The Microbial Marine Nitrogen Cycle

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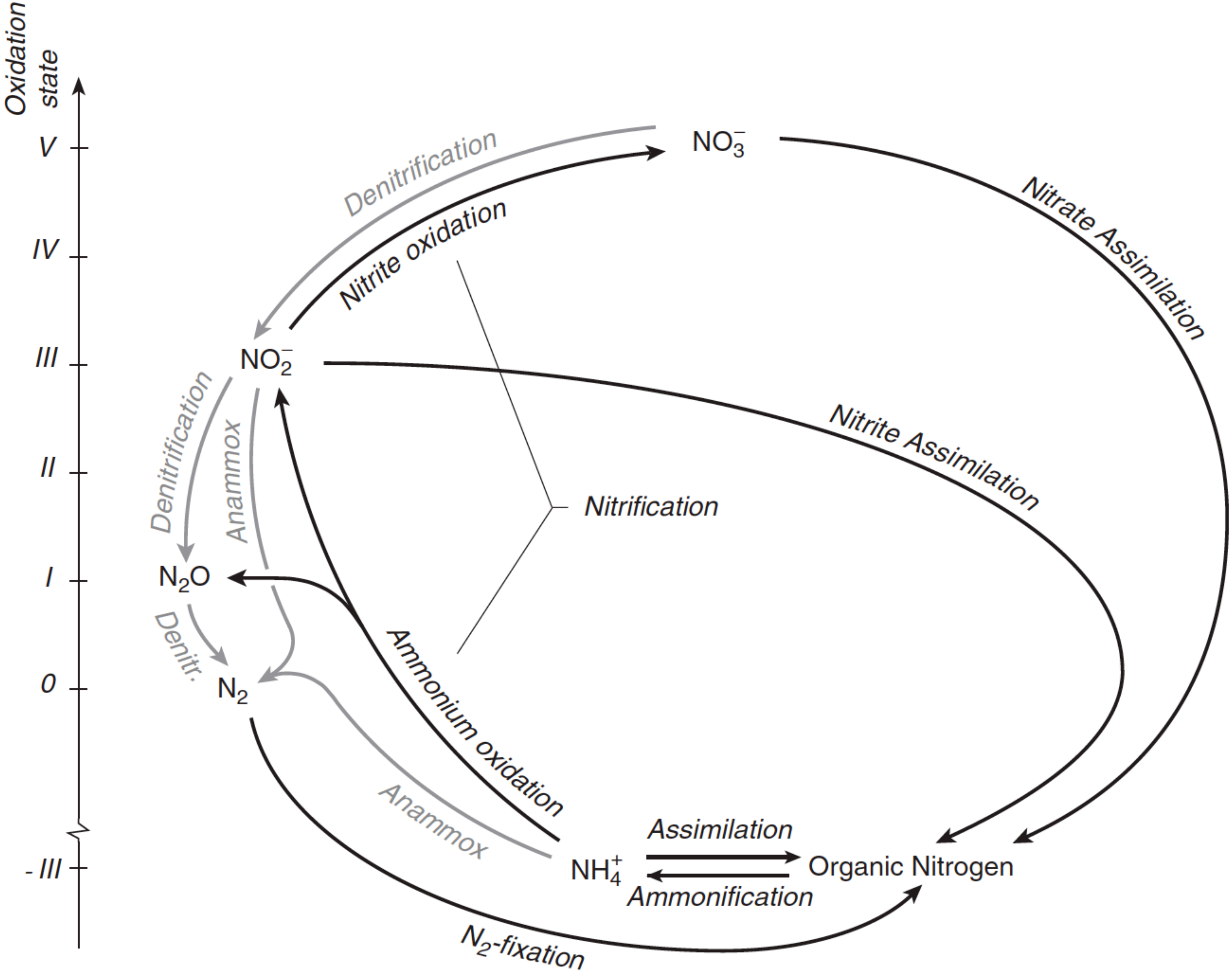
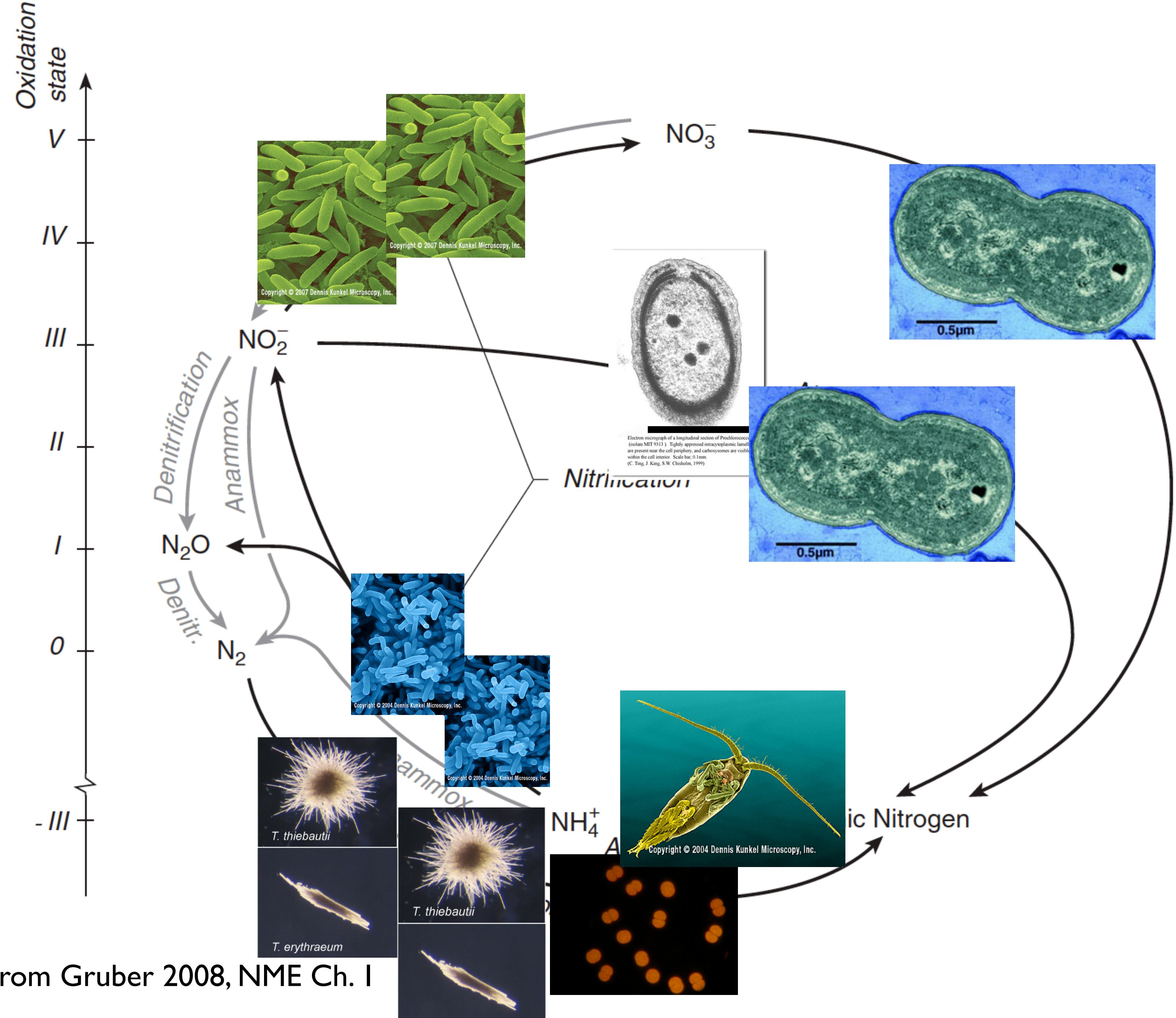
C-MORE Microbial Oceanography Course 2014



from Gruber 2008, NME Ch. 1



from Gruber 2008, NME Ch. I

Outline

- Overview of the marine nitrogen cycle - pools, depth distributions, and fluxes

- The cast of characters - select functional groups within the microbial nitrogen cycle

- Synthetic studies of the marine microbial nitrogen cycle *in situ* - case studies of oxygen minimum zones and the primary nitrite maximum

Key Ideas:

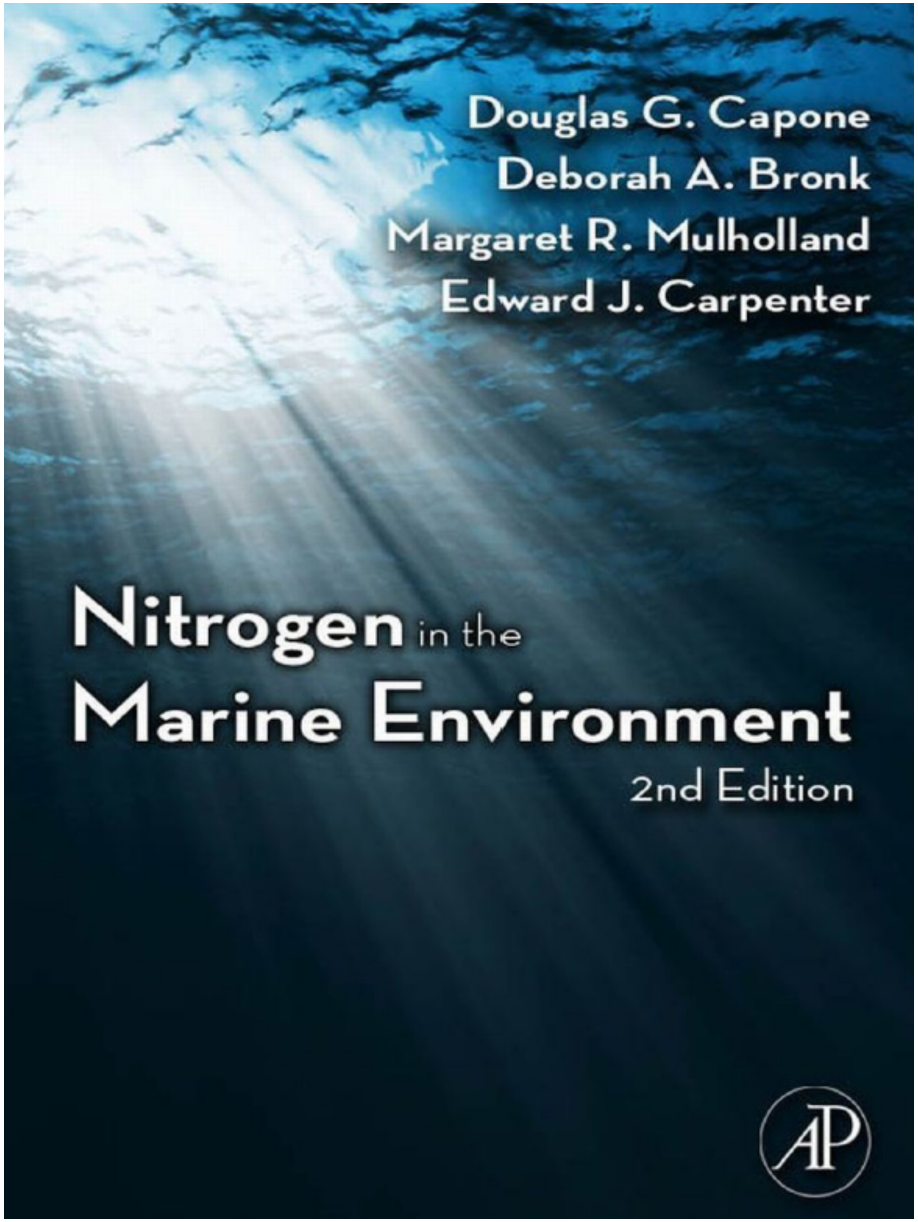
- Nitrogen cycle is complex because N has so many redox states.

- Marine nitrogen cycle is mainly mediated by biological reactions (microbes) with unique and varying ecologies and evolutionary histories.

- The nitrogen cycle has been a showcase for integrating biogeochemistry and molecular biology, greatly aided by the suite of available ‘functional genes’ and the existence of stable N isotopes.

Some bedtime reading. . .

Everything you ever wanted to know about the marine nitrogen cycle, plus 36 more chapters.

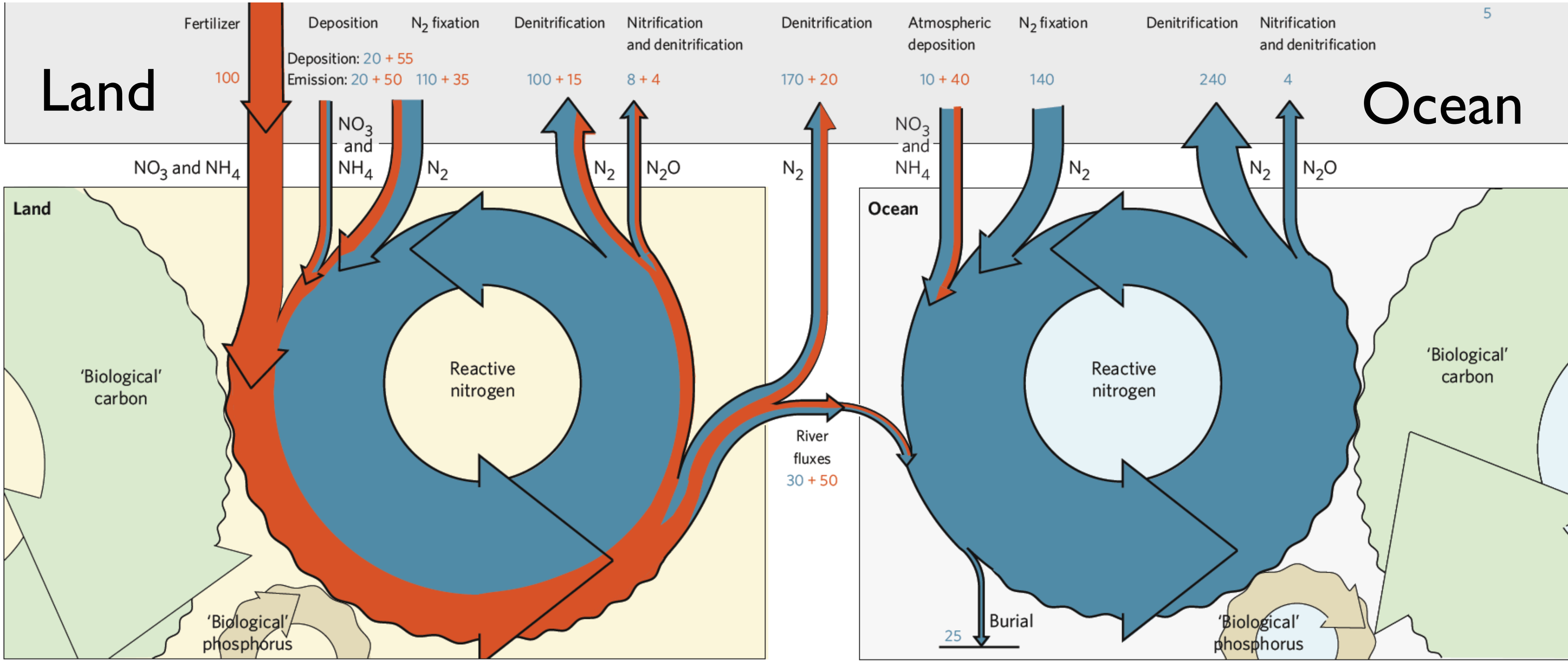


Douglas G. Capone
Deborah A. Bronk
Margaret R. Mulholland
Edward J. Carpenter

Nitrogen in the Marine Environment
2nd Edition

AP

We are altering the global N cycle



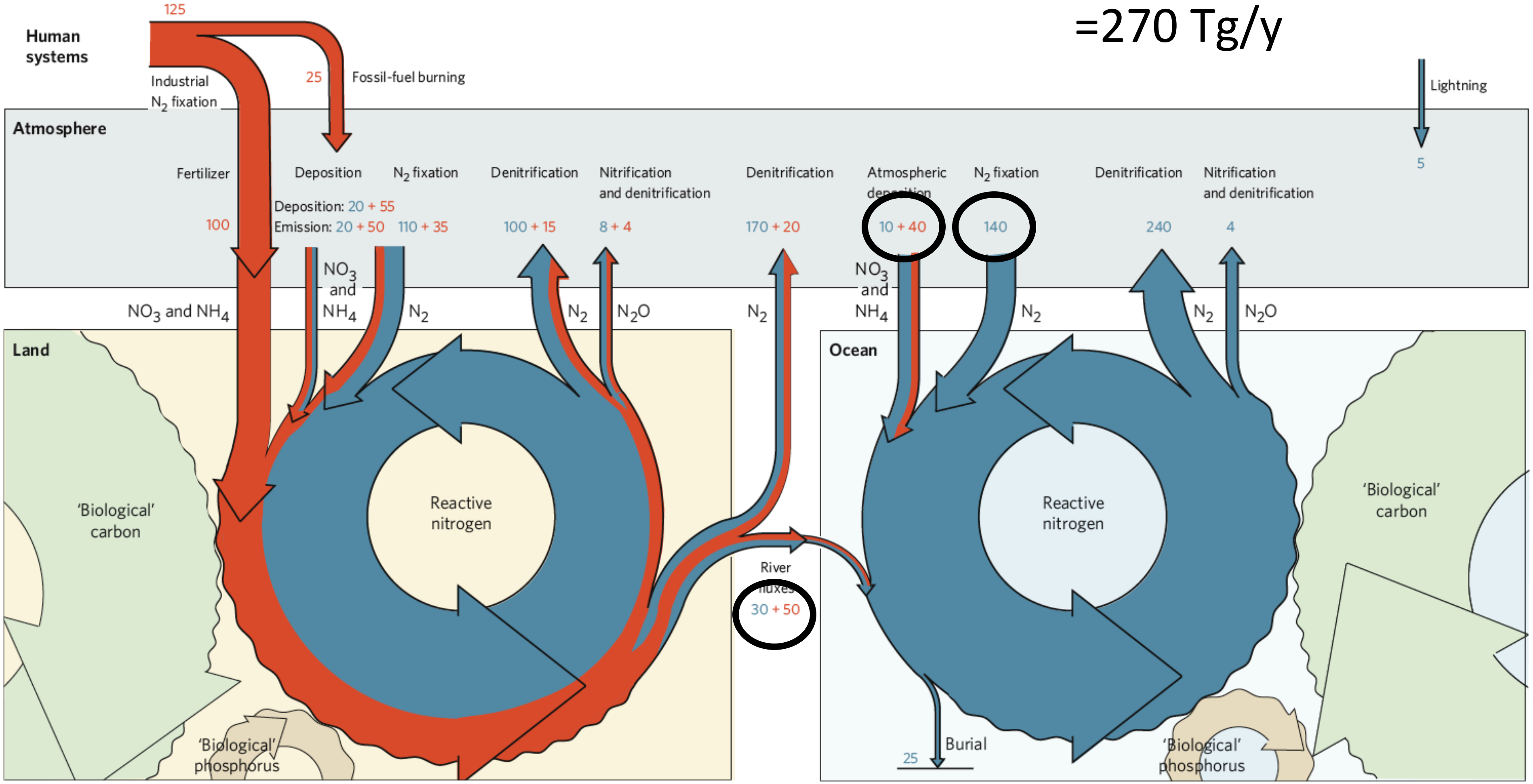
"Few of these flux estimates are known to better than 20%, and many have uncertainties of 50% or larger."

Gruber and Galloway 2008

Input terms (Tg/yr)

Biological N_2 fixation	140
Atm. Dep.	50
Runoff	80

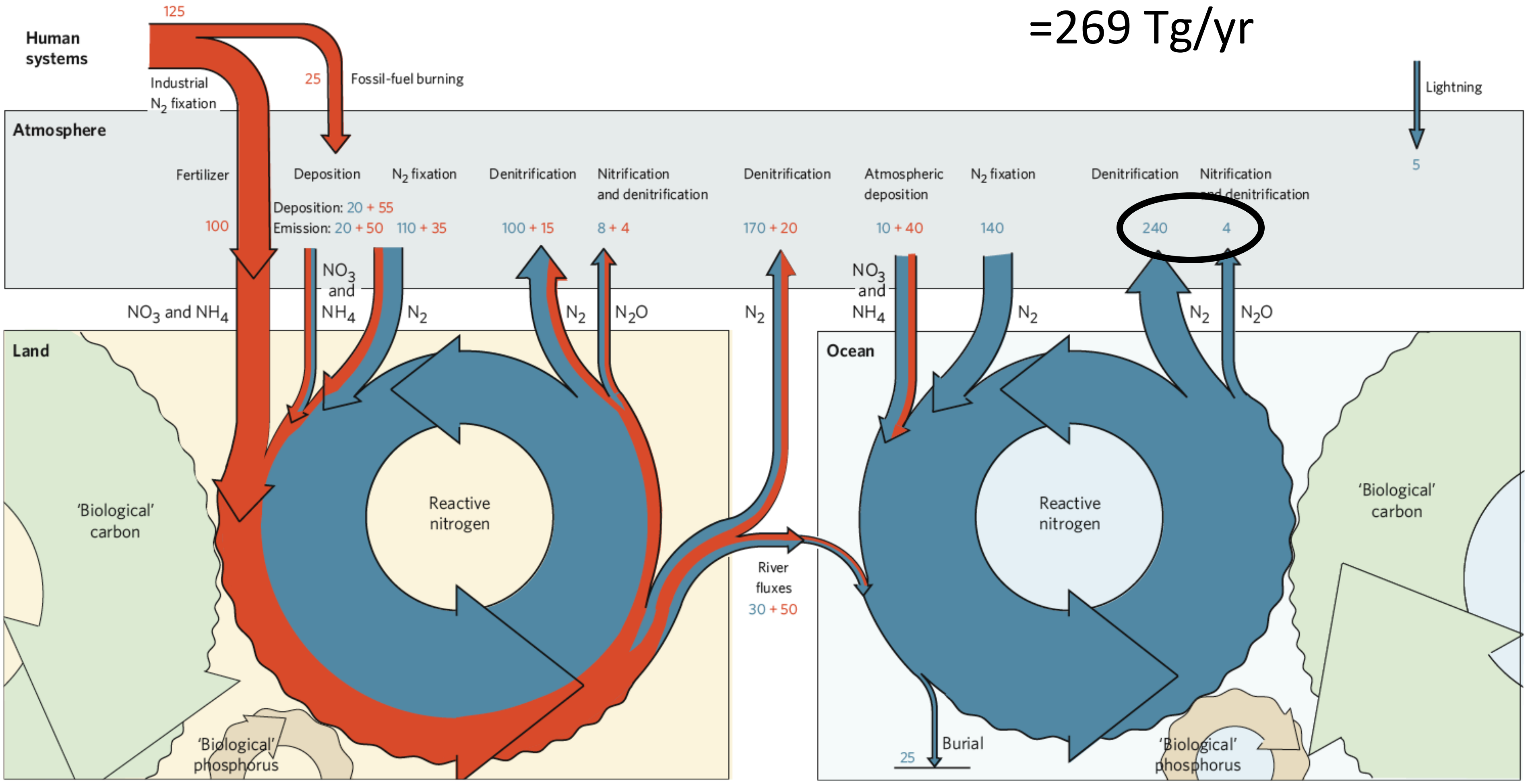
=270 Tg/y



Gruber and Galloway, 2008

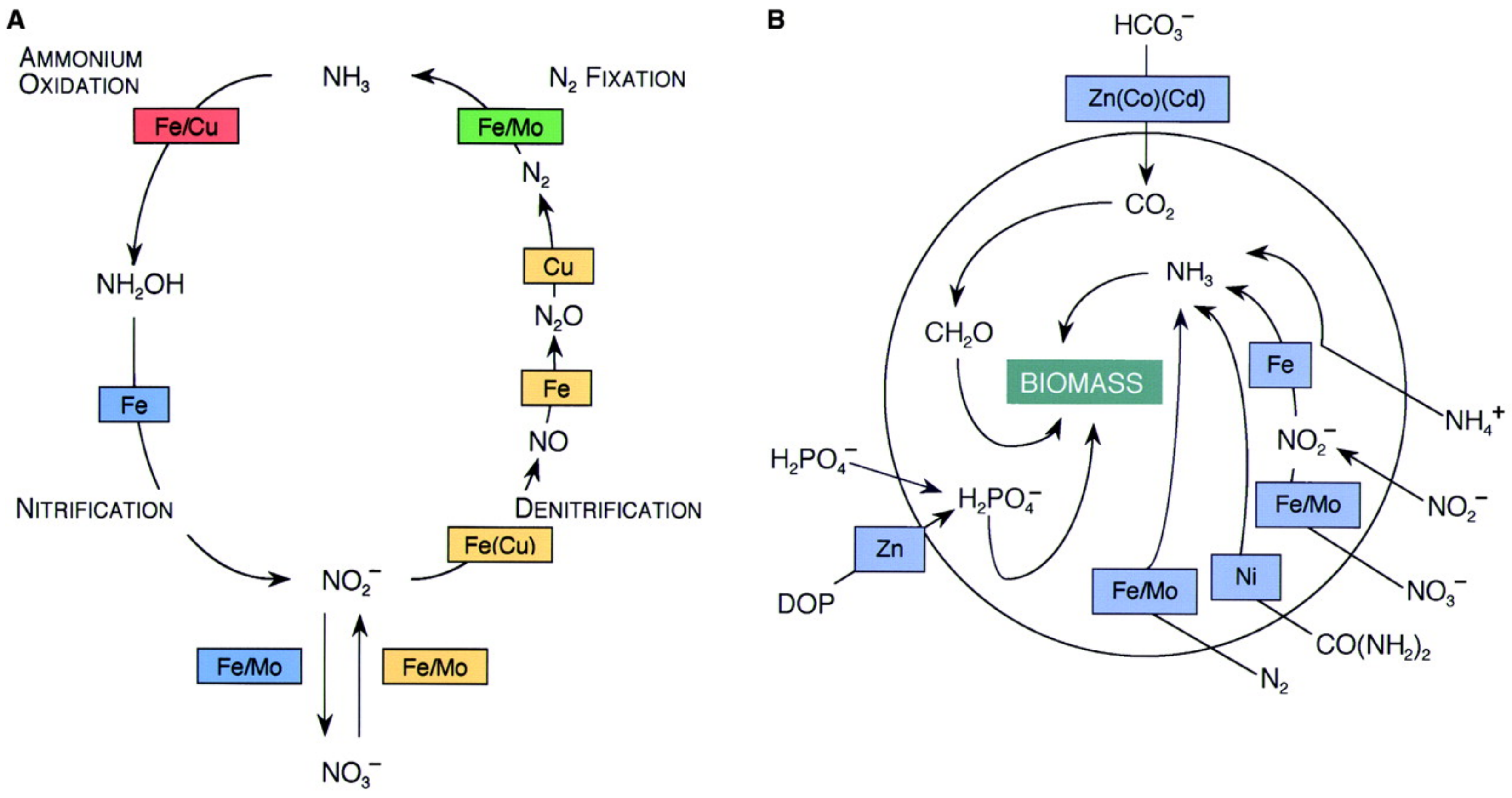
Output terms (Tg/y)

