



University of Naples "Federico II"

Marine Microbial Diversity

Tell me what you eat: Functional Diversity as a Key Concept

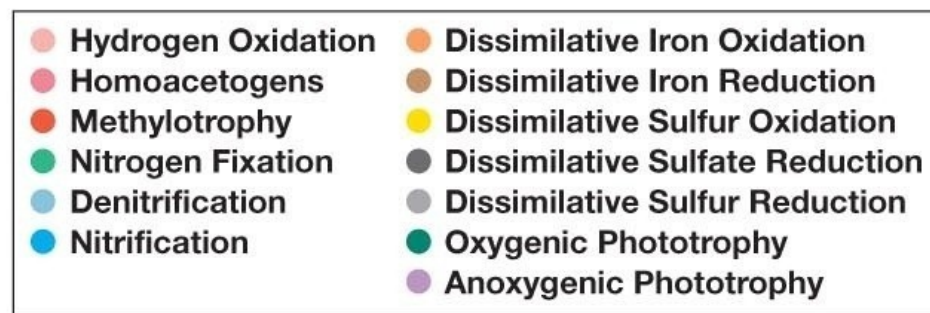
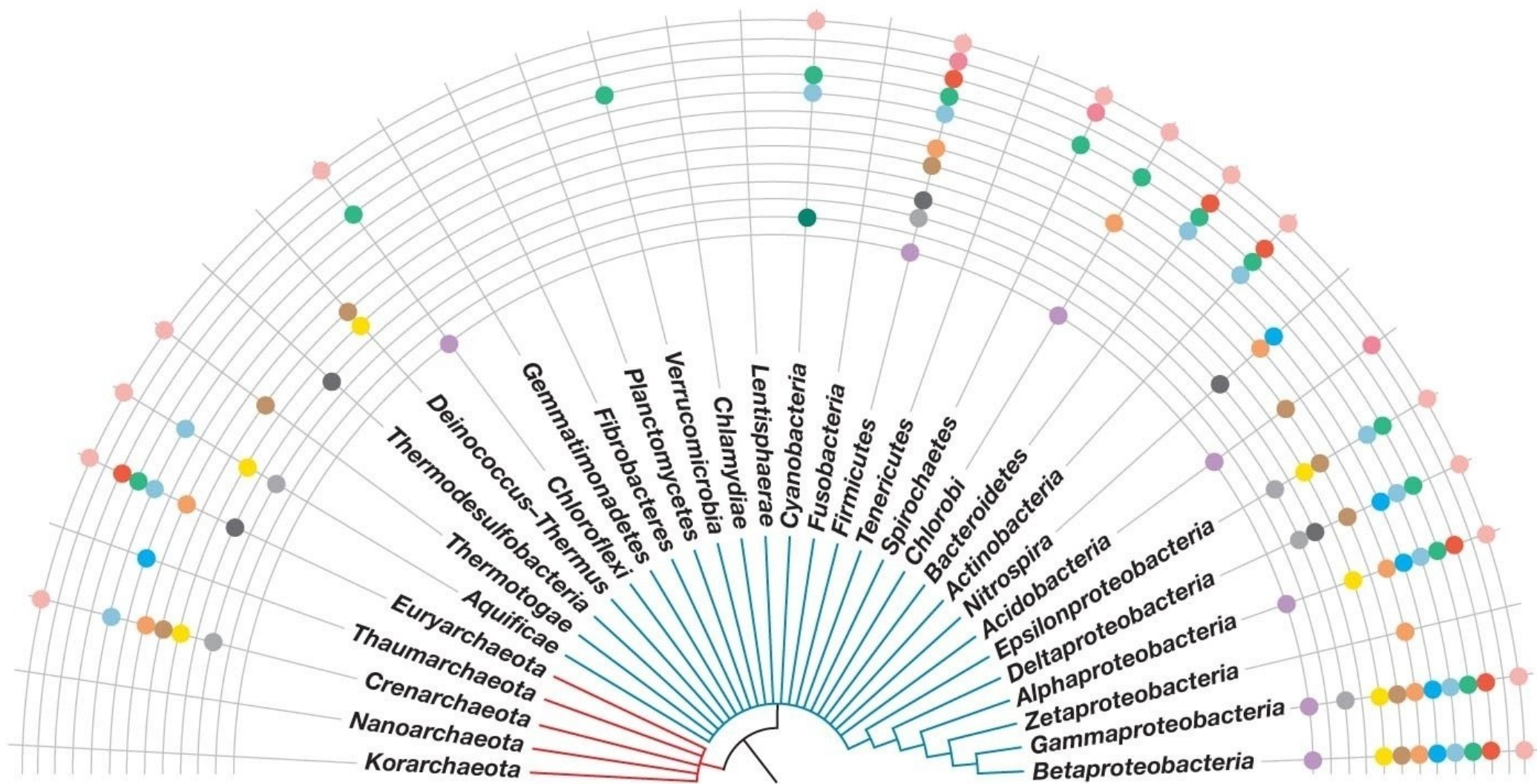
Functional diversity as a concept

- *Phylogenetic diversity* is the component of microbial diversity that deals with evolutionary relationships between microorganisms
- *Functional diversity* is the component of microbial diversity that deals with diversity in form and function as it relates to microbial physiology and ecology
- It is useful to consider microbial diversity in terms of functional groupings because organisms with common traits and common genes often share physiological characteristics and have similar ecological roles
- Functional diversity can be thus further defined in terms of *physiological diversity*, *ecological diversity*, and *morphological diversity*

Functional diversity as a concept

The **divergence** of functional diversity from phylogenetic diversity (i.e., divergent organisms with dissimilar 16S rRNA gene sequences share similar physiology/metabolism) can be explained by at least three reasons:

- The first is **gene loss**, a situation where a trait present in the common ancestor of several lineages is subsequently lost in some lineages but retained in others which over evolutionary time became quite divergent
- The second is **convergent evolution**, in which a trait has evolved independently in two or more lineages and is not encoded by homologous genes shared by these lineages
- The third is **horizontal gene transfer**, a situation where genes that confer a particular trait are homologous and have been exchanged between distantly related lineages



Ecological Redundancy

A characteristic of species within an ecosystem where certain species contribute in equivalent ways to an *ecosystem function* such that *one species may substitute for another*

Note that species that are redundant for one ecosystem function may not be redundant for others

Ecological redundancy is sometimes referred to as *functional compensation*

Ecosystem Function

Ecosystem function refers to one or more specific functions performed by an ecosystem (or group of organisms) in regard of its ability to provide goods and services to other organisms/ecosystems or human society

Examples of ecosystems functions include for example *primary productivity*, *cycling of elements*, providing *nursery to commercially relevant species* and *coastal erosion protection*

One or more species might contribute to a specific ecosystem function

Biogeochemical cycle

Biogeochemical cycle refers to the complex interaction of *physical, chemical, geochemical and biological* interactions and reactions controlling the distribution, form and movement of different *elements* across our planet

A biogeochemical cycle is thus a combination of *abiotic* and *biotic* processes that govern the movement of matter in space and time

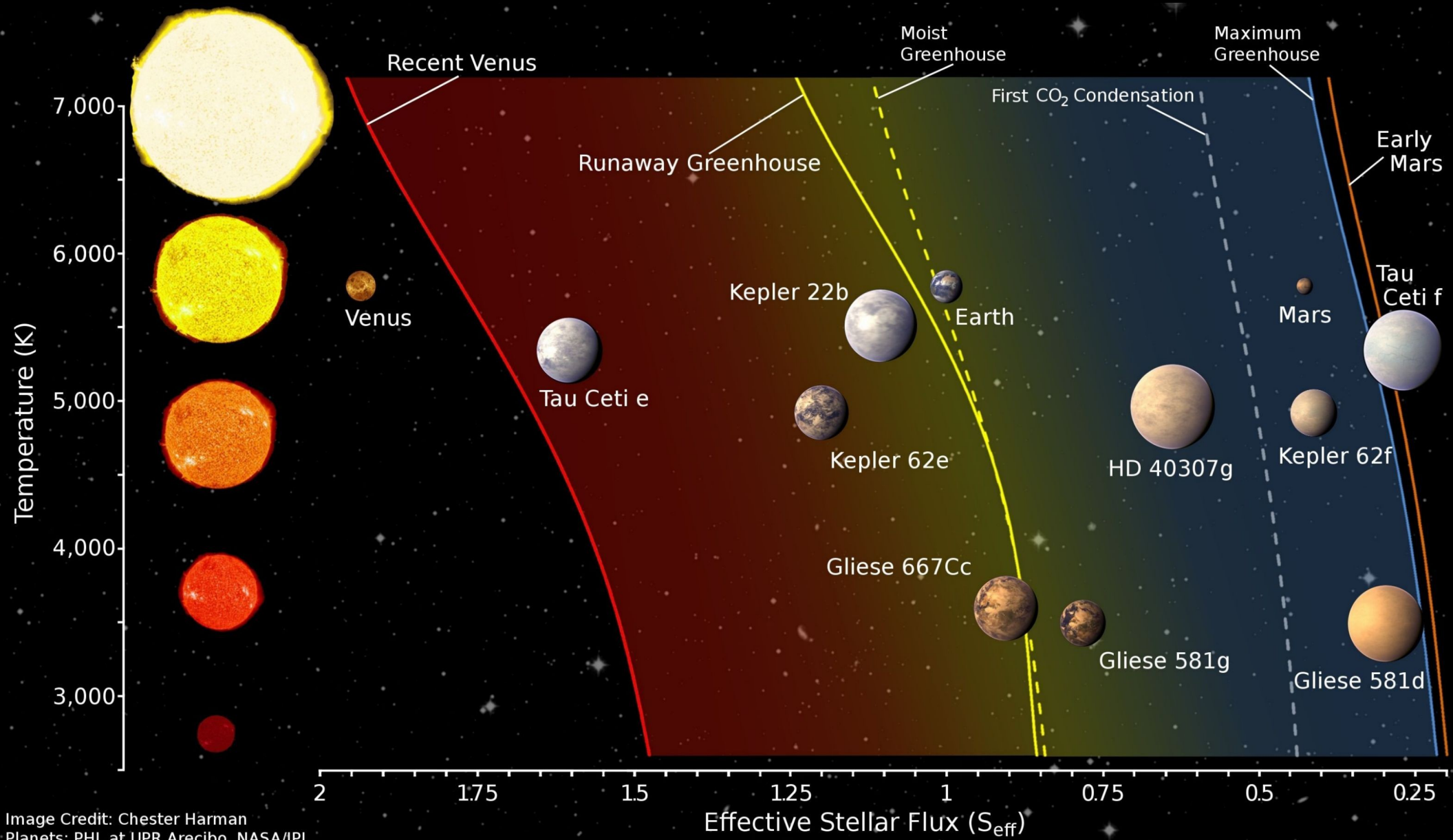
Biogeochemical cycles are *fundamental processes* controlling the *functioning of the planet* and ultimately its *habitability*

