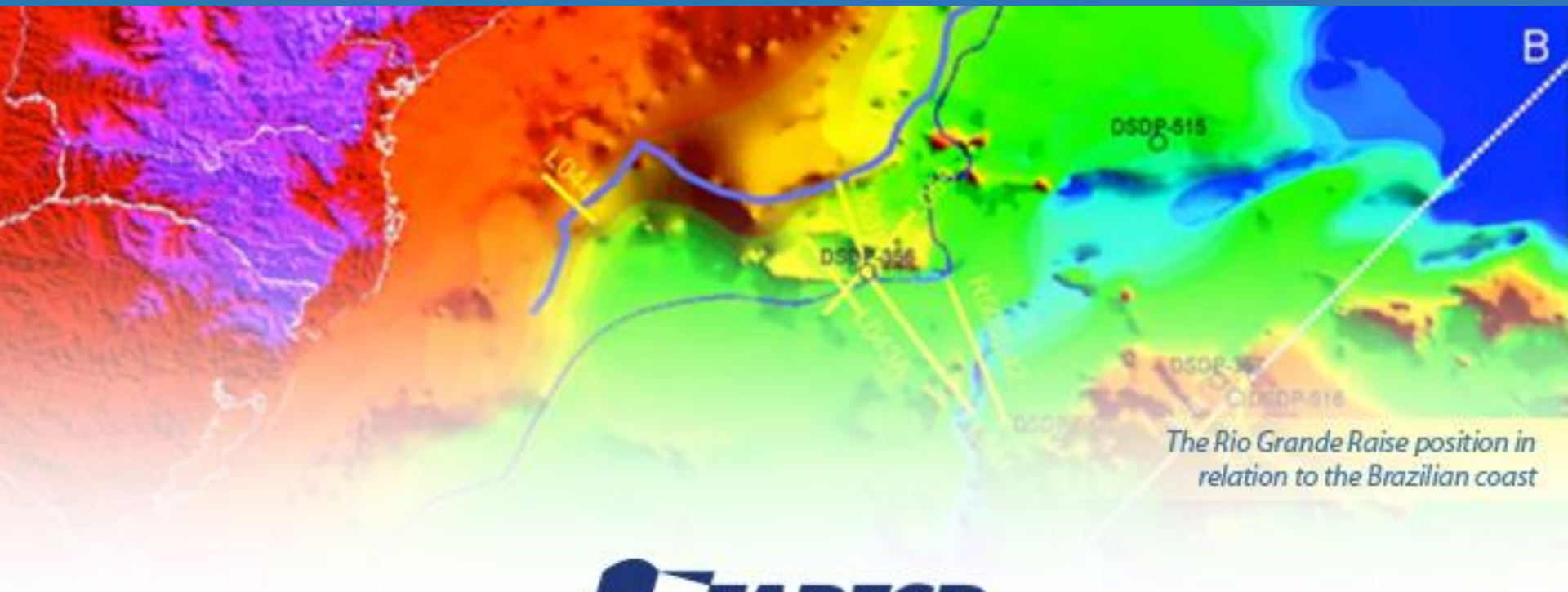
The E-tech Element Submarine Ferromanganese Crusts Research Workshop



FAPESP

FAPESP
December 8th 2015

The Role of Lithotrophyc Bacteria in the Formation of Deep-Ocean Polimetallic Deposits

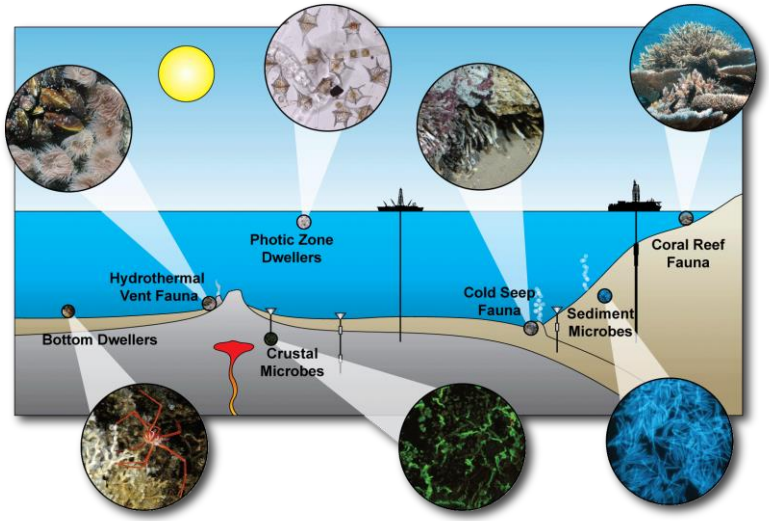
Vivian H. Pellizari

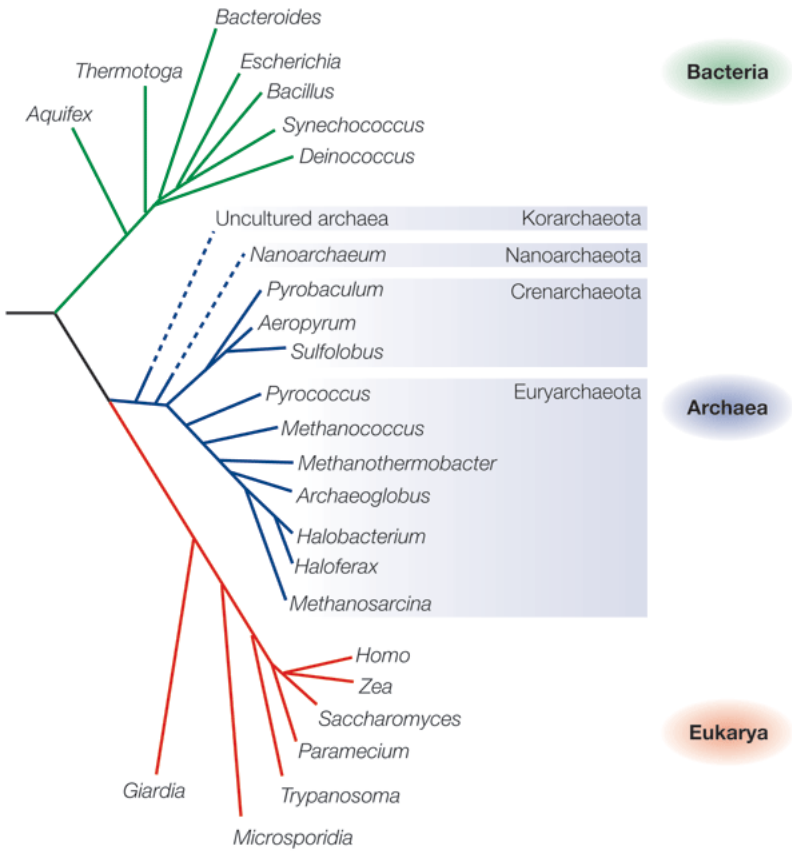
Instituto Oceanográfico - USP

(vivianp@usp.br)

What are the origin, composition, and global significance of subseafloor microbial communities?

