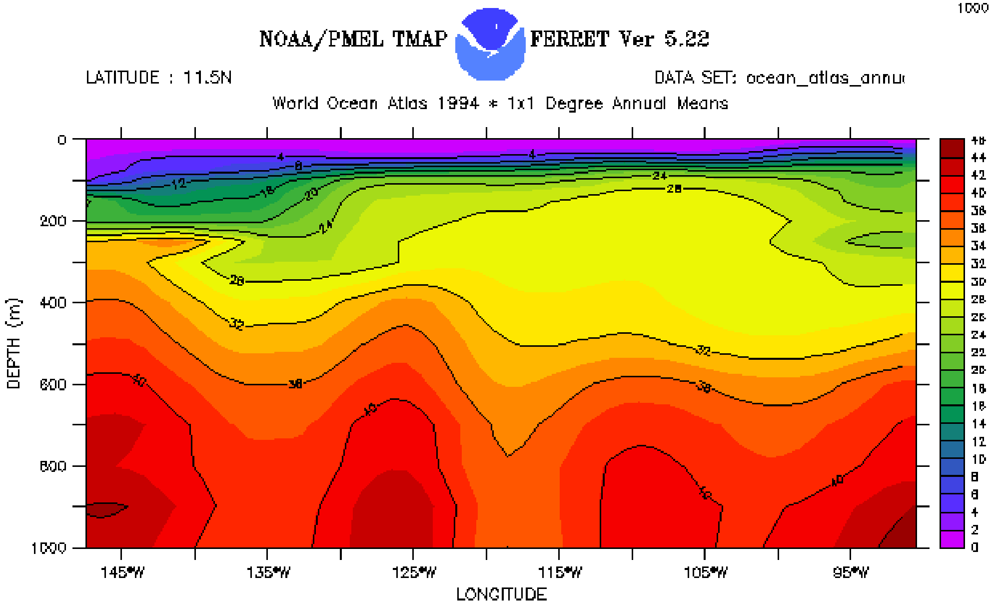
bacteria, higher orgs

bacterial, reductive

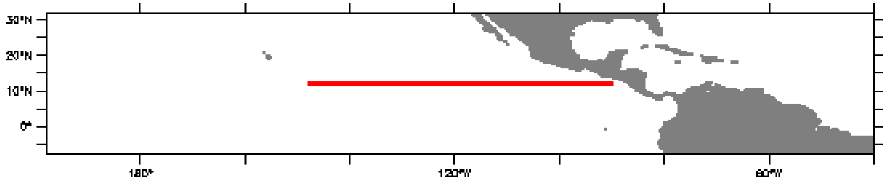
bacterial, oxidative



Dissolved Oxygen (ml/l)



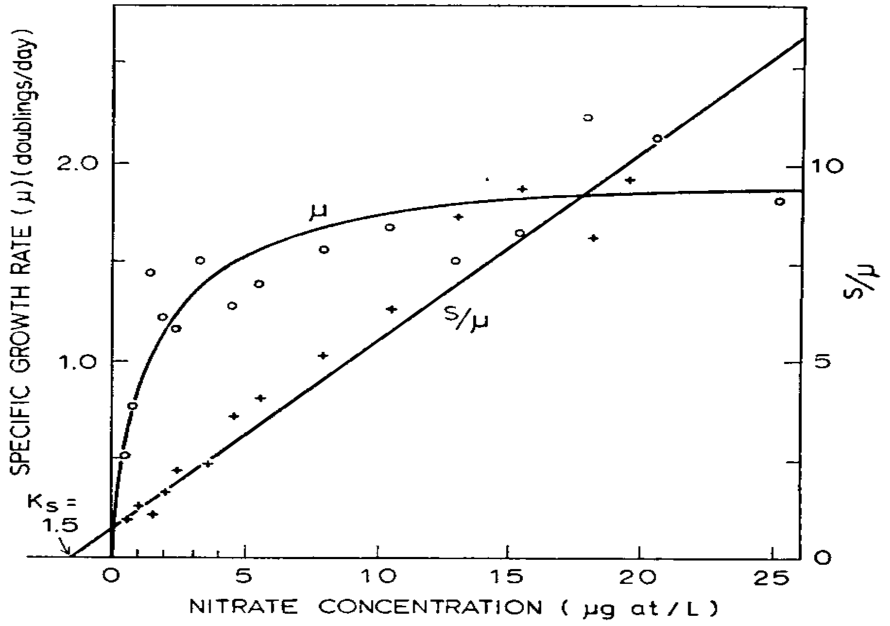
Nitrate (umol/l)

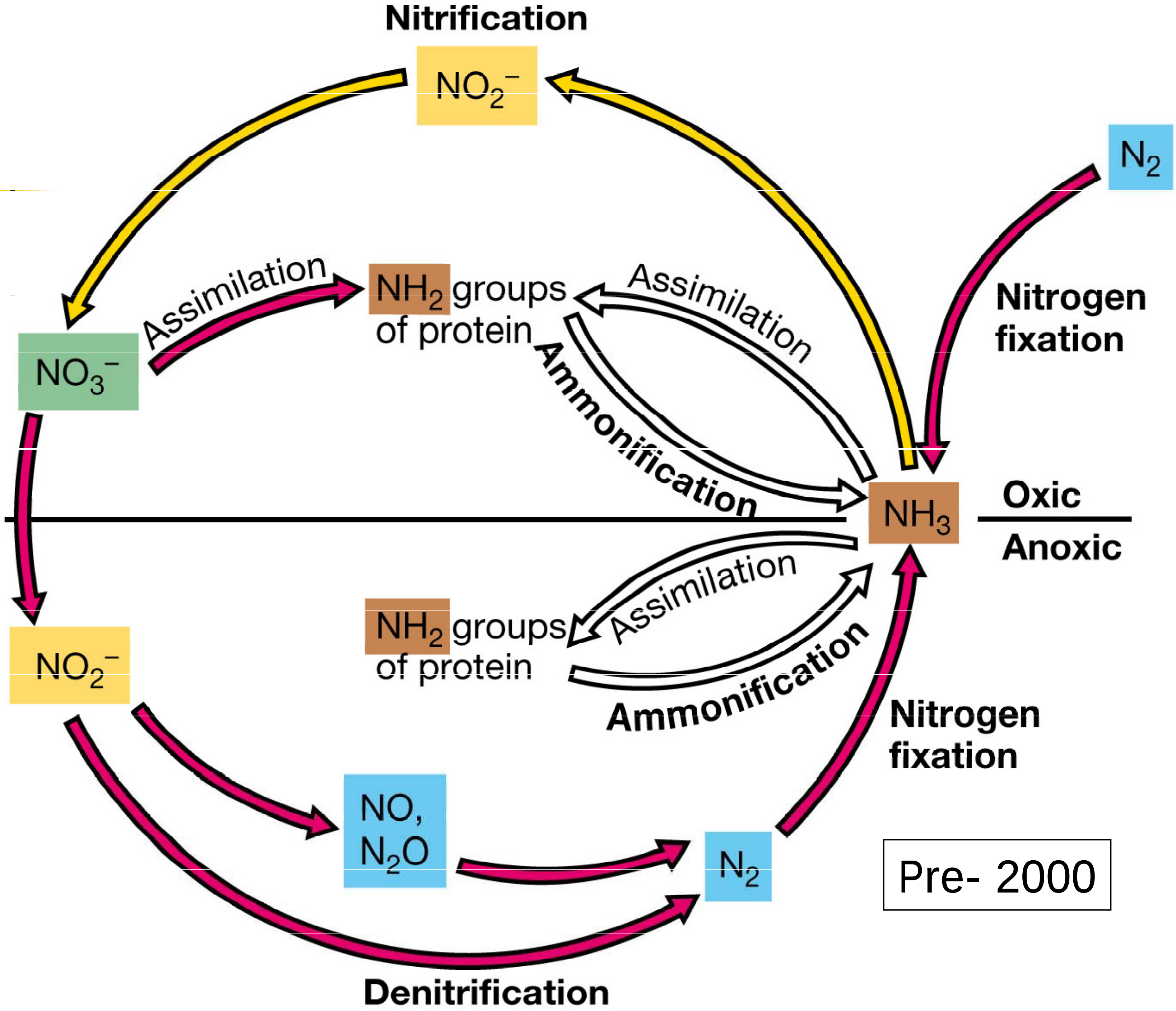


Nutrient Uptake

- Extend to growth (Monod):