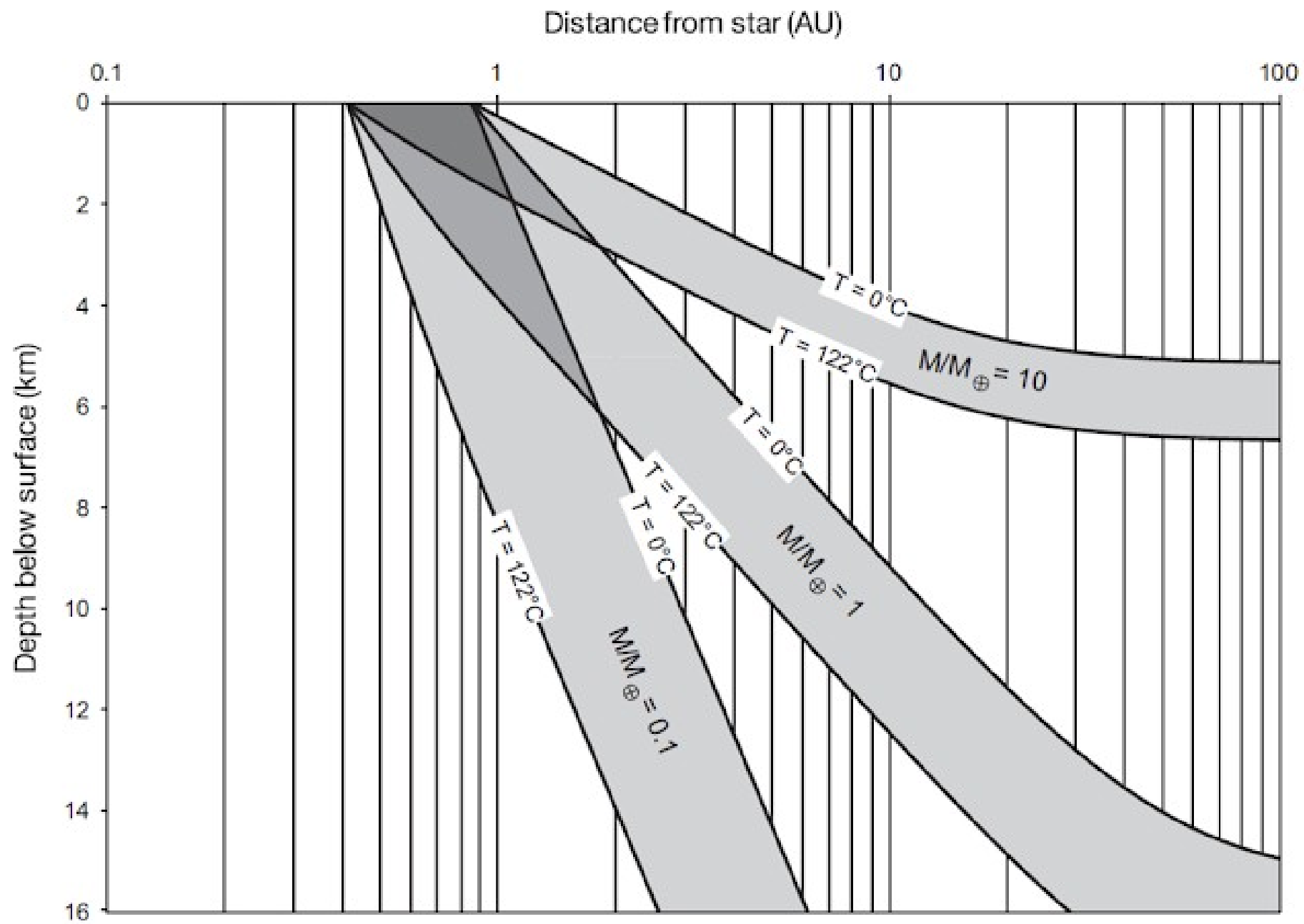
Habitability

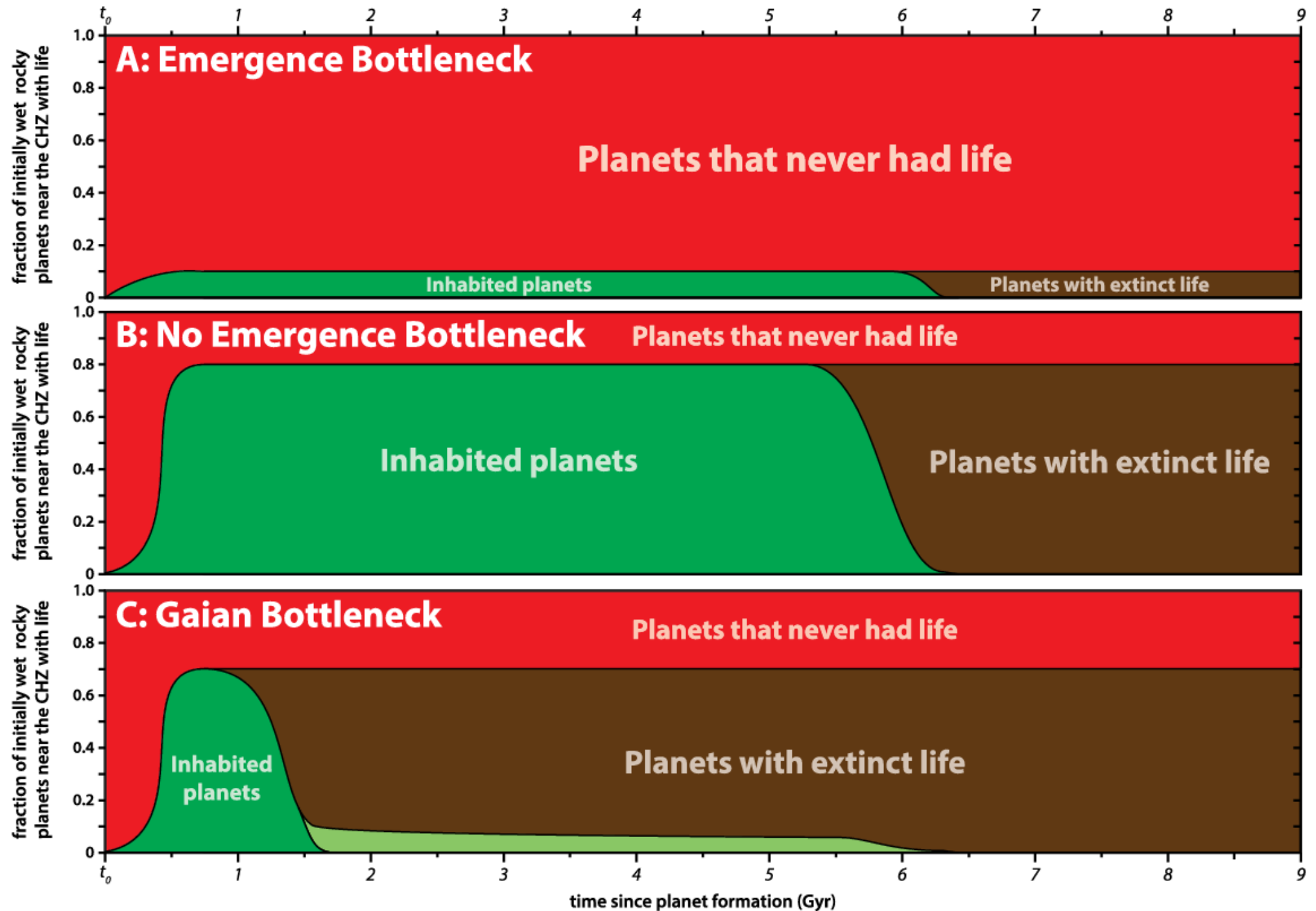
Habitability refers to the potential ability of a planet to sustain life. It is a complex concept that describes the potential for the *sustained presence of life* on a planetary body (planet, moon or other).

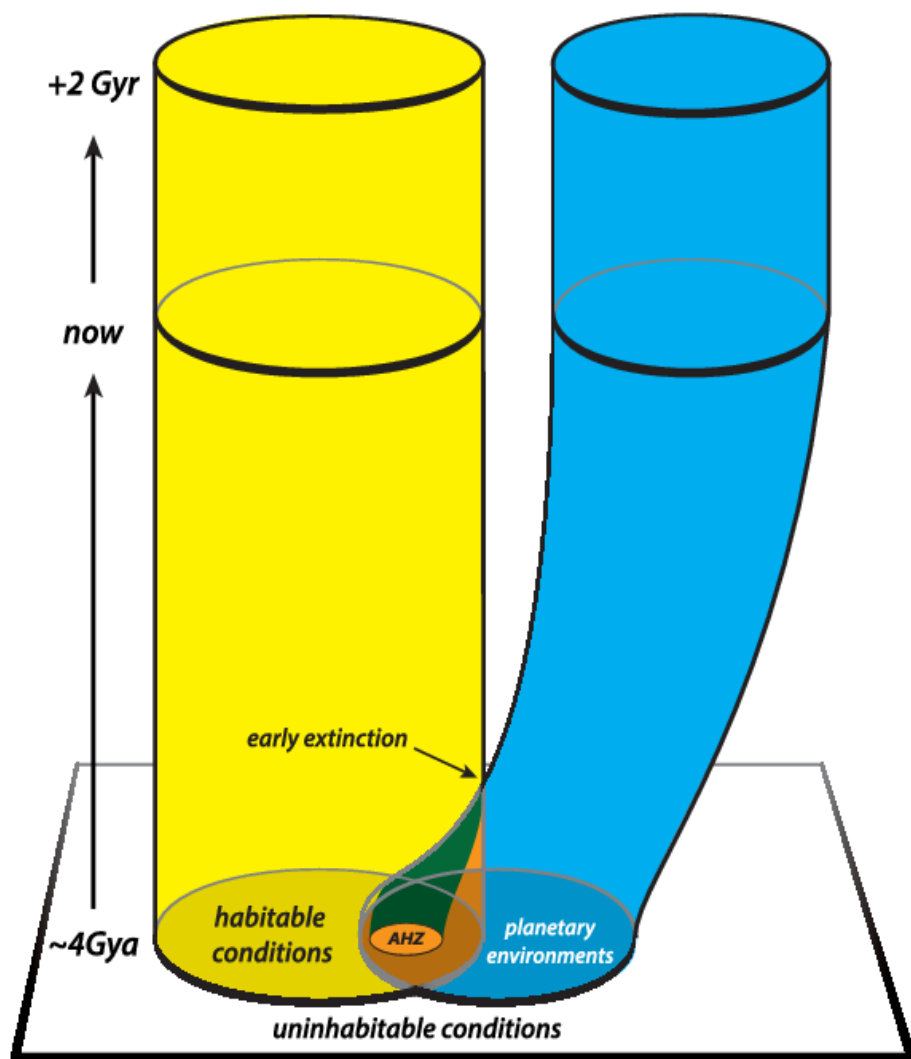
In practice, it is equaled to the *presence of liquid water* at the planet surface. This can be usually calculated from basic planetary parameters, and *changes depending on planet size and start type*.

An habitable planet *might* or *might not be inhabited* (have life on it).