- Drilling has revealed that the subsurface biosphere is widespread, large, and genetically and geochemically diverse.





Nature Reviews | Genetics

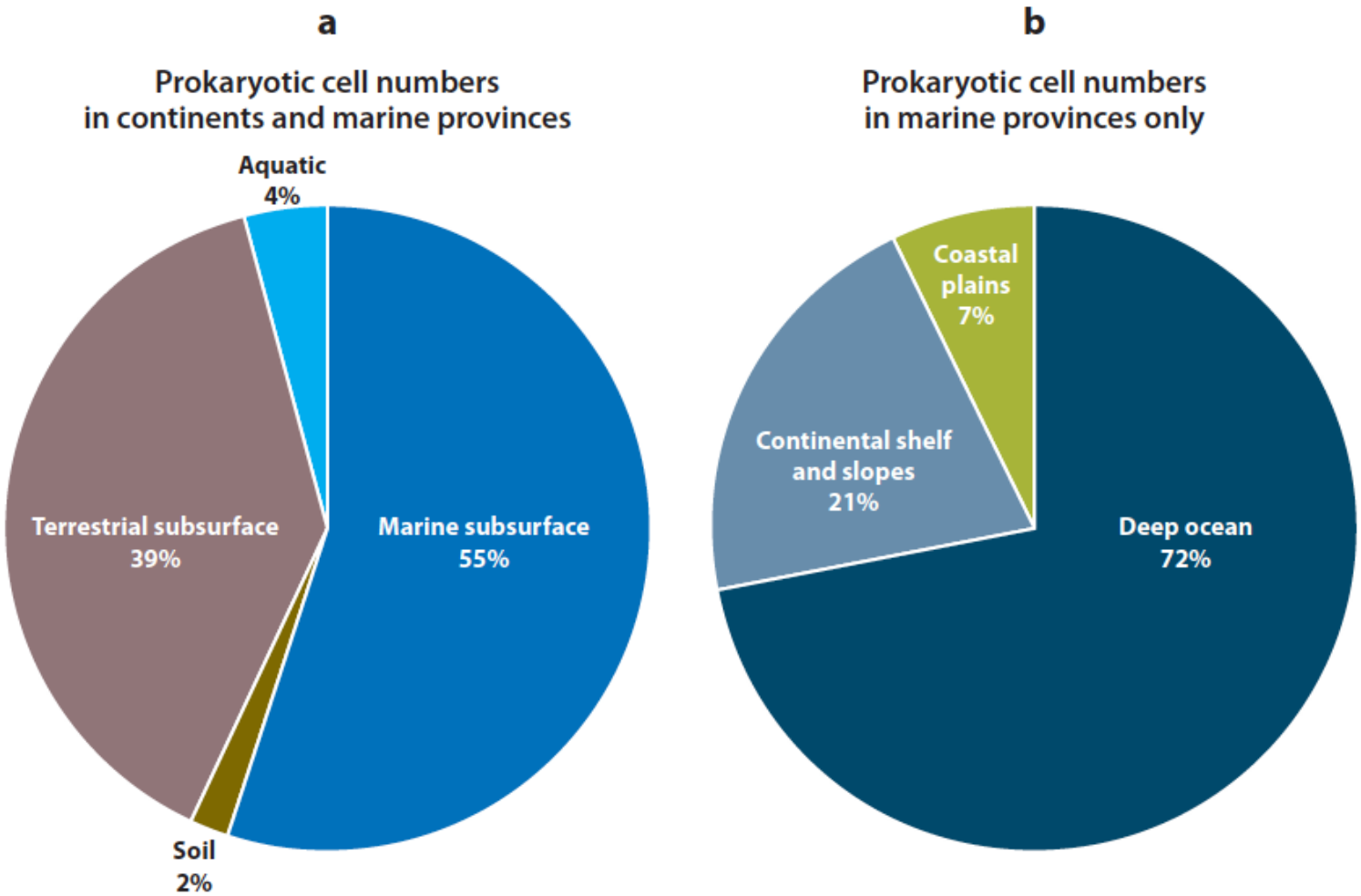


Figure 1

Data plotted from Whitman et al. (1998) for prokaryotic cell numbers represented in major geographical provinces: (*a*) the continents and marine provinces; (*b*) marine provinces only.

Colwell et al. 2012

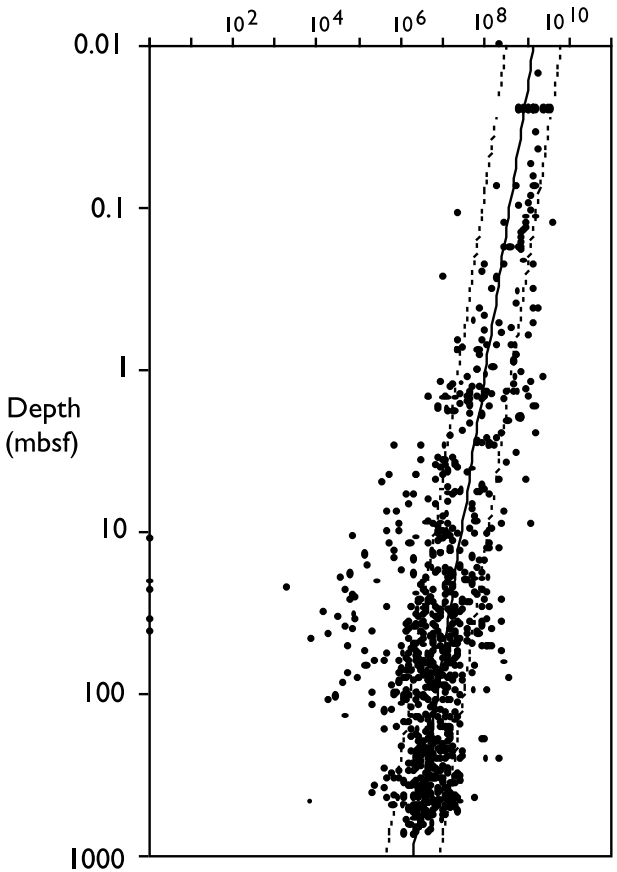


Figure 13. Bacterial abundances as a function of sub-bottom sediment depth as seen in ODP boreholes down to ~ 750 mbsf. Figure reprinted with permission from Parkes, R.J., B.A. Cragg and P.Wellsbury, Recent studies on bacterial populations and processes in subseafloor sediments: A review, Hydrogeol. Jour., 8, Figure 2b, p.15, copyright Springer-Verlag, 2000.

