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A brief introduction to microbial ecology and bacterial diversity

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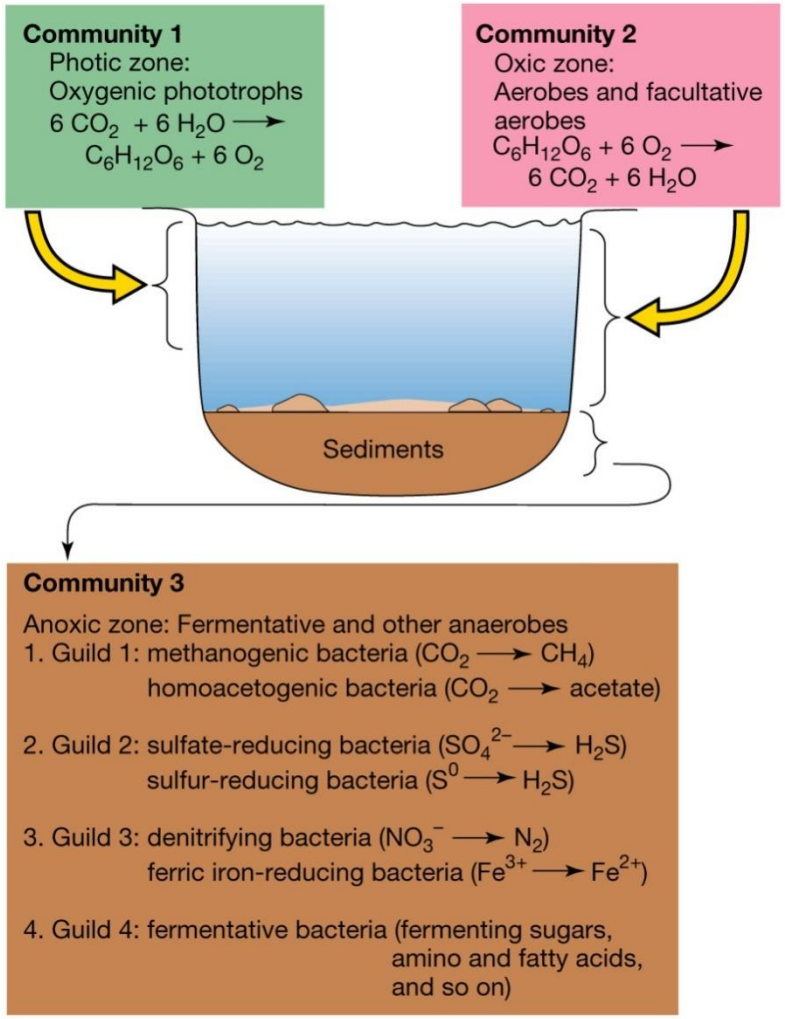
What is microbial ecology?
(or Environmental Microbiology)

A field concerned with answering such questions as:

- ✓ What microbes are present in a given habitat?
- ✓ What are they doing there?
- ✓ How do they interact with one another?
- ✓ How do they impact their environment?
- ✓ What impact does the environment have on their physiology, biochemistry and evolution?

