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**JAMSTEC** 国立研究開発法人  
海洋研究開発機構  
JAPAN AGENCY FOR MARINE-EARTH SCIENCE AND TECHNOLOGY

# Exploring the Limits of the Deep Subseafloor Biosphere

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# The first look of the subseafloor biosphere

## Deep bacterial biosphere in Pacific Ocean sediments

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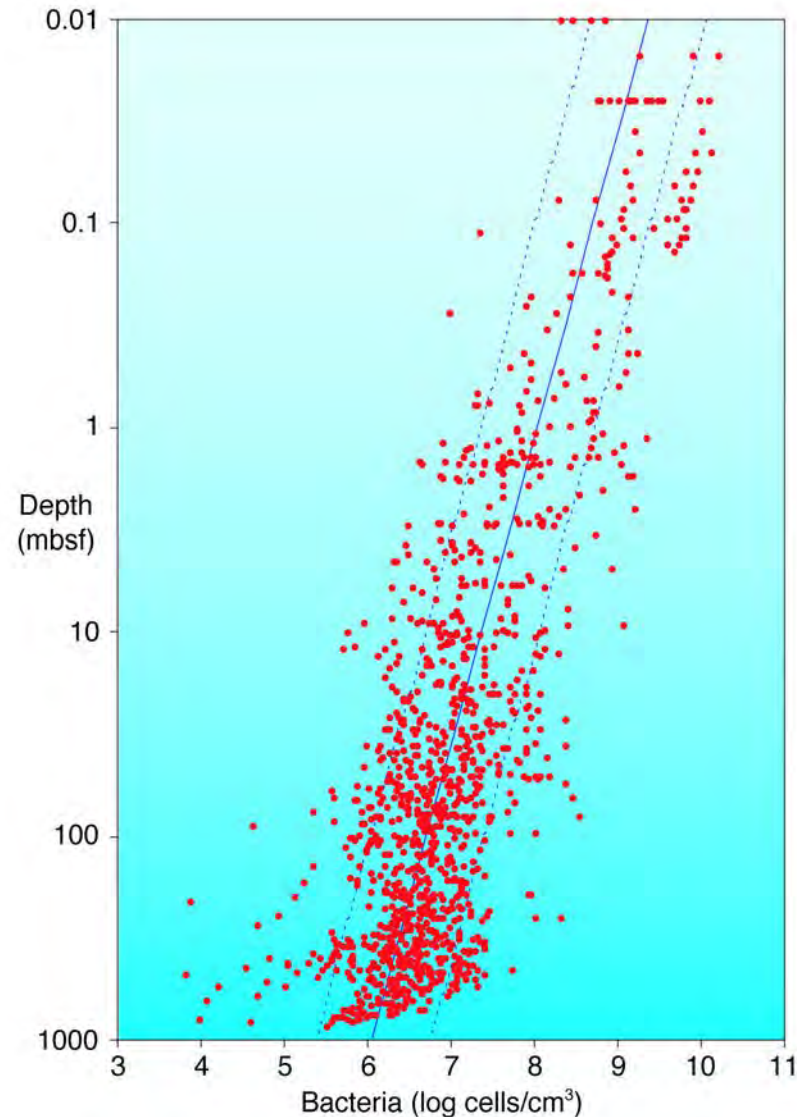
Argyll PA34 4AD, UK



John Parkes



Barry Cragg



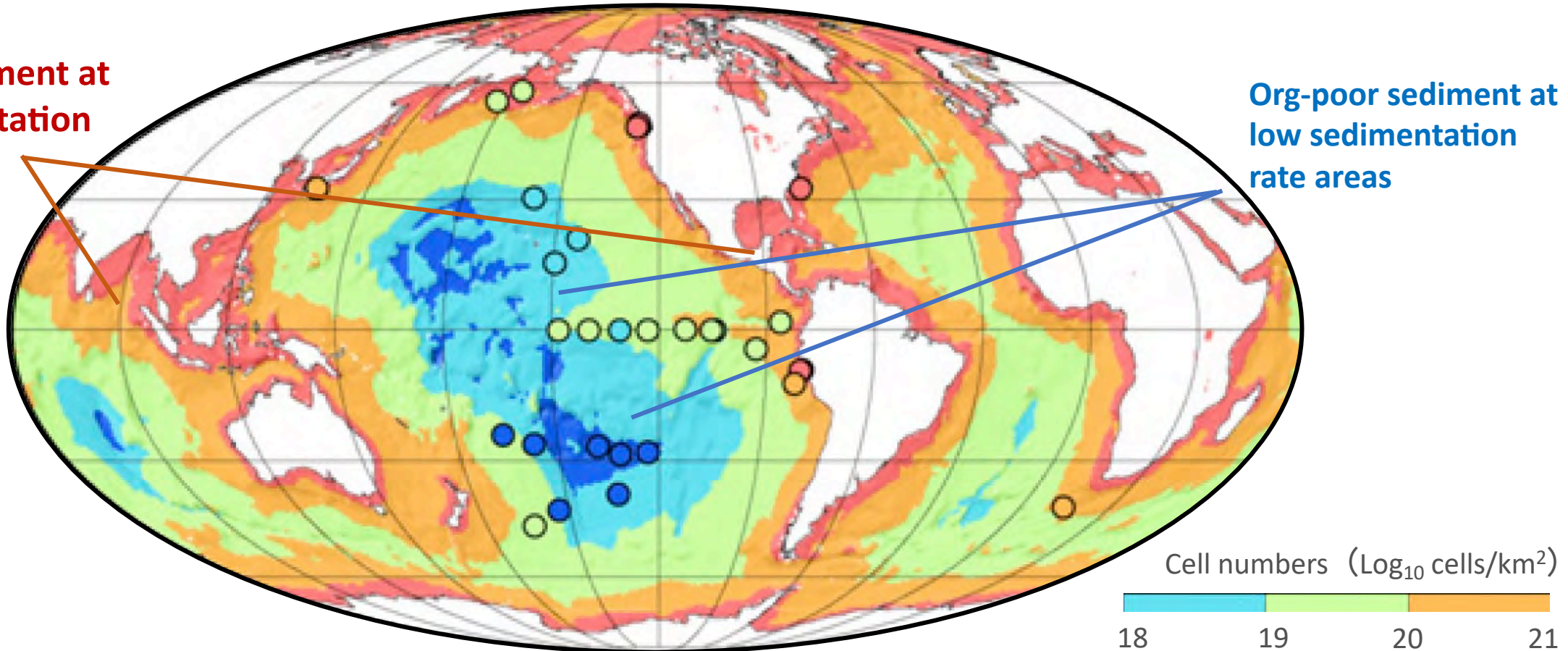
*“These findings significantly extend the depth of the marine biosphere and indicate that sedimentary organic matter, including molecular fossils, will undergo continued bacterial modification long after burial.”*



## Distribution of subseafloor sedimentary biomass

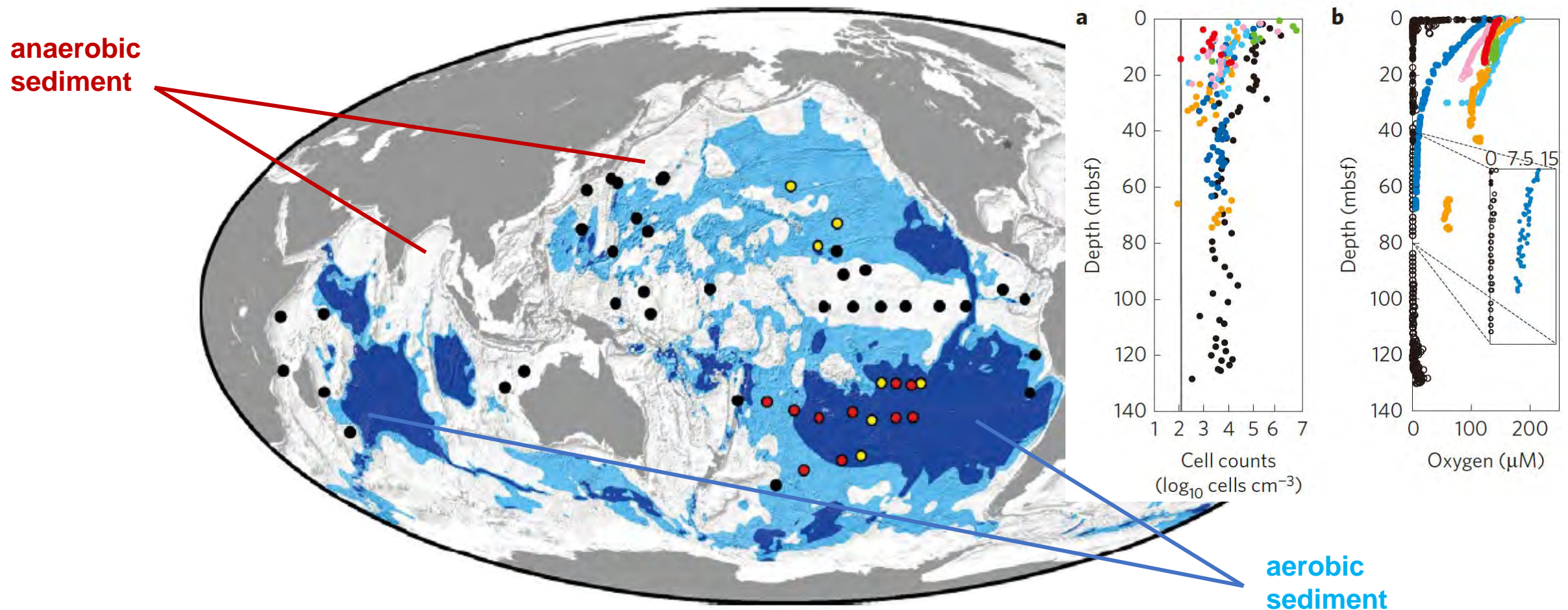
Org-rich sediment at  
high sedimentation  
rate areas

Org-poor sediment at  
low sedimentation  
rate areas



*A total microbial cell abundance in marine sediment is  $2.9 \times 10^{29}$  cells,  
corresponding to 4Gt of biomass carbon on Earth.*

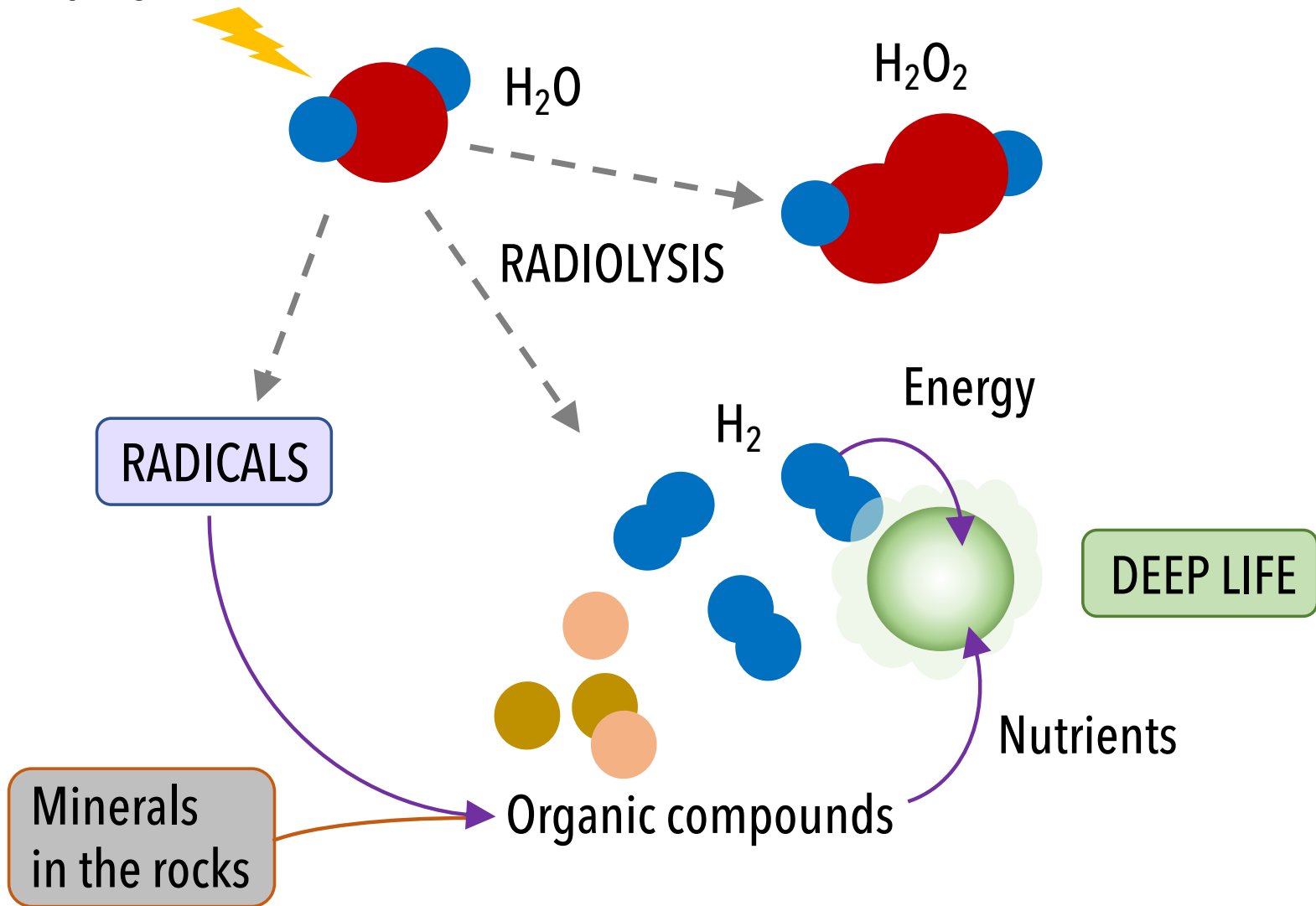
# Global distribution of aerobic and anaerobic sedimentary habitats



*"Oxygen and aerobic communities may occur throughout the entire sediment sequence in 15-44% of the Pacific and 9-37% of the global sea floor."*

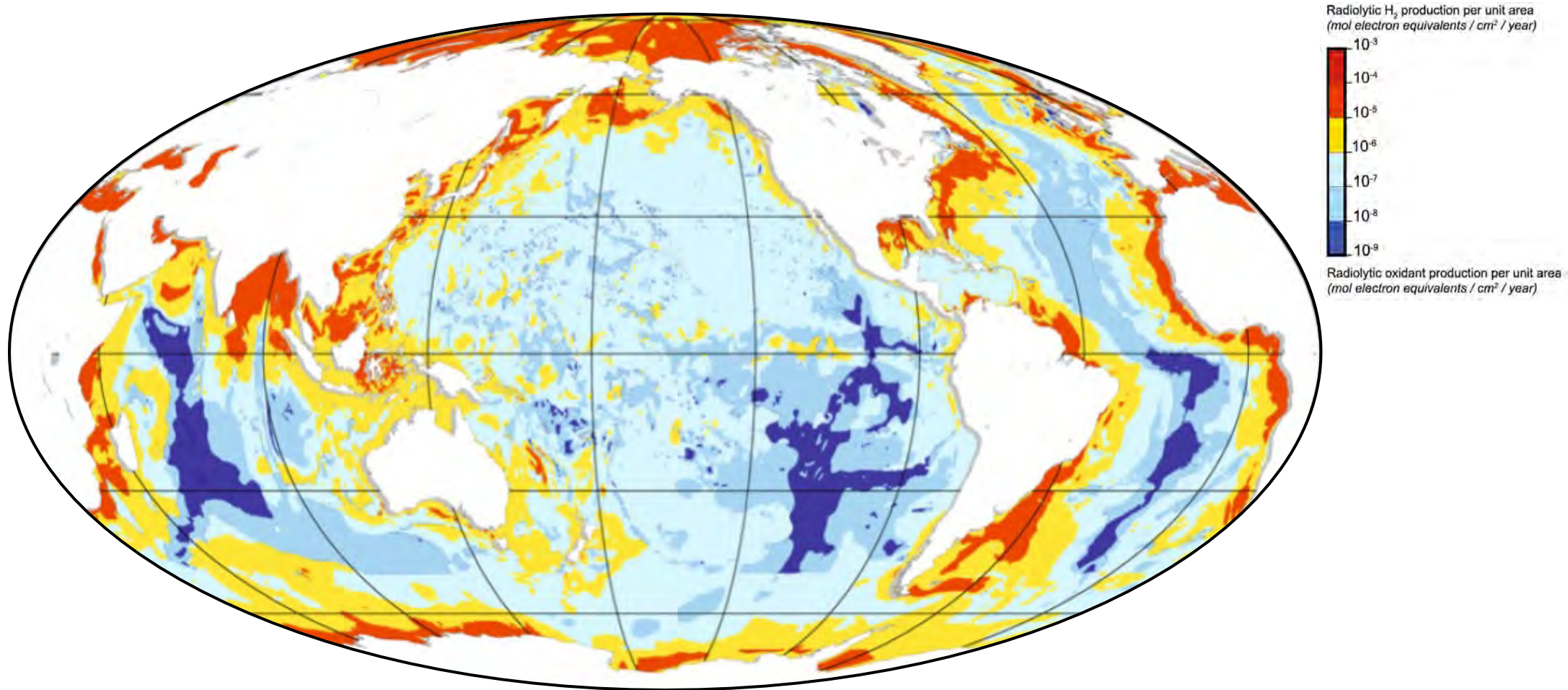
# The Deep Biosphere

Particles emitted from  
decaying elements (U, Th, K)