Denitrification: N_2 loss 240
N_2O loss 4
Sediment Burial 25
=269 Tg/yr



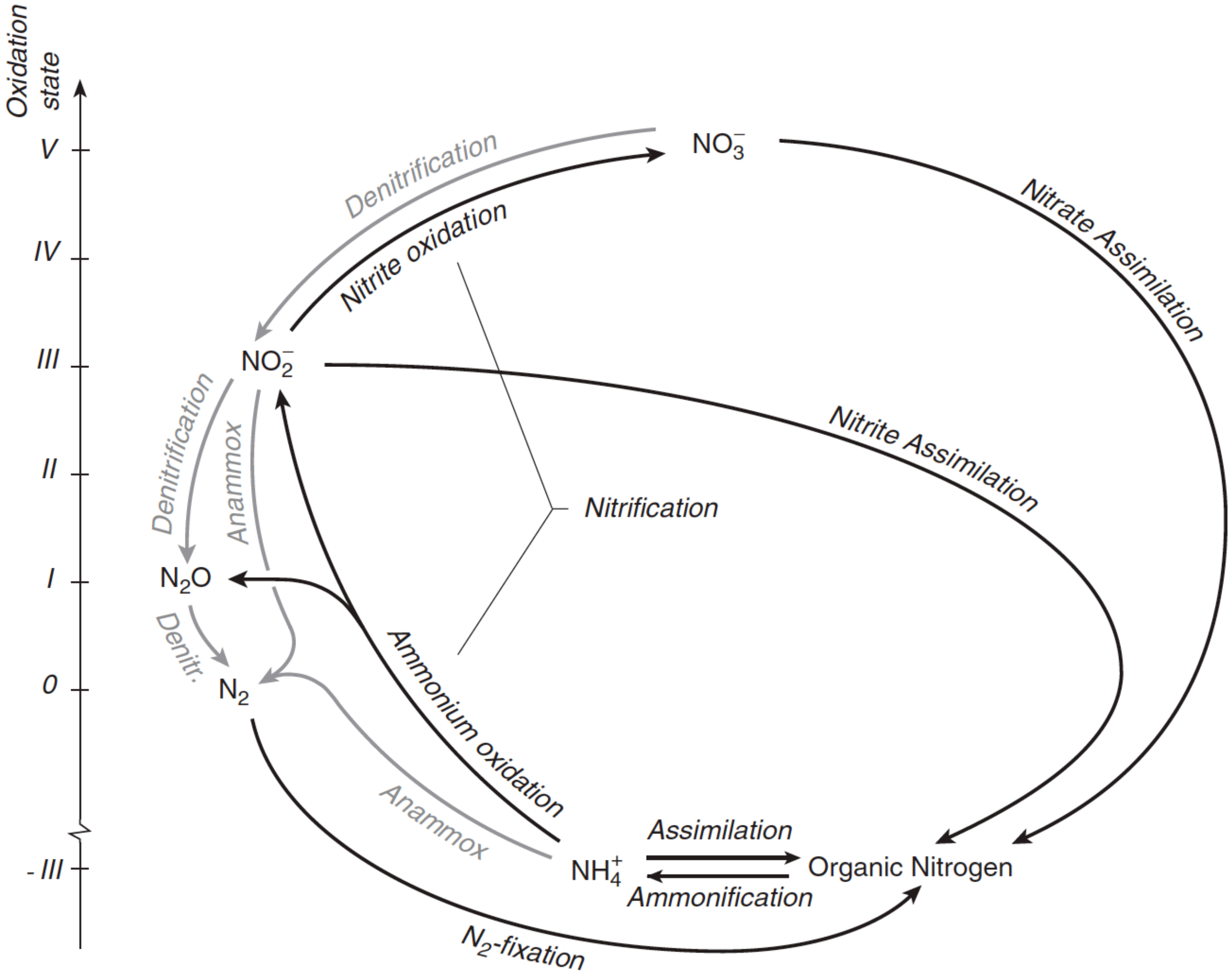
Gruber and Galloway, 2008

The nitrogen cycle is also inextricably linked to trace metal cycling



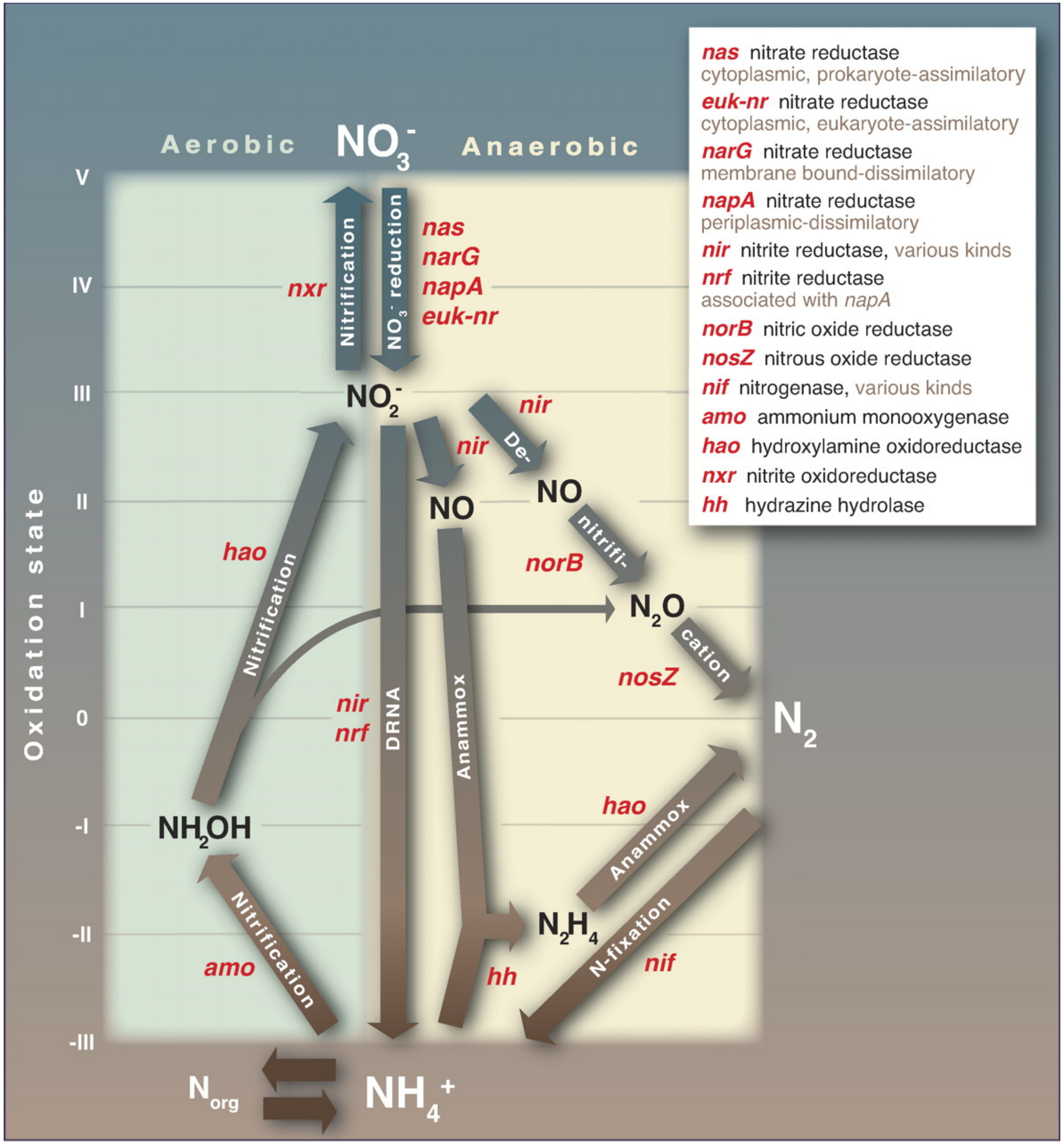
Morel and Price 2003, *Science*

Nitrogen exists in multiple redox states



from Gruber 2008

gray = reactions in low/no oxygen environments, black = oxic



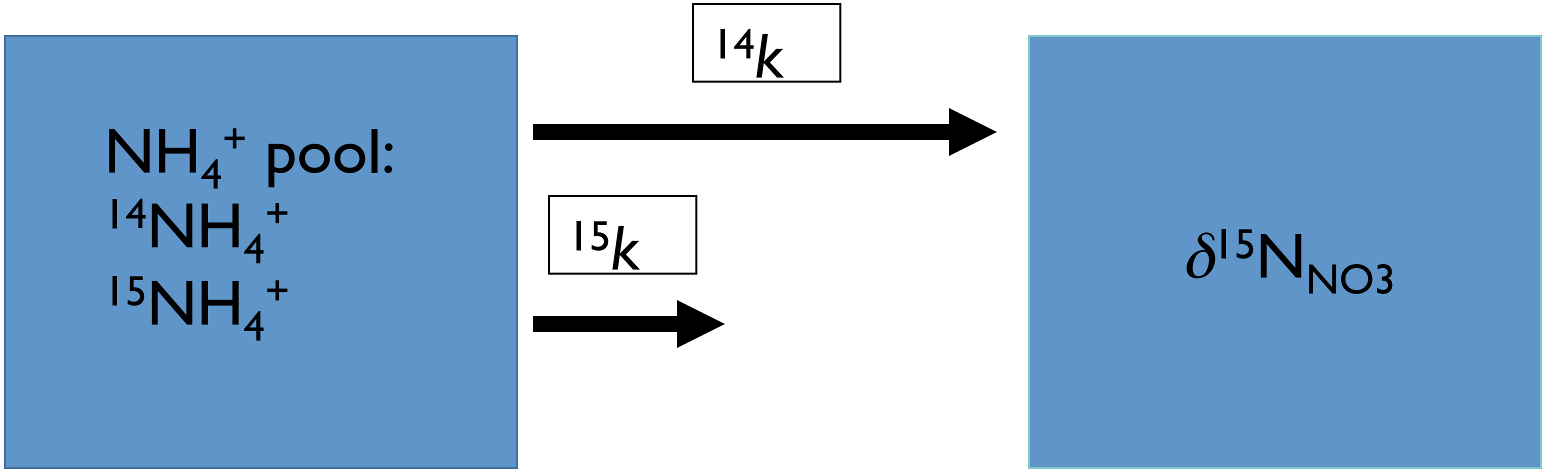
Many ‘functional genes’ available to study the nitrogen cycle

But, always remember that <u>genes come from cells.</u>

Canfield et al. 2010, *Science*

Another great thing about nitrogen: stable isotopes

Small differences in reaction rates leave an isotopic “signature” on compounds in the environment



NH_4^+ pool:

$^{14}NH_4^+$

$^{15}NH_4^+$

^{14}k

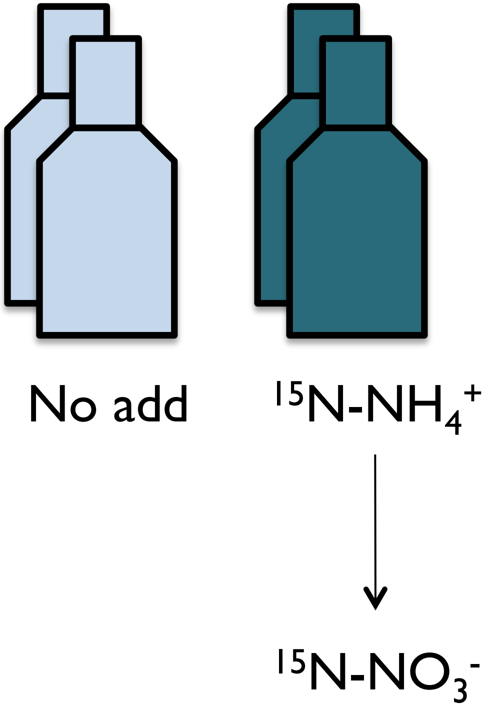
^{15}k

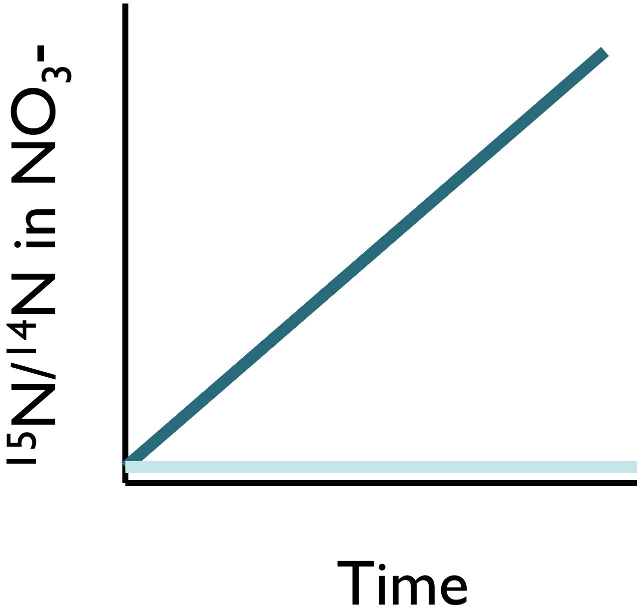
$\delta^{15}N_{NO3}$

Kinetic isotope effect:

$$\varepsilon = (^{14}k/^{15}k - 1) \times 1000$$

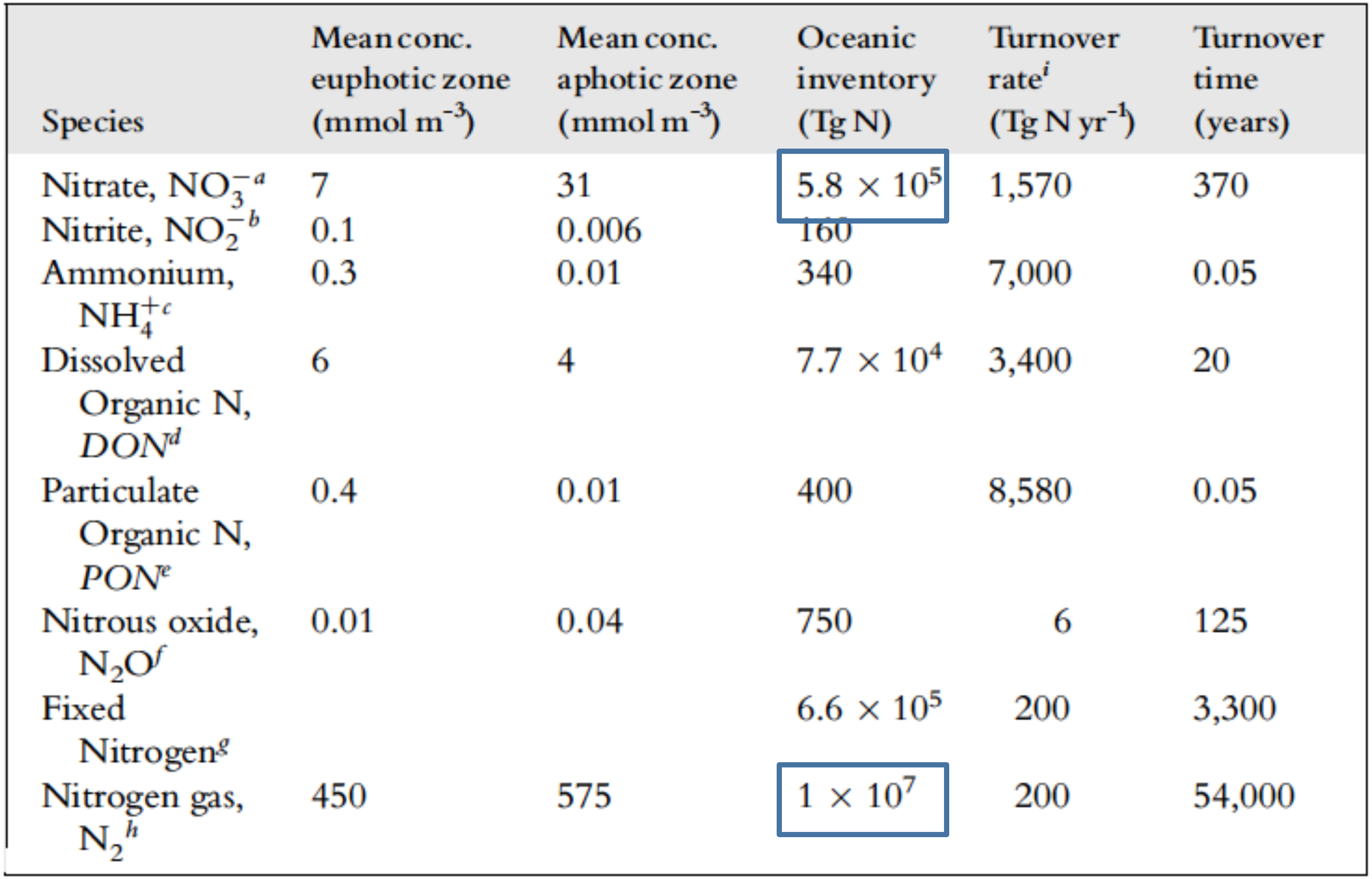
Stable isotopes can also be experimentally added to environmental systems as a tracer





See also Dugdale and Goering 1967; Lipschultz 2008

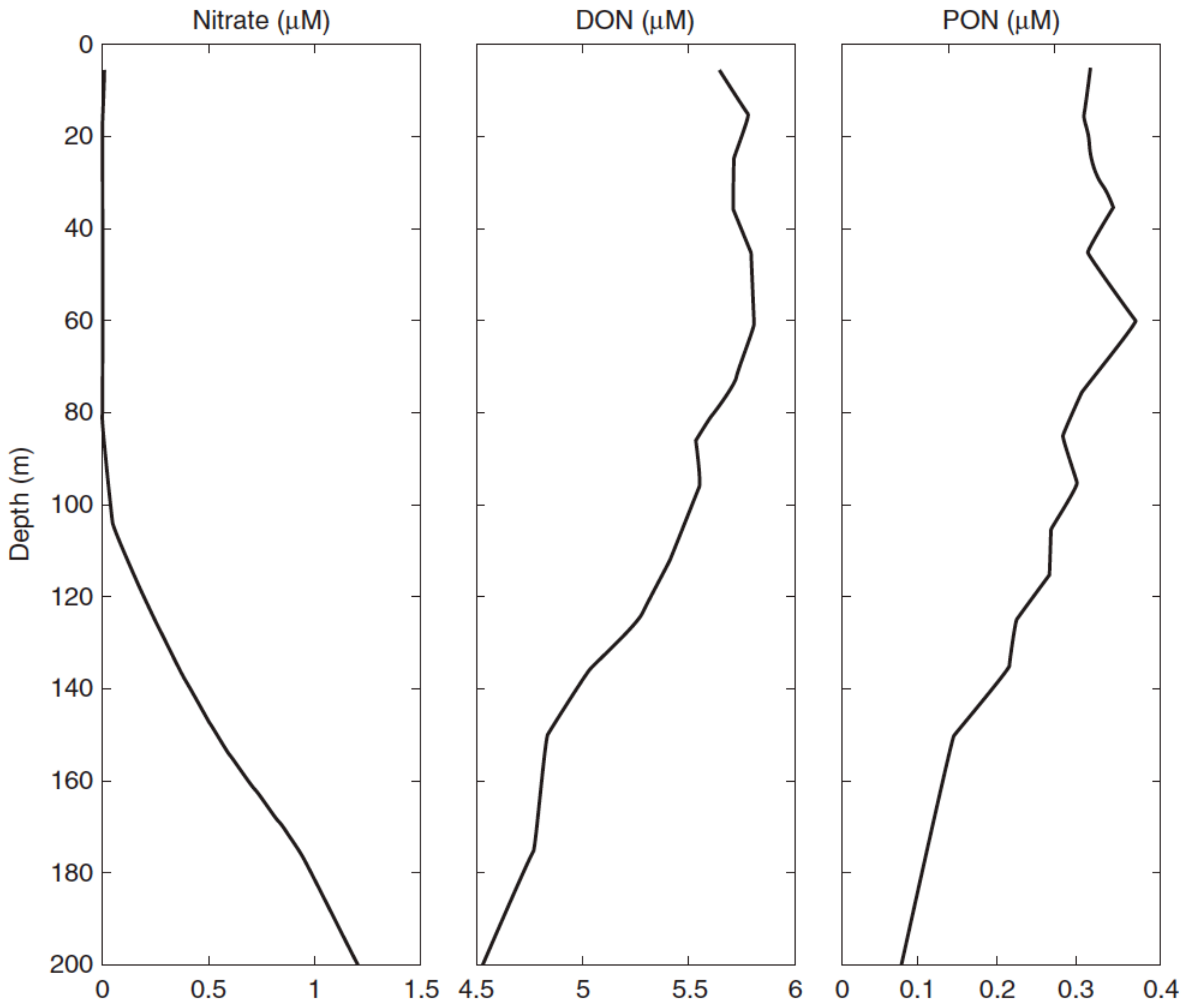
Vast majority of N in the ocean is N_2



Species	Mean conc. euphotic zone (mmol m^{-3})	Mean conc. aphotic zone (mmol m^{-3})	Oceanic inventory (Tg N)	Turnover rate[i] (Tg N yr^{-1})	Turnover time (years)
Nitrate, NO_3^-[a]	7	31	5.8×10^5	1,570	370
Nitrite, NO_2^-[b]	0.1	0.006	160		
Ammonium, NH_4^+[c]	0.3	0.01	340	7,000	0.05
Dissolved Organic N, *DON*[d]	6	4	7.7×10^4	3,400	20
Particulate Organic N, *PON*[e]	0.4	0.01	400	8,580	0.05
Nitrous oxide, N_2O[f]	0.01	0.04	750	6	125
Fixed Nitrogen[g]			6.6×10^5	200	3,300
Nitrogen gas, N_2[h]	450	575	1×10^7	200	54,000

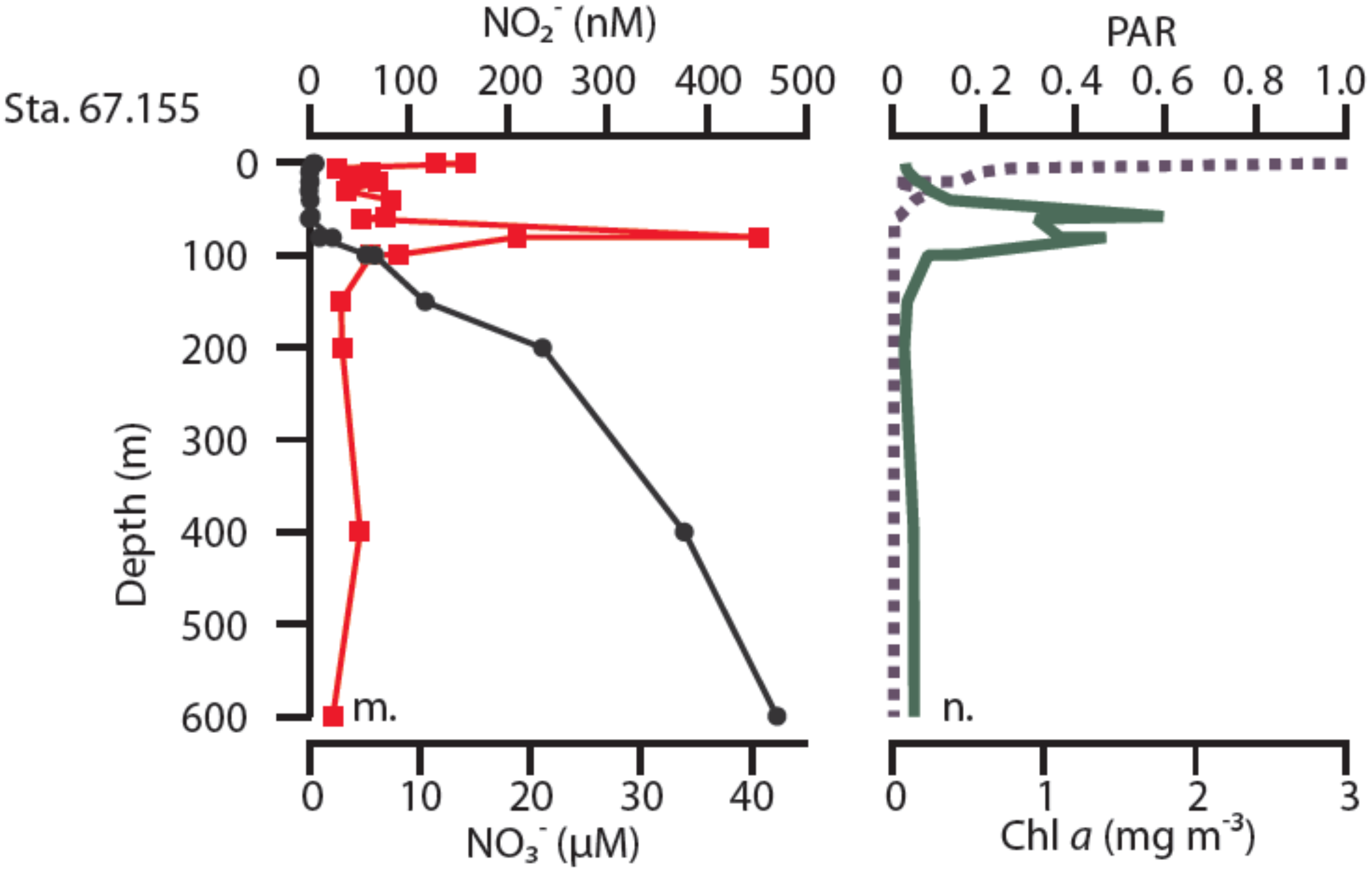
Gruber 2008
Nitrogen in the Marine Environment

