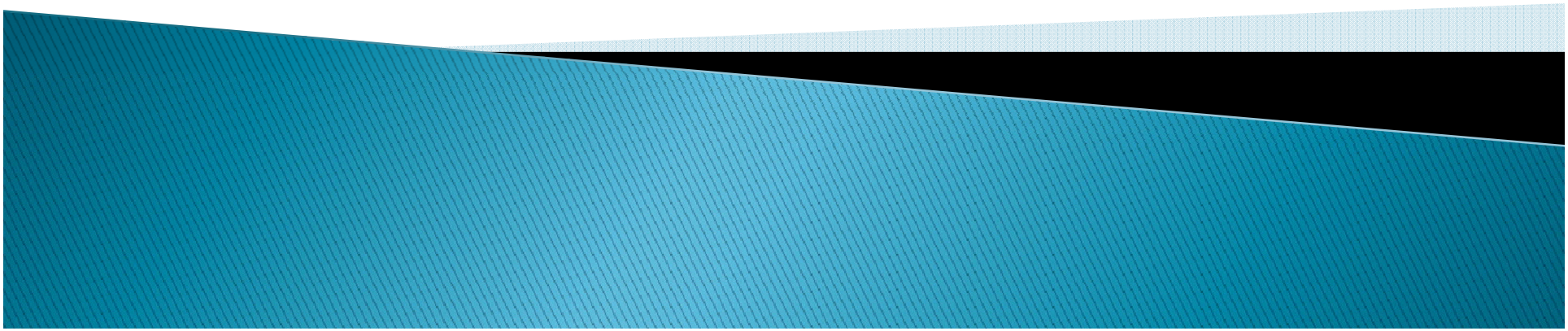
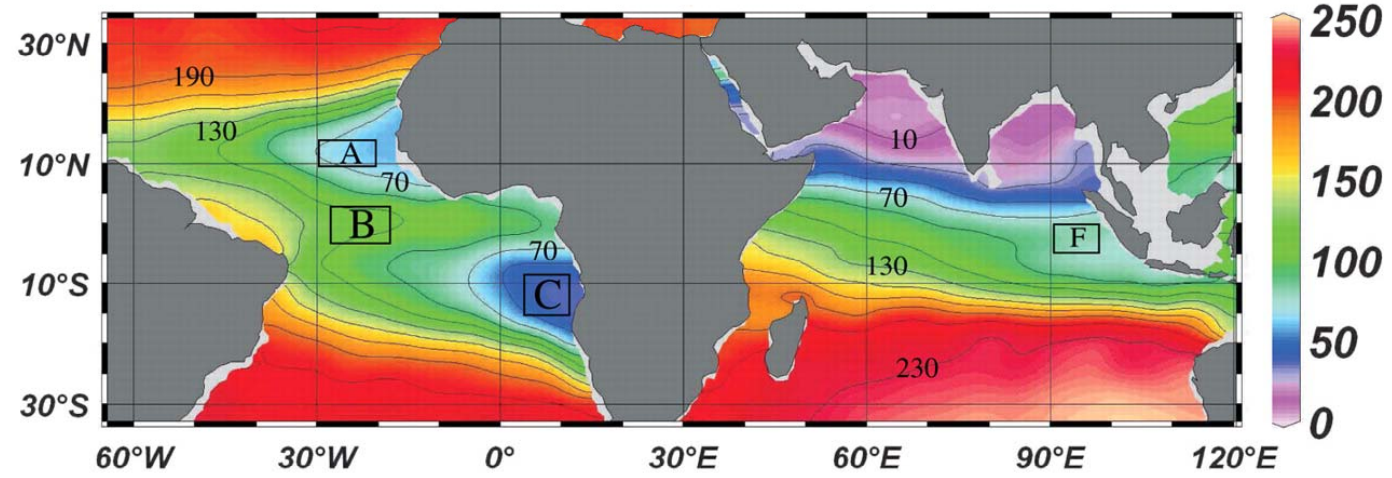
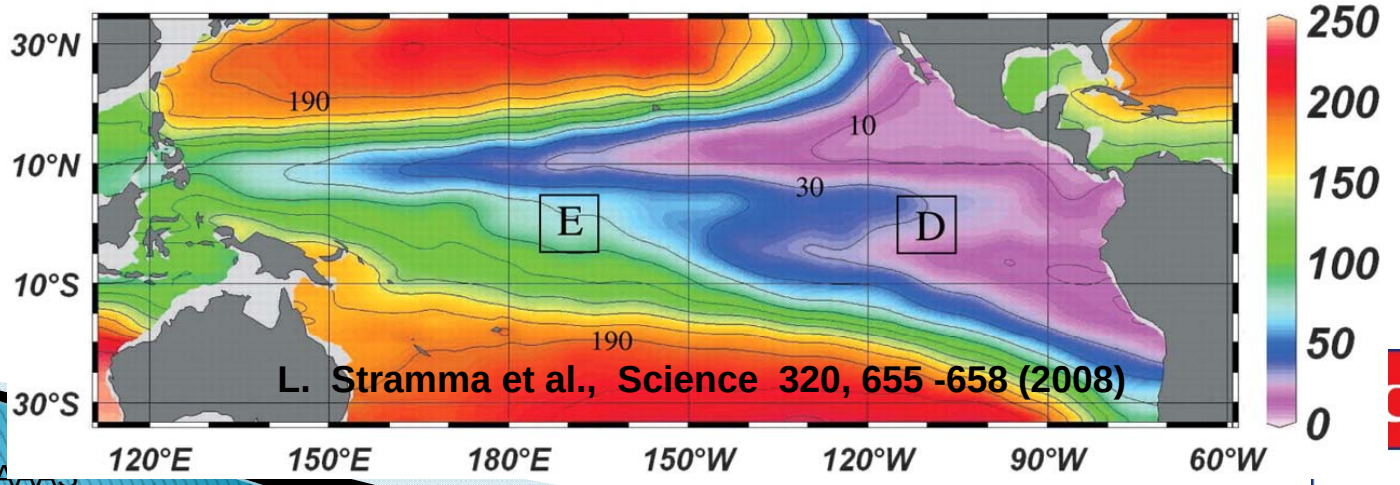
The evolving story of microbial nitrogen cycling processes in low oxygen zones



Oxygen minimum zones may be expanding

Local phenomenon with global consequences
Global balances of nitrogen



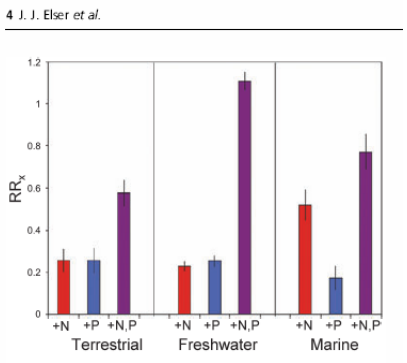


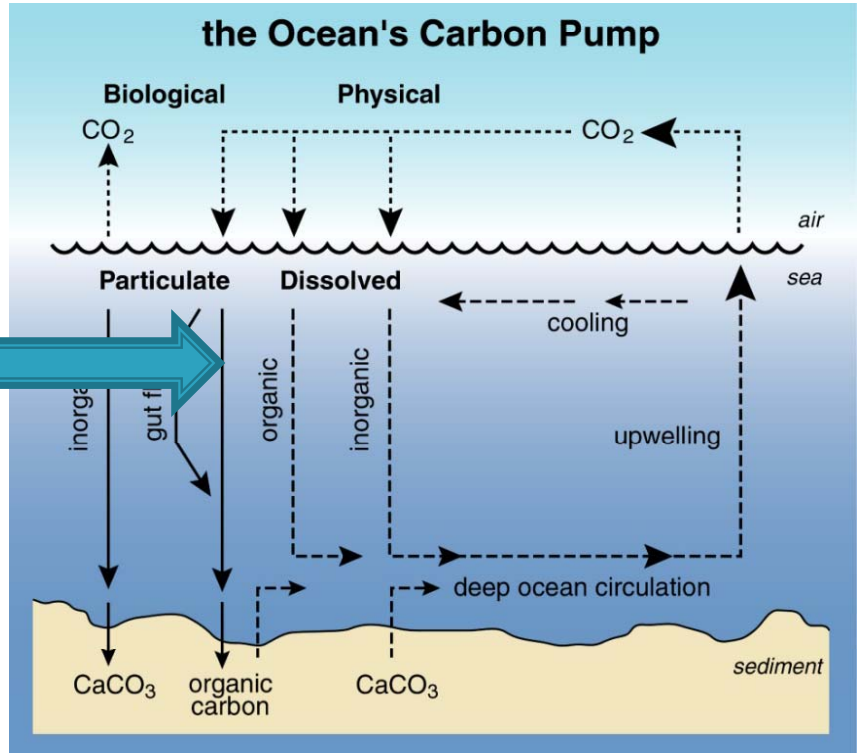
L. Stramma et al., Science 320, 655 -658 (2008)

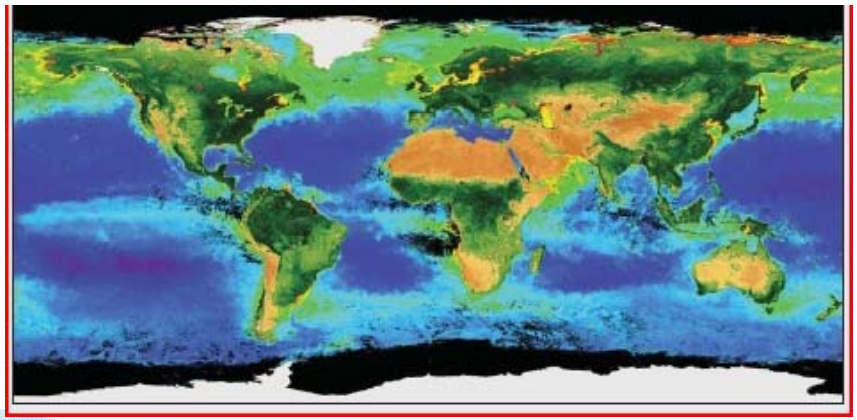
N important in global ocean carbon cycling

Nitrogen

Nitrogen fixation

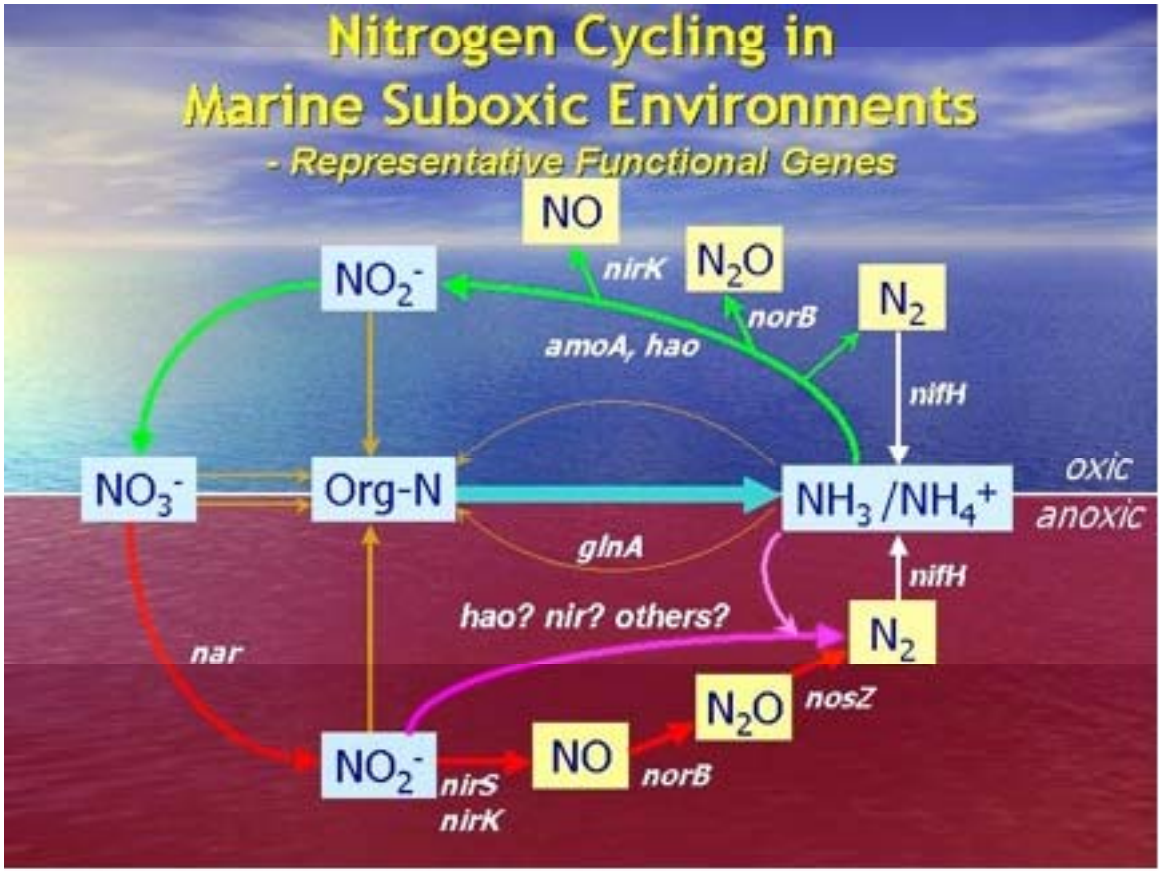






Low oxygen zones and the N cycle

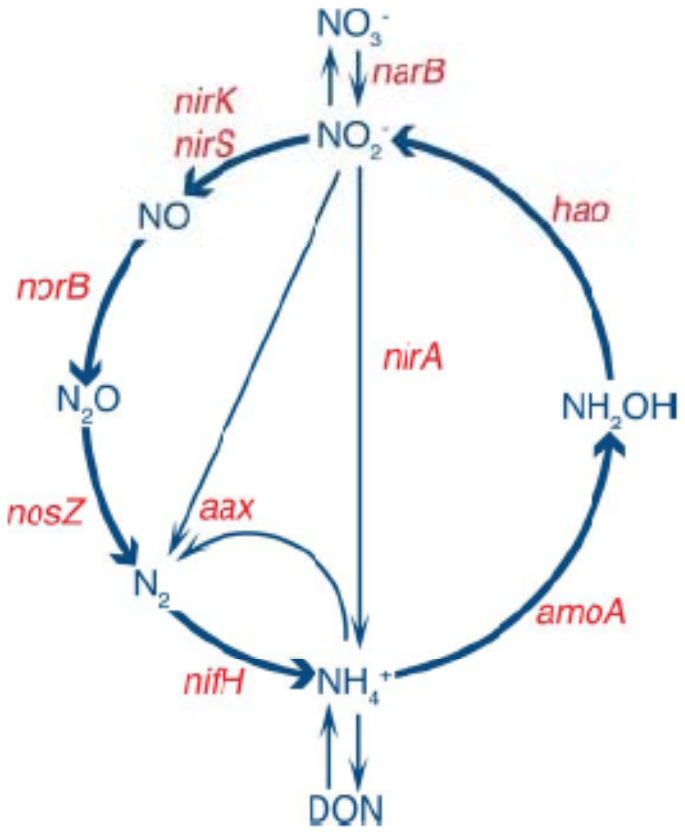
- Link between oxygen depletion and nitrogen
- Oxygen determines metabolic pathways involving N as electron acceptor or donor



http://www.mpi-bremen.de/Binaries/Binary2362/ncycle2.jpg

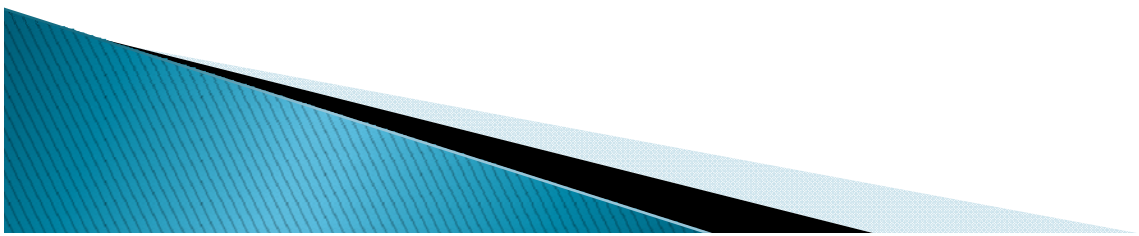
Microorganisms and genetic potential

- Identifying key genes
- Amo: responsible for deep water nitrate
- Annamox: important N removal process
- Nir: important N removal process
- Nrf: nitrate reduction to ammonium–important for recycling N in some environments
- Nar/Nas: important N uptake pathway for "new" production
- Nif: important N source