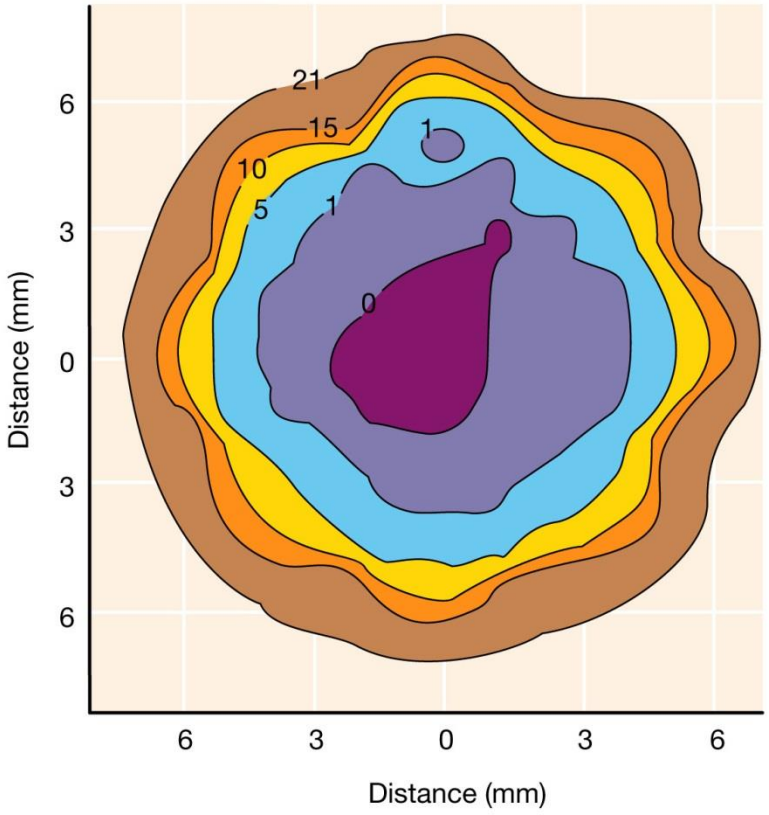
Populations, guilds and communities

- **Population** – several individuals of the same genotype in the same habitat
- **Guild** – several populations participating in the same geochemical cycle within the same habitat
- **Community** – all the populations present in the same habitat



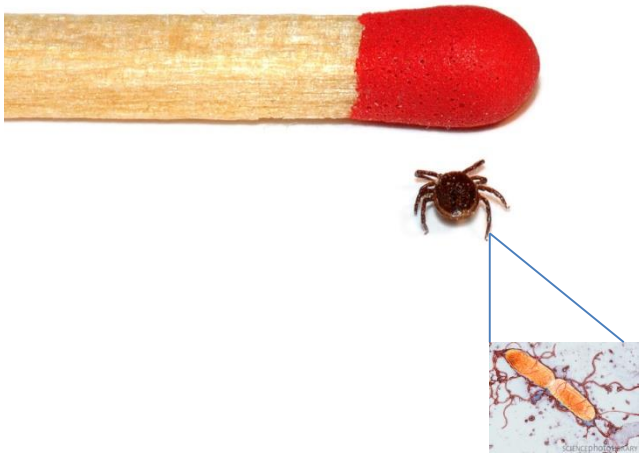
Niche, habitat, ecosystem

- **Habitat** – the place in which the organisms in question reside
- **Niche** – the set of constraints (physical, chemical, biotic) that a habitat must fulfill so that a given organism can reside in it
- **Ecosystem** – the environment and all the organisms residing in it