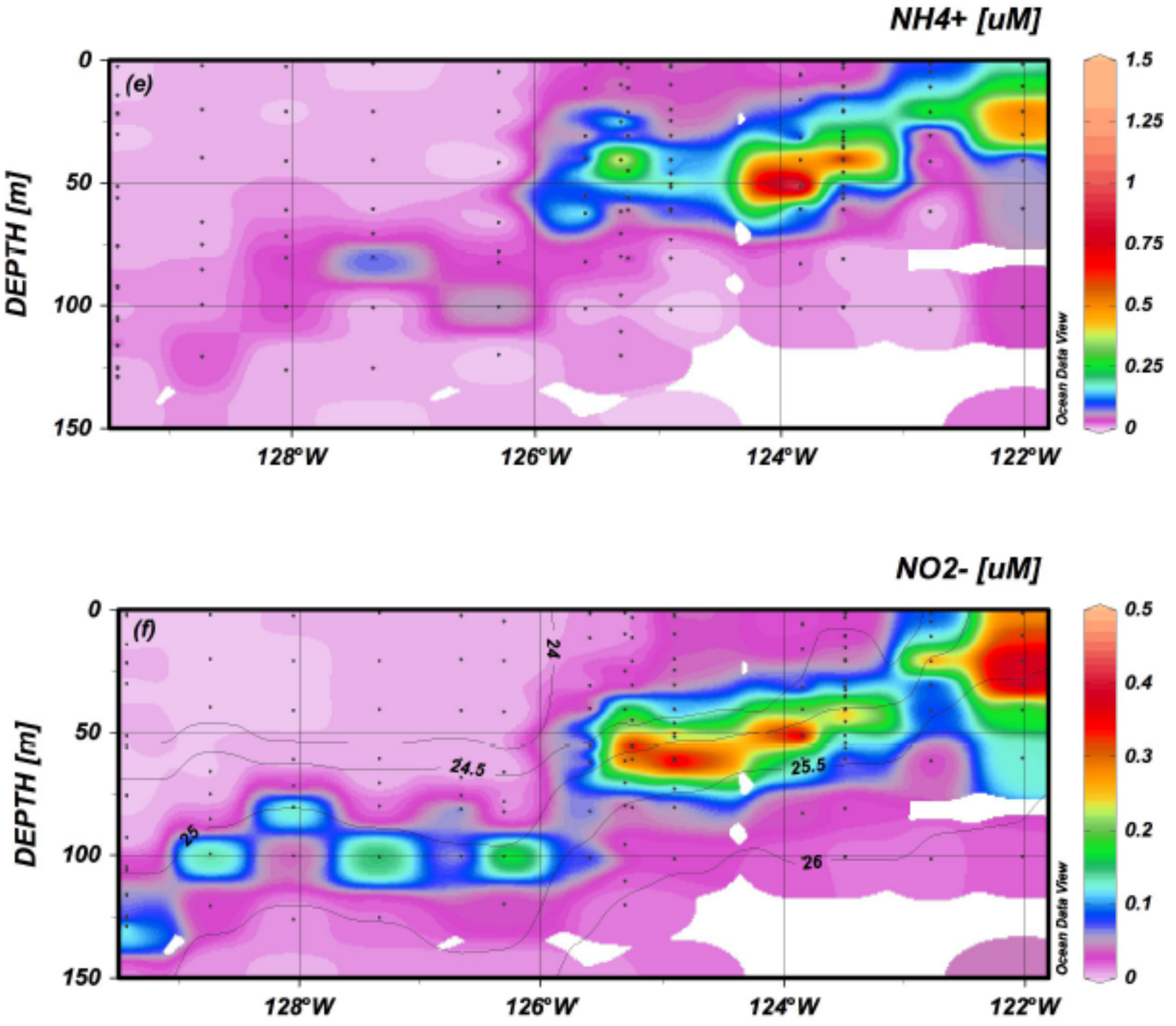
DIN increases with depth, DON is highest at the surface



Karl et al. 2008

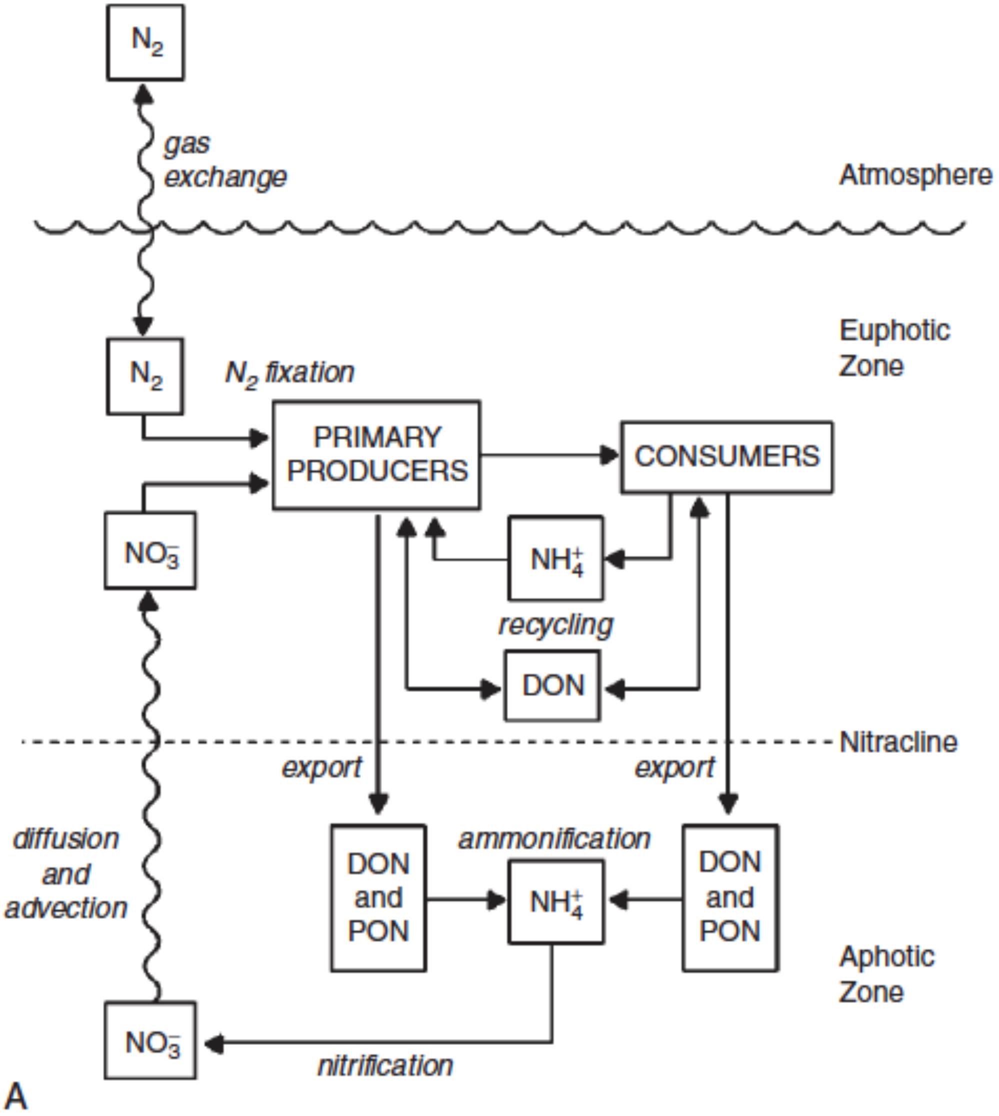
The primary nitrite maximum is a ubiquitous feature





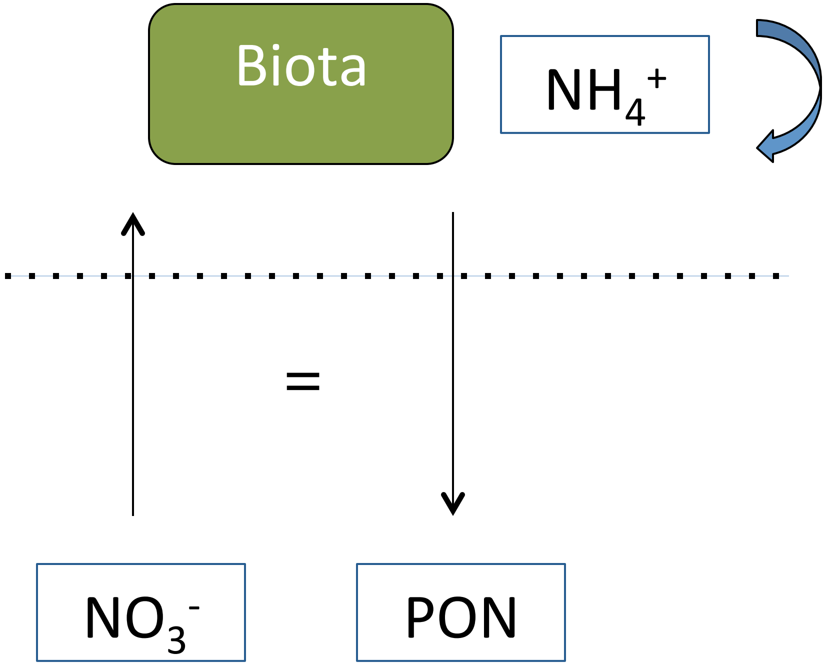
Santoro et al. 2013; *Biogeosciences*

Spatial distribution of the nitrogen cycle in the marine water column



Karl 2008 NME

Estimating export from N uptake: The new production paradigm



Biota

NH_4^+

=

NO_3^-

PON

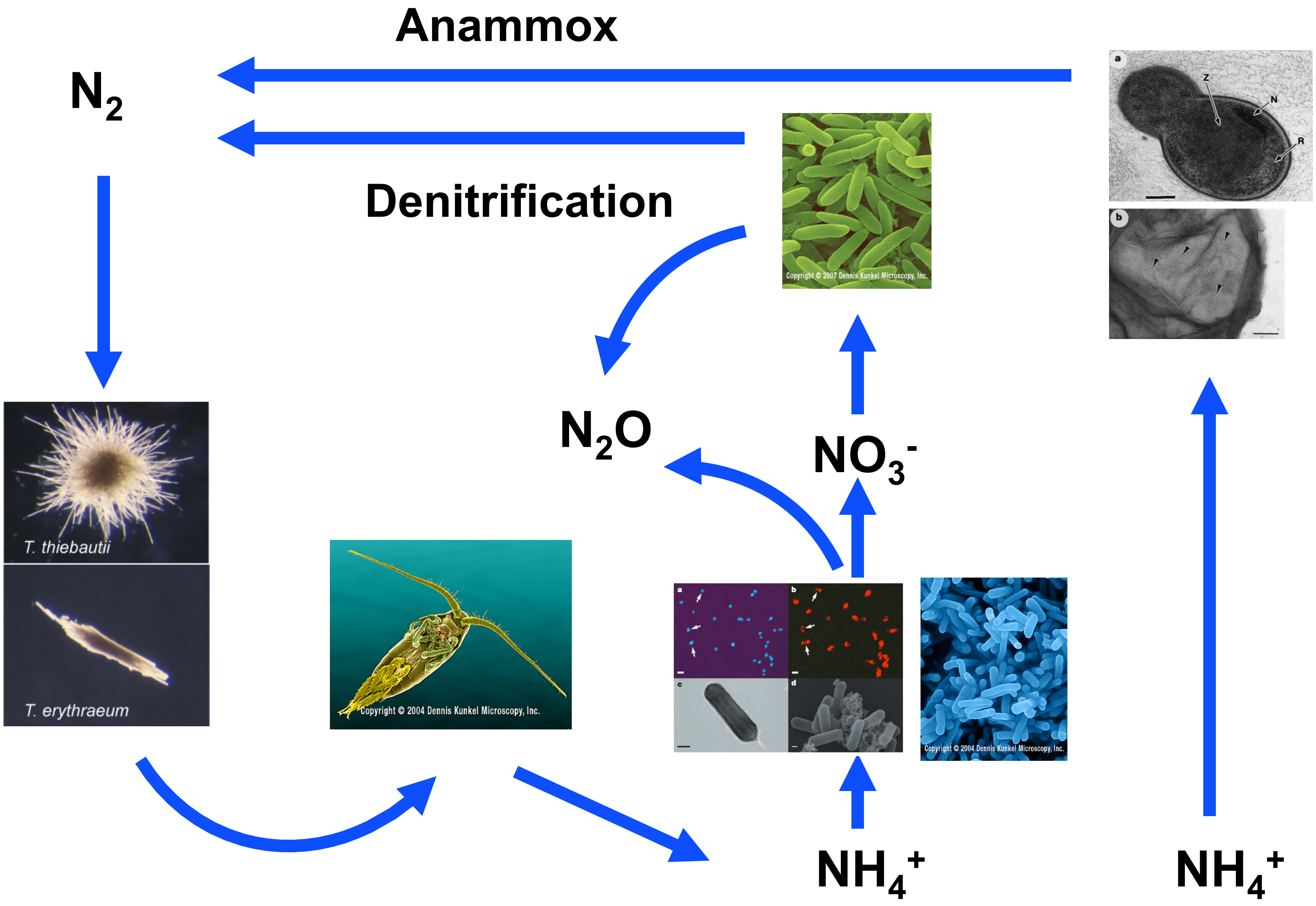
A net increase in phytoplankton biomass can only be supported by new NO_3- entering the euphotic zone.

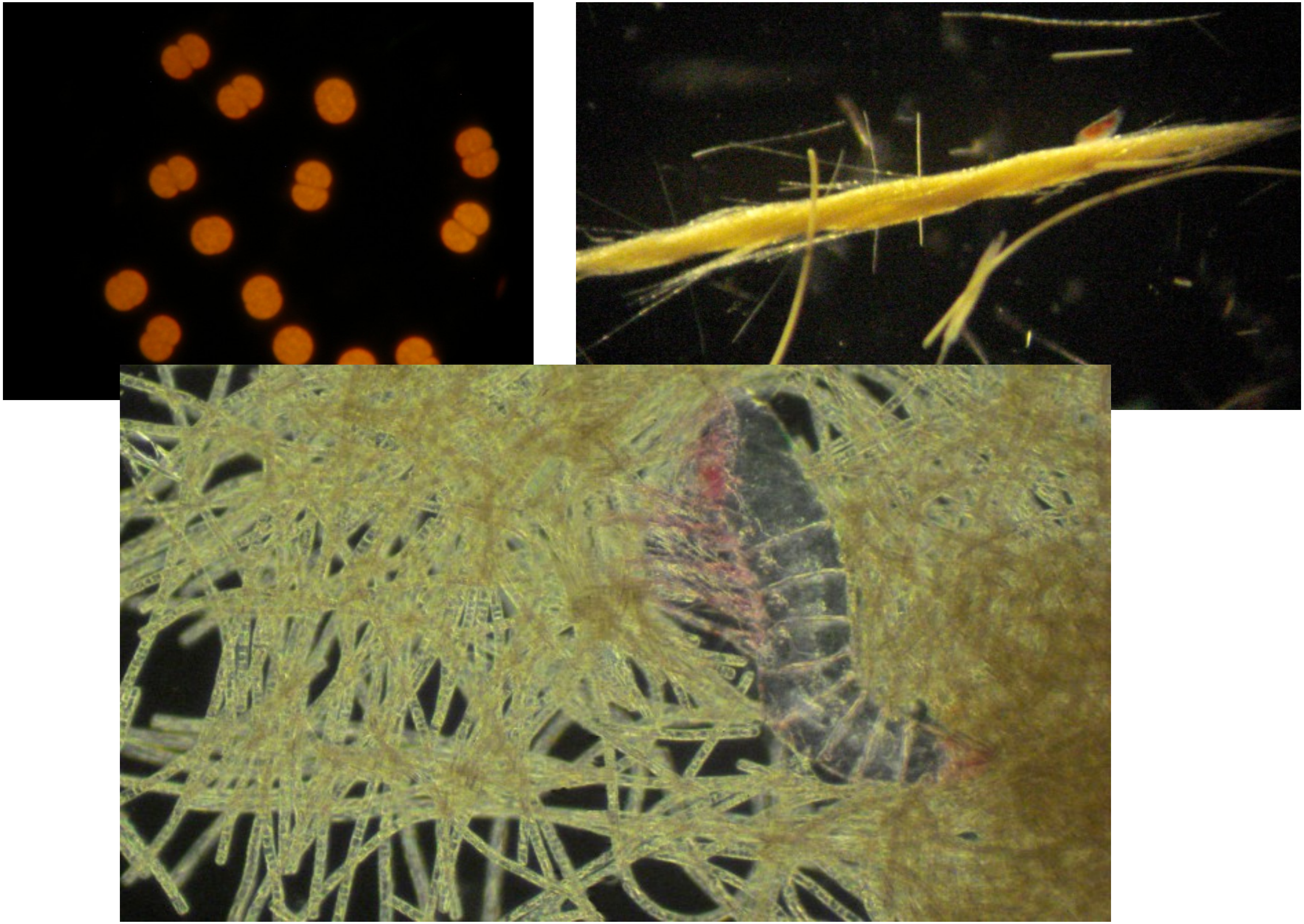
Complications:

- N_2 fixation: a source of non-NO_3- 'new' nitrogen
- Nitrification: a non-new source of NO_3^-
- DON: release as DON is not true export

Dugdale and Goering 1967; Eppley and Peterson 1979

A condensed N cycle for today





Images by Angel White, downloaded from C-MORE website.

Nitrogen fixation: Acquiring N in an N-limited ocean

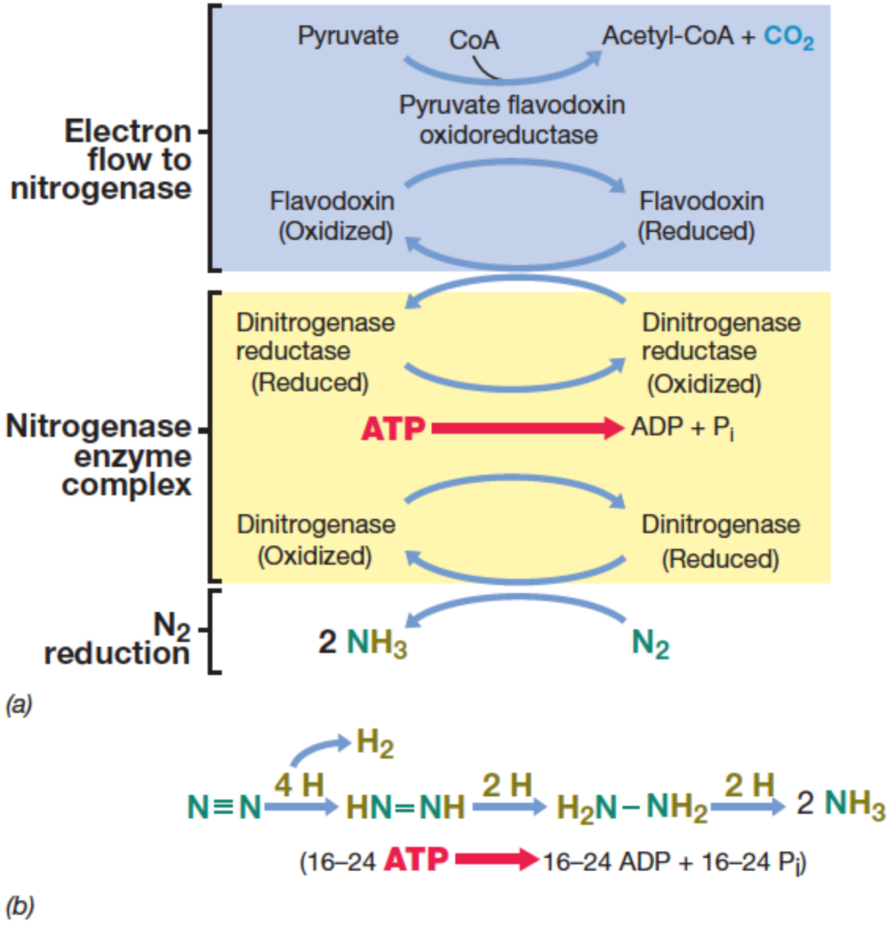
$$N_2 \longrightarrow NH_3$$

- Though thermodynamically favorable, not coupled to energy generation, and very energy intensive (16-29 ATP per N_2)
- Widespread among prokaryotes, and occurs in oxic and anoxic environments
- Diazotroph = "two nitrogen eater" = nitrogen-fixing organism

Nitrogenase: Breaking the N-N triple bond

- Nitrogenase enzyme complex = an Fe-enzyme (dinitrogenase reductase) and Fe-Mo-enzyme (dinitrogenase). Alternative forms contain Vanadium.

- Generates H_2



Electron flow to nitrogenase

Pyruvate → Acetyl-CoA + CO_2 (CoA)

Pyruvate flavodoxin oxidoreductase

Flavodoxin (Oxidized) ⇄ Flavodoxin (Reduced)

Nitrogenase enzyme complex

Dinitrogenase reductase (Reduced) ⇄ Dinitrogenase reductase (Oxidized)

ATP → ADP + P_i

Dinitrogenase (Oxidized) ⇄ Dinitrogenase (Reduced)

N_2 reduction

N_2 → $2\ NH_3$

(a)

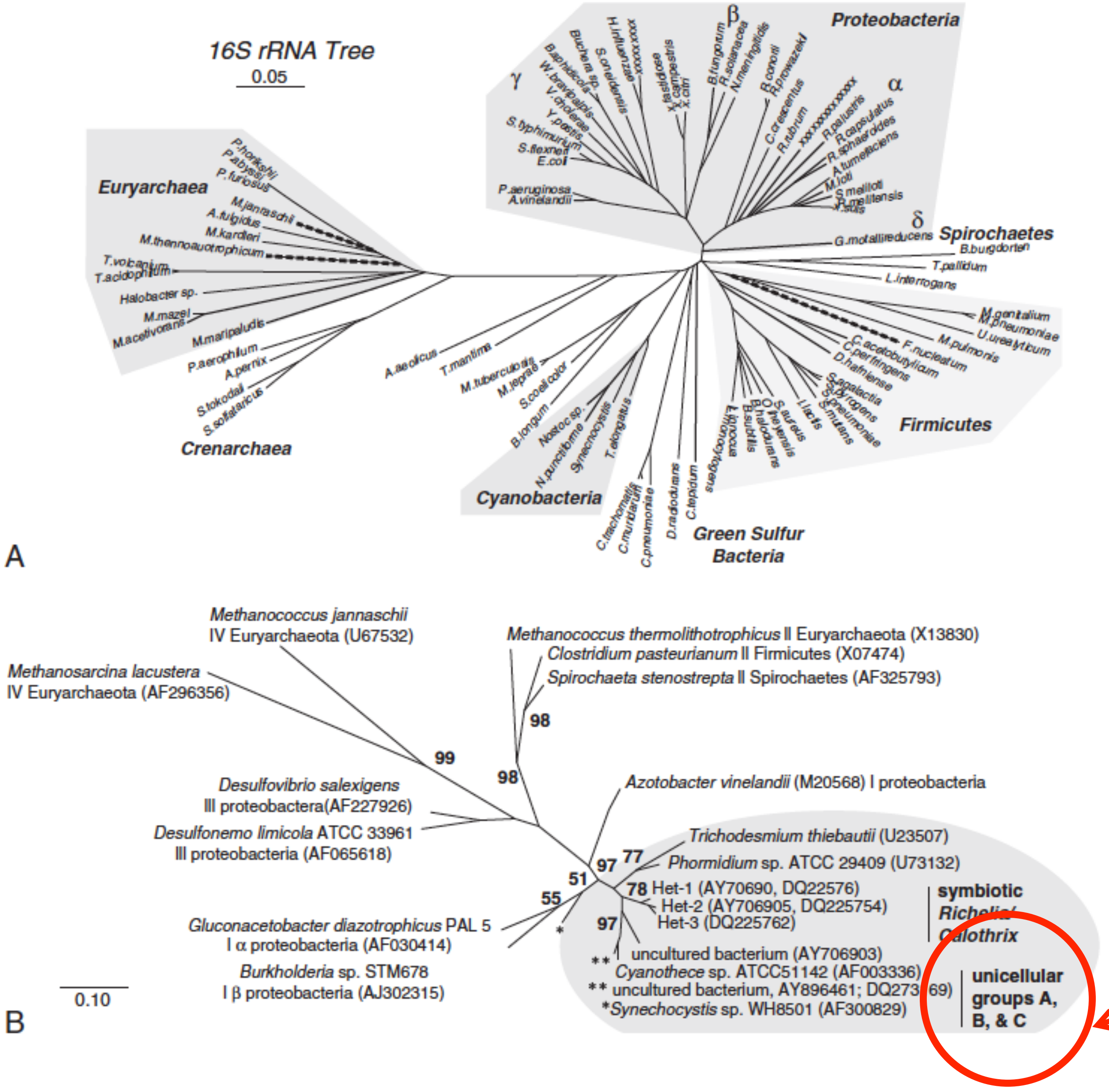
$$N{\equiv}N \xrightarrow{4\,H} HN{=}NH \xrightarrow{2\,H} H_2N{-}NH_2 \xrightarrow{2\,H} 2\ NH_3$$

H_2

(16–24 ATP → 16–24 ADP + 16–24 P_i)

(b)

Brock, 13th ed.



nifH and rRNA phylogenies are congruent.