$$\mu\ (1/t) = \frac{\mu_{max} * \quad [S]}{K_s + [S]}$$





Nitrification

NO_2^-

N_2

Assimilation

NH_2 groups of protein

Assimilation

NO_3^-

Ammonification

Nitrogen fixation

NH_3

Oxic

Anoxic

NH_2 groups of protein

Assimilation

NO_2^-

Ammonification

Nitrogen fixation

NO, N_2O

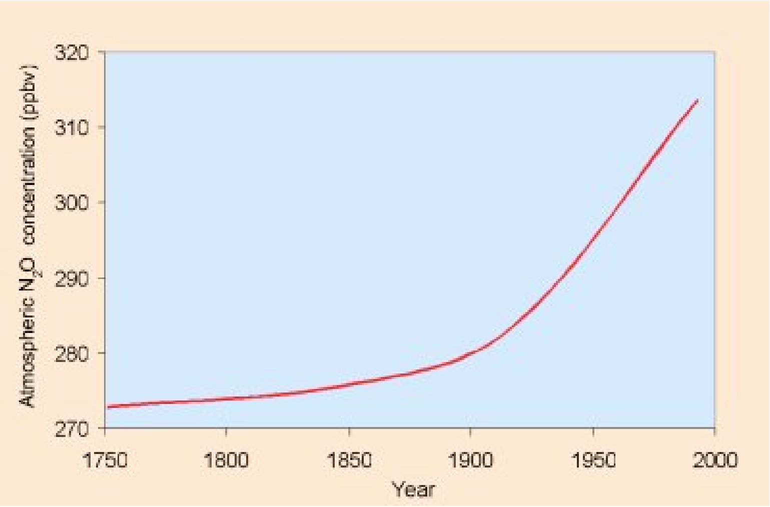
N_2

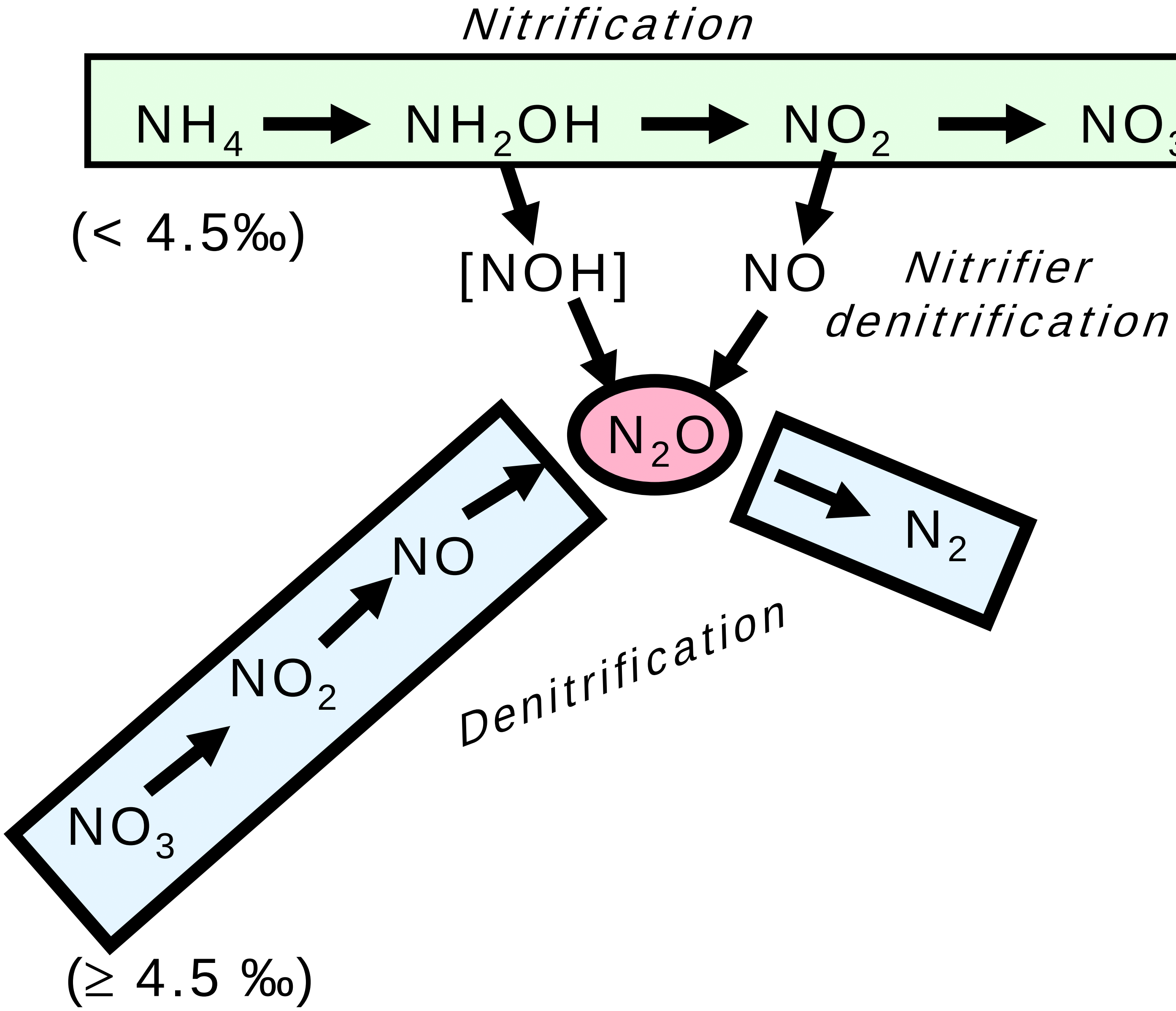
Denitrification

Pre- 2000

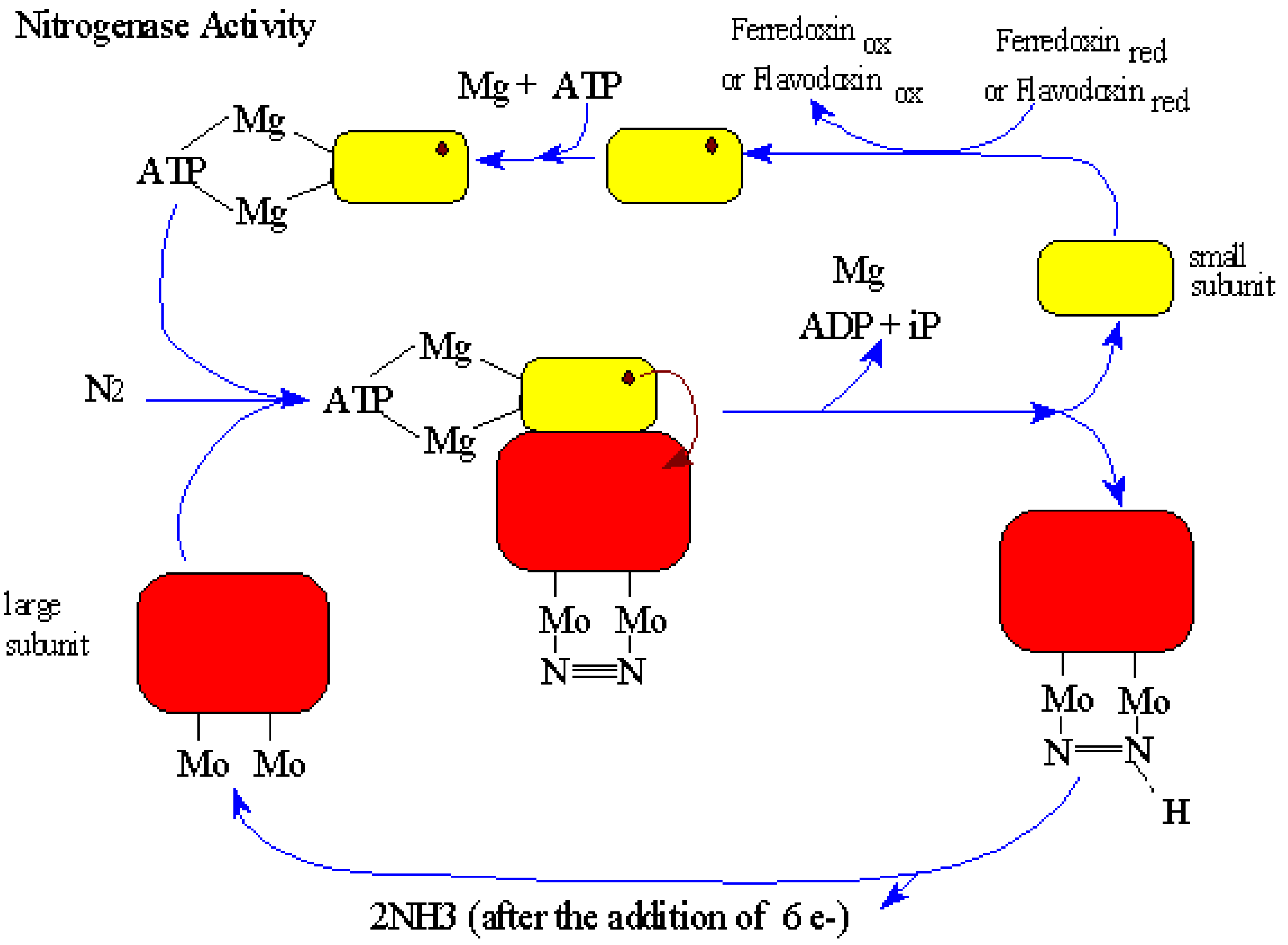
N_2O Production in the Sea

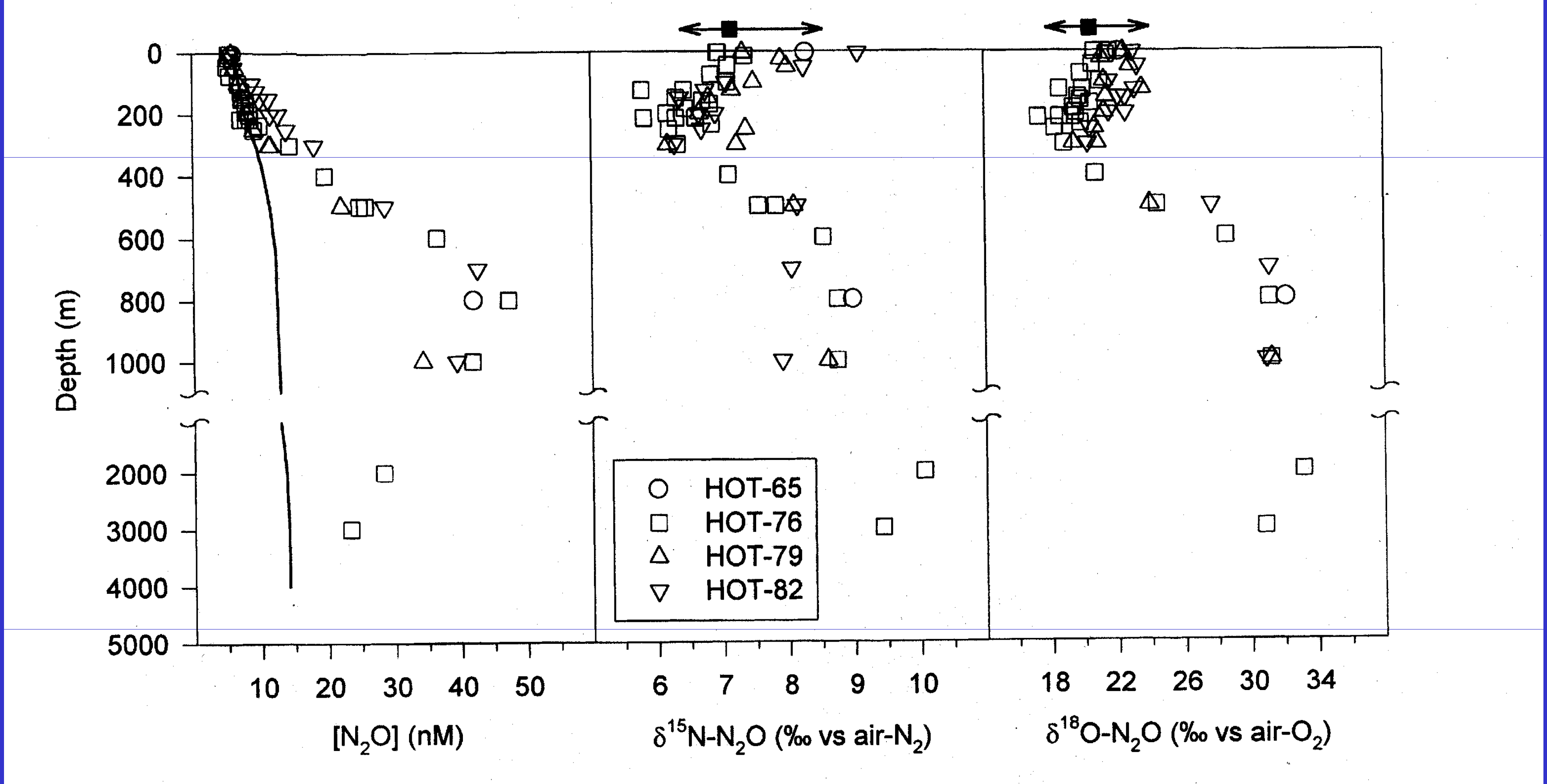
- Potent Greenhouse gas
  - Radiative forcing/ ozone depletion
    - About 300x as potent as CO_2
  - Increasing in atmosphere concentration - ppb
- Oceans are a slight source
  - Denitrification originally thought to be source
  - Nitrification also
  - denitrifying Nitrifiers??
  - Net Ocean flux ≈ 4 Tg; Global sum ≈ 16 Tg




Nitrification
NH₄ → NH₂OH → NO₂ → NO₃
(< 4.5‰)
[NOH]
NO
Nitrifier denitrification
N₂O
NO₃ → NO₂ → NO
N₂
Denitrification
(≥ 4.5 ‰)

Nitrogenase reaction





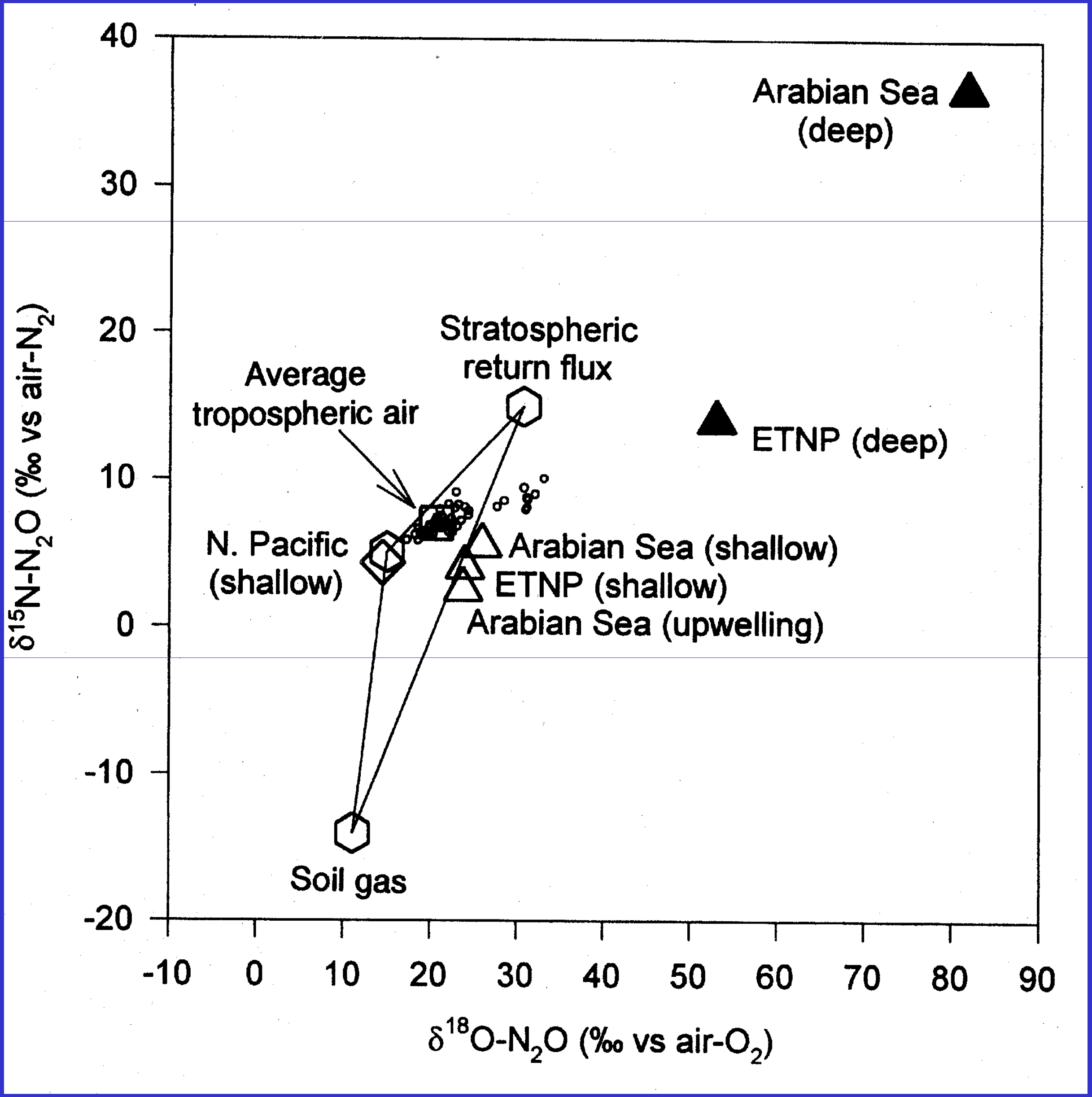
Source of “light” N_2O at base of euphotic zone: likely Nitrification

^{15}N Natural Abundance

- Natural abundance of ^{15}N = 0.3663 atm %
  - Slight natural variation through isotopic discrimination
  - Determined by isotope ratio mass spectrometry

- Many processes (e.g. $NO_{3\ \text{uptake}}$) discriminate strongly

- N_2 fixation effects little isotopic discrimination (relative to other N transformation pathways) and results in biomass with a $\delta^{15}N$ near “0”

