



Royal Netherlands Institute for Sea Research



[www.anaerobic-microbiology.eu](http://www.anaerobic-microbiology.eu)

# Let's dive in an ocean of microbes!

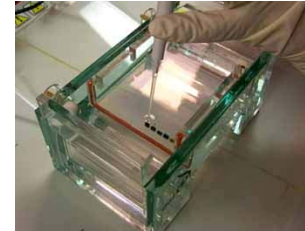
**Laura Villanueva**

Department of Marine Microbiology and Biogeochemistry (MMB)

[Laura.Villanueva@nioz.nl](mailto:Laura.Villanueva@nioz.nl)



Let me tell you about myself....



Since 2009 in  
the Royal  
NIOZ!



[https://www.youtube.com/watch?v=Sk3uy0jwh9o&list=PLN2ZUxZYhgCy\\_ywjZAJ1vqxsRQa0STceM](https://www.youtube.com/watch?v=Sk3uy0jwh9o&list=PLN2ZUxZYhgCy_ywjZAJ1vqxsRQa0STceM)



# Netherlands Institute for Sea Research NIOZ



Research in "De Keet" in the late 19th century



Zoological station, Den Helder 1890.

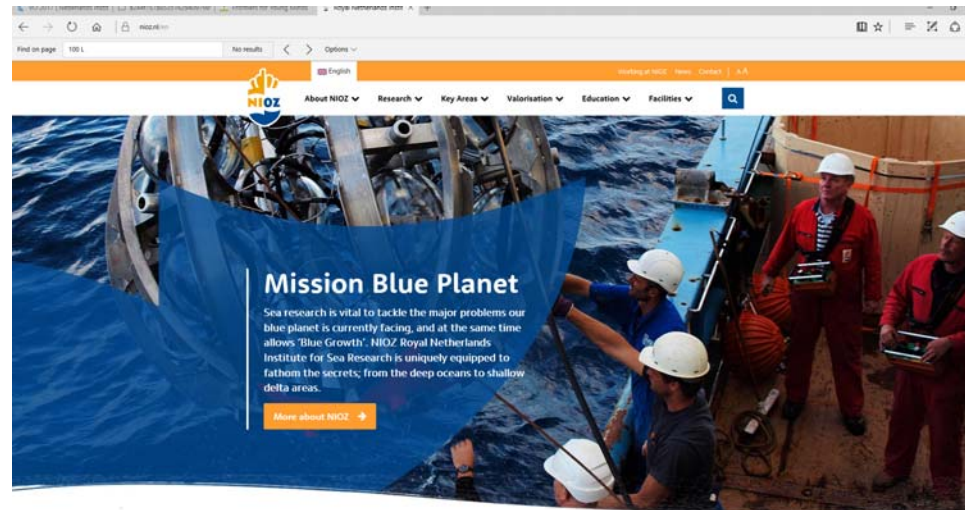


NIOZ in Texel



Research vessel R/V Pelagia

<http://www.nioz.nl>





## Netherlands Institute for Sea Research NIOZ

<https://www.youtube.com/watch?v=Ra2ilrdogNg>



# Evolution of Life on Earth



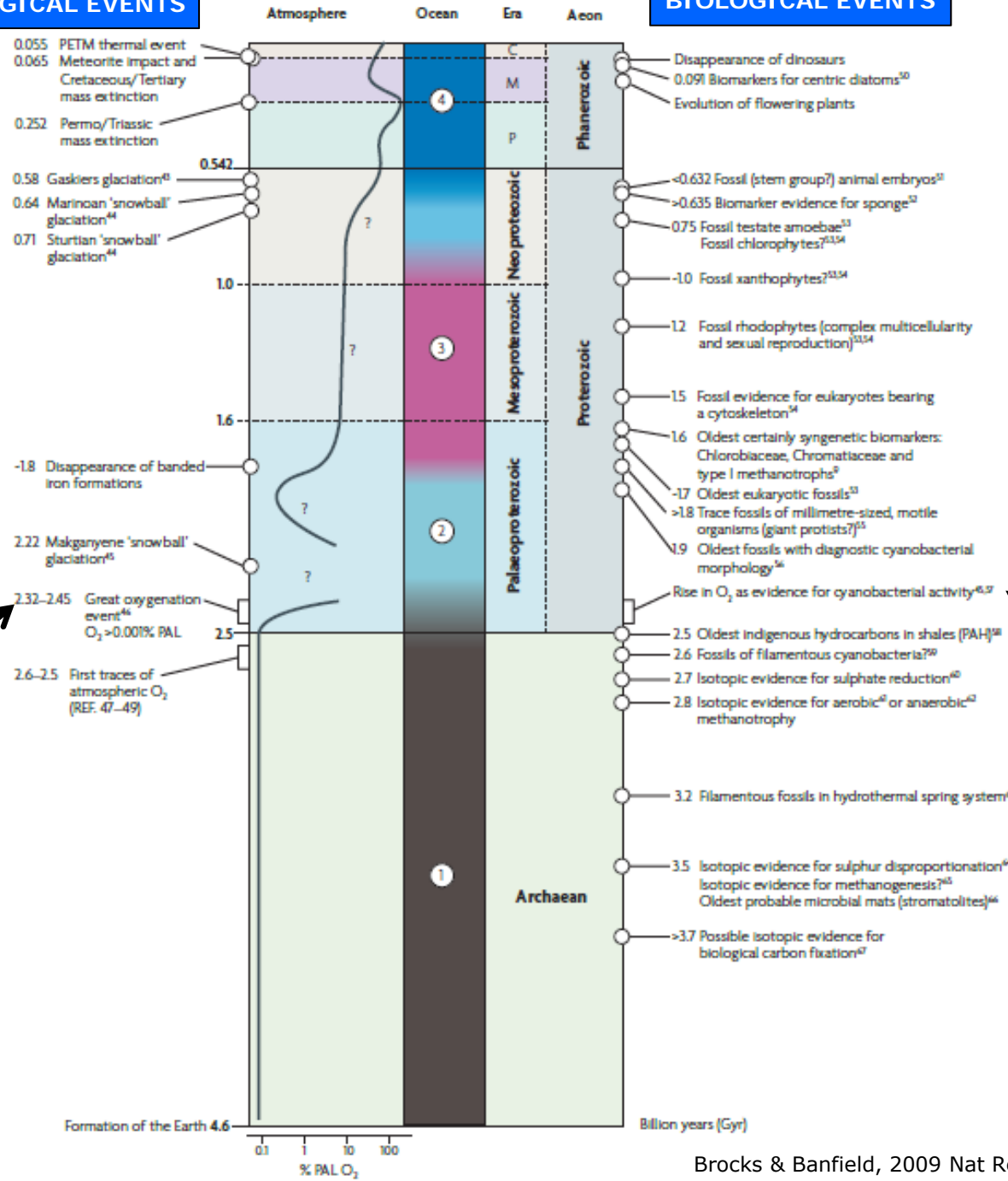


**GEOLOGICAL EVENTS**

**BIOLOGICAL EVENTS**

Great oxygenation event

Oxygenic phototrophs  
Cyanobacteria

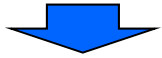






# 4 Ways Marine Microbes Changed Life on Earth Forever

How the Earth's  
Atmosphere Got  
Oxygen

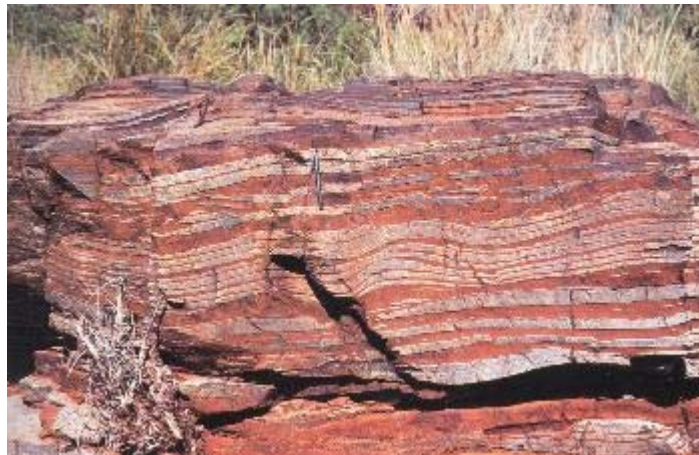


Great oxygenation  
event

**Stromatolites**



**Banded iron formations**





# 4 Ways Marine Microbes Changed Life on Earth Forever

The Worst Mass Extinction in the History of the Earth



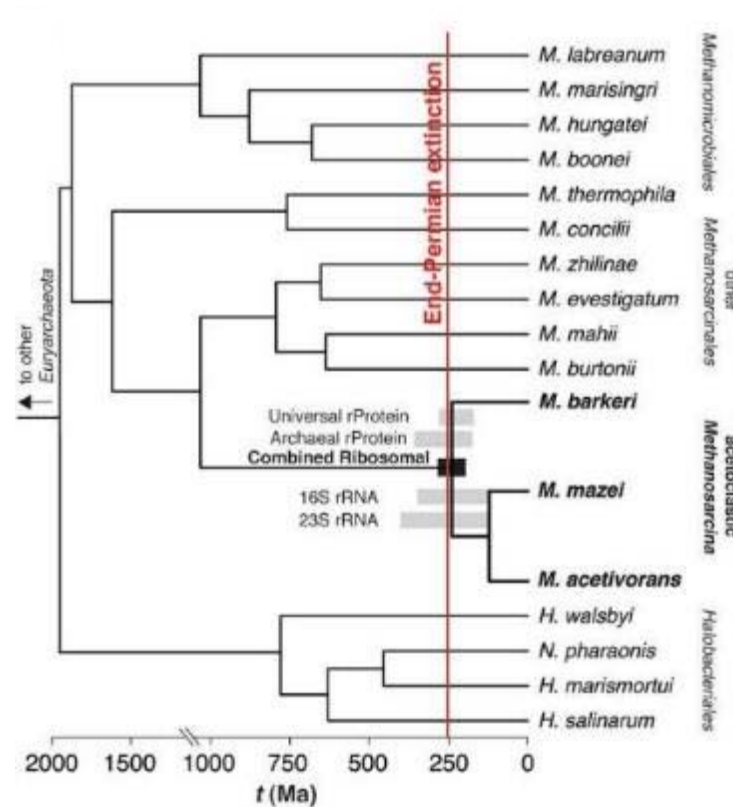
Great Dying  
end Permian period

Archaeageddon



- Disruption of Earth's Carbon cycle
- 250 million years ago
- Acetoclastic *Methanosarcina*
- Nickel availability

Acquisition of gene to grow on acetate by horizontal gene transfer



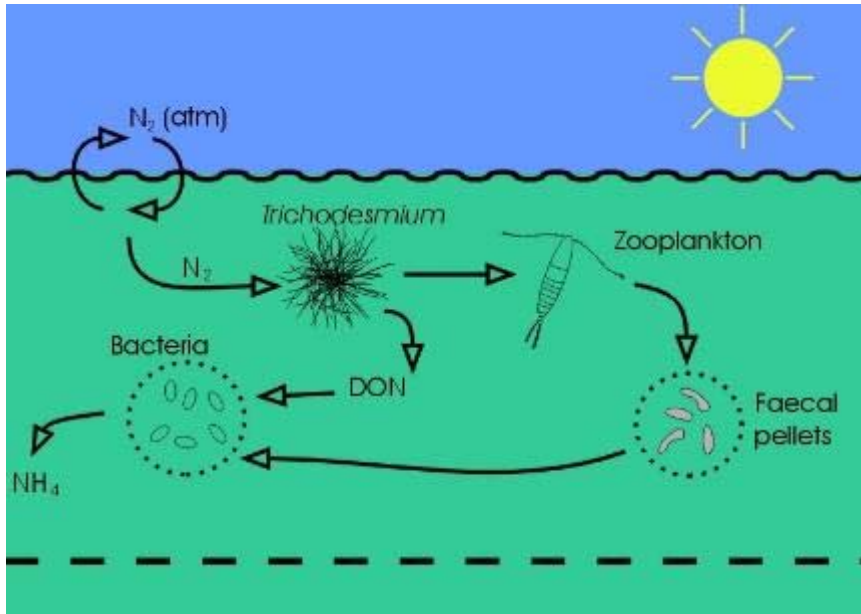
Rothman et al. PNAS 2014;111:5462-5467



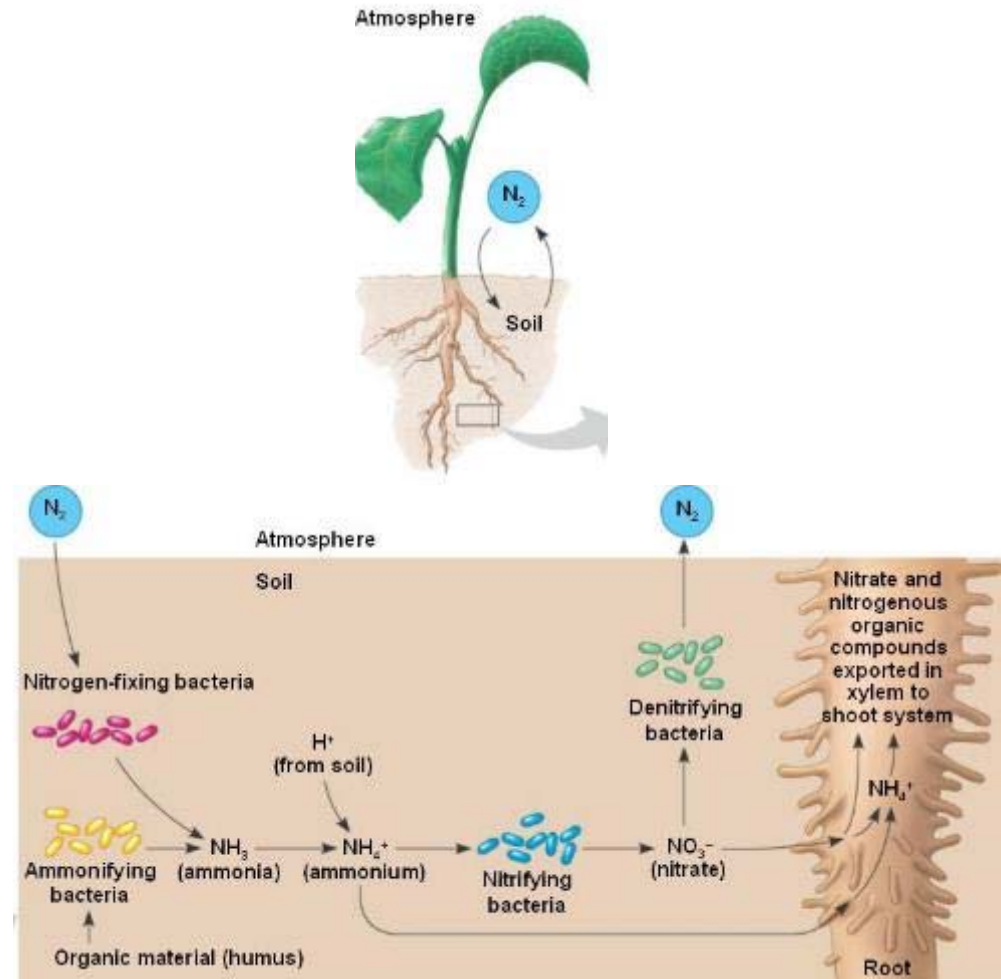


# 4 Ways Marine Microbes Changed Life on Earth Forever

## Nitrogen-Fixing Microbes in the seawater and soils >> Earth fertilization



[www.soes.soton.ac.uk](http://www.soes.soton.ac.uk)



[bio1152.nicerweb.com](http://bio1152.nicerweb.com)