Suggests that *nif* genes are ancient, inherited vertically over evolutionary time, and lost in some organisms

Great story! Zehr, Tripp, Thompson and coworkers

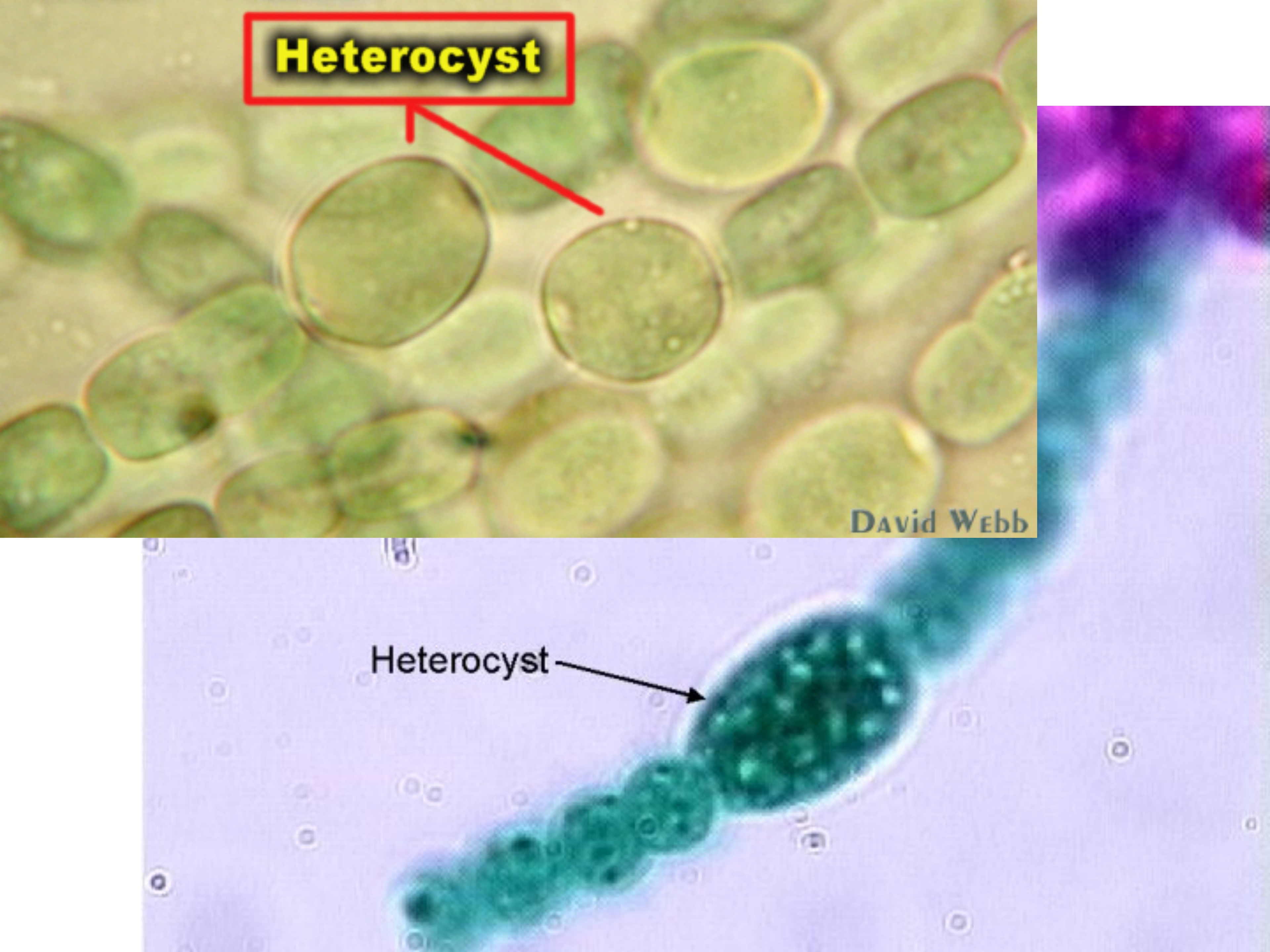
Oxygen is bad for N_2 fixation

O_2 is released by photosynthesis, but O_2 irreversibly inhibits nitrogenase. How can organisms that do both cope?

Dealing with O_2: Spatial segregation

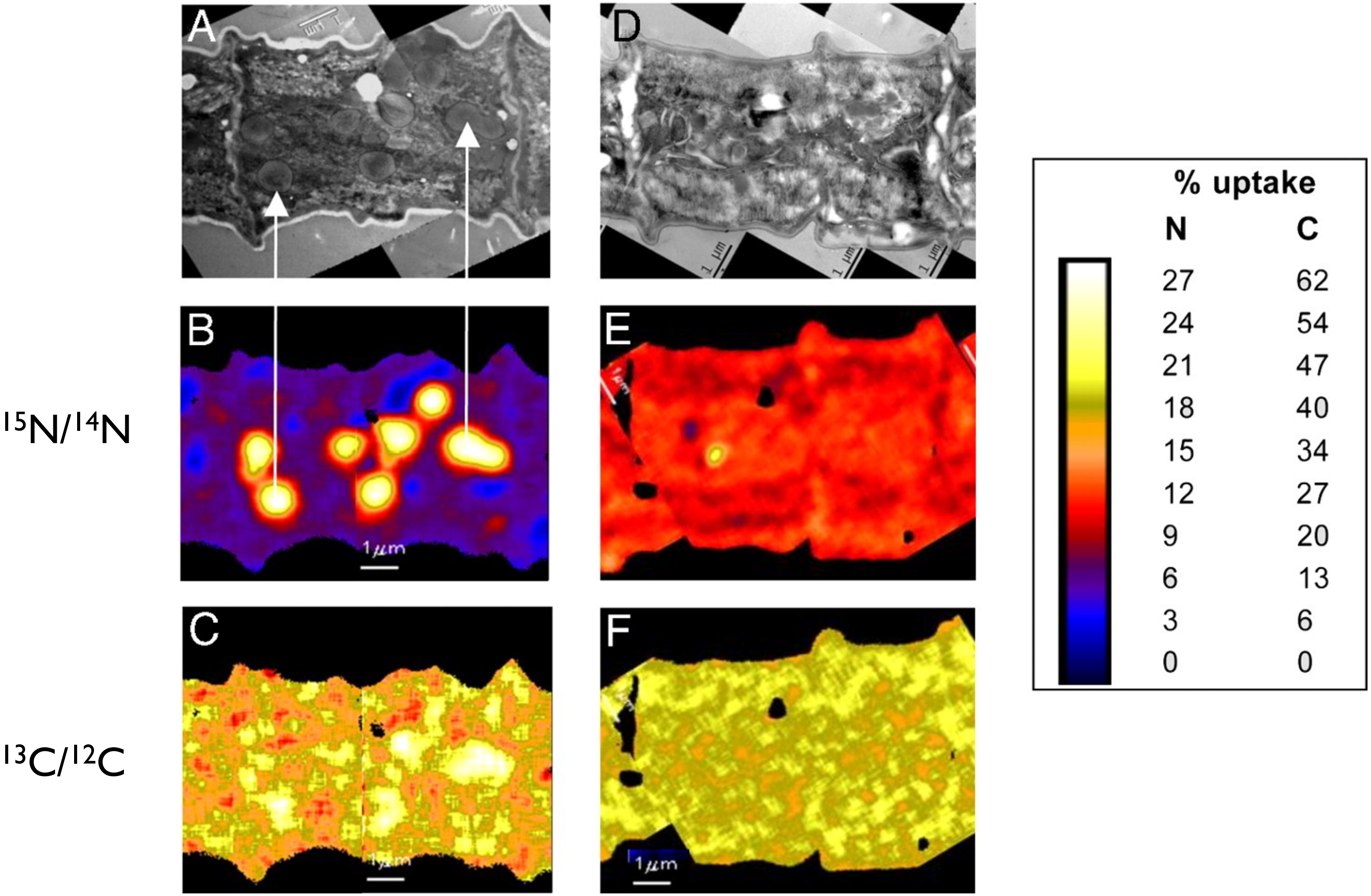
- Heterocysts: specialized cells, lack PSII. Common in freshwater cyanobacteria (*Anabena*) but relatively uncommon in marine waters, except in symbiosis with diatoms : *Richelia intracellularis.*

- Intracellular segregation: *Trichodesmium* (Finzi-Hart *et al.,* 2009); Polysaccharide capsule in aerobic heterotrophs that maintain a low internal oxygen concentration.



DAVID WEBB

NanoSIMS used to track the site of N_2 fixation



Finzi-Hart et al. 2009

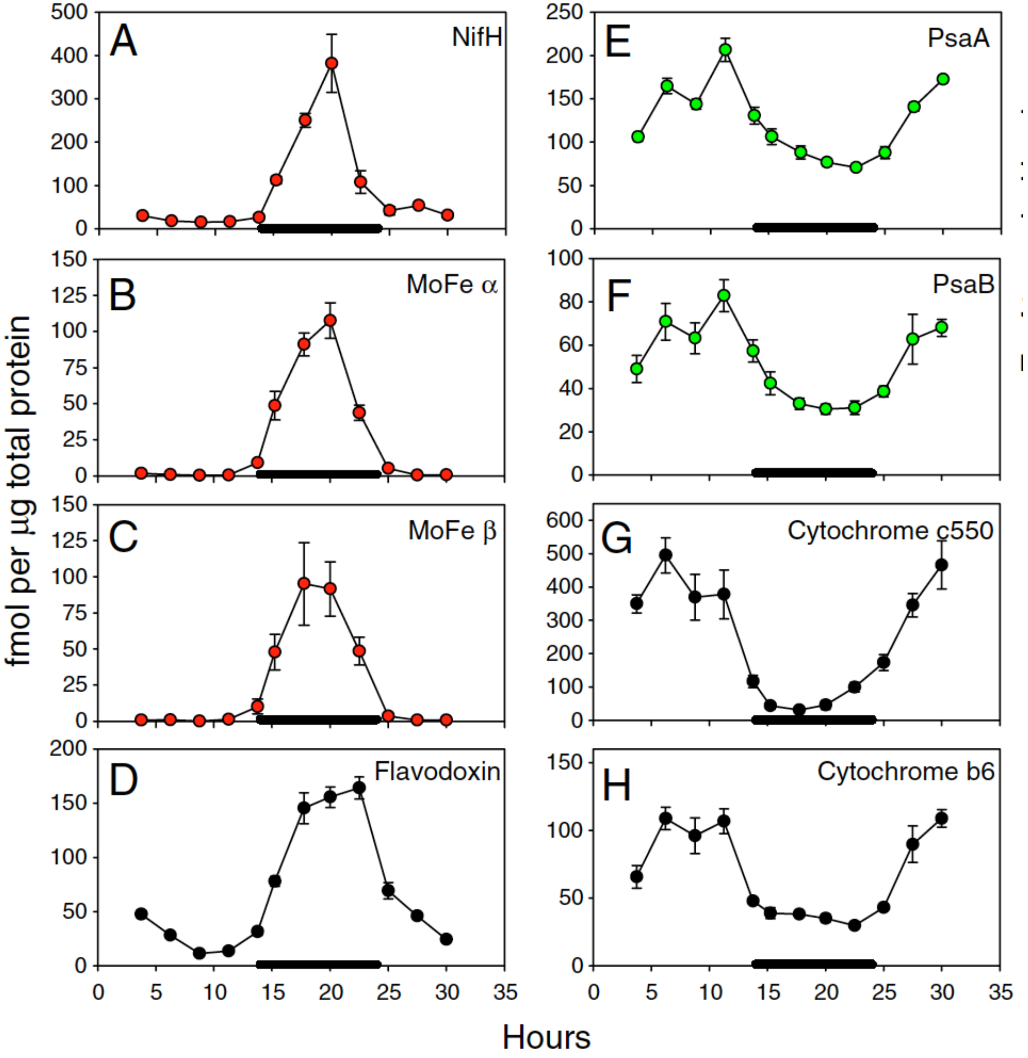
Dealing with O_2: Temporal segregation

Unicellular Cyanobacteria fix nitrogen at night when there is no photosynthesis and cellular oxygen is low.

Crocosphaera watsonii makes N_2 fixation proteins at night and photosynthetic proteins during the day to save Fe.

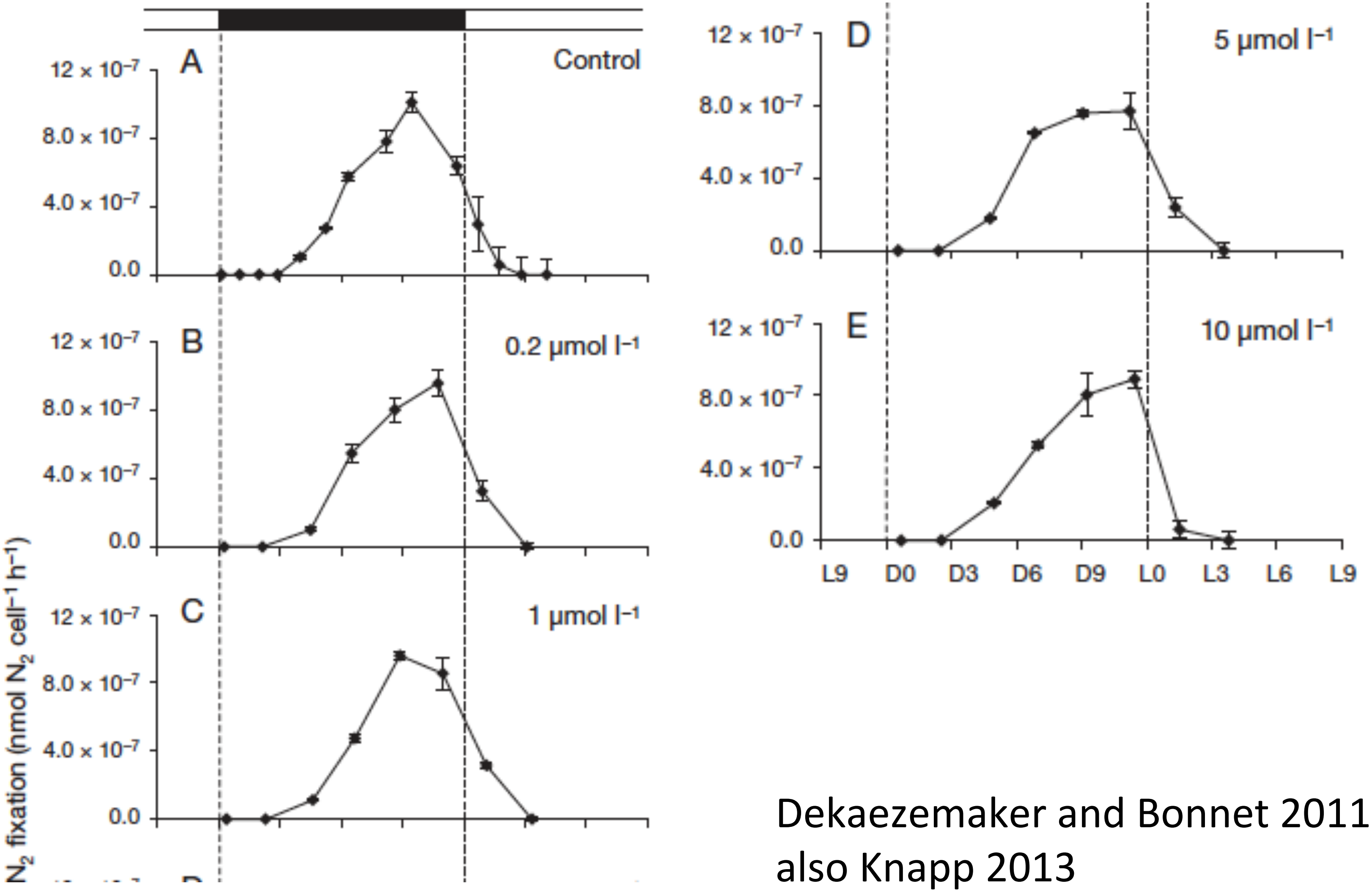
N_2 fixation proteins

Photosynthetic proteins



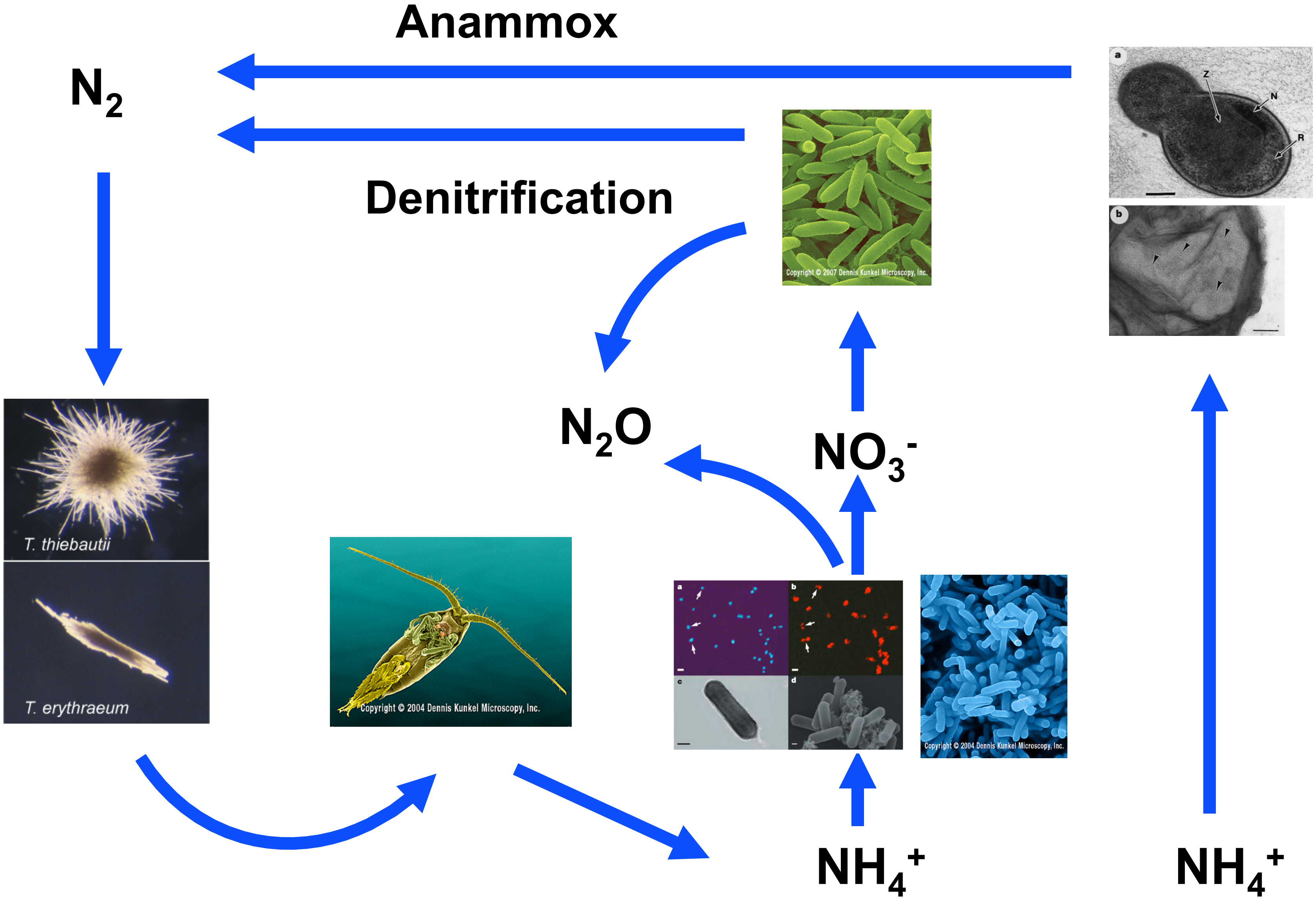
Saito et al. 2011

N_2 fixation can occur in the presence of DIN



Dekaezemaker and Bonnet 2011; see also Knapp 2013

A condensed N cycle for today



Anammox

N_2

Denitrification

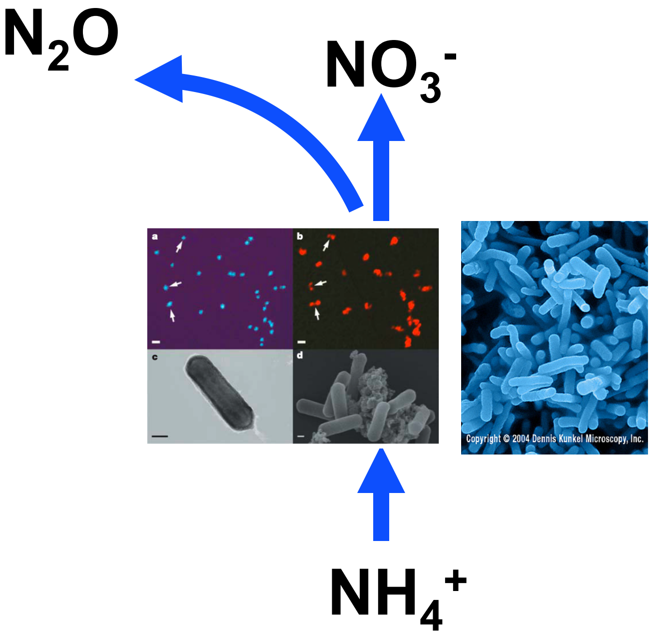
N_2O

NO_3^-

NH_4^+

NH_4^+

A condensed N cycle for today



N_2O

NO_3^-

NH_4^+

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Nitrification is a two step process

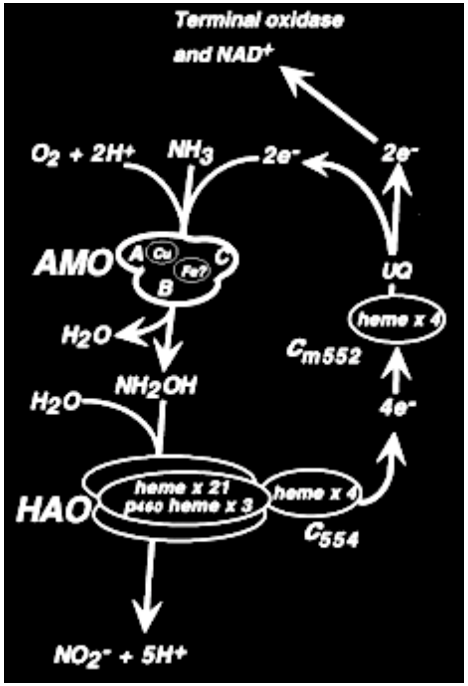
ammonia oxidation: $NH_3 \xrightarrow{\text{AMO}} NH_2OH \xrightarrow{\text{HAO}} NO_2^-$

nitrite oxidation: $NO_2^- \longrightarrow NO_3^-$

No organisms (yet?) known that can catalyze the complete oxidation of NH_3 to NO_3^-.

Some contention about whether NH_3 or NH_4^+ is transported into the cell and is the actual enzymatic substrate.

Model organisms are all autotrophic . . .fussy, slow growing, bastards.