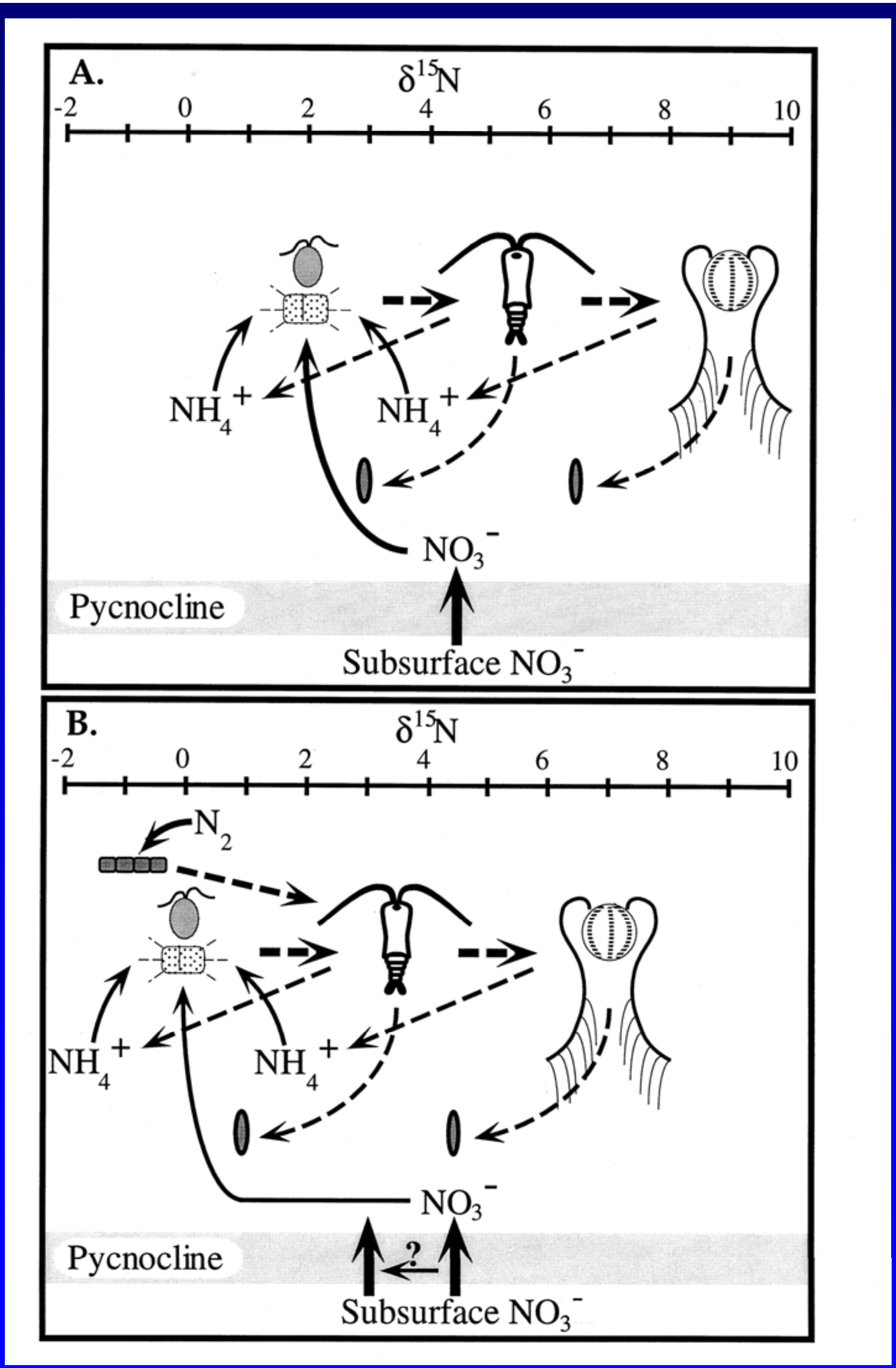

Arabian Sea (deep)
Stratospheric return flux
Average tropospheric air
ETNP (deep)
N. Pacific (shallow)
Arabian Sea (shallow)
ETNP (shallow)
Arabian Sea (upwelling)
Soil gas
40
30
20
10
0
-10
-20
-10
0
10
20
30
40
50
60
70
80
90
$\delta^{15}N-N_2O$ (‰ vs air-N_2)
$\delta^{18}O-N_2O$ (‰ vs air-O_2)

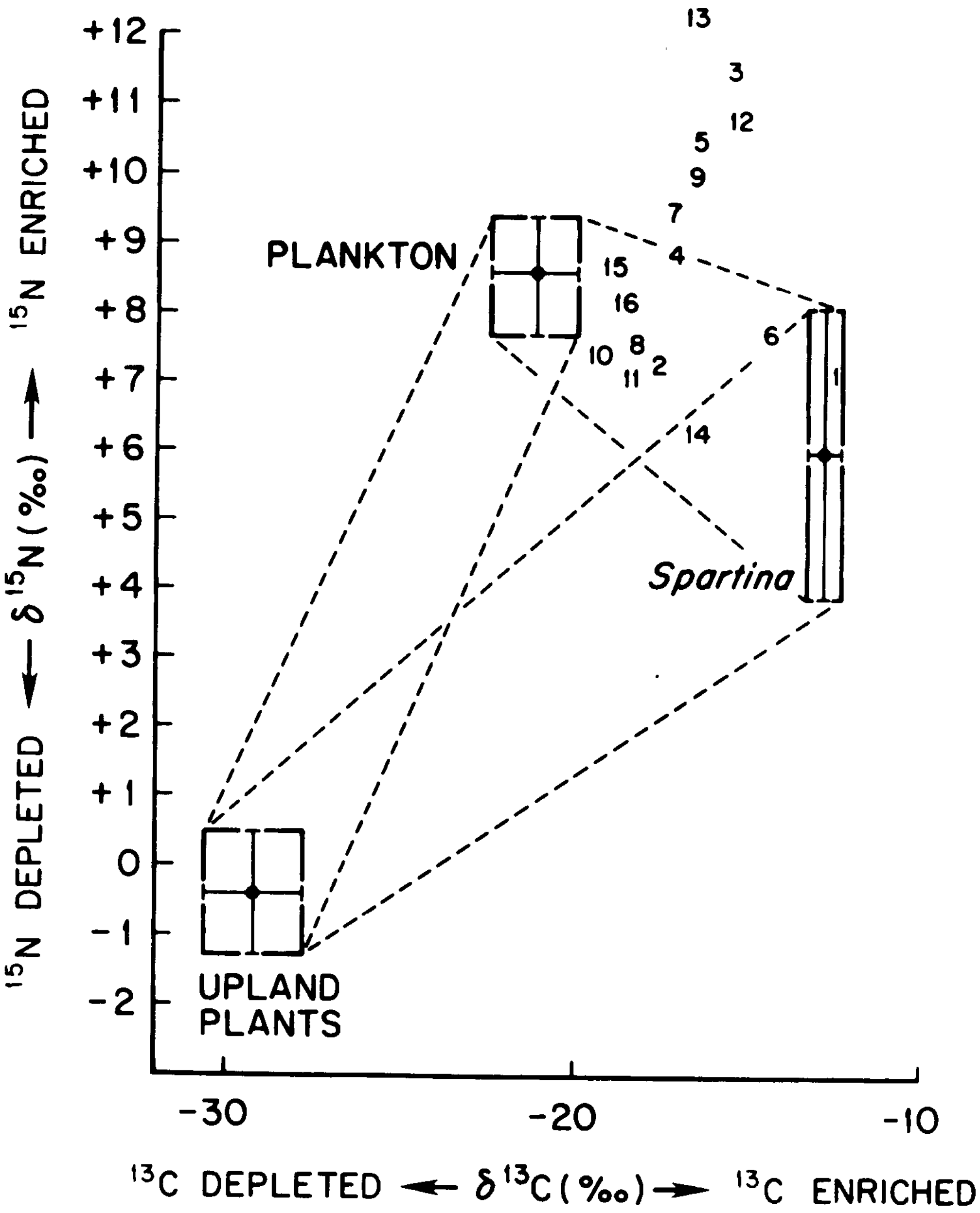
Nitrogen Isotope Fractionation

Process	Enrichment factor (ε) (w/r to products)	Comments
NO_3 assim: culture, field	**0 to -24 ‰ ; -10 ‰ -4 to -5 ‰**	**mM NO_3; µM NO_3 µM NO_3**
NH_4 assim: culture field	**0 to -15 ‰ ; -3 to -27 ‰ -10 ‰**	**mM NH_4; µM NH_4 µM NH_4**
Nitrification	**0 to -20 ‰**	**Concentration dependent**
Denitrification, pelagic Sediment	**-20 to -40 ‰ ~ 0 ‰**	**Concentration dependent**
N_2 Fixation	**~ -3 to +1 ‰**	**Little enzymatic fractionation**
Ammonification	**-3 to -5 ‰ (?)**	**Hard to isolate NH_4^+ at low levels**