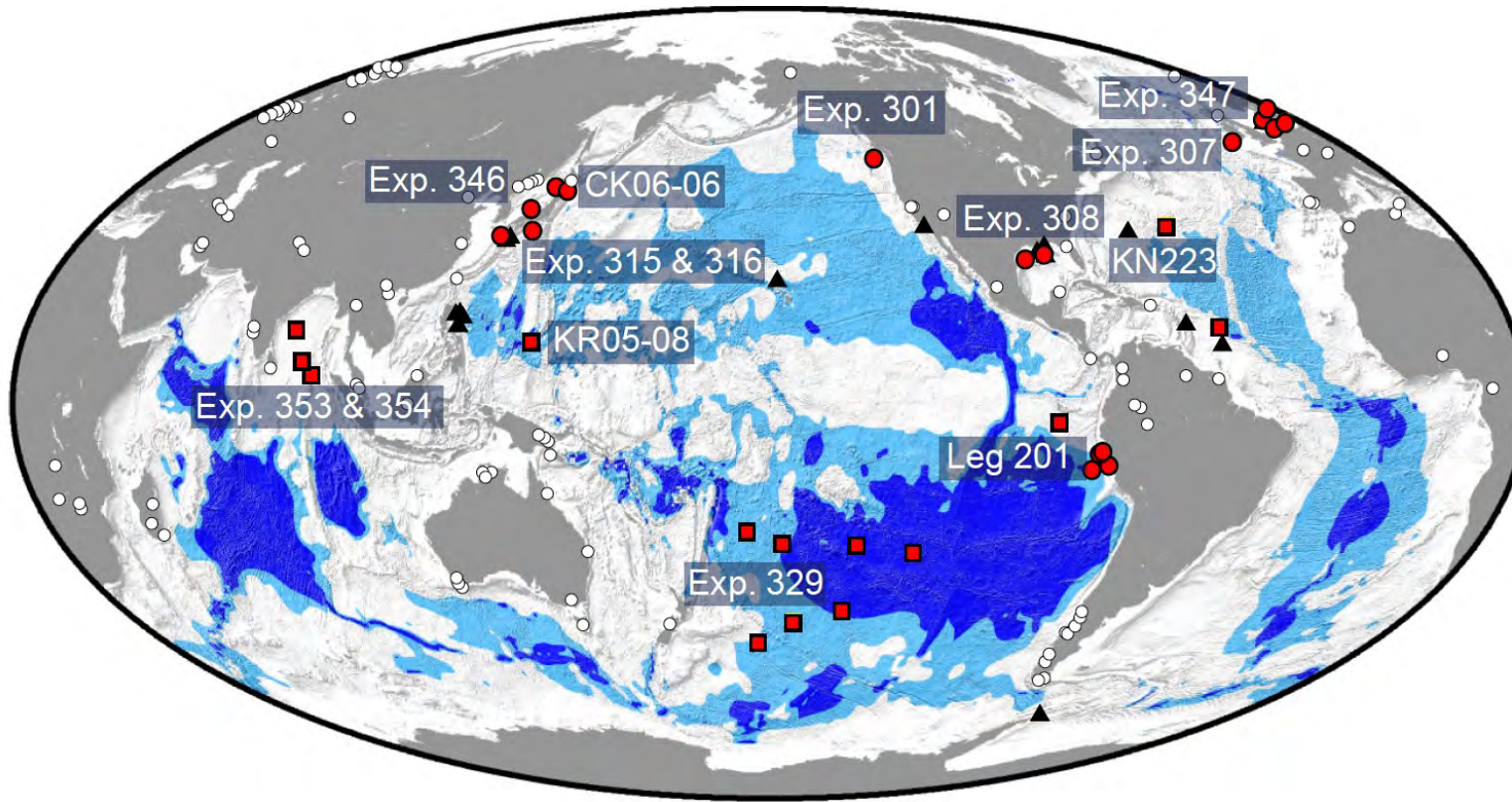
# Global distribution of radiolytic H<sub>2</sub> production rates in marine sediment



*“...water radiolysis is the principal source of biologically accessible energy for microbial communities in marine sediment older than a few million years.”*



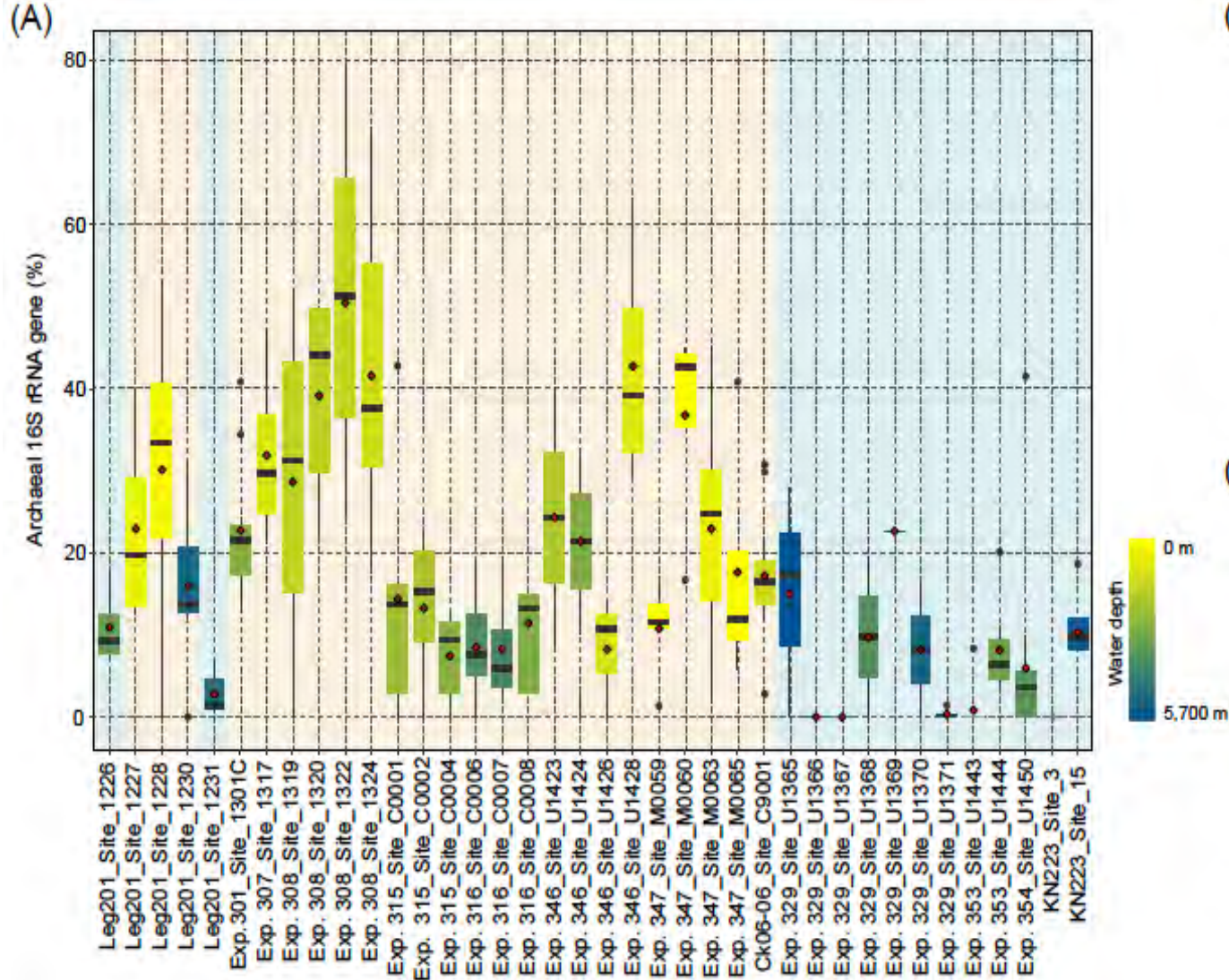
# Global survey of sedimentary microbiomes beneath the Ocean



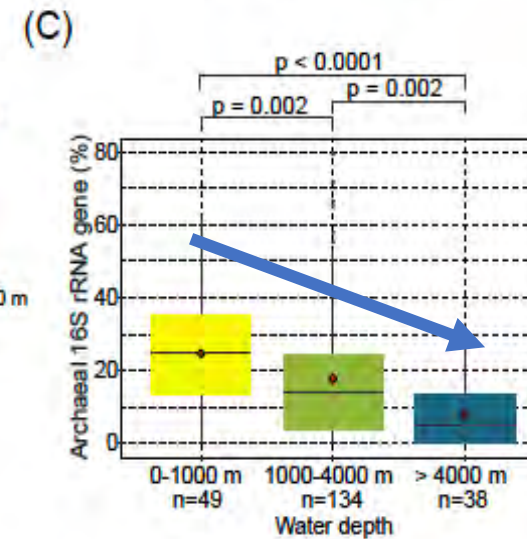
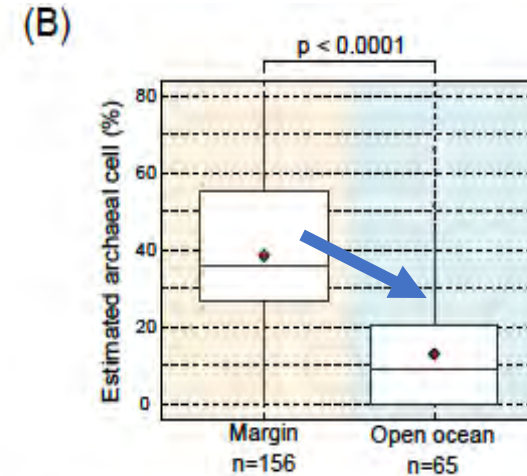
~300 deep-frozen sediment core samples collected from 40 different sites were analyzed by using the same sub-sampling and analytical approach



# Distribution and abundance of Archaea in the marine sedimentary biosphere



Boxplot of archaeal proportion in microbial 16S rRNA gene (%) at each drilling site determined by microfluidic digital PCR.

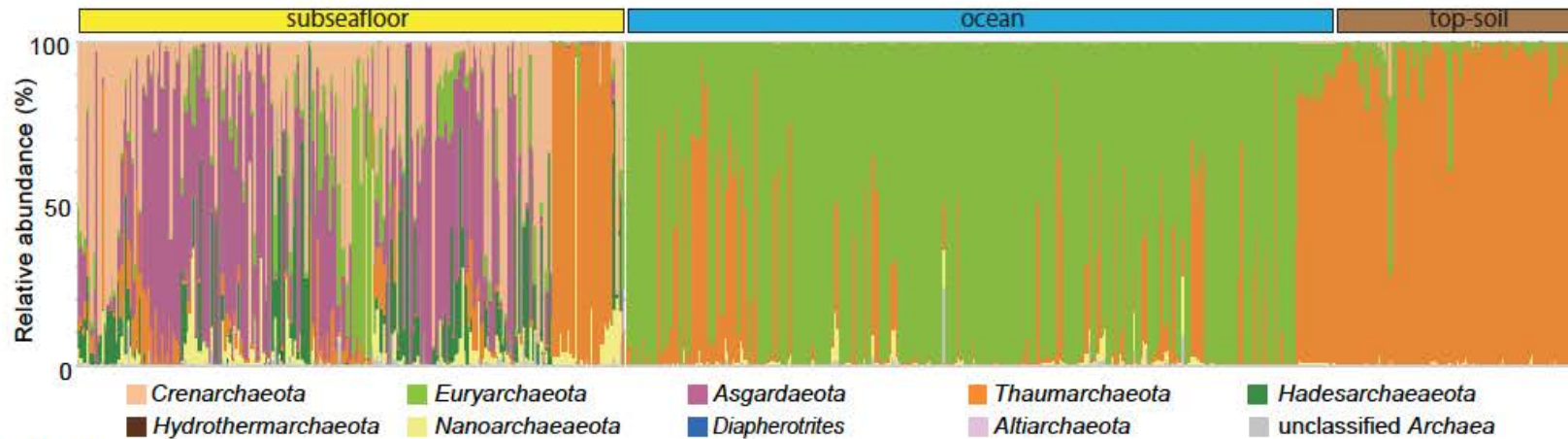


*“We estimated that archaeal cells constitute 37.3% of the total microbial cells, corresponding to  $1.1 \times 10^{29}$  cells in the global subseafloor sedimentary biosphere.”*

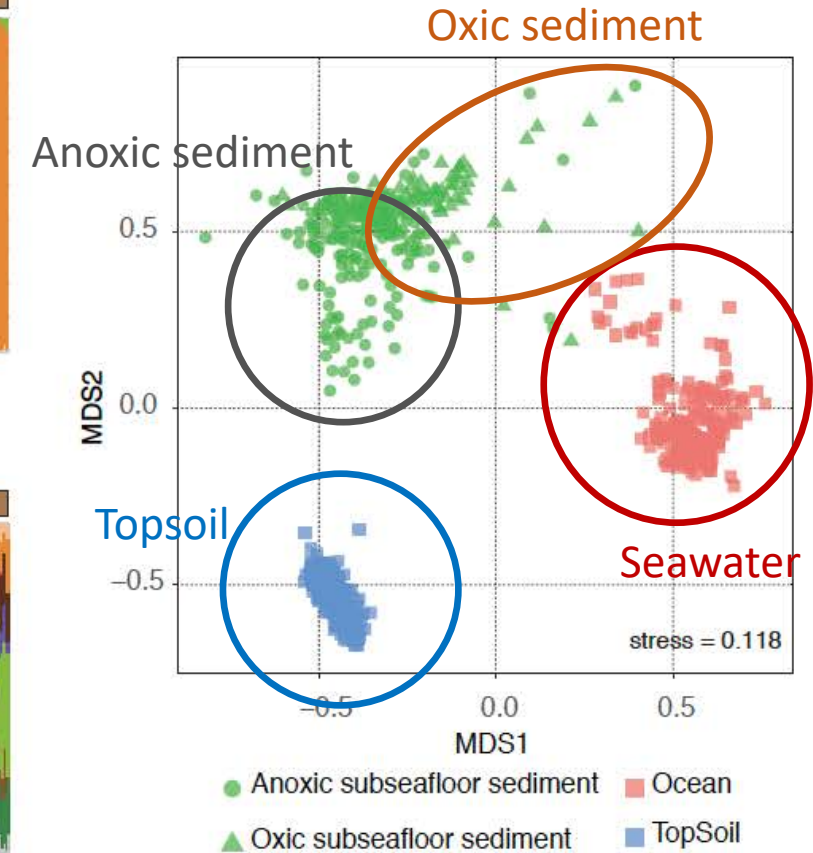
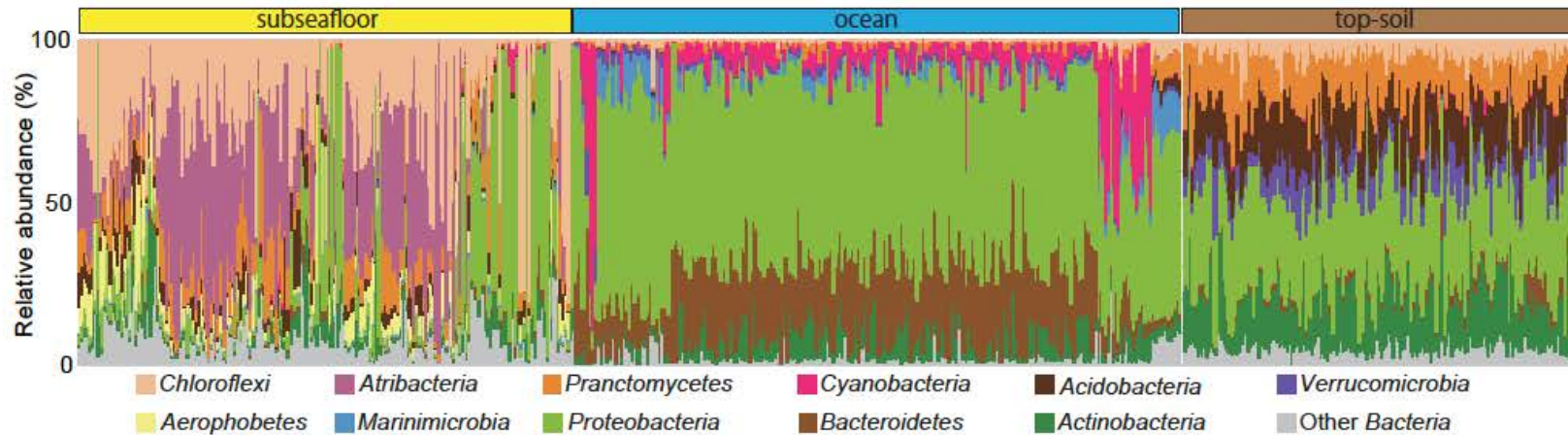


# Comparison of the microbial community compositions of marine sediment, seawater, and topsoil samples

## Archaea

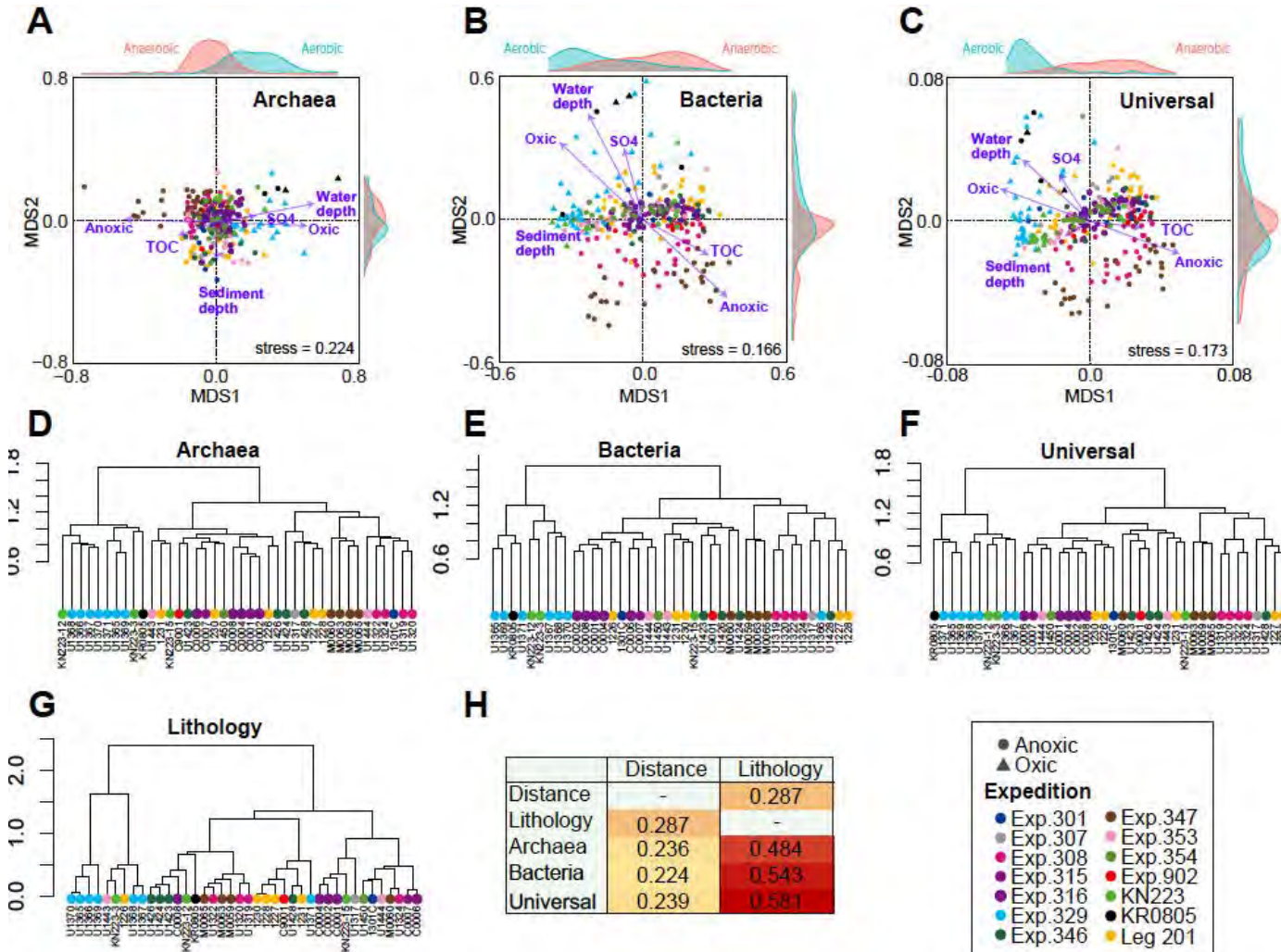


## Bacteria



The Earth's microbiome contains a distinctly different community.