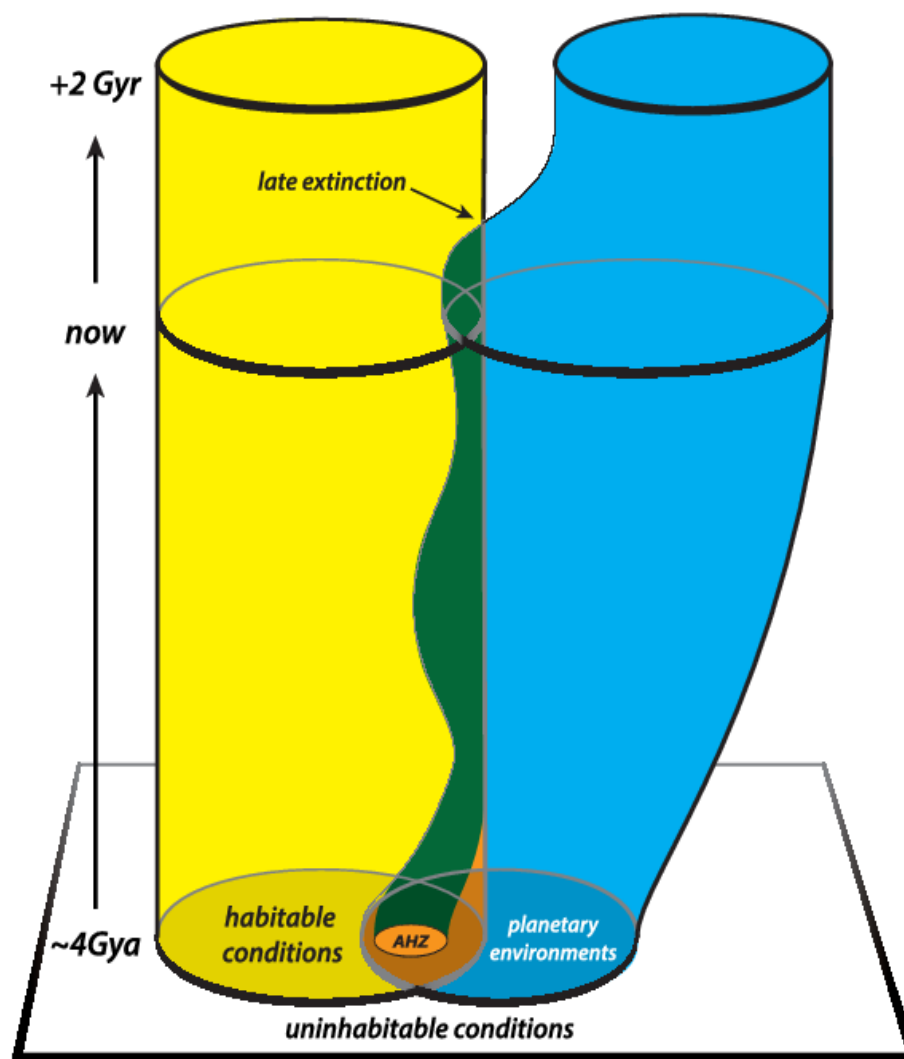








Gaian Bottleneck: Early Extinction



Gaian Regulation: Late Extinction

Biogeochemical cycles

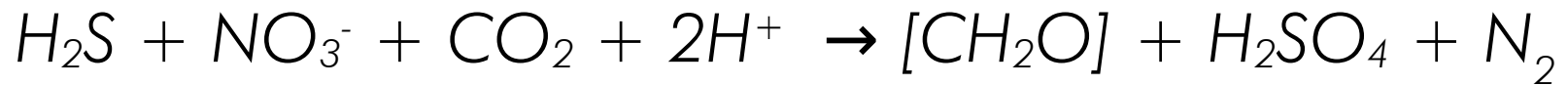
Every element used by *life* has its own biogeochemical cycle. Traditionally, some elements have well studied cycles that include a large number of possible reservoirs and transformation reactions.

Carbon, Nitrogen, Sulfur and *Iron* are the most studied biogeochemical cycles with the addition of the *water cycle* (not an element!)

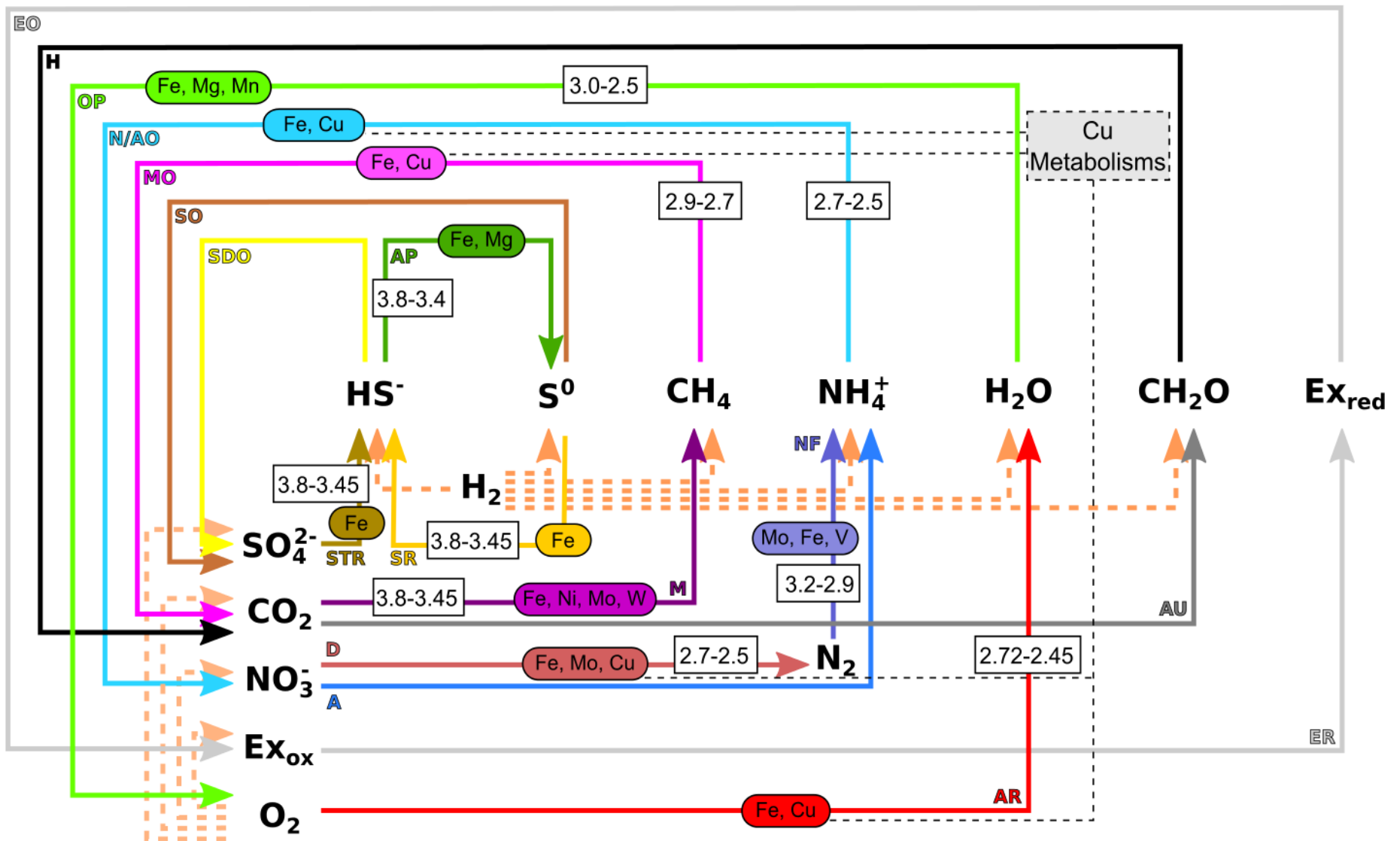
The cycling of other elements (e.g. arsenic) has been investigated much less comparatively despite its importance in human and ecosystem health.

Biogeochemical cycles

Biogeochemical cycles, while often approached individually, *are indissolubly linked to each other*. Organisms involved in cycling one element are also using many other elements in the process, *impacting different biogeochemical cycles* at once and with different magnitude:



Identifying the key role of each organism in a specific ecosystem is fundamental in defining the key players in each trophic group and the key provider of specific metabolic functions



Carbon cycle

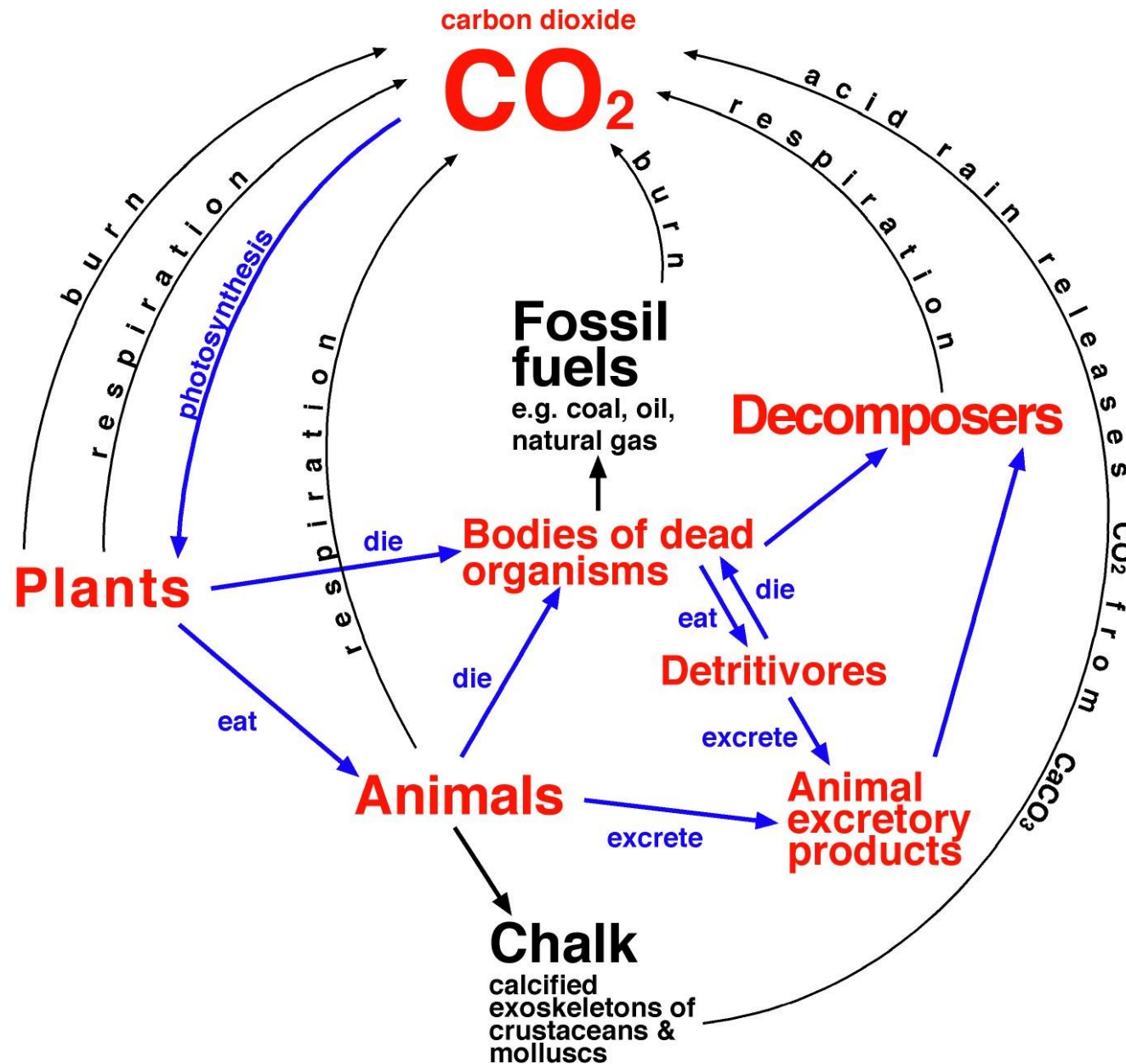
Carbon Cycle

The series of processes by which carbon compounds are interconverted in the environment, involving the incorporation of inorganic carbon into living tissue by photosynthesis and chemosynthesis and its return to the atmosphere through respiration, the decay of dead organisms, and the burning of fossil fuels.