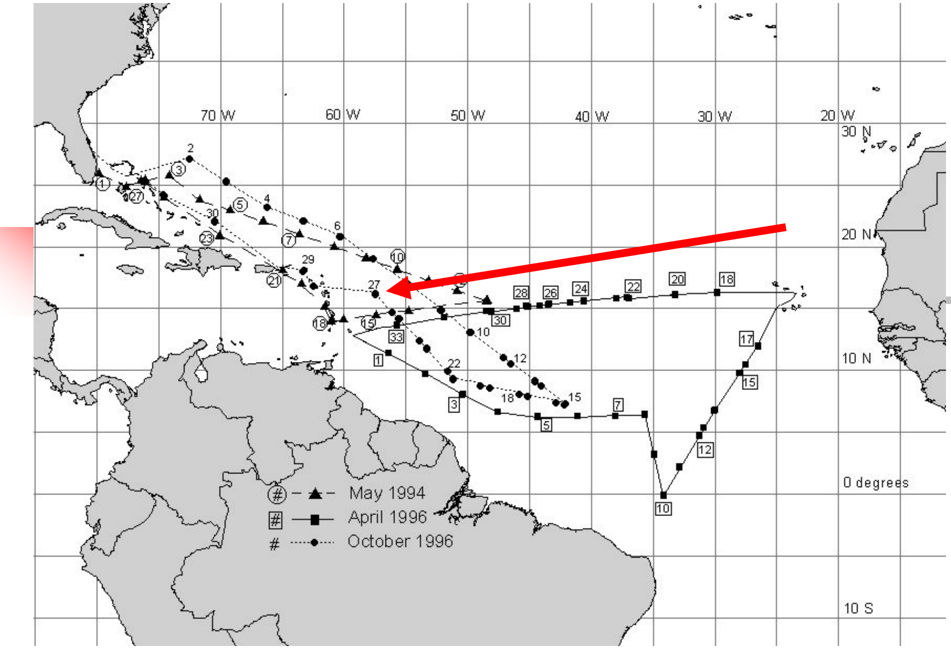
Classical food chain

N_2 fixation-based food chain

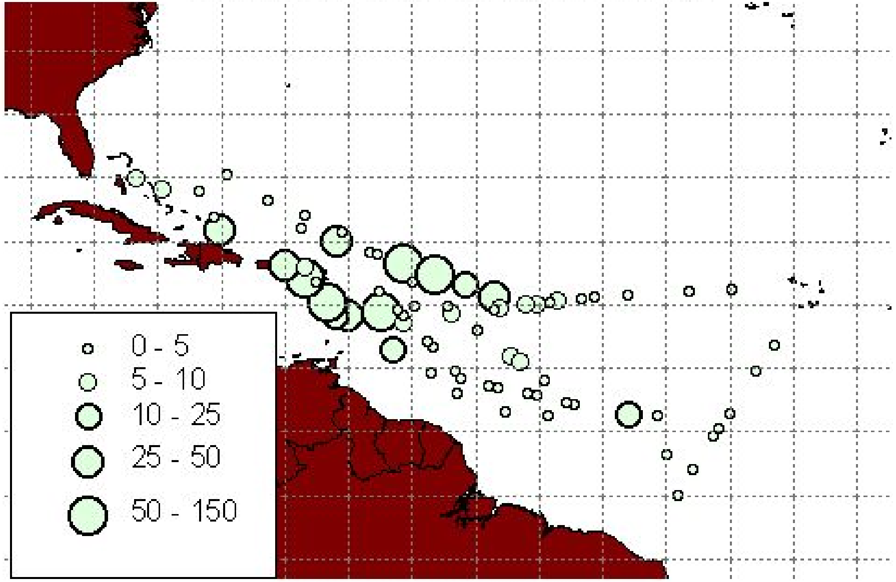


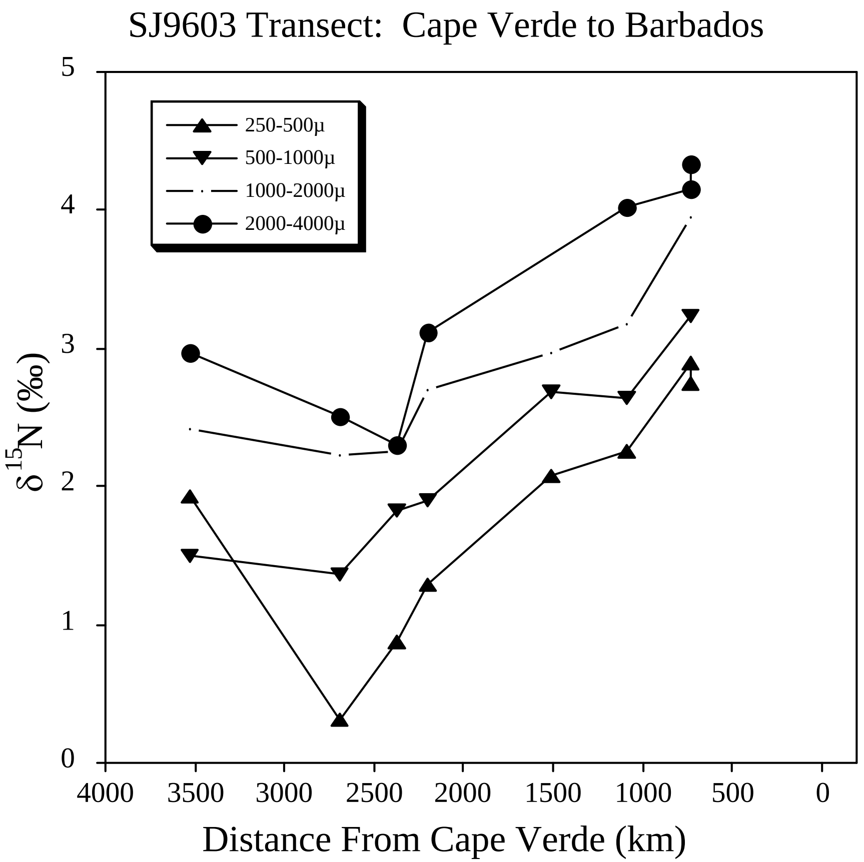

+12
+11
+10
+9
+8
+7
+6
+5
+4
+3
+2
+1
0
-1
-2
^{15}N DEPLETED ← δ^{15}N (‰) → ^{15}N ENRICHED
13
3
12
5
9
7
4
15
16
10
8
2
11
6
1
14
PLANKTON
Spartina
UPLAND PLANTS
-30
-20
-10
^{13}C DEPLETED ← δ^{13}C (‰) → ^{13}C ENRICHED

δ^{15}N in zooplankton Lighter moving towards western boundary: (Montoya, Carpenter & Capone, 2002)

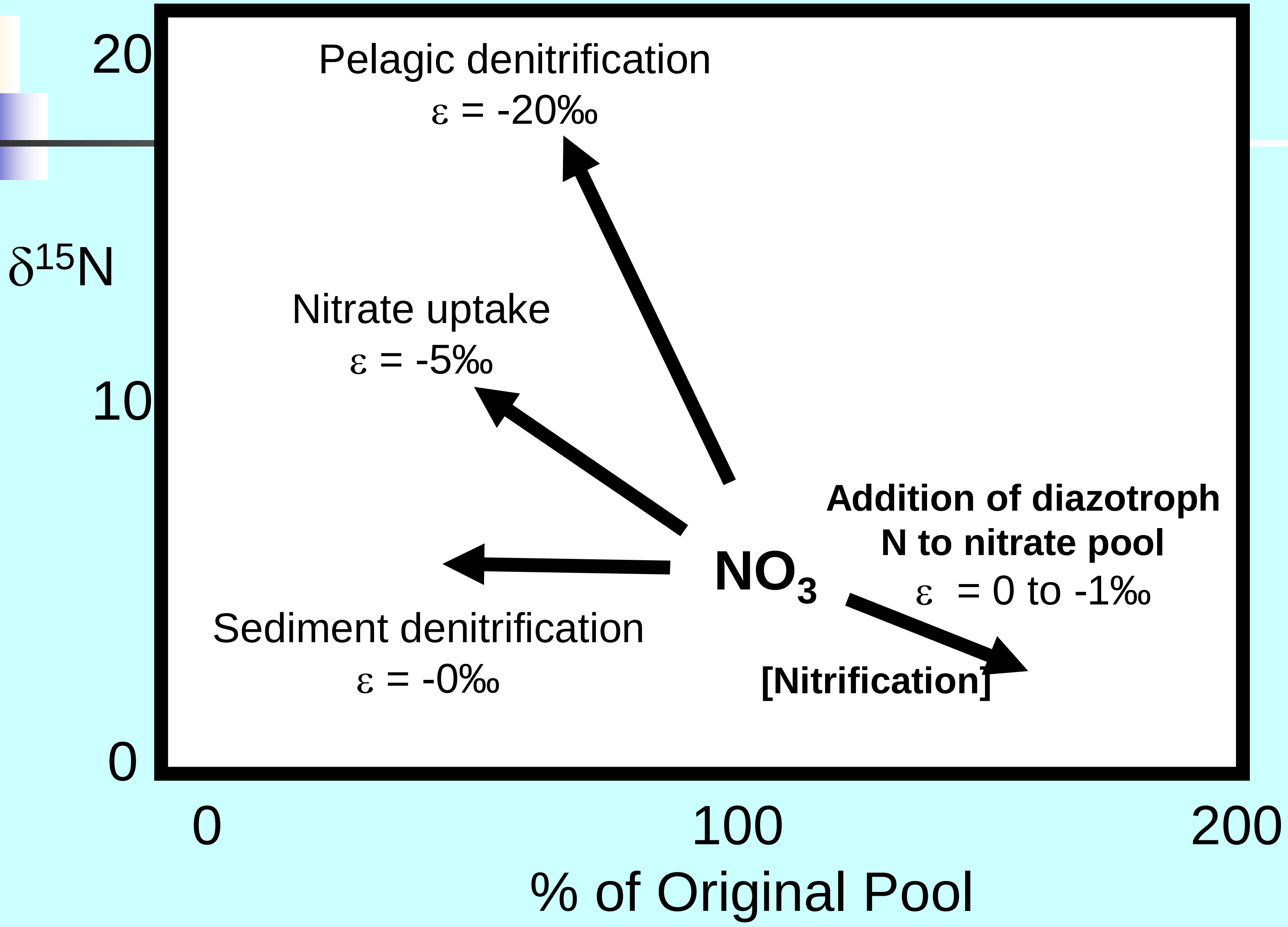


Trichodesmium Chlorophyll (mg/m2)





Global average = 4.5 to 5.0 ‰



Pelagic denitrification
ε = -20‰

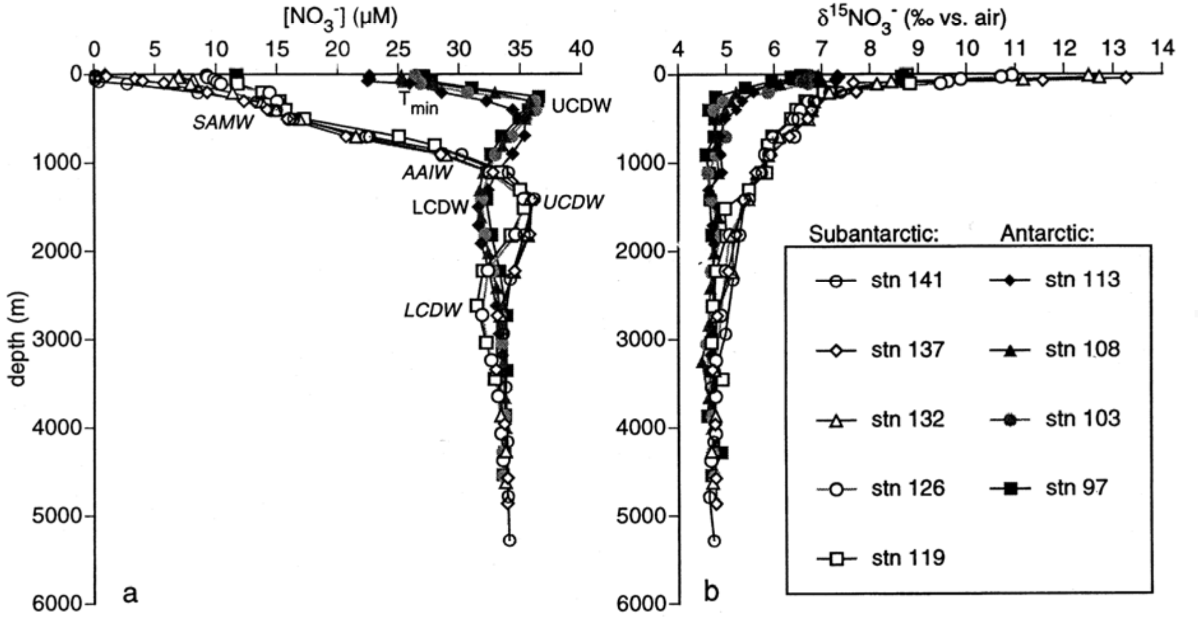
Nitrate uptake
ε = -5‰

Sediment denitrification
ε = -0‰

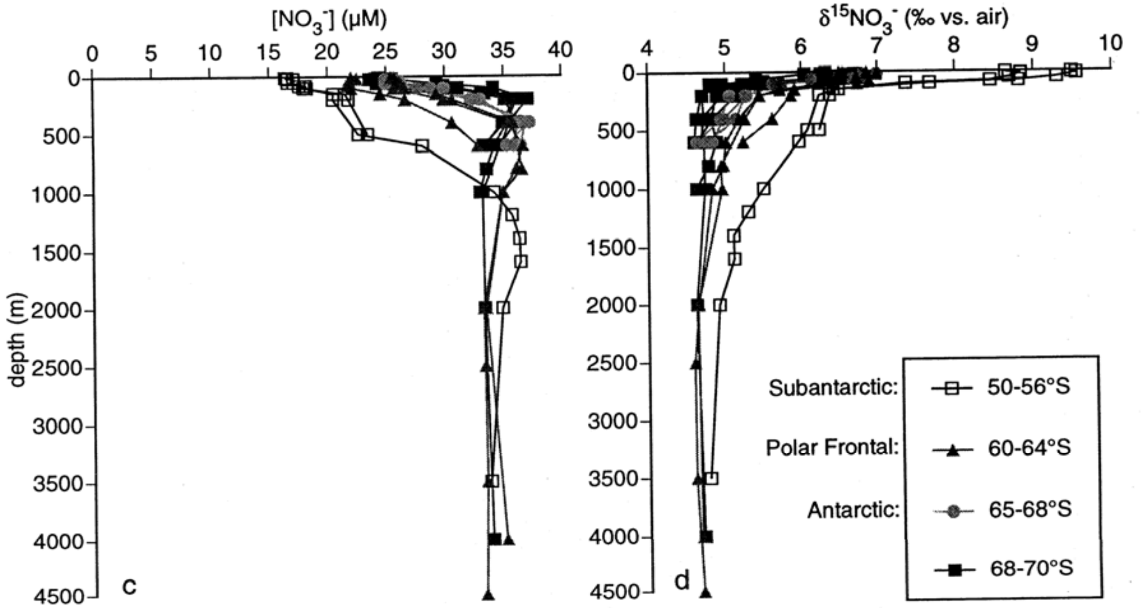
Addition of diazotroph N to nitrate pool
ε = 0 to -1‰

[Nitrification]