# Global microbial amplicon sequence variants (ASVs) predicted using different models



- The asymptotic relation model best describe our data, predicting total species diversity in marine sediment to be  $7.8 \times 10^3$  for Archaea and  $3.2 \times 10^4$  for Bacteria.
- Comparative analysis of marine sediment, topsoil, and seawater microbiomes showed roughly similar levels of global microbial richness.
- This result indicates that Bacteria are more diverse than Archaea in Earth's global biosphere.

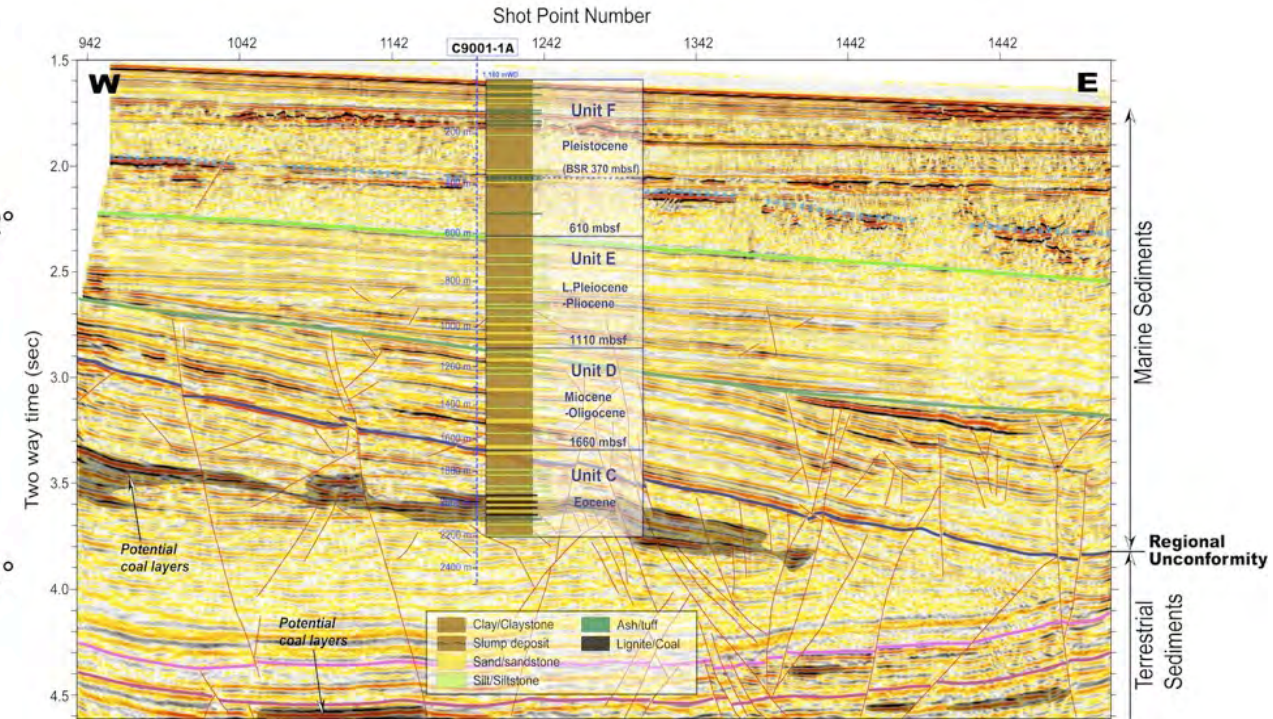
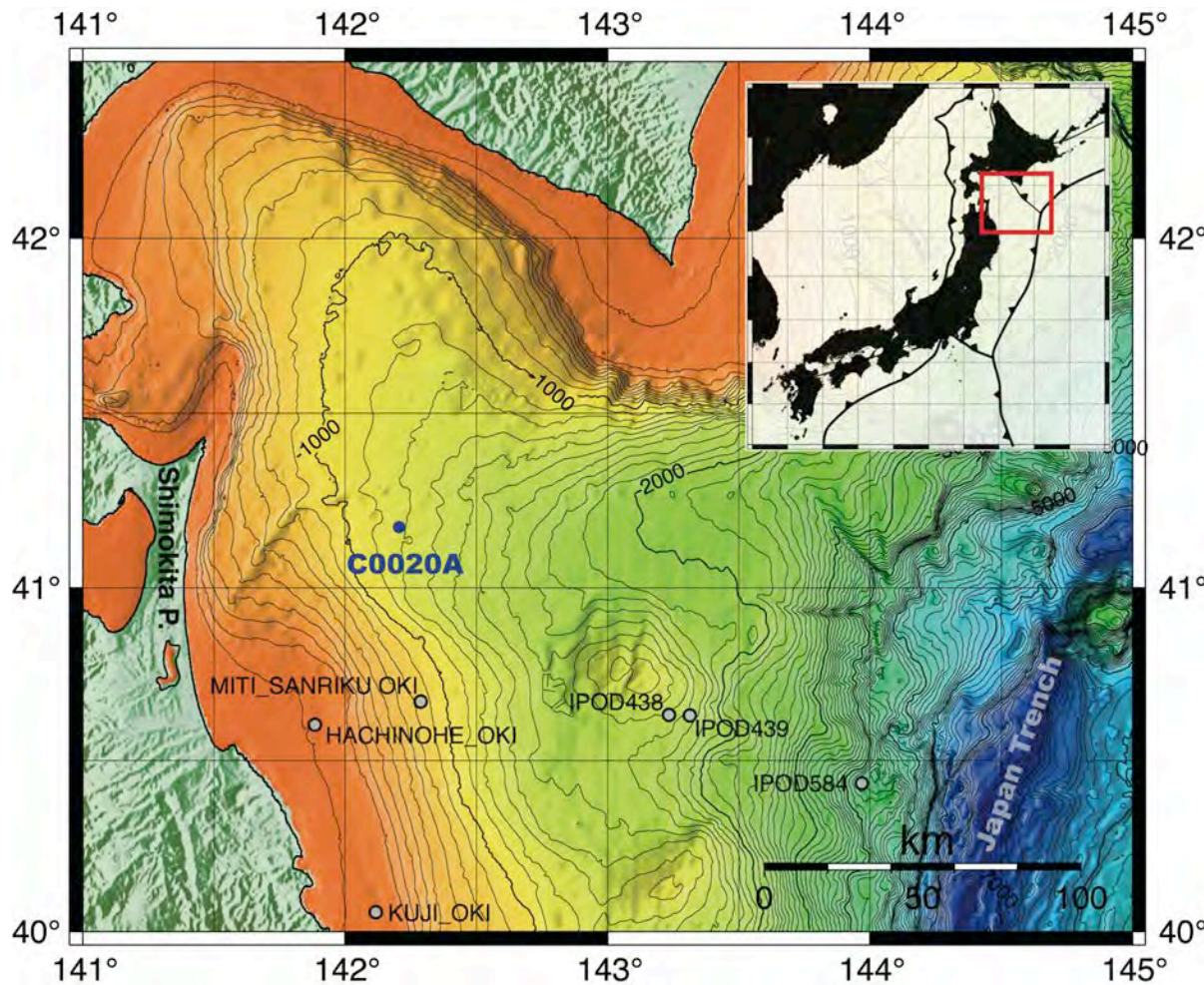


What are the ecological roles and limits of the deep seafloor sedimentary biosphere?



# IODP Expedition 337: The deep coalbed biosphere off Shimokita

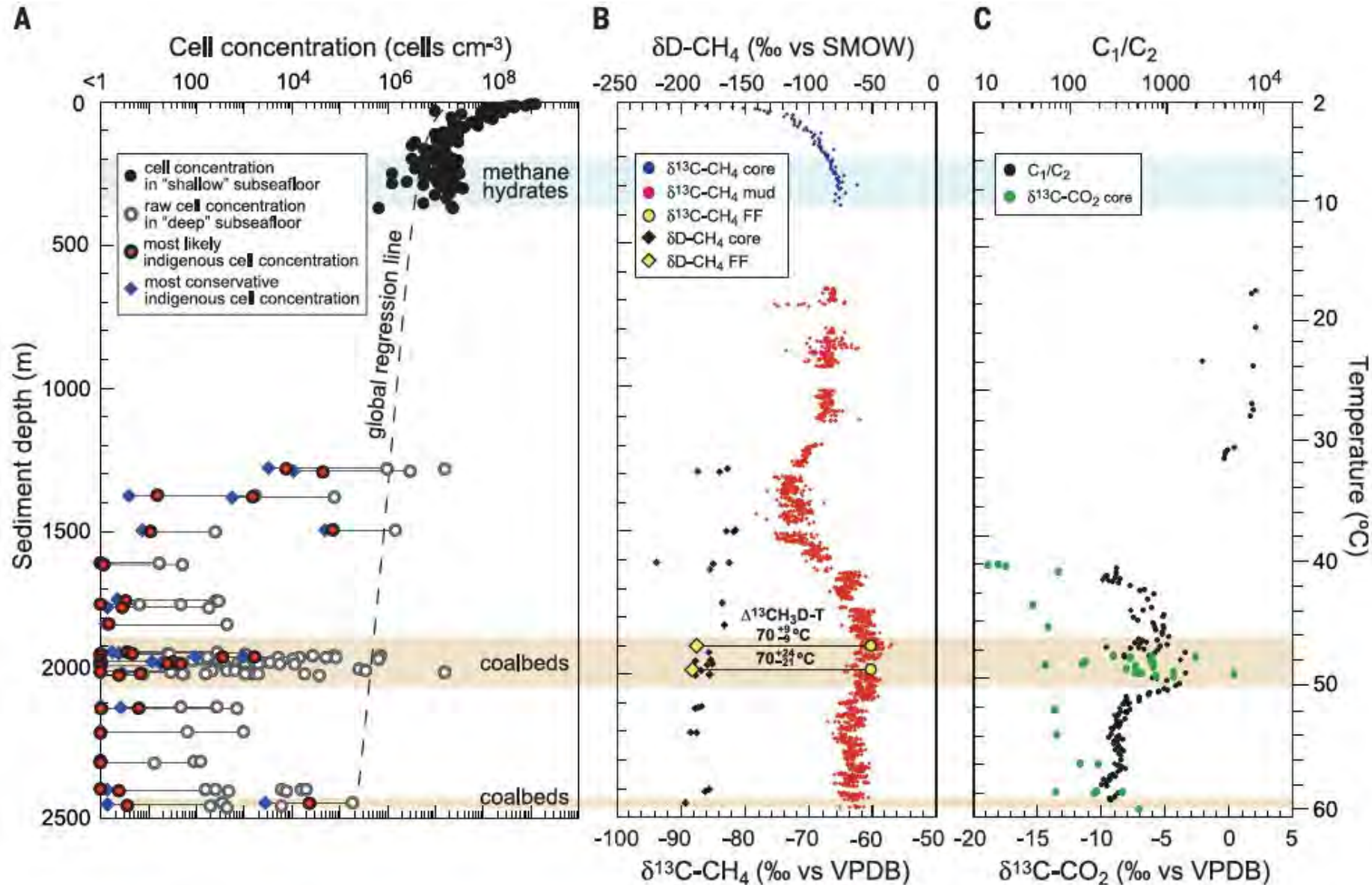
## The first deep biosphere-dedicated riser drilling expedition targeting deeply buried coalbeds in the Ocean



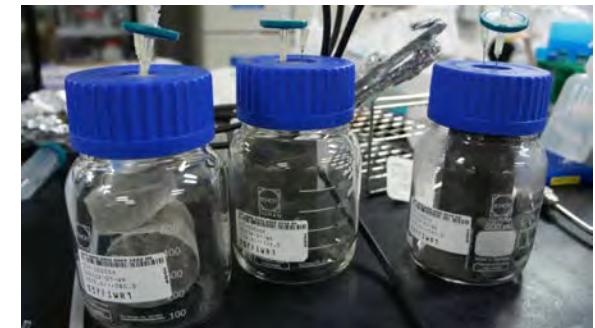
Water depth: 1200 m  
Drilling depth: 2,466 mbsf



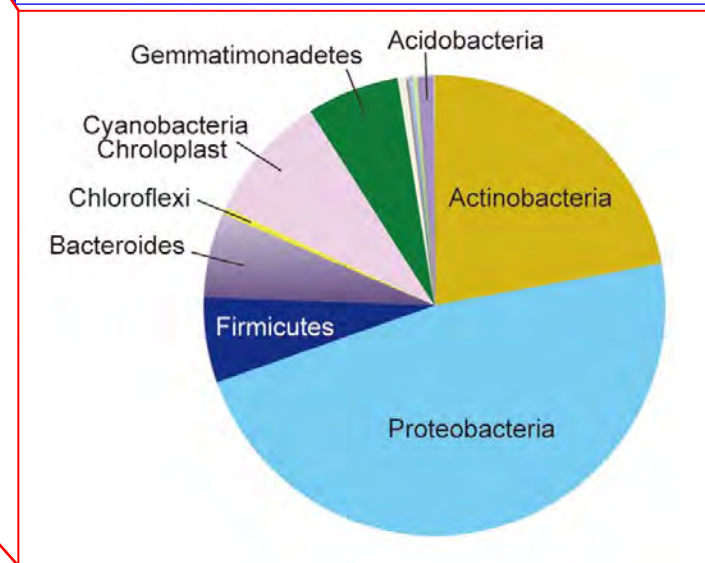
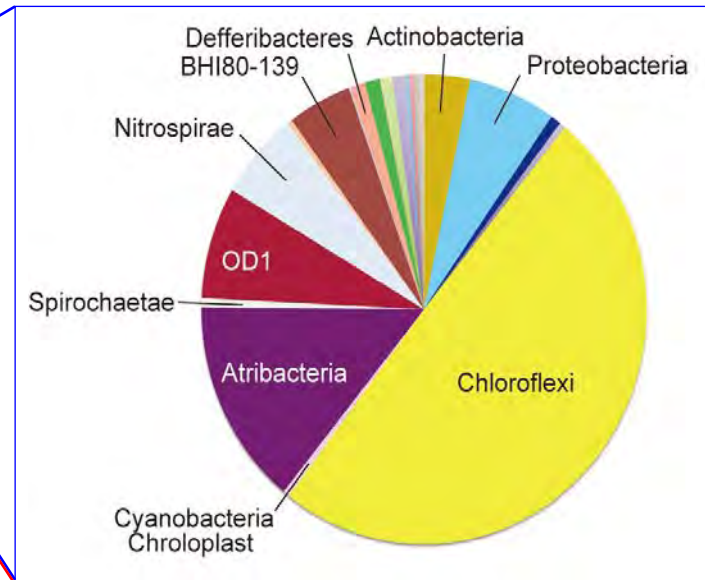
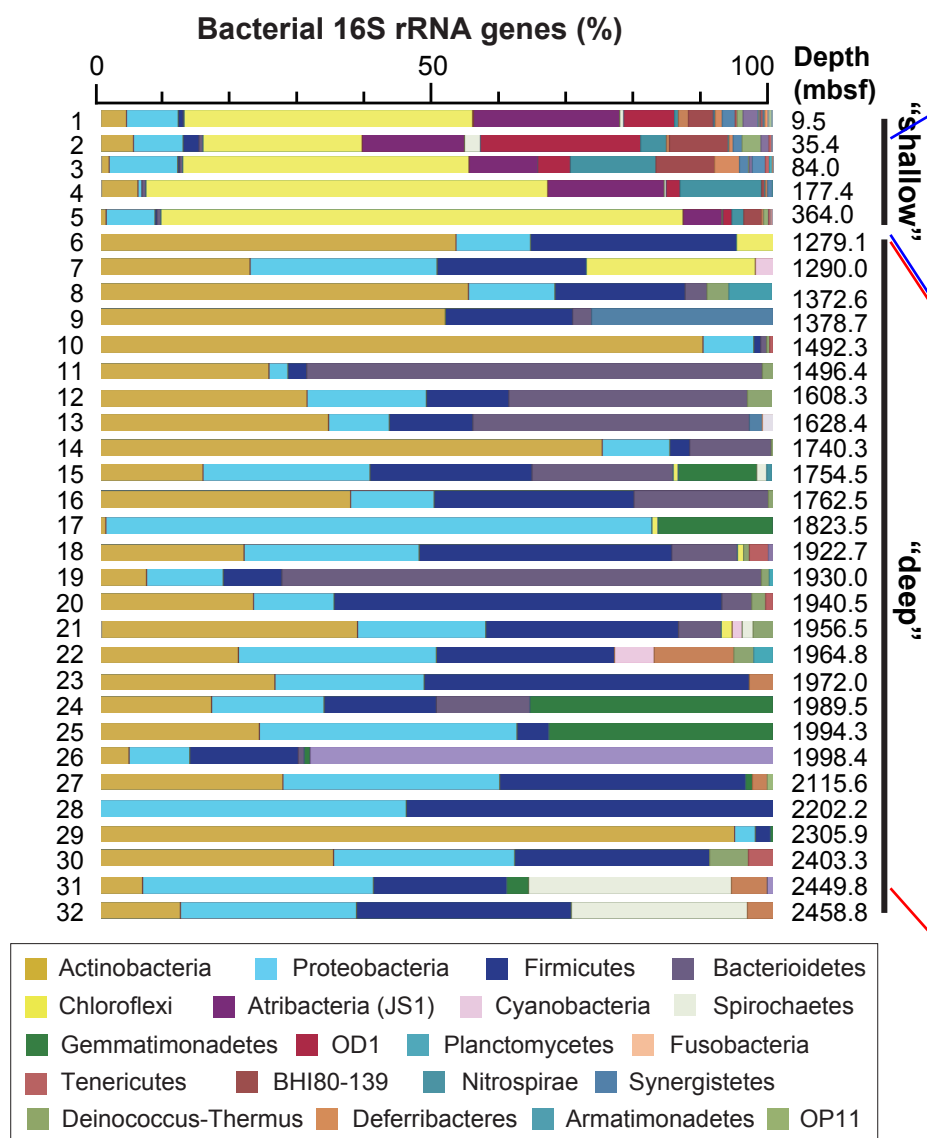
# Cell abundance and geochemical profiles at Site C0020 off Shimokita



*"Multiple lines of geochemical, sedimentological, and microbiological evidences indicate that microbial life is present even down to 2.5 km below the ocean floor and may play an important role in carbon cycling."*