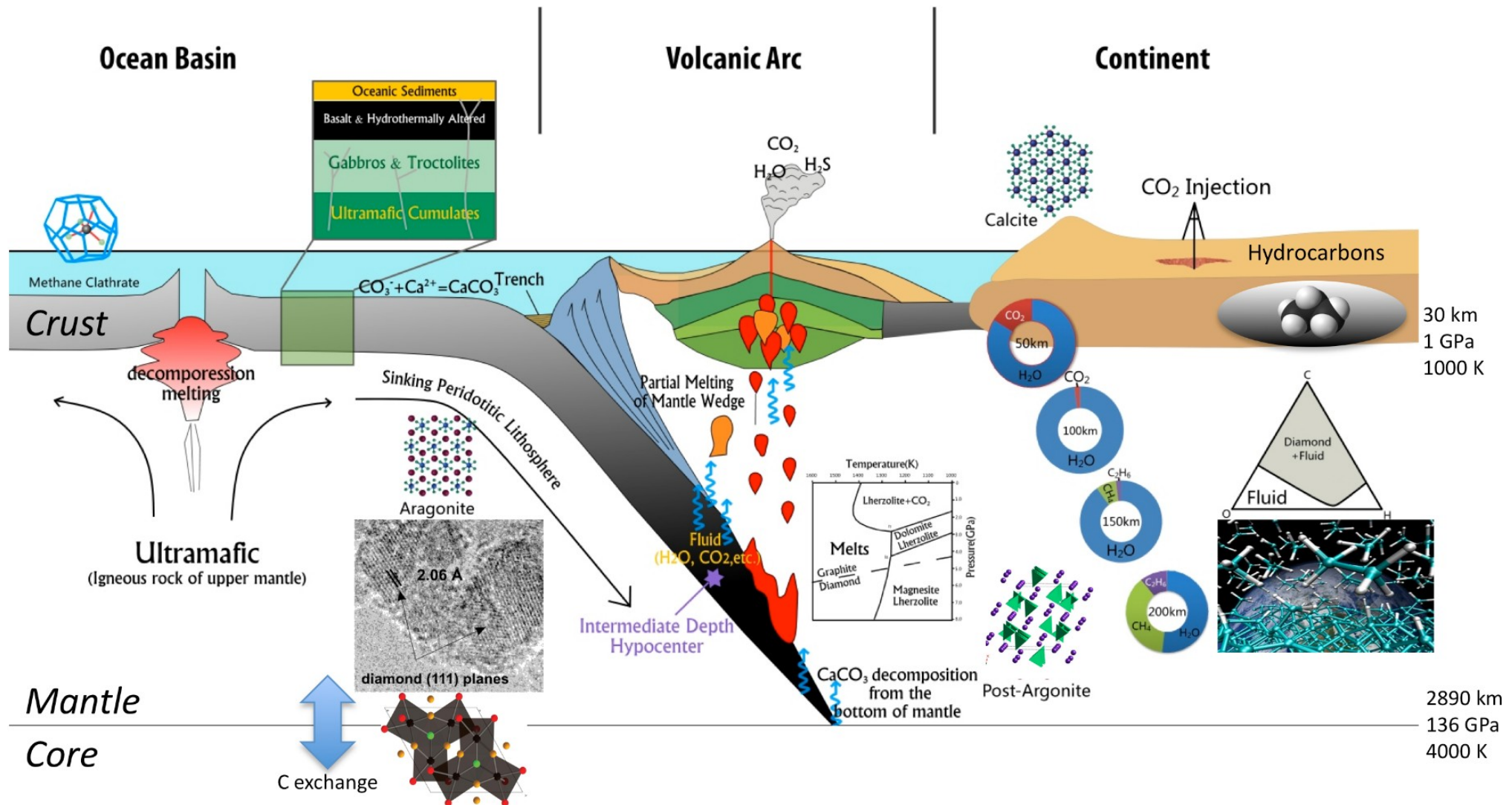
The **carbon cycle** is divided in **fast carbon cycle** and **deep** (or slow) carbon cycle.

The fast carbon cycle happens at the **surface of the planet** and involves all the biological contribution to carbon cycling. The deep carbon cycle, while also including biology in the subsurface, is dominated by the rock cycle.

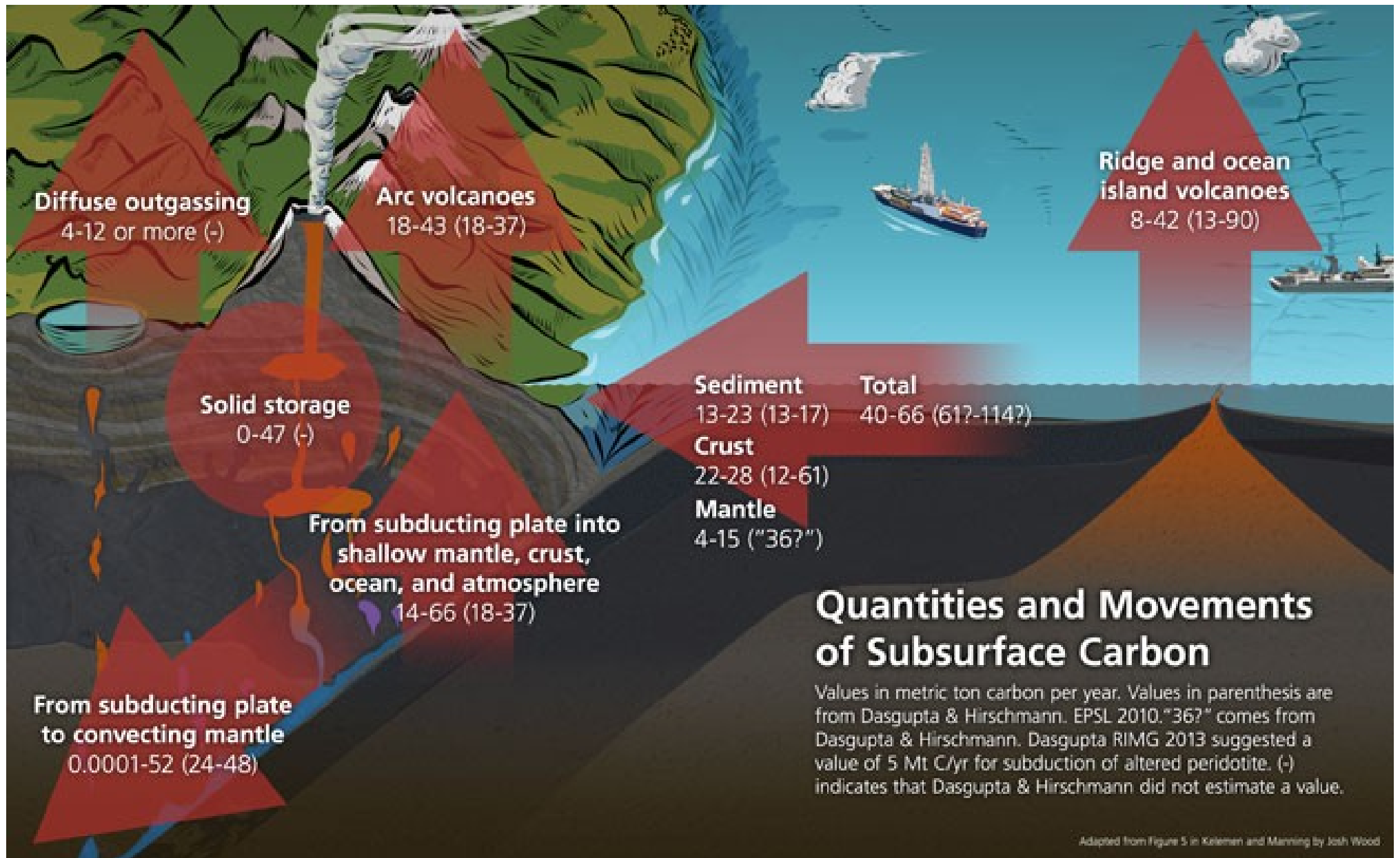
Carbon Cycle (fast)