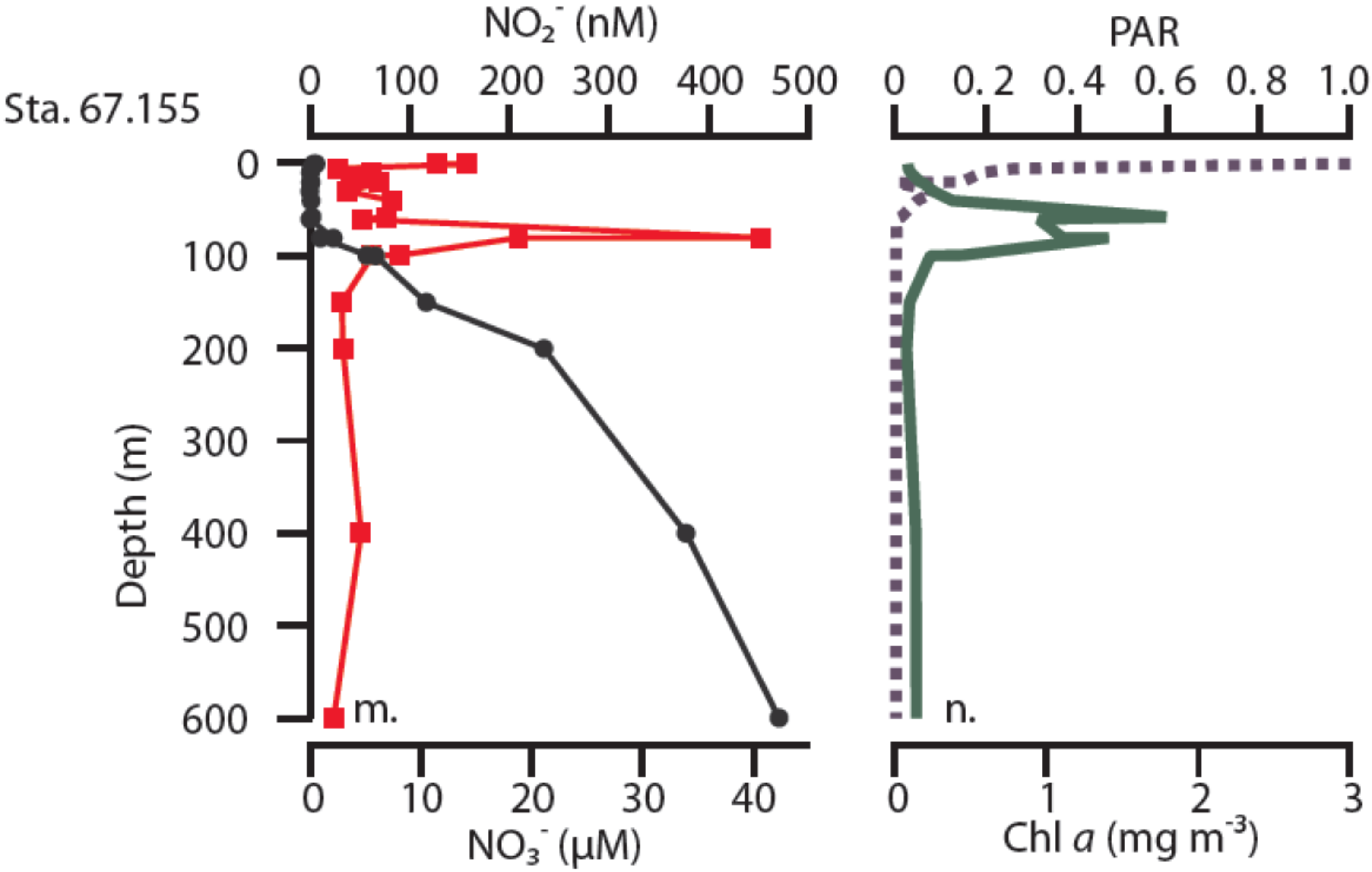


Arp 2002

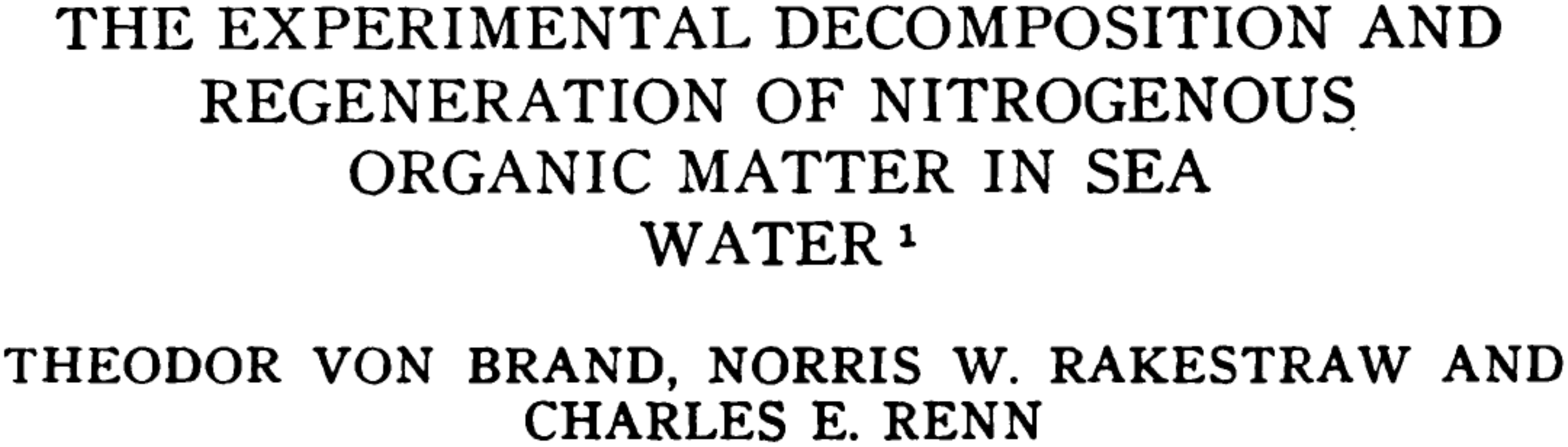

A NEW KIND OF CLEAN
BACTERIA!
NOW WITH
"NITROSOMONAS EUTROPHA"
ROUND-THE-CLOCK MICROBIAL ACTION
BACTERIA!

NYT, 5/25/14

The primary nitrite maximum is a ubiquitous feature

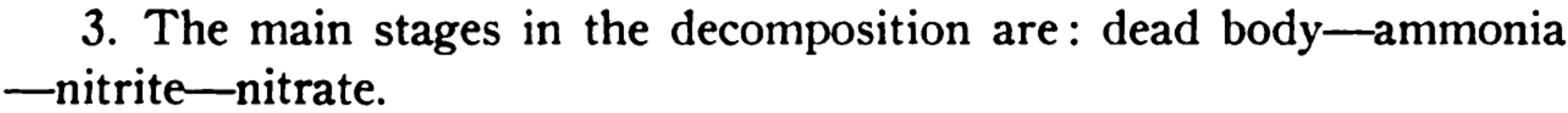


Nitrification = nitrogen remineralization



THE EXPERIMENTAL DECOMPOSITION AND REGENERATION OF NITROGENOUS ORGANIC MATTER IN SEA WATER[1]

THEODOR VON BRAND, NORRIS W. RAKESTRAW AND
CHARLES E. RENN



3. The main stages in the decomposition are: dead body—ammonia—nitrite—nitrate.

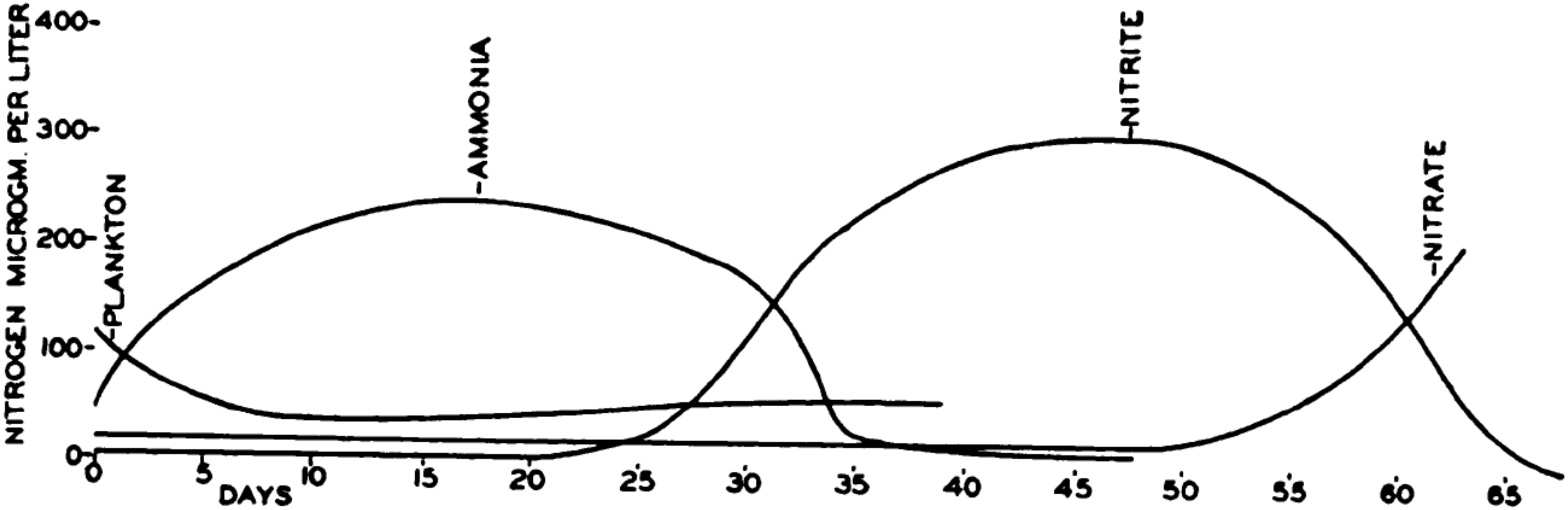
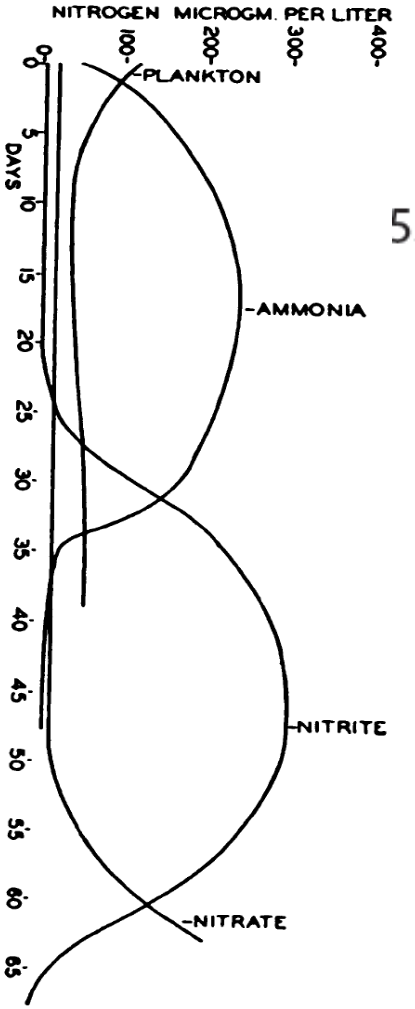
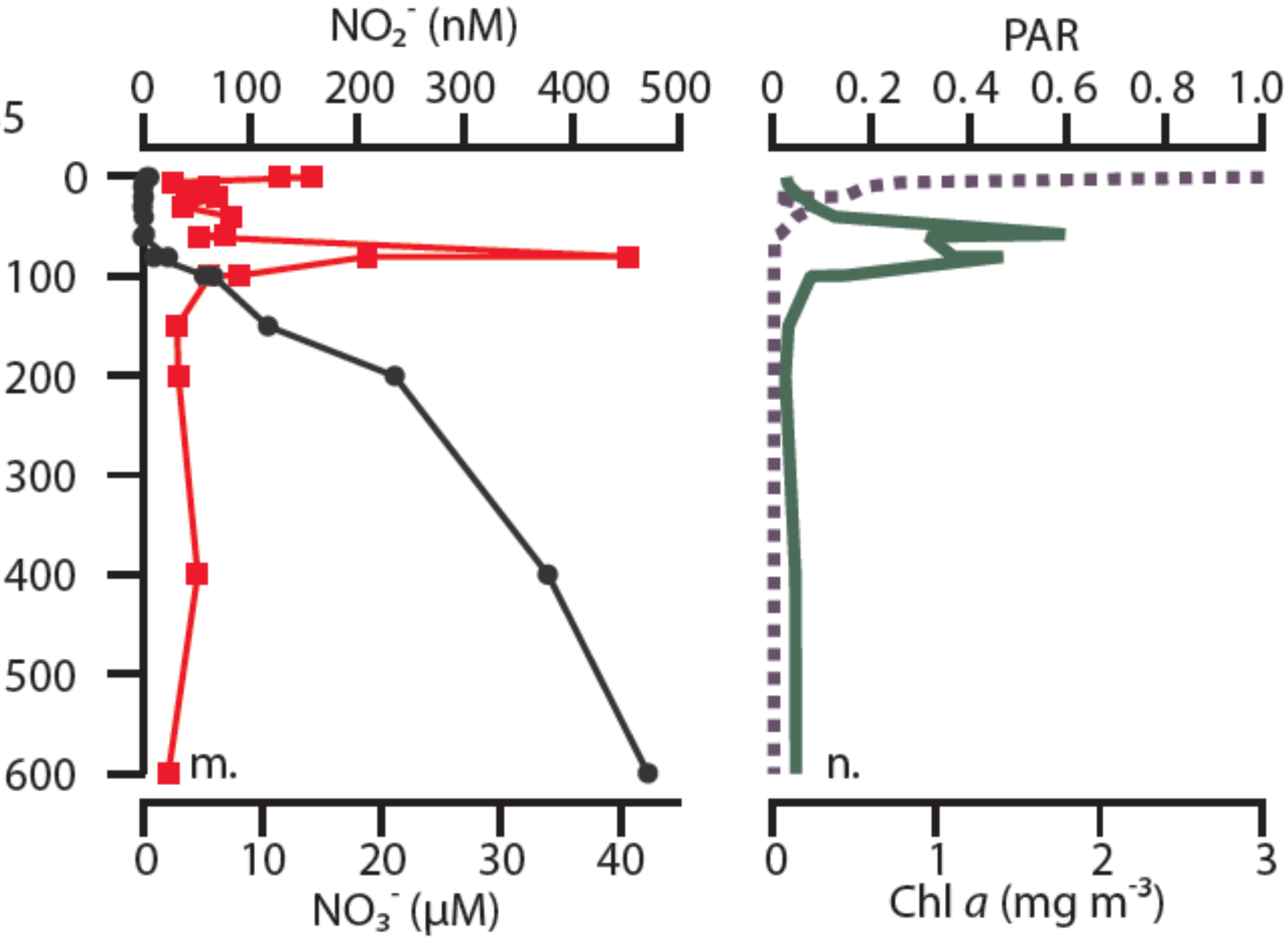


FIG. 2. Series IV. The decomposition of nitrogenous organic matter in mixed plankton, showing the appearance of soluble nitrogen compounds in the water in which it is suspended. Plankton previously filtered through No. 8 bolting silk.


NITROGEN MICROGM. PER LITER
0
100
200
300
400
DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
65
PLANKTON
AMMONIA
NITRITE
NITRATE

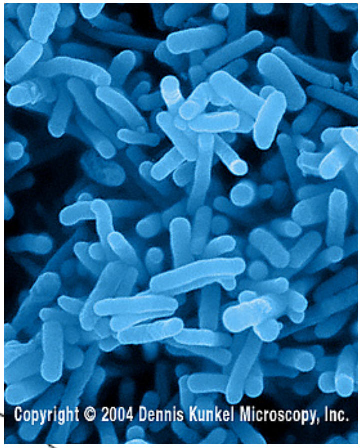
55


NO_2^- (nM)
0
100
200
300
400
500
0
100
200
300
400
500
600
m.
0
10
20
30
40
NO_3^- (μM)
PAR
0
0.2
0.4
0.6
0.8
1.0
n.
0
1
2
3
Chl *a* (mg m^{-3})

Nitrifiers perform a very specific task

- Fix CO_2 or bicarbonate for anabolic reactions, oxidize NH_3 for energy
- Slow growth and somewhat inflexible nutritional requirements (more on this in next lecture)
- Historic view of nitrifiers in the ocean as being inhibited by light due to photobleaching of cytochrome c

amoA phylogeny of AOB is highly congruent with 16S tree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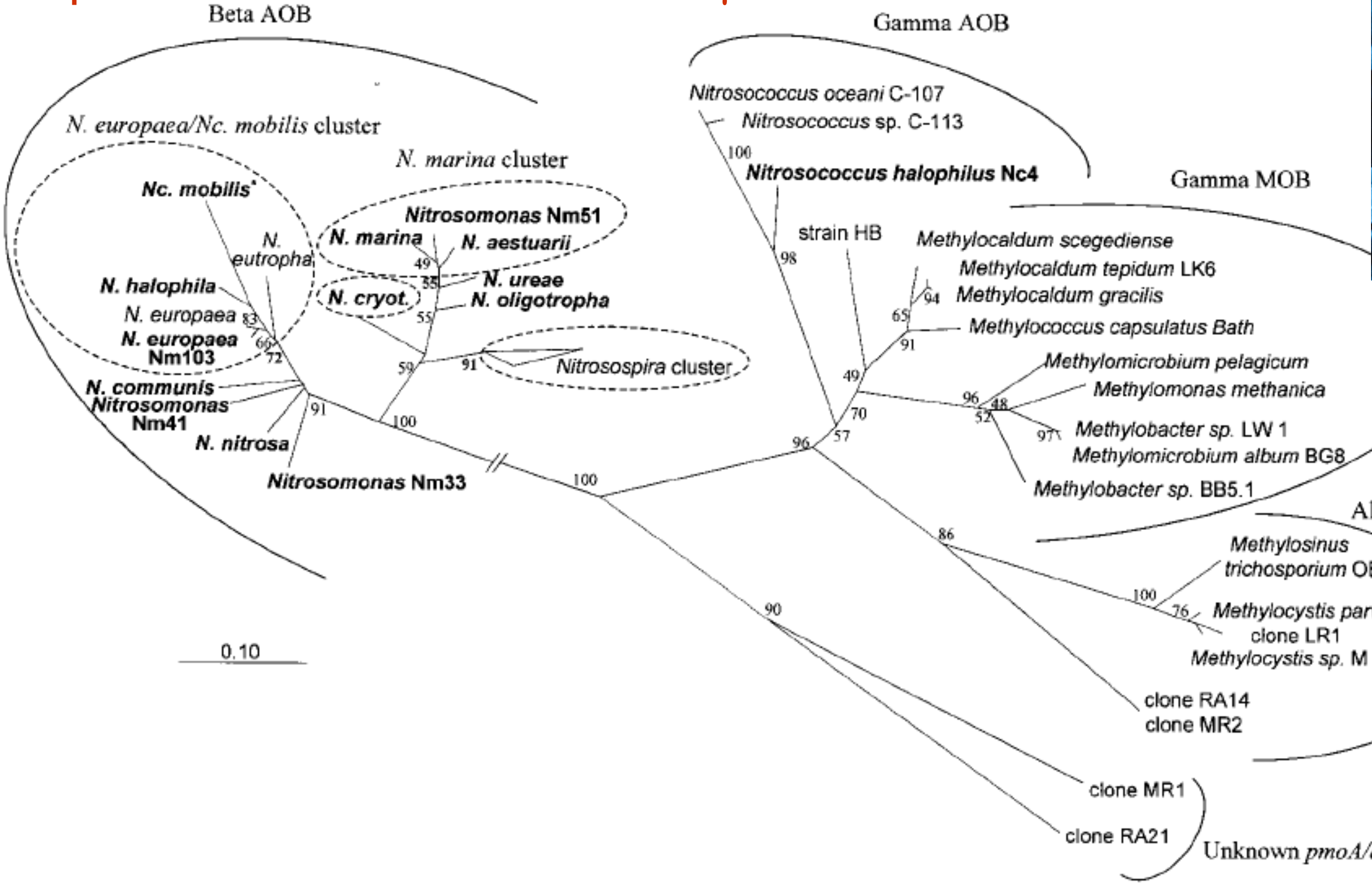
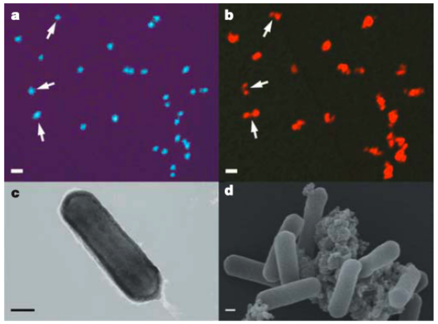
β-Proteobacteria

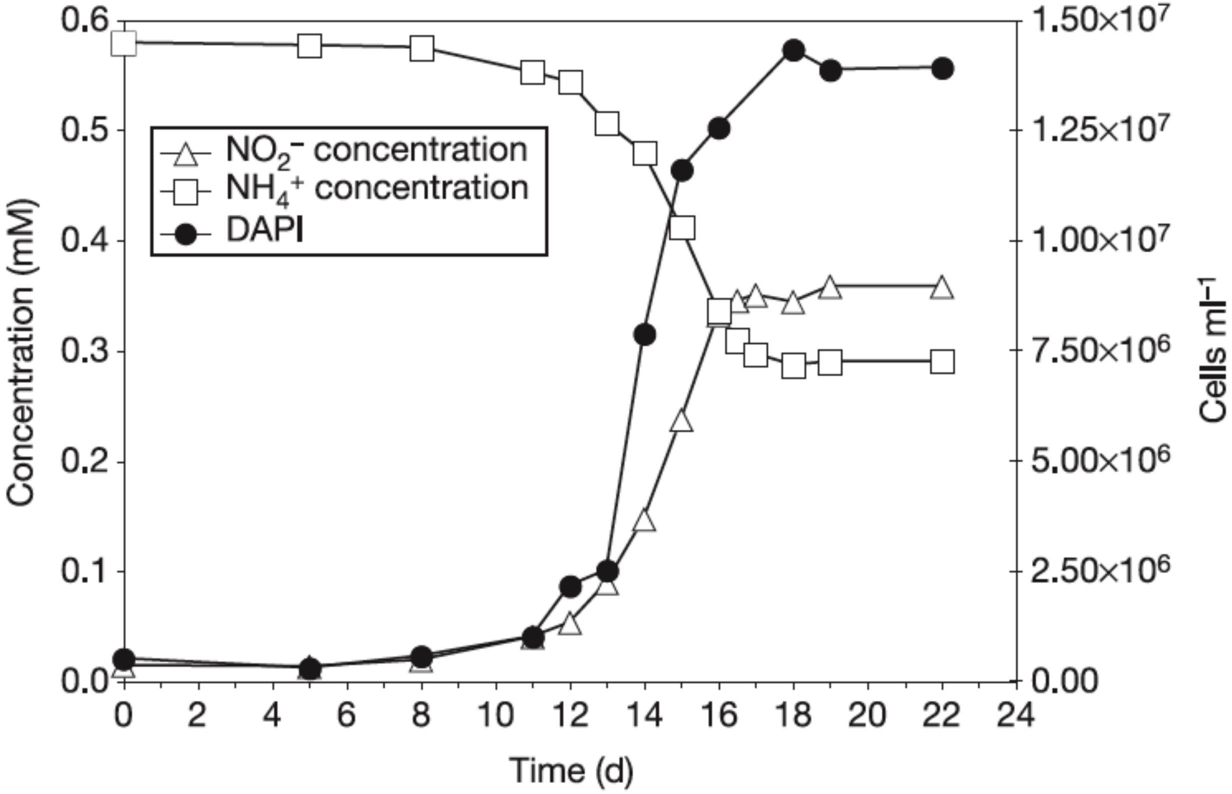
γ-Proteobacteria



Purkhold et al, AEM, 2000; image: Dennis Kunkel Microscopy



The first culture of a mesophilic marine crenarchaeon, isolated from gravel at the Shedd Aquarium.



Archaea can oxidize NH_3

Konneke et al. (2005), Nature

Ecology of ammonia oxidizers

- *amoA* phylogeny is congruent with 16S rRNA phylogeny. Not much lateral gene transfer.

- At low O_2 tension ammonia oxidizers produce N_2O. Some ammonia oxidizers contain *nirK* genes (dissimilatory nitrite reductase)

- Potentially inhibited by light (photobleaching of cytochrome C). Hypothesis that NOB are more inhibited than AOB.