# Taxonomic composition of indigenous bacterial communities

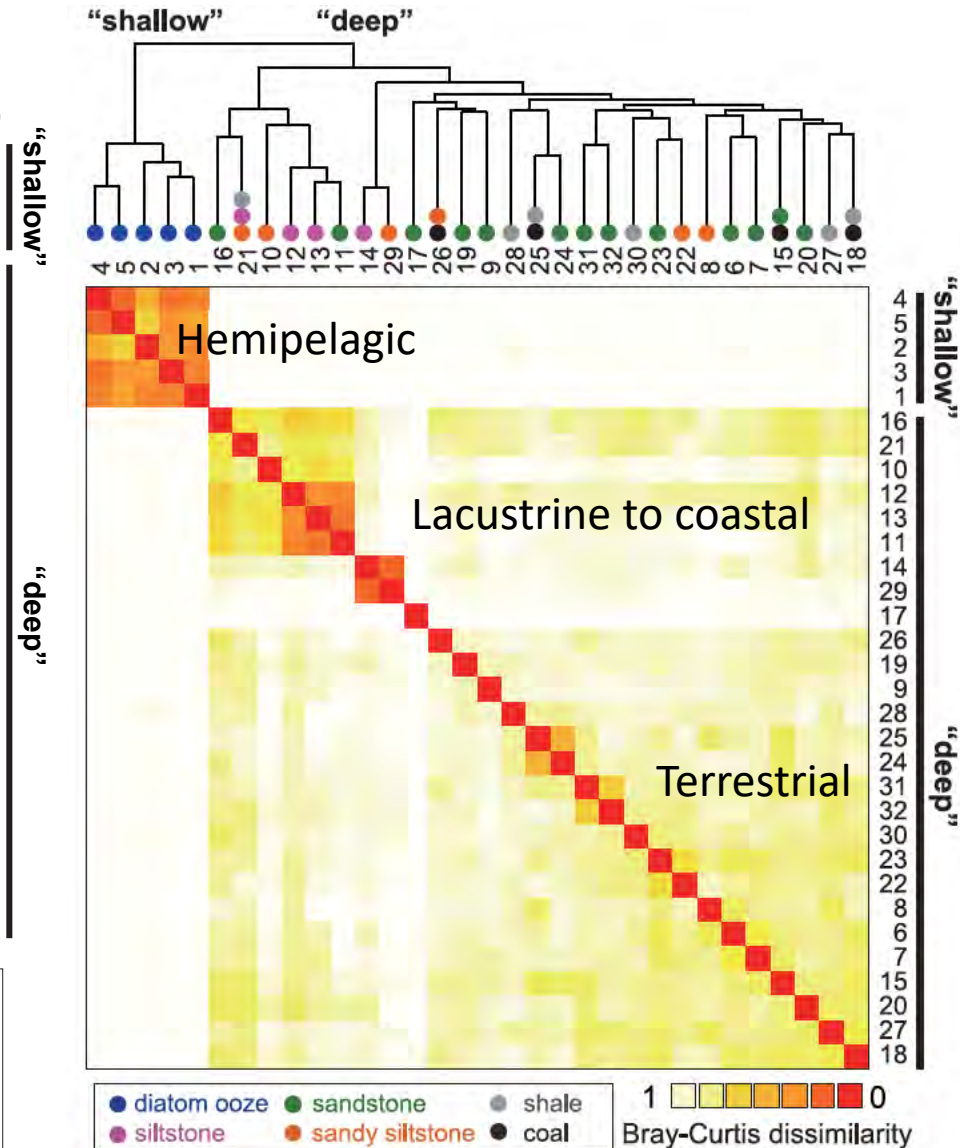
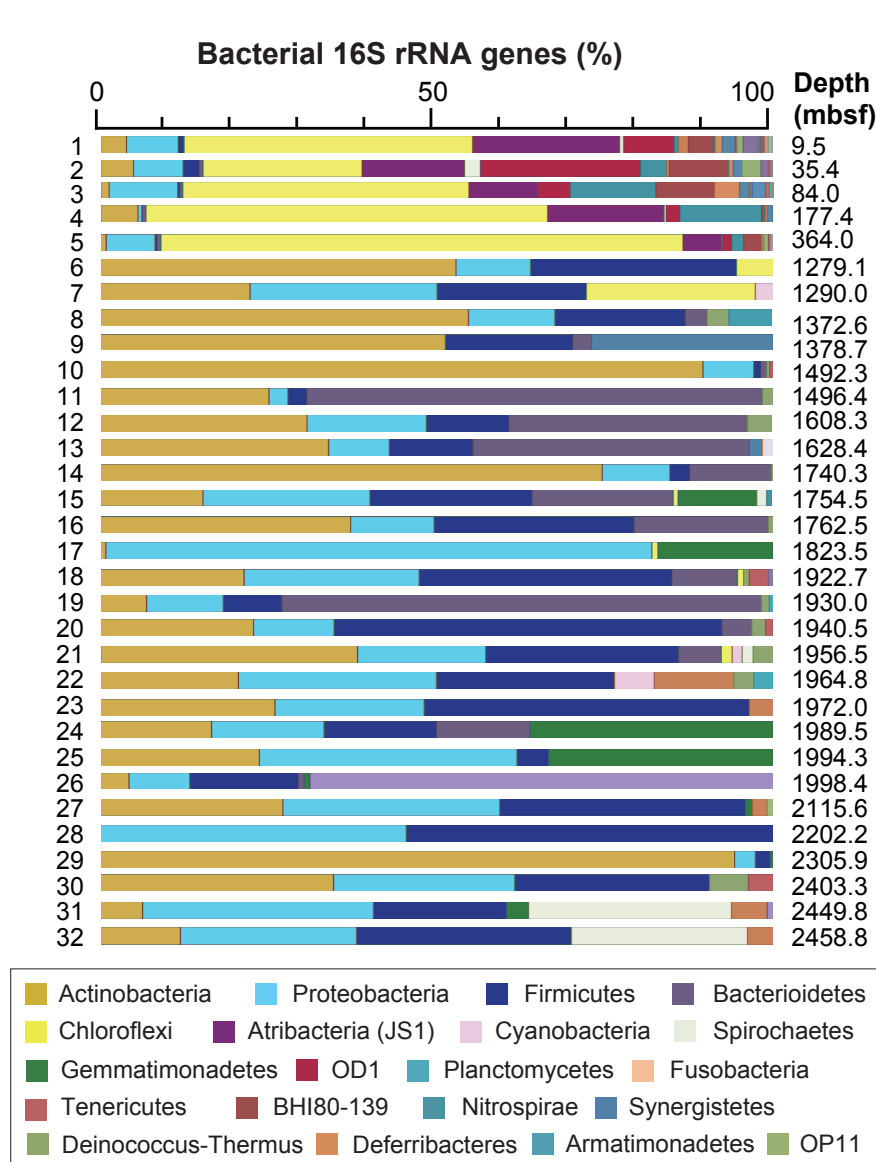


*“...communities differed markedly from shallower subseafloor communities and instead **resembled organotrophic communities in forest soils.**”*





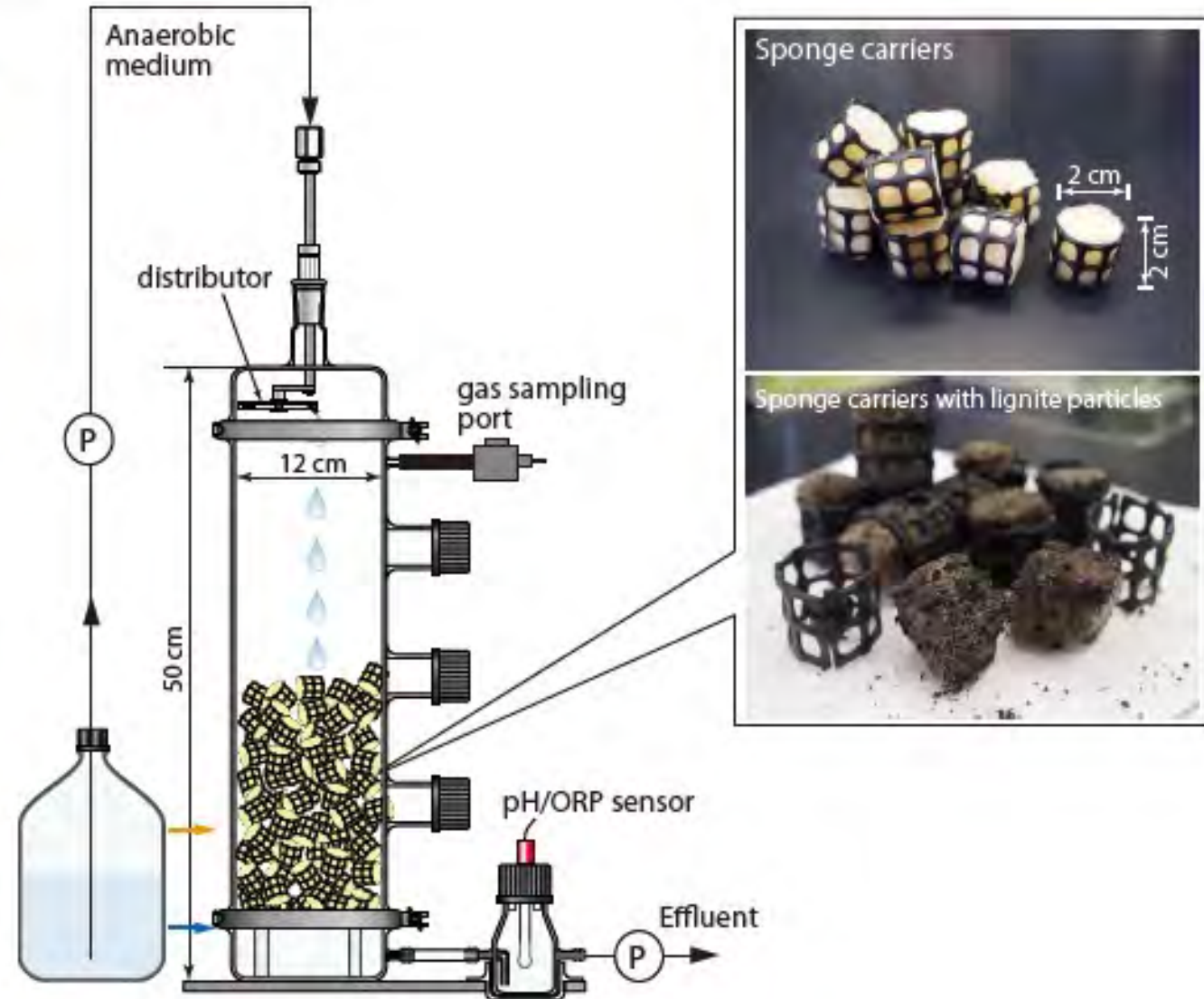
# Taxonomic composition of indigenous bacterial communities



*This suggests that terrigenous sediments retain indigenous microbial communities over 20 millions of years after burial in the seabed.*

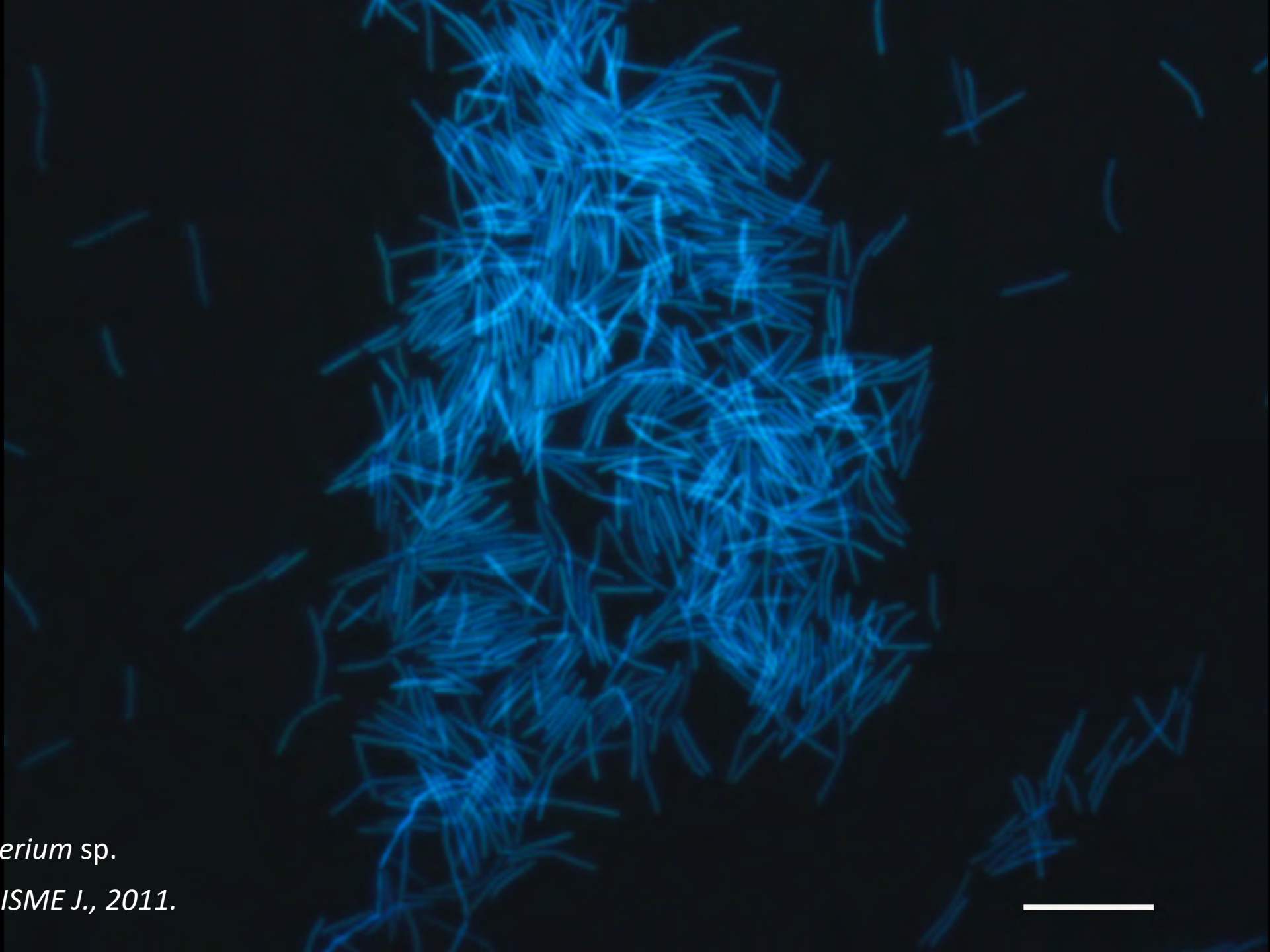


# Down-hanging sponge (DHS) flow-through bioreactor system





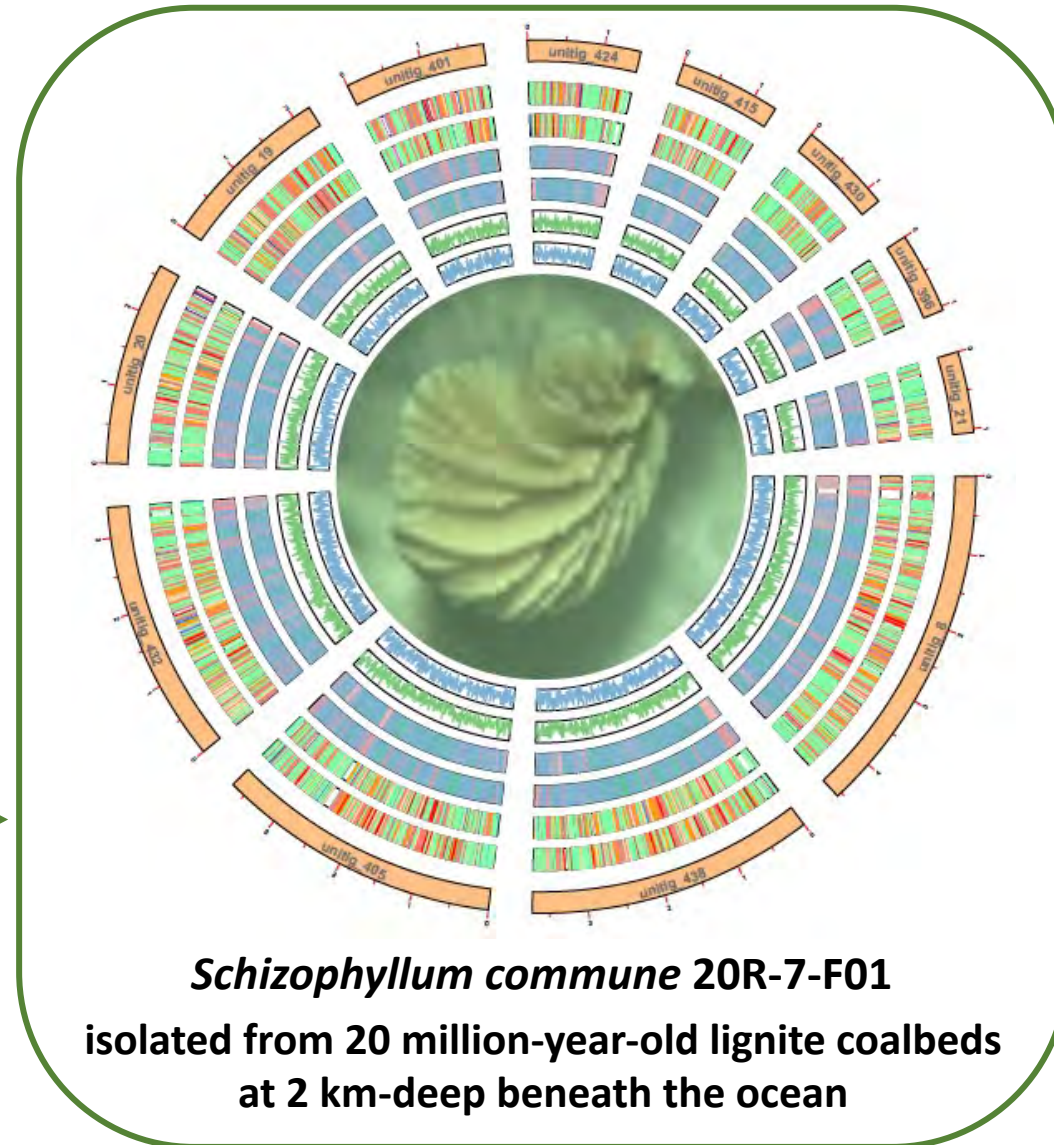
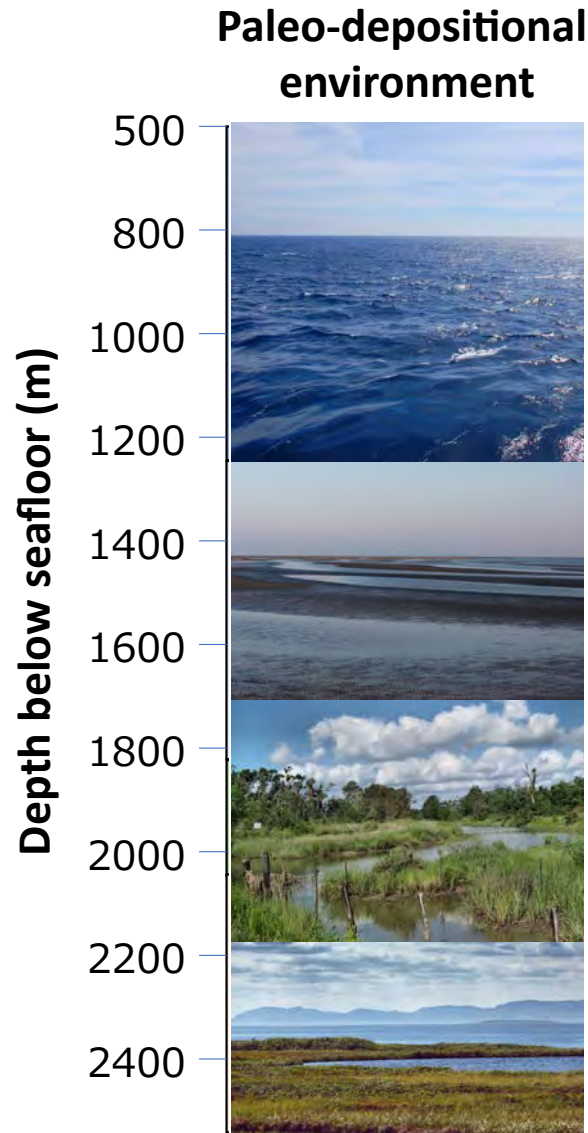