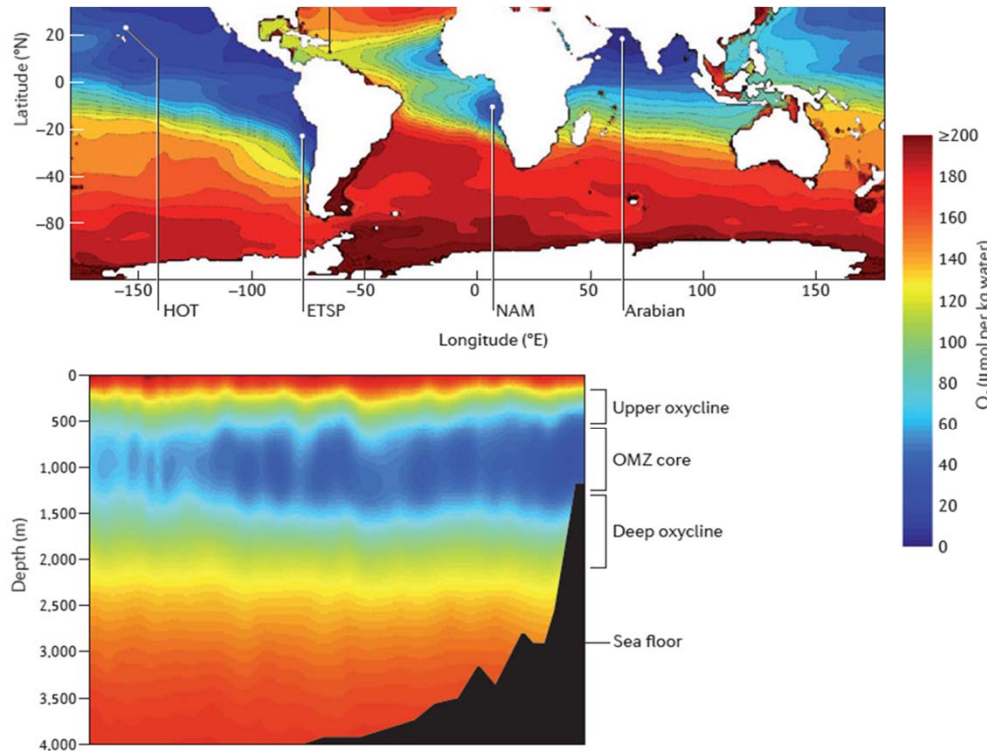
# 4 Ways Marine Microbes Changed Life on Earth Forever

What Microbes Mean for Climate Change



Carbon & Nitrogen cycles

## Oxygen minimum zones



### WHY do we study them?

- Expansion of anoxic zones
- Active areas of denitrification and release of greenhouse gases
- Source of unknown marine microbes

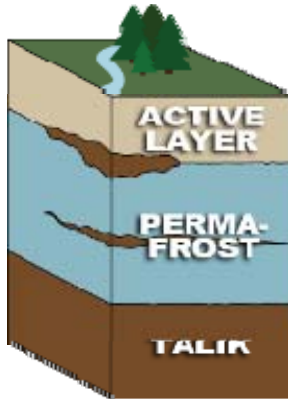


# 4 Ways Marine Microbes Changed Life on Earth Forever

What Microbes Mean for Climate Change



Carbon & Nitrogen  
cycles



Thawing of the permafrost



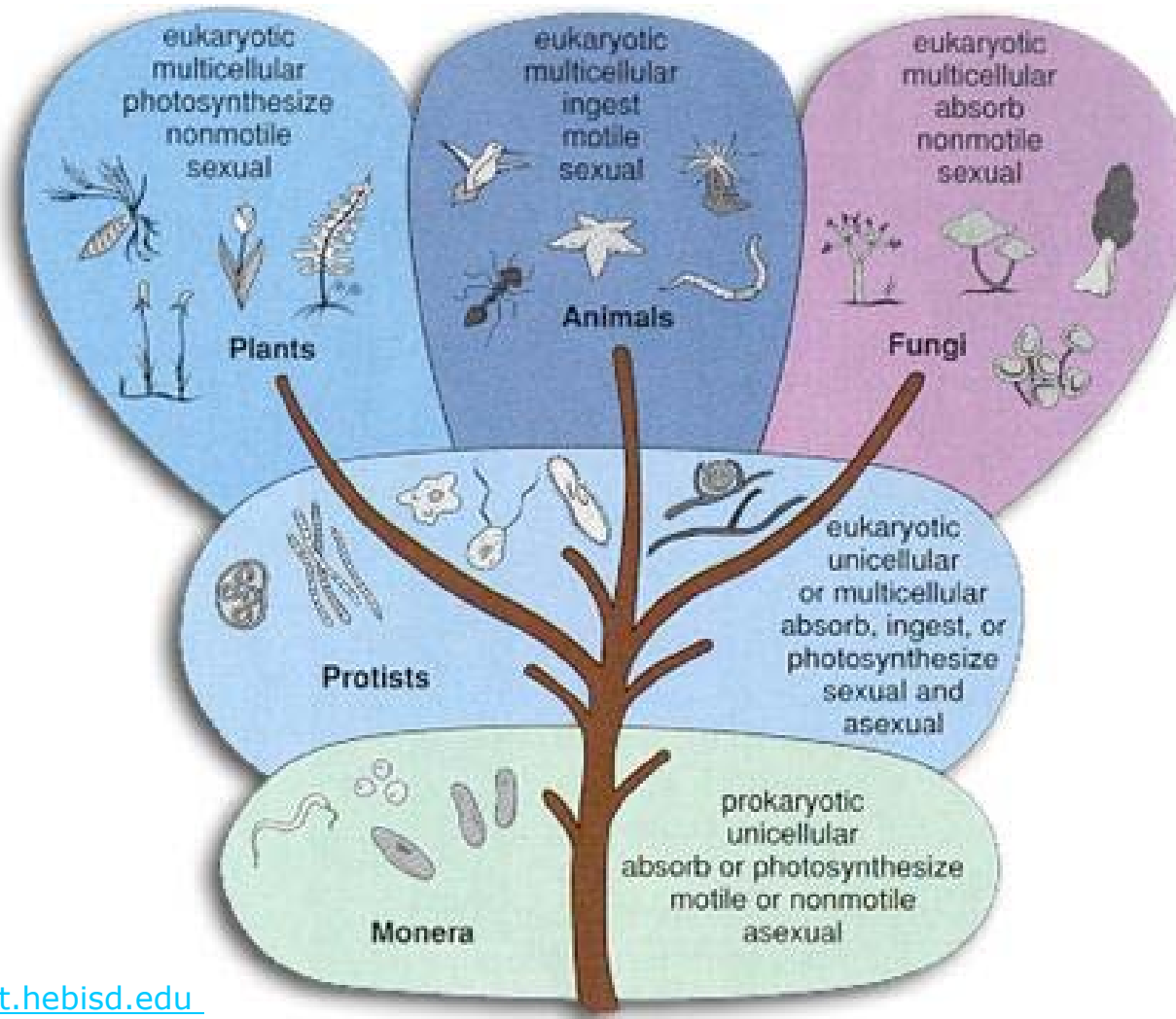
[www.johnshawphoto.com](http://www.johnshawphoto.com)





# Microbial diversity & classification

## “old” classification of organisms

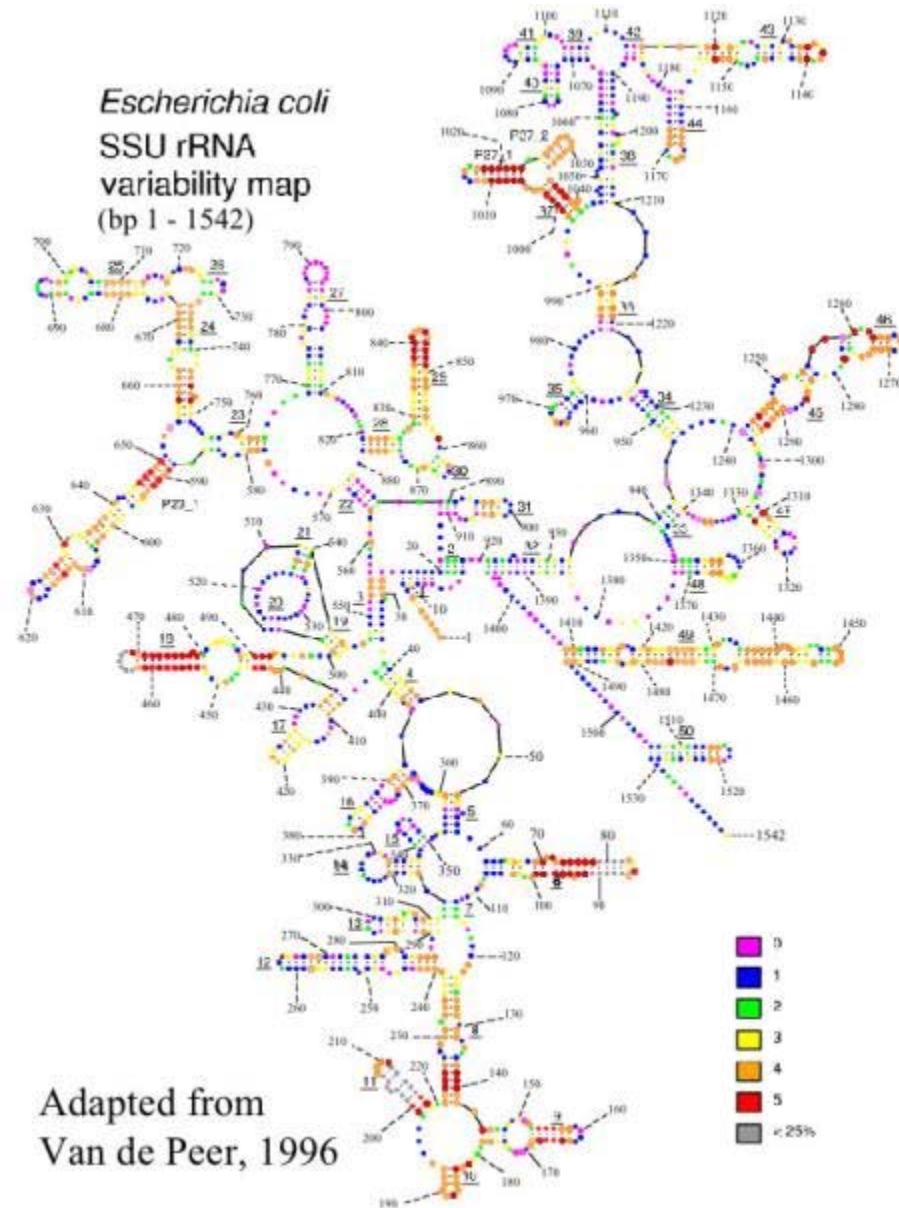
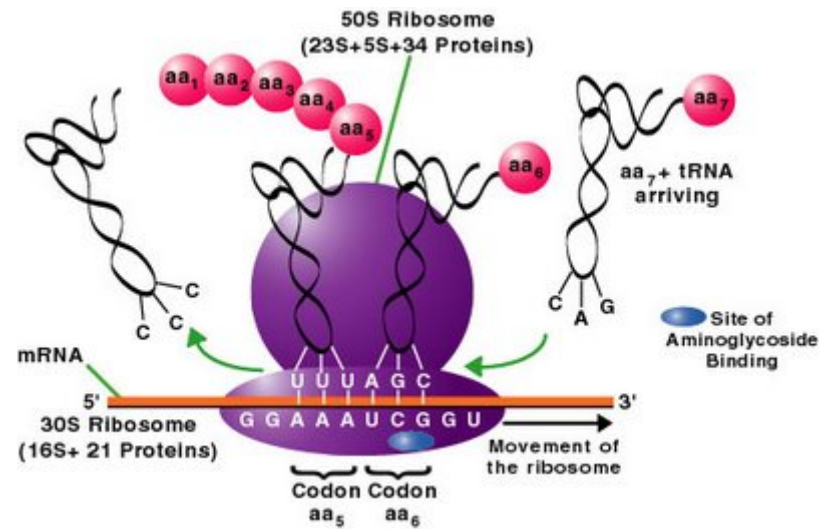