AOA and AOB have different life history strategies

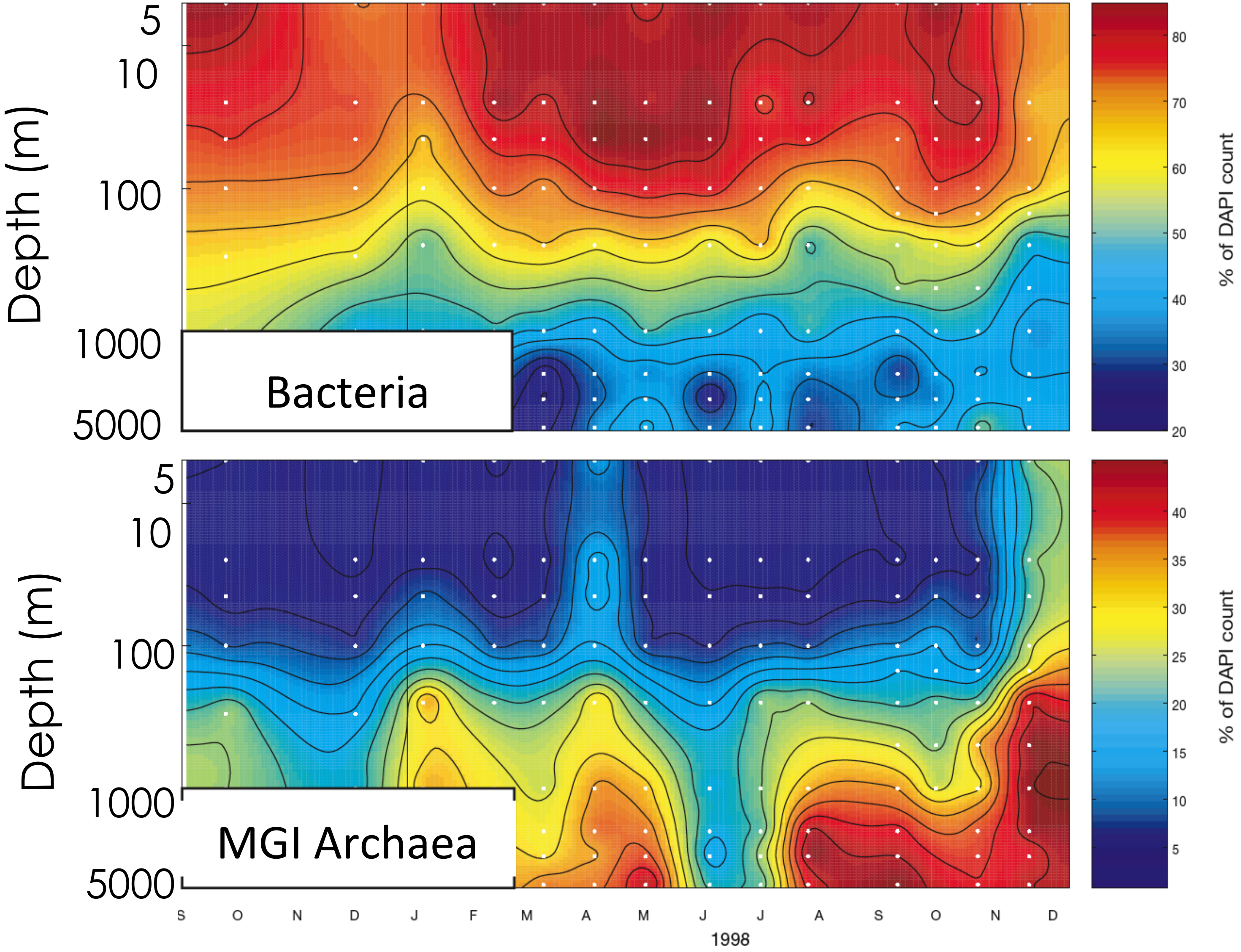
AOB

- Larger cells
- Easier to culture
- High K_m for NH_3
- Faster growth
- Big genomes
- Fe-based electron transport
- Very light sensitive

AOA

- Small cells
- Difficult to culture
- Low K_m for NH_3
- Slow growth
- Reduced genomes
- More tolerant of low O_2
- Cu-based electron transport
- Highly efficient C fixation pathway

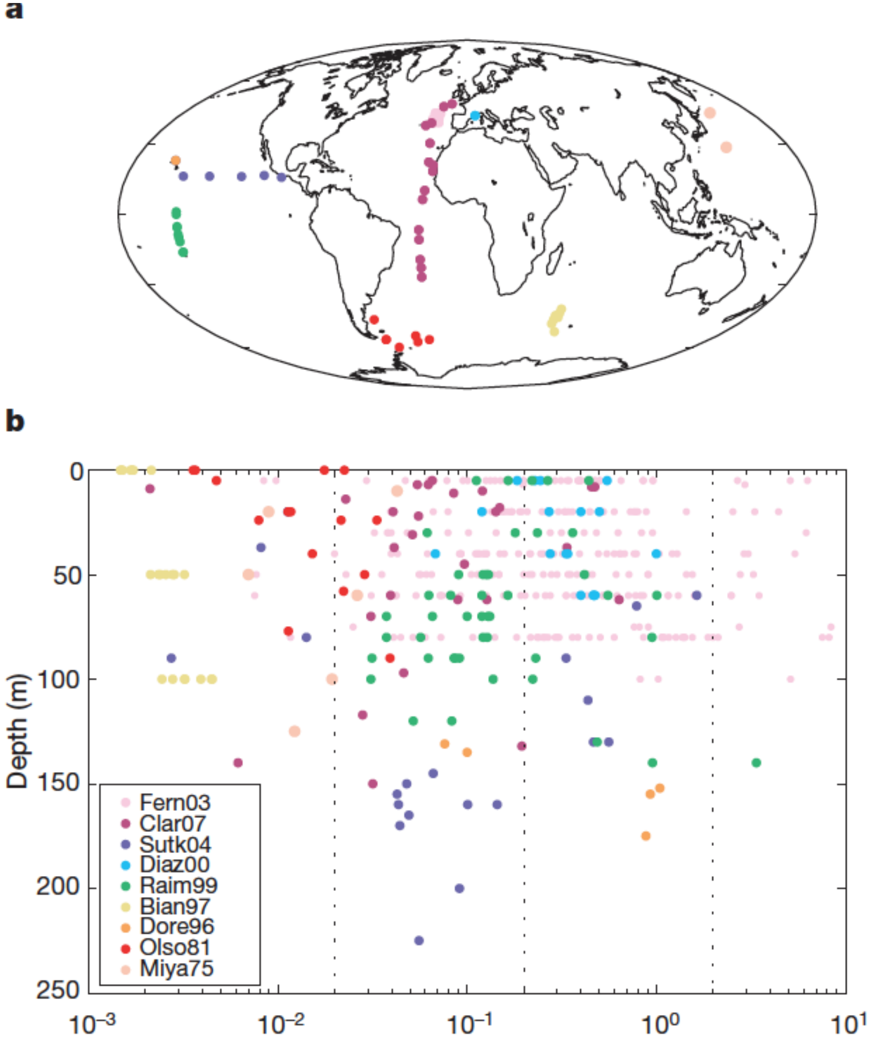
Putative ammonia oxidizers are among the most abundant cells in the deep ocean.



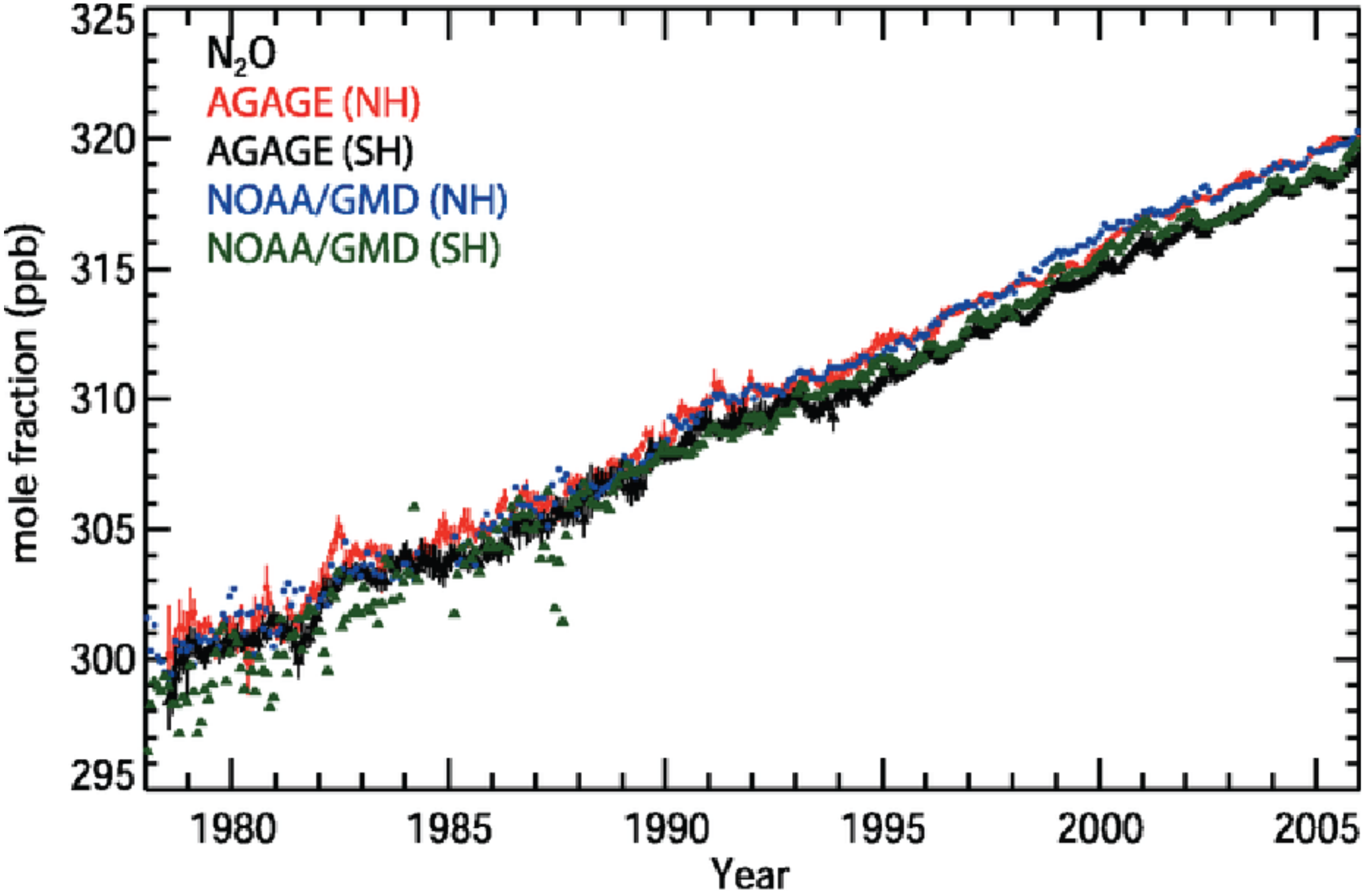
modified from Karner et al., Nature, 2001

The significance of nitrification for oceanic new production

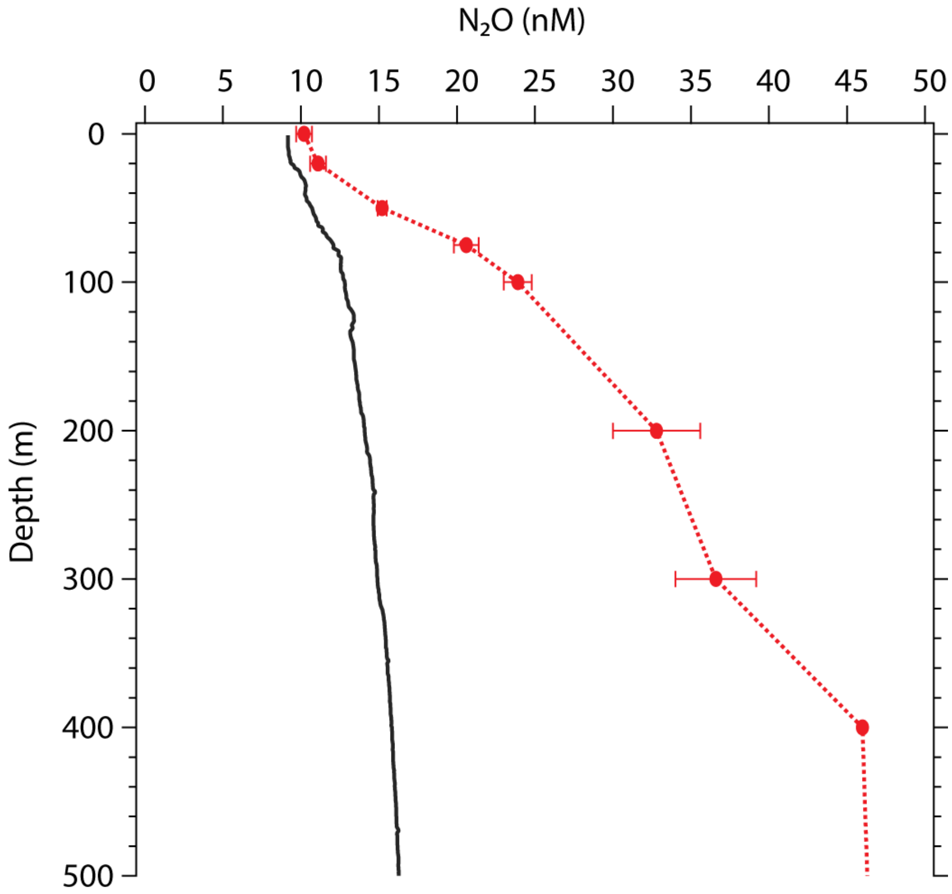
Andrew Yool[1], Adrian P. Martin[1], Camila Fernández[2,3] & Darren R. Clark[4]



N_2O is a greenhouse gas whose concentration in the atmosphere is increasing



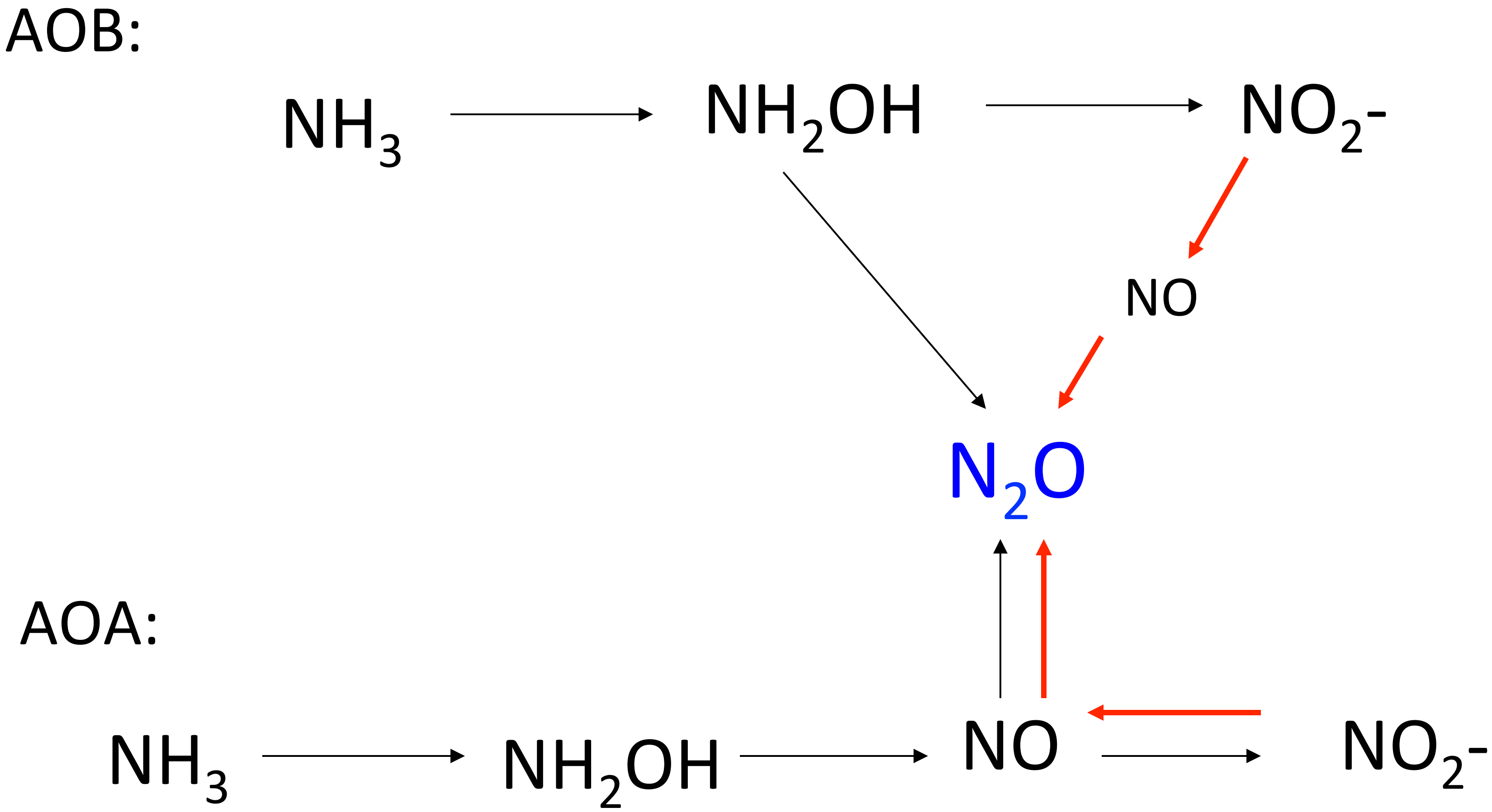
N_2O is supersaturated in most of the ocean



Santoro et al. 2010, EM

Central California Current

Ammonia oxidation is a potential source of marine N_2O



AOB:

NH_3

NH_2OH

NO_2-

NO

N_2O

AOA:

NH_3

NH_2OH

NO

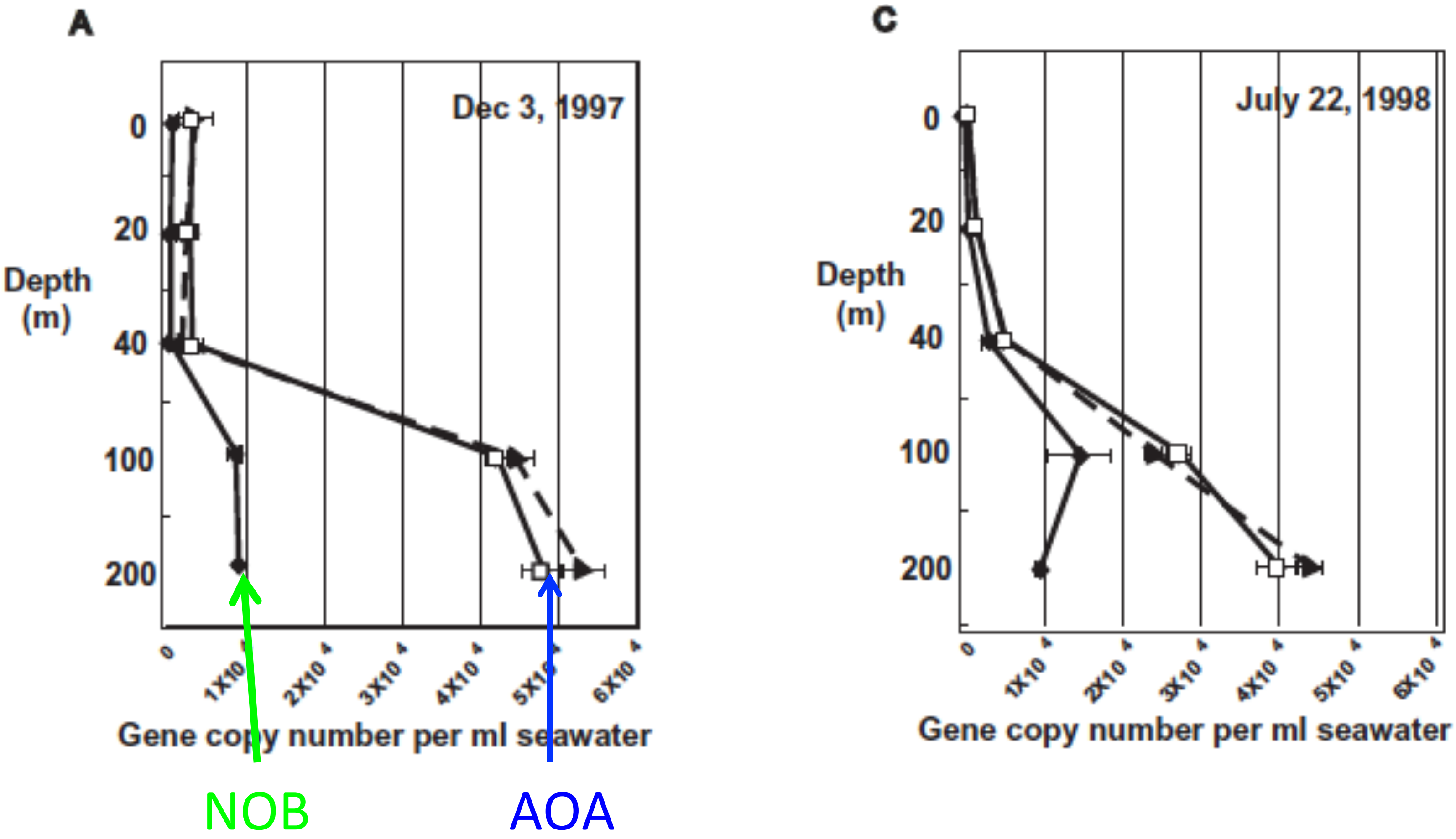
NO_2-

Nitrite oxidizers carry out the second step of nitrification

$$NO_2^- \longrightarrow NO_3^-$$

- Limited to only a few genera (*Nitrospira*, *Nitrospina*, *Nitrobacter*)
- Mostly autotrophs, but some capacity for mixotrophy
- Growing as autotrophs, they are living near the threshold for life ($\Delta G^{0'}$ = -74 kJ/mol)

Covariation of ammonia oxidizers (AOA) and nitrite oxidizers (NOB)

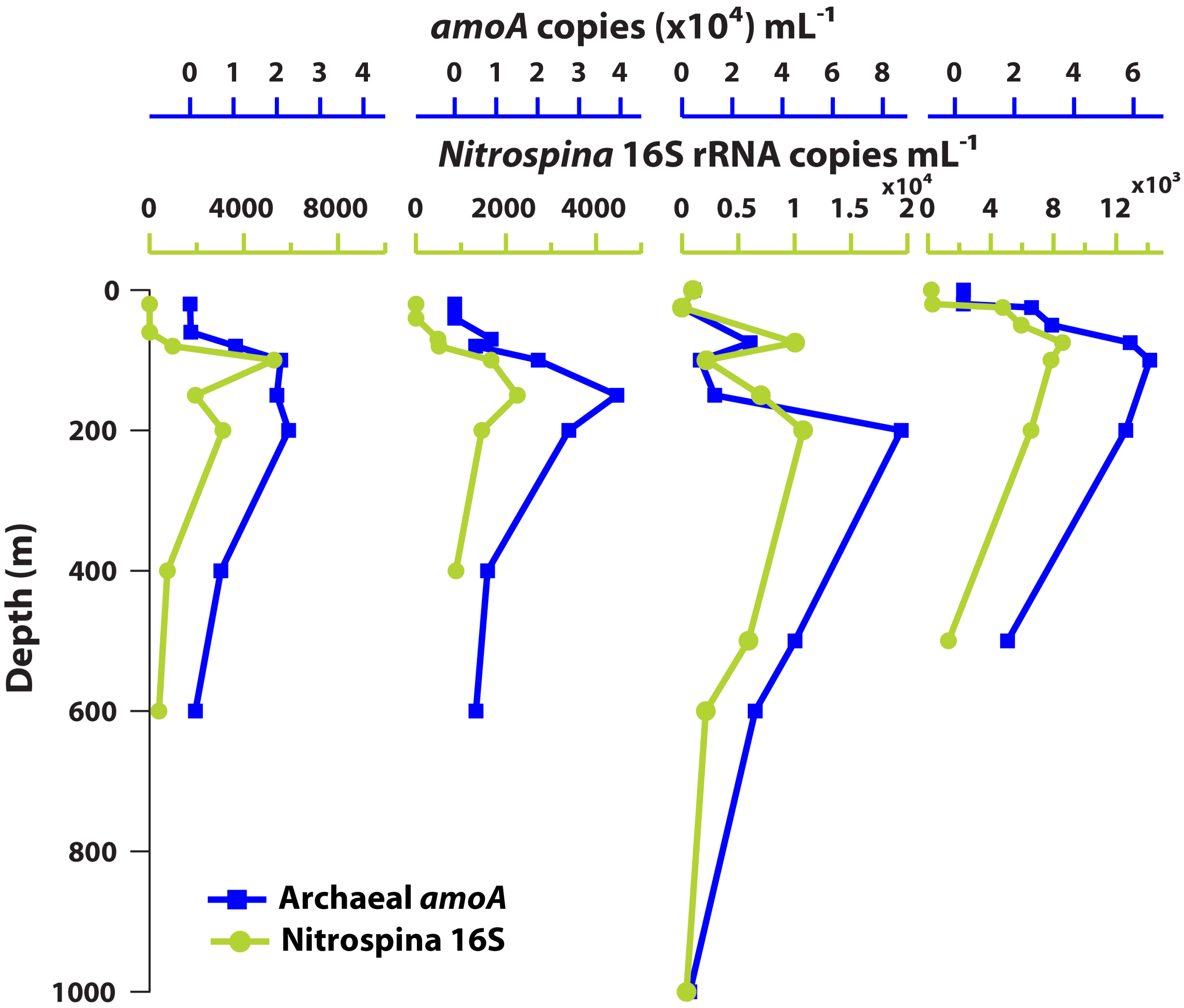


NOB

AOA

Mincer et al. 2007

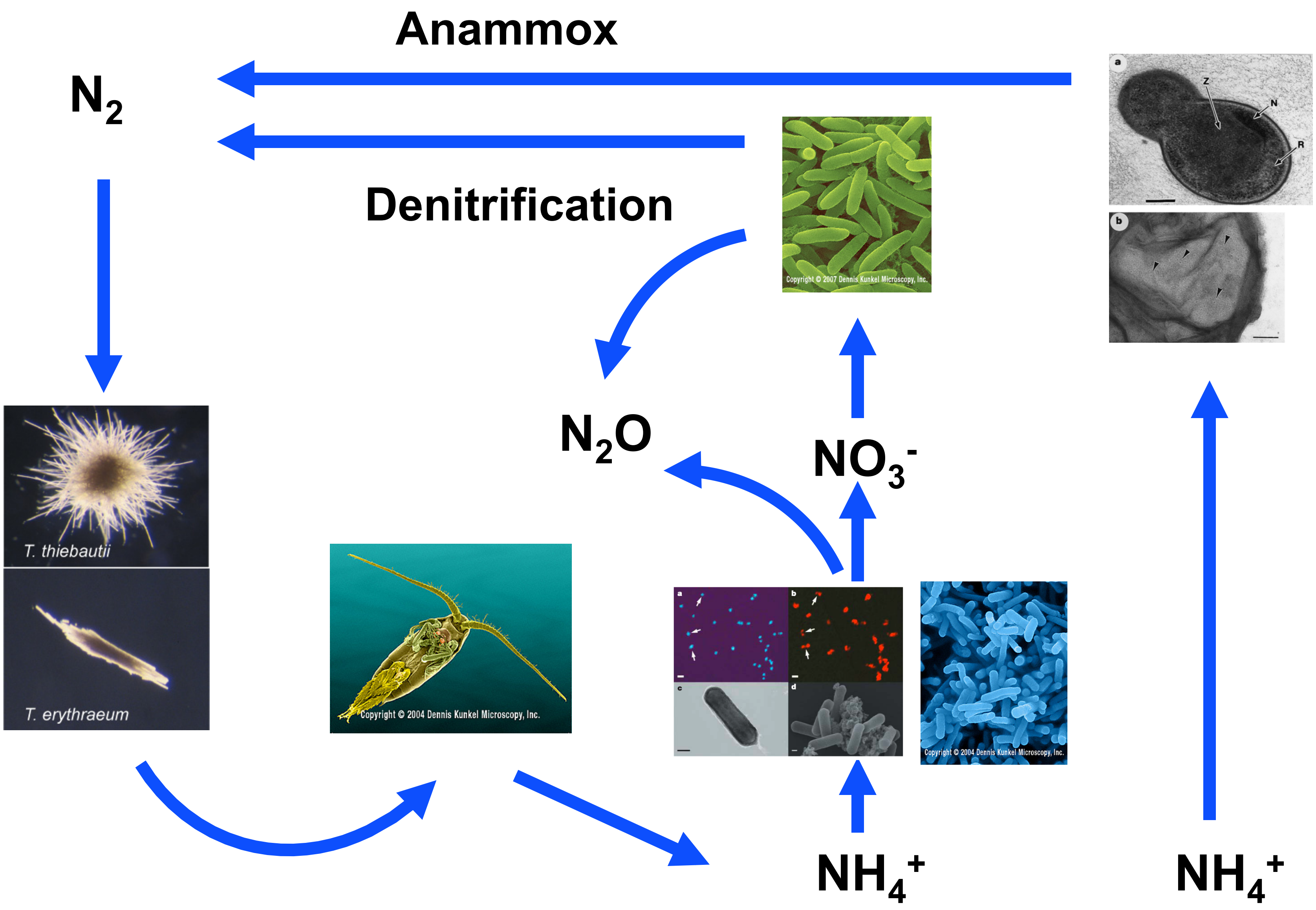
Covariation of AOA and NOB



data from
CA current

Santoro et al. 2010

A condensed N cycle for today



Anammox

N_2

Denitrification

N_2O

NO_3^-

NH_4^+

NH_4^+

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Denitrification is a multi-step process

$$\mathrm{NO_3^-} \xrightarrow[\text{Nap}]{\text{Nar}} \mathrm{NO_2^-} \xrightarrow[\text{NirK}]{\text{NirS}} \mathrm{NO} \xrightarrow{\text{Nor}} \mathrm{N_2O} \xrightarrow{\text{Nos}} \mathrm{N_2}$$