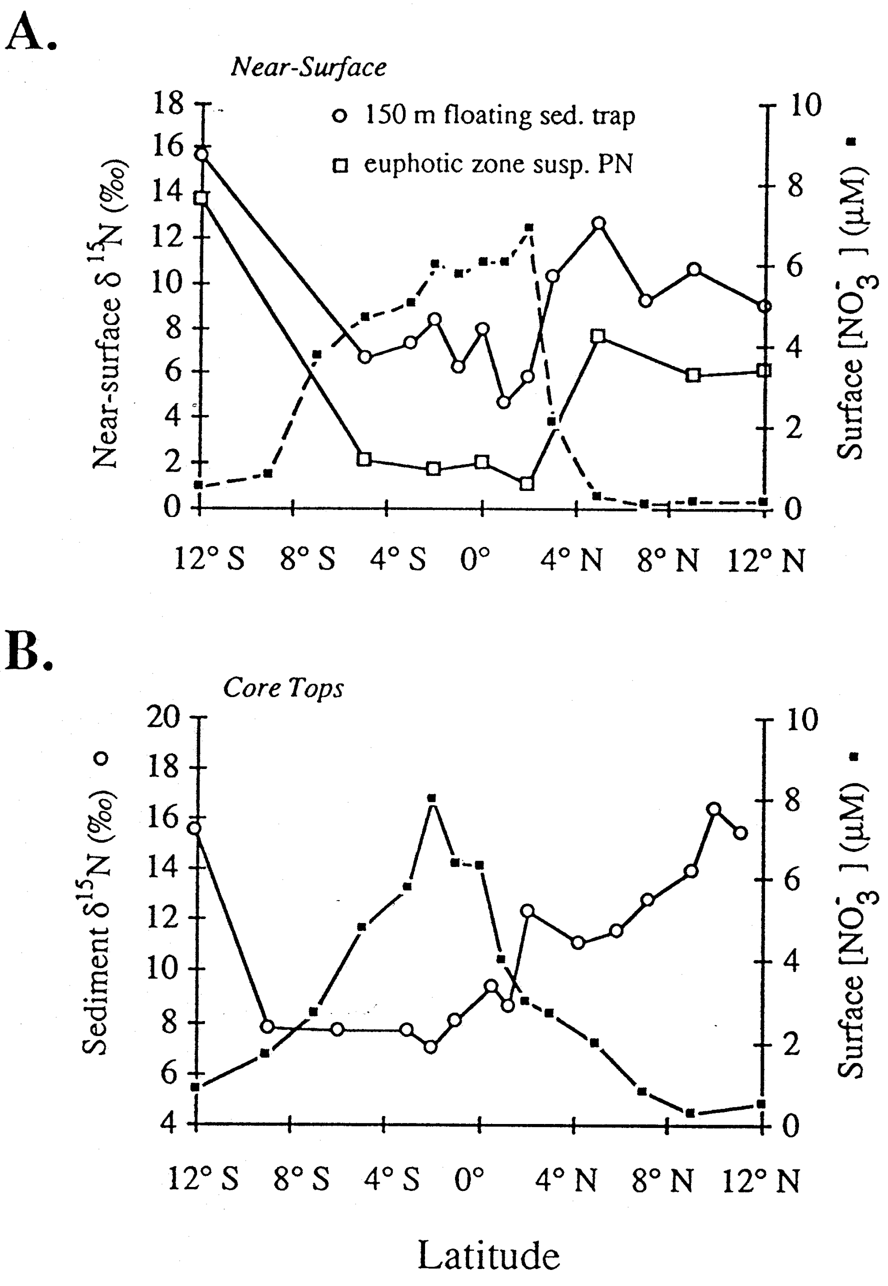
WOCE I9 depth profiles

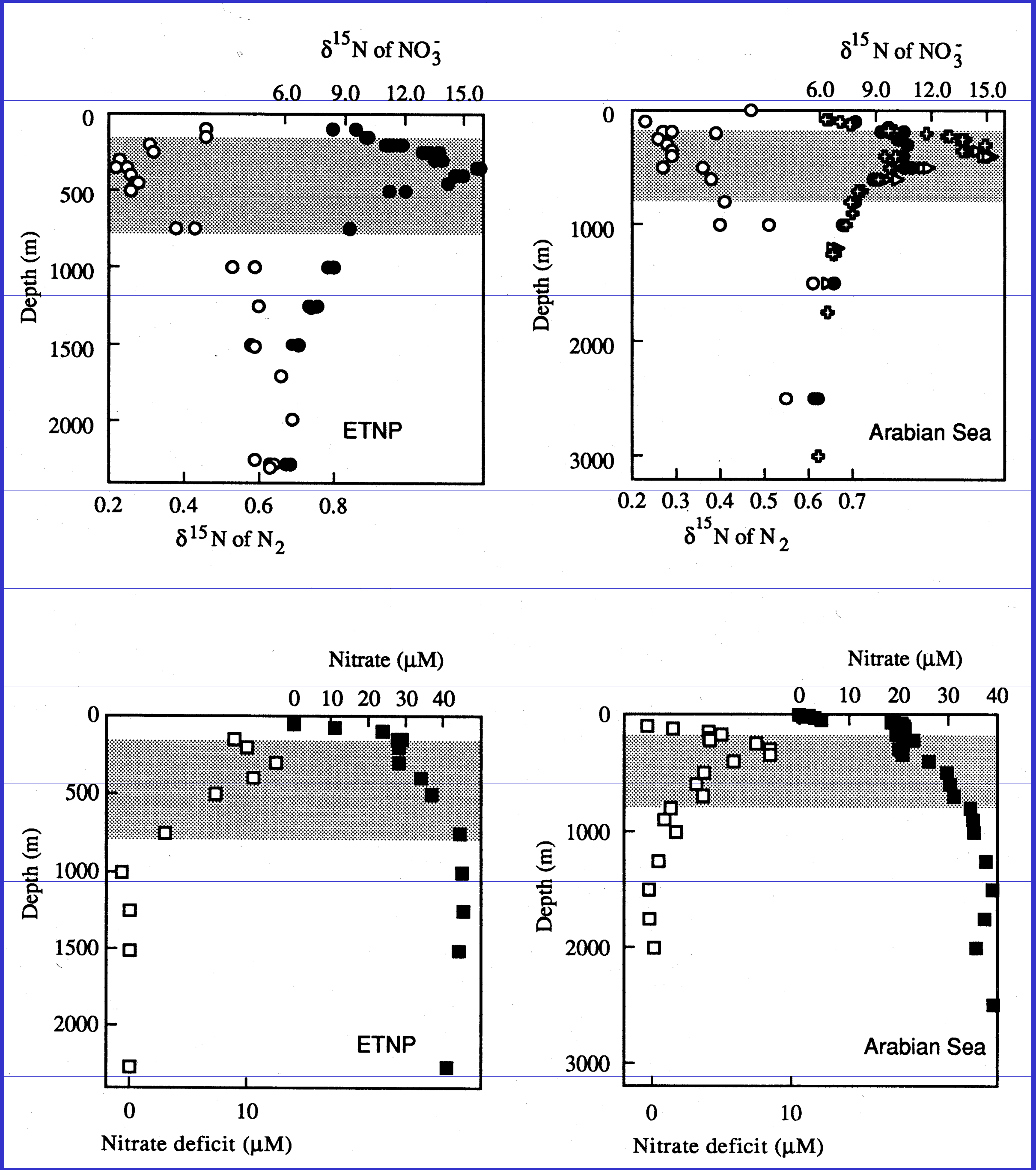


ANT XII/4 depth profiles



Equatorial Pacific





In tropical waters surface nitrate depletion may be accompanied by lighter nitrate pools

Subsurface waters depleted of O_2 may be very enriched in ^{15}N by denitrification

Geological Aspects

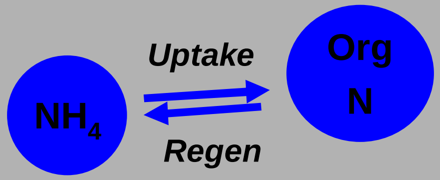
- Evolution of the N Cycle
- Glacial-Interglacial Dynamics
  - Relationships with C cycle
  - Fe inputs
- Pre-Industrial- Post Industrial

Evolution of the N Cycle

- Archaen Ocean- reducing, high NH_4^+
  - NH_4^+ uptake and regeneration
- Oxygenation of Atmosphere- 2.2 BYA
  - cyanobacteria
- Nitrification
- Denitrification
- N_2 Fixation- However….