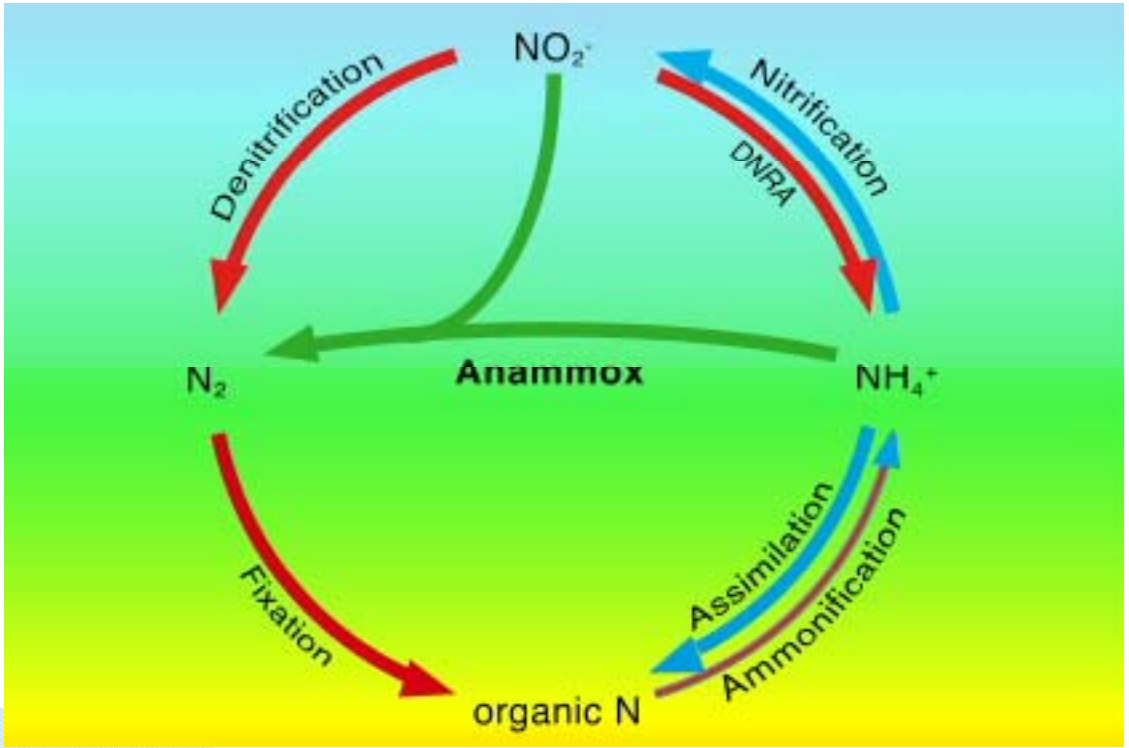
Two controversies

- Denitrification vs anammox as major N loss pathways in oxygen depleted waters
- OMZs directly linked to nitrogen fixation



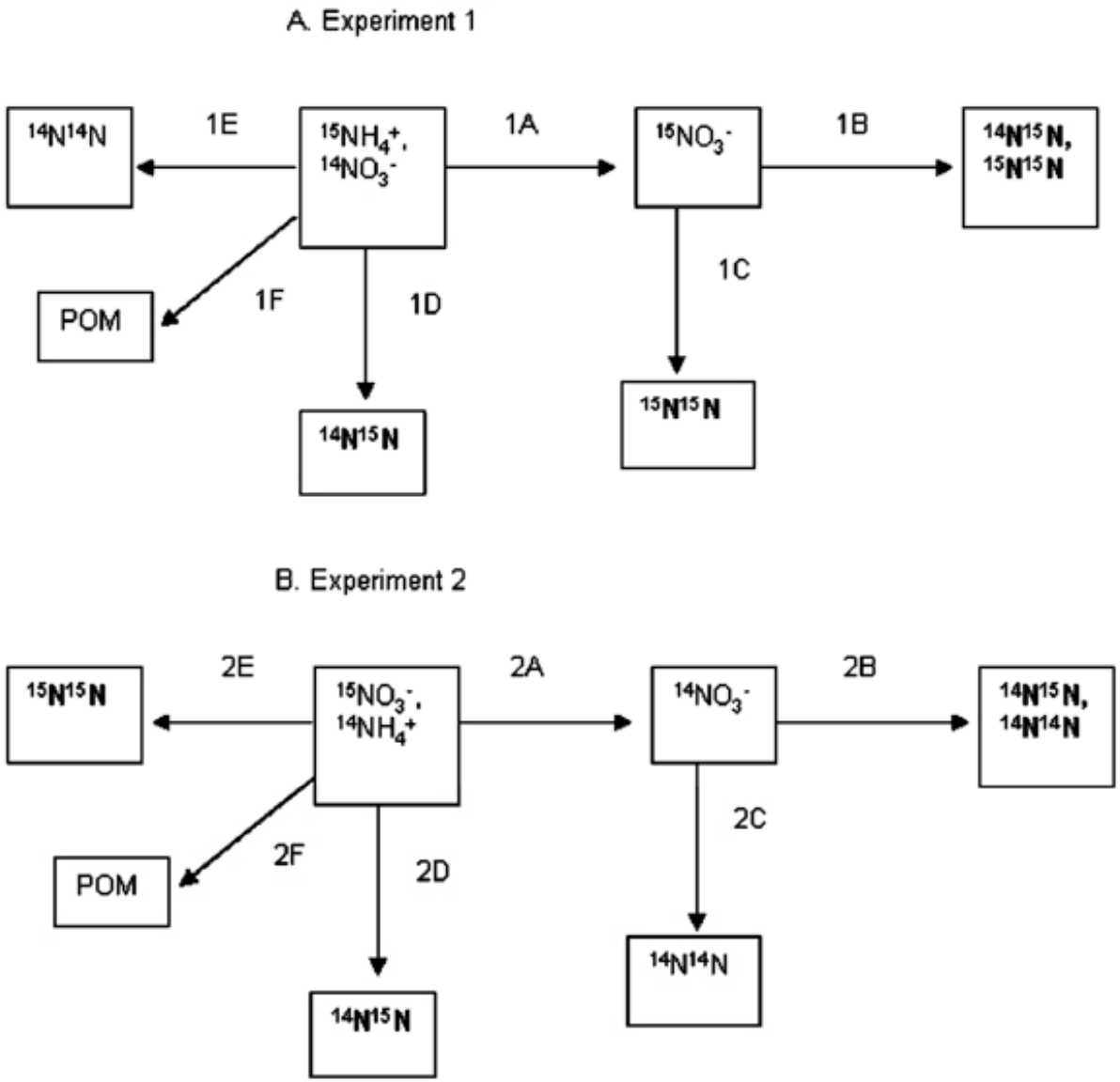
Paradox of N loss pathways in low oxygen zones

- Anammox recently shown to be important
- Requires nitrite, ammonium and low oxygen
  - Explaining how anammox can be supported in OMZs major challenge



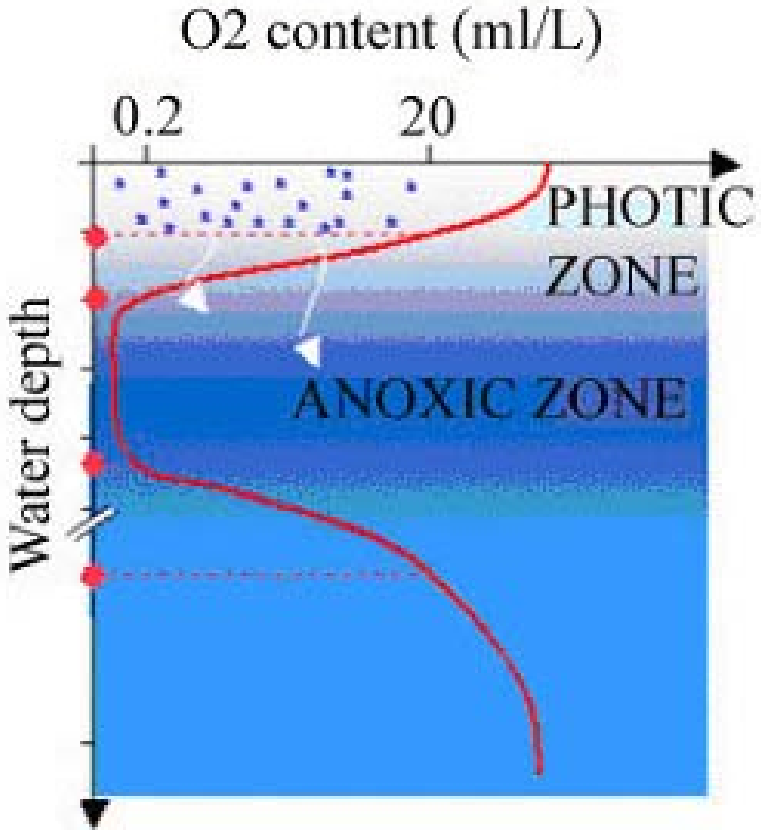
Stable isotope methods: difficult to separate transformations

- Dalsgaard
- Thamdrup
- Kuypers

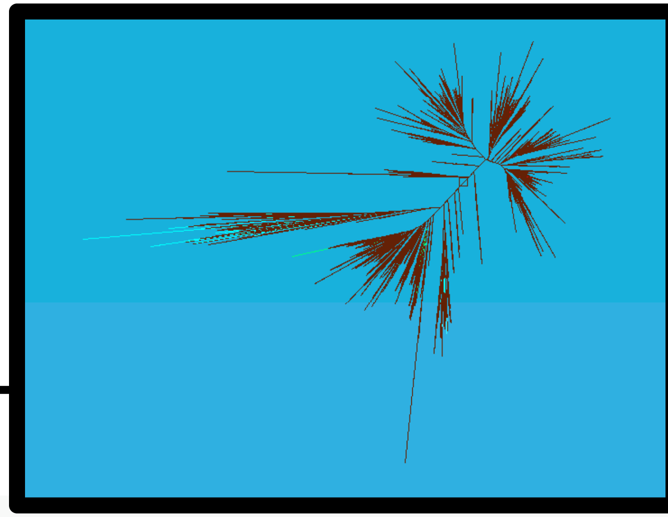


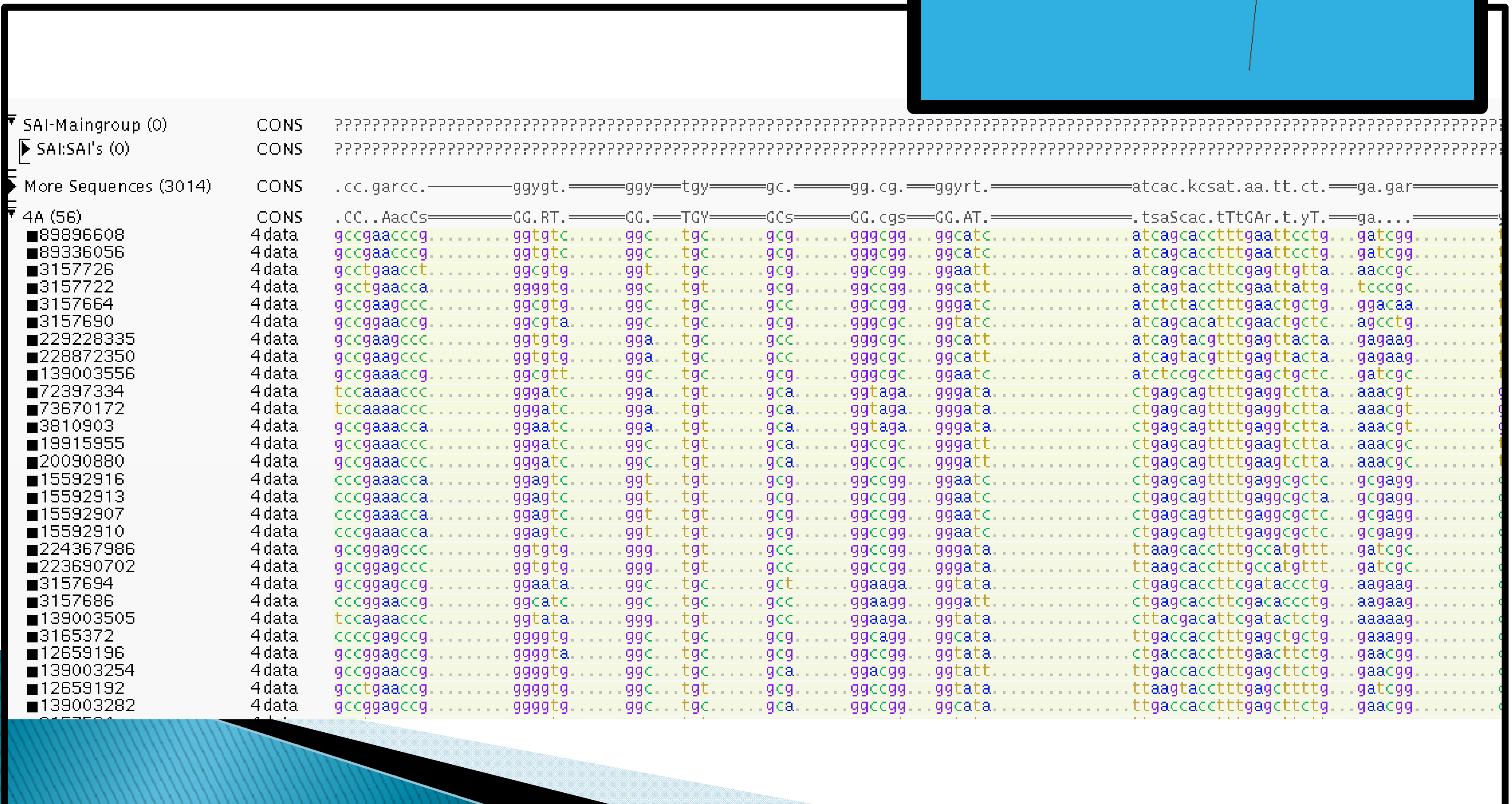
Identification of N pathways with gene expression