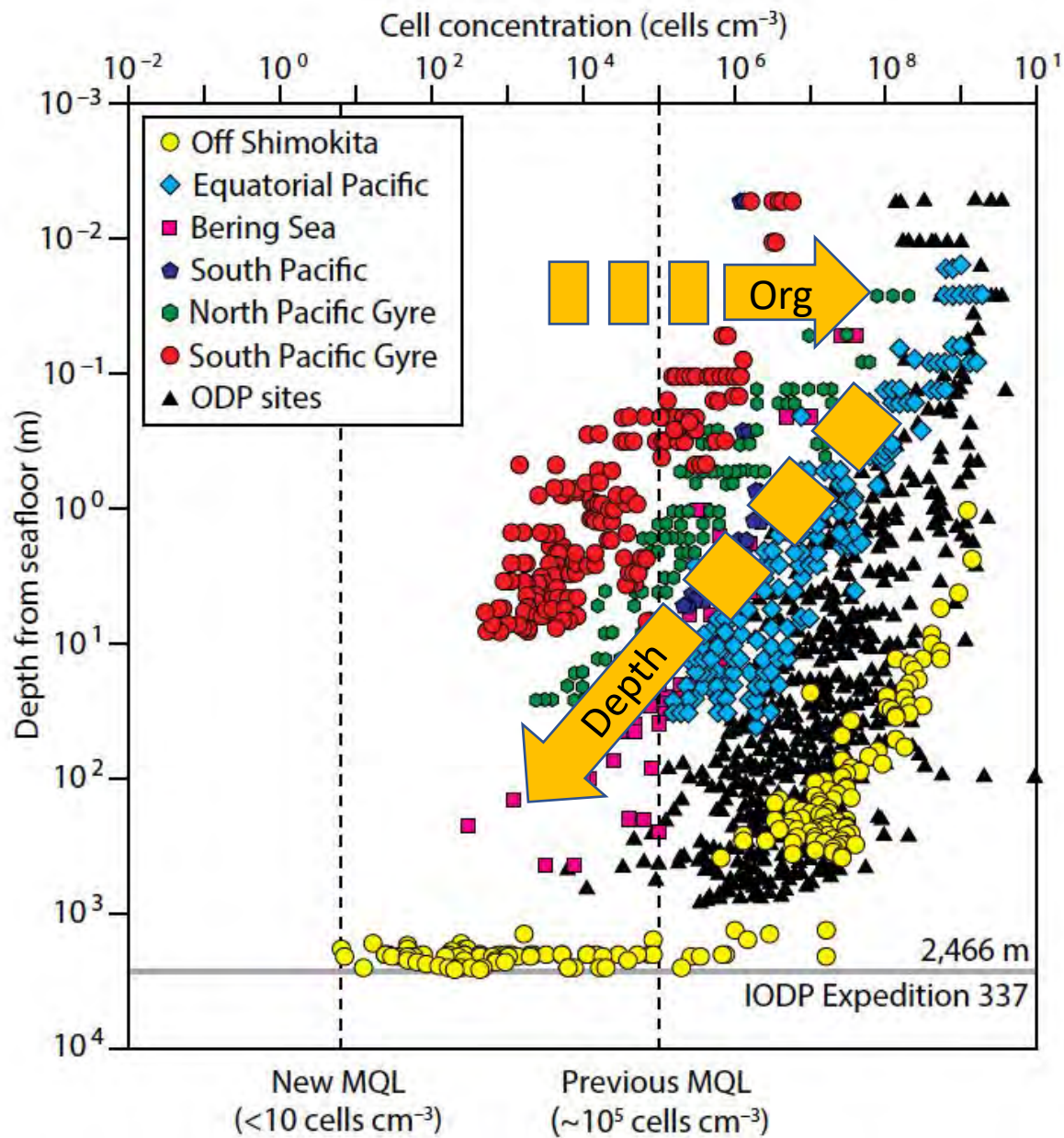
*Methanobacterium* sp.  
*Imachi et al., ISME J., 2011.*



# The sedimentary fungi have experienced an evolutionary stasis



Genomes of subseafloor fungal isolates have 3 orders of magnitude lower nucleotide diversity, substitution and homologous recombination rates than other terrestrial strains.



Cell concentrations in marine sediment.

## The limits to the deep biosphere

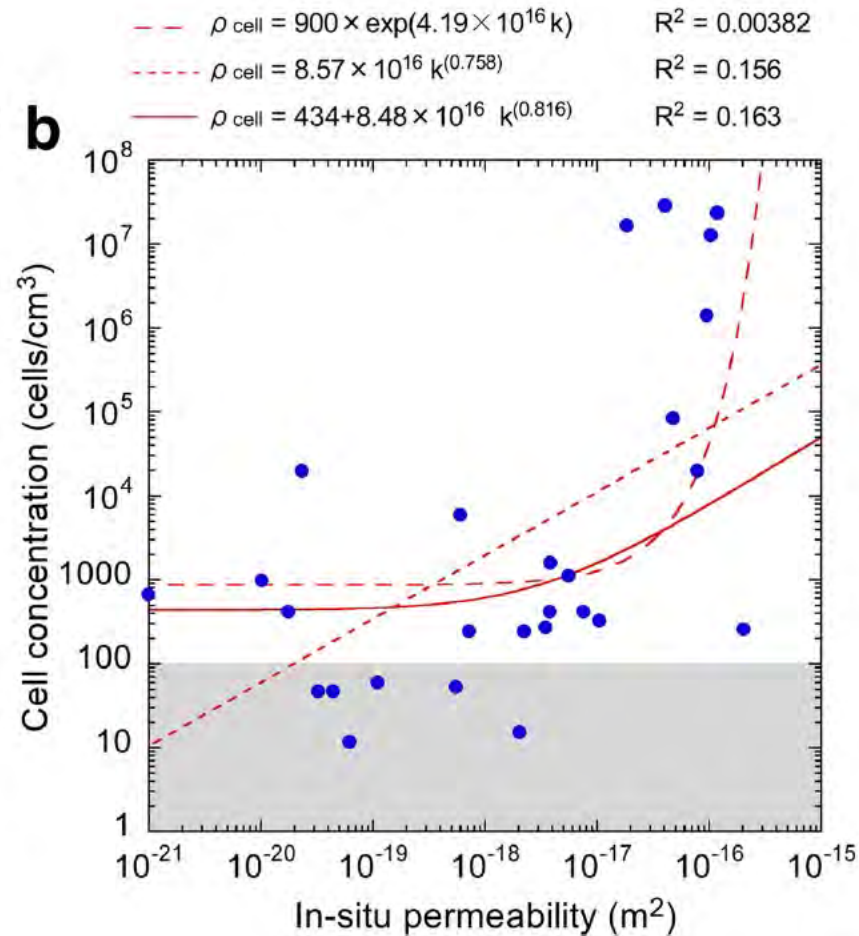
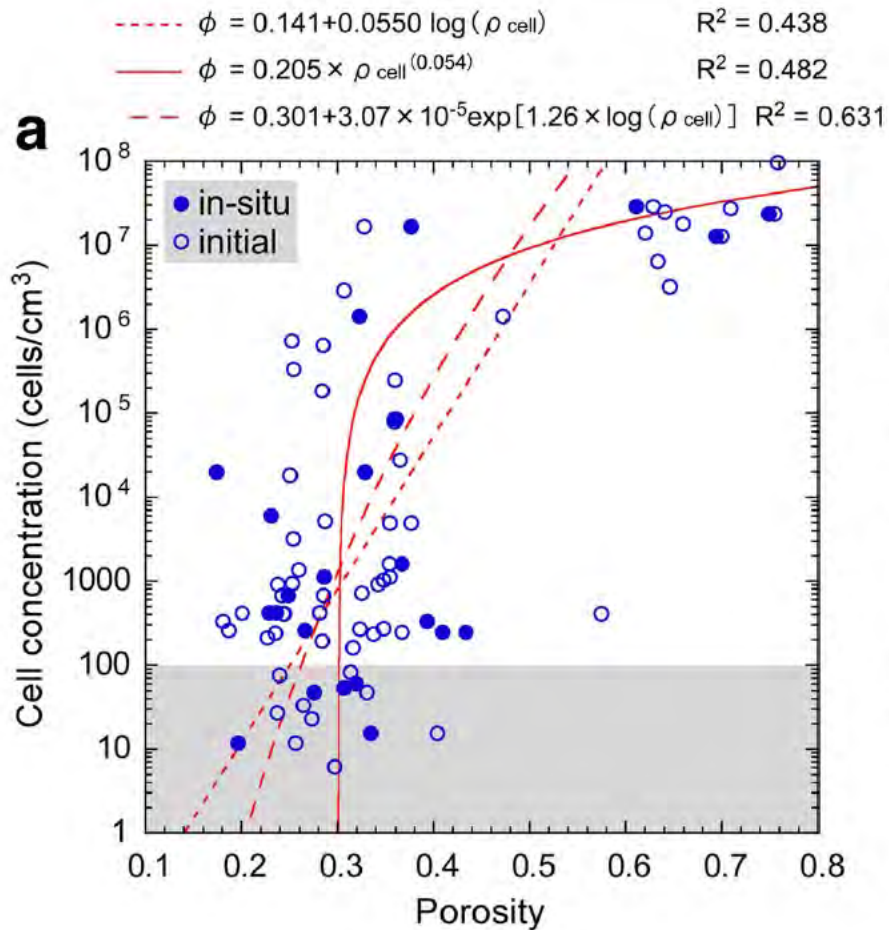
- Temperature
- Pressure
- pH
- Water
- Nutrients
- Energy substrates
- Age
- Porosity
- Permeability

*“Although the new study constrains some of the environmental factors that control the population density of subseafloor microbes, critical gaps remain before we can confidently model the size and extension of deep subseafloor life. For example, the limits of life that define the habitable zones remain poorly constrained.”*

*Hinrichs & Inagaki, Science, 2012:  
D'Hondt, Inagaki, Orcutt, and Hinrichs, Oceanography, 2019.*



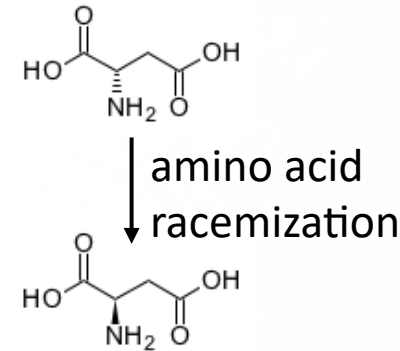
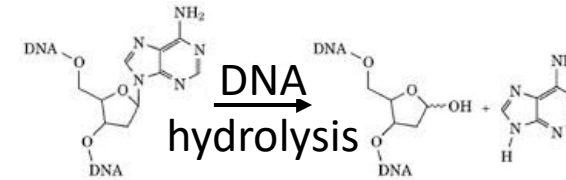
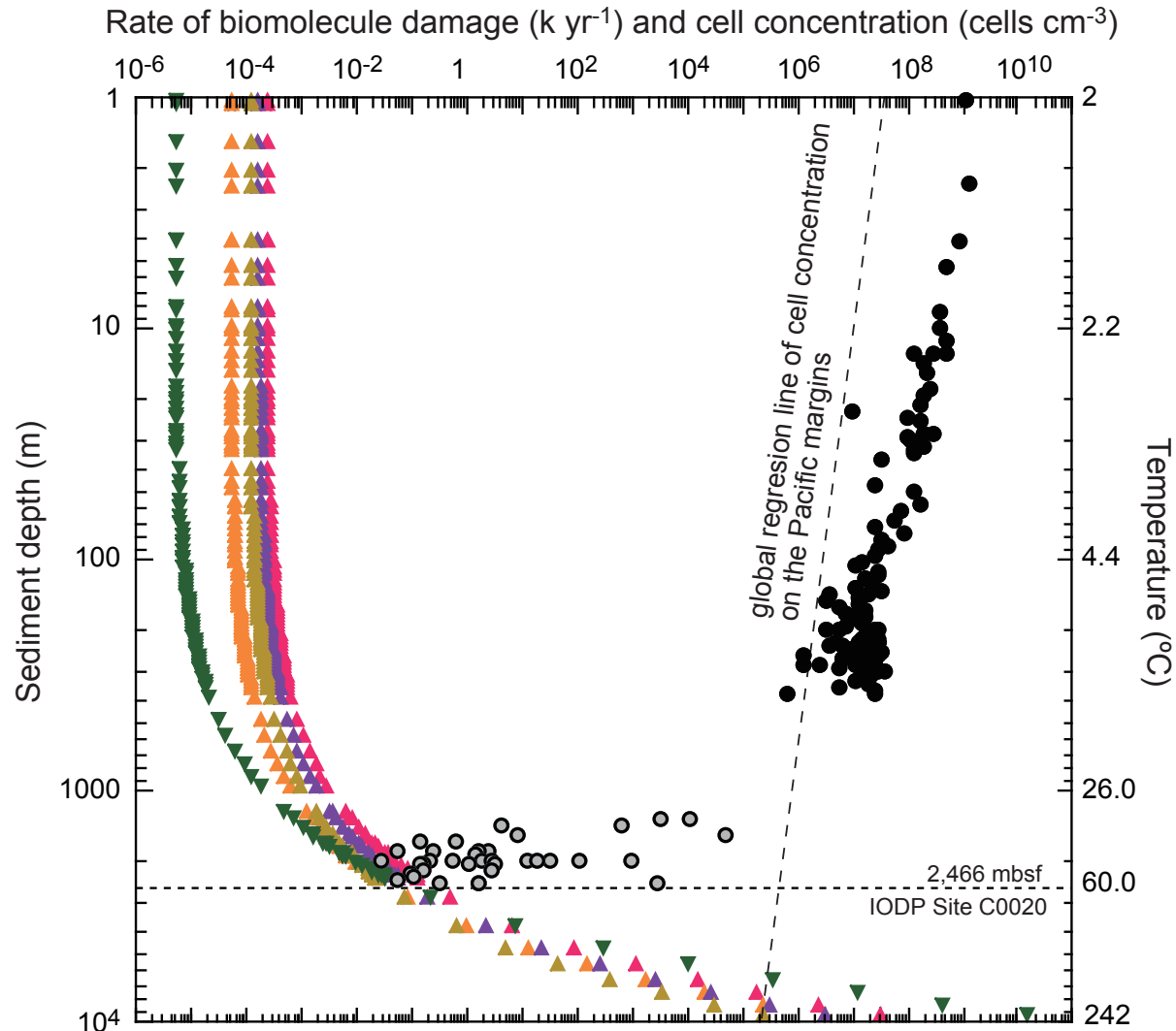
# Geophysical limits to the deep coalbed biosphere: porosity and permeability



*“...one of the factors affecting the decrease in microbial cell abundance with increasing depth was a reduction in nutrient and water supply to indigenous microbial communities as a result of a decrease in porosity and permeability due to sediment compaction.”*

*“...impermeable shale and siltstone with small pores (<0.2 μm) may act as barriers to water and energy-yielding substrates for deep microbial life.”*

# Temperature is a critical factor for microbial persistence

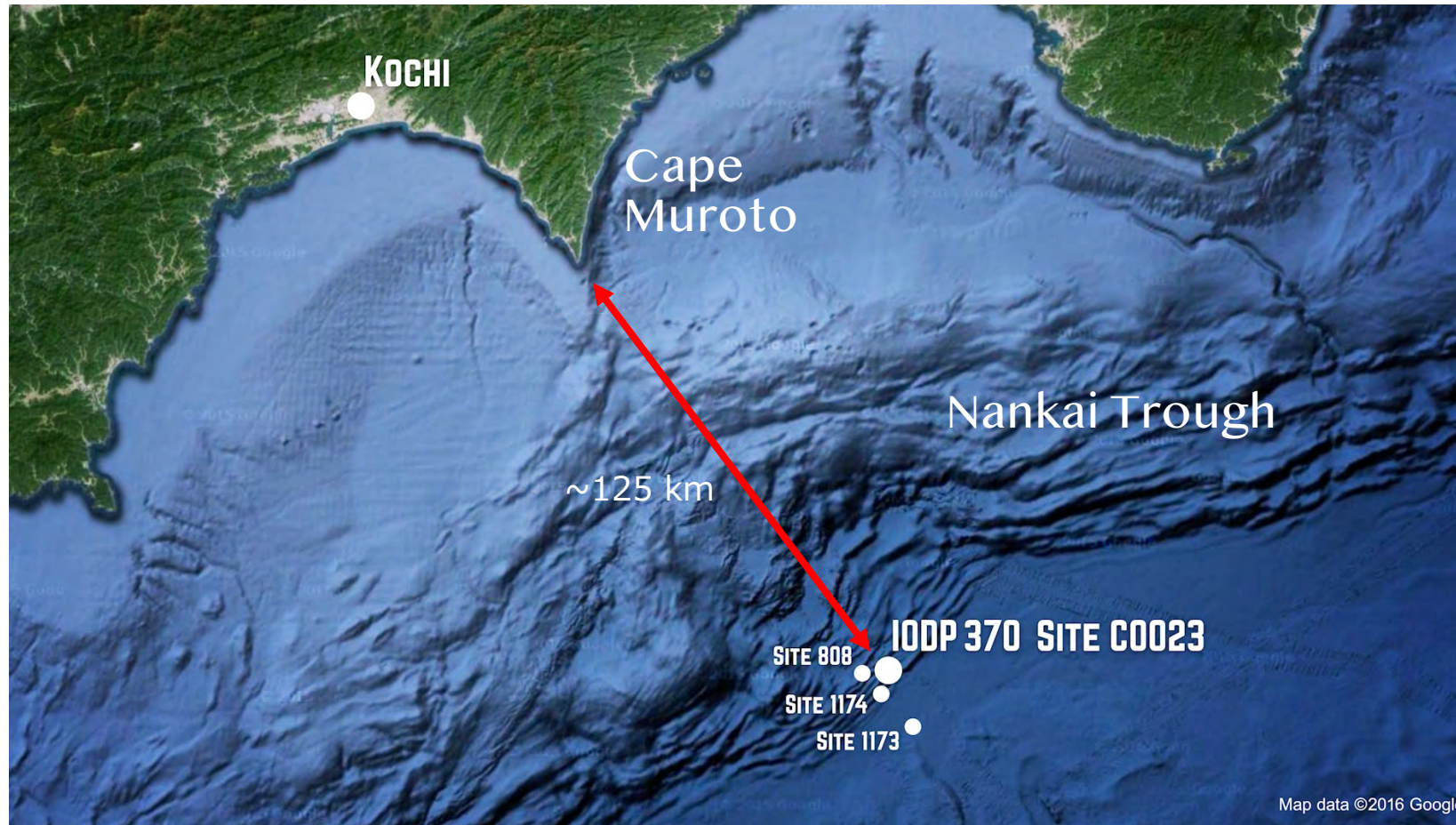


The energy requirement for biomolecule damage fixation increases with temperature *in situ*.