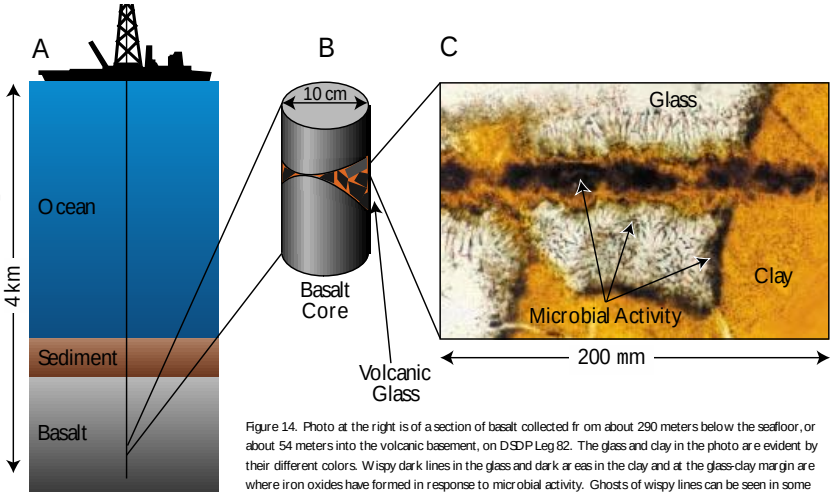


Figure 14. Photo at the right is of a section of basalt collected fr om about 290 meters below the seafloor, or about 54 meters into the volcanic basement, on DSDP Leg 82. The glass and clay in the photo are evident by their different colors. Wispy dark lines in the glass and dark ar eas in the clay and at the glass-clay margin are where iron oxides have formed in response to micr obial activity. Ghosts of wispy lines can be seen in some areas of clay near the glass. ODP samples have established that this type of alteration exists thr oughout the

Deep microbial communities play an important role in global biogeochemical cycles, mineral alteration, and hydrocarbon production and destruction.

Sediment Biogeochemical Zonation

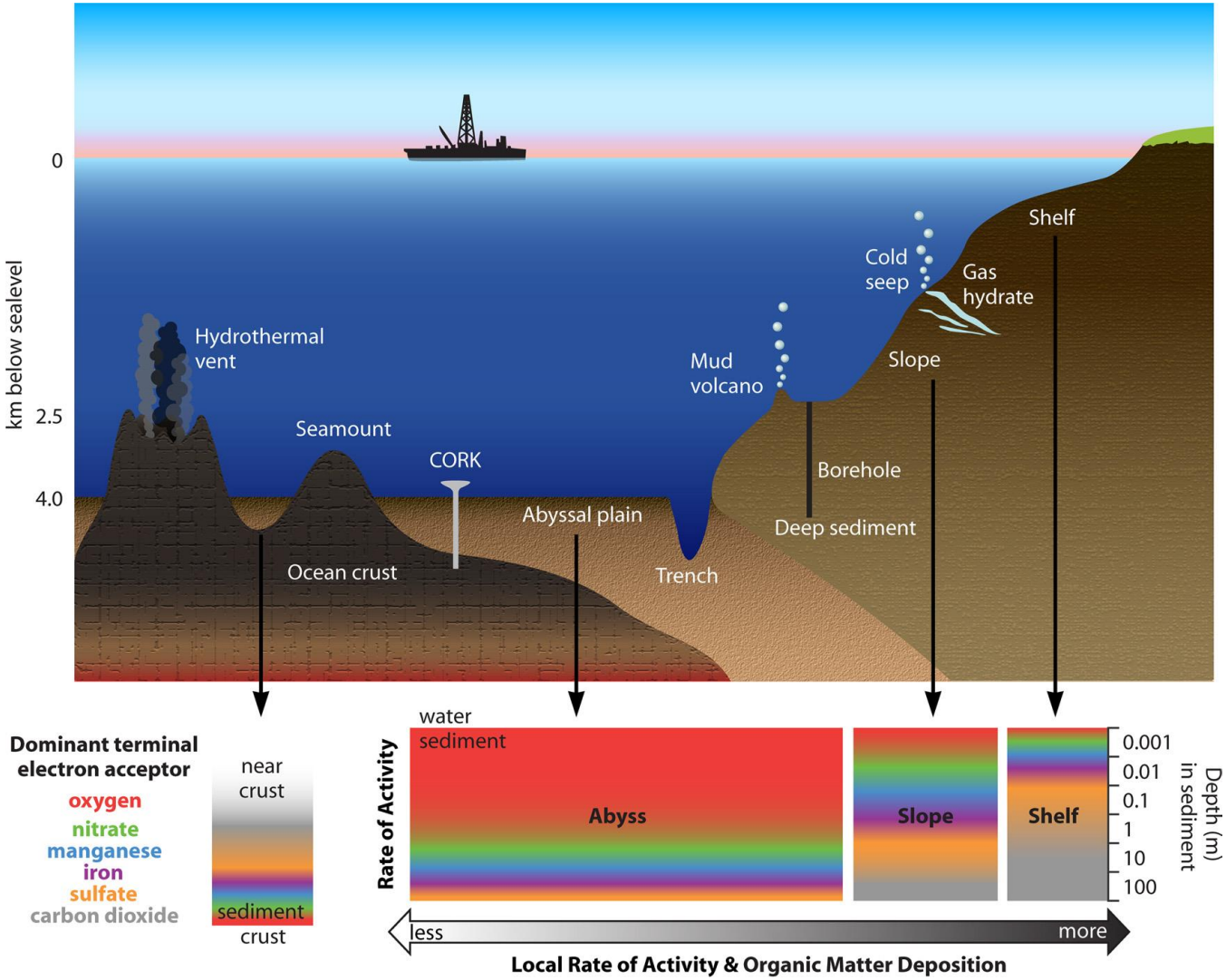


FIG. 2. Schematics depicting a stylized cross section of dark ocean habitats (top; adapted from reference 259 by permission of Macmillan Publishers Ltd., copyright 2007) and representations of sediment biogeochemical zonation (bottom). Note that the upper panel is not drawn to scale. In the lower panel, dominant electron acceptors in the various sediment habitats are indicated by vertical depth into sediment (note the logarithmic sediment depth scale). The relative quantity of organic matter deposited in each sediment type and the scale of metabolic rates in sediment are indicated by the grayscale bar, with dark shades indicating higher rates.

Biomineralization

- Biomineralization is an important component for the initiation of both nodule and crust formation.
- The processes of mineralization follow exclusively chemical and physical principles and reactions and involve the accumulation of inorganic materials from solution without any participation of organic molecules.