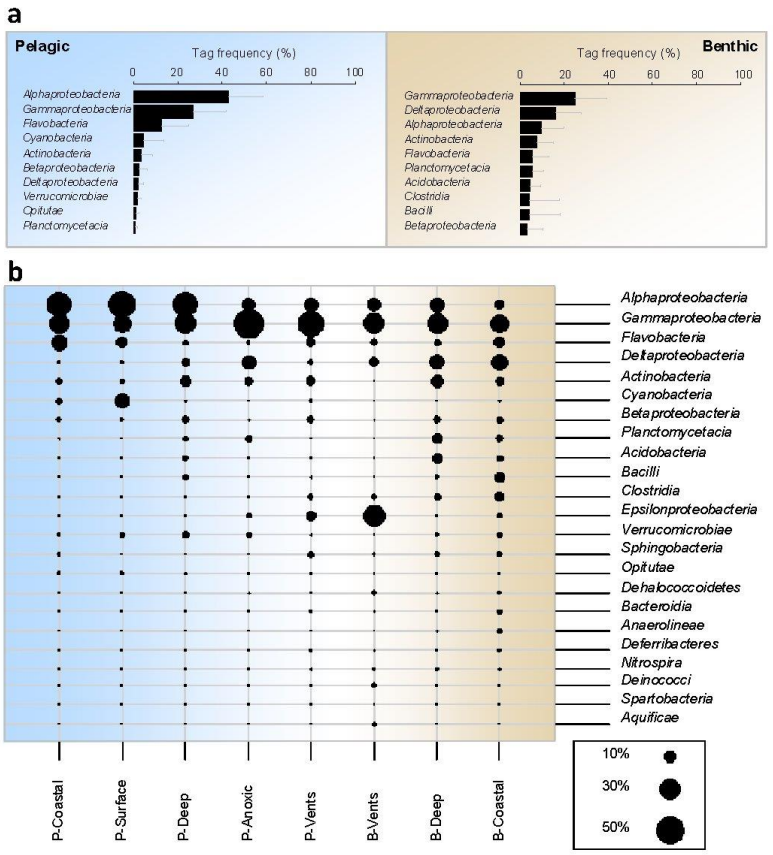
The problem of scale

- While important and numerous, bacteria are very, very small, each individual weighing about 10^{-12} g (1 pg), occupying about 10^{-18} m^3 (1 fL) of space
  - ⇒A very populous community of bacteria can fit in a very small space
  - ⇒A very small sample can contain a large number of (micro)habitats
  - ⇒A very small sample can contain a large diversity of bacteria
  - It is virtually impossible to accurately and completely describe the habitats and populations of a single g of soil



To this dividing bacterium, the tick is roughly the size of Mt. Súlur.

- Look hard enough and you'll find representatives of nearly all bacterial and archaeal phyla and classes in a single m^3 of seawater
- … so, what's the point of microbial ecology if "everything is everywhere"?? (the Baas Becking hypothesis)

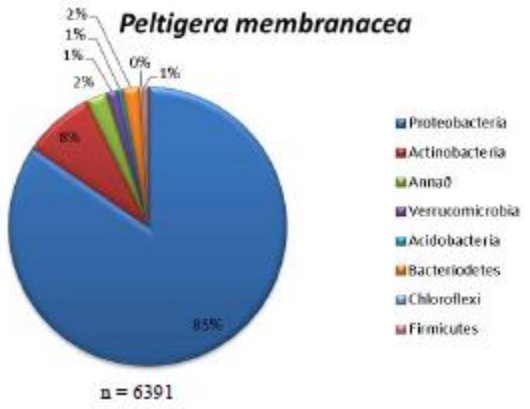


Zinger *et al*. (2011) found enormous diversity of bacteria in seawater. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0024570

So, is "everything" really "everywhere"?

- No, not really, but bacteria do disperse very easily and widely
  - *Beijerinck*: „Everything is everywhere..."
  - *Baas Becking*: „... but the environment selects" ("Alles is overal, maar het milieu selecteert"- Baas Becking 1934)
- And, besides, the populations of different species in a given sample vary greatly in size, sometimes by many orders of magnitude

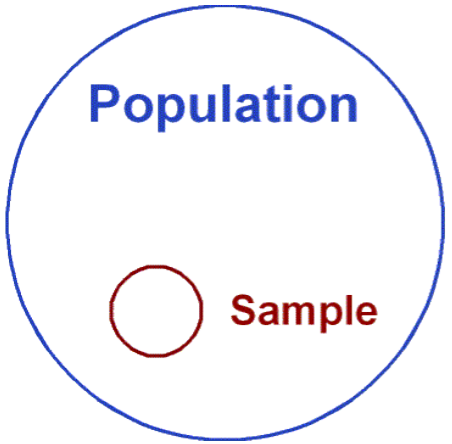
Martinus Beijerinck (1851-1931) and Lourens Baas Becking (1895-1963) were pioneers of environmental microbiology.