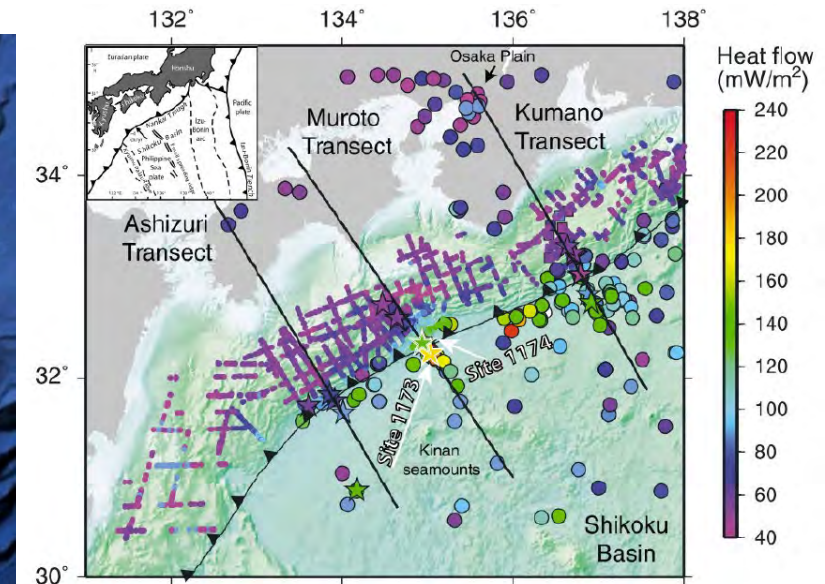
The cell abundance and the energy supply should be balanced in the deep biosphere.



# IODP Expedition 370: Temperature limits to the deep biosphere off Muroto (T-Limit)

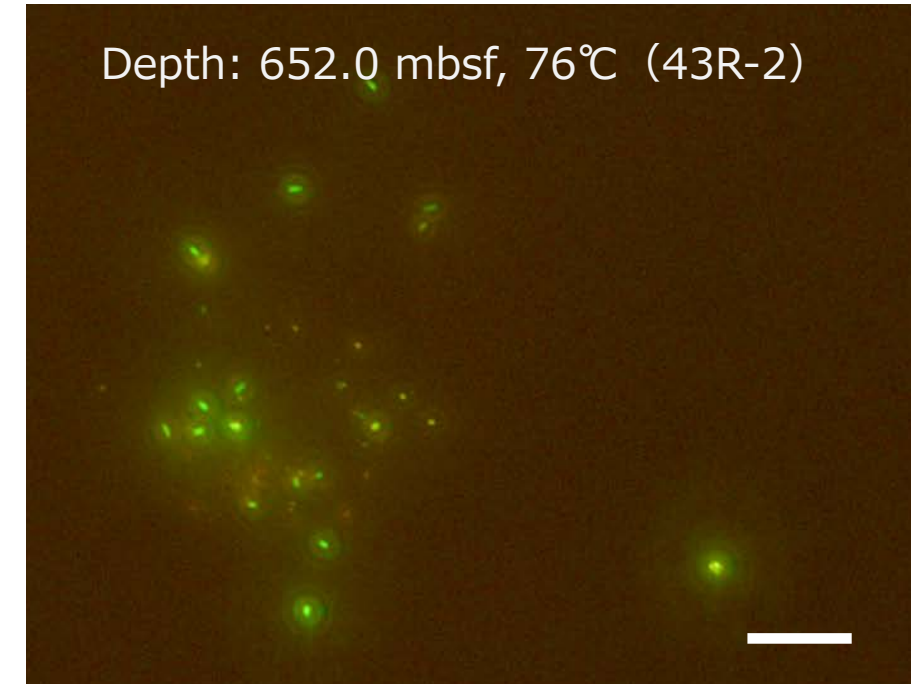
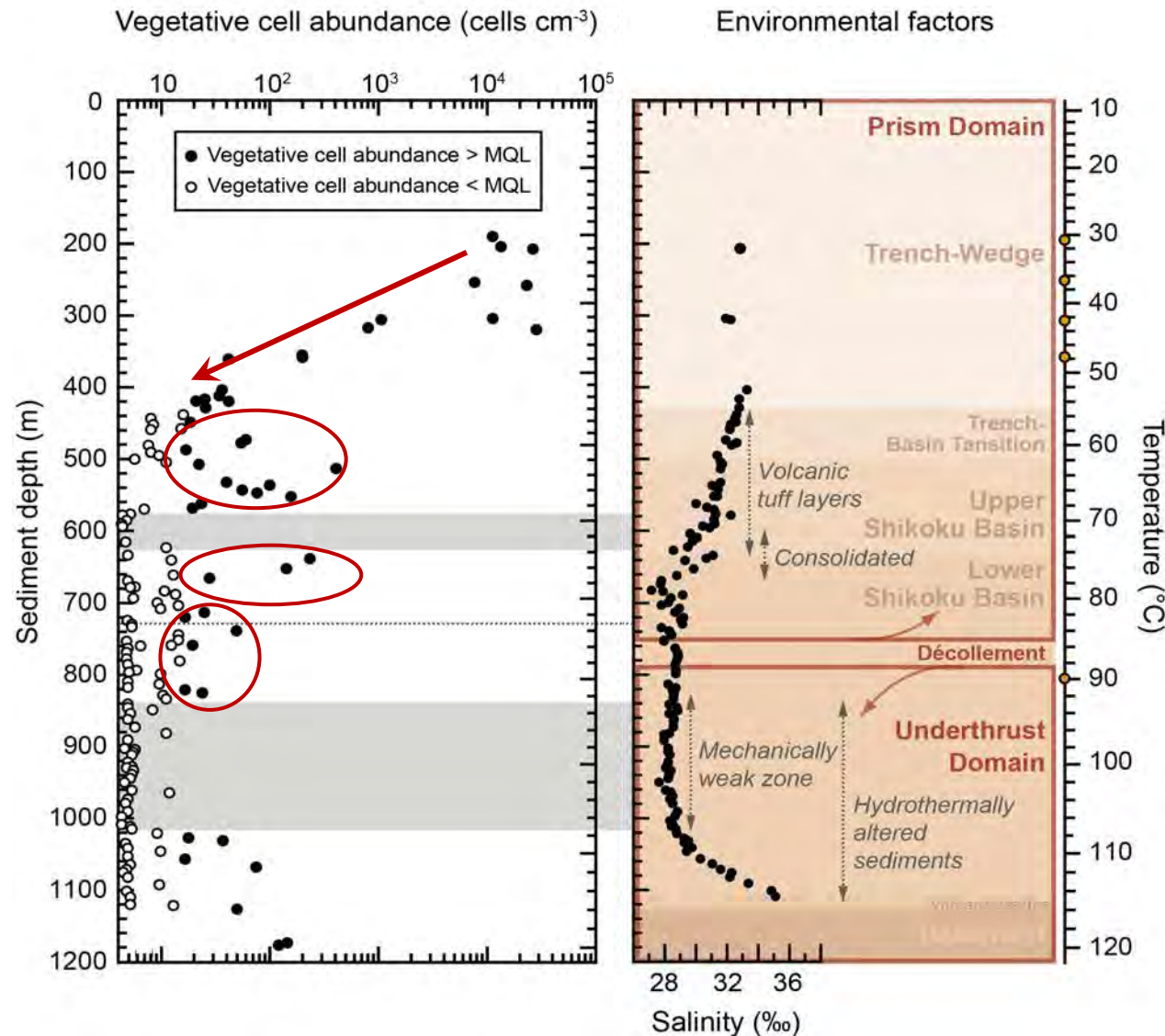


Site C0023: Water depth: 4776 m, Drilling depth: 1177 mbsf





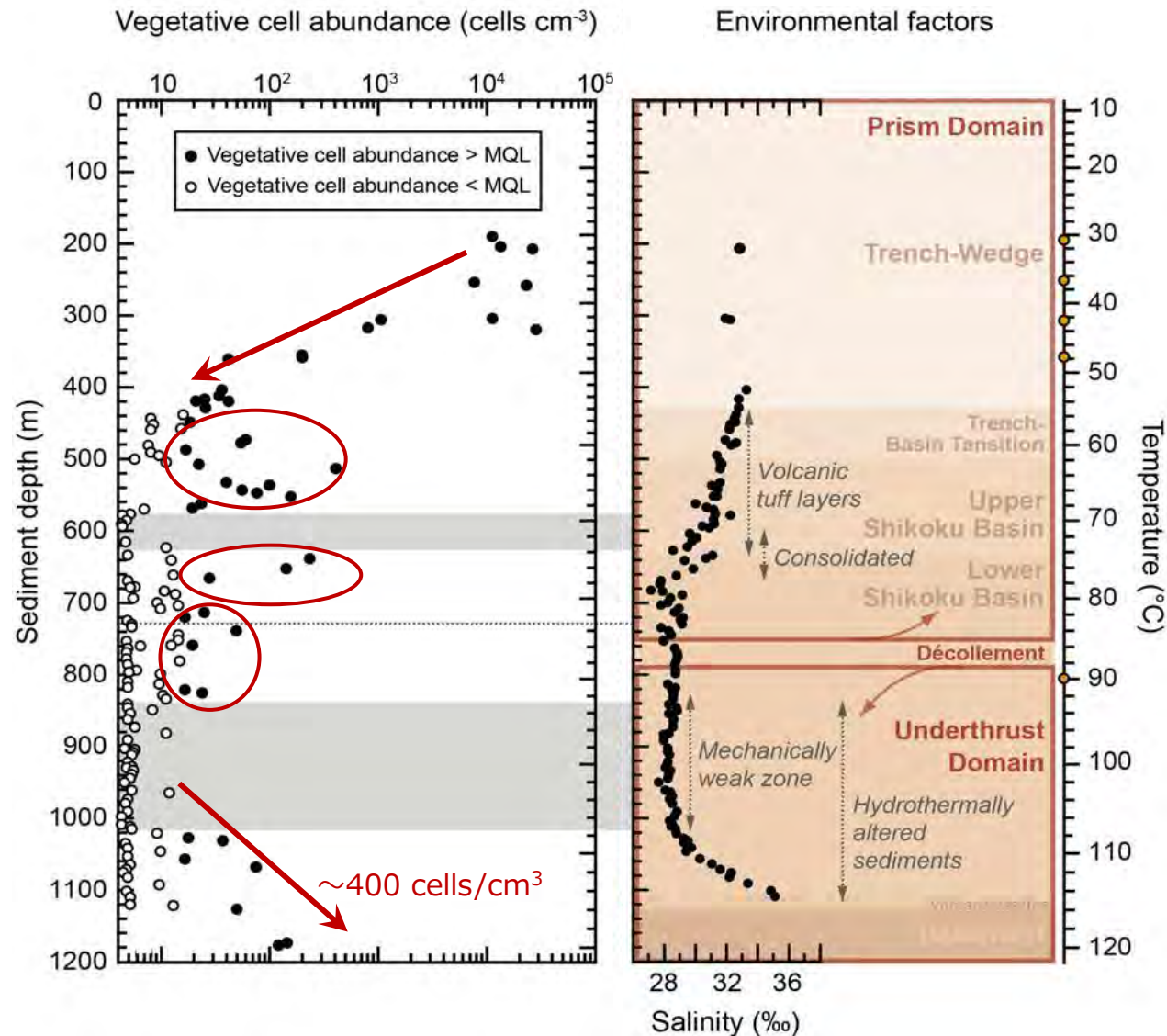
# Substantial impact of temperature on microbial abundance and activity



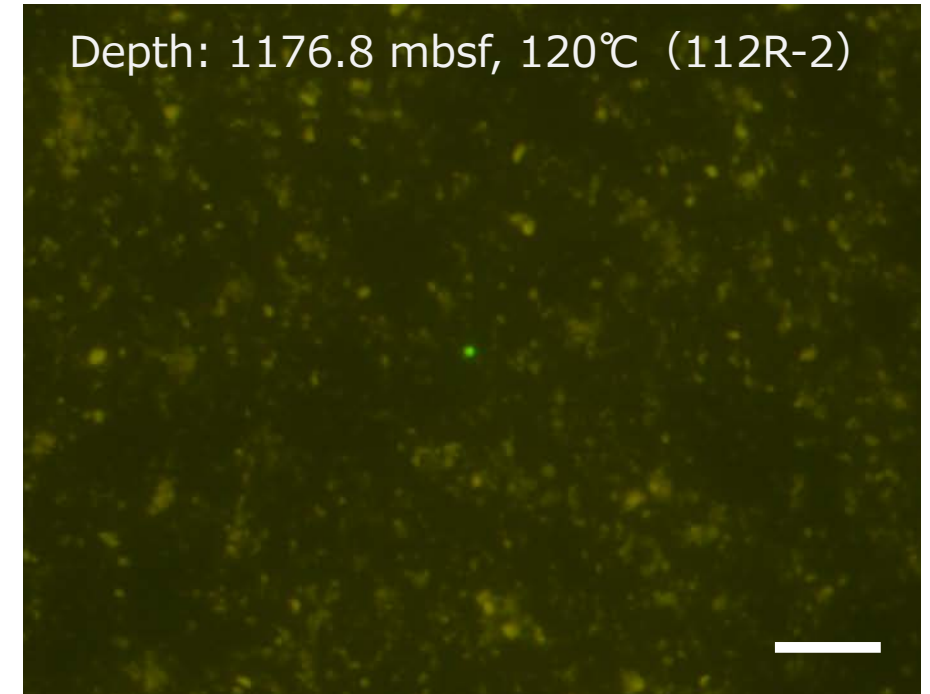
*"Multiple lines of geochemical and microbiological evidence show that temperature is a critical environmental factor for defining the abundance, activity, and distribution of microbial cells in the deep subseafloor biosphere."*



# Substantial impact of temperature on microbial abundance and activity

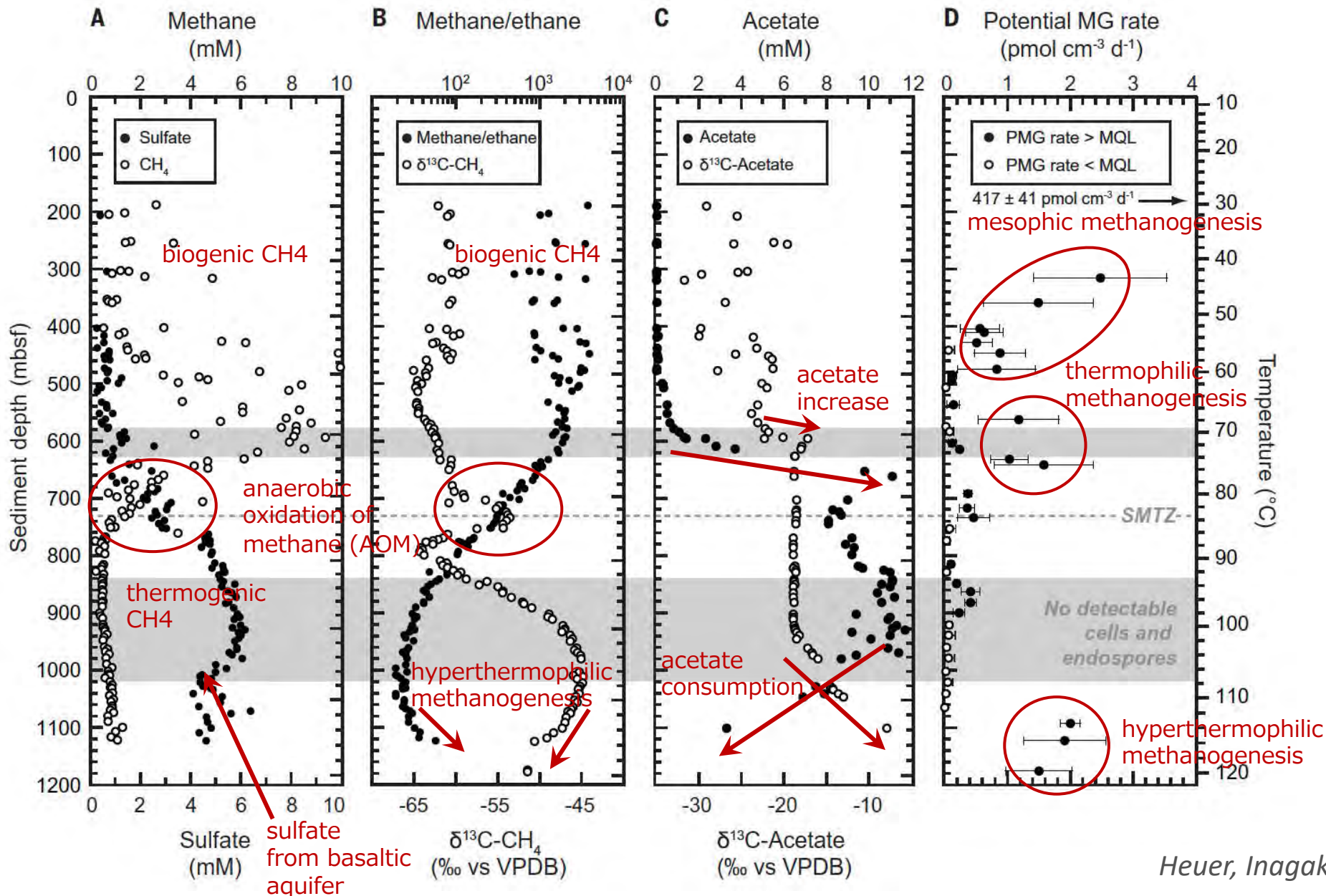


Depth: 1176.8 mbsf, 120°C (112R-2)



*"..it moreover shows that life in the deep subseafloor is **NOT** constrained by an upper temperature limit below 120°C."*