- N loss in OMZs
- Denitrification
  - Nitrate→ N2
- Anaerobic ammonia oxidation
  - Nitrite+ammonium→N_2



"Probes" from gene sequences
Quantitative PCR





Lam P et al. PNAS 2009;106:4752-4757

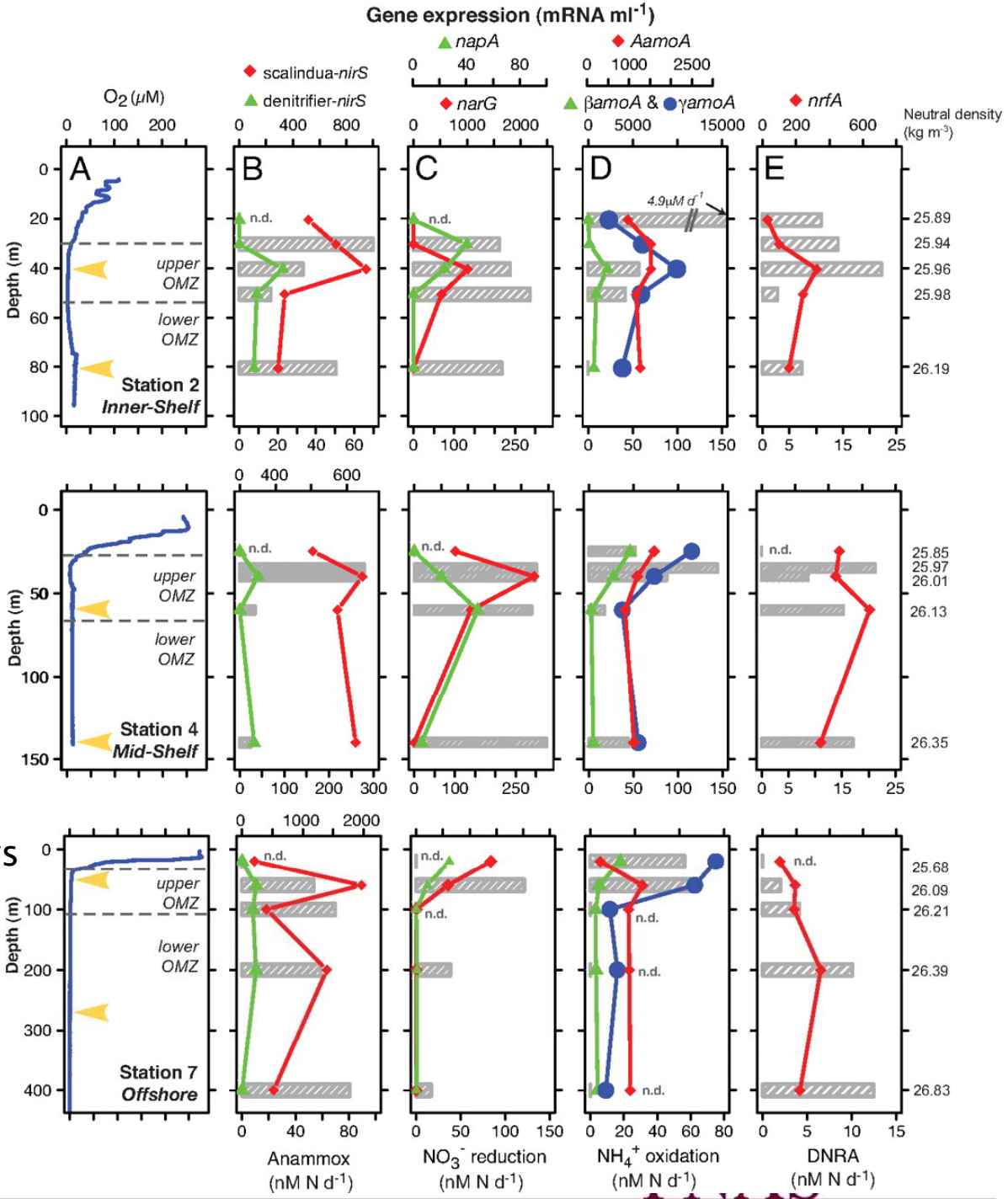
Vertical distribution of oxygen (A) and the various measured 15N rates along with corresponding functional gene expression (B–E) at Stations 2 (inner shelf), 4 (mid-shelf), and 7 (offshore).

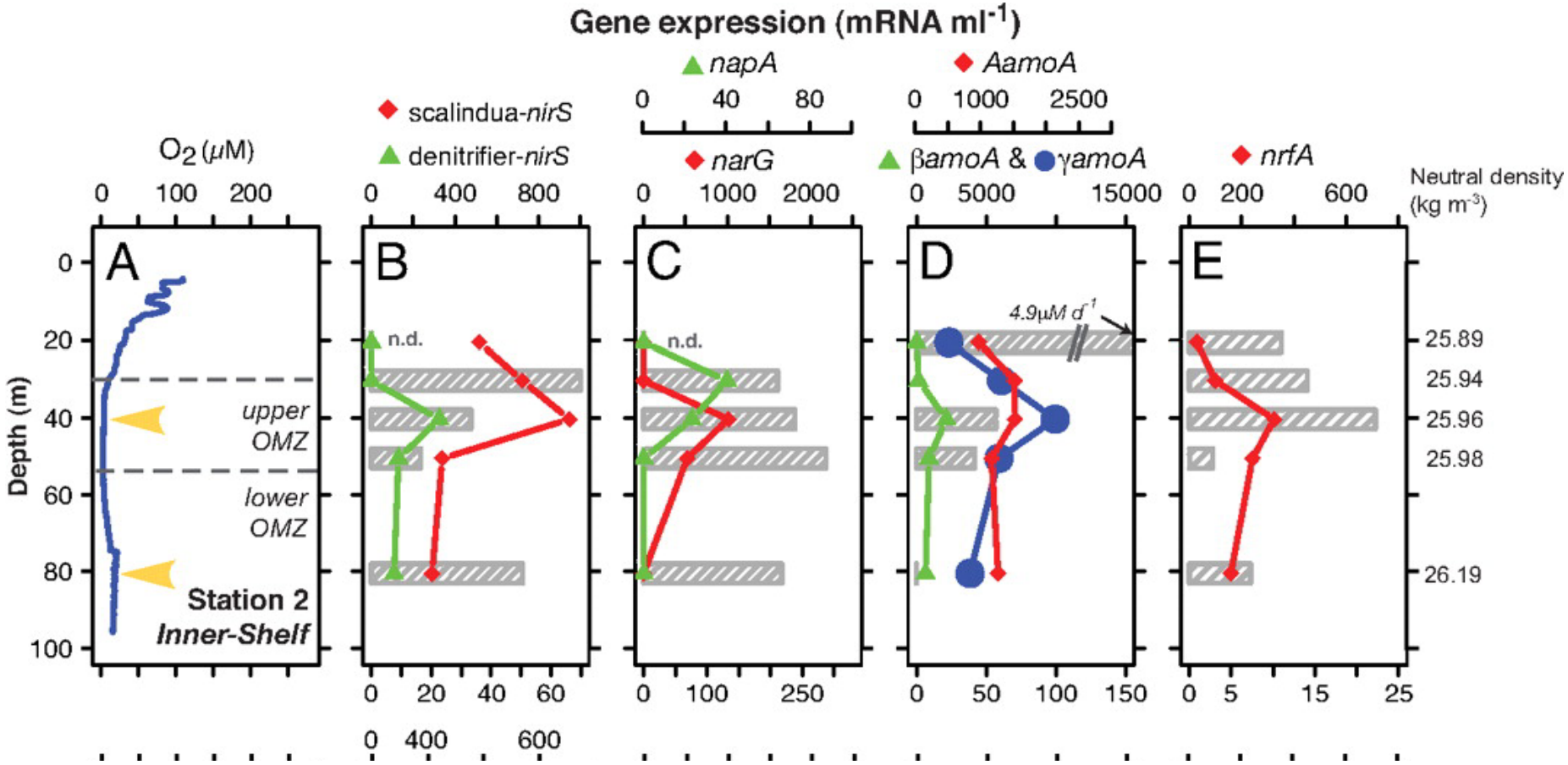
Genes

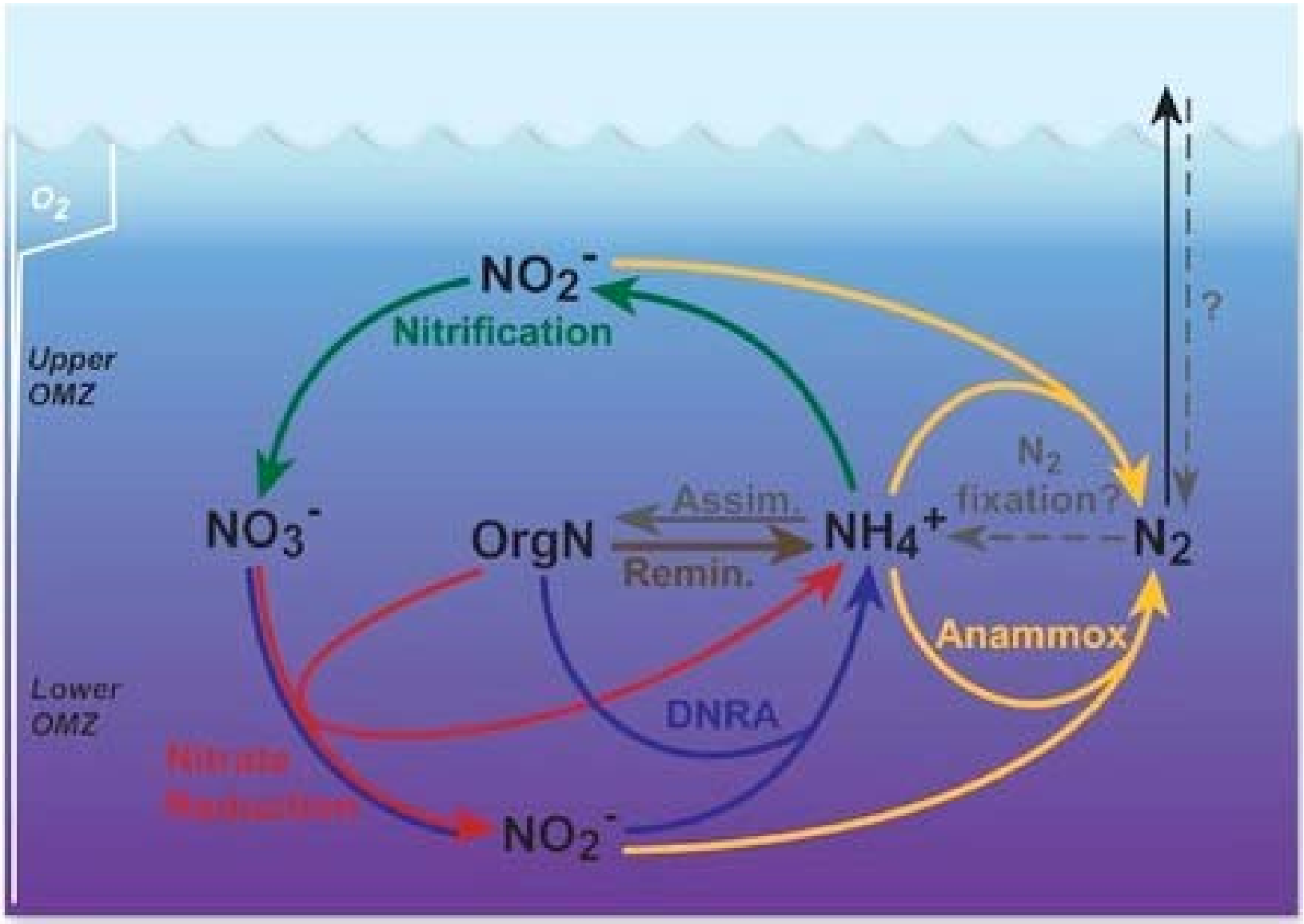
Nir
Nar
Amo
Nrf

Gene expression suggests relative Importance of different N pathways

Role of dissimilatory reduction of nitrate in providing ammonium

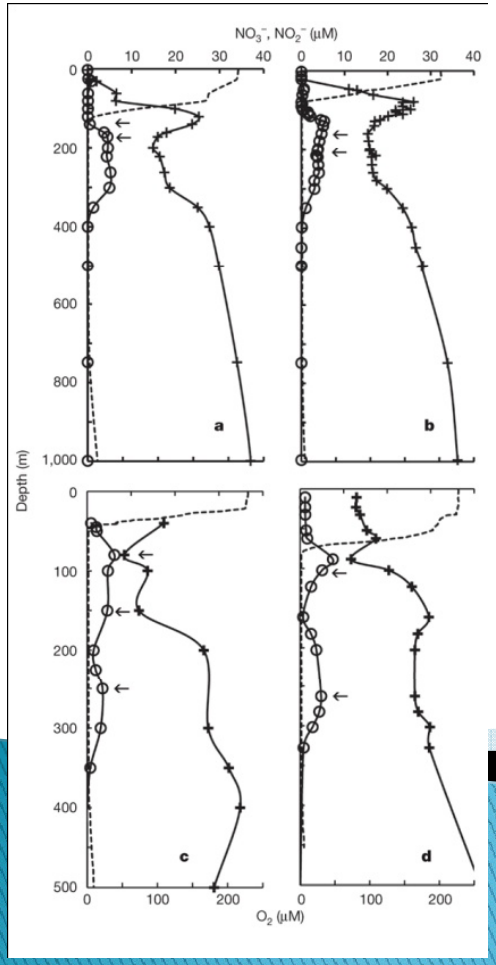


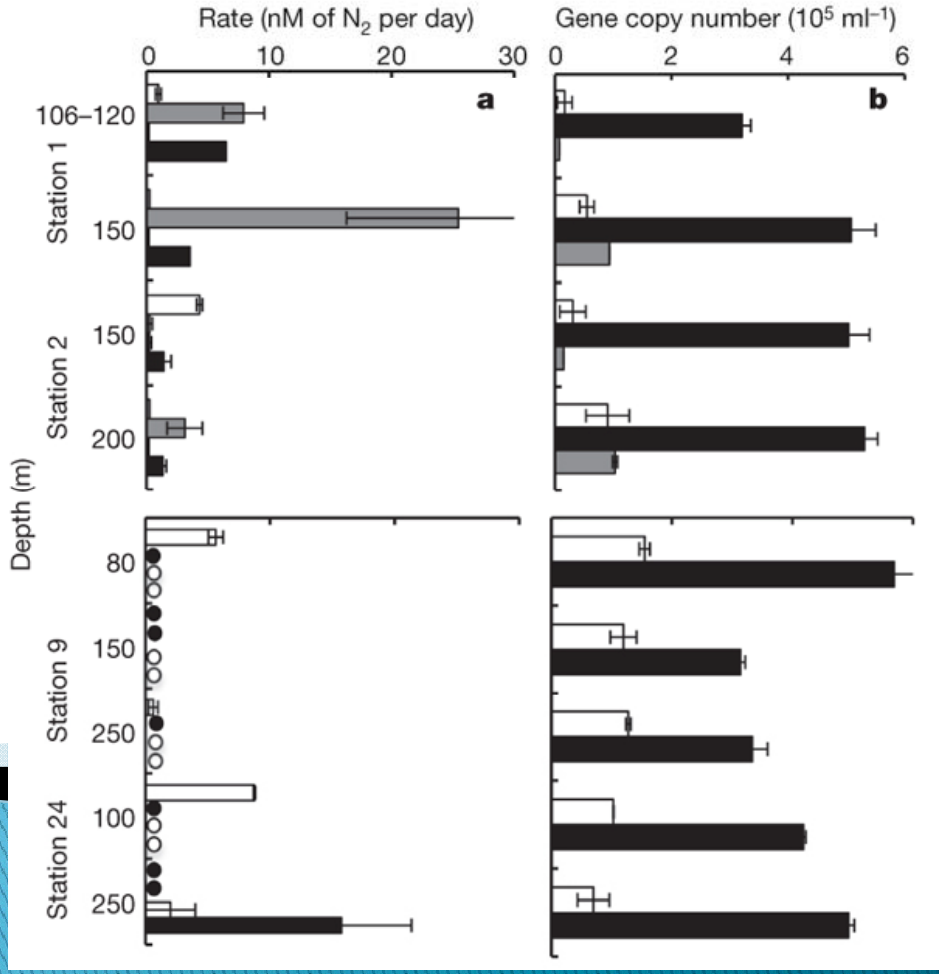

Gene expression (mRNA ml-1)
napA
AamoA
scalindua-nirS
denitrifier-nirS
narG
βamoA & γamoA
nrfA
O2 (μM)
Neutral density (kg m-3)
Depth (m)
A
B
C
D
E
upper OMZ
lower OMZ
Station 2
Inner-Shelf
n.d.
n.d.
4.9μM d-1
25.89
25.94
25.96
25.98
26.19


O2
Upper OMZ
Lower OMZ
NO2-
Nitrification
NO3-
OrgN
Assim.
Remin.
NH4+
N2 fixation?
N2
?
Anammox
DNRA
NO2-

Chemical profiles for experimental stations in the Arabian Sea and ETSP.