# The three domains of life: rRNA

## Ribosomal rRNA classification

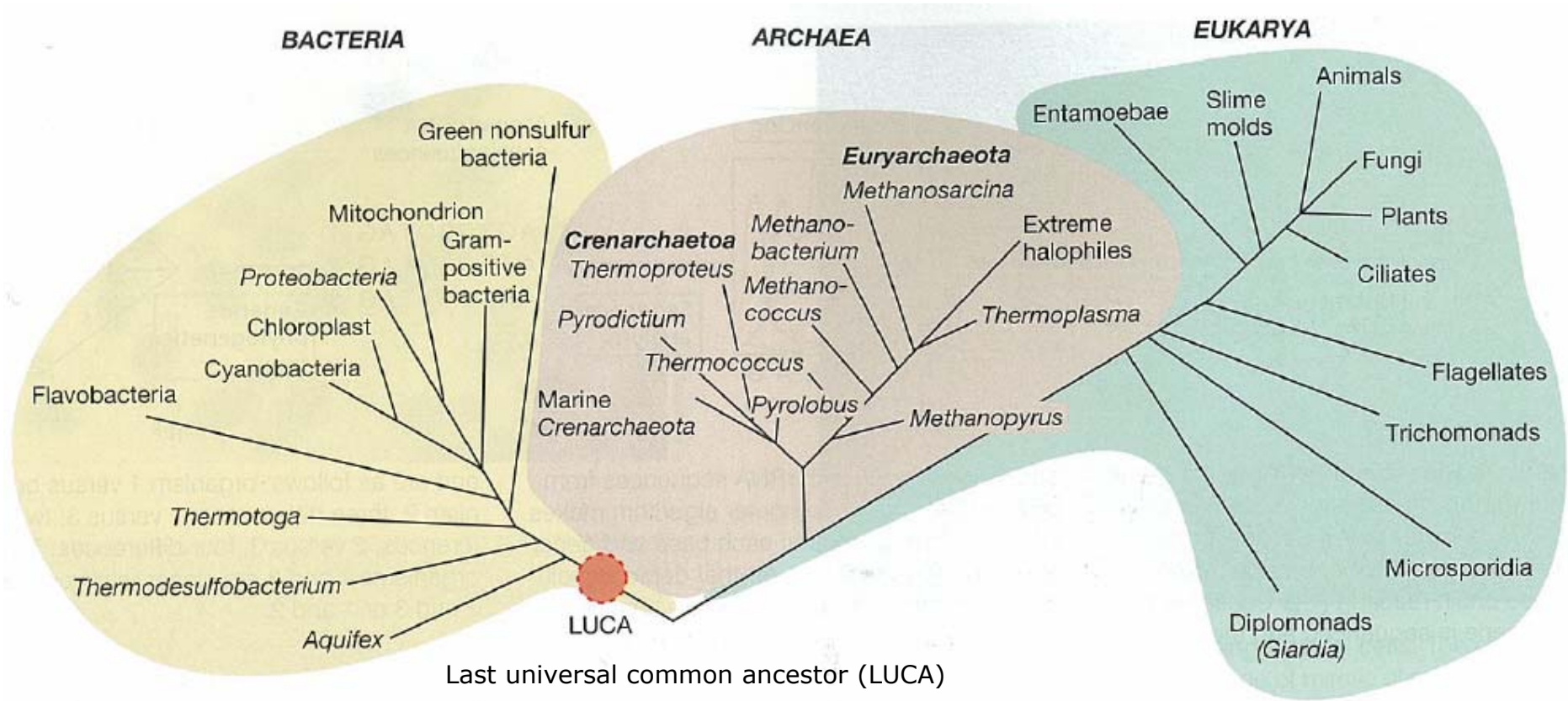


Adapted from Van de Peer, 1996



# The three domains of life (by Woese)

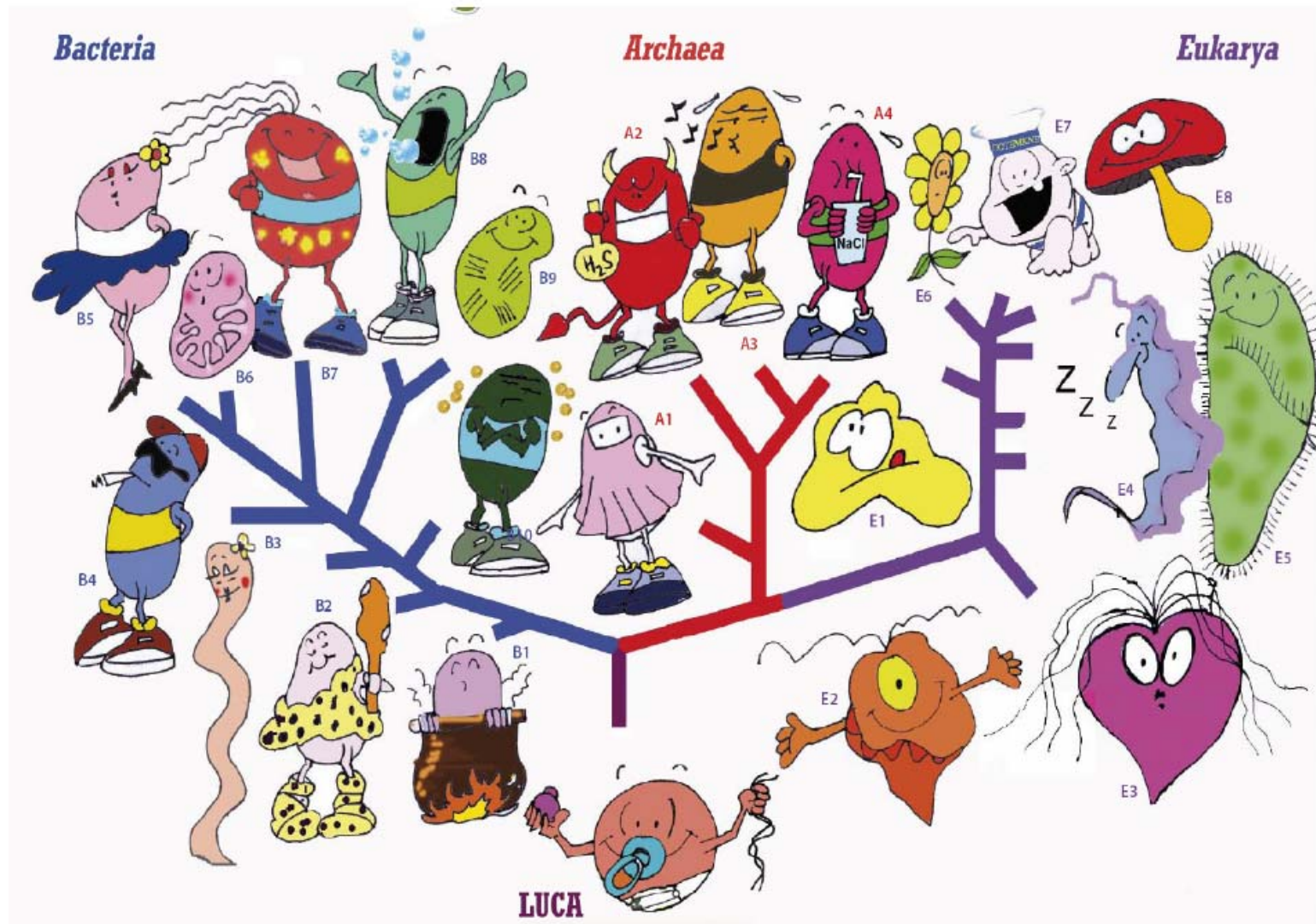
Tree of life: 3 domain system based on molecular phylogeny







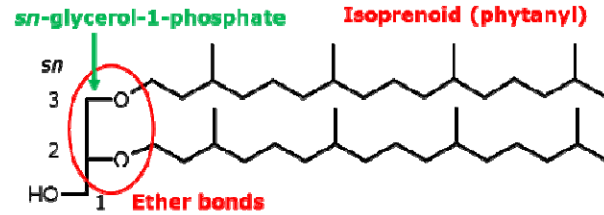
# The three domains of life (Woese)



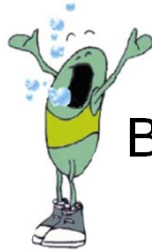
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# Three domains of life and membrane lipids



- Cell wall of peptidoglycan
- One RNA polymerase



Bacteria

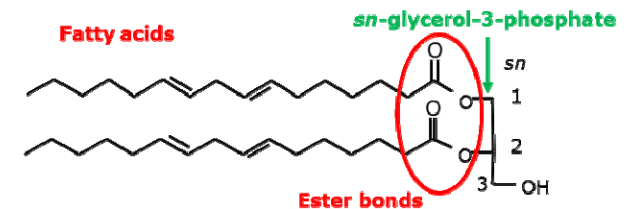
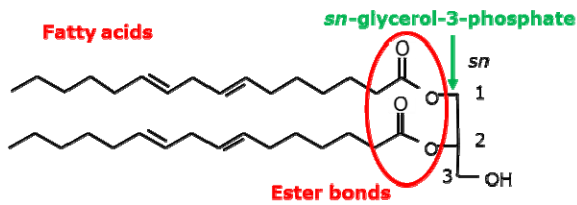


- No peptidoglycan
- several RNA polymerases

Archaea

- No peptidoglycan
- Three RNA polymerases

Eukaryotes

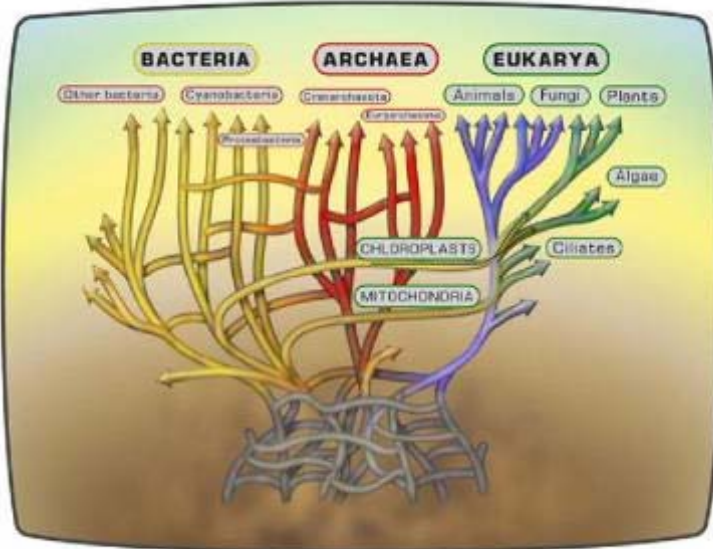


LUCA

Last universal common ancestor

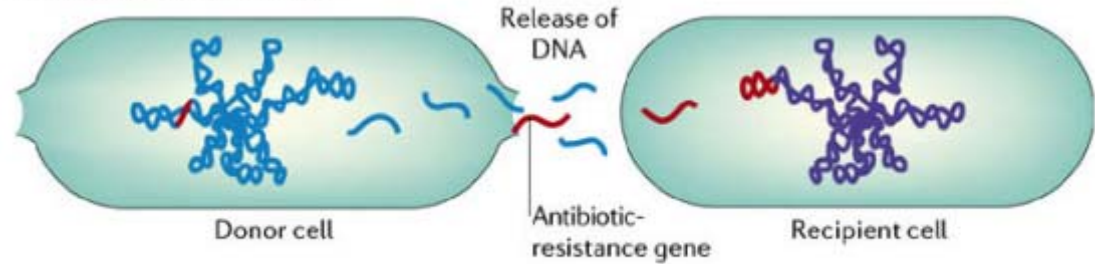


# Horizontal gene transfer

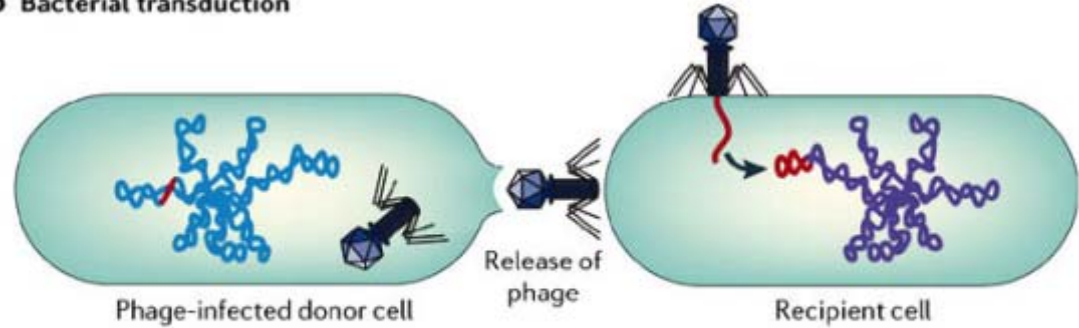


Doolittle (1999)

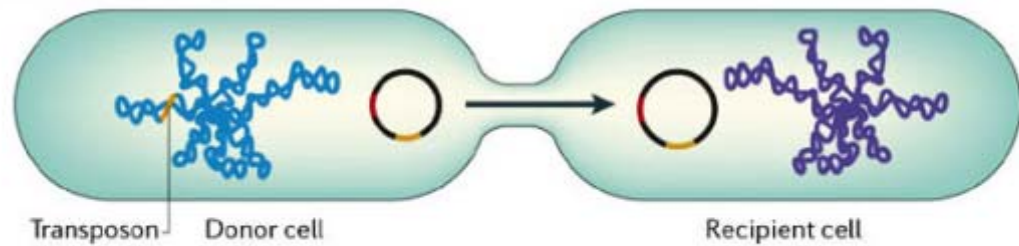
## a Bacterial transformation



## b Bacterial transduction



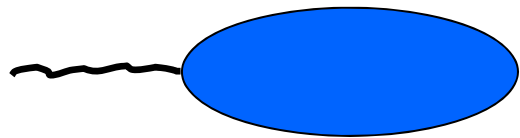
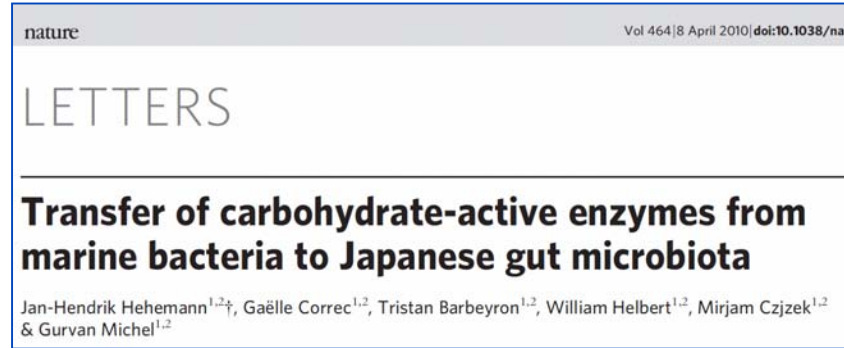
## c Bacterial conjugation







# Example: Horizontal Gene Transfer gut microbiota



*Zobellia galactivorans*



*porphyranases*

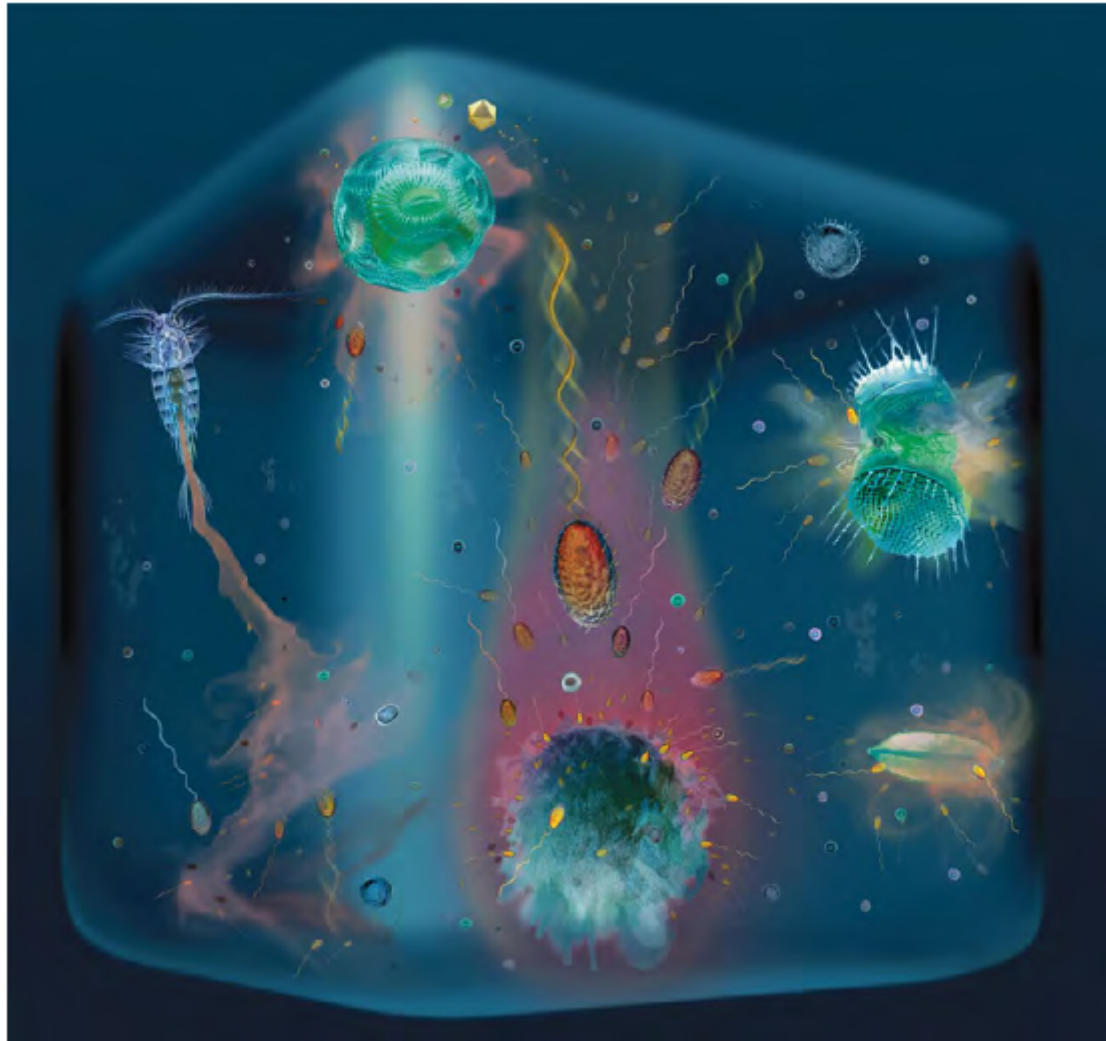


Porphyra (nori)





## Marine Microbes

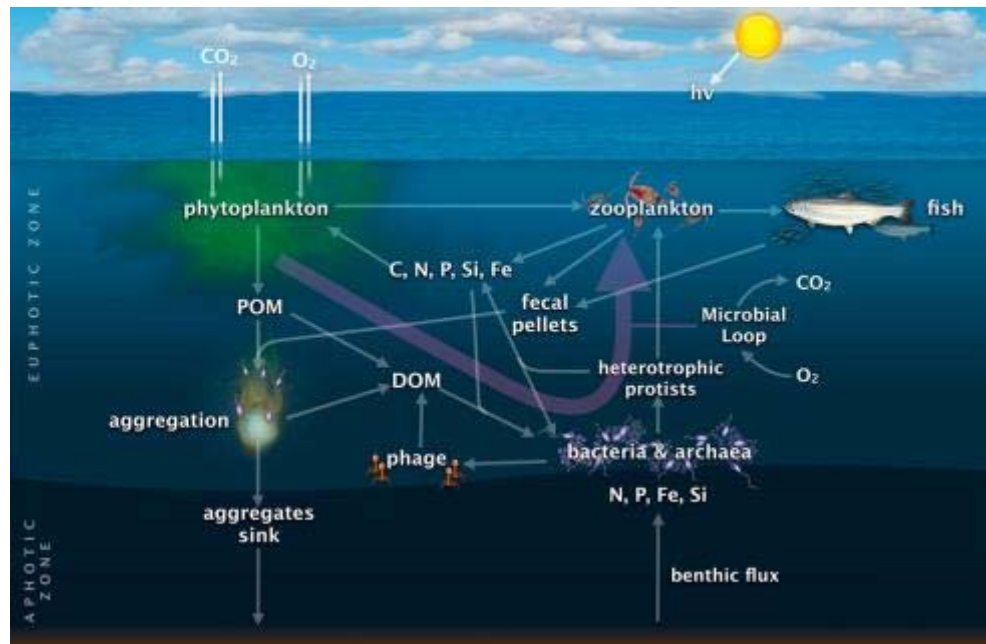


One drop of seawater contains: 10 million viruses, one million bacteria and about 1000 protozoans and microalgae!