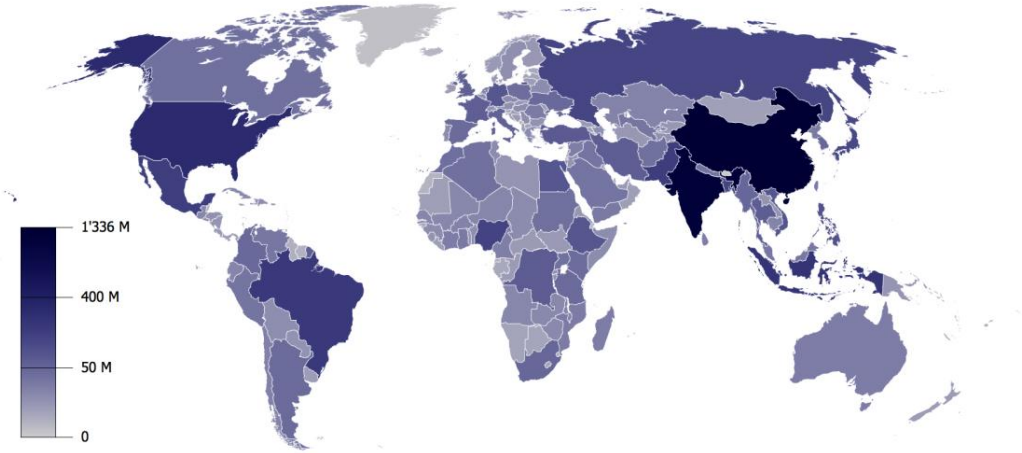


The community of bacteria within lichen thalli is quite diverse, but the Proteobacteria are strongly dominant (Sigurbjörnsdóttir *et al.*, unpublished).

Sampling and population structure

- If we were to sample the Earth's human population at random, sampling 100 individuals, we would expect to find:
  - 19 Chinese, 17 Indians, 4 Americans, 3 Indonesians, ... perhaps 1 Briton (~90% chance), ... but not a single Icelander (~0.5% chance that one would be included)
- Are Icelanders for real?? If an Icelander were to be found in such a sample, would we consider her
  - a fluke?
  - contamination?
  - noise?
  - a sampling artefact?
  - an error?
- In any case, we'd probably want to re-sterilize our sampling gear and repeat the experiment

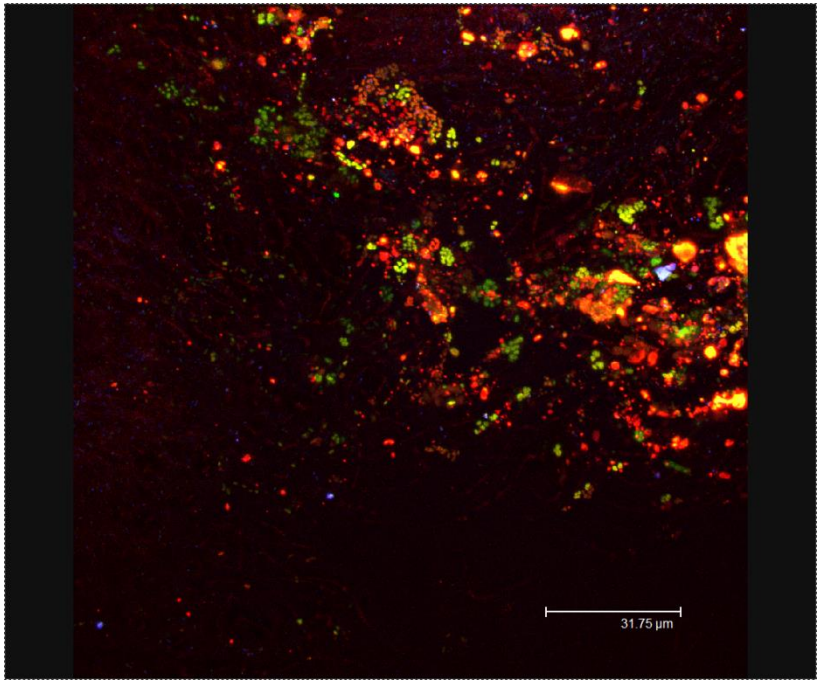




⇒Microbial ecology labs are increasingly relying on "deep sequencing" metagenomics in order to tease out the "real" structure of natural populations.

Small population = less importance?

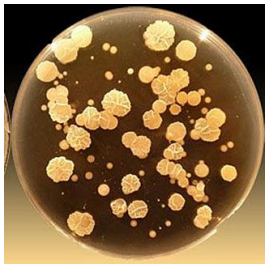
- No, absolutely not!
  - For every lion in Africa, there are ~100 wildebeest. That does not make the wildebeest 100× as important as lions.
- However ...
  - "Local" (autochthonous, endemic or indigenous) species are likely to be more numerous than the "foreign visitors" (allochthonous species).
  - Bacteria present in very low numbers are unlikely to affect the local environment to a great extent.
  - Not everything present in a given habitat is active, viable or even alive!



In this FISH-CLSM image of a lichen thallus, bacteria in general stain red, whereas ***Alphaproteobacteria*** are yellow or green. The 2-3 blue dots are ***Firmicutes*** ... are they present as active, viable cells or as inactive spores? Do they contribute to the lichen symbiosis or are they simply allochthons from a nearby environment?