⌘Evolution of N Cycle

1. Archean ocean, anoxic



NH_4

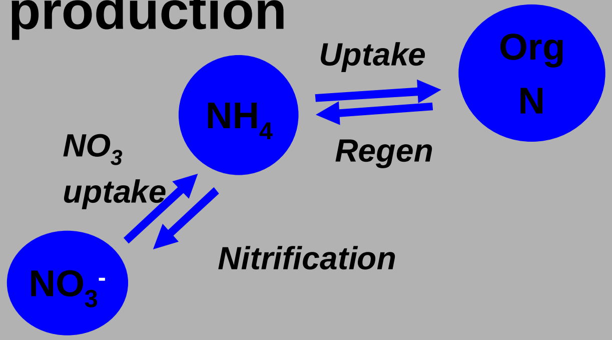
Uptake

Regen

Org N

2. Oxygenation, 2.2. BYA:

NO_3^- production



NH_4

Uptake

Regen

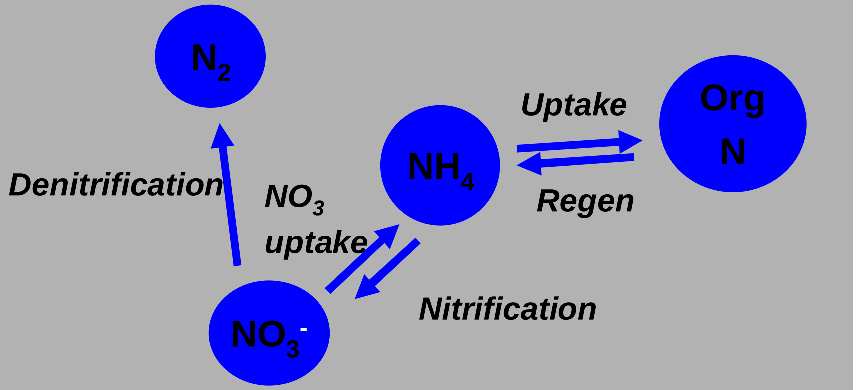
Org N

NO_3 uptake

Nitrification

NO_3^-

3. Denitrification



N_2

Denitrification

NO_3 uptake

NH_4

Uptake

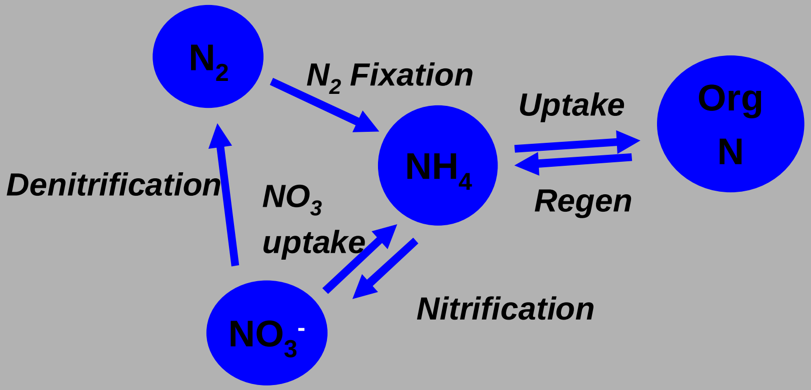
Regen

Org N

Nitrification

NO_3^-

4. N_2 Fixation



N_2

N_2 Fixation

Uptake

NH_4

Org N

Regen

Denitrification

NO_3 uptake

Nitrification

NO_3^-

Garvin et al. Science 323: 1045, 2009

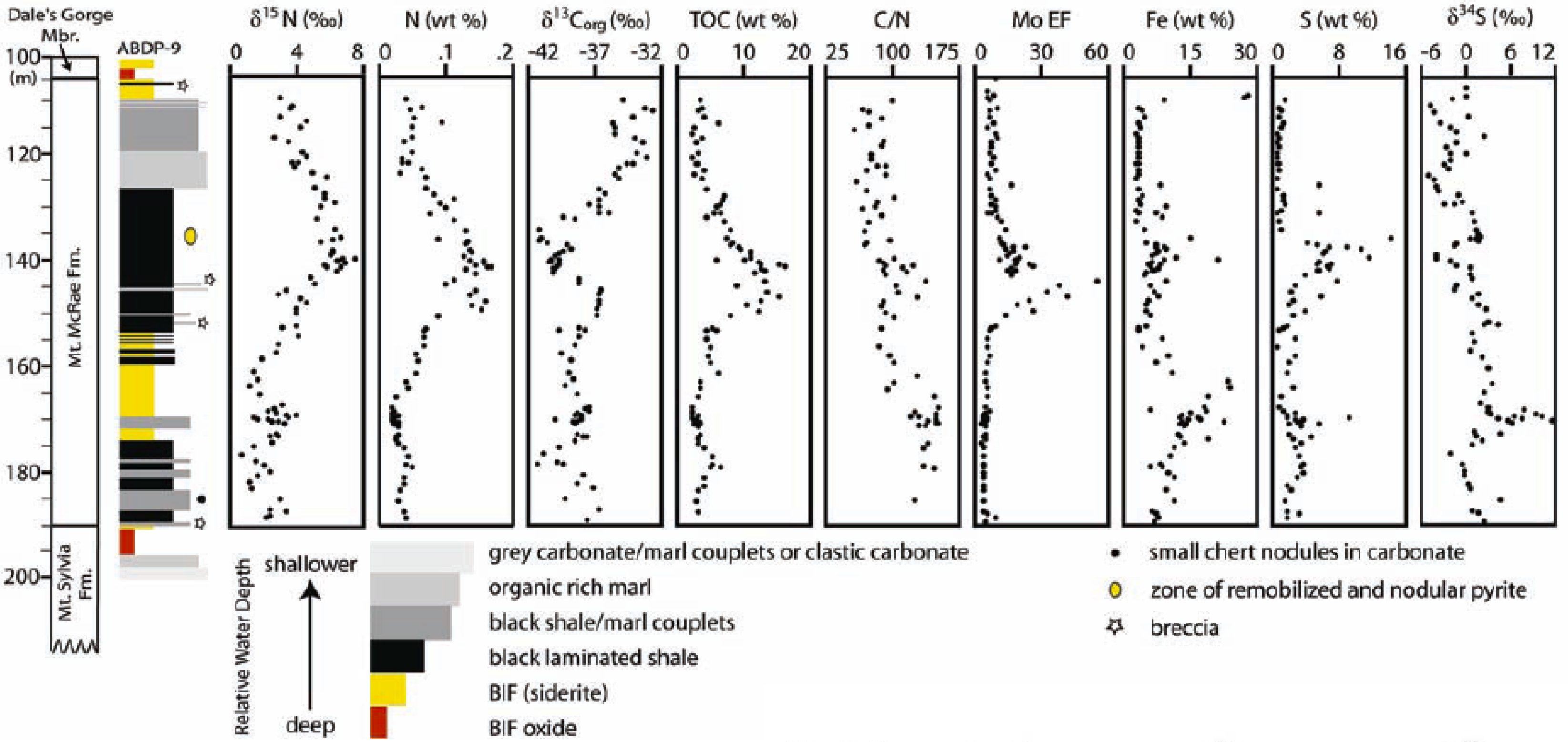
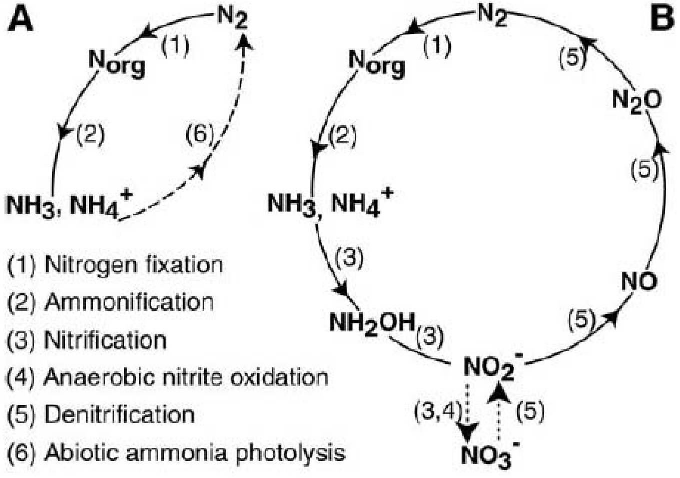
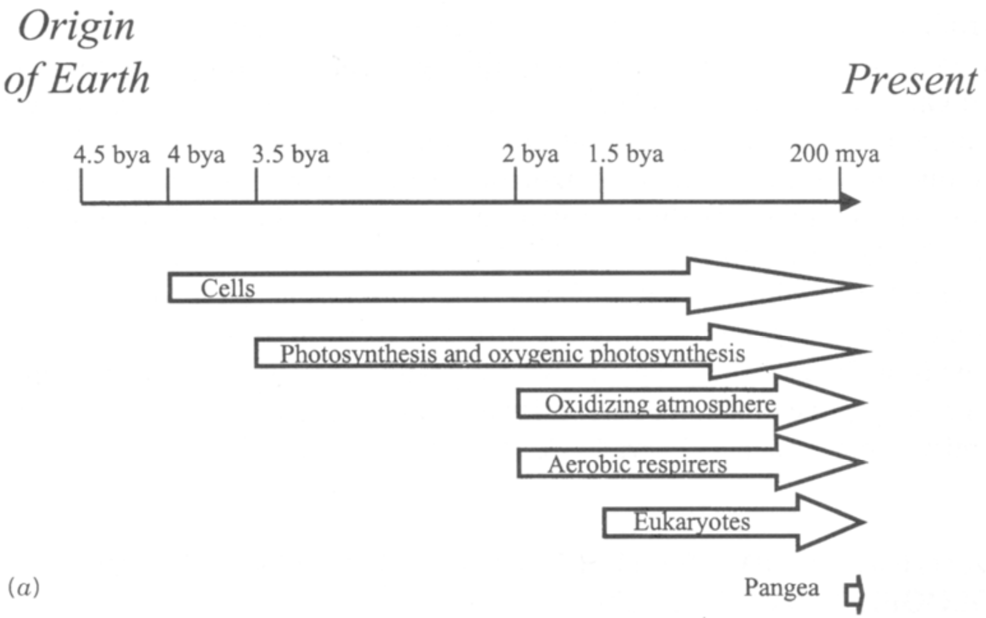
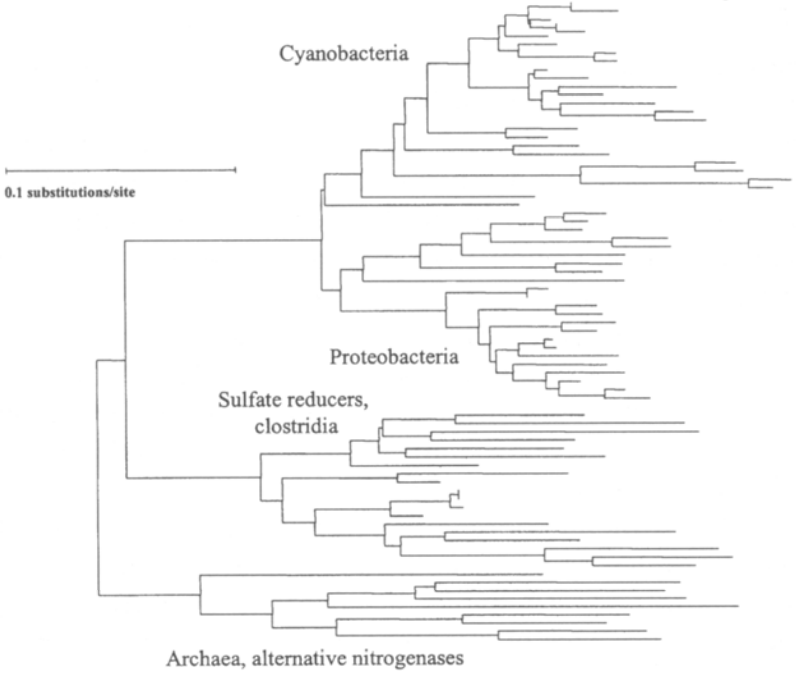


Fig. 2. Nitrogen cycle transformations. **(A)** Hypothesized anaerobic N cycle before Mount McRae $\delta^{15}N$ excursion and **(B)** hypothesized suboxic aerobic N cycle at peak of Mount McRae $\delta^{15}N$ excursion. The broken line indicates abiotic processes, and the dotted line indicates plausible but unproven processes.



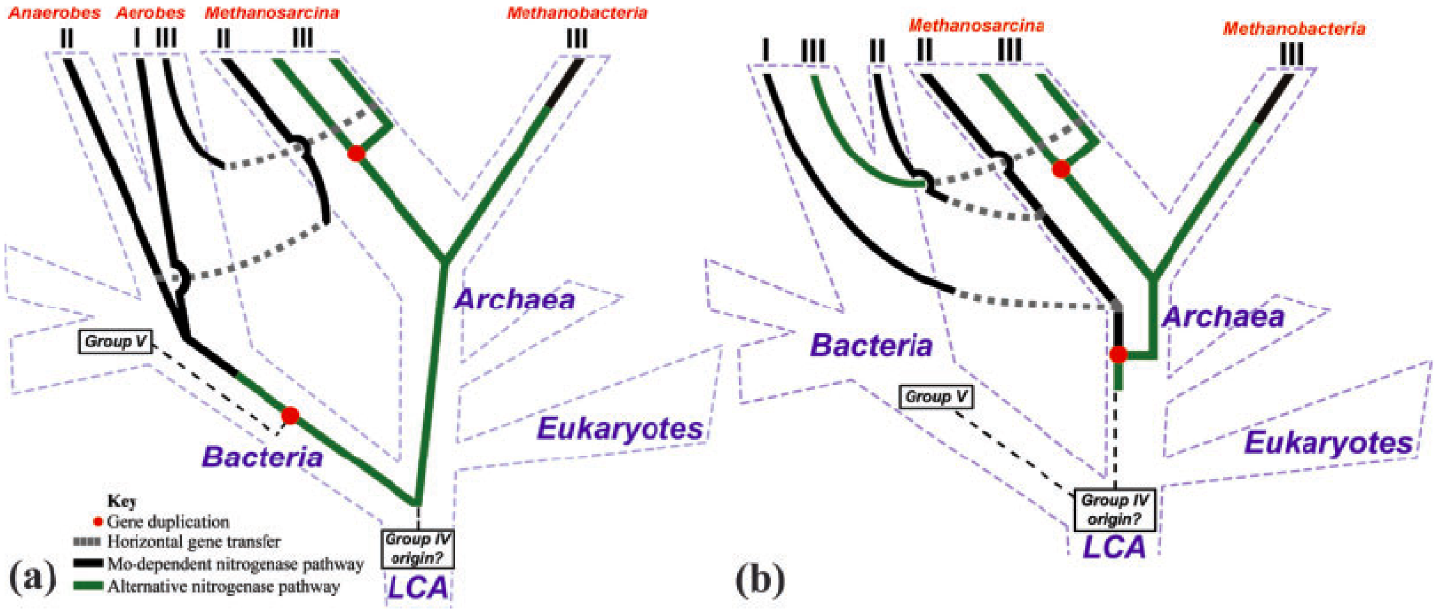

Origin of Earth
Present
4.5 bya
4 bya
3.5 bya
2 bya
1.5 bya
200 mya
Cells
Photosynthesis and oxygenic photosynthesis
Oxidizing atmosphere
Aerobic respirers
Eukaryotes
Pangea

(*a*)


Cyanobacteria
0.1 substitutions/site
Proteobacteria
Sulfate reducers, clostridia
Archaea, alternative nitrogenases

(*b*)

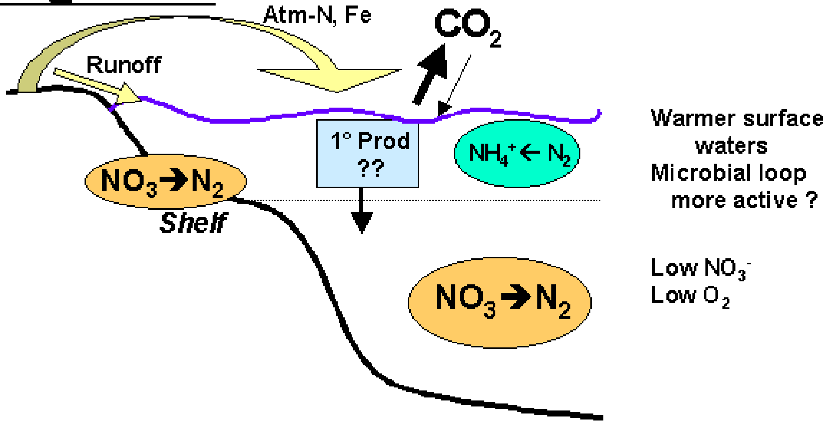
Raymond & Blakenship 2004



Enigmas

- Nitrogenase- evolved early
  - in many anaerobes
  - alternative function- detox ?
- Recent lateral transfer ??
- Cyanobacteria- e.g. Tricho
  - very ancient

Interglacial



Atm-N, Fe

CO_2

Runoff

$NO_3 \rightarrow N_2$

Shelf

1° Prod
??

$NH_4^+ \leftarrow N_2$

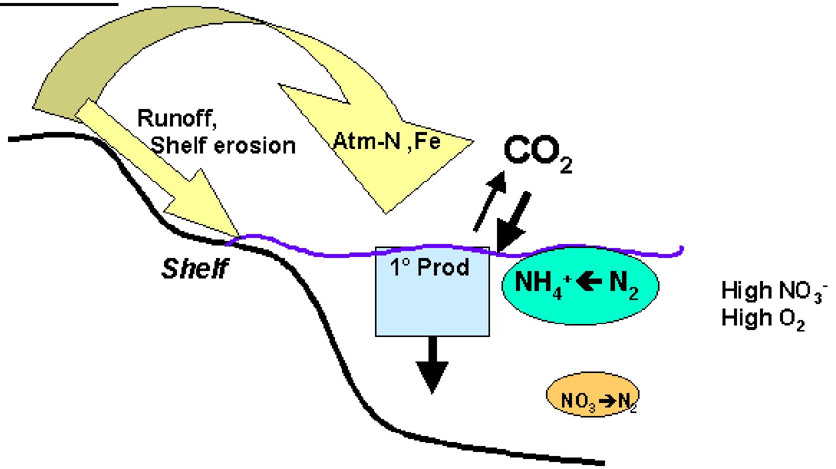
Warmer surface
waters
Microbial loop
more active ?