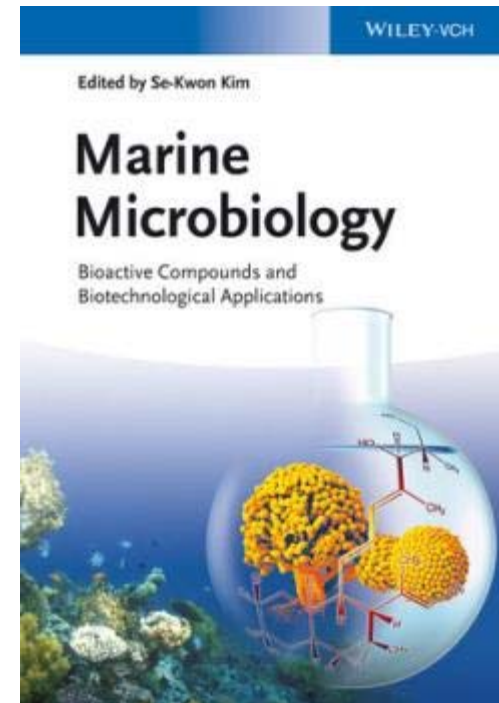


# Marine Microbiology

- Role of marine microorganisms in oceanic biogeochemical cycles
- Beneficial microbes: Marine biotechnology (antibiotics, vitamins, growth factors), bioremediation, climate change



[www.sciencemag.org](http://www.sciencemag.org)

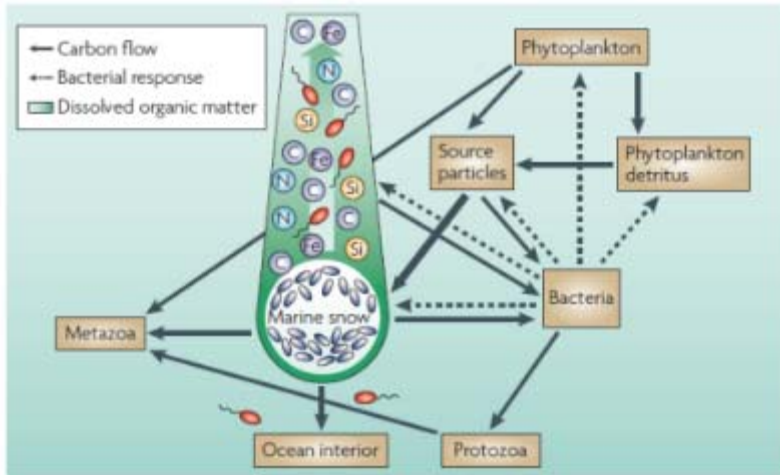






# Distribution and niche of marine microbes

## Marine snow particles

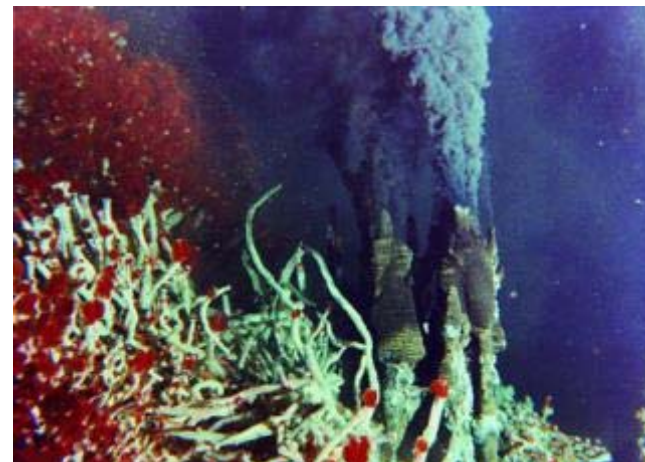


Azam & Malfatti Nat Rev Microbiol 2007

## Microbes in polar regions

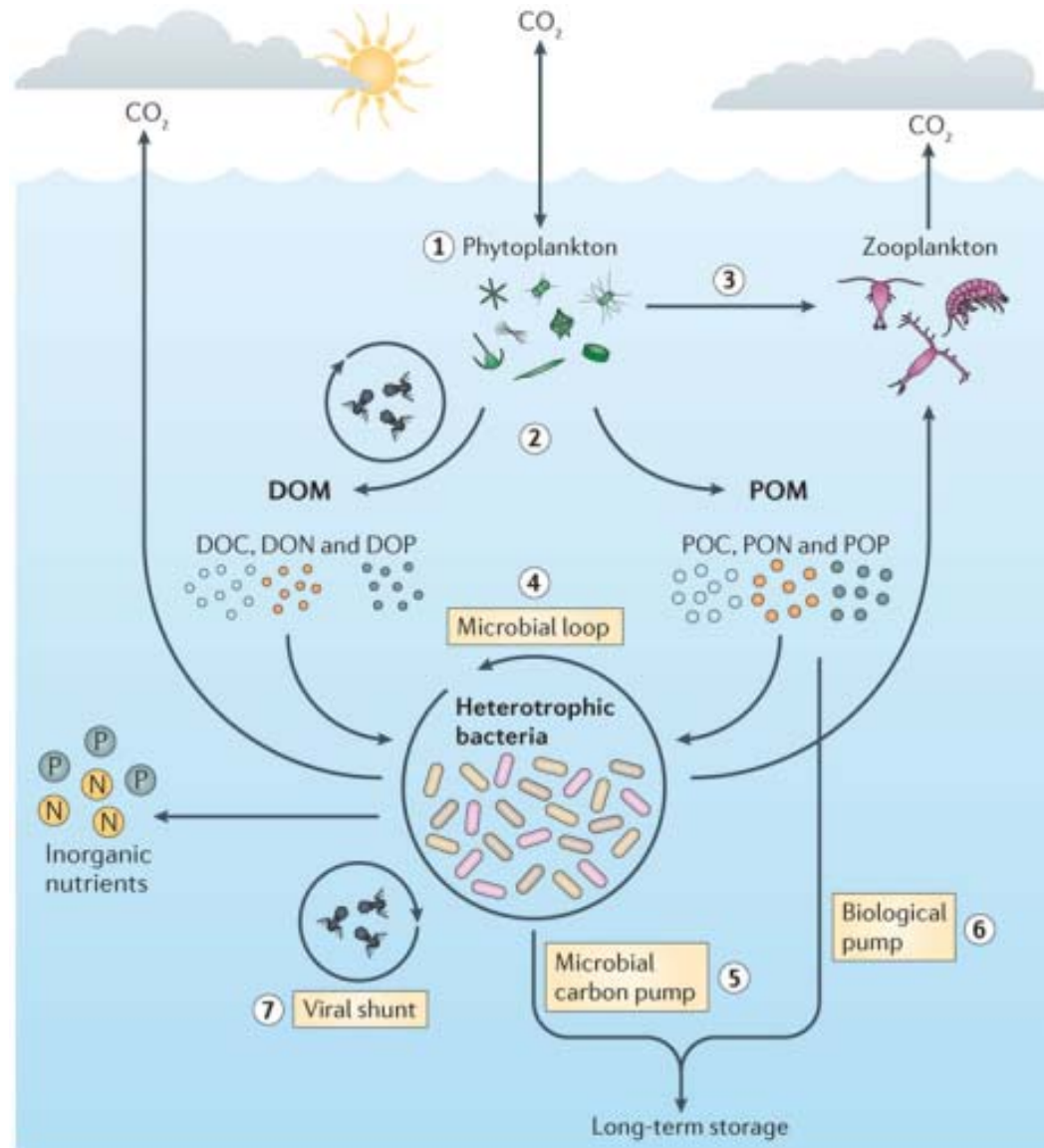


## Microbes in hydrothermal vents





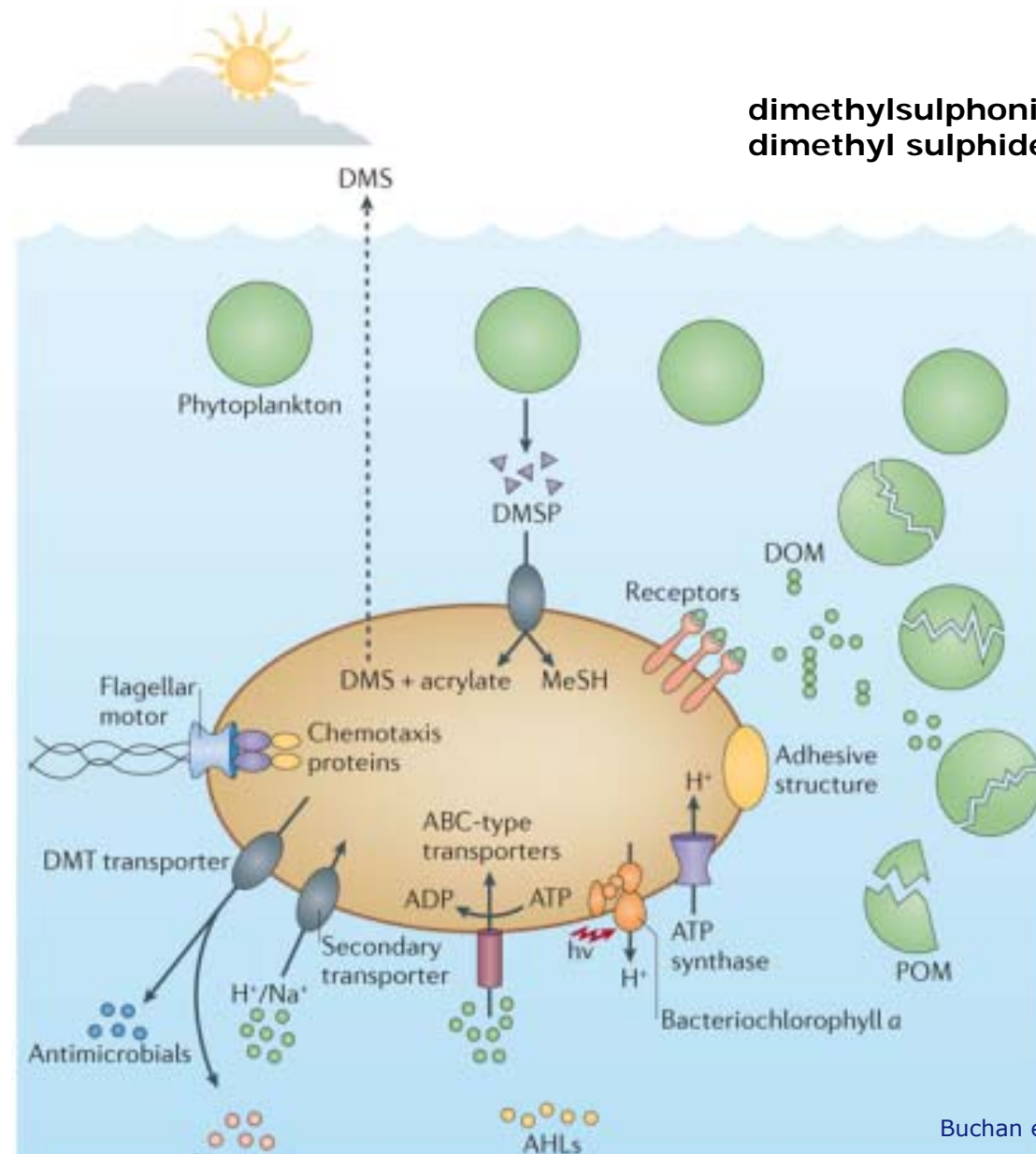
# Marine Carbon cycle



Buchan et al., Nat Rev Microbiol 2014



# Roseobacter and organic sulfur compounds

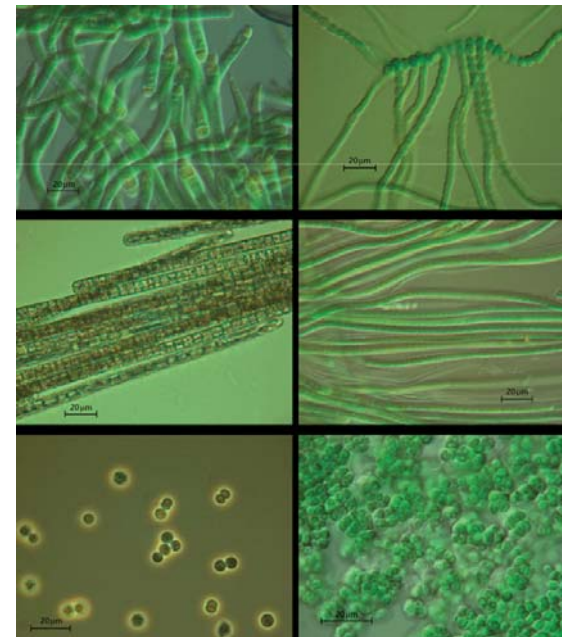
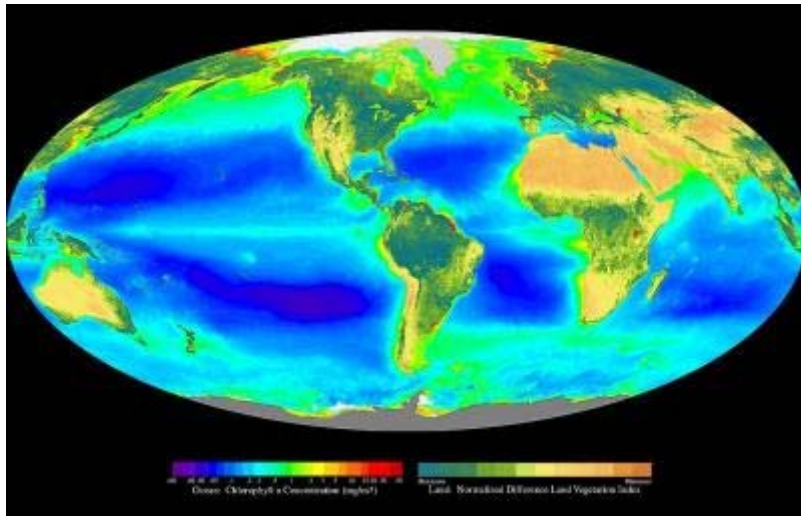


dimethylsulphoniopropionate (DMSP)  
dimethyl sulphide (DMS)

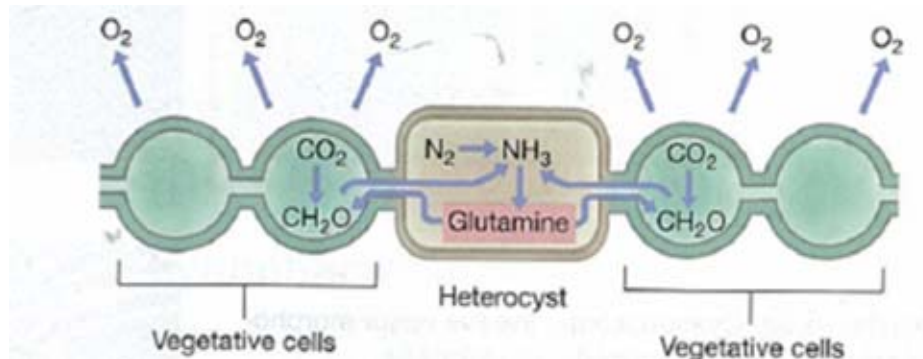


# Marine Carbon cycle

- Oxygenic phototrophs: Phytoplankton (algae and cyanobacteria)



Photosynthesis  
N<sub>2</sub>-fixation!