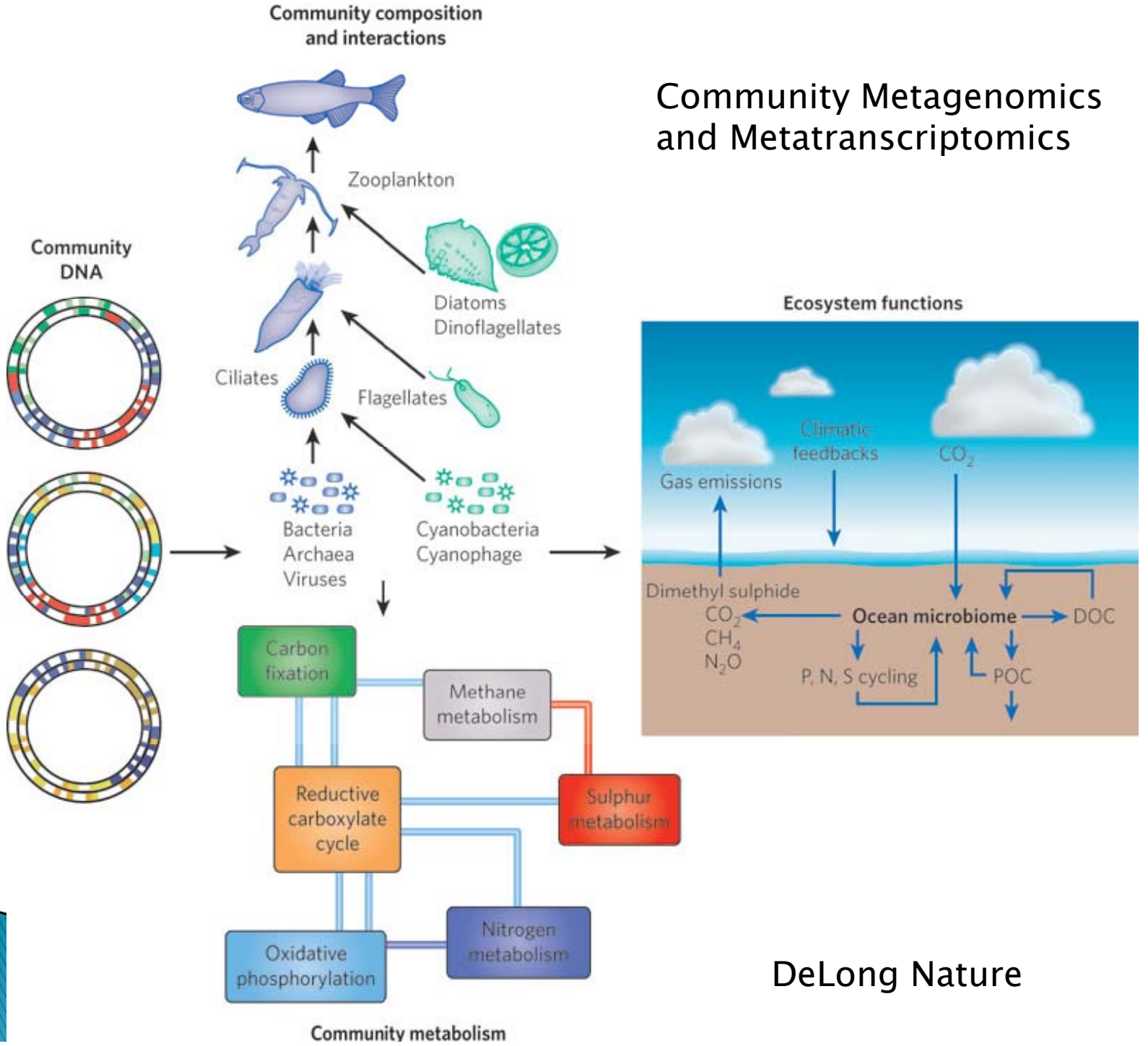
Conflicting results of





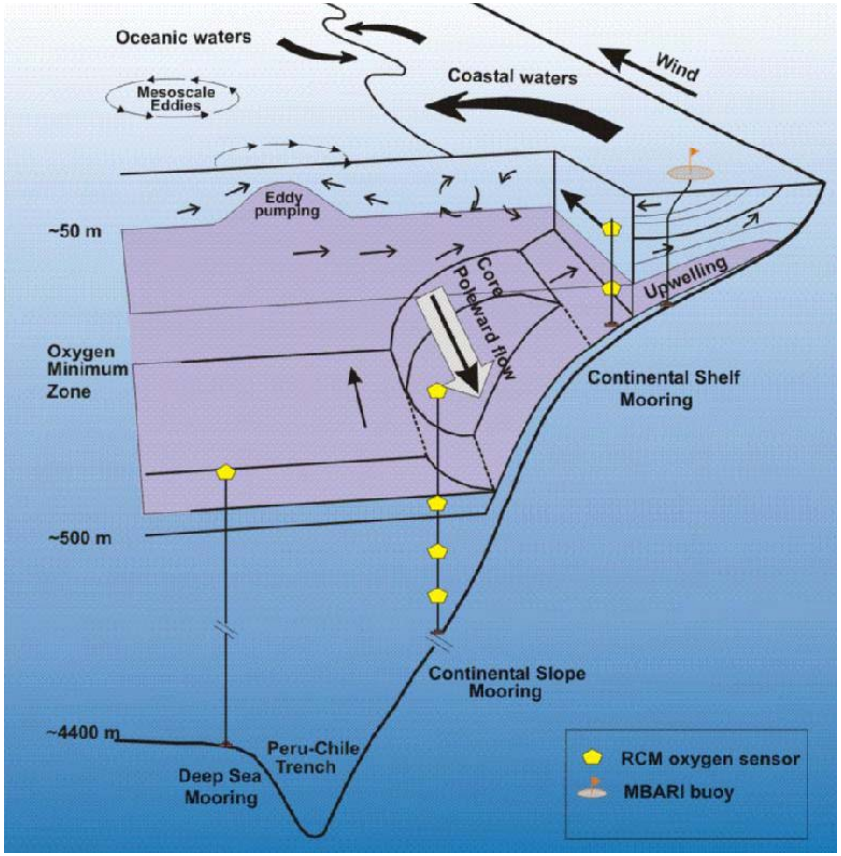
BB Ward *et al. Nature* 461, 78–81 (2009) doi:10.1038/nature08276

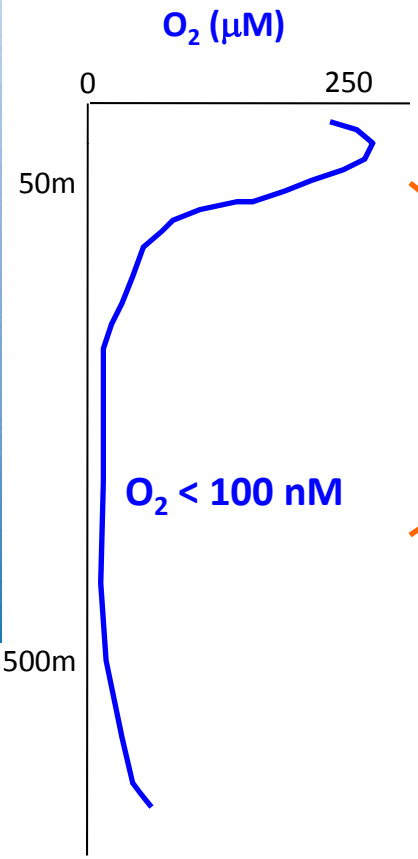
Community Metagenomics and Metatranscriptomics

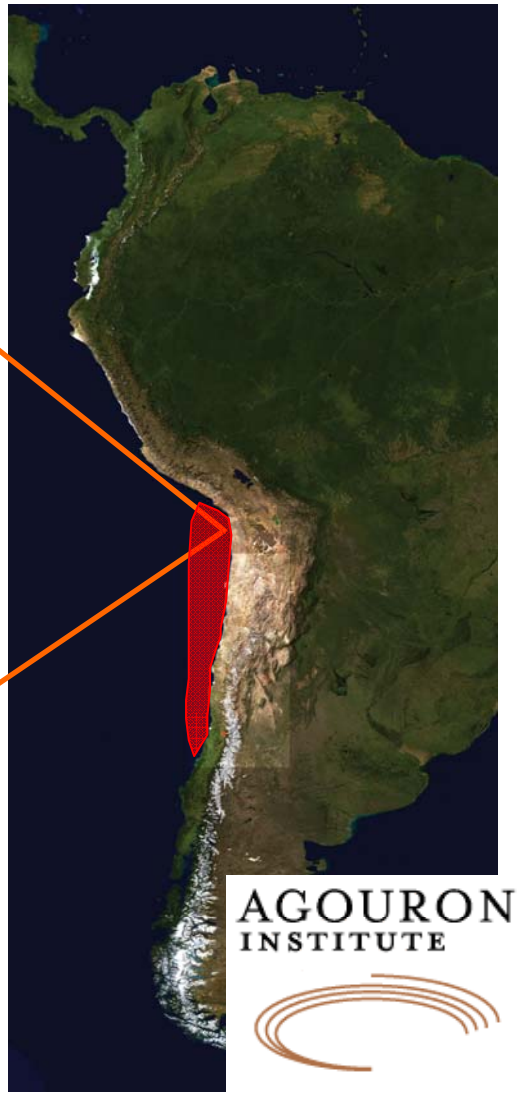


DeLong Nature

Eastern Tropical South Pacific OMZ







- Perennial
- Strong nutrient-rich upwelling
- Adjacent to productive fisheries

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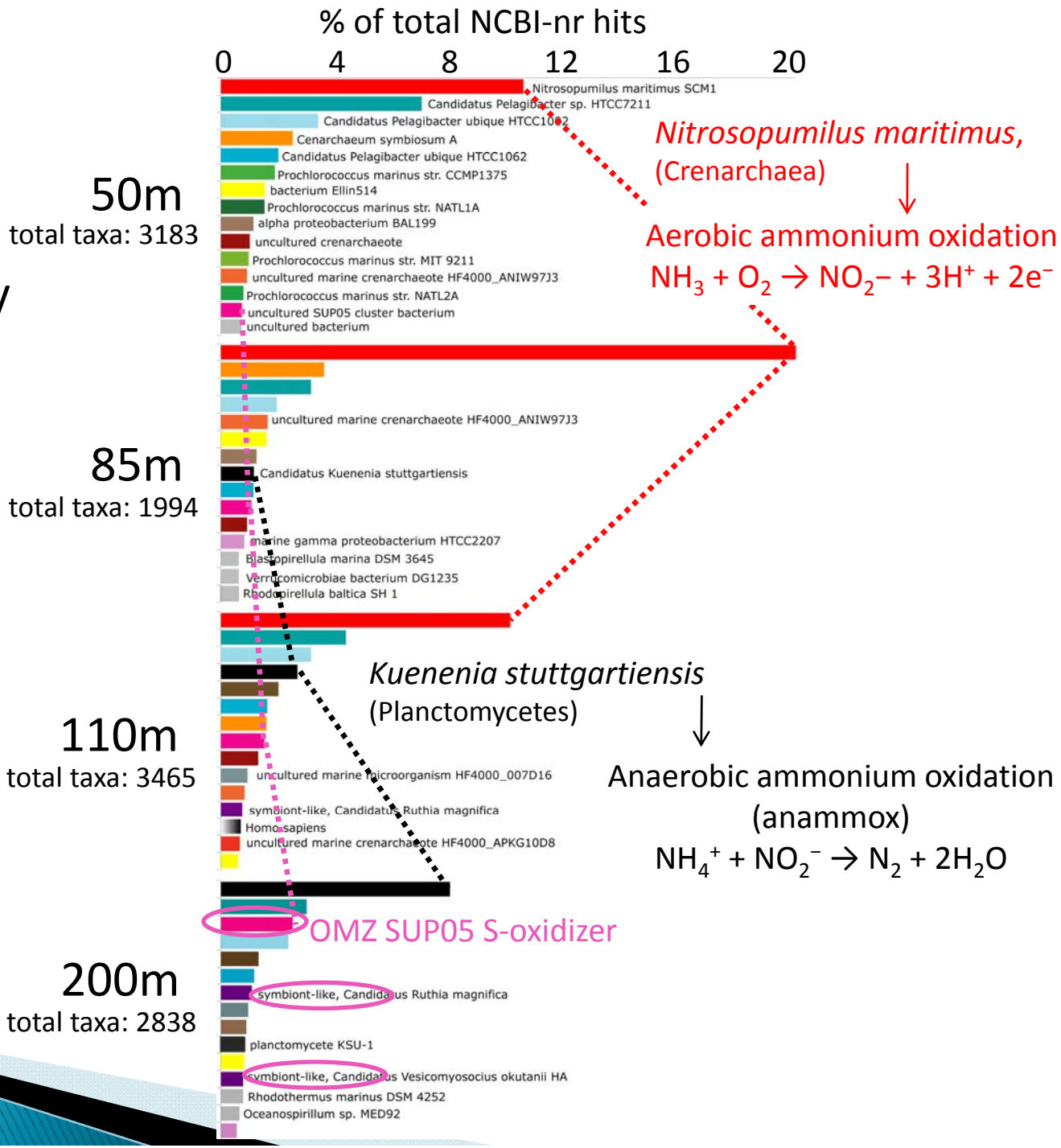
Who's there?
Annotations of protein-coding cDNA reads informs taxonomy