Culture-independent methods revolutionized understanding of microbial ecosystems

- Culture-dependent methods are biased towards easily culturable microbes. (There may be other biases, though!)

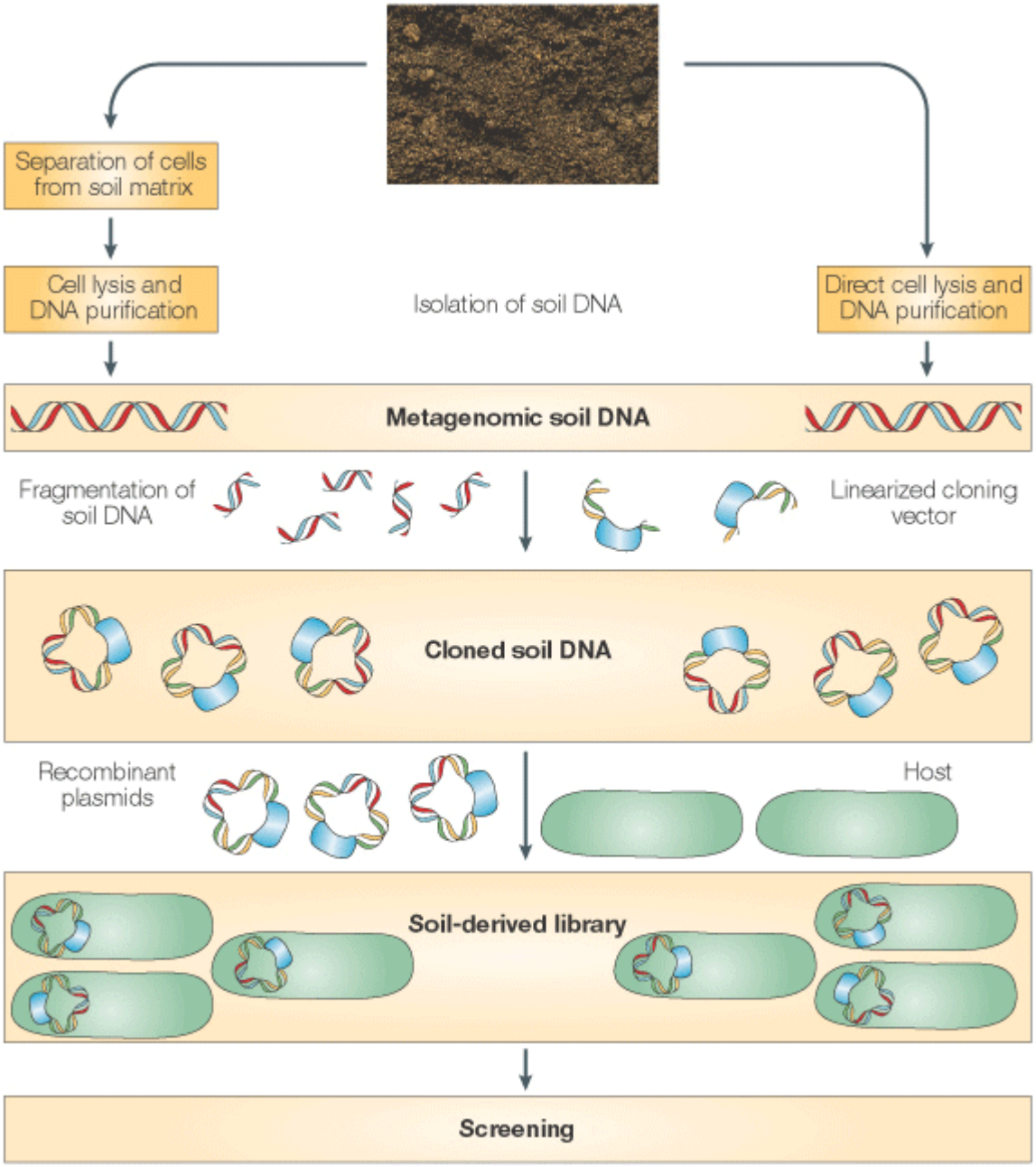


Culture-independent surveys