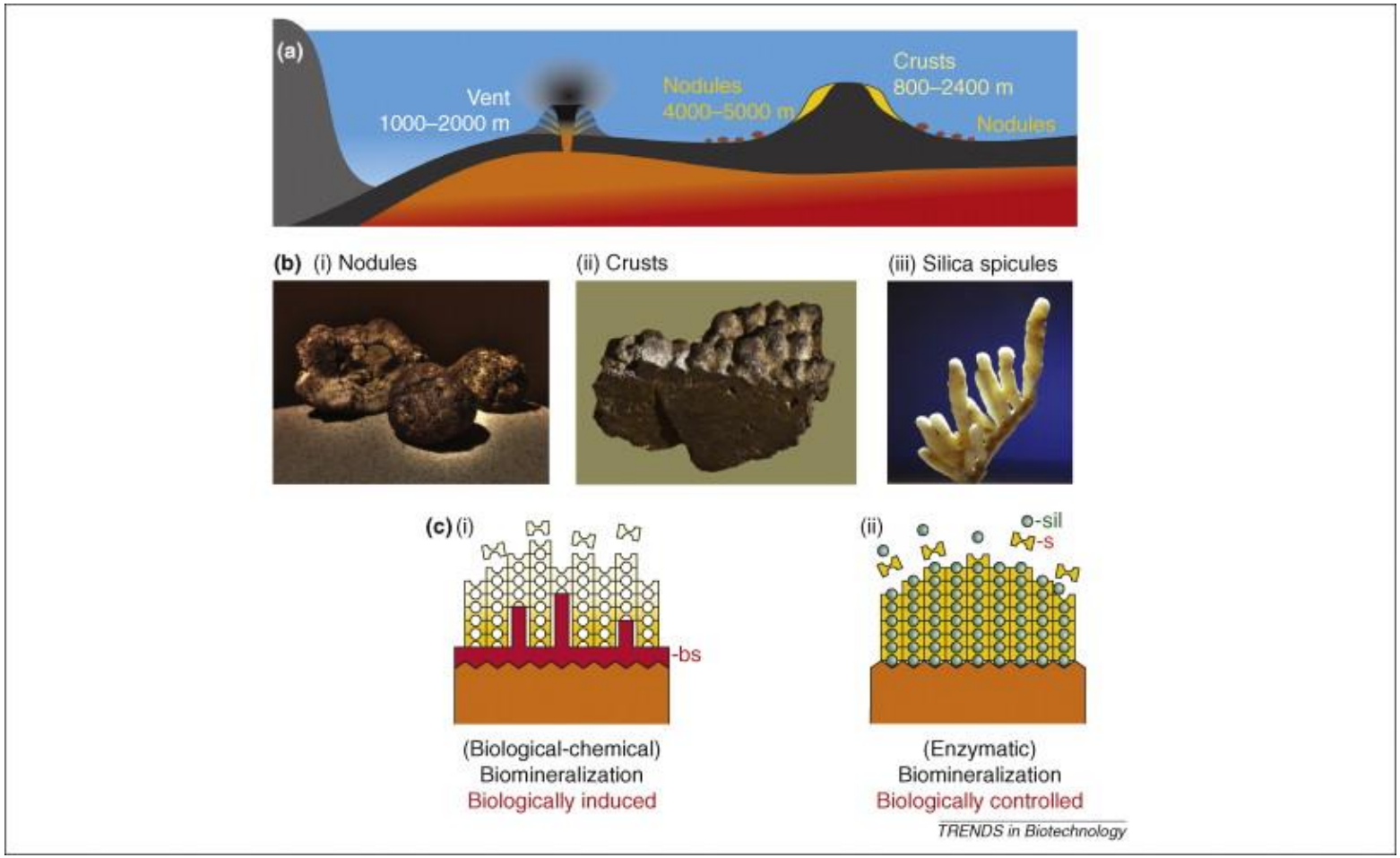


Figure 1. Categories of biomineralization. (a) Main types of seabed mineral resources and their deposition sites. (b) Examples of biominerals; shown are nodules (i), crusts (ii) and silica spicules (iii). (c) Schematic illustrations of biomineralization from inorganic buikding blocks (yellow)

http://dx.doi.org/10.1016/j.tibtech.2009.03.004 **Marine biominerals: perspectives and challenges for polymetallic nodules and crusts**

What is the role of microorganisms in deep-sea Fe-Mn deposit formation and E-tech element concentration?

The role of microorganisms in precipitation of Fe-Mn nodules was proposed shortly after the nodules were and that hypothesis has persisted and gained experimental support.