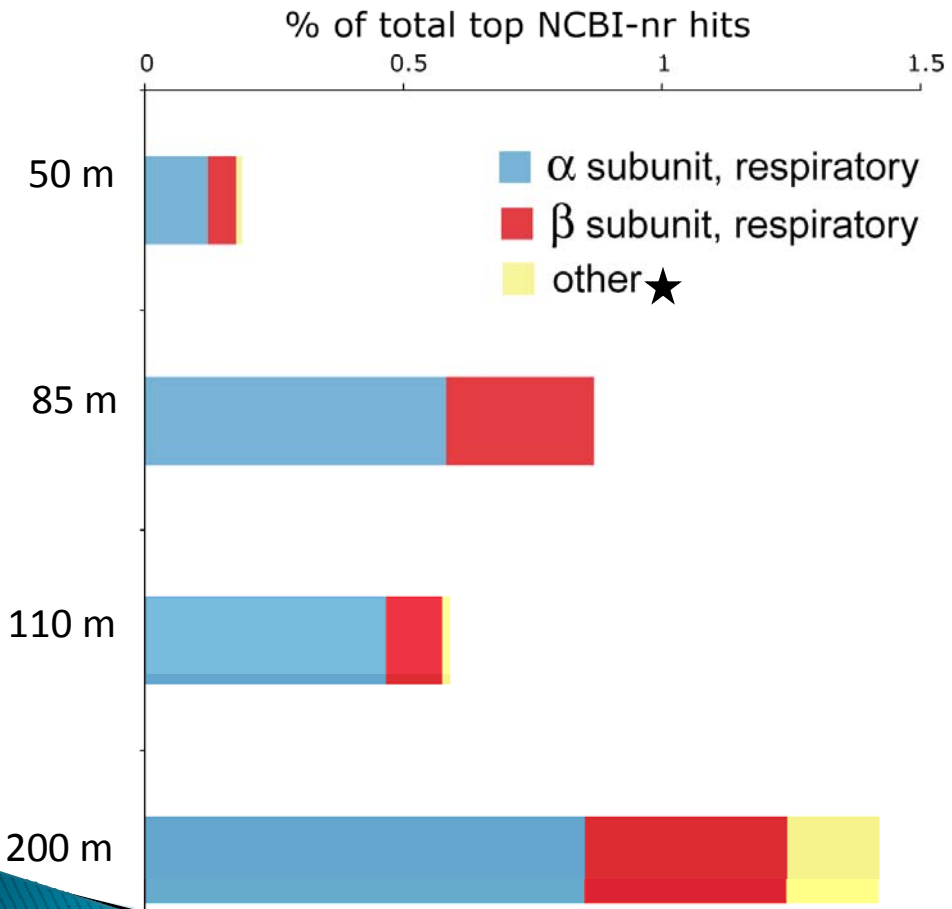


DeLong et al. unpublished

Nitrate reductase expression

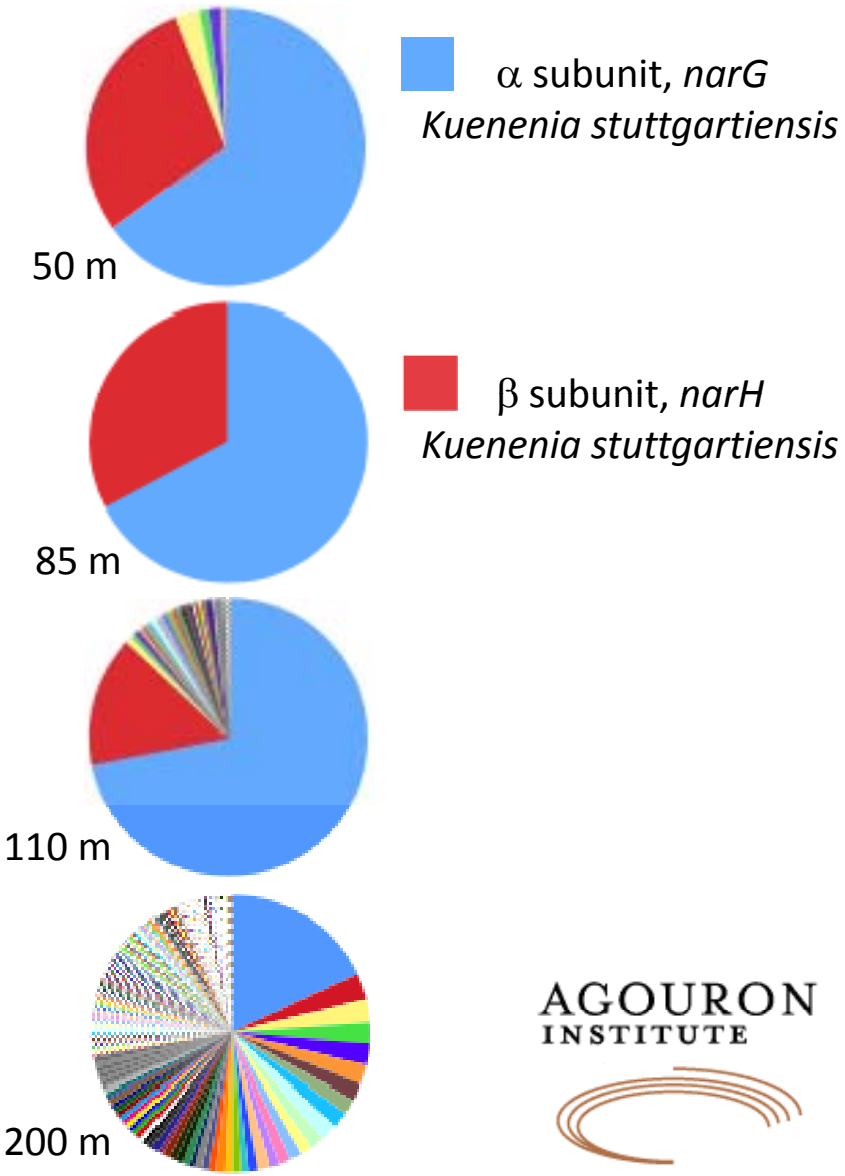
denitrification

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$



★ Includes a) other subunits, b) genes identified as nitrate reductase but not differentiated by subunit, c) and assimilatory nitrate reductase genes

taxonomic composition



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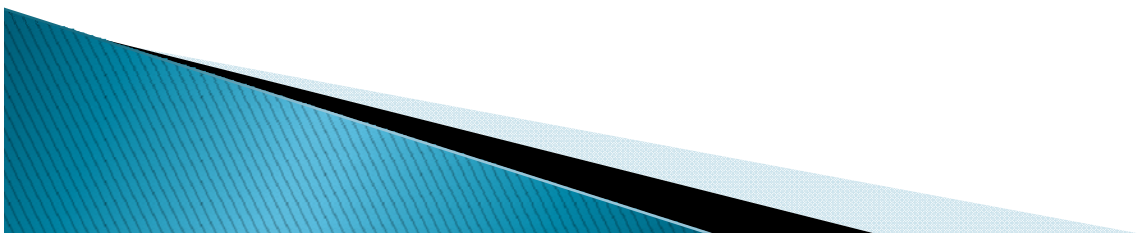
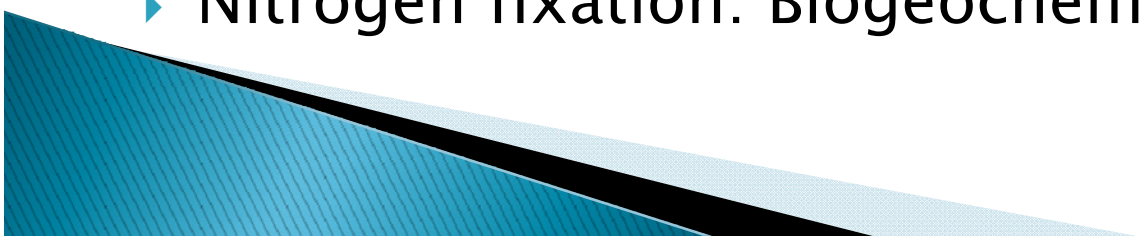
- Limitations in interpretation of molecular techniques
  - Gene abundance-representation of cell abundance?
  - Gene expression-representation of enzyme activity?
- Temporally dynamic populations may influence relative importance
- May be cross feeding between microorganisms
  - Difficulty in integrating across time and space



- OMZs directly linked to nitrogen fixation
  - Due to effect of oxygen

- Global balance between N_2 fixation and denitrification hard to determine
- Limited measurements in space and time
- Nitrogen fixation: Biogeochemical inference



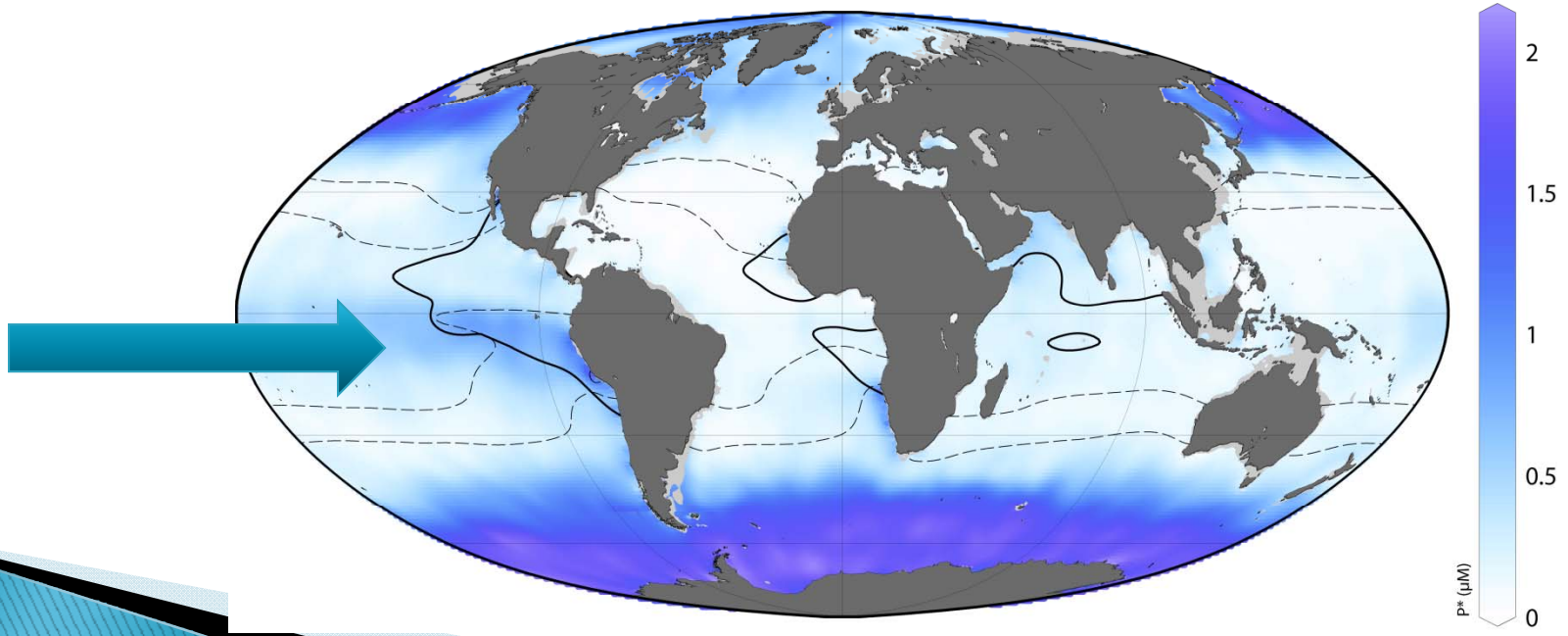
Nitrogen fixation enhanced in OMZ due to removal of N (denitrification)

$$P^* = PO_4^{3-} - NO_3^{-}/r_n,$$

Biogeochemically inferred processes

P* → regions selecting for N_2 fixation

"N_2 fixation will be revealed as a reduction in P* along the transport path of a surface water mass, and its rate can be estimated by combining the observed distributions of nutrients with information about the rate of ocean circulation and mixing."

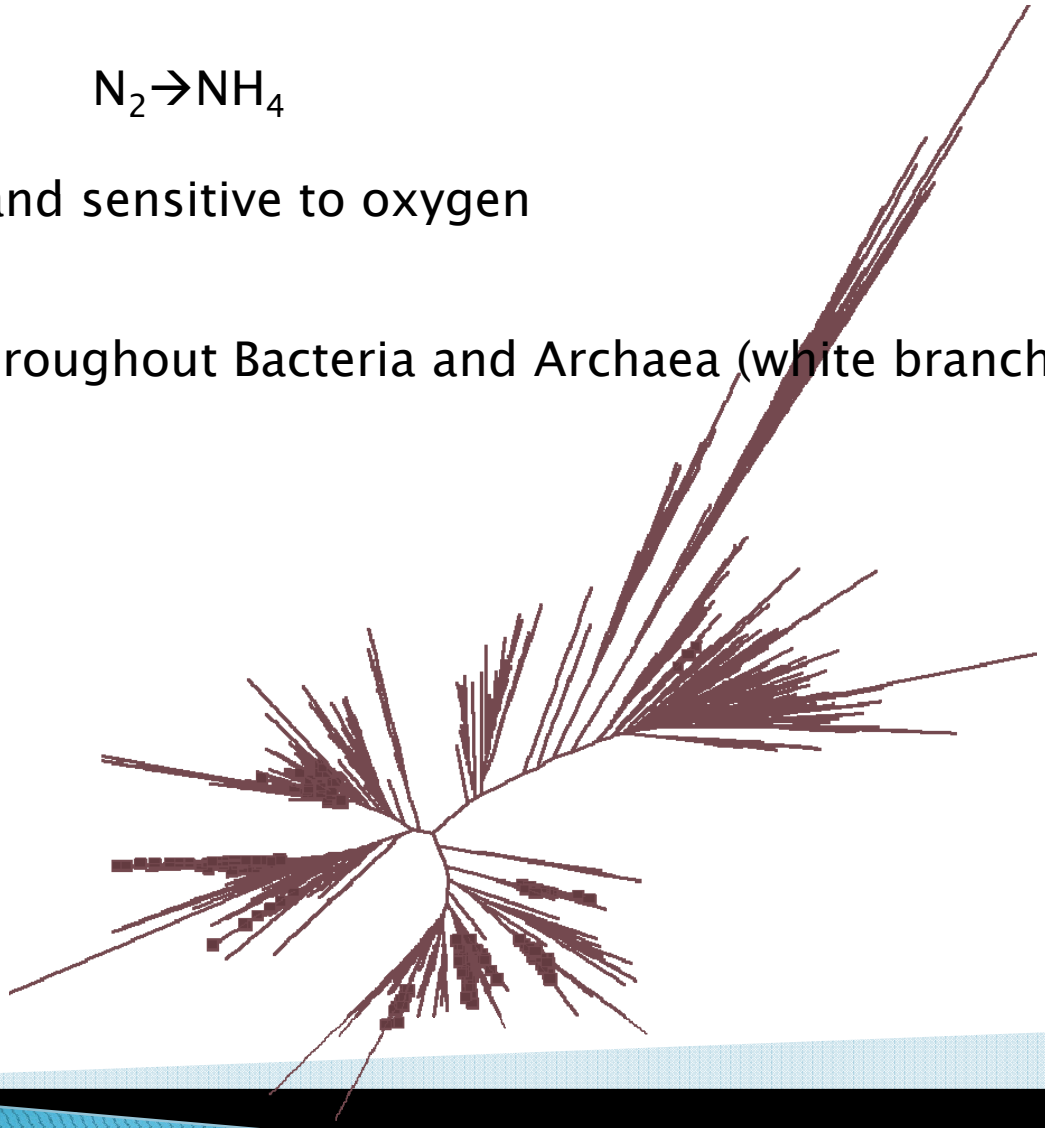


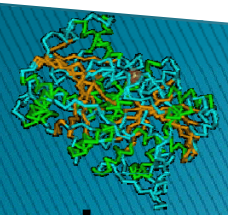
Biological nitrogen fixation $N_2 \rightarrow NH_4$