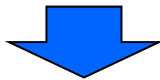




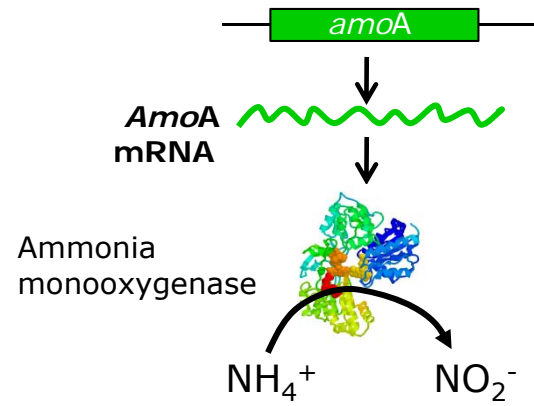
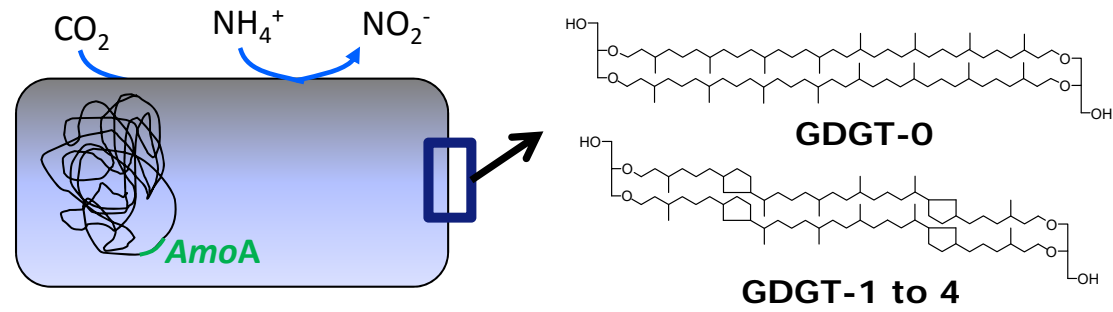
# Marine Carbon cycle

- Marine Thaumarchaeota: Involved in the C and N cycles

Marine Crenarchaeota = Thaumarchaeota



Not all Archaea are extremophiles  
20% picoplankton ocean  
Evolutionary importance

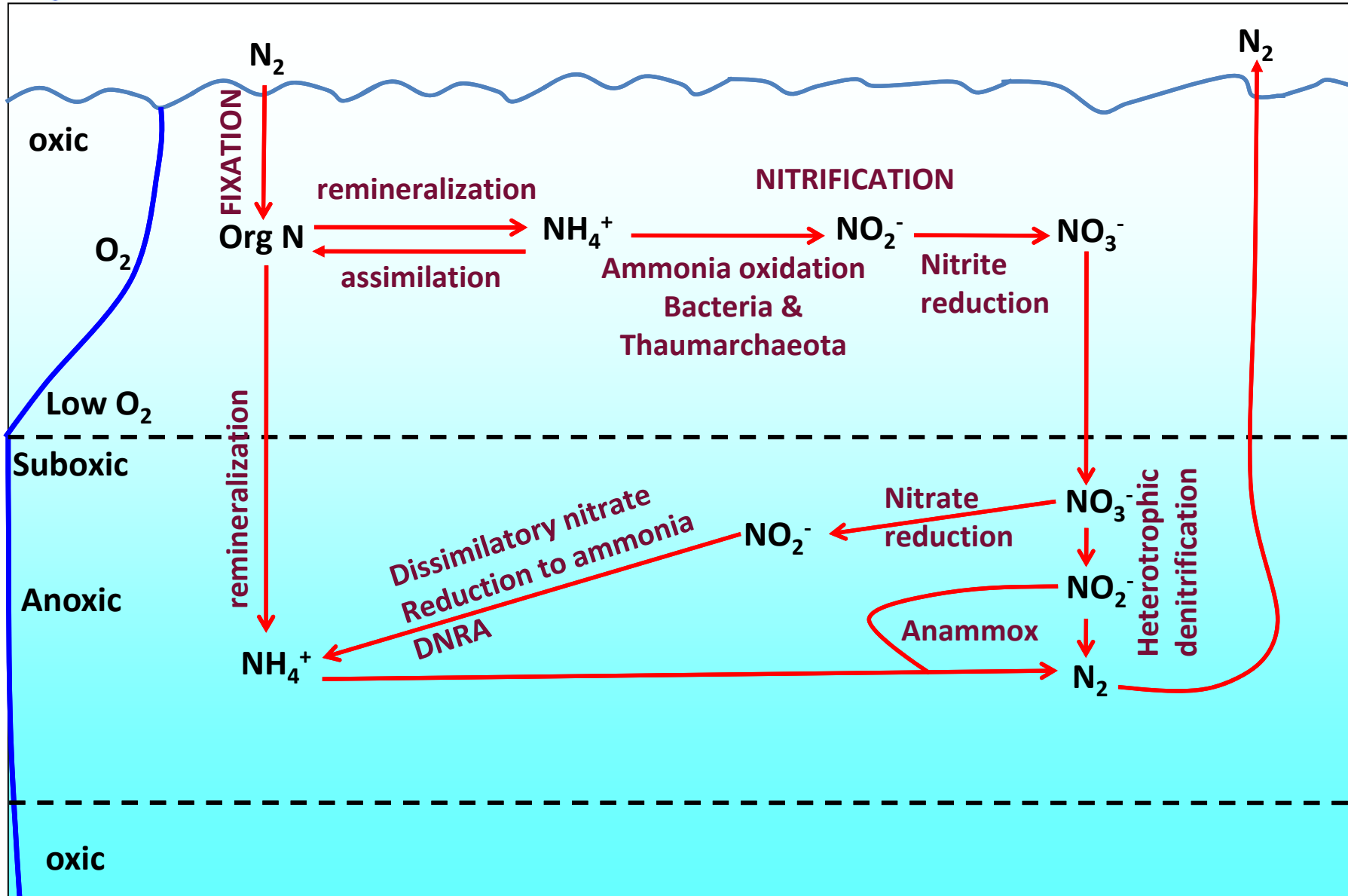


**Ammonia-oxidizing Archaea**





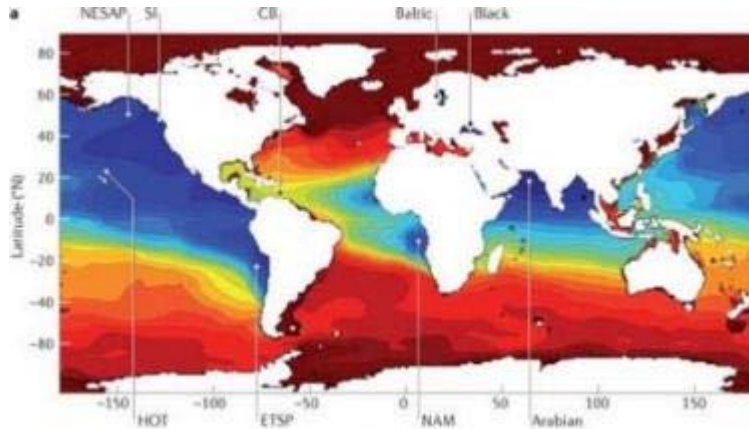
# Marine Nitrogen cycle



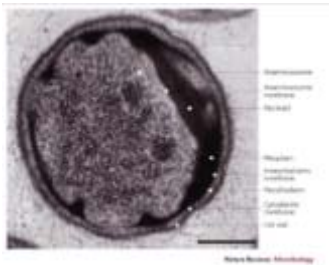




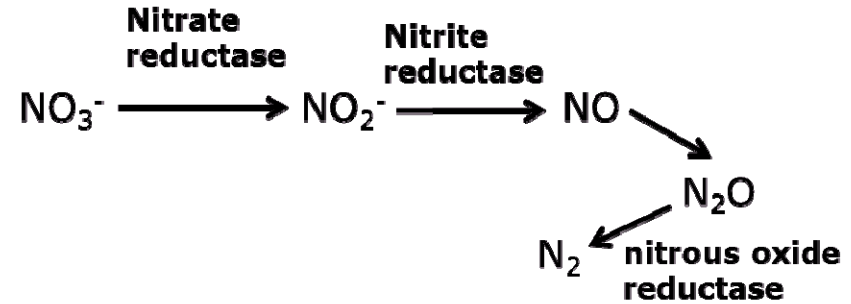
# Denitrifying bacteria in oxygen minimum zones



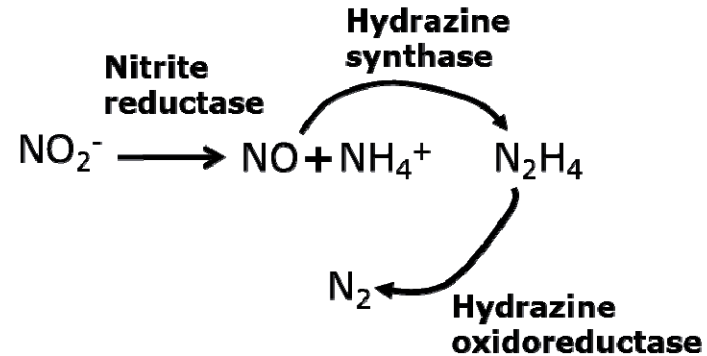
## Anammox bacteria



## Heterotrophic denitrifiers

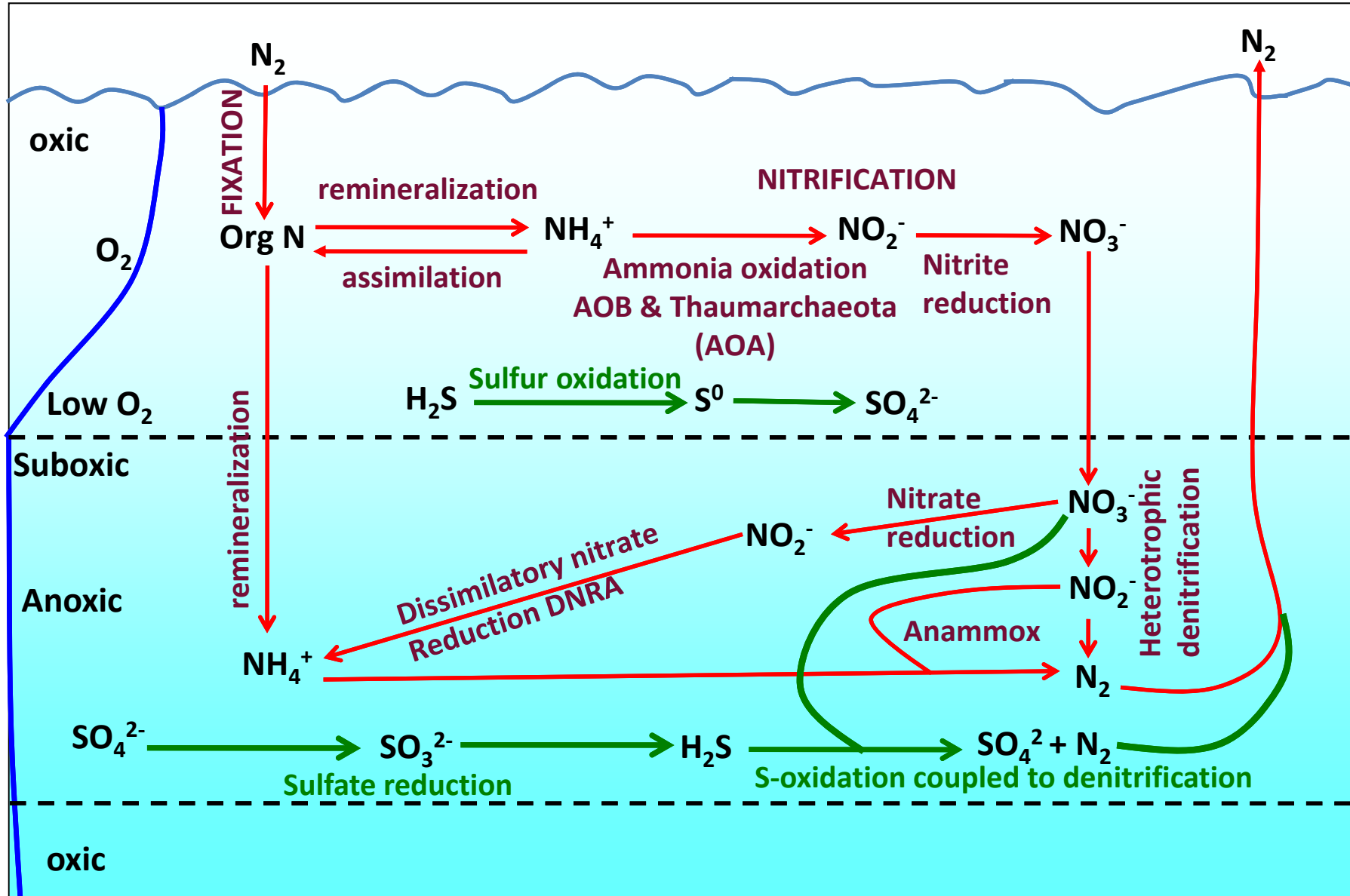


## Anammox bacteria





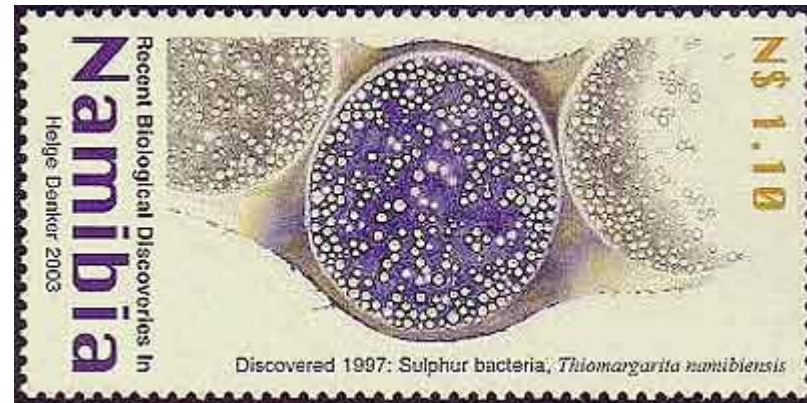
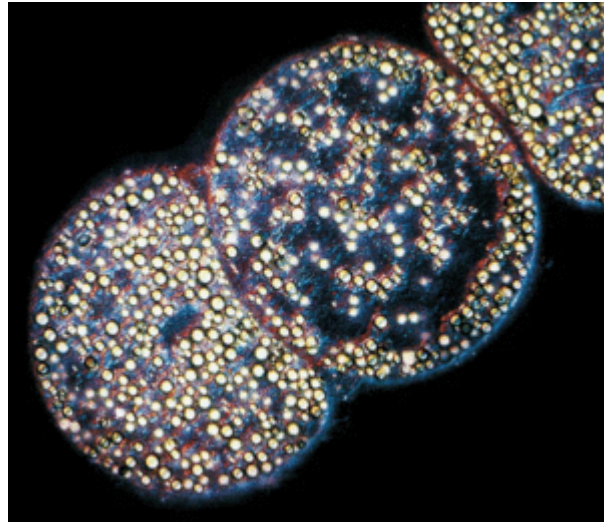
# The Marine Nitrogen & Sulfur cycles: Interactions