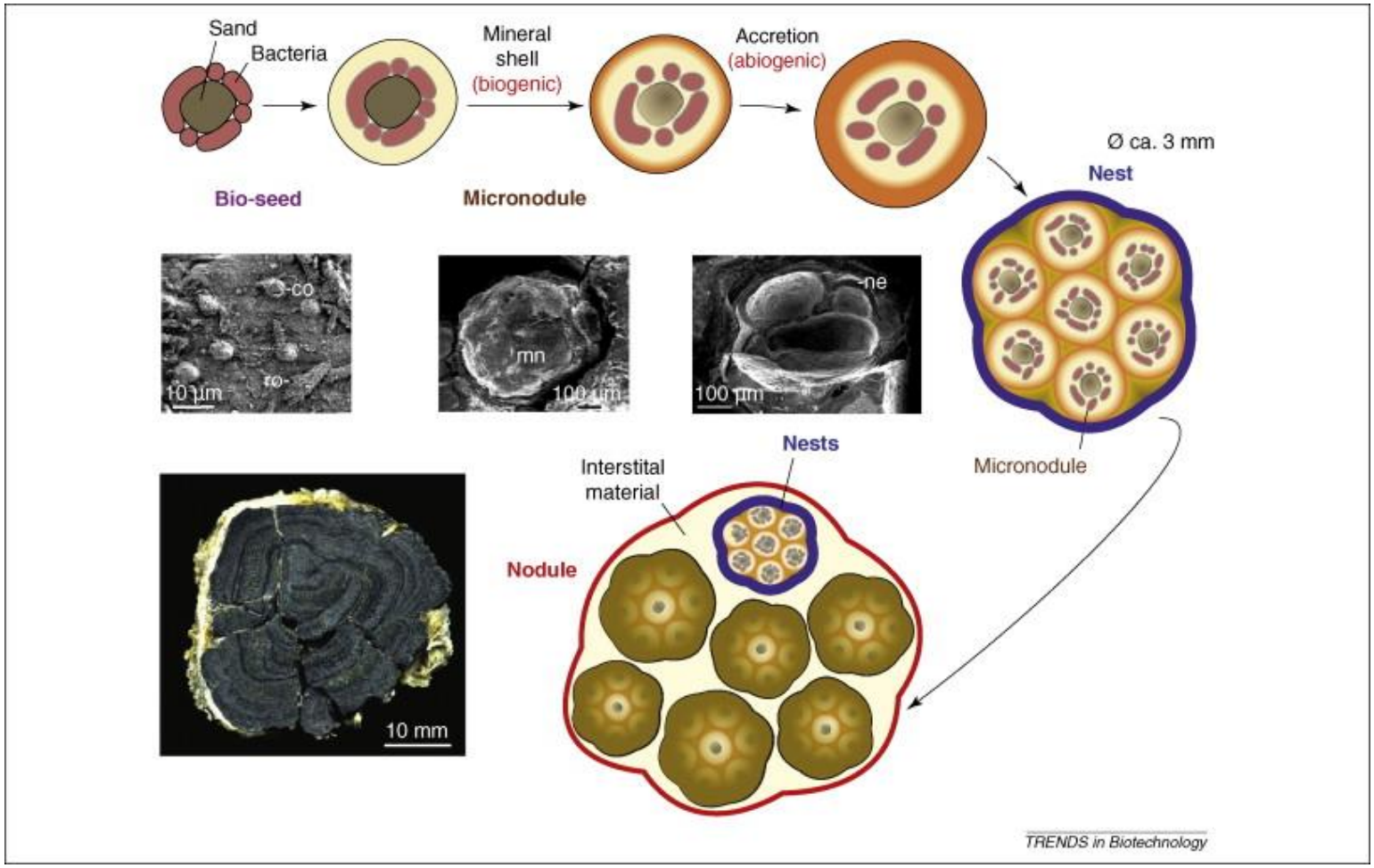


Figure . Schematic illustration of the formation of polymetallic nodules from micronodules. Initially, a bio-seed is formed when bacteria adhere to a substrate formed of minute clay/sand aggregates. This bio-seed (magenta) functions as a template and allows the progression of mineralization through abiogenic processes, which lead to the formation of micronodules (brown) that are further assembled into nests (blue) and, finally, into a nodule (red) formed of many nests. Nodules are formed under (rotating) movements caused by, for example, water currents on the sea floor, and additional inorganic abiogenic material is incorporated. The HR-SEM images shown exemplify, from left to right: bio-seeds formed from bacteria (rods [ro] and cocci [co]); micronodules (mn); and a nest (ne).

Marine biominerals: perspectives and challenges for polymetallic nodules and crusts

http://dx.doi.org/10.1016/j.tibtech.2009.03.004

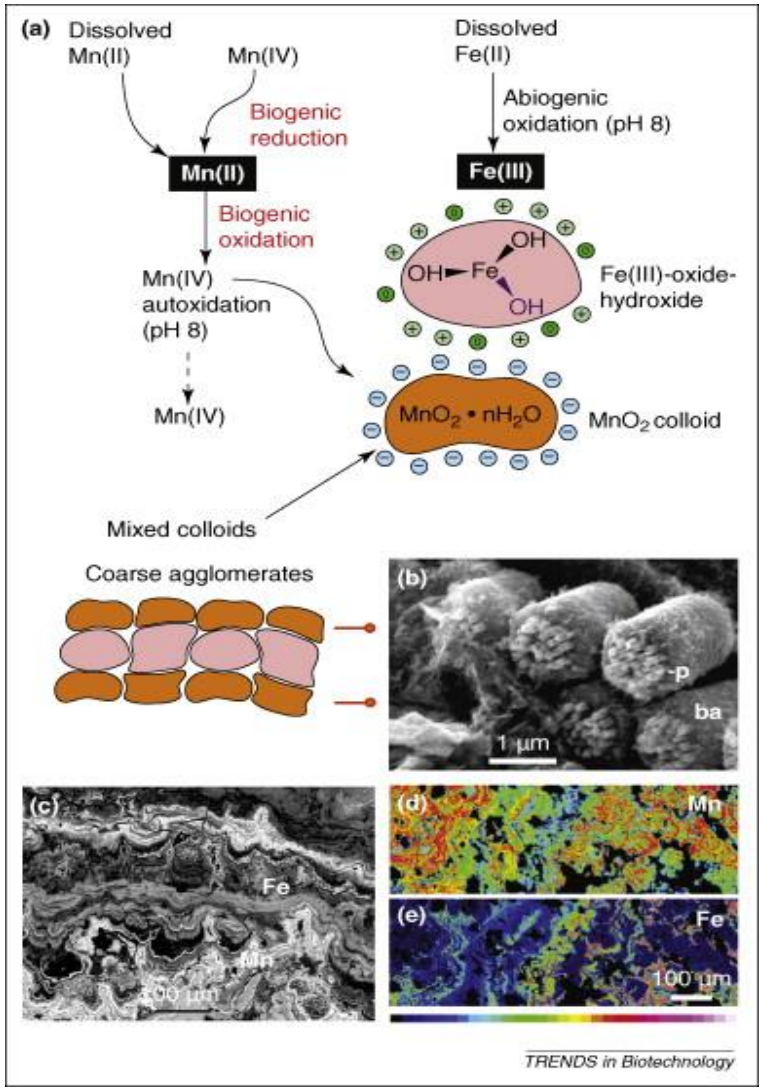


Figure . Formation of mixed colloids of Mn- and Fe-oxide-hydroxides through biogenic oxidation of Mn(II) and abiogenic oxidation of Fe(II). (a) Schematic illustration of the steps leading to the formation of Mn(II) and Fe(III), to Fe(III)- oxide-hydroxides and to Mn(IV)-oxides and, finally, to their respective colloids. These colloids increase in size until layers of Fe(III)-colloids (pink) and Mn(IV)-colloids (orange) are formed. (b) The formation of Mn-colloids is promoted by endolithic bacteria (ba) that are covered with S-layer. Individual bacteria are decorated with pillar-shaped protrusions (p) that are embedded into the Mn-rich region of the metallic zone (see panel (d)). (c) This polished cut through a nodule shows the concentric arrangement of Fe-rich and Mn-rich layers. Mn: Mn-rich region; Fe: Fe-rich region. (d,e) X-ray mapping of a nodule crosscut confirms that separate layers of Mn (d) and Fe (e) exist. Blue indicates low levels of Mn or Fe and red indicates high levels of Mn or Fe.