$NO_3 \rightarrow N_2$

Low NO_3^-
Low O_2

Denitrification >> DIN input

Glacial



Runoff,
Shelf erosion

Atm-N ,Fe

CO_2

Shelf

1° Prod

$NH_4^+ \leftarrow N_2$

High NO_3^-
High O_2

$NO_3 \rightarrow N_2$

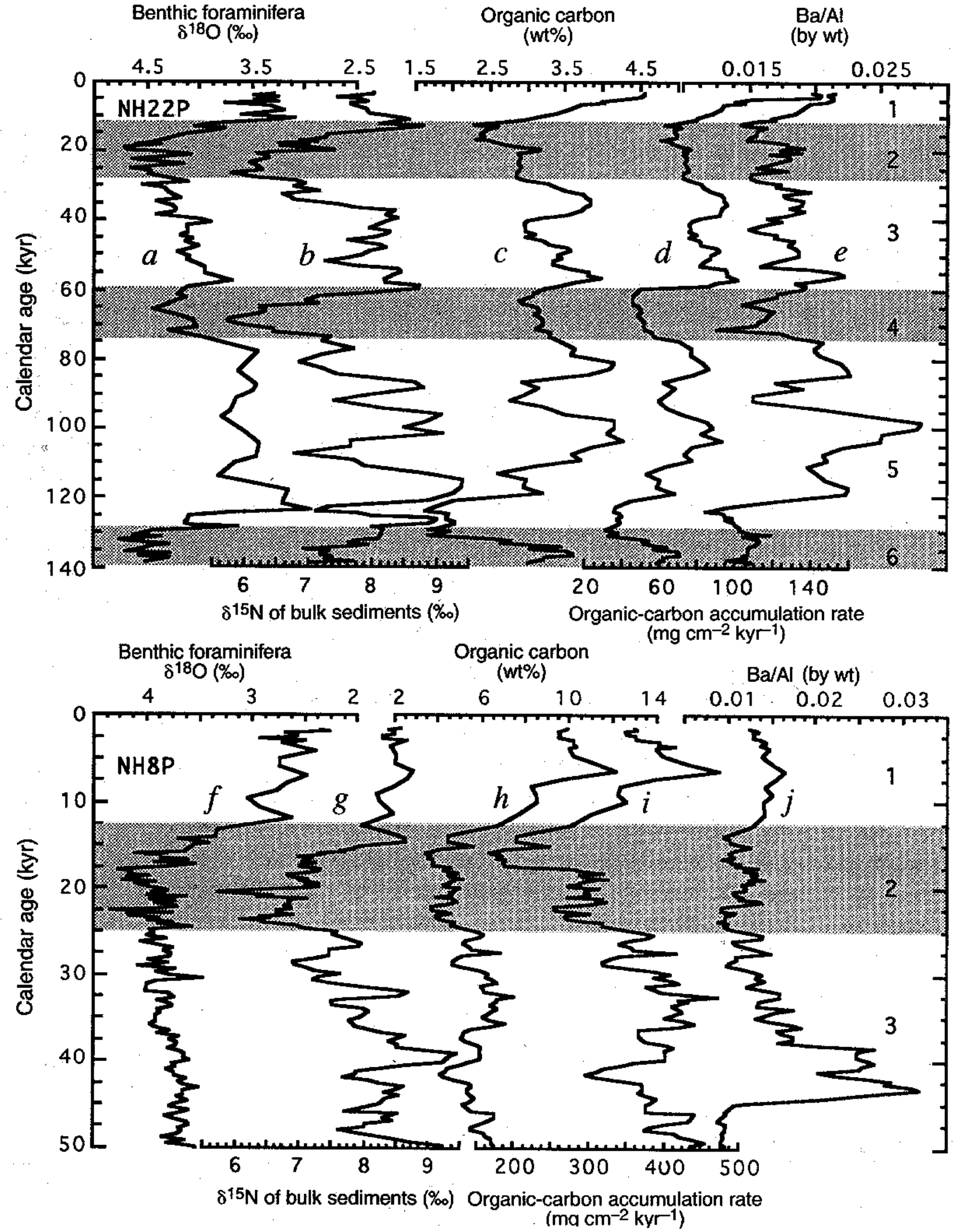
Denitrification << DIN input

Ganeshram et al. 1995

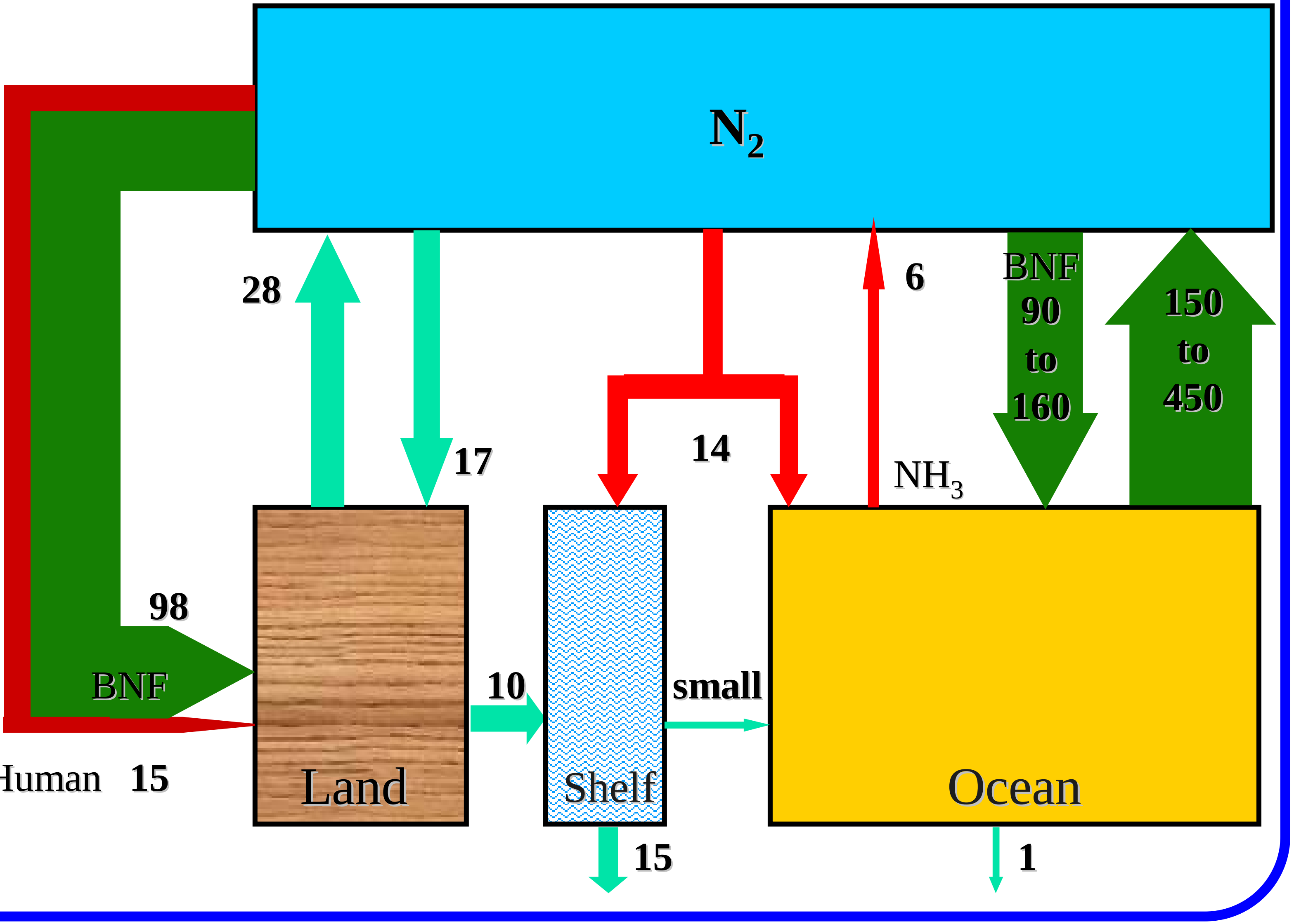
Stronger denitrification during interglacials