- The dissimilatory reduction of nitrogen oxides to gaseous products under low-oxygen conditions
- A series of one electron transfers, not necessarily carried out by a single organism
- Frequently found in organisms capable of using other electron acceptors, including oxygen

Denitrification genes are a "necklace"

Genes are not congruent with 16S rRNA phylogeny (laterally transferred)

from Zumft 1997

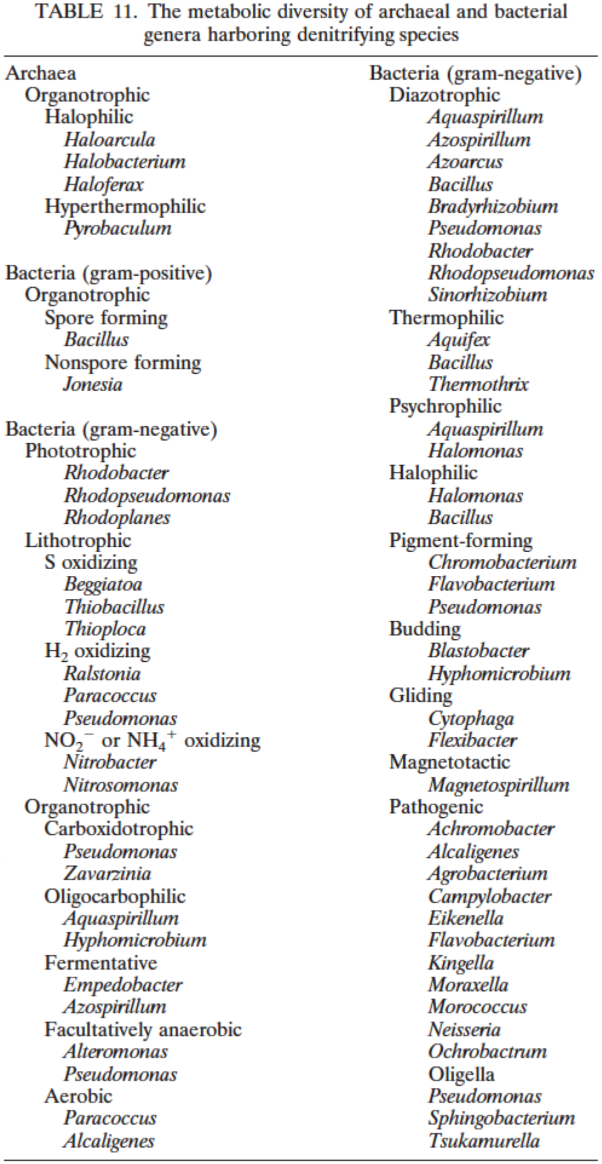


TABLE 11. The metabolic diversity of archaeal and bacterial genera harboring denitrifying species

- Archaea
  - Organotrophic
    - Halophilic
      - *Haloarcula*
      - *Halobacterium*
      - *Haloferax*
    - Hyperthermophilic
      - *Pyrobaculum*
- Bacteria (gram-positive)
  - Organotrophic
    - Spore forming
      - *Bacillus*
    - Nonspore forming
      - *Jonesia*
- Bacteria (gram-negative)
  - Phototrophic
    - *Rhodobacter*
    - *Rhodopseudomonas*
    - *Rhodoplanes*
  - Lithotrophic
    - S oxidizing
      - *Beggiatoa*
      - *Thiobacillus*
      - *Thioploca*
    - H_2 oxidizing
      - *Ralstonia*
      - *Paracoccus*
      - *Pseudomonas*
    - NO_2^- or NH_4^+ oxidizing
      - *Nitrobacter*
      - *Nitrosomonas*
  - Organotrophic
    - Carboxidotrophic
      - *Pseudomonas*
      - *Zavarzinia*
    - Oligocarbophilic
      - *Aquaspirillum*
      - *Hyphomicrobium*
    - Fermentative
      - *Empedobacter*
      - *Azospirillum*
    - Facultatively anaerobic
      - *Alteromonas*
      - *Pseudomonas*
    - Aerobic
      - *Paracoccus*
      - *Alcaligenes*
- Bacteria (gram-negative)
  - Diazotrophic
    - *Aquaspirillum*
    - *Azospirillum*
    - *Azoarcus*
    - *Bacillus*
    - *Bradyrhizobium*
    - *Pseudomonas*
    - *Rhodobacter*
    - *Rhodopseudomonas*
    - *Sinorhizobium*
  - Thermophilic
    - *Aquifex*
    - *Bacillus*
    - *Thermothrix*
  - Psychrophilic
    - *Aquaspirillum*
    - *Halomonas*
  - Halophilic
    - *Halomonas*
    - *Bacillus*
  - Pigment-forming
    - *Chromobacterium*
    - *Flavobacterium*
    - *Pseudomonas*
  - Budding
    - *Blastobacter*
    - *Hyphomicrobium*
  - Gliding
    - *Cytophaga*
    - *Flexibacter*
  - Magnetotactic
    - *Magnetospirillum*
  - Pathogenic
    - *Achromobacter*
    - *Alcaligenes*
    - *Agrobacterium*
    - *Campylobacter*
    - *Eikenella*
    - *Flavobacterium*
    - *Kingella*
    - *Moraxella*
    - *Morococcus*
    - *Neisseria*
    - *Ochrobactrum*
    - Oligella
    - *Pseudomonas*
    - *Sphingobacterium*
    - *Tsukamurella*

Maybe it's more of a charm bracelet . .

COUPLED TO GLUCOSE UTILIZATION

Aerobic respiration:

$(CH_2O)_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

$\Delta G^{o\prime}$ = - 2870 kJ (-686 kcal)

1. Complete denitrification:

$(CH_2O)_6 + 4.8\ HNO_3 \rightarrow 6\ CO_2 + 2.4\ N_2 + 8.4\ H_2O$

$\Delta G^{o\prime}$ = - 2669 kJ (-638 kcal)

2. Nitrate respiration (to nitrite only)

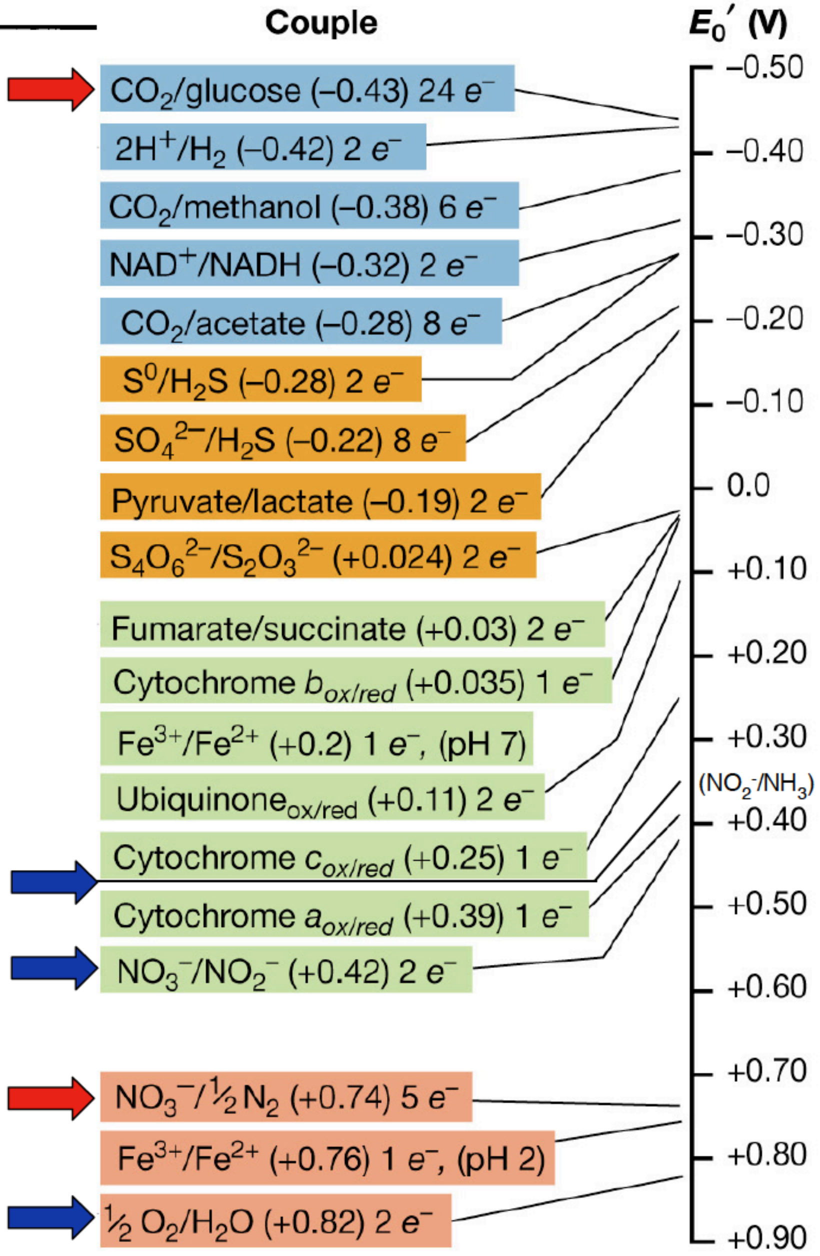
$(CH_2O)_6 + 12\ NO_3 \rightarrow 6\ CO_2 + 12\ NO_2 + 6\ H_2O$

$\Delta G^{o\prime}$ = - 1766 kJ (-422 kcal)

3. Nitrate reduction to ammonia

$(CH_2O)_6 + 3\ HNO_3 \rightarrow 6\ CO_2 + 3\ NH_3 + 3\ H_2O$

$\Delta G^{o\prime}$ = - 1796 kJ (-429 kcal)



Couple

CO_2/glucose (–0.43) 24 e^-

$2H^+/H_2$ (–0.42) 2 e^-

CO_2/methanol (–0.38) 6 e^-

NAD^+/NADH (–0.32) 2 e^-

CO_2/acetate (–0.28) 8 e^-

S^0/H_2S (–0.28) 2 e^-

SO_4^{2-}/H_2S (–0.22) 8 e^-

Pyruvate/lactate (–0.19) 2 e^-

$S_4O_6^{2-}/S_2O_3^{2-}$ (+0.024) 2 e^-

Fumarate/succinate (+0.03) 2 e^-

Cytochrome $b_{ox/red}$ (+0.035) 1 e^-

Fe^{3+}/Fe^{2+} (+0.2) 1 e^-, (pH 7)

Ubiquinone$_{ox/red}$ (+0.11) 2 e^-

Cytochrome $c_{ox/red}$ (+0.25) 1 e^-

Cytochrome $a_{ox/red}$ (+0.39) 1 e^-

NO_3^-/NO_2^- (+0.42) 2 e^-

$NO_3^-/\frac{1}{2}N_2$ (+0.74) 5 e^-

Fe^{3+}/Fe^{2+} (+0.76) 1 e^-, (pH 2)

$\frac{1}{2}\ O_2/H_2O$ (+0.82) 2 e^-

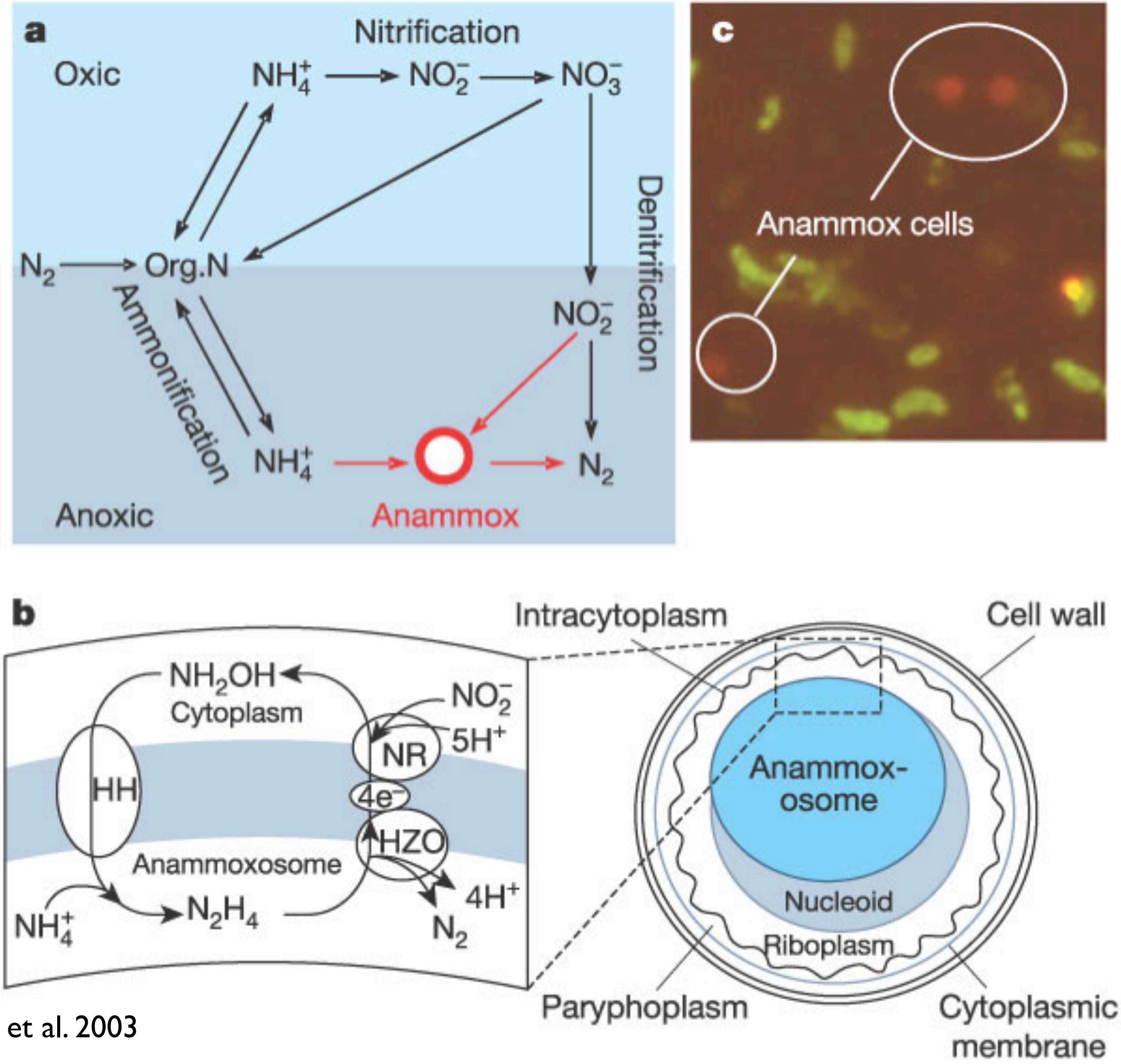
Anaerobic ammonia oxidation (anammox) solves a marine conundrum (?)

$$NH_4^+ + NO_2^- \longrightarrow N_2$$

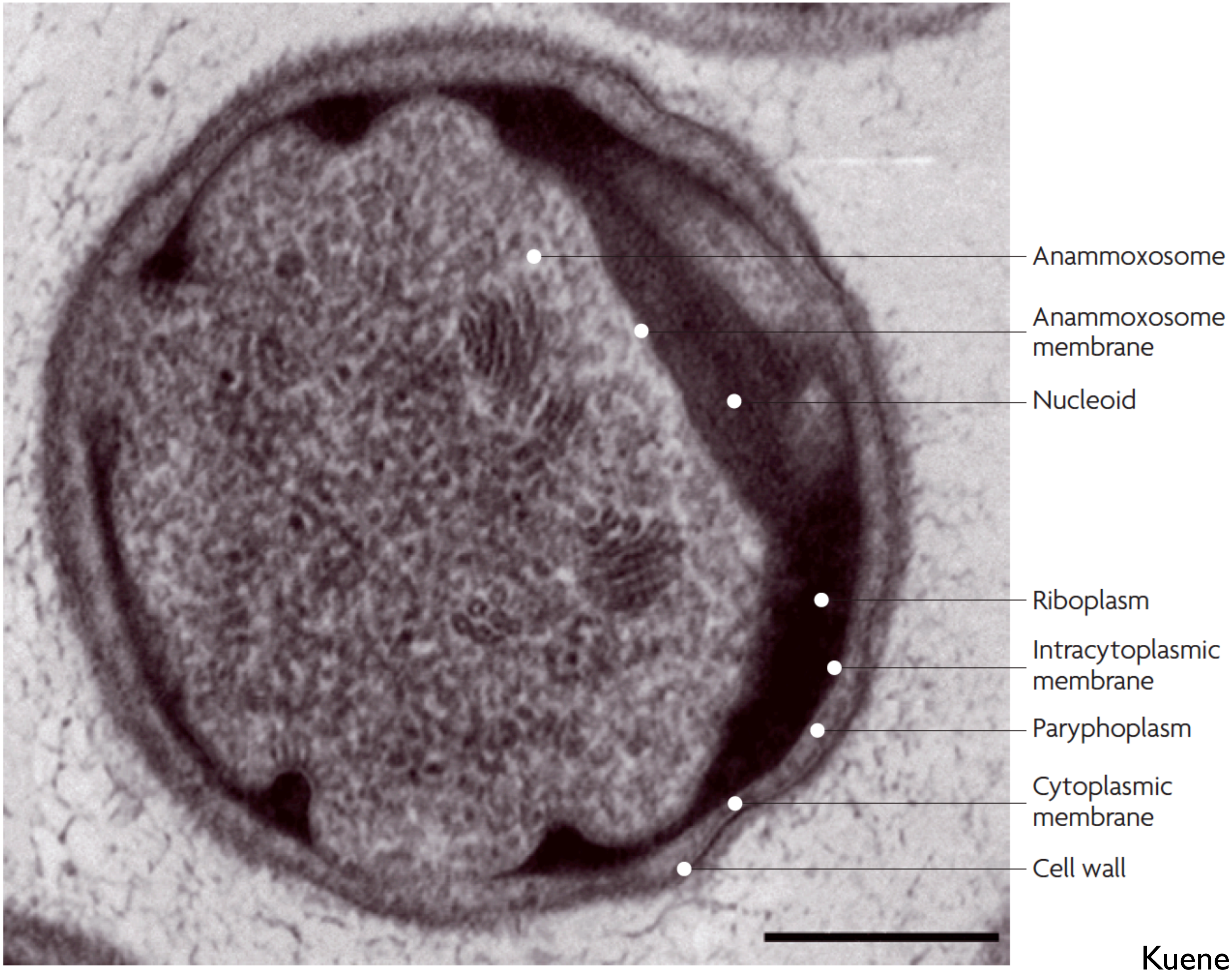
- Based on stoichiometric breakdown of Redfield organic matter, there should be a lot more NH_4^+ in anoxic environments then there actually is.

- 'Discovered' in 1985, but the responsible bacteria were not identified until 1999

- Chemoautotrophic organisms with an enormous iron requirement.

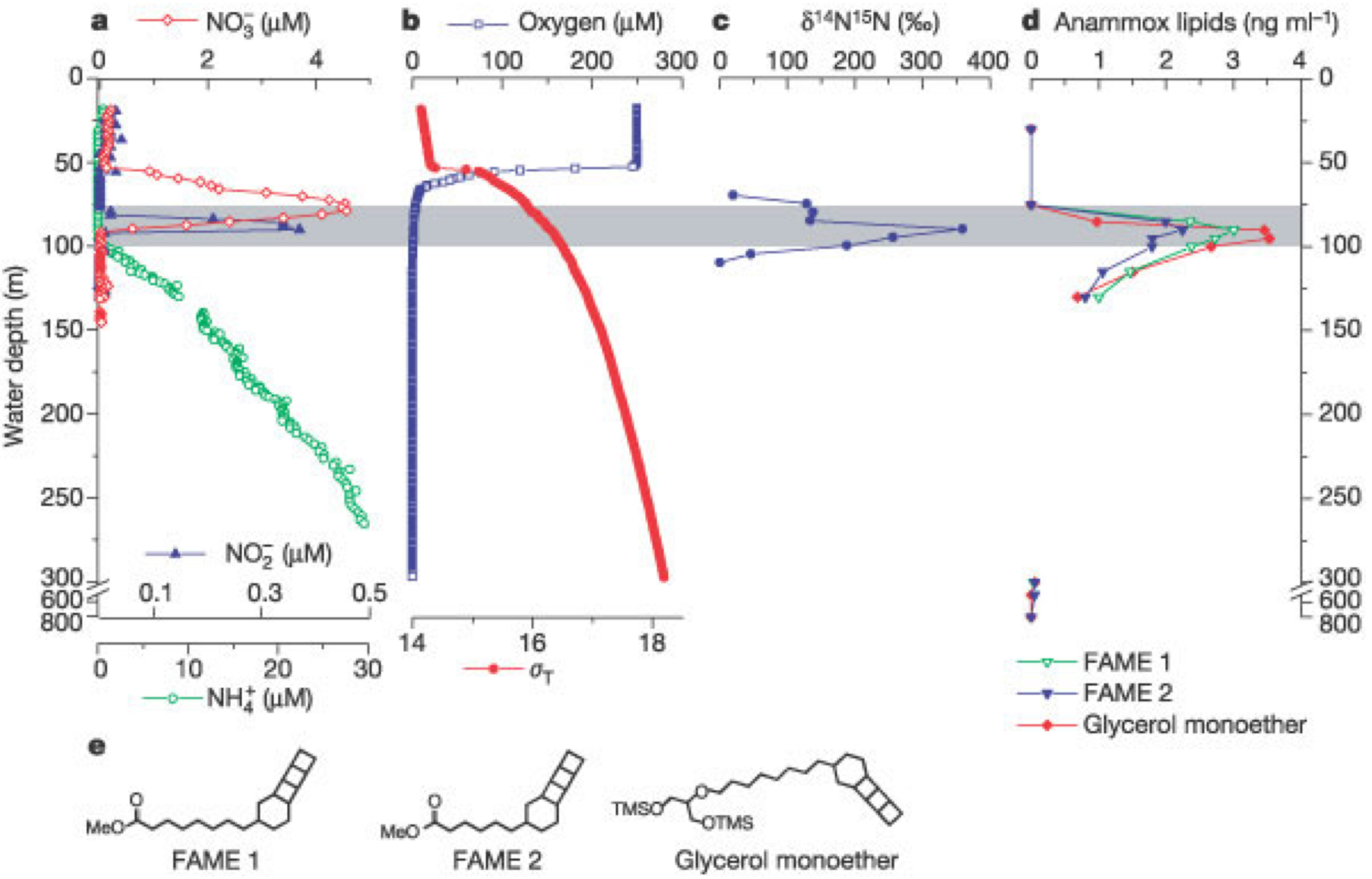


Kuypers et al. 2003

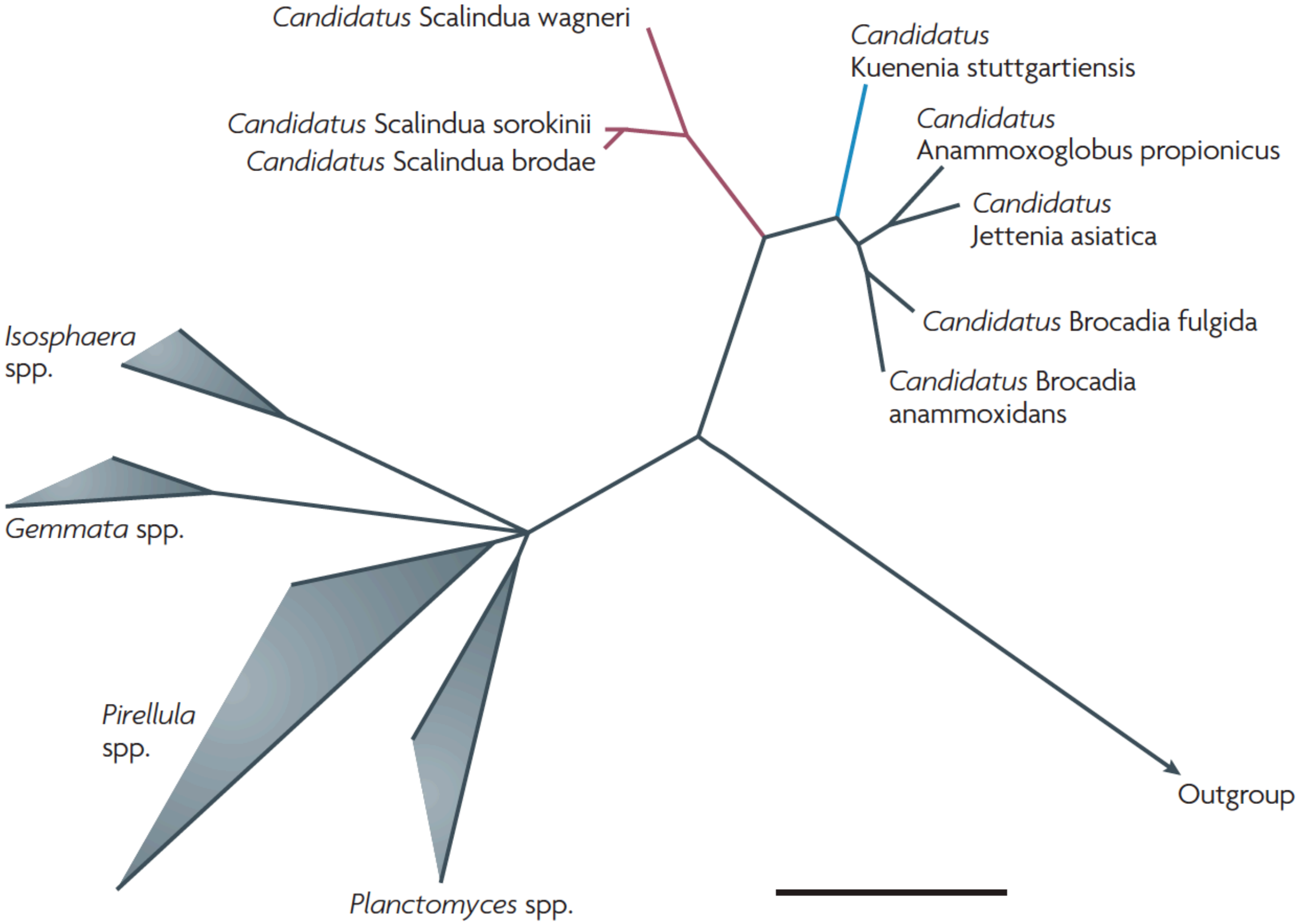

Anammoxosome
Anammoxosome membrane
Nucleoid
Riboplasm
Intracytoplasmic membrane
Paryphoplasm
Cytoplasmic membrane
Cell wall