Energetically expensive and sensitive to oxygen

nif genes widely distributed throughout Bacteria and Archaea (white branches)

MoFe protein

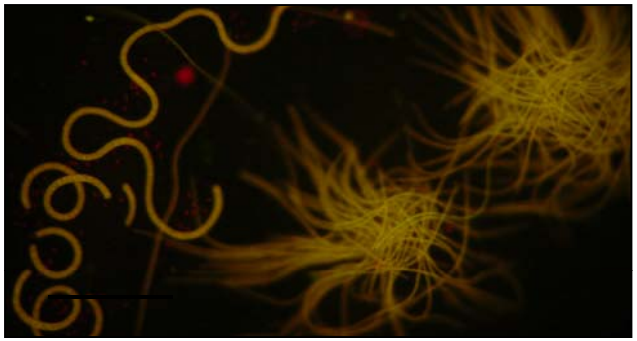


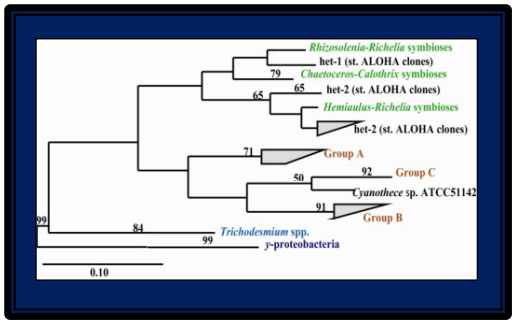


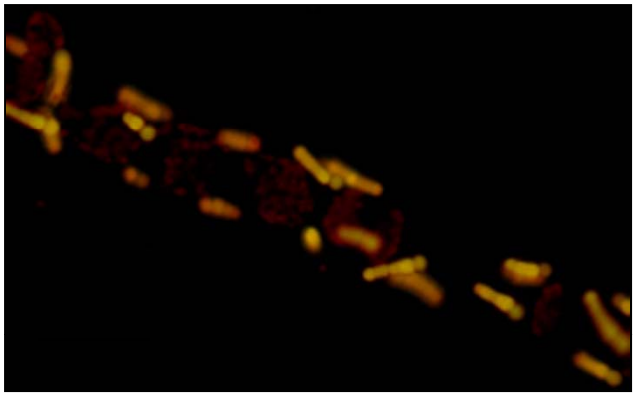
Fe protein

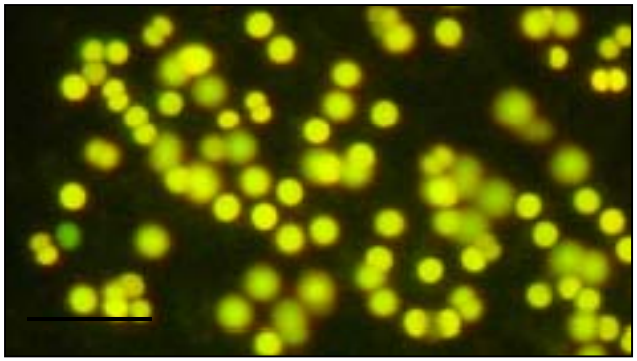
>15,500 nitrogenase (*nifH*)genes sequenced

Open ocean N_2-fixing cyanobacteria:microscopy and gene amplification (PCR)

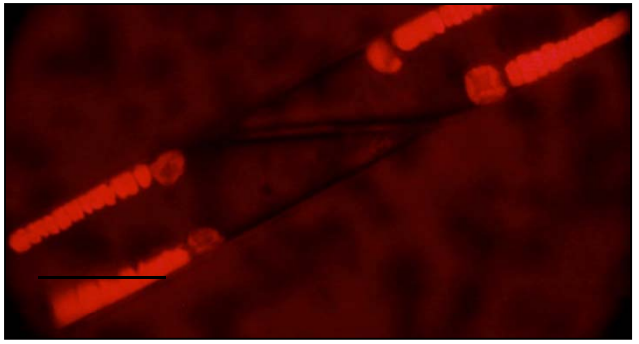


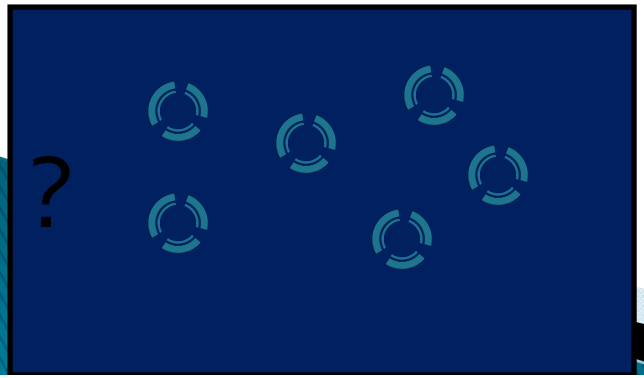




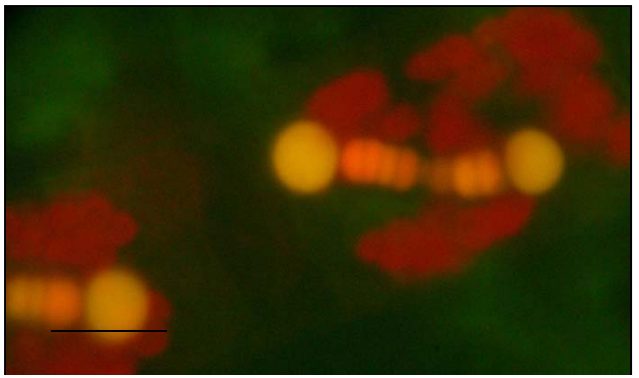


Crocosphaera
-cultivated

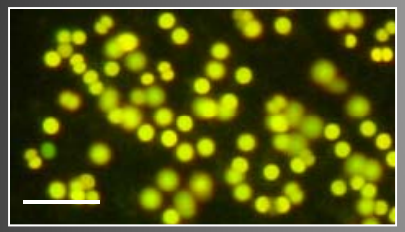


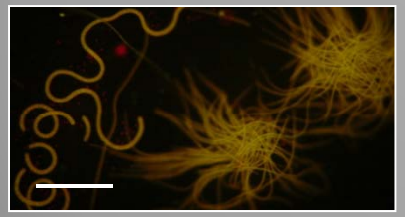


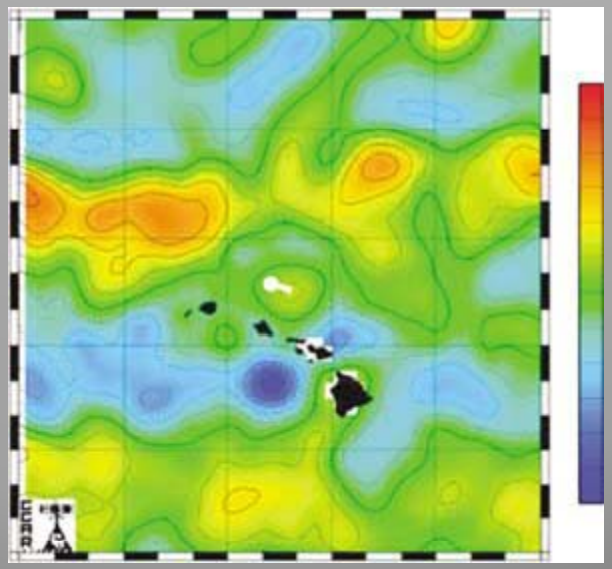
"Group A"
-uncultivated



N_2-fixers highly variable in space and time







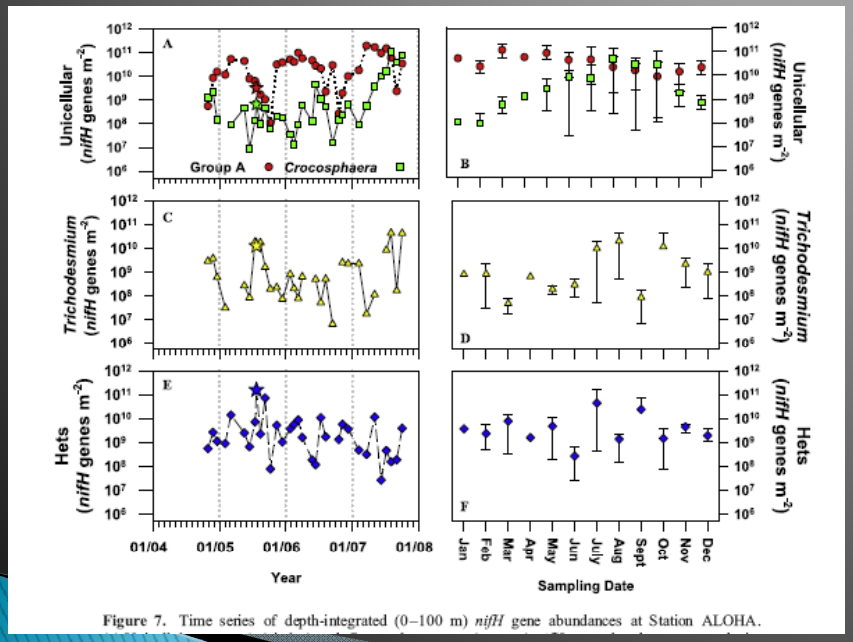
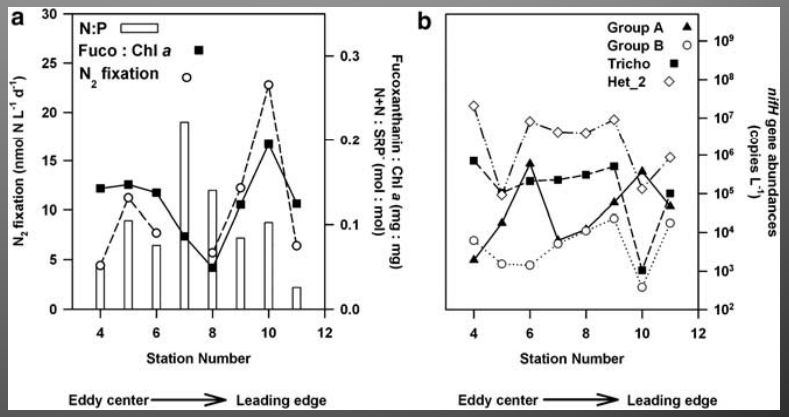


Figure 7. Time series of depth-integrated (0–100 m) *nifH* gene abundances at Station ALOHA.