# Geochemical signals of microbial metabolisms at Site C0023

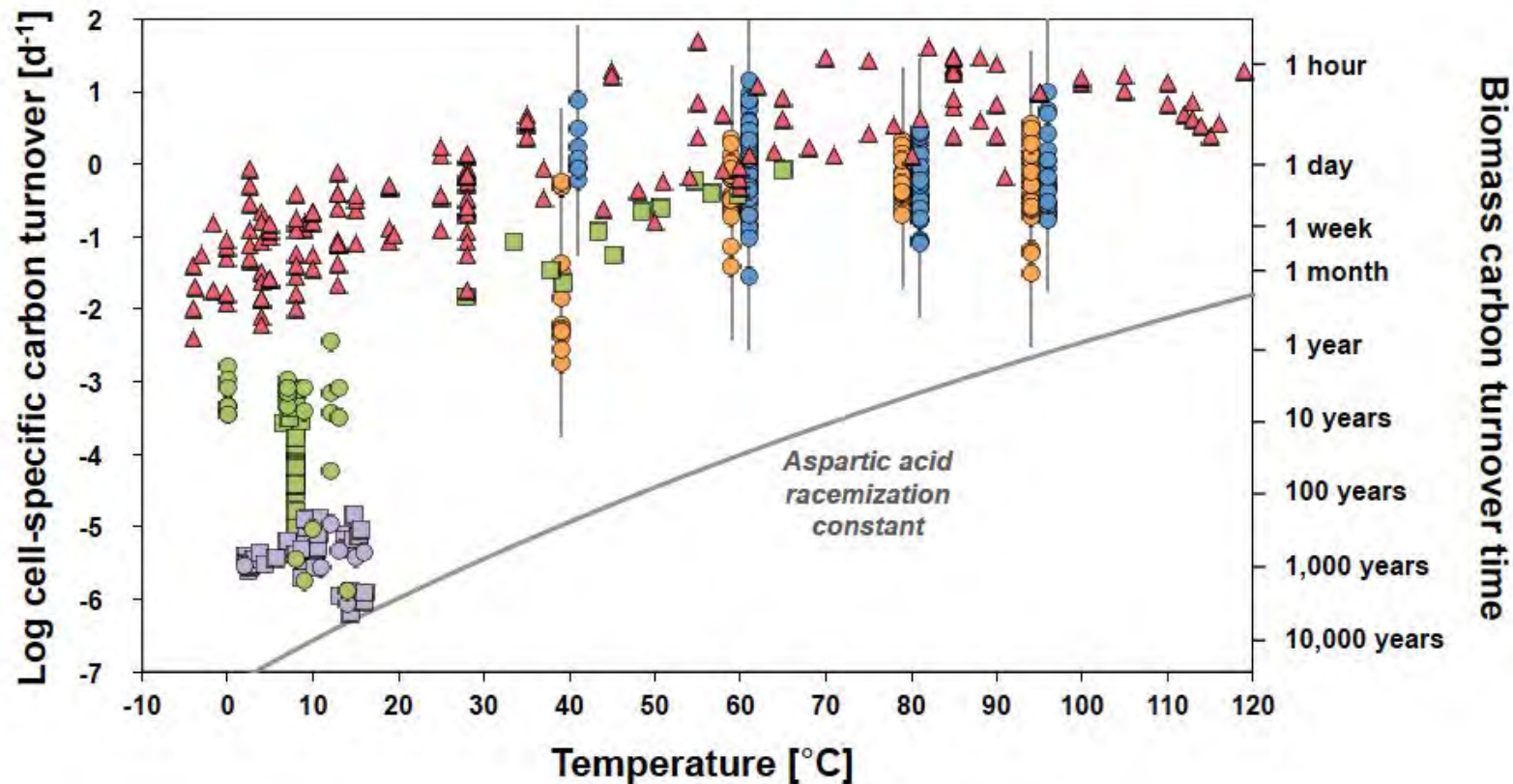


Geochemical profiles of microbial substrates and products provide evidence for microbial activity down to the ~16-million-year-old oceanic crust.

The transient heating events may have locally sterilized sediment, but microbial cells, acetate consumption, and methanogenic activity prevail again in >100°C sediments toward the sediment-basement interface.



# Rapid microbial life in the deep and hot subseafloor biosphere

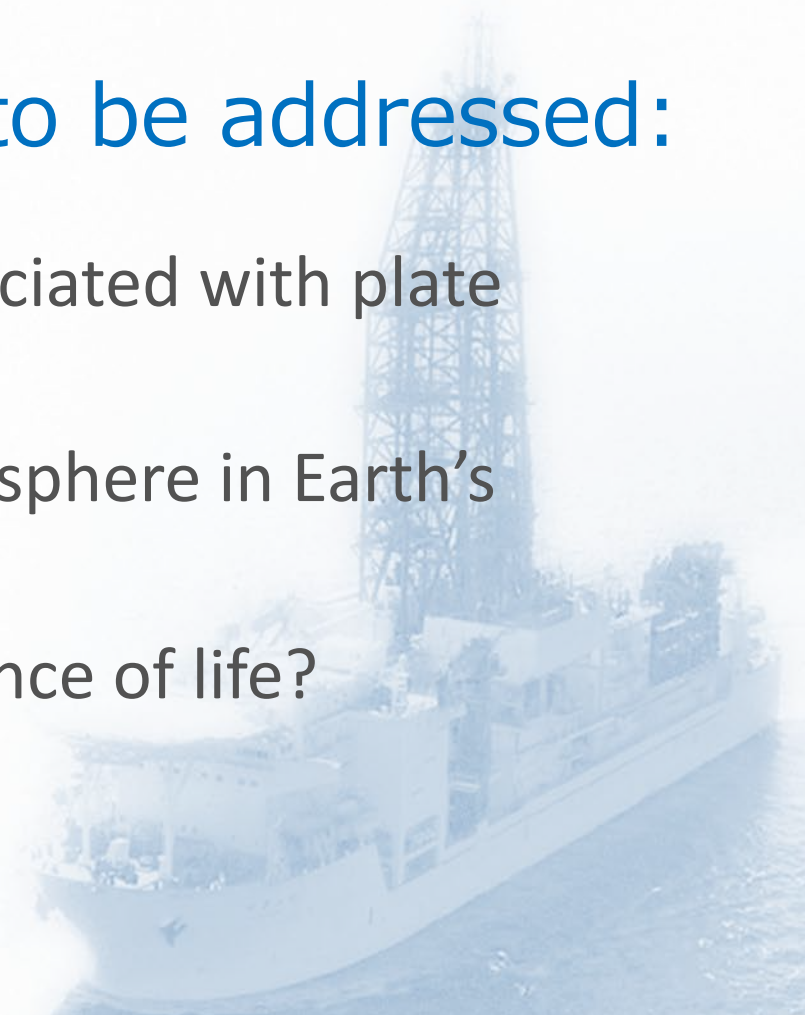


“At this high temperature, a small community of microorganisms subsisted with exceptionally high cell-specific rates of energy metabolism, similar to rates in surface sediments and laboratory cultures.”

“This discovery is in stark contrast to the extremely slow microbial life otherwise observed in the deep subseafloor, but is consistent with a high energy demand needed to withstand and repair thermal cell damage.”

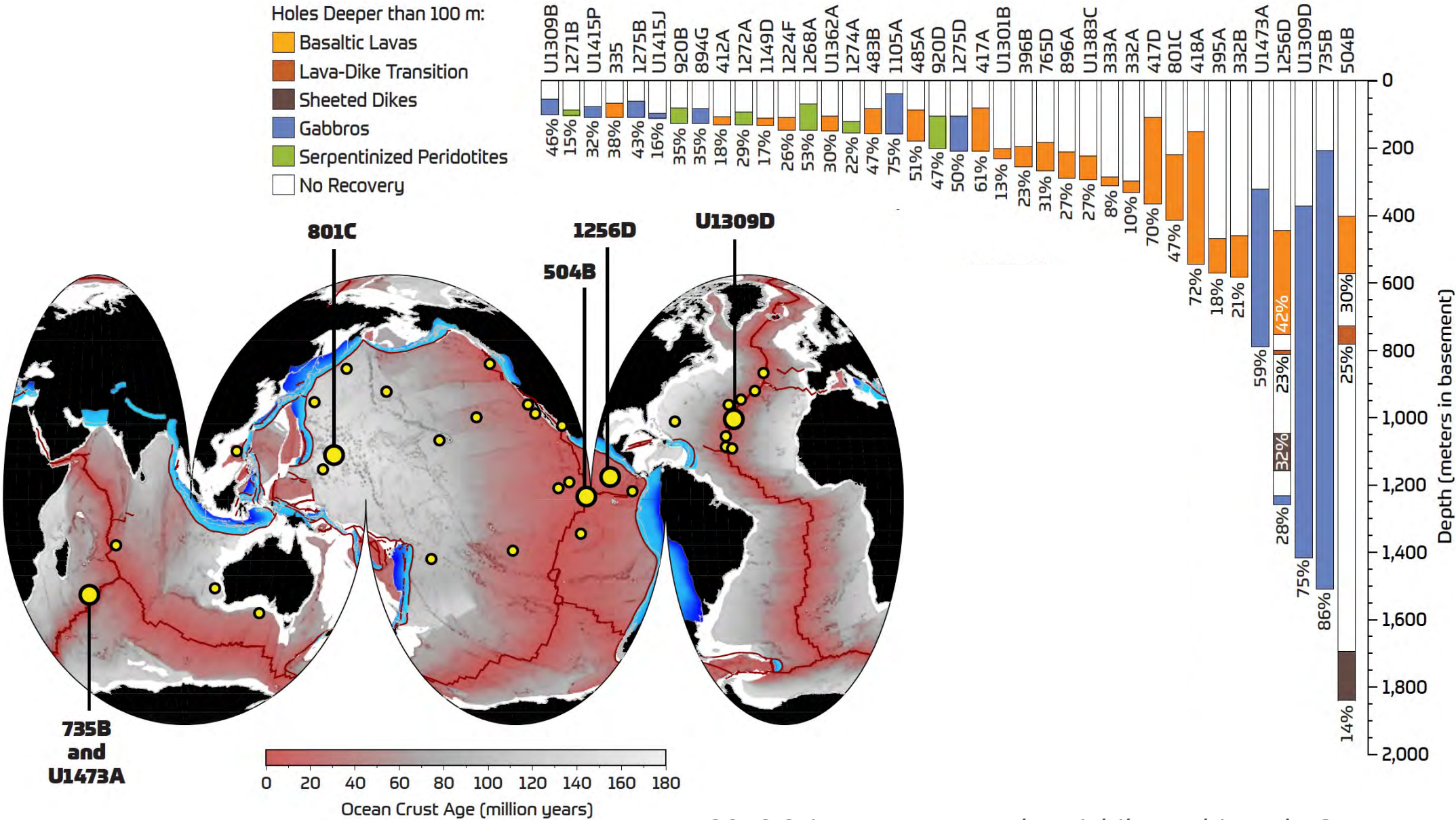
## Some significant questions remain to be addressed:

- How is the deep biosphere evolution associated with plate tectonics and mantle convection?
- What are the roles of the deep crustal biosphere in Earth's element cycling?
- Is plate tectonics required for the emergence of life?
- Why the planet Earth is habitable?

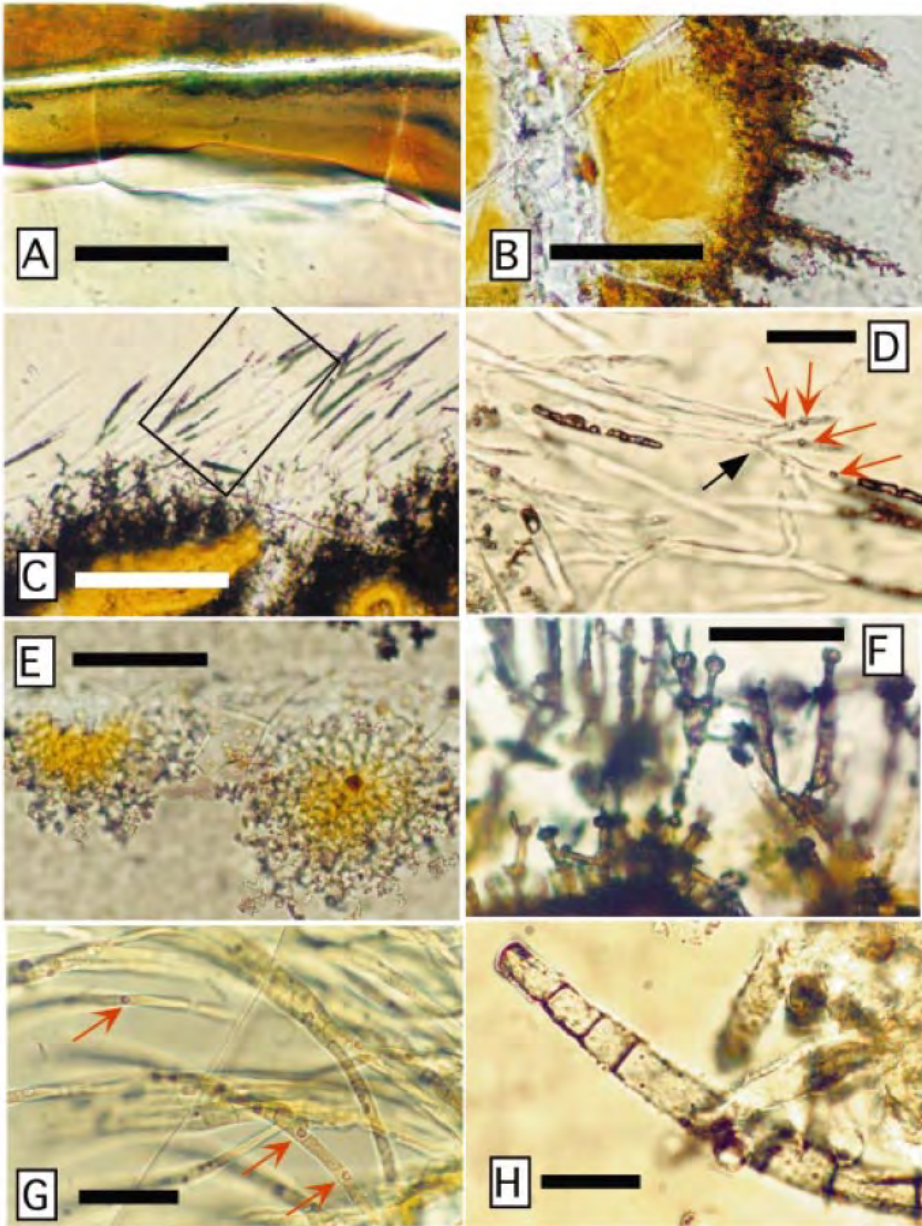




# The oceanic lithosphere has been largely unexplored



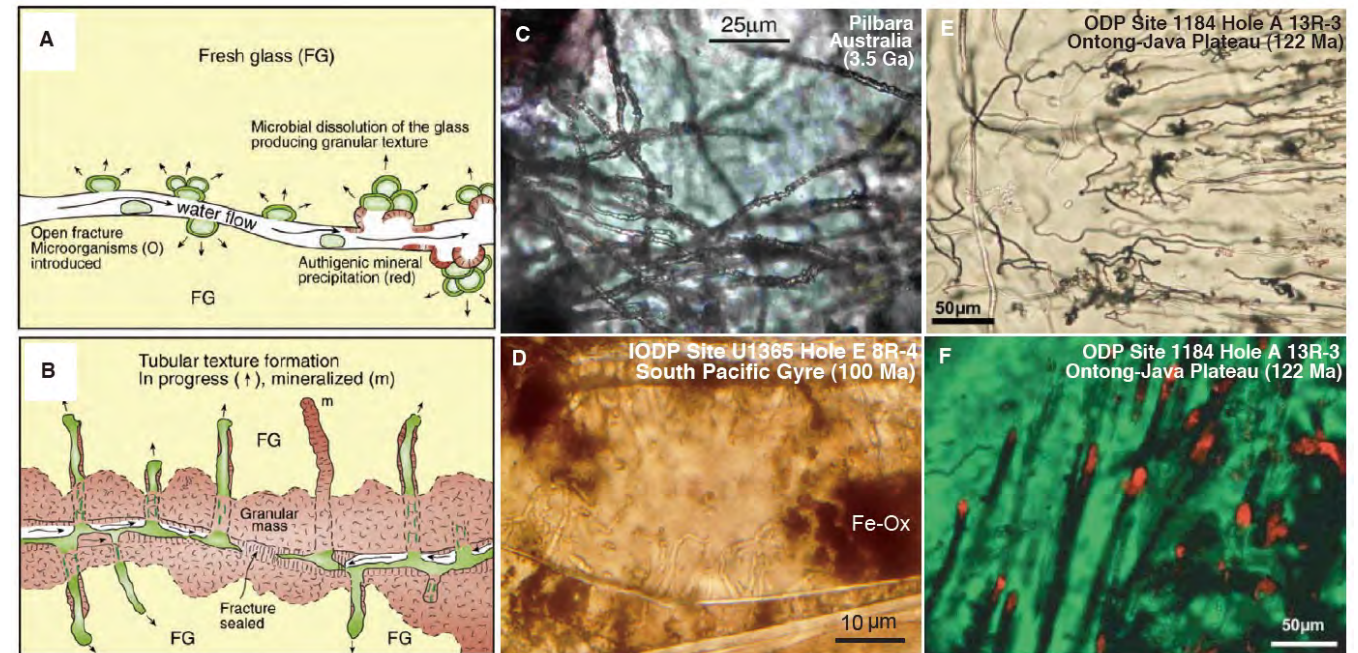




Fisk et al., Science, 1998.