Deep Carbon Cycle

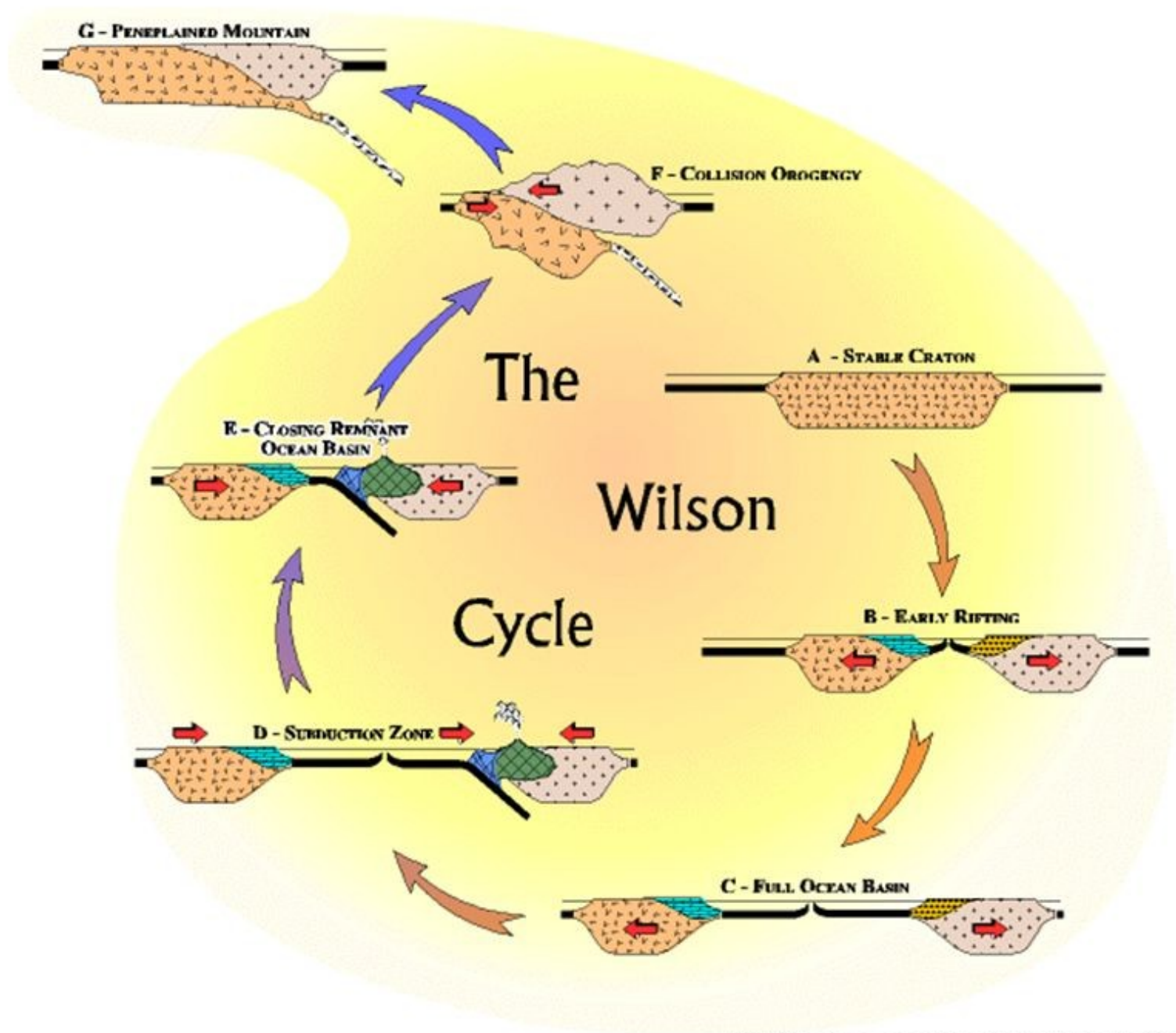


Deep Carbon Cycle

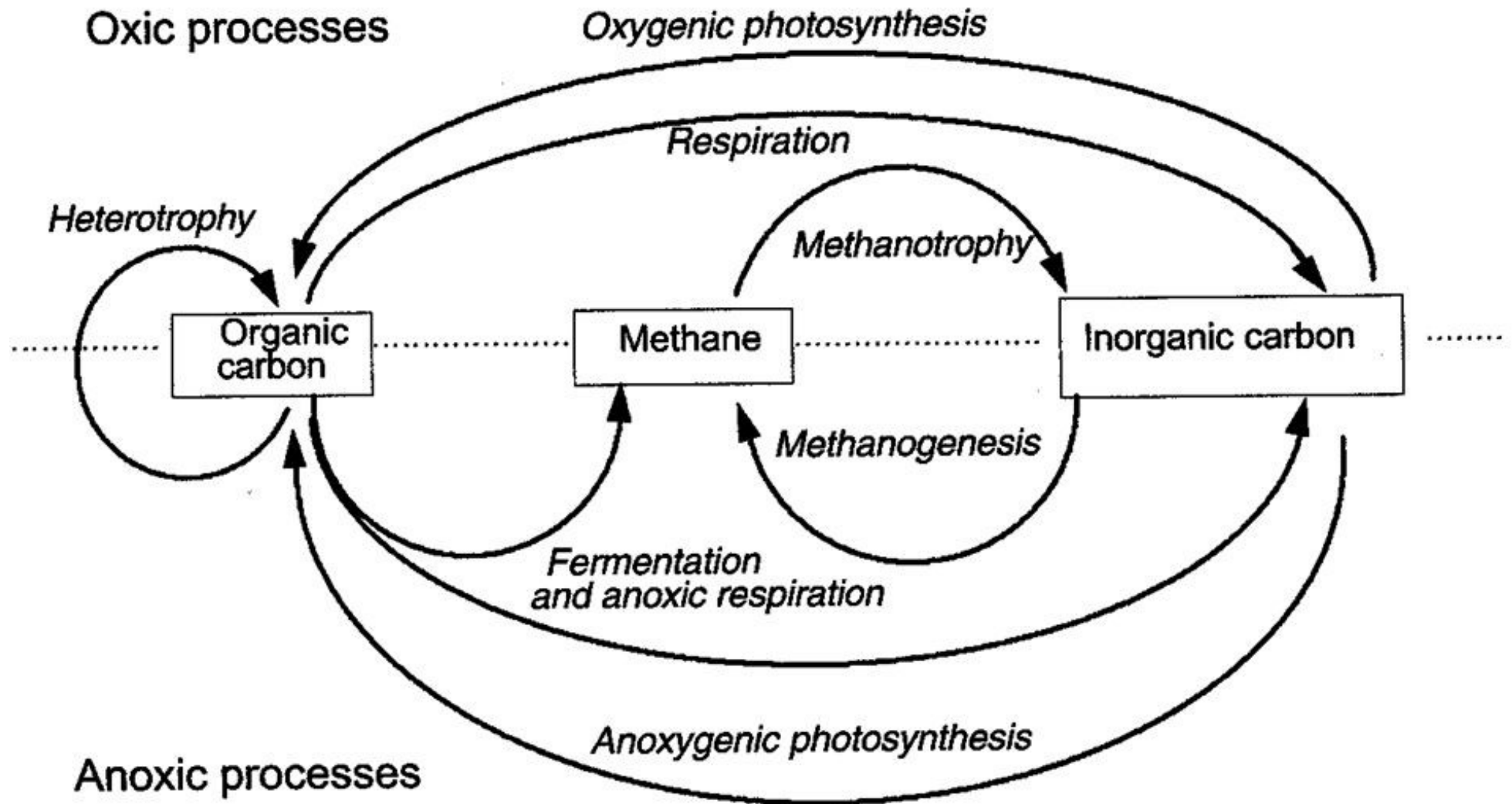


Historical Geology

The Wilson Cycle



Carbon Cycling



Carbon cycling: Primary producers

- *Primary production* is responsible for the biological conversion of inorganic carbon (CO and CO₂) in organic compounds. *Abiotic* formation of organics is possible under certain conditions, however the vast majority of organic compounds on the extant Earth is biotic in origin
- Primary production in extant Earth is primarily performed by *oxygenic phototrophs*
- Despite this, primary producers diversity and metabolic strategy is *largely dependent on the environmental conditions*
- *Anoxygenic phototrophs* and *chemolithoautotrophs* can also constitute the main primary producers in several environments
- Examples of ecosystems dominated by alternative types of primary production are *deep-sea, deep-sea hydrothermal vent* and *cold seeps, geothermal environments* and the *deep subsurface*

Diversity of phototrophic prokaryotes

Oxygenic Photosynthesis

- *Cyanobacteria*

Anoxygenic photosynthesis

- *Purple Sulfur Bacteria (Proteobacteria)*
- *Purple Nonsulfur Bacteria (Proteobacteria)*
- *Green Sulfur Bacteria (Chlorobi)*
- *Green Nonsulfur Bacteria (Chloroflexi)*

Cyanobacteria

Oxygenic phototrophs using H_2O as electron donor and fixing CO_2 using the CBB cycle

Many genera can also switch to anoxygenic photosynthesis

Widely distributed in aquatic ecosystems and key primary producers in the photic zone

Impressive morphological diversity (Unicellular, Filamentous, Branching)

Key genera: *Prochlorococcus*, *Crocosphaera*, *Synechococcus*, *Oscillatoria*, *Anabaena*



Daniel H. Buckley

(c)



Daniel H. Buckley

(e)

Purple Sulfur Bacteria

Found in illuminated anoxic zones of lakes and other aquatic habitats where H_2S accumulates, as well as sulfur springs

Use hydrogen sulfide (H_2S) as an electron donor for CO_2 reduction in photosynthesis

Sulfide oxidized to elemental sulfur (S_0), which is stored as globules either inside or outside cells. Sulfur later disappears as it is oxidized to sulfate (SO_4^{2-}). Many can also use other reduced sulfur compounds, such as thiosulfate ($S_2O_3^{2-}$)

Key genera: *Chromatium*, *Ectothiorhodospira* (Class Gammaproteobacteria)



Purple Nonsulfur Bacteria

Morphologically and phylogenetically diverse can synthesize wide array of carotenoids. Able to use H_2S as an electron donor for CO_2 reduction. Most can grow aerobically in the dark as chemoorganotrophs, some can also grow anaerobically in the dark using fermentation or anaerobic respiration

Most can grow photoheterotrophically using light as an energy source and organic compounds as a carbon source

Key genera: *Rhodospirillum*, *Rhodoferrax*, *Rhodobacter* (Class Alphaproteobacteria and Betaproteobacteria)



Green Sulfur Bacteria

Phylogenetically distinct, nonmotile, anoxygenic phototrophs. Green sulfur bacteria inhabit anoxic environments rich in H_2S . Can use H_2 , H_2S , S as electron donor and oxidize it to SO_4^{2-} and fix CO_2 using the rTCA cycle.

Model organisms for the study of anoxygenic photosynthesis and rTCA cycle

Key genera: *Chlorobium*, *Chlorobaculum* (phylum Chlorobi)



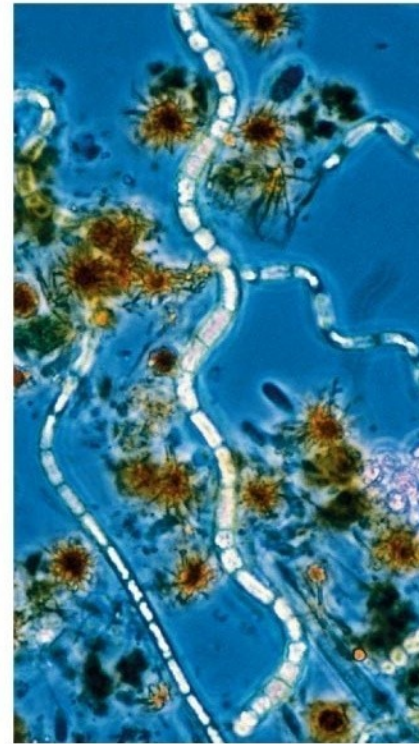
Green Nonsulfur Bacteria

Thermophilic filamentous bacteria that form thick microbial mats in neutral to alkaline hot springs

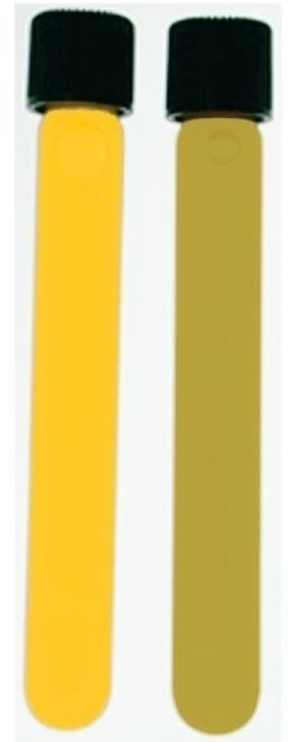
Grows best phototrophically, some can grow photoorganotrophically.

The pigment is found in specialized photosynthetic bodies called chlorosomes.

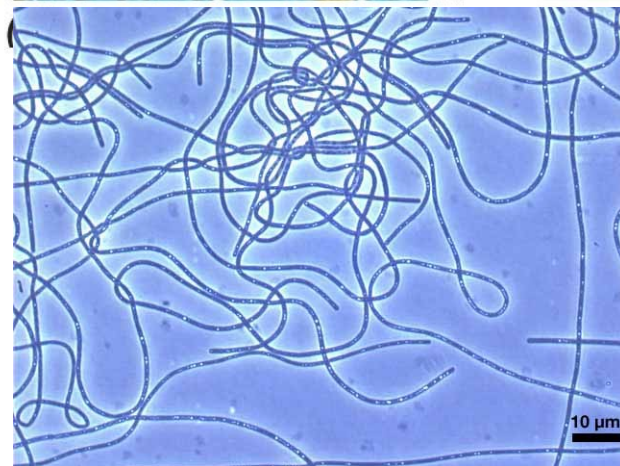
Key genera: *Chloroflexus*, *Heliothrix*, *Roseiflexus* (Phylum Chloroflexi)



Charles A. Abella



Deborah O. Jung



Heterotrophs

Heterotrophy is the major biotic pathway of organic carbon degradation, and it is ultimately responsible for the conversion of diverse organic carbons to CO₂.

Heterotrophs are a polyphyletic group of diverse organisms capable of degrading organic compounds as energy and carbon source using a variety of different biochemical pathways.

The major modes of heterotrophy (chemoorganotrophy) include *aerobic respiration*, *anaerobic respiration* and *fermentation*. Special cases of chemoorganotrophy include acetoclastic methanogens and photoheterotrophy.