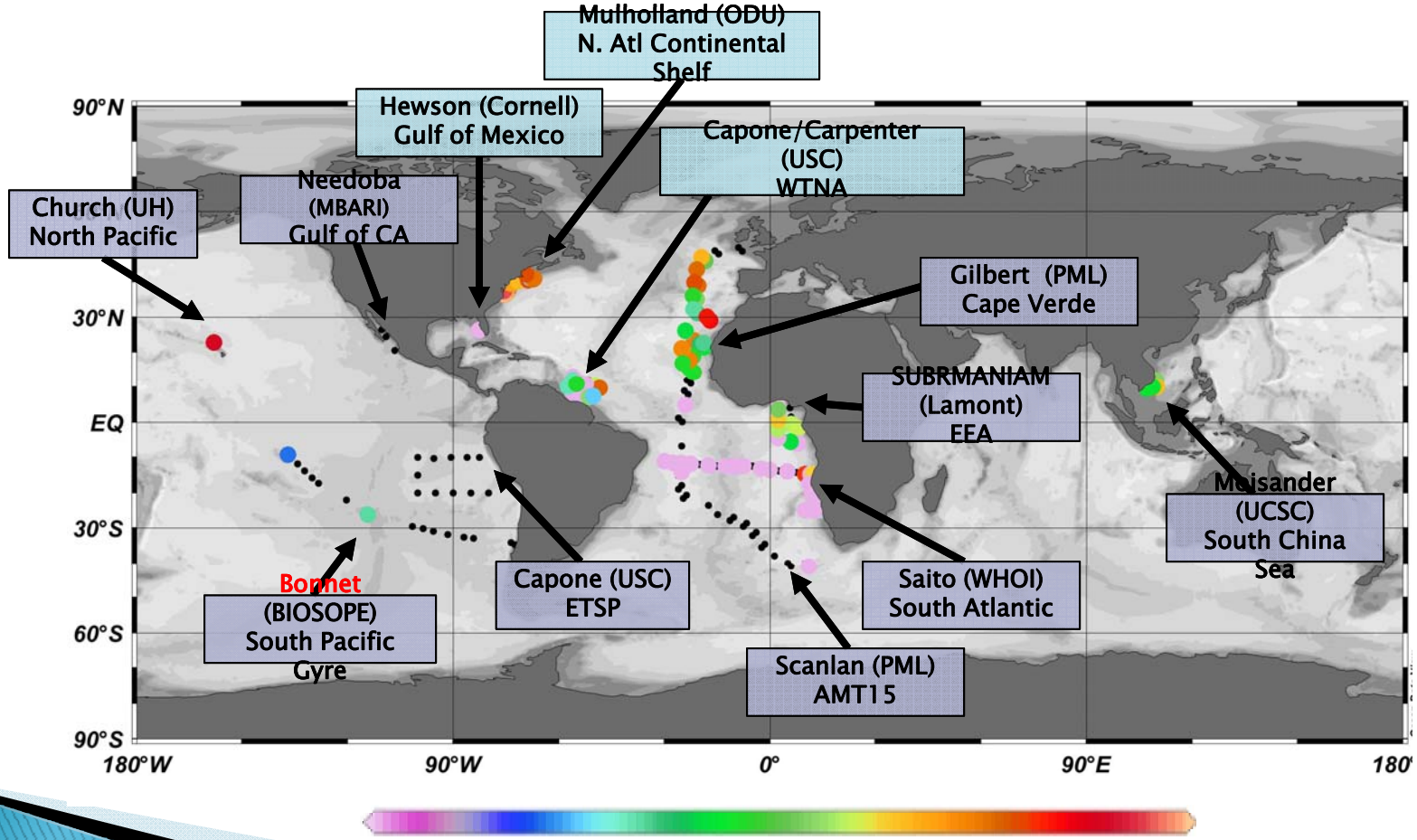
Church et al. 2009 GBC



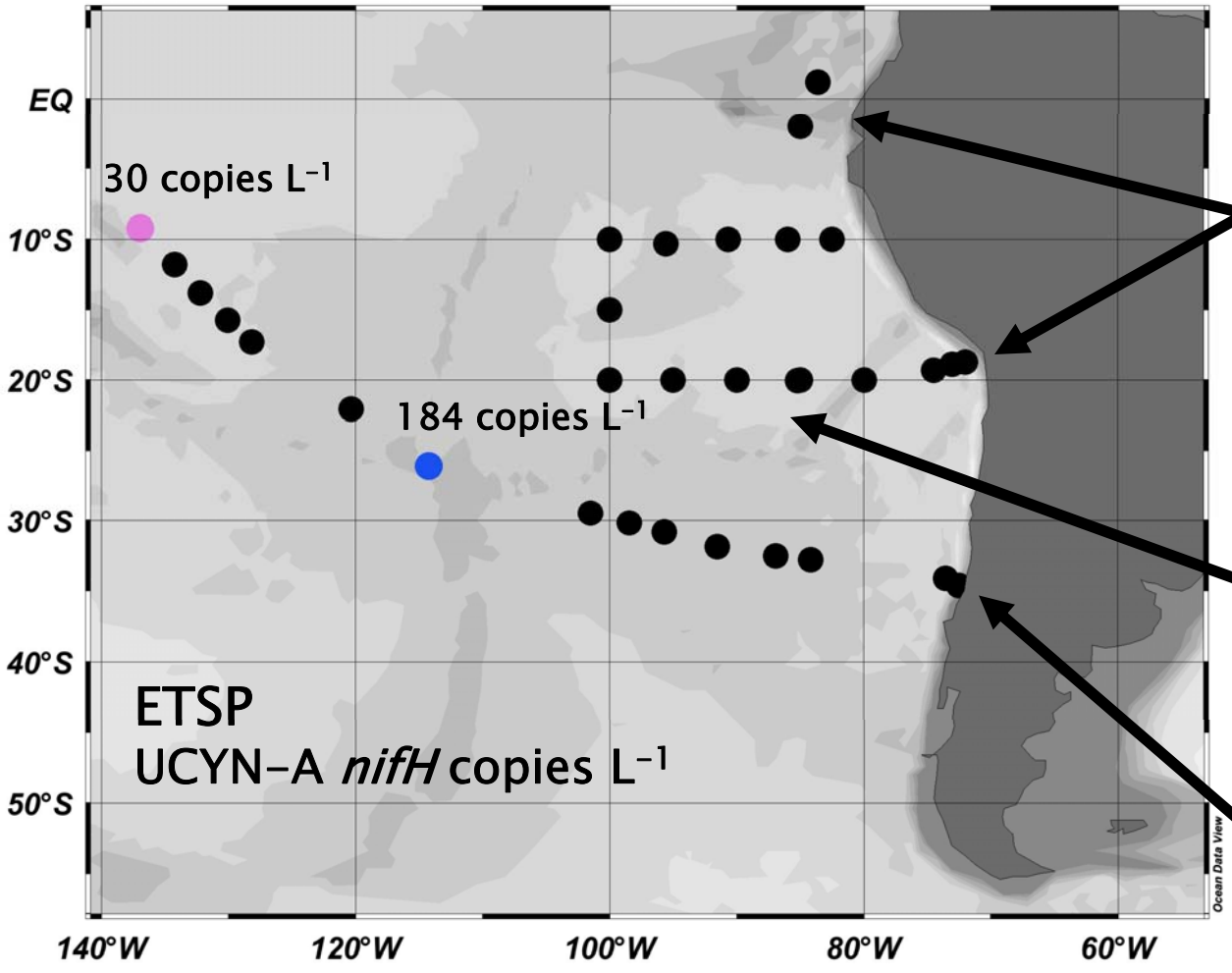
Fong et al. 2008 ISME Journal

From genomes to ecosystems

Global collaborative efforts



Eastern Tropical South Pacific
Very few diazotrophs detected using qPCR assays on DNA extracts



Collaboration with Univ. de Concepcion:

No UCYN-A, γ24744A11 or α24809A06 detected in any samples.

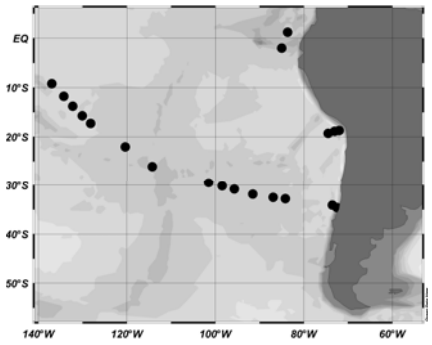
ETSP1 2010

No UCYN-A, UCYN-B, *Trichodesmium*, *Richelia* in *Rhizosolenia*, γ24744A11 or α24809A06 detected at any station/depth.

BIOSOPE 2006

UCYN-A, UCYN-B and *Trichodesmium*: very low detection at a few stations

Eastern Tropical South Pacific
All *nifH* sequences characterized are proteobacterial



~50% sequences

Phylotype also observed in N. and S. Pacific, N. Atlantic, Arabian Sea and South China Sea

Phylotype also observed in N. Atlantic

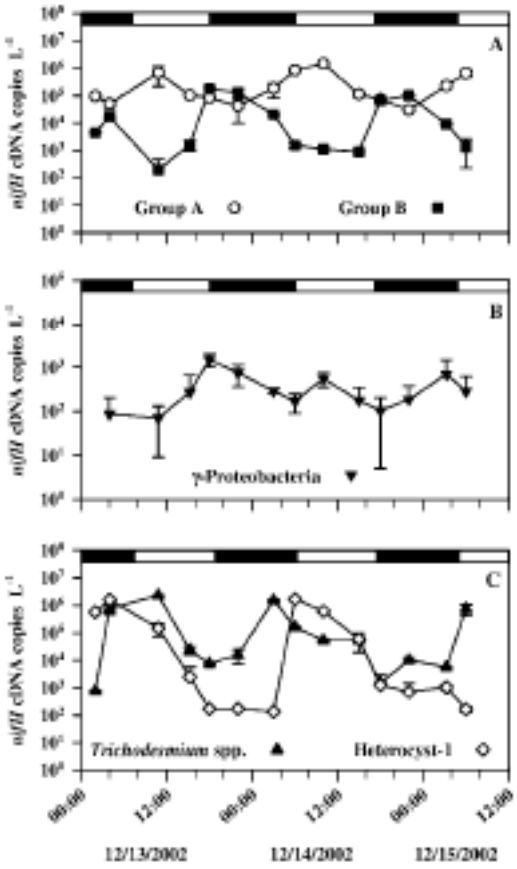
No Cyanobacterial diazotrophs

Open ocean N_2-fixing heterotrophs: gene amplification (PCR & qPCR)

Understanding heterotrophic diazotrophs contribution to N cycle is complicated due to prevalence of contaminants

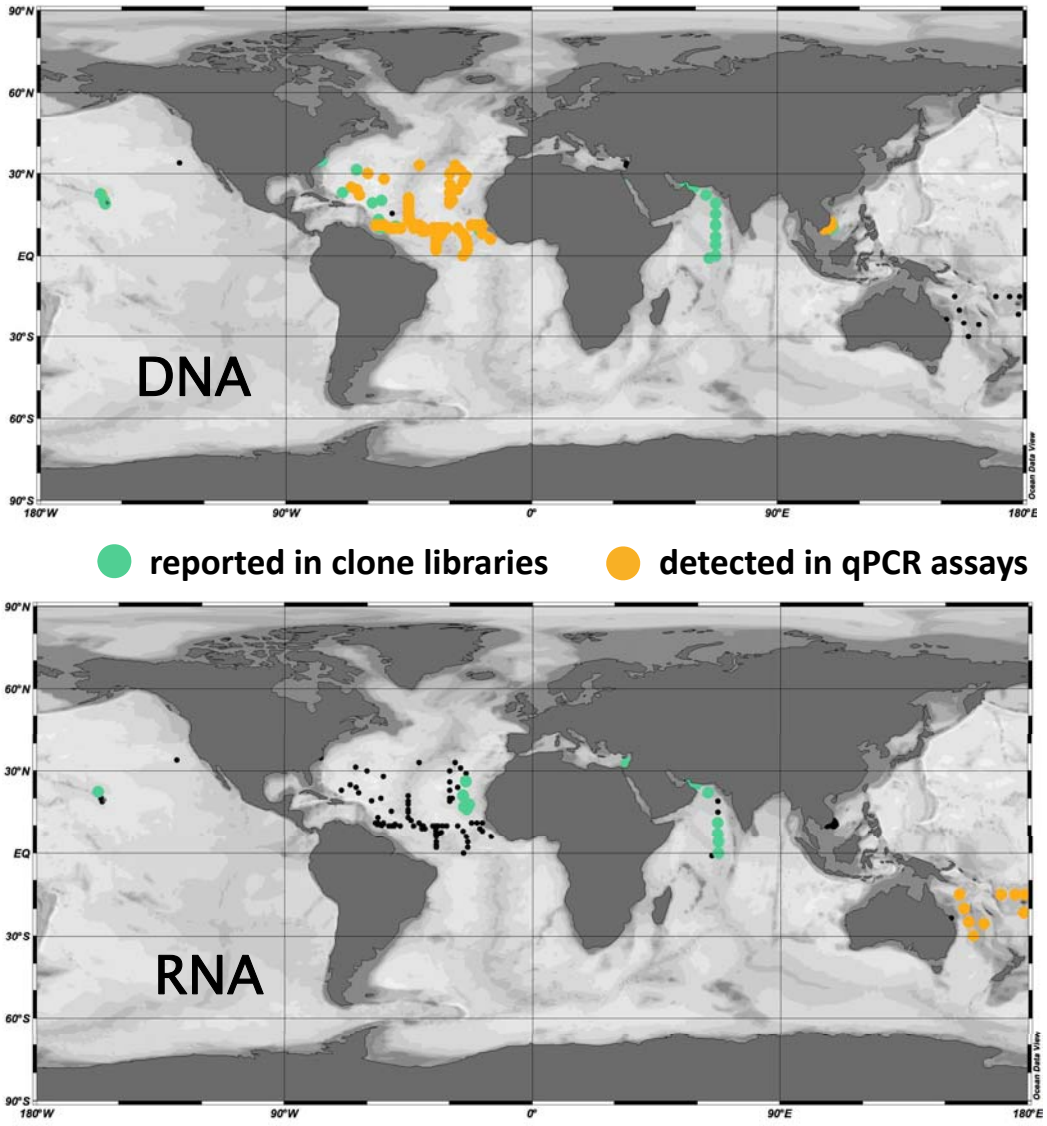
Open ocean N_2-fixing heterotrophs: gene amplification (PCR & qPCR)

Some heterotrophic diazotrophs have widespread distributions but low abundances – implications for global N cycle are unclear.

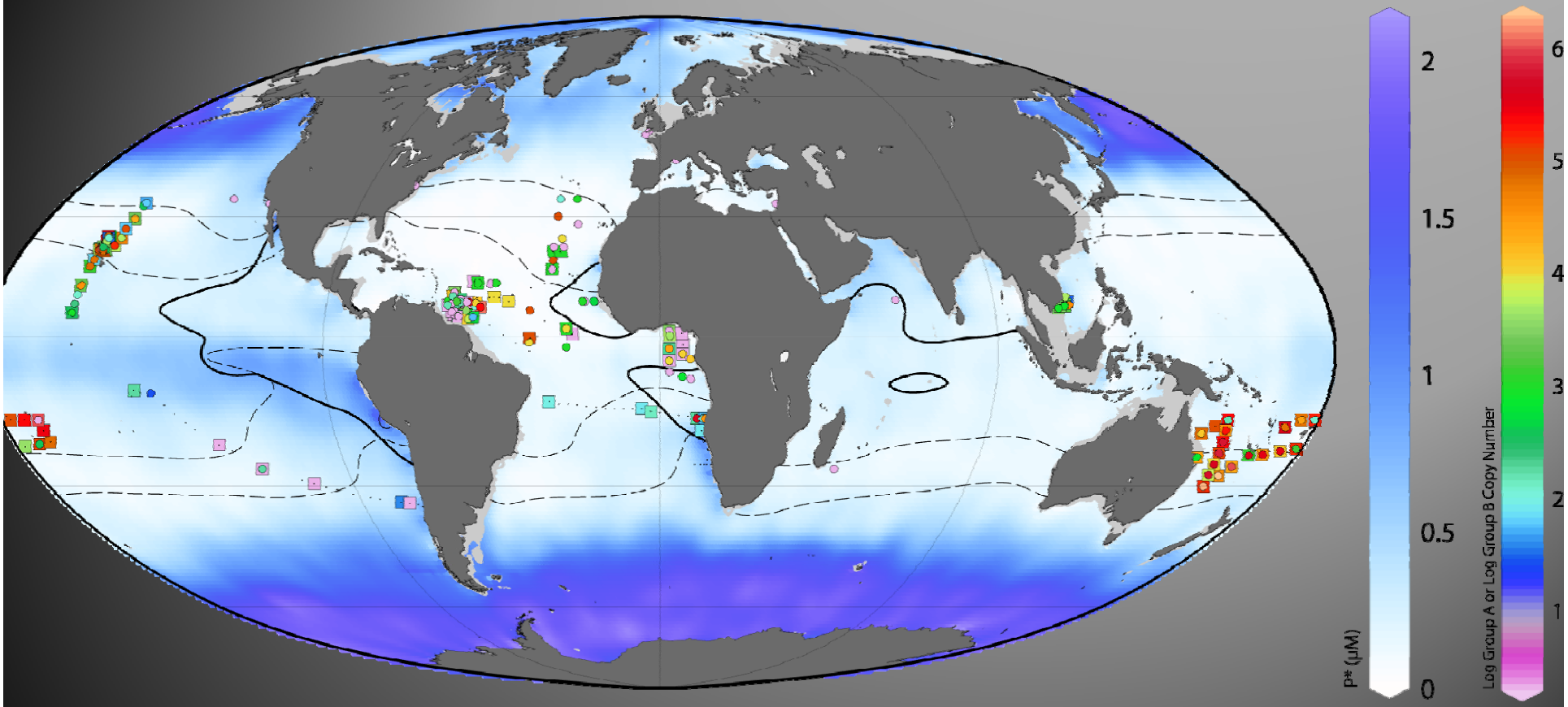


From Church et al, 2005

Global distribution of **γ-proteobacteria 24774A11**



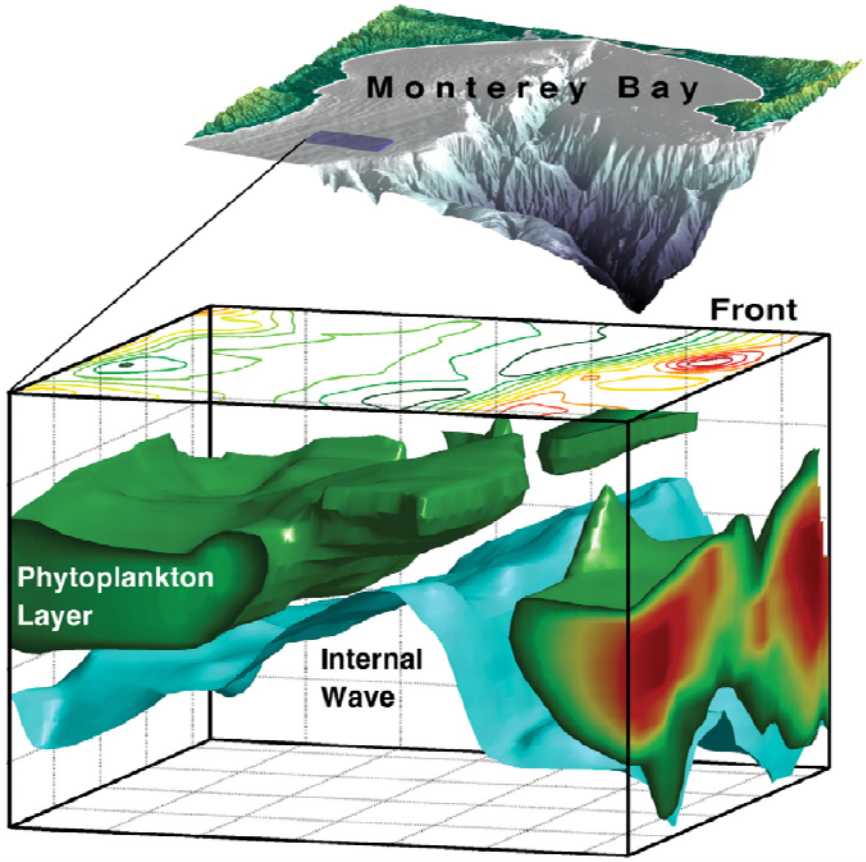
Global map–P* with N_2 fixer distributions