## Marine Sulfur cycle

### Sulfur oxidizing bacteria (anaerobic)



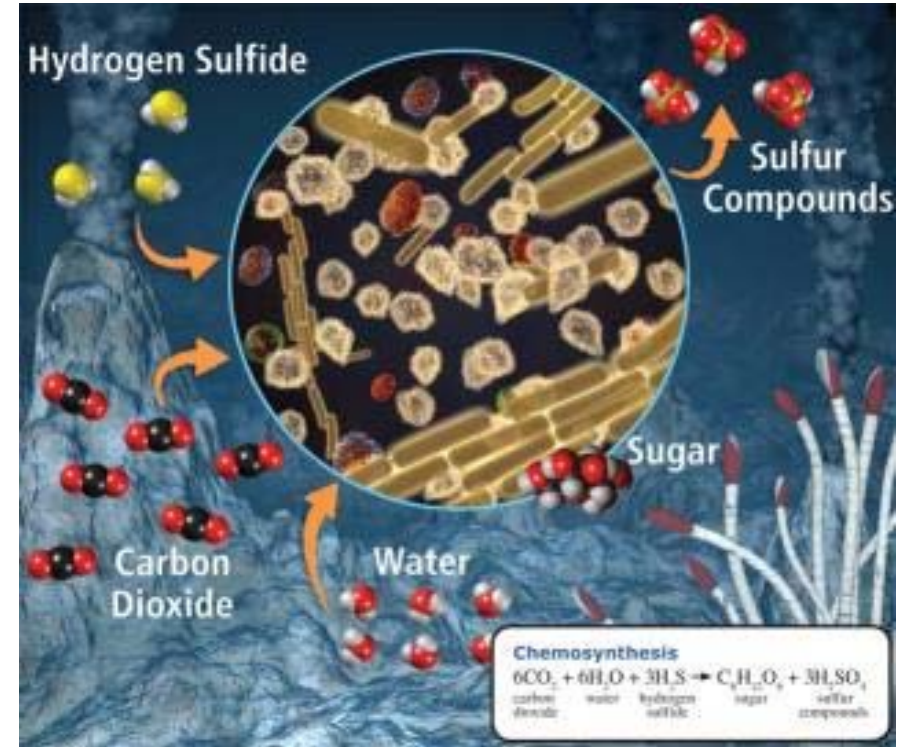
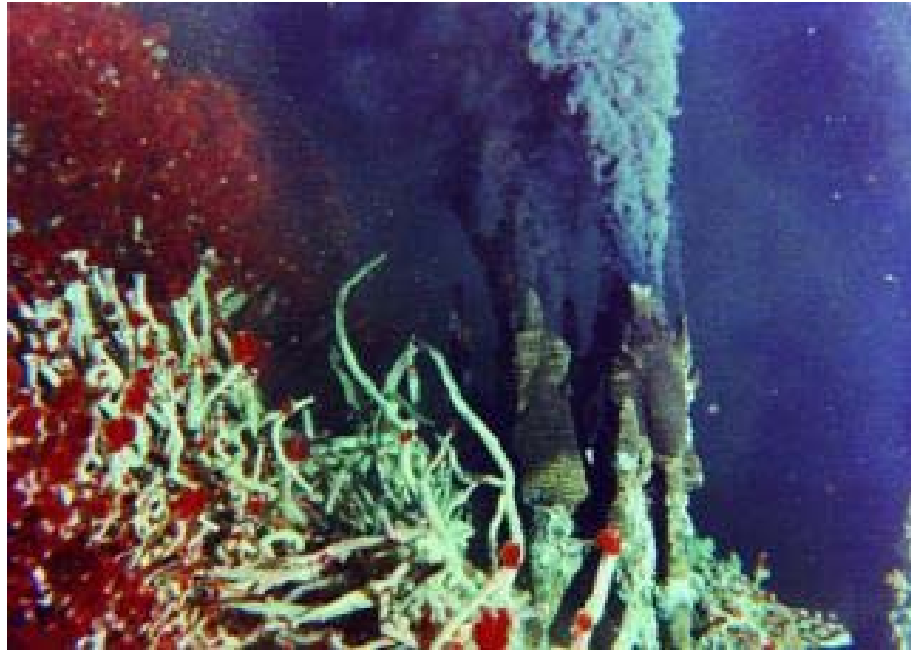
### ***Thiomargarita namibiensis.***

Image from Oceanus Online Magazine

- ocean sediments of the continental shelf of Namibia
- Reduce nitrate and oxidize sulfide.
- store both sulfur and nitrate
- 0.1-0.75 mm diameter



# Hydrothermal vents: C, N and S cycles



electron donors (e.g.,  $\text{H}_2$ ,  $\text{H}_2\text{S}$ ,  $\text{Fe}^{2+}$ , and  $\text{CH}_4$ )  
and acceptors (e.g.,  $\text{O}_2$ ,  $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ ,  $\text{SO}_4^{2-}$ , and  $\text{CO}_2$ )

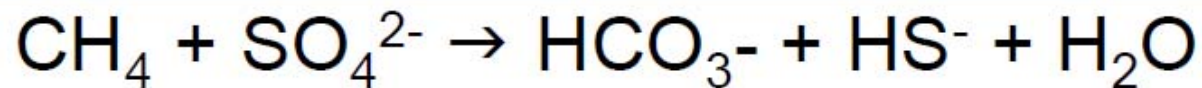
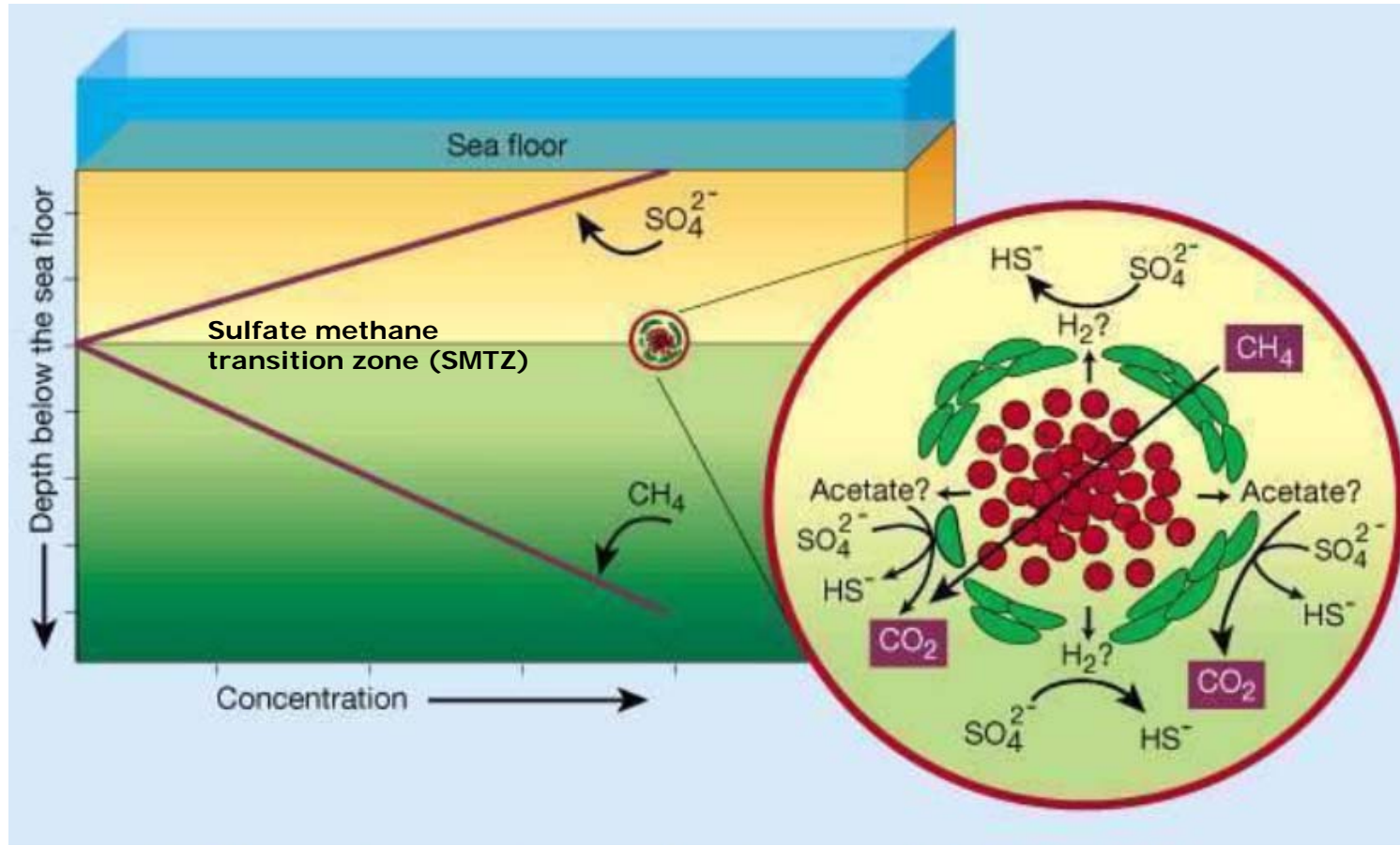




# Marine Carbon and Sulfur cycles

## Microbial anaerobic oxidation of methane (AOM)

Anaerobic methanotrophic archaea (ANMEs) & Sulfate reducing bacteria



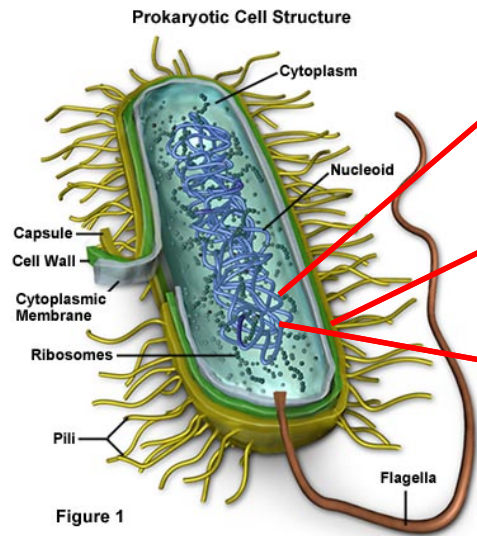
Boetius et al 2000



# How can we study marine microbes?

Biogeochemical cycles

Evolution  
Geological events  
Early life



Nucleic acids

Lipids

Proteins

DNA  
RNA

Lipids

Proteins/  
peptides

**Biomolecule**

**Biomarker**

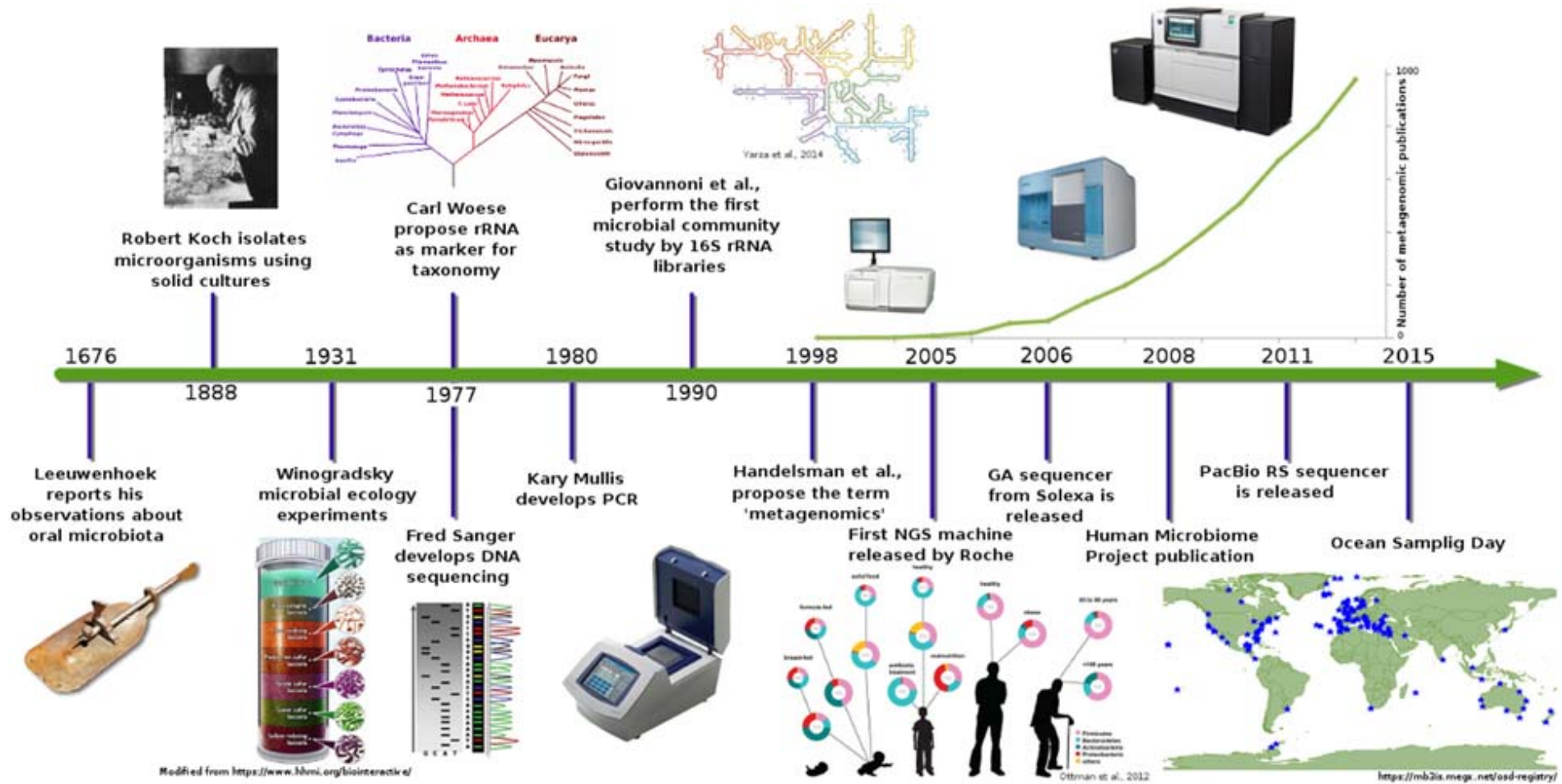
Ancient DNA

Lipid biomarker

??