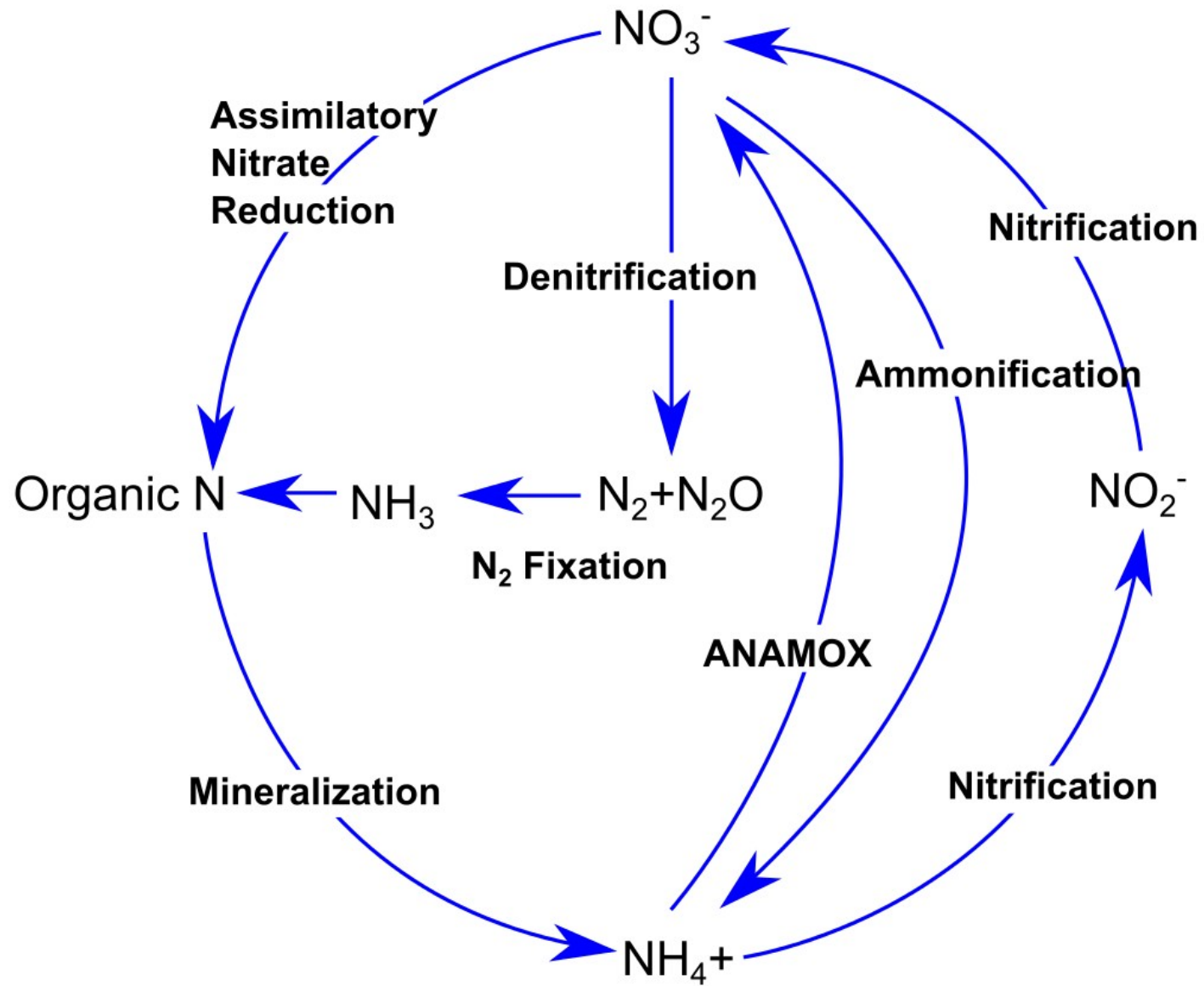
Heterotrophs are ubiquitous on our planet and in certain ecosystems may constitute the bulk of the biomass.

Major group of heterotrophs: *Gammaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, *Firmicutes*, *Bacteroidetes*, *Planctomycetes*

Nitrogen cycle

Nitrogen Cycle

- The *nitrogen biogeochemical cycle* is divided in numerous reactions, many of which can be exclusively carried by biology.
- Most of the nitrogen in the ocean and atmosphere is in the form of N_2 (g). This nitrogen is biologically inaccessible except to those few microbes, called *diazotrophs* (nitrogen fixers), that can break N_2 strong triple bond.
- Most of the fixed nitrogen in the ocean is dissolved and present as deepwater *nitrate and DON*. The marine biota contain less than 0.05% of the marine N.
- *Denitrification* is a major sink of organic carbon in marine sediments, while *ammonia oxidation (nitrification)* can be a powerful contributor to primary productivity in the deep ocean
- Some of these reservoirs are also likely to be in a state of change due to anthropogenic impacts on the global nitrogen cycle.



Nitrogen Cycle

Table 24.1 Common Species of Marine Nitrogen.

Species	Molecular Formula	Oxidation Number of Nitrogen
Nitrate ion	NO_3^-	+ V
Nitrogen dioxide gas	NO_2	+ IV
Nitrite ion	NO_2^-	+ III
Nitrous oxide gas	N_2O	+ I
Nitric oxide gas	NO	+ II
Nitrogen gas	N_2	0
Ammonia gas	NH_3	– III
Ammonium ion	NH_4^+	– III
Organic amine	RNH_2	– III

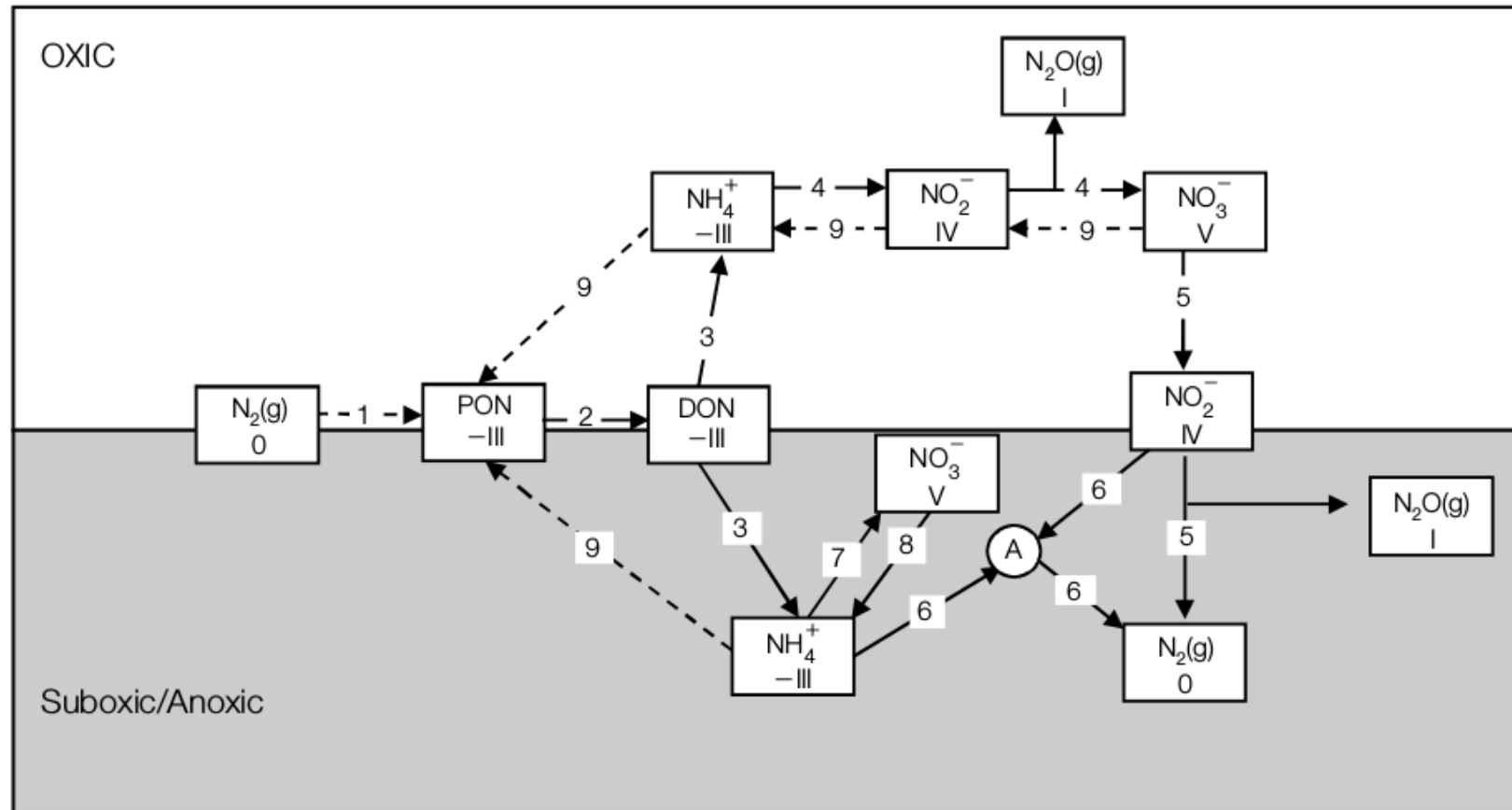


FIGURE 24.3

A simplified depiction of the marine nitrogen cycle illustrating redox and phase transitions mediated by microbes. The boxes contain the nitrogen species and its oxidation number. The arrows represent transformation reactions as follows: (1) nitrogen fixation, (2) solubilization, (3) ammonification, (4) nitrification, (5) denitrification, (6) anammox, (7) anaerobic nitrification mediated by manganese reduction, (8) dissimilatory nitrate reduction to ammonia (DNRA), (9) assimilatory nitrogen reduction. A = anammox microbes.

Diversity of Nitrogen Fixers

Nitrogen fixation is the key pathway allowing for the transformation of N_2 to forms that can be used by a wide diversity of other organisms.