## Alternation of oceanic volcanic glass: Textural evidence of microbial activity

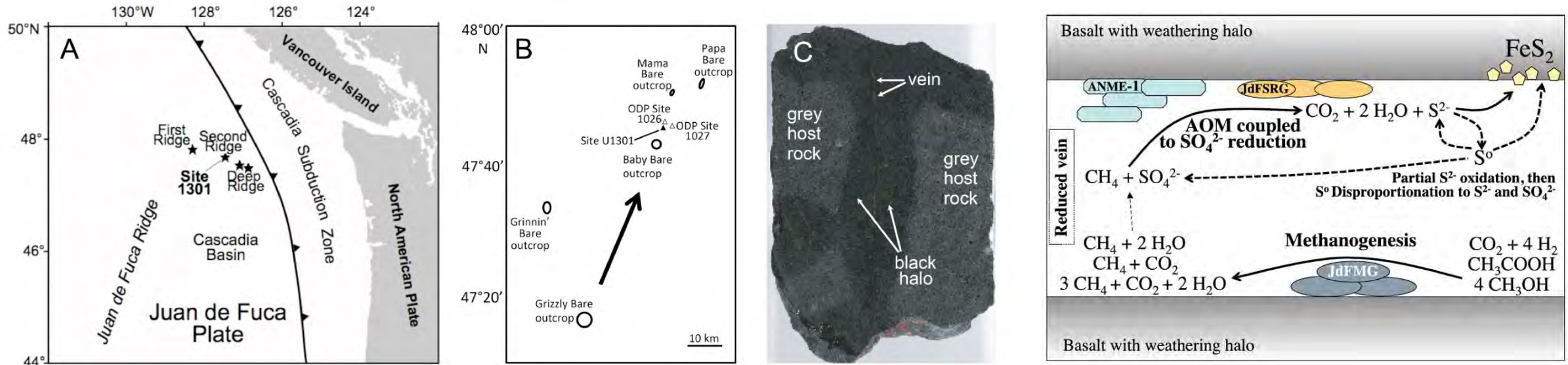
*“...autotrophic microbes living on the chemical energy of Earth’s volcanic rocks could also exist within other planetary bodies where fluid flow establishes gradients in redox potential, as long as there are sources of required nutrients, water, and carbon.”*



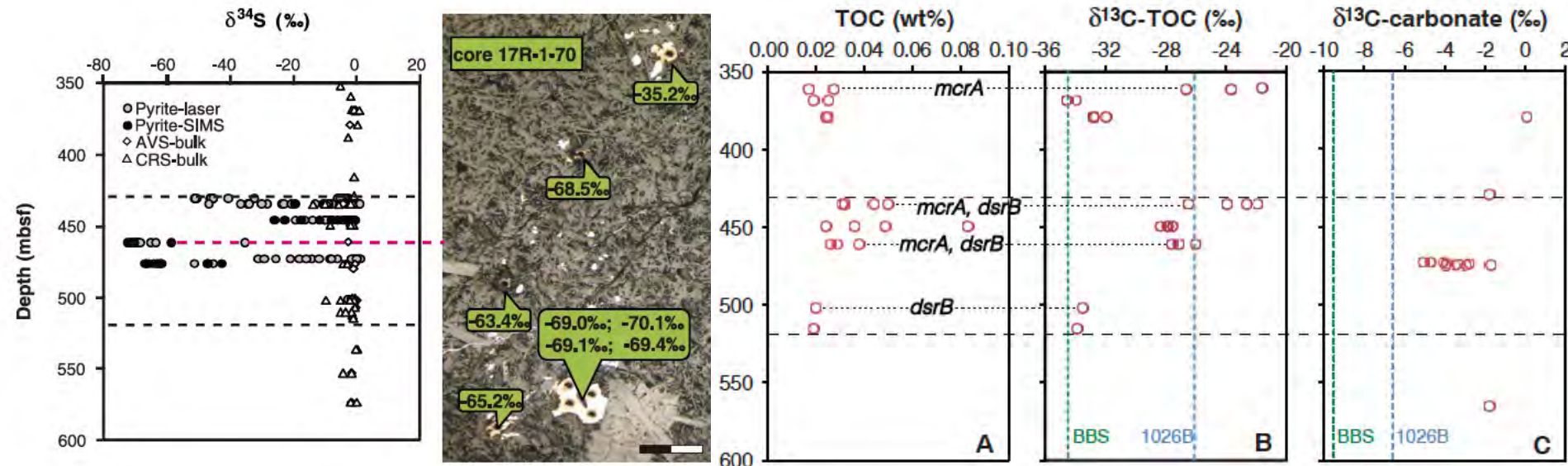
IODP Science Plan 2013-2023.



# Direct evidence for microbial activity in 3.5 Ma-oceanic basalt



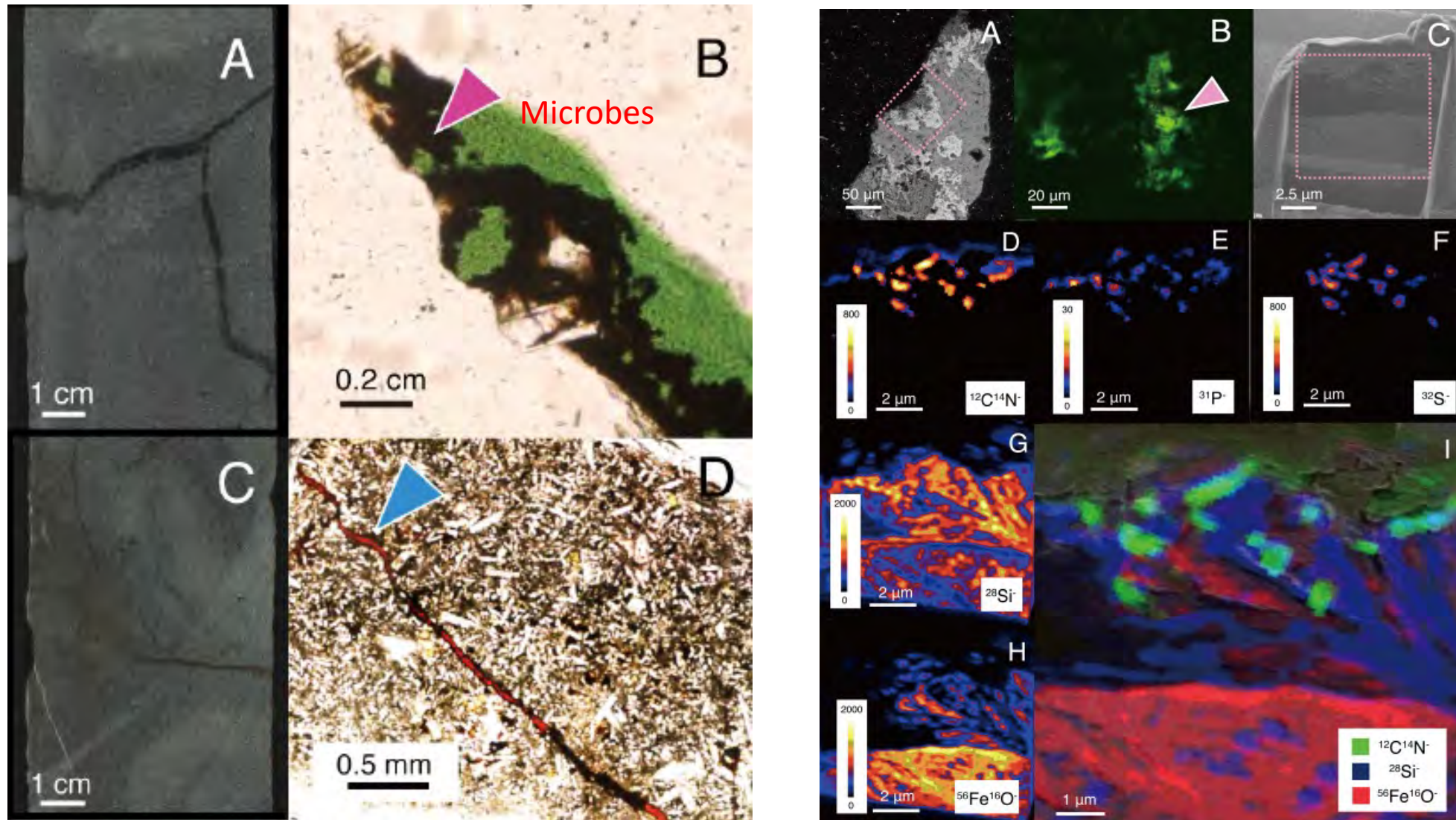
Site 1301, IODP Expedition 301



"Downcore changes in carbon and sulfur cycling show **discrete geochemical intervals** with chemoautotrophic  $\delta^{13}\text{C}$  signatures locally attenuated by heterotrophic metabolism."



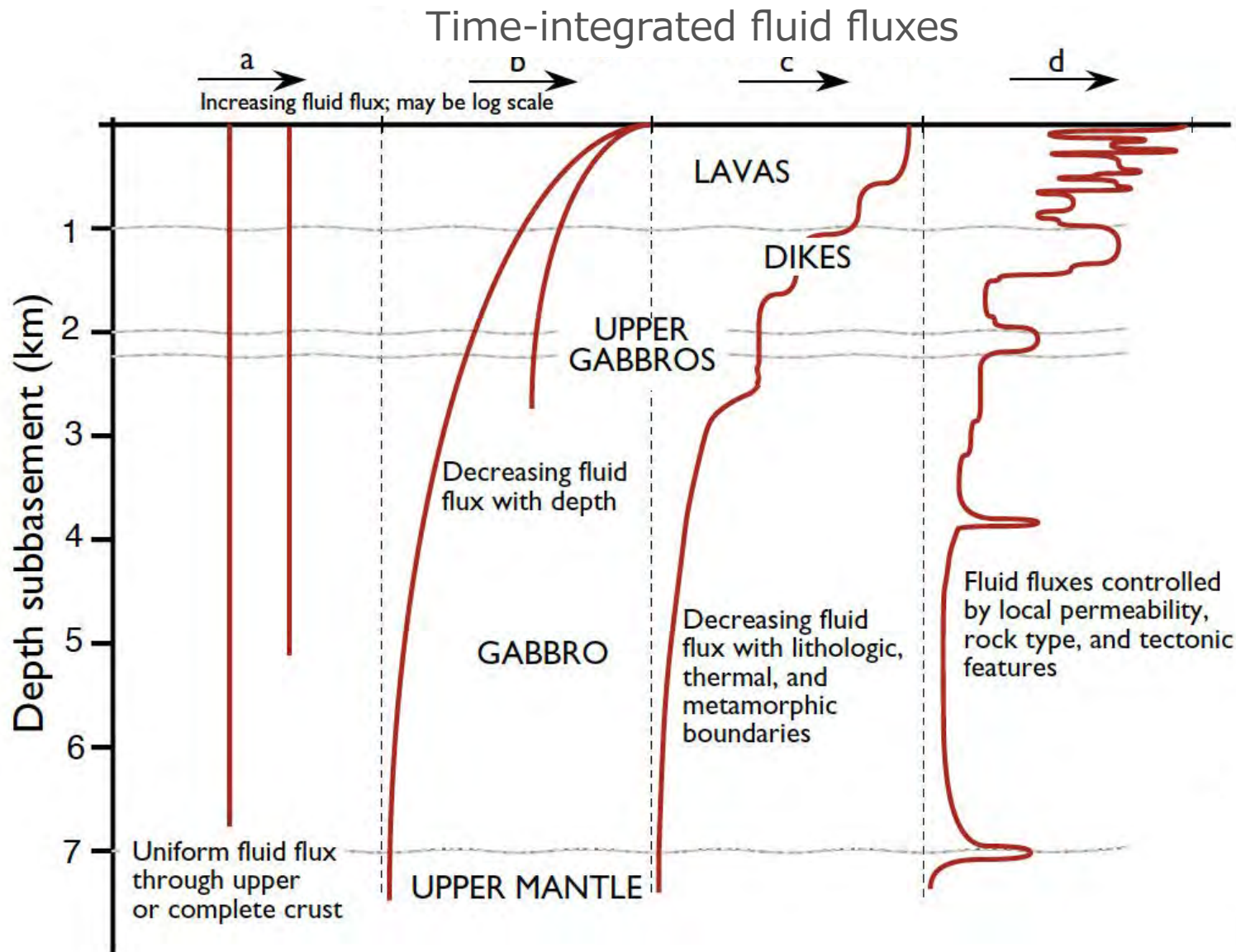
## Expansive microbial habitats in ~100 Ma-aged crustal fractures



Dense microbial populations within nontronite-filling veins (like biofilms)



# Is there life in the deep oceanic crust (and even in the upper mantle)?



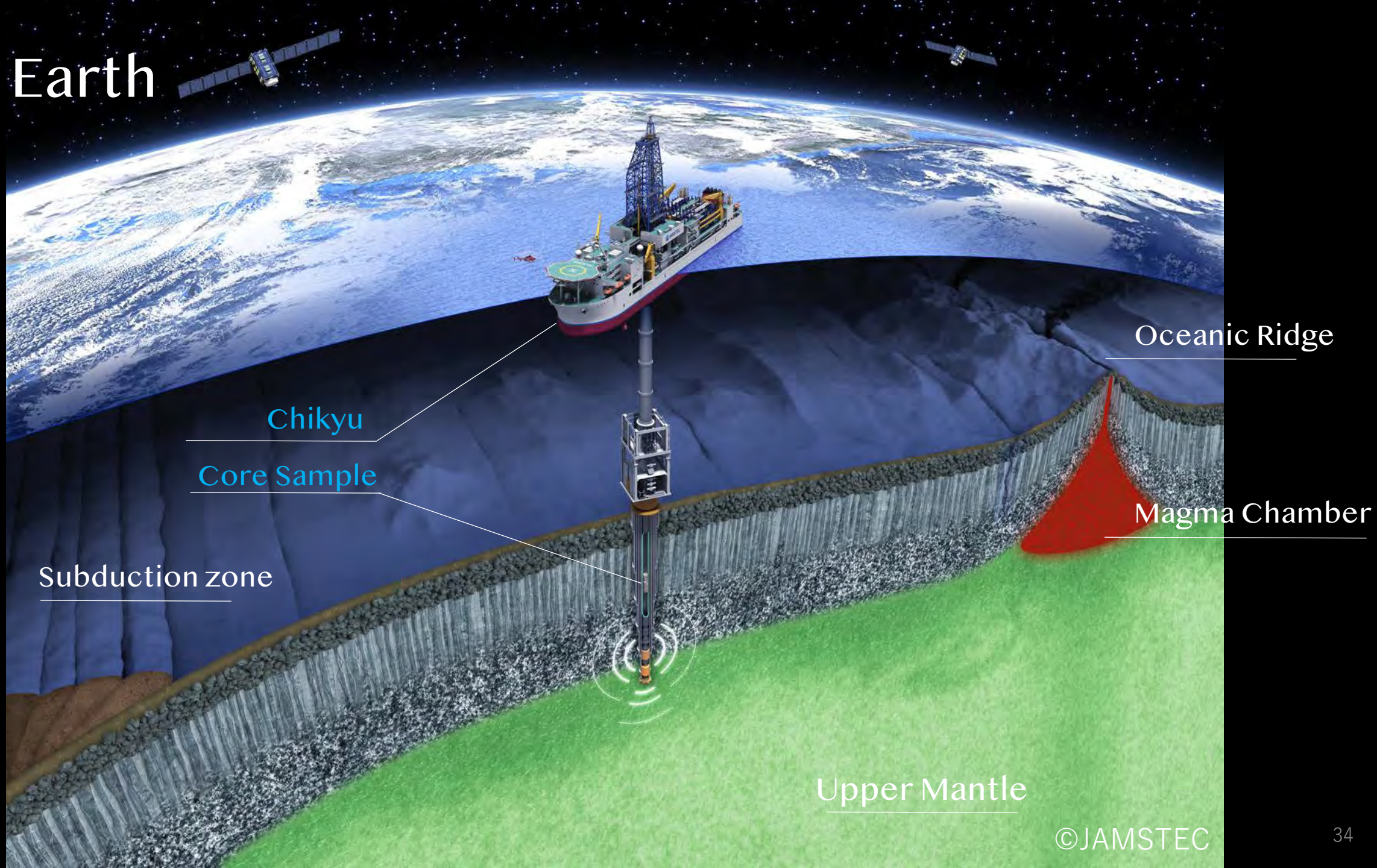
## Primary hypothesis

In oceanic crust cooler than the thermal limit to life, water-rock interactions sustain microbial life as deeply as seawater-derived fluid penetrates.

- ✓ Microbes in deep oceanic crust are strongly associated with specific secondary mineral phases.
- ✓ The composition of microbial communities and the processes that sustain the communities change with depth in the crust, in response to lithologic boundaries and/or chemical and physical gradients.



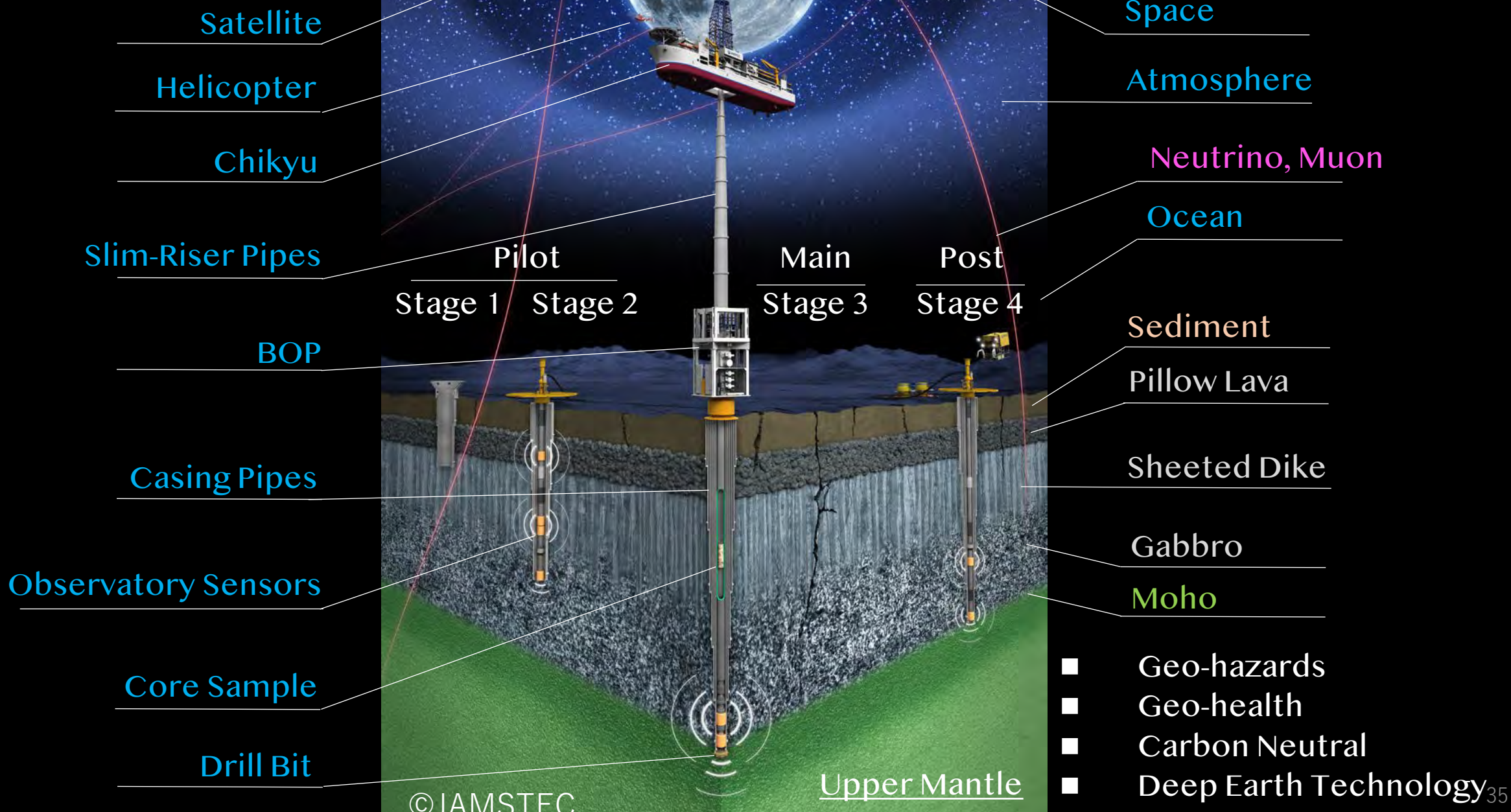
# The Earth





# Technologies

# Nature





## Acknowledgement

- ODP/IODP, JR and Chikyu crews/operators
- Globally distributed collaborators and friends (esp. ODP Leg 201, IODP Exp. 329, 337, 370)
- Kochi Core Center, MarE3, Mantle Drilling Promotion Office, JAMSTEC
- J-DESC, Deep Carbon Observatory (DCO), CIFAR Earth4D Program
- Funding agencies: MEXT/JSPS, NSF