Also
Farrell et al. 1995
ETSP

Altabet et al. 1995
Arabian Sea

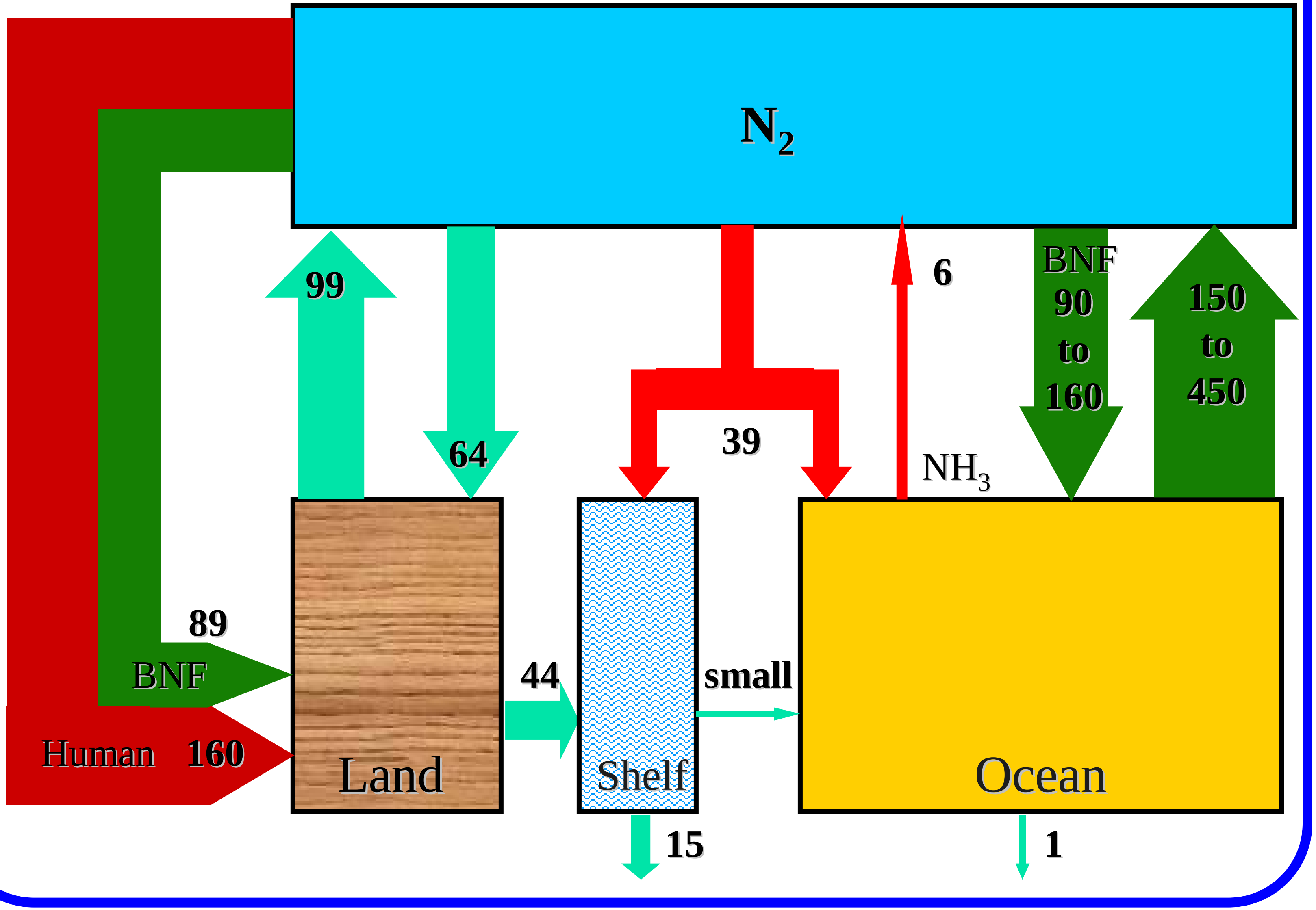


1860: Land-Atmosphere-Ocean Connections, Tg N/yr



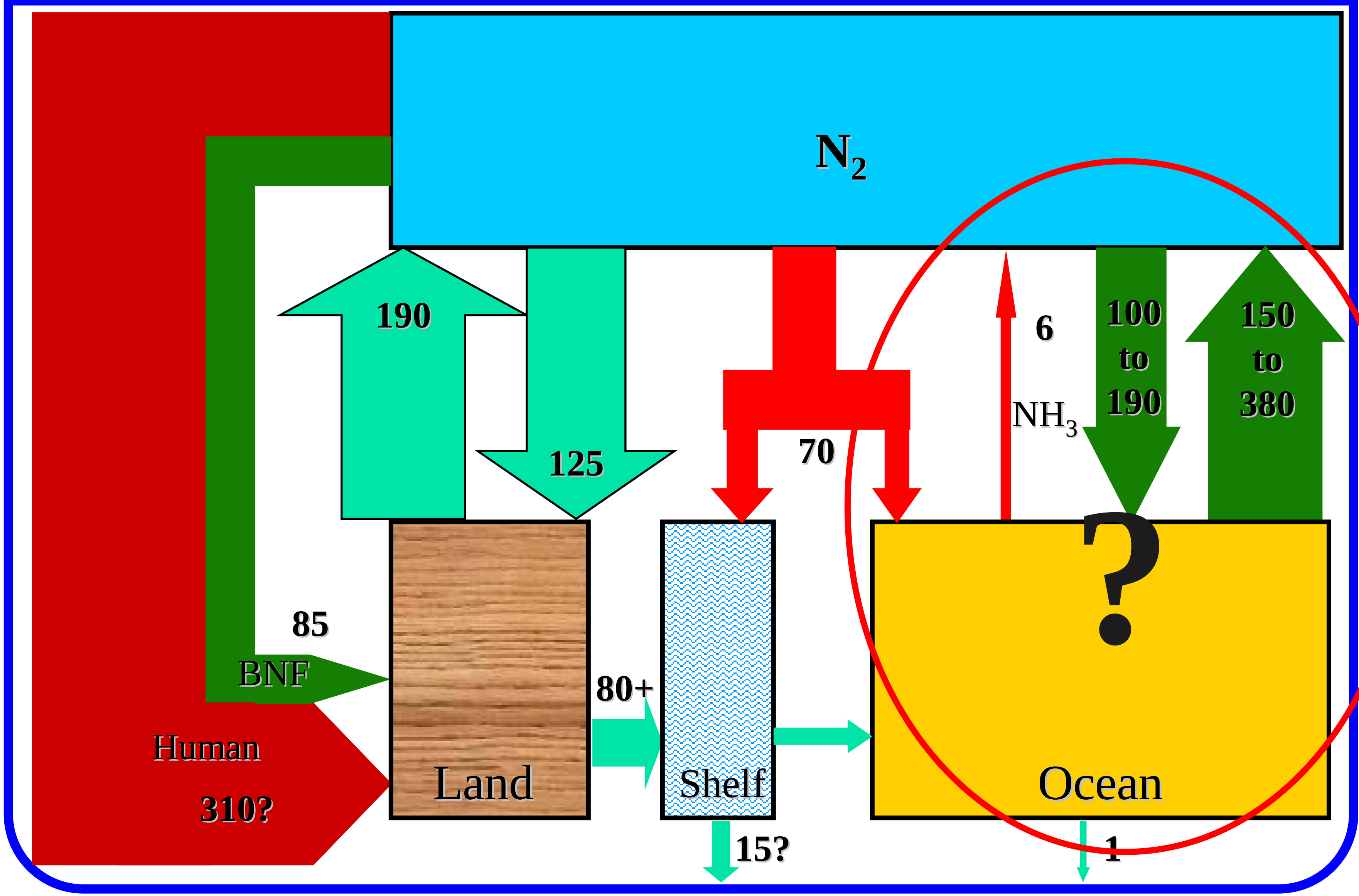
Galloway et al, 2005

1993: Land-Atmosphere-Ocean Connections, Tg N/yr



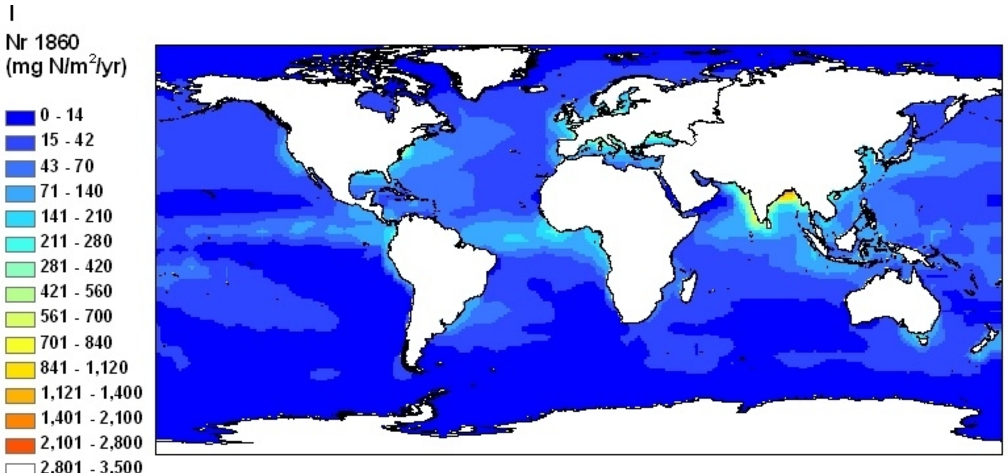
Galloway et al, 2005

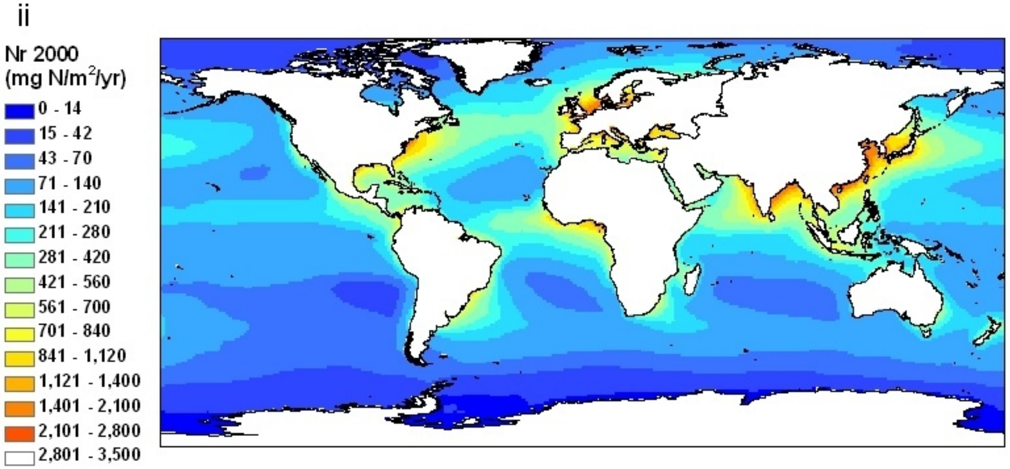
2050--Land: Ocean Global Connections for Nitrogen, Tg N/yr

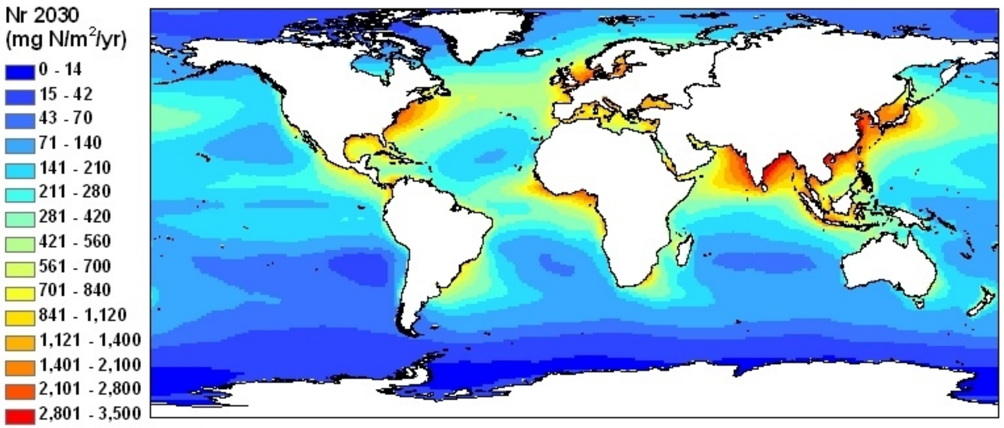


Galloway et al, 2005

Year	Total	AAN
	Tg N yr^{-1}	
1860	20	5.7
2000	67	54
2030	77	62

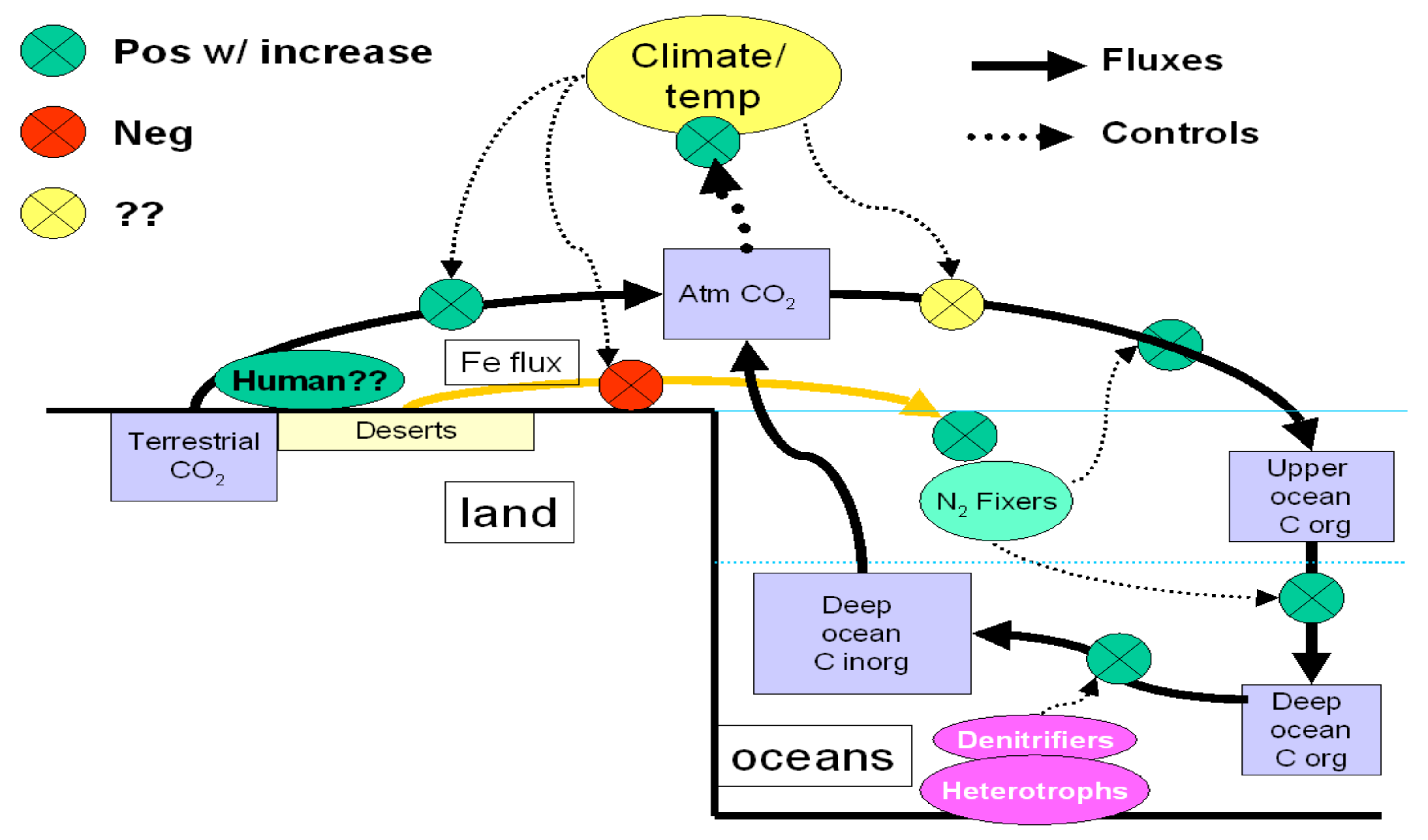






Duce et al. 2008

Figure 1. Conceptual model of linkages and feedbacks

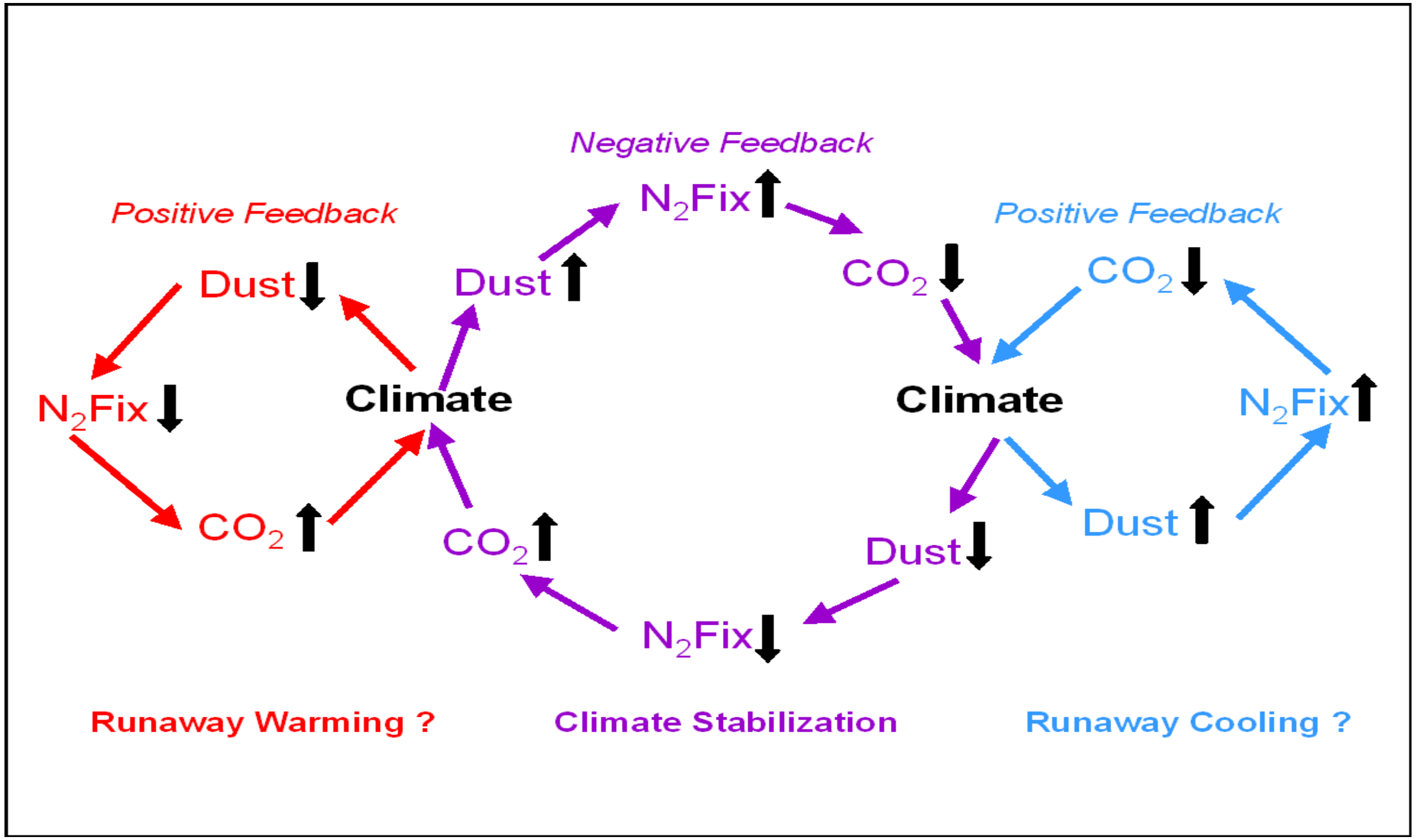


Trace Nutrient/ Trace Metal Limitation

- Evidence of Fe limitation- but limited data
  - Rueter, Raven- Fe quota
  - Paerl - Fe stimulation of N_2 fixation, growth (EDTA??)
  - Berman-Frank et al. – Fe quotas, limitation

- Intriguing correlations: e.g. dust inputs and N*

Global feedback loops involving nitrogen fixation, climate and dust



The History of Nitrogen

--Cropland--