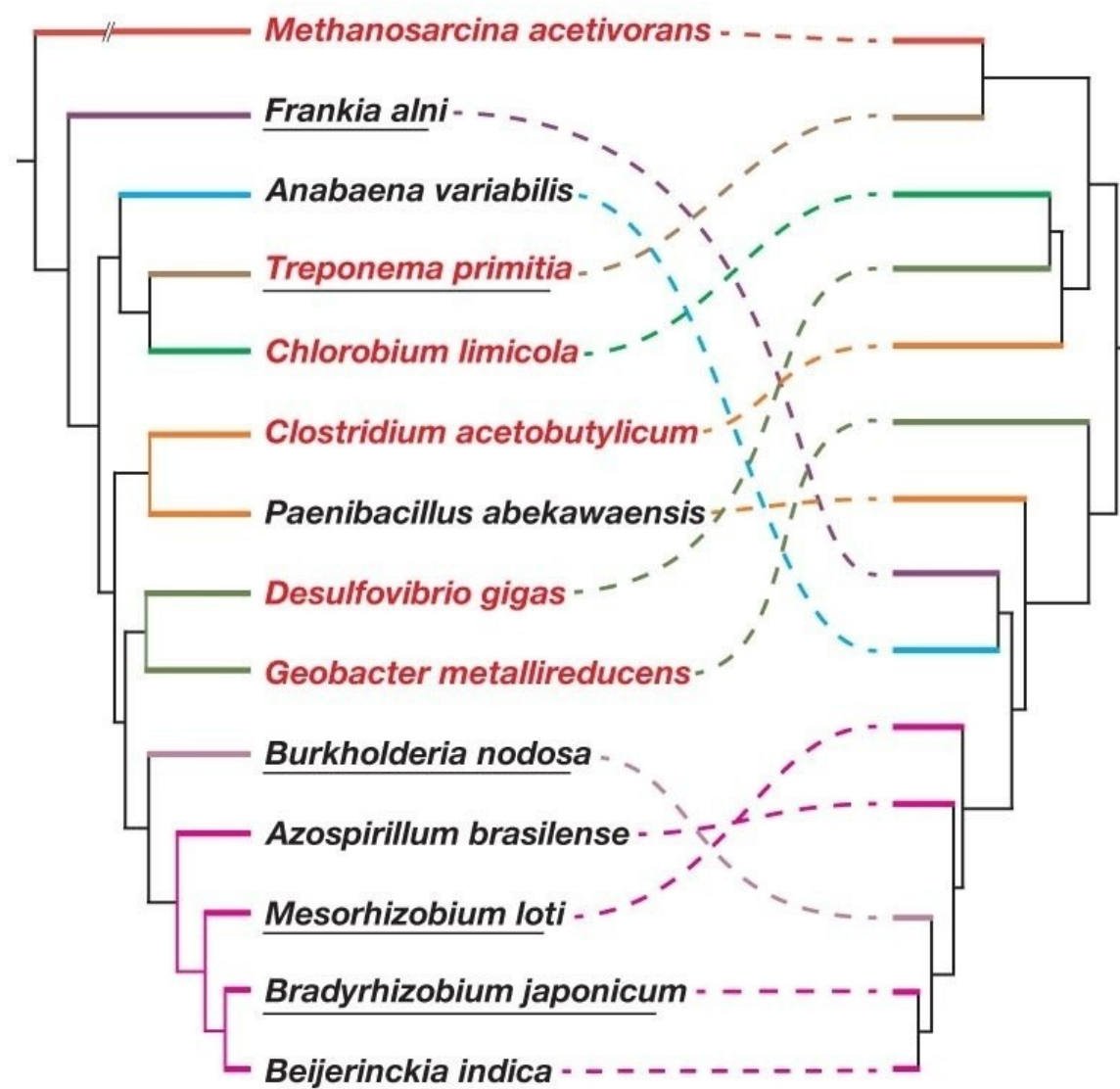
Nitrogen fixation is widespread among microorganisms and it is carried out by the enzyme nitrogenase. Nitrogenase genes have been found in *nine bacterial phyla and one archaeal phylum*. The phylogeny of nitrogenase gene sequence is inconsistent with phylogeny of 16S rRNA.

Diazotrophs are both free living and symbionts. Several diazotrophs form symbiotic relationships with plants, animals, and fungi. Symbiosis between rhizobia and leguminous plants (*Alphaproteobacteria* and *Betaproteobacteria*); Other symbioses: Shipworms and *Teredinibacter*, Termite guts and *Treponema*, Fungi, algae, and plants with *Cyanobacteria*.

Major genera capable of fixing N_2 nonsymbiotically are *Azotobacter*, *Azospirillum*, and *Beijerinckia*

16S rRNA Gene Phylogeny

NifH Phylogeny



Euryarchaeota	Spirochaetes	Deltaproteobacteria
Actinobacteria	Chlorobi	Betaproteobacteria
Cyanobacteria	Firmicutes	Alphaproteobacteria

Diversity of Nitrifiers

Nitrification is the biological conversion of ammonia to nitrite and nitrate. It is widespread in soil and water. Several nitrifiers are *chemolithotrophs*. Found in *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Thaumarchaeota*. Nitrification (oxidation of ammonia to nitrate) occurs as two separate reactions by different groups of bacteria: Ammonia oxidizers (nitrosifiers; e.g., *Nitrosococcus*) and Nitrite oxidizer (e.g., *Nitrobacter*)

Ammonia oxidizers

Ubiquitous in the marine environments in oxygenated areas. Is a major contributor to deep ocean primary production.

Found in *Betaproteobacteria* and *Gammaproteobacteria* and in the *Thaumarchaeota* (*Archaea*). Key genera: *Nitrosomonas*, *Nitrospira*, *Nitrosopumilus*

Nitrite oxidizers

Most are *obligate chemolithotrophs* and aerobes. One exception is *annamox* organisms, which oxidize ammonia anaerobically

Found in *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria*, *Nitrospirae* and *Planctomycetes* (*Anammox*). Key genera: *Nitrospira*, *Nitrobacter*

Diversity of Denitrifiers

Widely distributed in the tree of life. Found in *six bacterial phyla and two archaeal phyla*

Phylogenetically and metabolically diverse. Growth by anaerobic respiration of nitrate or nitrite. Facultative aerobes or strict anaerobes. Can be a *major sink of organic carbon in marine sediments*.

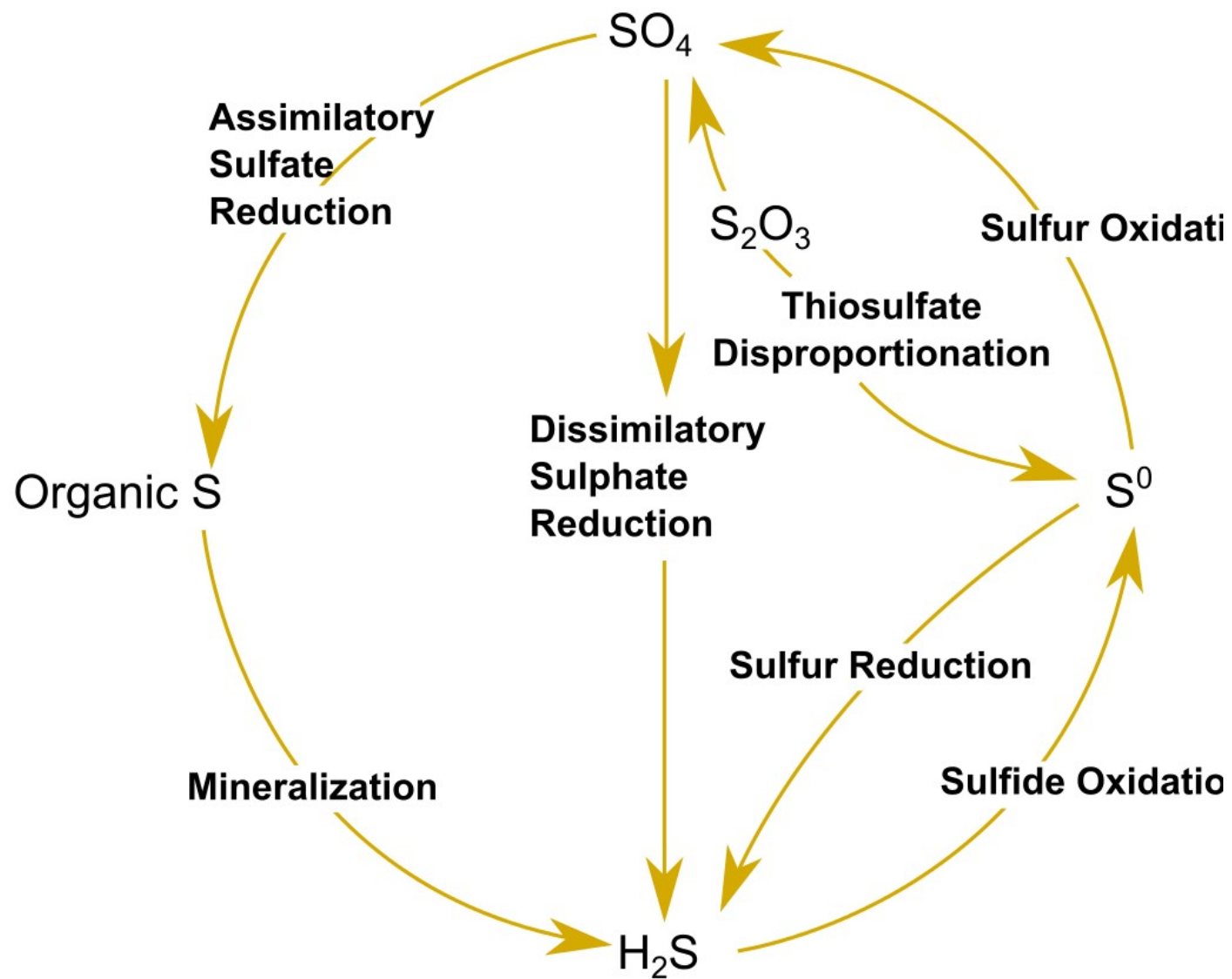
Capable of reducing nitrite or nitrate *to different partially reduced compounds* (NO_2 , N_2O , NO , N_2 , NH_4). Those performing NO_3 to NH_4 reduction are termed DNRA (*Dissimilatory Nitrate Reduction to Ammonia*)

Key genera (many): *Sulfurovum*, *Sulfurimonas*, *Thermovibrio*, *Paracoccus*, *Pseudomonas*

Sulfur cycle

Sulfur Cycle

- The *sulfur biogeochemical cycle* similarly to the nitrogen cycle is divided in numerous reactions, but *does not contain reaction exclusively* carried by biology.
- Most of the sulfur in the ocean is in the form of *sulfate* (SO_4^{2-}) and *pyrite* (Fe_2S) in the marine sediments.
- Sulfate is a powerful electron acceptor and *sulfate reduction* is a *major sink of organic carbon* in marine sediments, and through the partnership with Anaerobic Methane Oxidizers (Archaea of the ANME divisions) *contributes also significantly to methane consumption*.
- The *biological oxidation of sulfur species* is globally less relevant (in oxic systems competes with abiotic rates) but is of primary importance in several extreme environments (deep-sea hydrothermal vents, cold seeps, sulfidic sediments).



Sulfur Cycle

TABLE 19.1
Geomicrobially Important Forms of Sulfur and Their Oxide States

Compound	Formula	Oxidation State(s) of Sulfur
Sulfide	S^{2-}	-2
Polysulfide	S_n^{2-}	-2, 0
Sulfur ^a	S_8	0
Hyposulfite (dithionite)	$S_2O_4^{2-}$	+3
Sulfite	SO_3^{2-}	+4
Thiosulfate ^b	$S_2O_3^{2-}$	-1, +5
Dithionate	$S_2O_6^{2-}$	+4
Trithionate	$S_3O_6^{2-}$	-2, +6
Tetrathionate	$S_4O_6^{2-}$	-2, +6
Pentathionate	$S_5O_6^{2-}$	-2, +6
Sulfate	SO_4^{2-}	+6

^a Occurs in an octagonal ring in crystalline form.

^b Outer sulfur has an oxidation state of -1; the inner sulfur has an oxidation state of +5.

Dissimilative Sulfate-Reducers

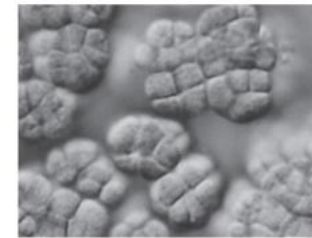
Over 30 genera of sulfate-reducers across five phyla of Bacteria and Archaea. Most are *Deltaproteobacteria*, some are *Firmicutes*, *Thermodesulfobacteria*, *Nitrospira* and *Archaeoglobus* in Archaea.

Use SO_4^{2-} and S^0 as electron acceptors, and organic compounds or H_2 as electron donors. H_2S is an end product. Most are obligate anaerobes. Widespread in aquatic and terrestrial environments and a *major sink of organic carbon*.