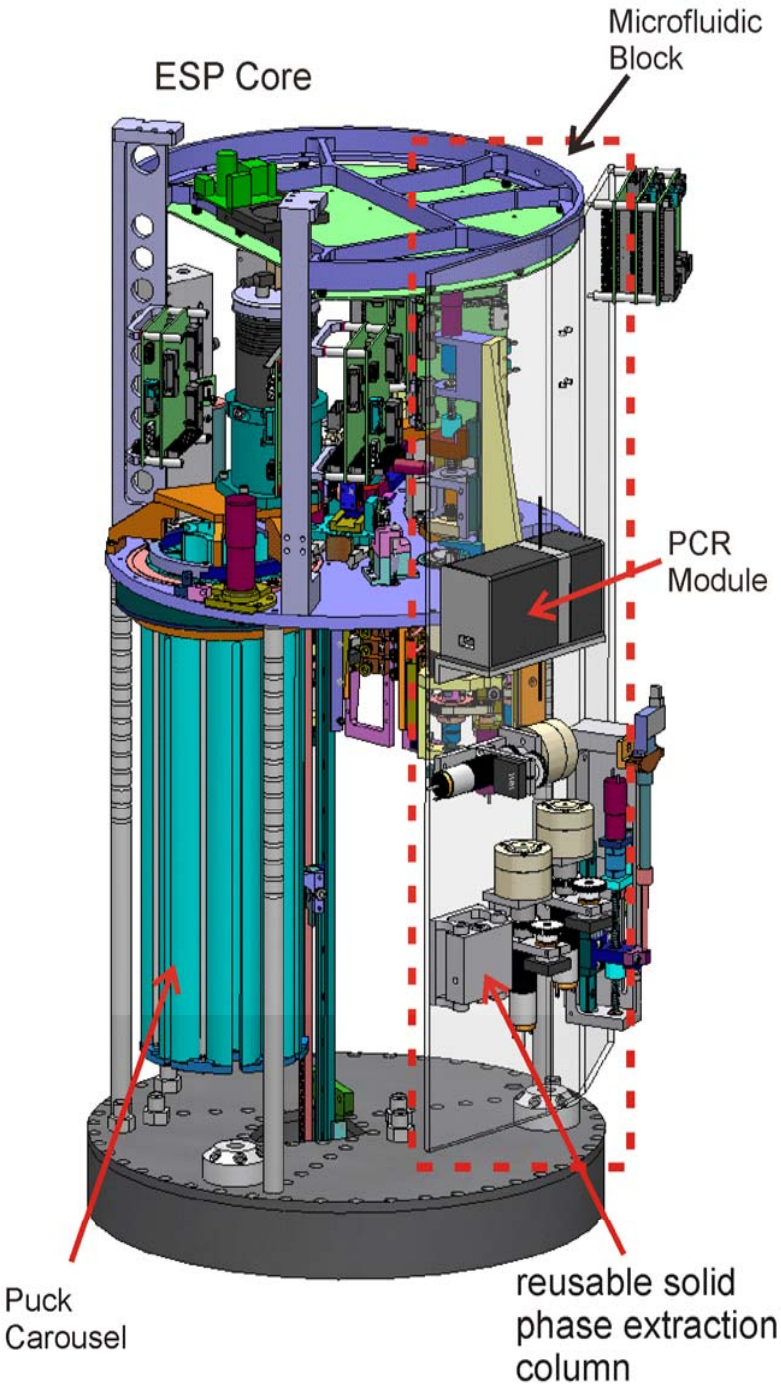
N_2 fixer distributions are not correlated with P*

Molecular tools provide information on potential for N cycle processes, and whether they are active

- Understanding dynamics of N cycles in time and space requires higher resolution data
- Remote instrumentation increases sampling resolution
- Detection of microorganisms by genes, mRNA, etc.
- Environmental Sample Processor (ESP)–Chris Scholin/Chris Preston (MBARI), Julie Robidart (UCSC–MBARI)



John Ryan



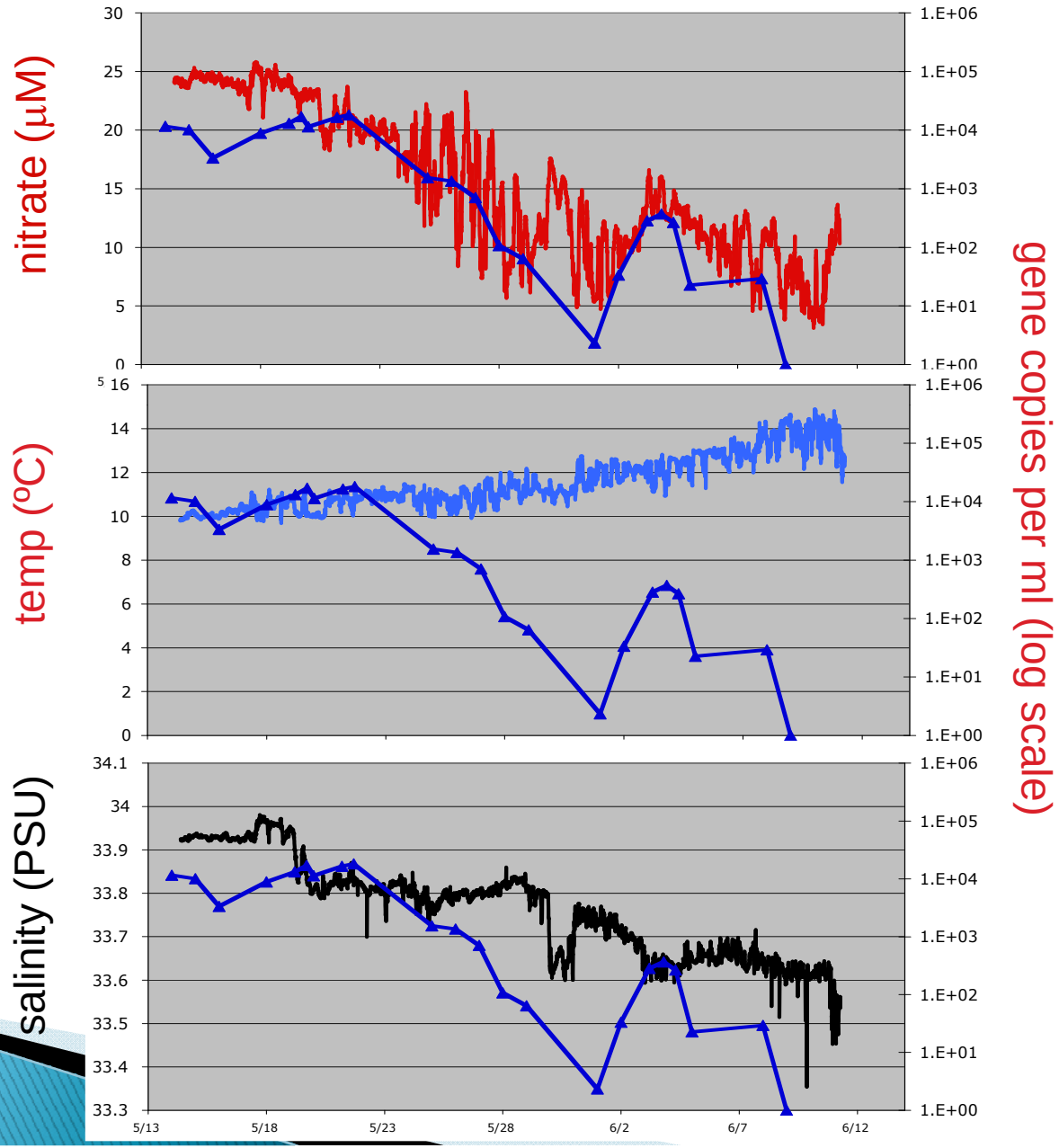
Remote instrumentation that can perform molecular biological measurements

Few molecular biological samplers- ESP and AMG

Can enumerate strains from genes- subspecies/ecotypes

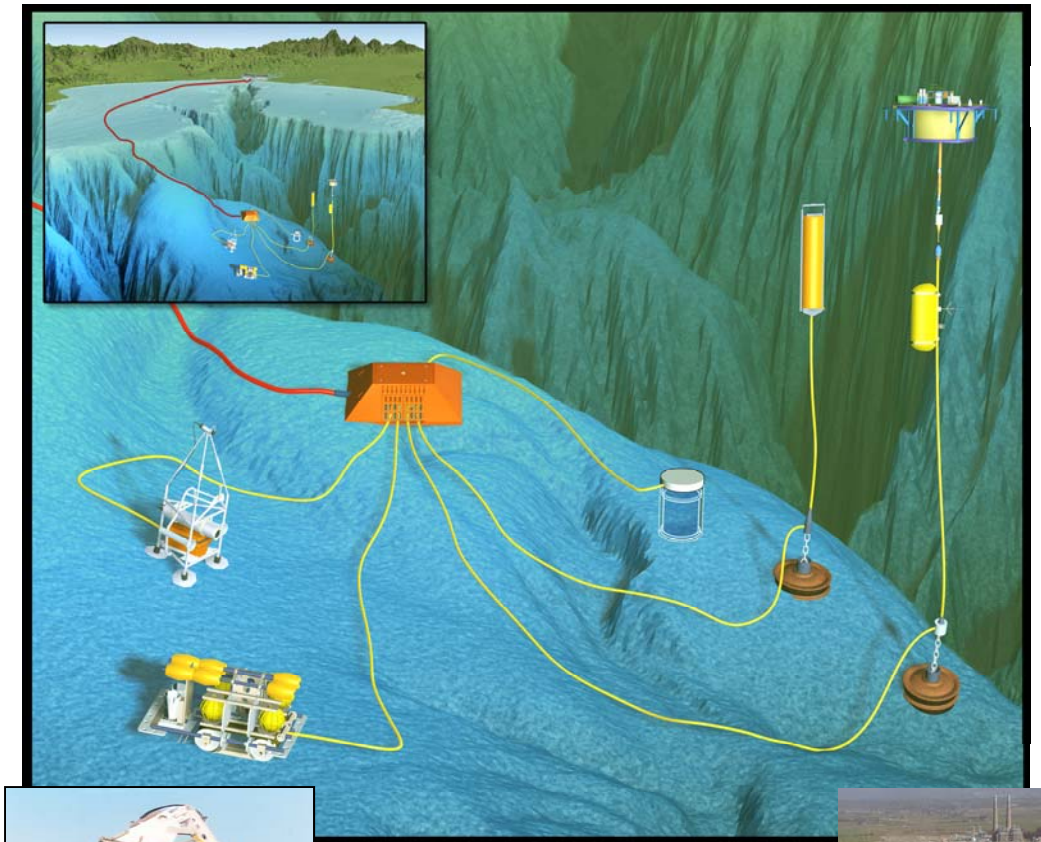
Crenarchaeota vs. nitrate, temp, salinity

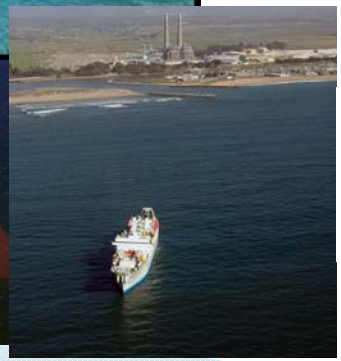
Spring ESP Deployment



C. Preston

The Monterey Accelerated Research System (MARS)



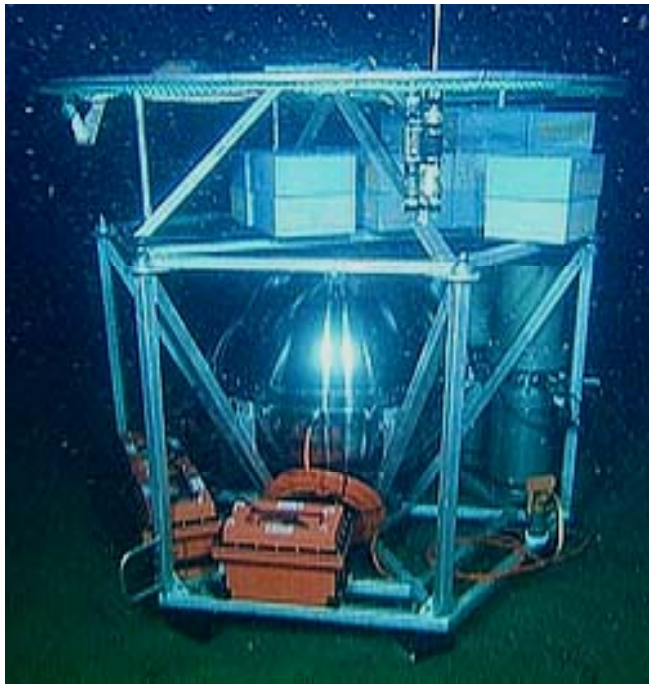


- 9 kW of power for science
- 8X 100 Mbit/sec Ethernet ports
- Precision time distribution
  - ~5 uSec
- Deep water – 890 meters
- Accessible – 2 hrs from MBARI
- Extended geographic coverage with extension cables
- Extensive shoreside support
  - Staging, ships, ROVs, expertise

http://www.mbari.org/mars

D-ESP Deployment on MARS

Scholin et al. MBARI



900 m

Summary

- Molecular biology needed for disentangling the N cycle
- Higher resolution data needed
- Need integration of models, remote instrumentation and molecular biology

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