Kuenen 2008

Marine anammox ‘discovered’ in the Black Sea



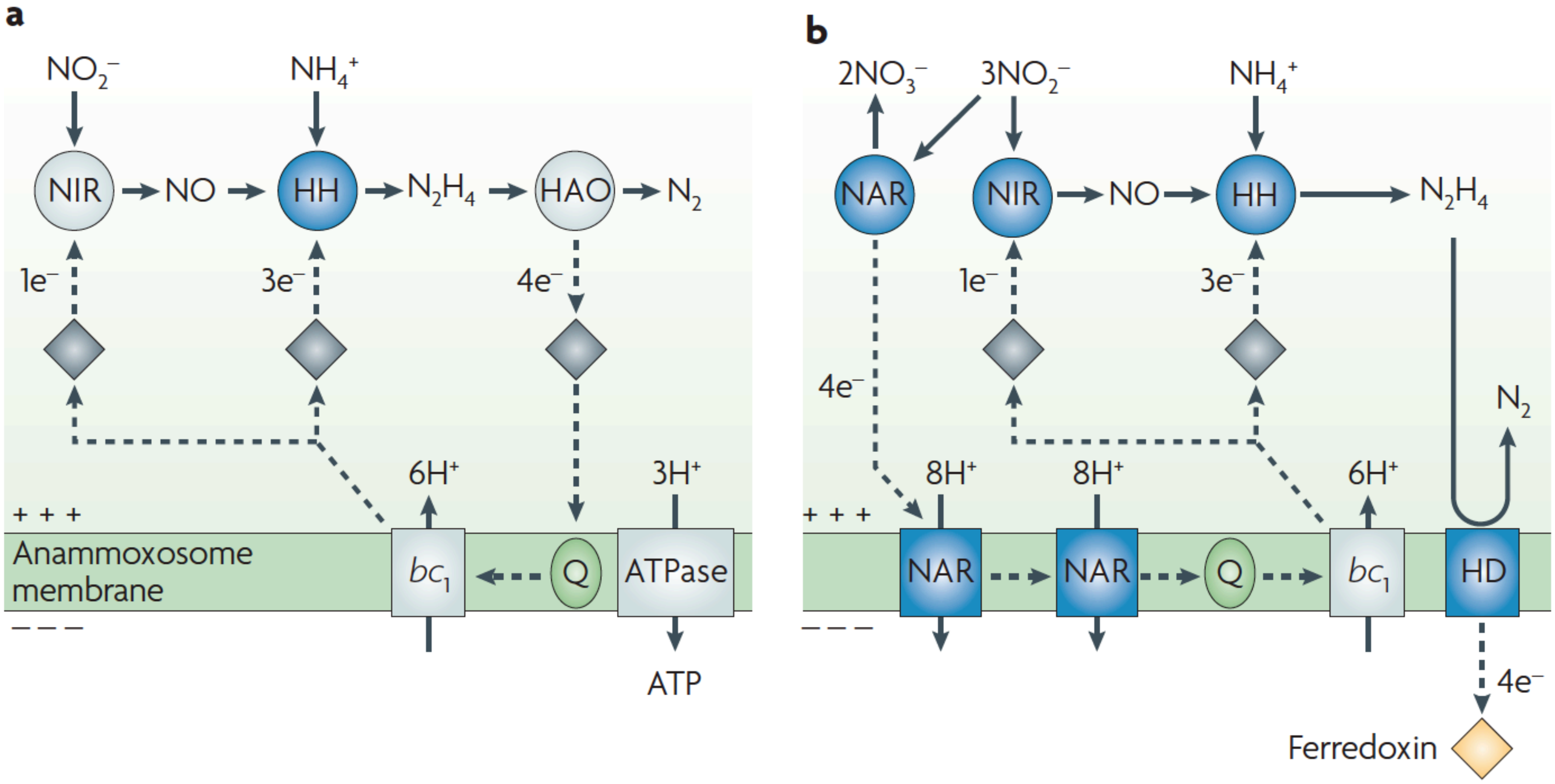
Anammox bacteria are highly divergent from their nearest neighbors



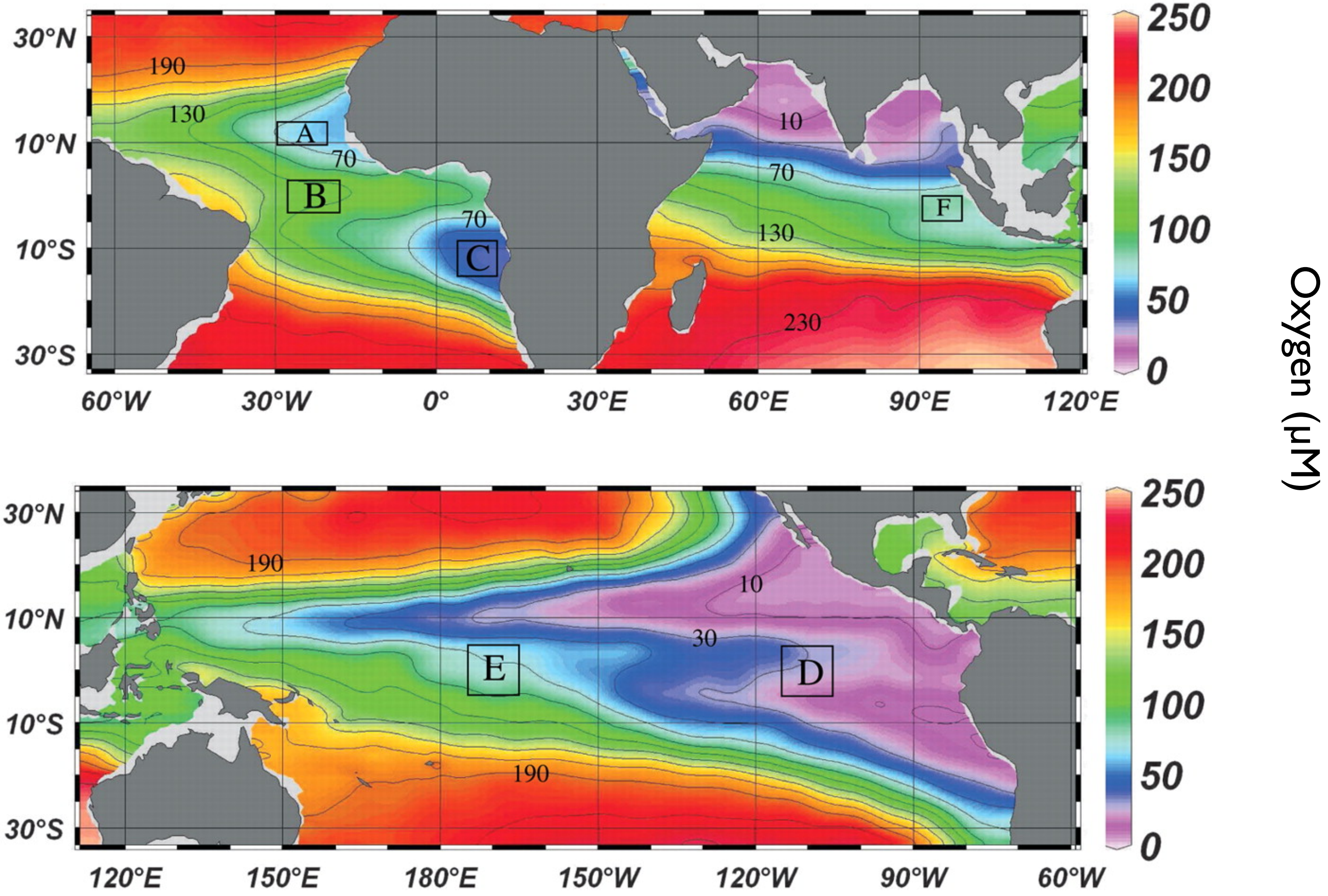
Kuenen 2008

Several functional gene targets for anammox bacteria

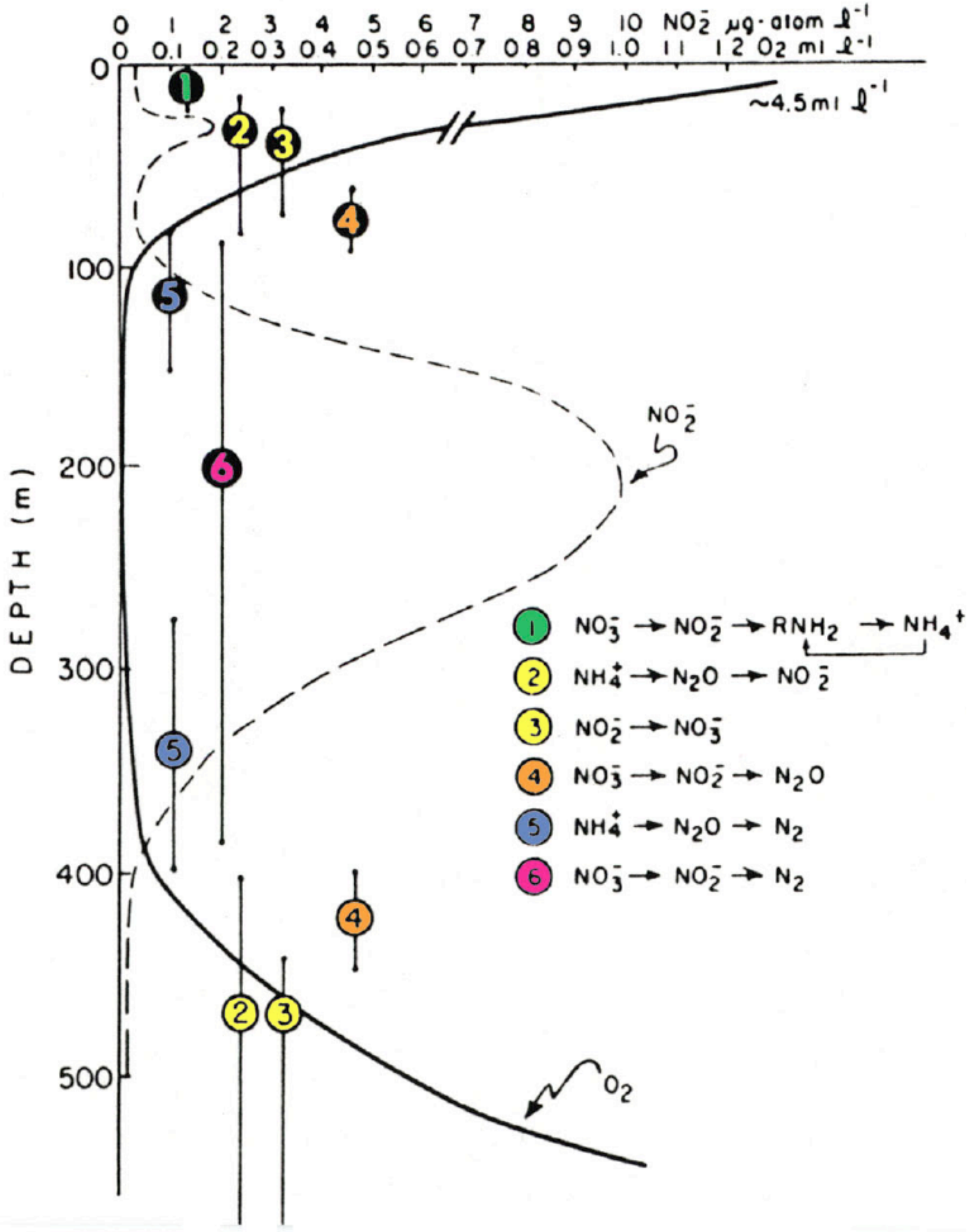
Candidates: *hao* (hydrazine oxidoreductase); *nirS*



Putting it all together: Synthetic studies of the marine nitrogen cycle



Stramma et al. 2008



(Codispoti and Christiansen, 1985)

Oxygen minimum zones were ripe for integrated molecular and biogeochemical studies.

Revising the nitrogen cycle in the Peruvian oxygen minimum zone

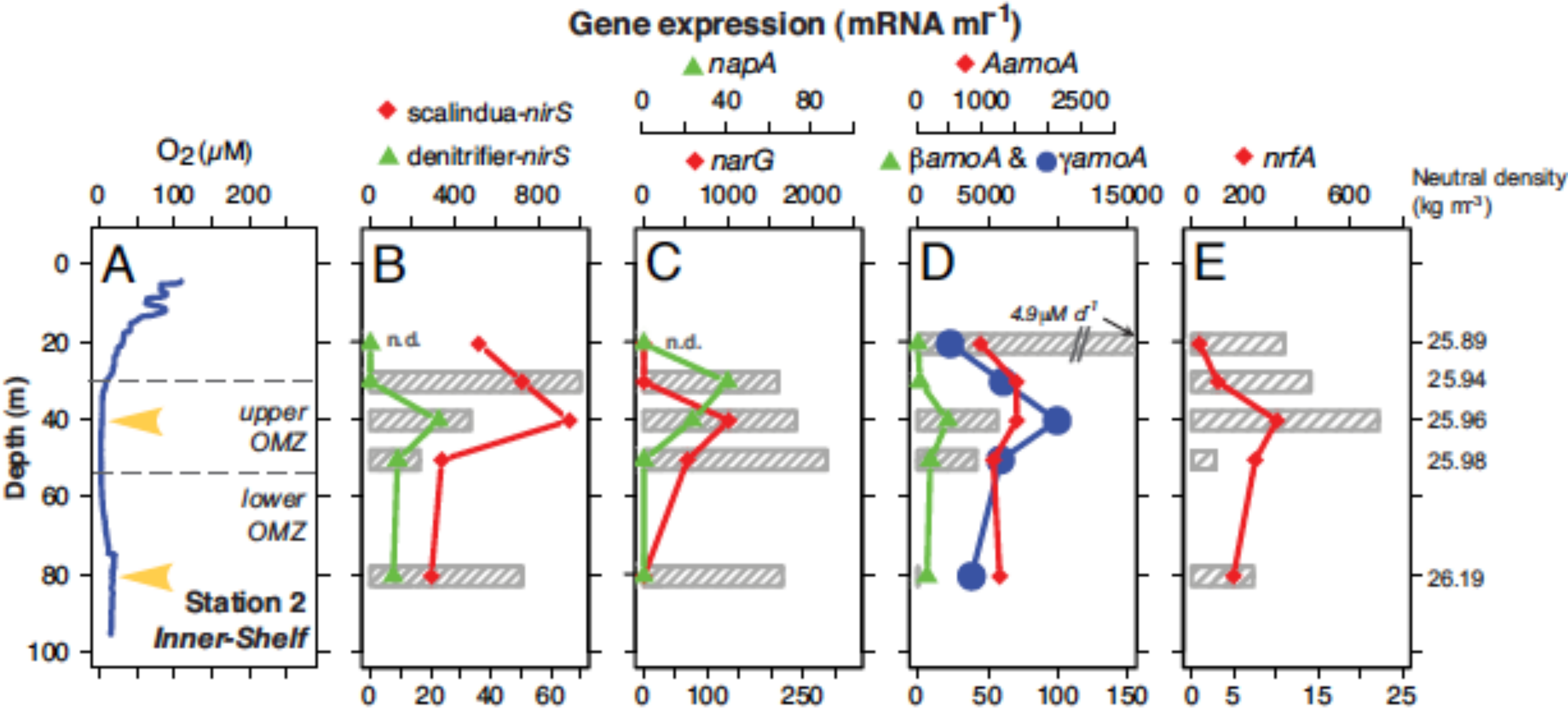
Phyllis Lam[a,1], Gaute Lavik[a], Marlene M. Jensen[a,2], Jack van de Vossenberg[b], Markus Schmid[b,3], Dagmar Woebken[a,4], Dimitri Gutiérrez[c], Rudolf Amann[a], Mike S. M. Jetten[b], and Marcel M. M. Kuypers[a]

[a]Max Planck Institute for Marine Microbiology, D-28359 Bremen, Germany; [b]Department of Microbiology, IWWR, Radboud University Nijmegen, 6500 HC Nijmegen, The Netherlands; and [c]Dirección de Investigaciones Oceanográficas, Instituto del Mar del Perú, Esquina Gamarra y General Valle S/N, Chucuito, Callao 22, Peru

LETTERS

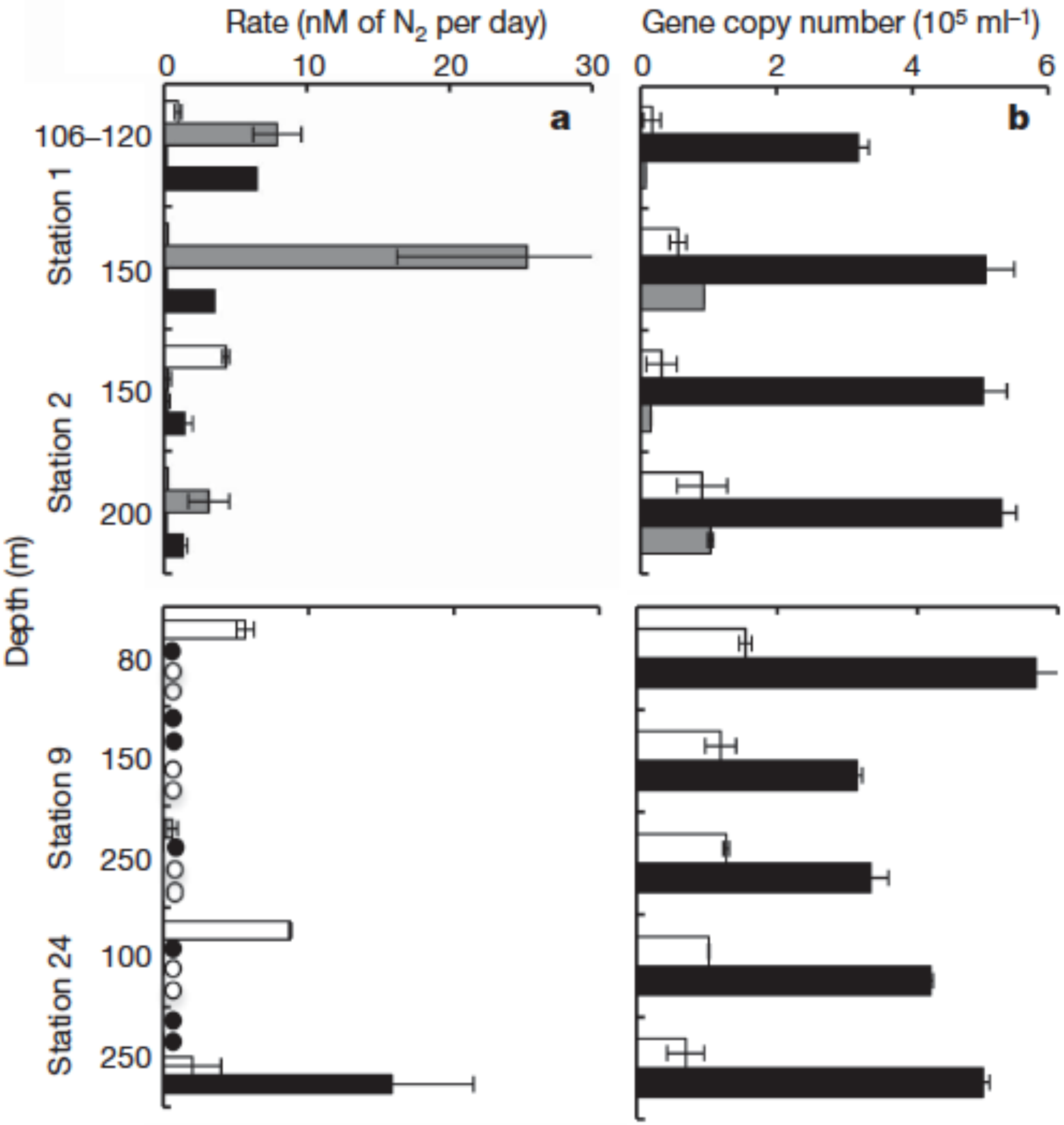
Denitrification as the dominant nitrogen loss process in the Arabian Sea

B. B. Ward[1], A. H. Devol[2], J. J. Rich[3], B. X. Chang[2], S. E. Bulow[1], Hema Naik[4], Anil Pratihary[4] & A. Jayakumar[1]



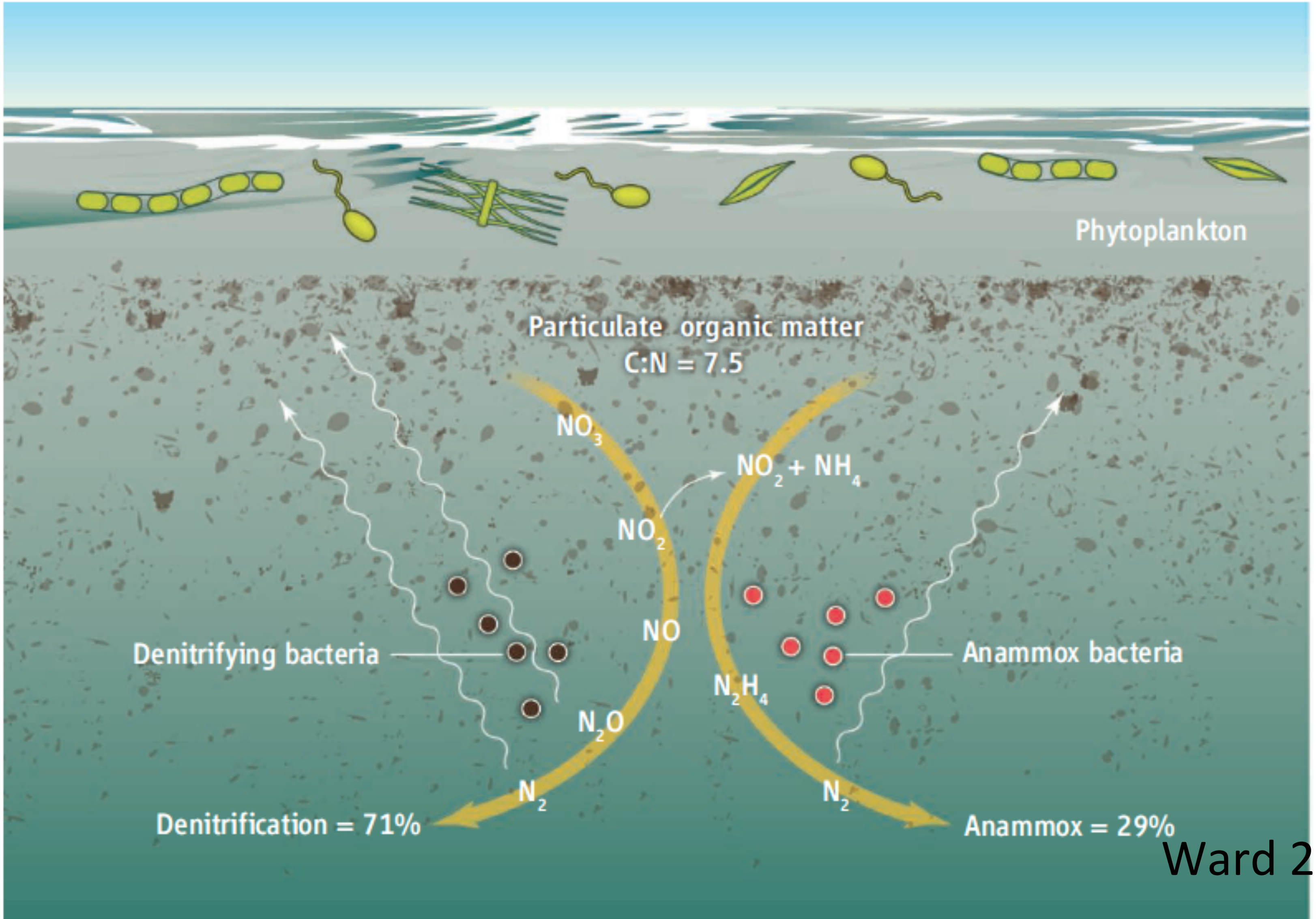
But beware the ‘invisible present’ . . .

Lam et al. 2009



Ward et al. 2009

Organic matter C:N sets nitrogen loss pathway



Ward 2013