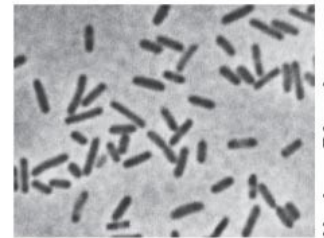
Physiology of sulfate-reducing bacteria

Group I Include *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum*, and *Desulfobulbus*. Oxidize lactate, pyruvate, or ethanol to acetate

Group II: Include *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, and *Desulfonema*. Oxidize fatty acids, lactate, succinate, and benzoate to CO_2



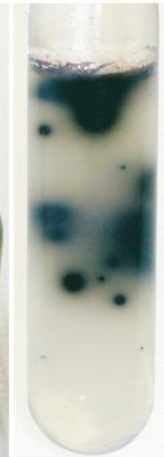
Fritz Widdel



Norbert Pfennig

(e)

(f)



Matthew Sattley and Deborah O. Jung

(g)

Dissimilative Sulfur-Reducers

Over 25 genera of sulfur-reducers across five phyla of Bacteria and Archaea

Most are *Deltaproteobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Aquificae*, several in *Crenarchaeota*

Widespread in aquatic and terrestrial environments, although limited in numbers compared to sulfate reducers. Numerically abundant in geothermal environments.

Most are obligate anaerobes, some are facultative aerobes. Some completely oxidize acetate and fatty acids to CO_2

Key genera: *Desulfuromonas*, *Sulfolobus*, *Desulfurobacterium*

Dissimilative Sulfur-Oxidizers

Sulfur-oxidizers are found in three phyla of Bacteria and one phylum of Archaea. Most are *Betaproteobacteria*, *Gammaproteobacteria*, and *Epsilonproteobacteria*, several in *Crenarchaeota*.

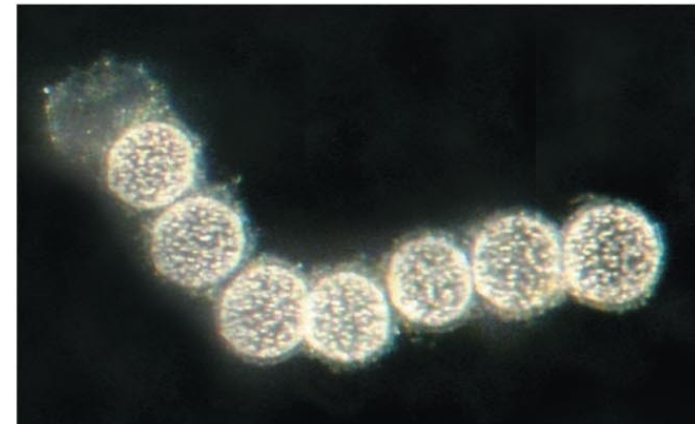
Grow chemolithotrophically on reduced sulfur compounds. Couple oxidation of H_2S with reduction of NO_3^- or O_2

Two broad classes: *Neutrophiles* and *Acidophiles*. Some acidophiles able to use ferrous iron (Fe^{2+})

Sulfur compounds most commonly used as electron donors are H_2S , S^0 , $\text{S}_2\text{O}_3^{2-}$; generates sulfuric acid.

Some obligate chemolithotrophs possess special structures that house Calvin cycle enzymes. Found in habitats rich in H_2S

Examples: sulfur springs, decaying seaweed beds, mud layers of lakes, sewage-polluted waters, and hydrothermal vents



Other distinctive chemotrophic microbes

Dissimilative Iron-Reducing Bacteria

Dissimilative Iron-Oxidizing Bacteria

Hydrogen-Metabolizing Bacteria

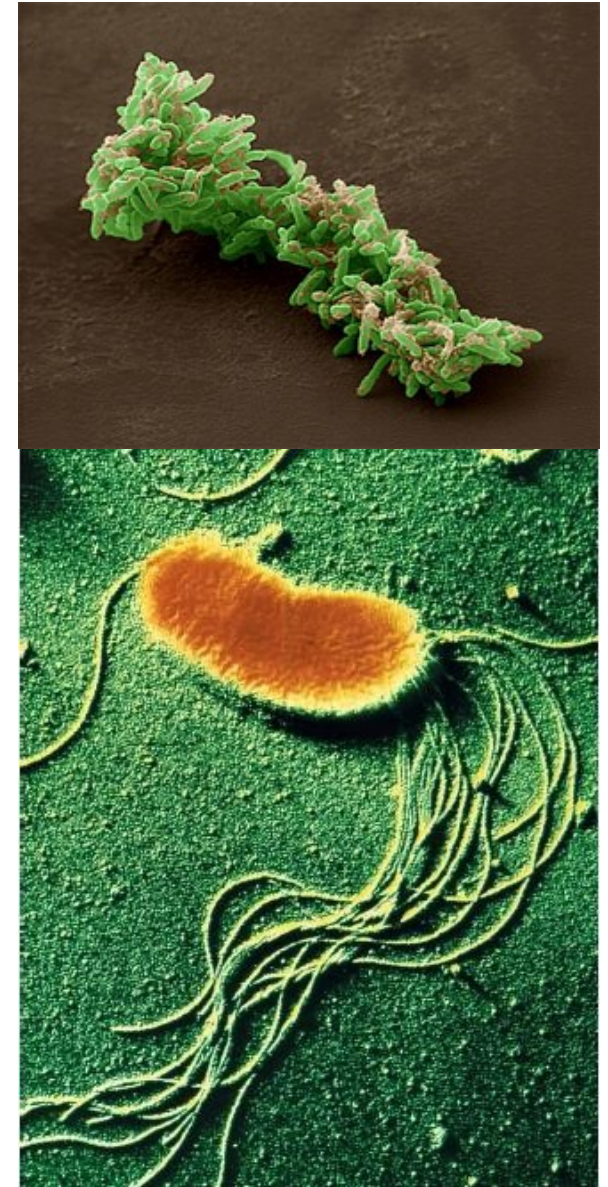
Dissimilative Iron-Reducers

Phylogenetically diverse. Found in
Proteobacteria, *Acidobacteria*, *Deferribacteres*,
Deinococcus-Thermus, *Thermotogae*,
Firmicutes and *Crenarchaeota*

Most are obligate *anaerobes*. Electron donors
are typically organic compounds.

Common in anoxic freshwater and marine
sediments

Key genera: *Geobacter*, *Shewanella*,
Deferribacter



Dissimilative Iron-Oxidizers

Found in five bacterial phyla and two archaeal phyla

Iron oxidizers are divided into four functional groups: acidophilic aerobic iron oxidizers, neutrophilic aerobic iron oxidizers, anaerobic chemotrophic iron oxidizers, anaerobic phototrophic iron oxidizers

Common in freshwater and marine environments

Producing typical structures indicative of iron oxidation (*twisted stalk*)

Key genera: *Acidithiobacillus*, *Gallionella*, *Mariprofundus*



W. C. Ghiorse

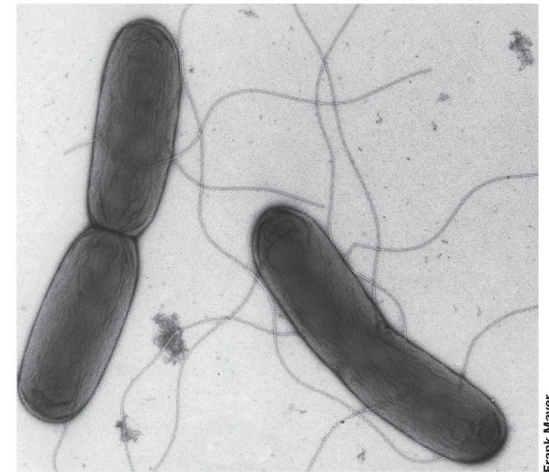
Hydrogen-Oxidizing Bacteria

Phylogenetically and metabolically diverse group of prokaryotes. Include "knallgas" metabolism and numerous other hydrogen oxidizer utilizing different electron acceptors as well as methanogens

Contains
chemolithoheterotrophs
phototrophs
chemolithoautotrophs,
and
anaerobic

One or more hydrogenase enzymes are usually present

Key genera: *Caminibacter*, *Aquifex*, *Ralstonia*, *Paracoccus*

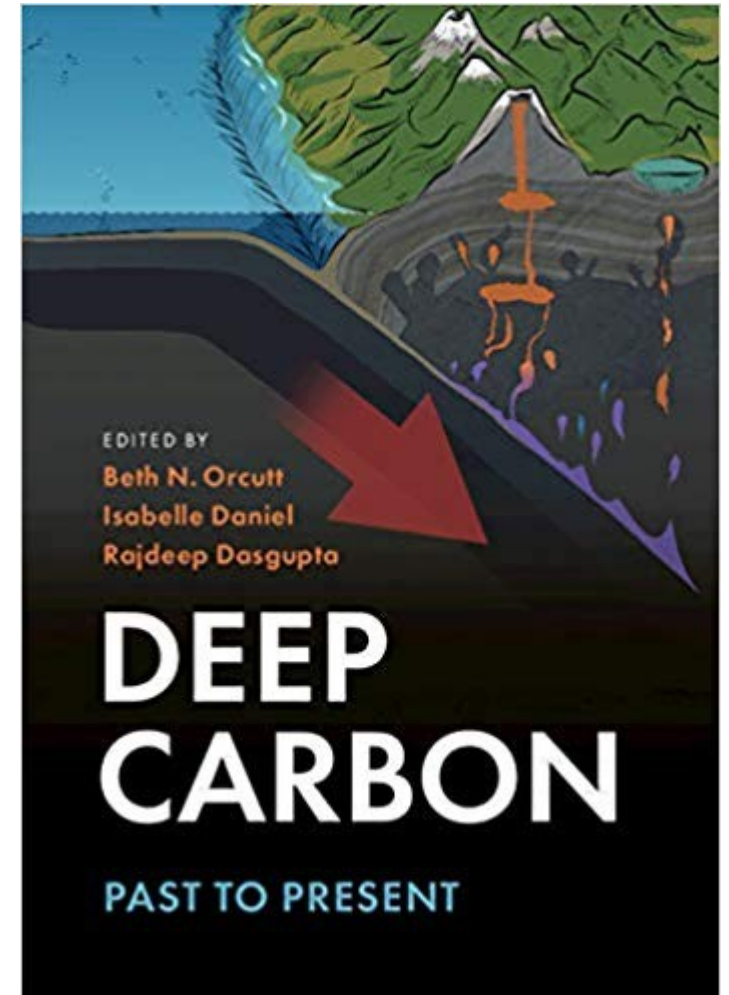


Frank Mayer

Open Access Book on Deep Carbon

“Deep Carbon: Past to Present”

bit.ly/2CVqHQe



This week reading

Shock, E. L., and Boyd, E. S. (2015). *Principles of Geobiochemistry*. Elements 11, 395–401. doi:10.2113/gselements.11.6.395

Falkowski, P. G., Fenchel, T., and Delong, E. F. (2008). *The Microbial Engines That Drive Earth's Biogeochemical Cycles*. Science 320, 1034–1039. doi:10.1126/science.1153213

Suggested:

Chopra, A., and Lineweaver, C. H. (2016). *The Case for a Gaian Bottleneck: The Biology of Habitability*. Astrobiology 16, 7–22. doi:10.1089/ast.2015.1387

Offre, P., Spang, A., and Schleper, C. (2013). *Archaea in Biogeochemical Cycles*. Annual Review of Microbiology 67, 437–457. doi:10.1146/annurev-micro-092412-155614

Jetten, M. S. M. (2008). *The microbial nitrogen cycle*. Environmental Microbiology 10, 2903–2909. doi:10.1111/j.1462-2920.2008.01786.x