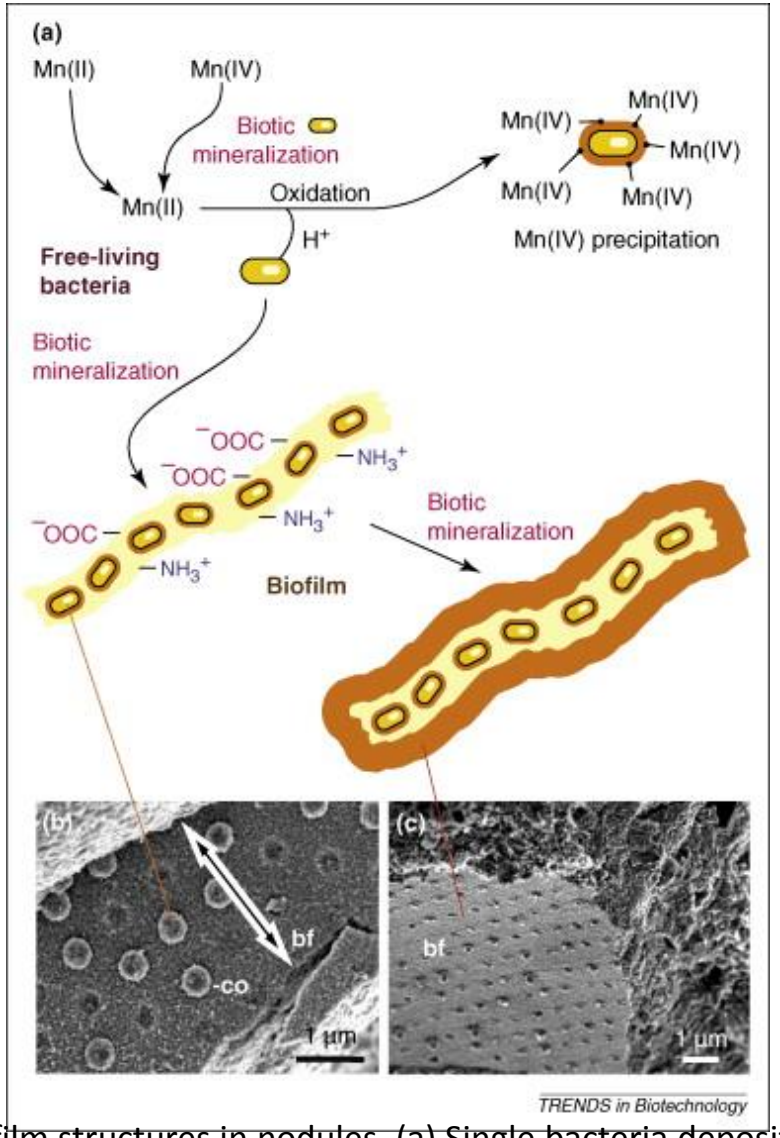


Figure . Formation of biofilm structures in nodules. (a) Single bacteria deposit Mn(IV) on their surface through biogenic oxidation. Subsequently, isolated bacteria form an extracellular biofilm carrying both negative (carboxyl-groups) and positive (amino-groups) residues. (b) HR-SEM image of a biofilm (bf; indicated by double arrow head) into which cocci (co) are embedded. (c) HR-EM image of an empty biofilm (bf) from which the bacteria have been detached, leaving behind holes.

Marine biominerals: perspectives and challenges for polymetallic nodules and crusts

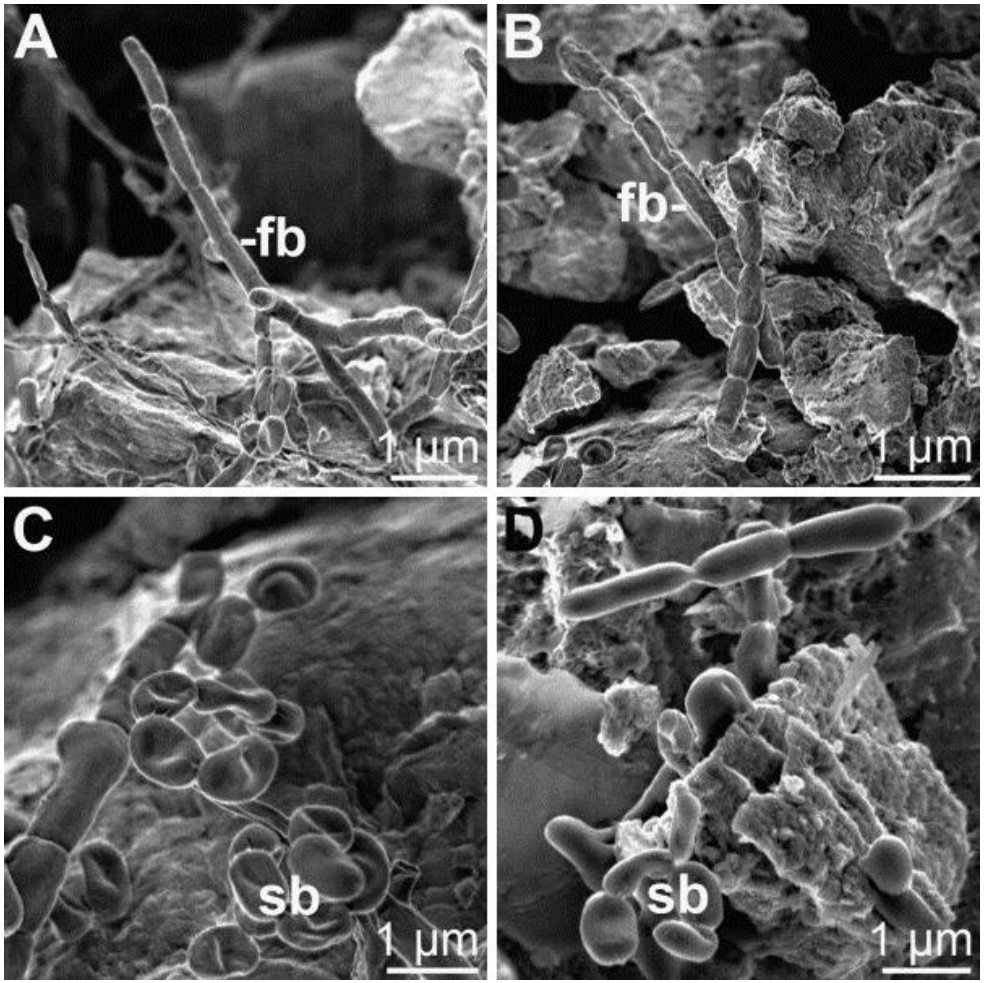
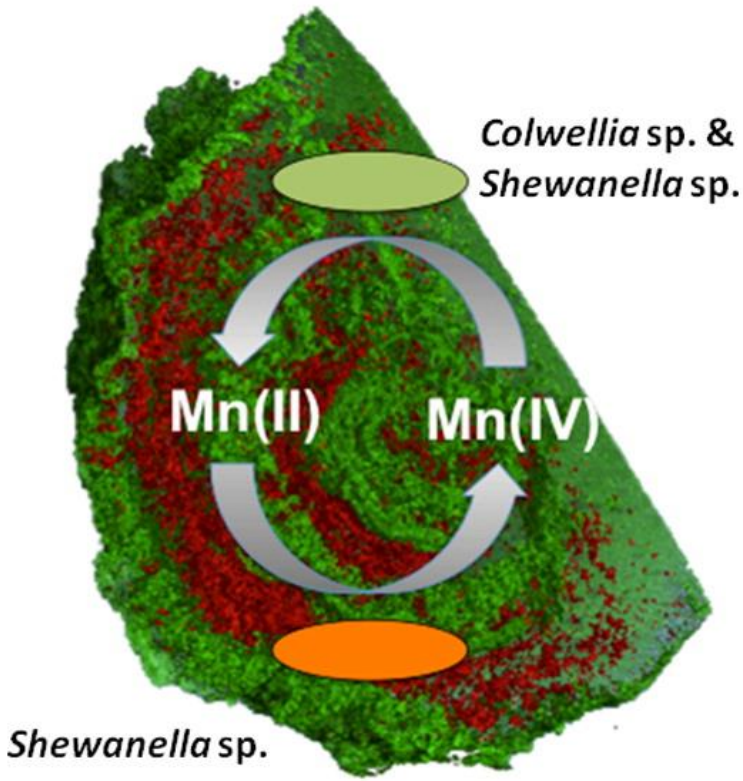