# From Microbiology to molecular approaches





# Marine metagenomic expeditions

## Global Ocean Sampling expedition



<https://www.youtube.com/watch?v=uexFwQGhsYU>





# Marine metagenomic expeditions



## Tara Oceans expedition

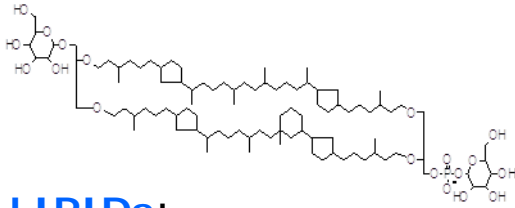


<https://www.youtube.com/watch?v=mNXj7pFvHDo>



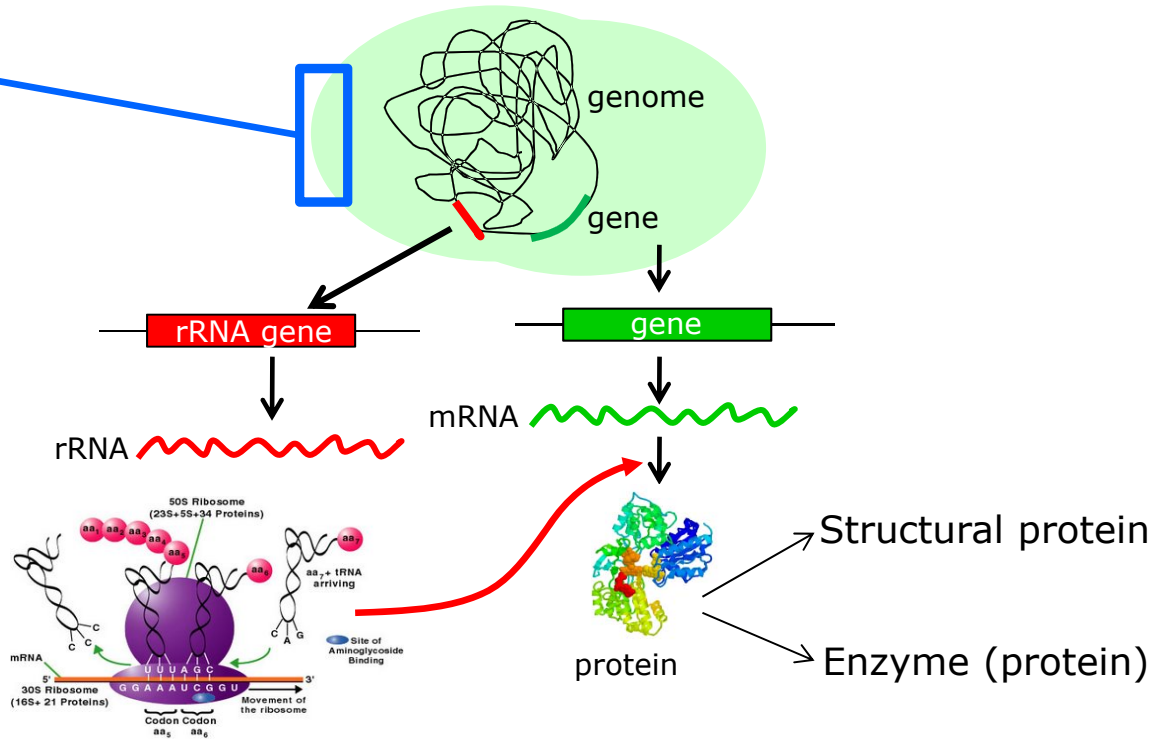
# Lipids and Nucleic acids as biomarkers

## Membrane lipids



### LIPIDS:

- Abundance
- Viability
- Marker of specific groups



### DNA:

What is the cell capable of?

Who is there? **ABUNDANCE**

### RNA:

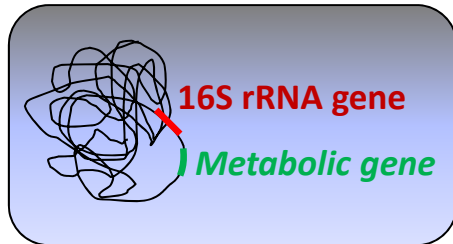
Is the cell taking advantage of its capability?

What are they doing? **ACTIVITY**



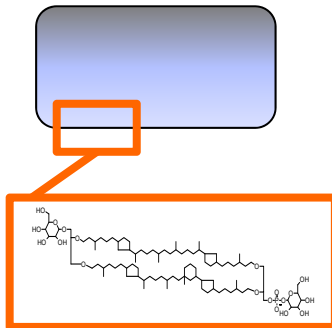
# How to estimate abundance of microorganisms?

## Quantify how many copies of a gene in a sample



1 microbe = 1 genome = 1 gene copy

## Quantify other cellular components: lipids!



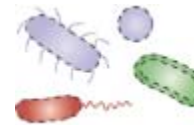
1 microbe = x amount of membrane lipids  
More lipids = more microbes?

## Cell counting

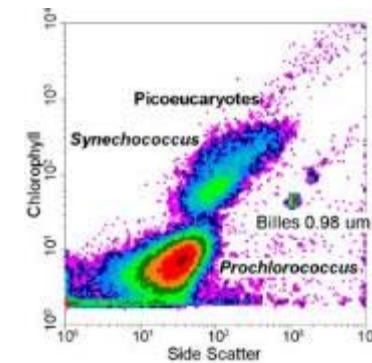
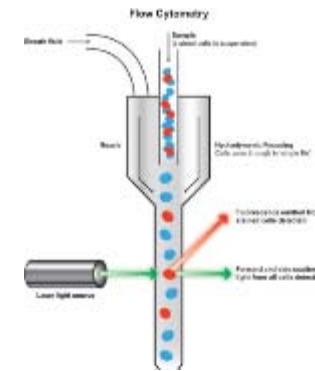
### Microscopy-based methods



Epifluorescence microscopy



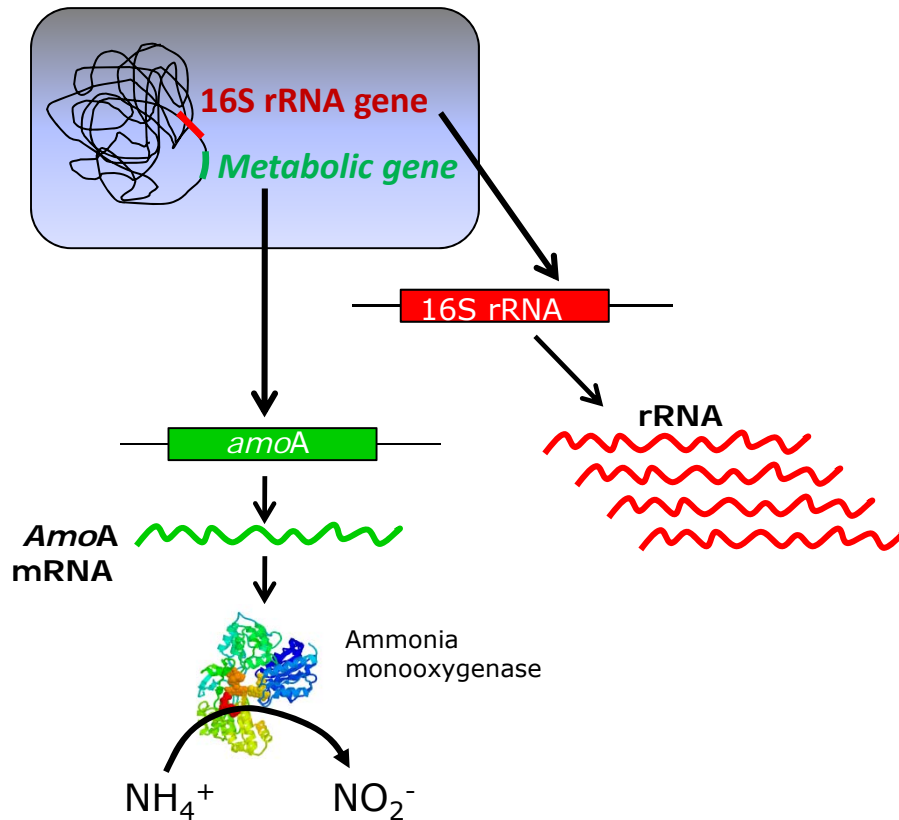
### Flow cytometry





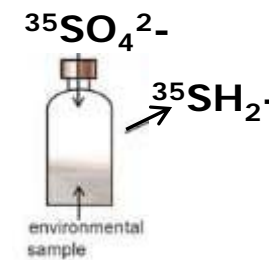
# How to estimate activity?

## Quantify abundance of RNA molecules



Microbes synthesize RNA only when they are active either to make more ribosomes (rRNA) or to eventually make proteins/enzymes for a metabolic/catabolic activity

## Activity measurements

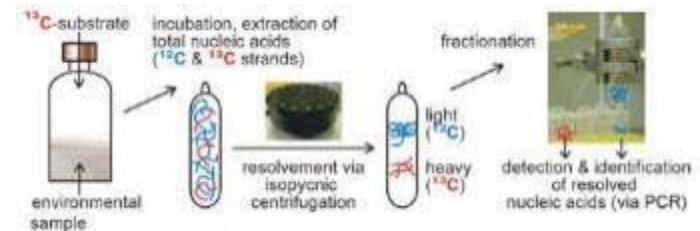


### Sulfate reduction rate

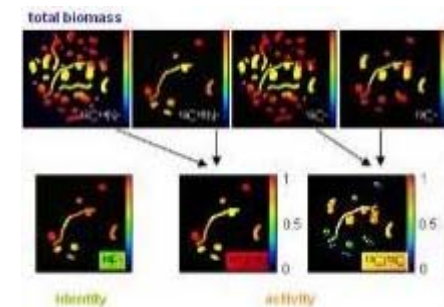
$$SRR = [SO_4^{2-}] \cdot \frac{\alpha}{t} \cdot \frac{^{35}S_{red}}{^{35}SO_4^{2-}}$$

## Incorporation of labeled substrates

### Stable Isotope probing (DNA-SIP, PLFA-SIP)



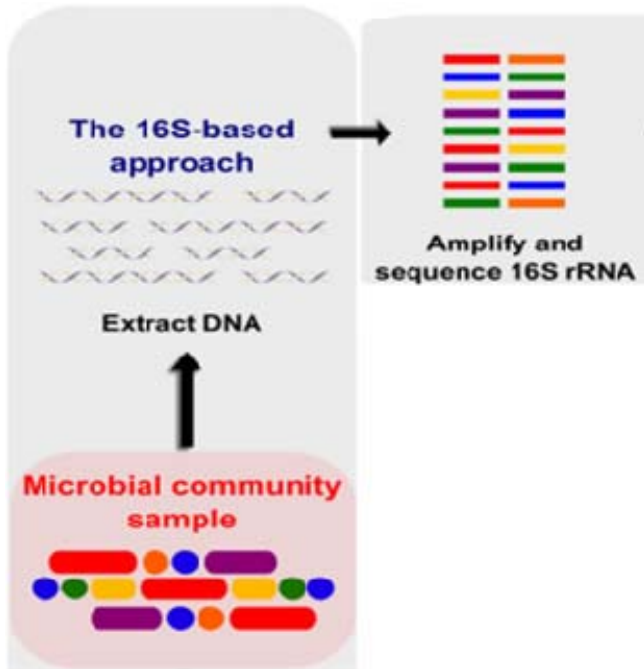
**NanoSIMS**  
Nanometer-scale secondary ion mass spectrometry



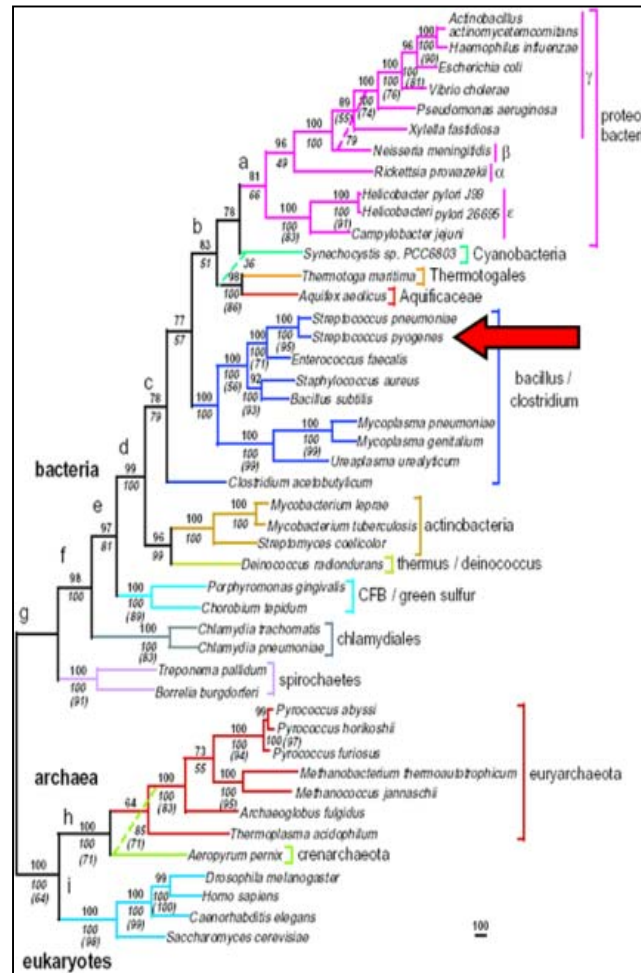




# How to estimate microbial diversity?

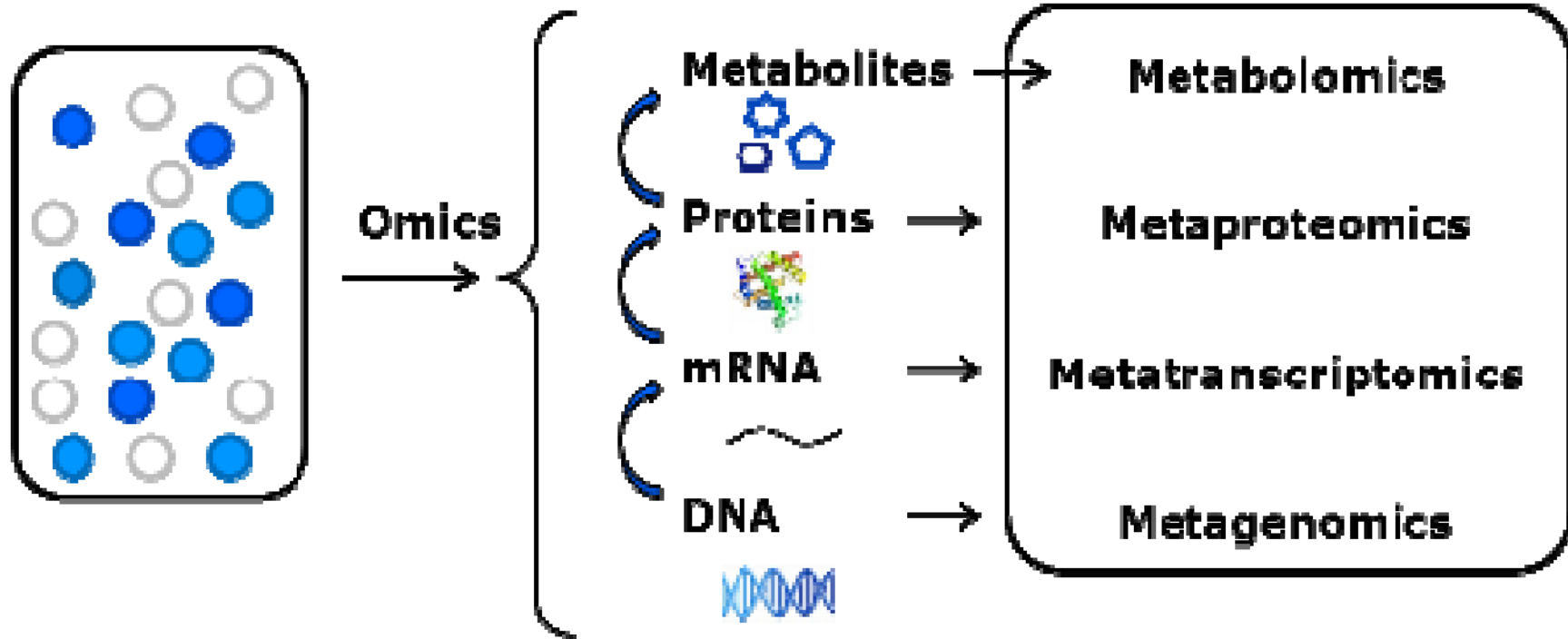


## Phylogeny



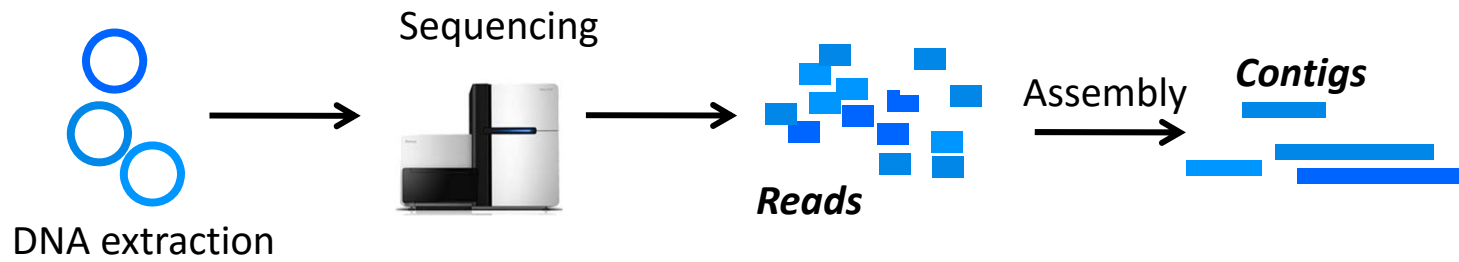


# Omics techniques: Analysis of the entire community





# Metagenomics



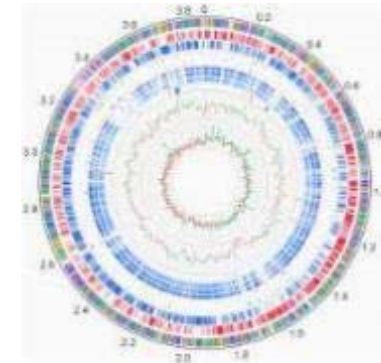
Complex sample

"Binning"