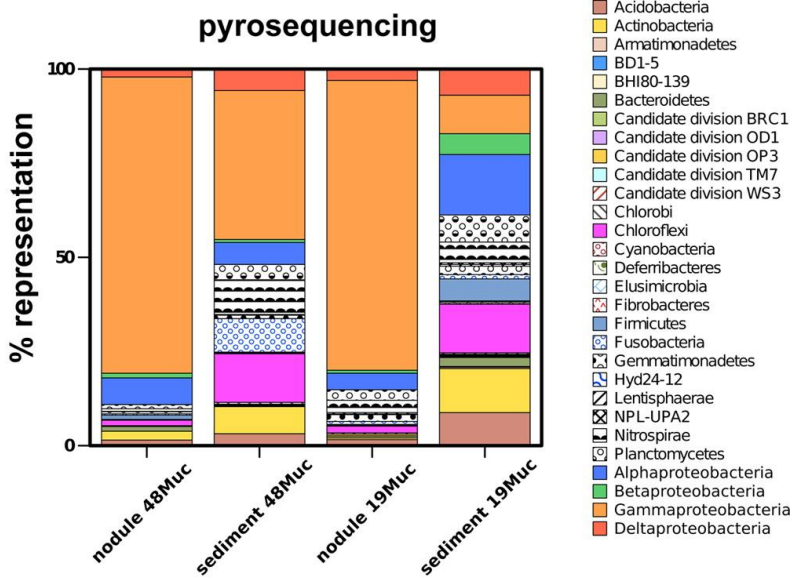
Fig. 3. Microbial assemblages in the outer region of the nodules [exolithobiontic colonization]. (A and B) Within the surface zone, in the micro-canals, filamentous (likely to be) bacteria/actinobacteria (fb) exist which comprise a smooth surface. (C and D) In...

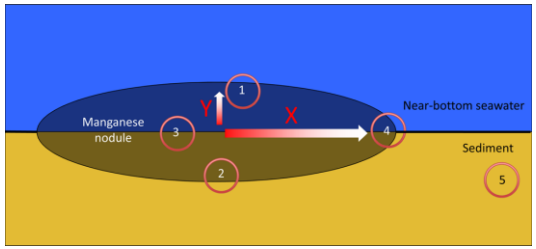
Manganese/polymetallic nodules: Micro-structural characterization of exolithobiontic- and endolithobiontic microbial biofilms by scanning electron microscopy ⋆

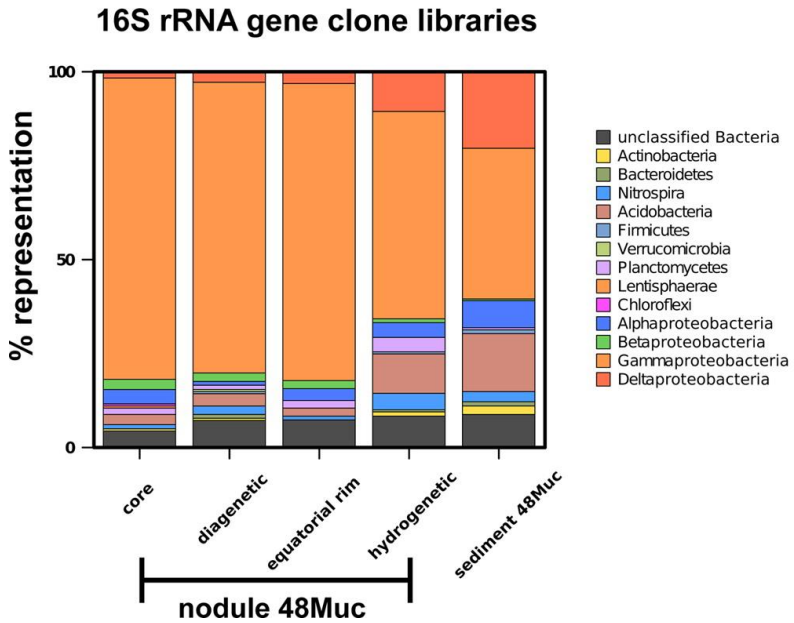


Polymetallic nodules provide a suitable habitat for prokaryotes with an abundant and diverse prokaryotic community dominated by nodule-specifi c Mn(IV)-reducing and Mn(II)-oxidizing bacteria. The high abundance and dominance of Mn-cycling bacteria in the manganese nodules argue for a biologically driven closed manganese cycle inside the nodules relevant for their formation and potential degradation.

Published in: Marco Blöthe; Anna Wegorzewski; Cornelia Müller; Frank Simon; Thomas Kuhn; Axel Schippers; *Environ. Sci. Technol.* **2015,** 49, 7692-7700.
DOI: 10.1021/es504930v







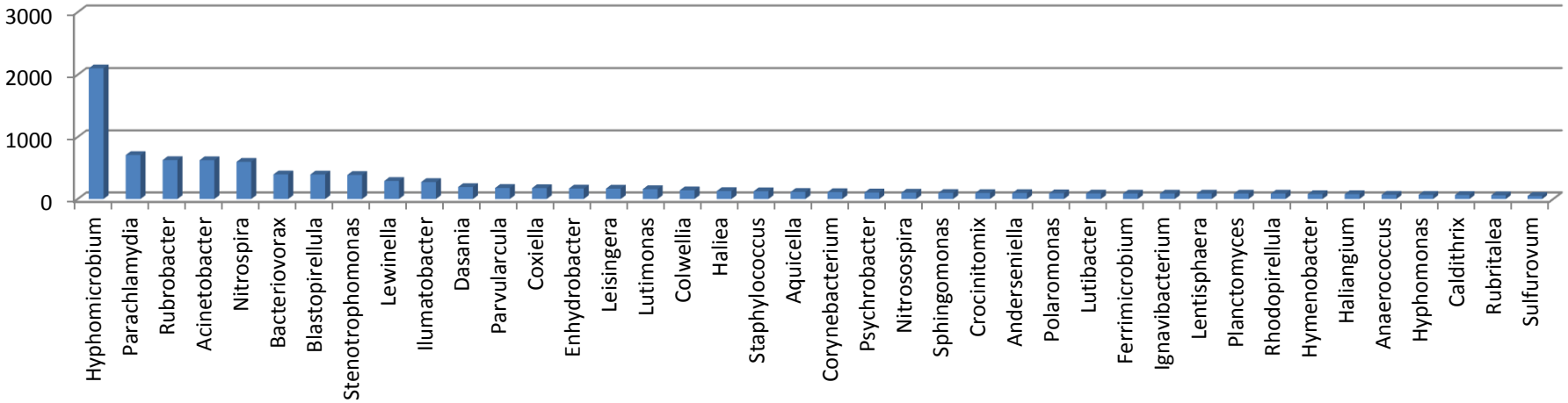
Representation of bacterial lineages obtained via pyrosequencing of DNA from whole nodules and 16S rRNA gene clone libraries from different parts of a manganese nodule (hydrogenetic, diagenetic, core, and equatorial rim; Figure 1) and the surrounding sediment from the Clarion Clipperton Zone.

Published in: Marco Blöthe; Anna Wegorzewski; Cornelia Müller; Frank Simon; Thomas Kuhn; Axel Schippers; *Environ. Sci. Technol.* **2015,** 49, 7692-7700.
DOI: 10.1021/es504930v

Prokaryotic abundance and community diversity of sediment samples of Sao Paulo Plateau

Leg 2- Iatá-piuna cruise

Metagenomic (16S rRNA) Consensus Ranking of Classified Bacteria (> 50 reads)



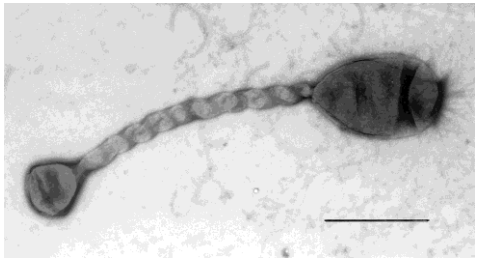
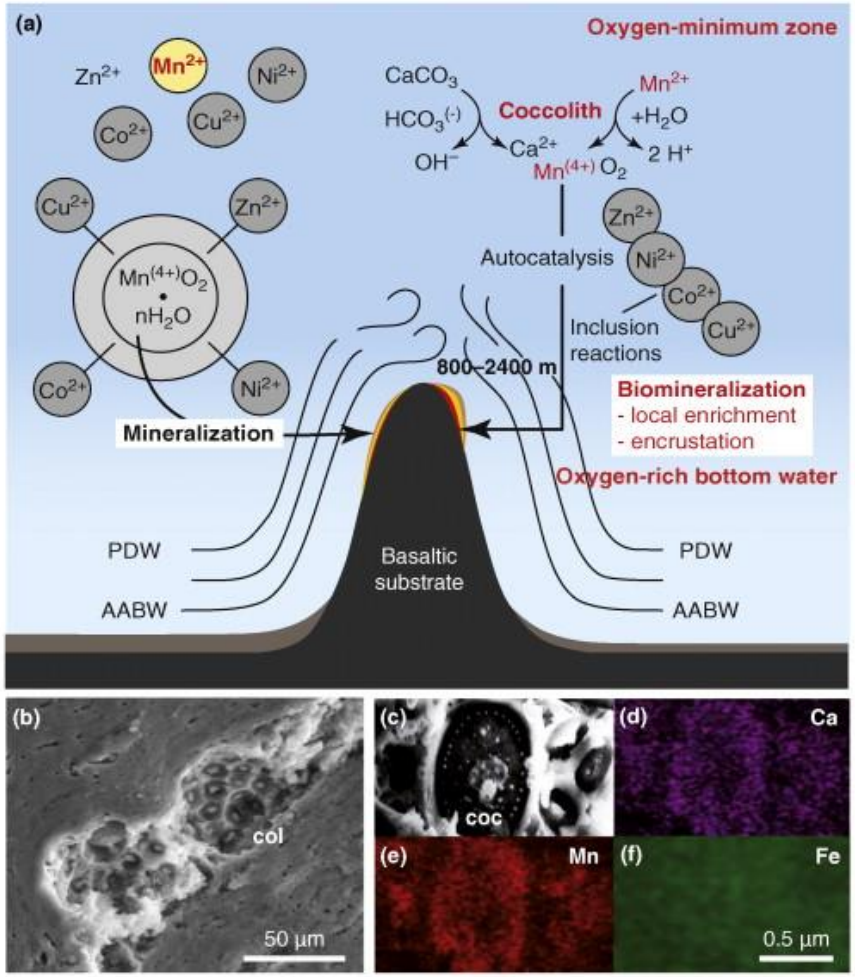


FIG. 2. *Hyphomicrobium* sp. strain IFAM 1465, from a sewage treatment plant, showing helically twisted hypha. Bar, 1 μm.

Hyphomicrobium.
Mn(II) oxidation



Co-rich crust formation. (a) The crusts are formed at the interface between the upper oxygen-minimum zone (OMZ) and the lower oxygen-rich bottom zone (ORZ) in deep water. The bottom oxygen-rich layer originates from two sources, the Pacific Deep Water (PDW) and the Antarctic Bottom Water (AABW). On the left, colloid-chemical processes are indicated, which result in the adsorption of heavy metals by Mn-oxyhydroxide (Mn(IV)) colloids, resulting in their mineralization at the seamount surface. On the right, the chemical transformation processes are shown that are likely to occur in coccolithophores during their sinking from the photic zone to the region of crust formation. Their skeletal CaCO3 is replaced by Mn-oxide (Mn(IV)). Thereafter, precipitation of Mn(IV)-oxide proceeds. Finally the particles attach to the basalt (shown in red), initiating large-scale encrustation (indicated in yellow].

Assessment of the role of microbes in E-tech element concentration and cycling and implications for bioprocessing

- **to compare abundance and composition of microorganisms (prokaryotes / eukaryotes):**
  - flow cytometry, microscopy, phylogenetic genomic analyses including fluorescence in situ hybridizations, enrichment cultures inhabiting intact surfaces of Fe-Mn crusts and overlaying seawater at selected sites across the sea-mount
- **In situ seafloor experiments will assess microbial re-colonization of perturbed crust surfaces after cutting**.
- **Crusts, crust-overlaying seawater and colonized surfaces will be collected for metagenomic analyses and isolation/cultivation of associated microorganisms.**
  - Follow-up laboratory experiments on enrichment and pure cultures of isolated microorganisms will examine their potential role in E-tech element co-precipitation dependence on nutrient conditions (Isotope tracer, cell sorting, analytical microscopic and spectroscopy techniques)

Biotechnology

- Microbial-driven manganese cycling may also provide a chance for developing a biomining process for the recovery of relevant metals from manganese nodules.
- The selection of suitable S-layer proteins will be a crucial step in any attempt to use recombinant bacteria to efficiently concentrate minor mineral components from seawater and precipitate them onto organic surfaces

Assessment of the impact of crust extraction on the surface of ocean

- Focus on the effect of slurries of studied crusts as well as of crust-overlaying seawater on the biological process – CO2 fixation by the dominant microscopic plants – cyanobacteria and smallest algae.
- How their growth is affected by slurry additions using recently developed radiotracer approach (Zubkov 2014)

Agradecimentos

Mike Zubkov NERC- NOC