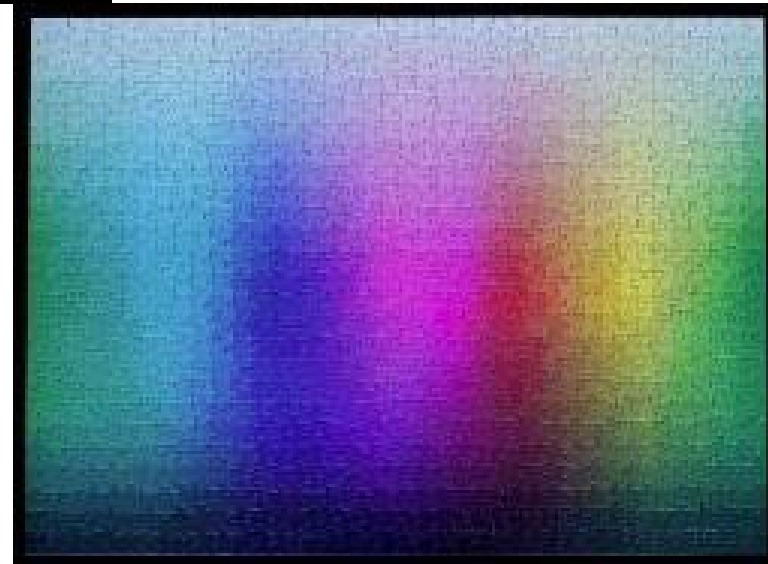
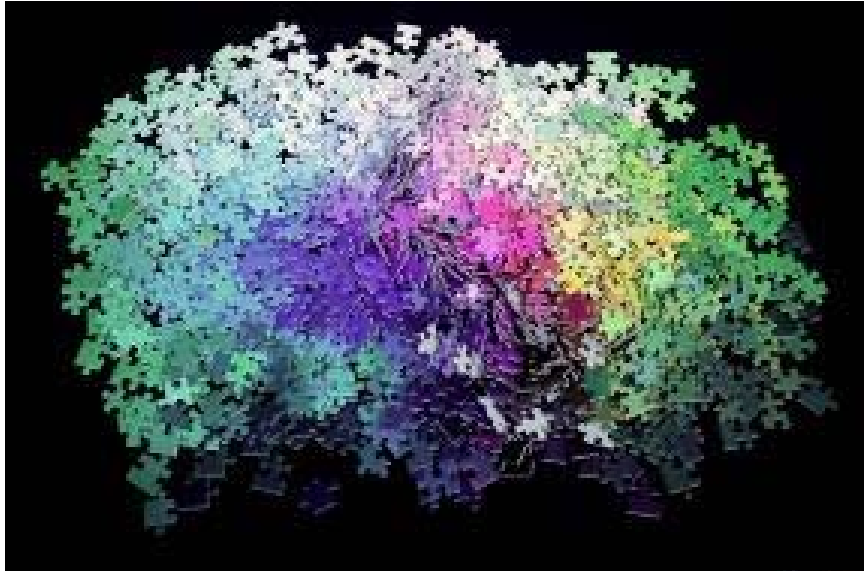
Who are they? Phylogenetic genes  
What are they doing? Metabolic genes

Genomes (bins)





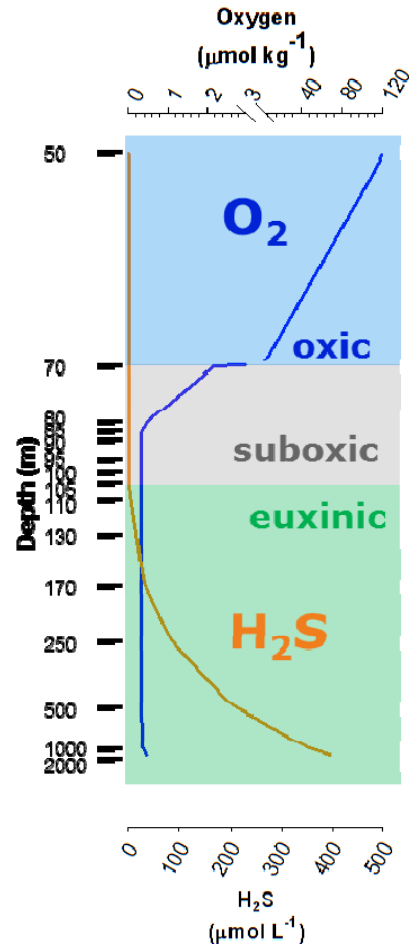
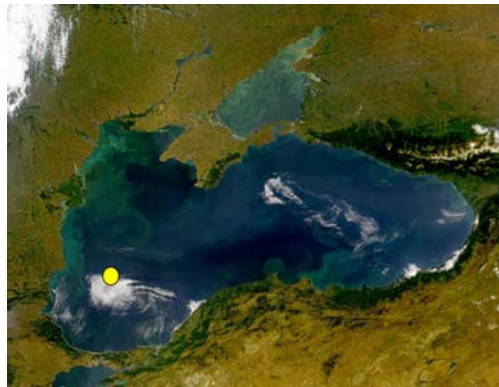
## Binning metagenomes: the ultimate puzzle!







# Study site: Black Sea

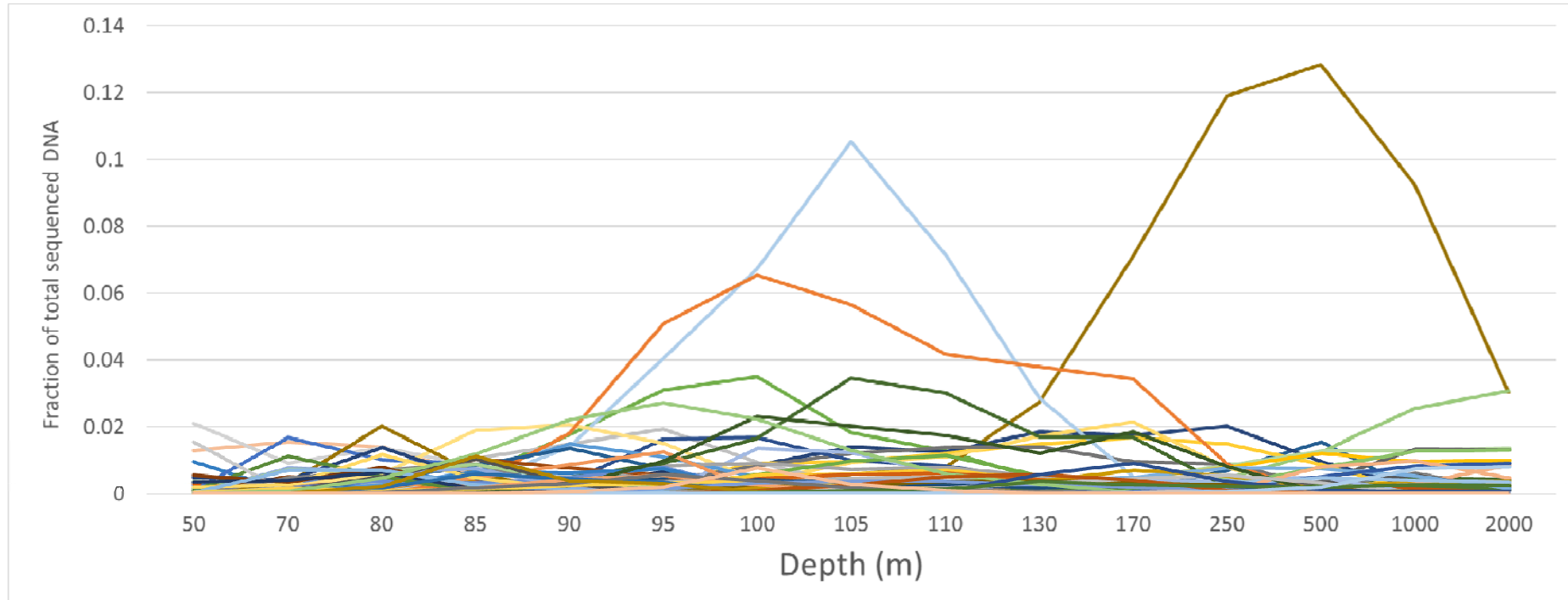


## Sampling





# Metagenomes in the Black Sea water column

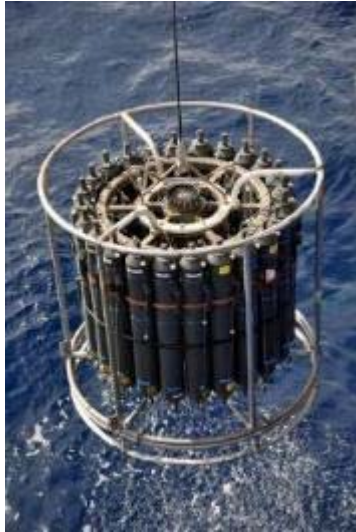


95 bacterial bins and 9 archaeal bins  
with >40% completeness and <20% contamination!!

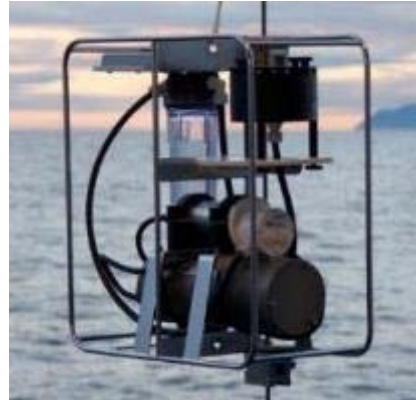


# How do we collect samples in a research vessel?

## Water samples



rosette



In situ pump

## Sediment samples



Multicore



Piston core



<https://www.youtube.com/watch?v=mAn3GDNu9yY>

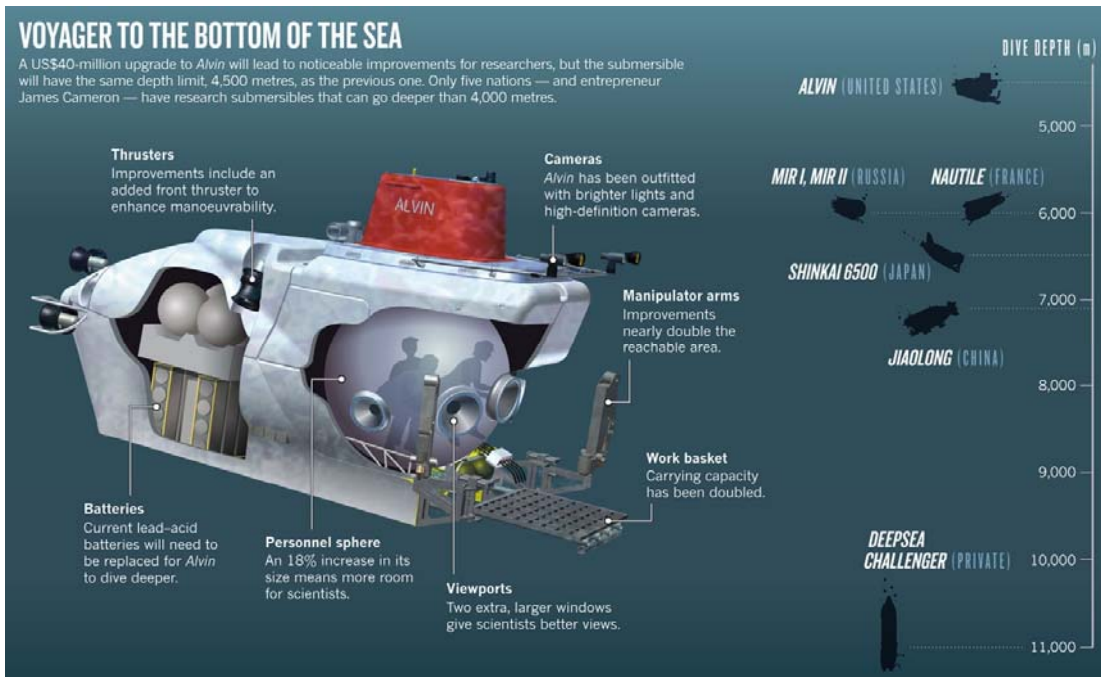


# How do we collect samples in a research vessel?

## ROV (Remote Operated Vehicles)



Landers



Sediment trap





Busy at work....



ROYAL  
NIOZ  
IN 60  
SECONDS

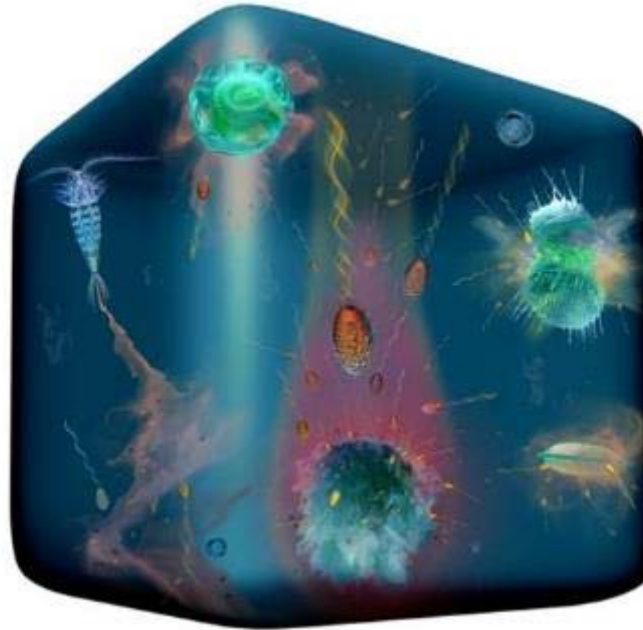


## Marine Microbes: Wonder in a drop of water

Involvement in biogeochemical cycles (nutrient recycling)

Involvement in climate change (greenhouse gases and DMS)

Capable of evolve and acquire novel skills (HGT)



Responsible for oxygenic atmosphere

Capable of induce global extinctions!

**The oceans are an exciting source of novel microbes to be discovered!**



Thank you!