1. **Extract DNA from environmental samples.**

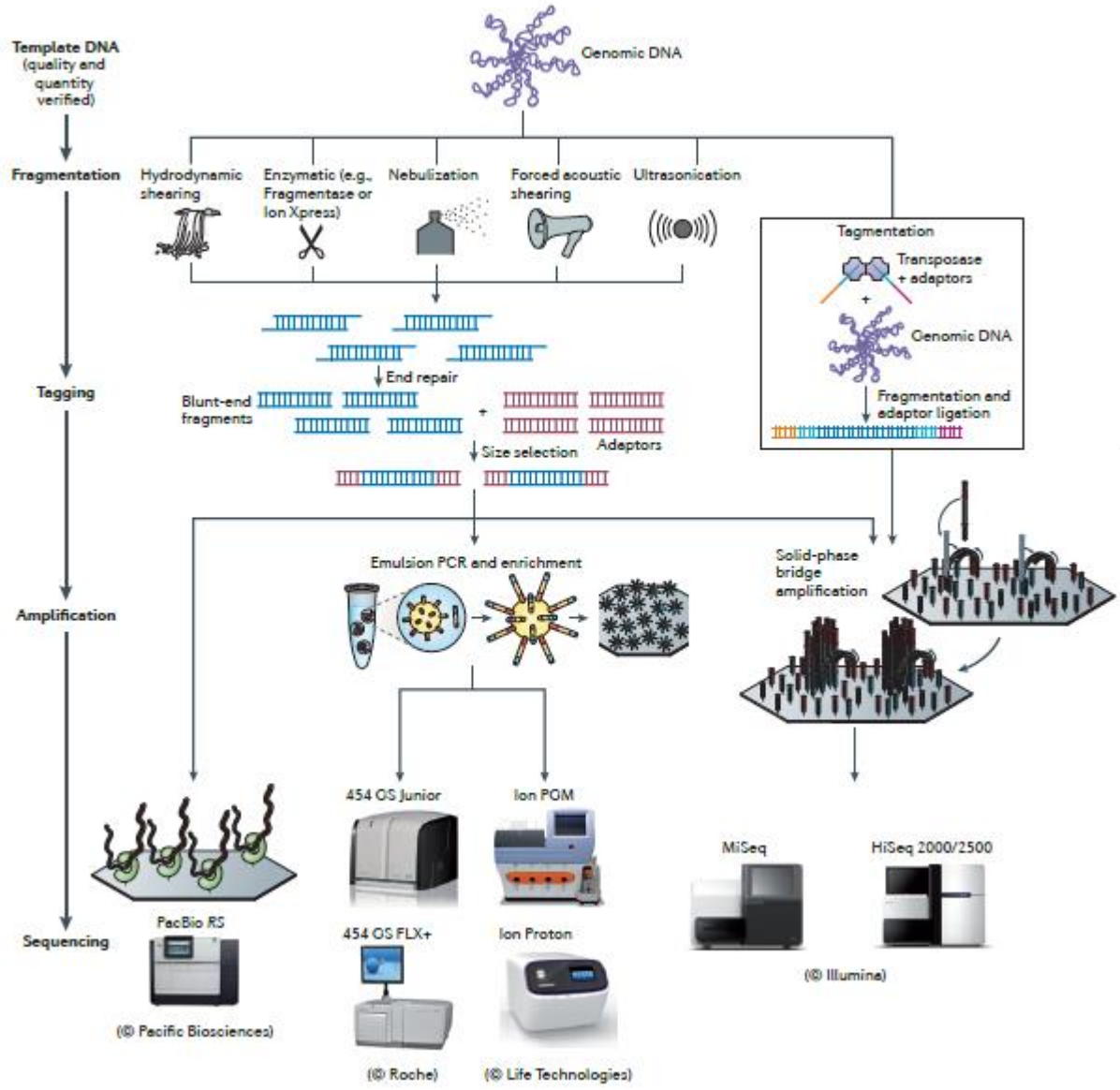
2. ◆PCR-amplify SSU rRNA gene (which species?)
◆Sequence random fragments (which function?)

3. Evaluate Sequences



„Classical metagenomics“ is based on clone libraries.

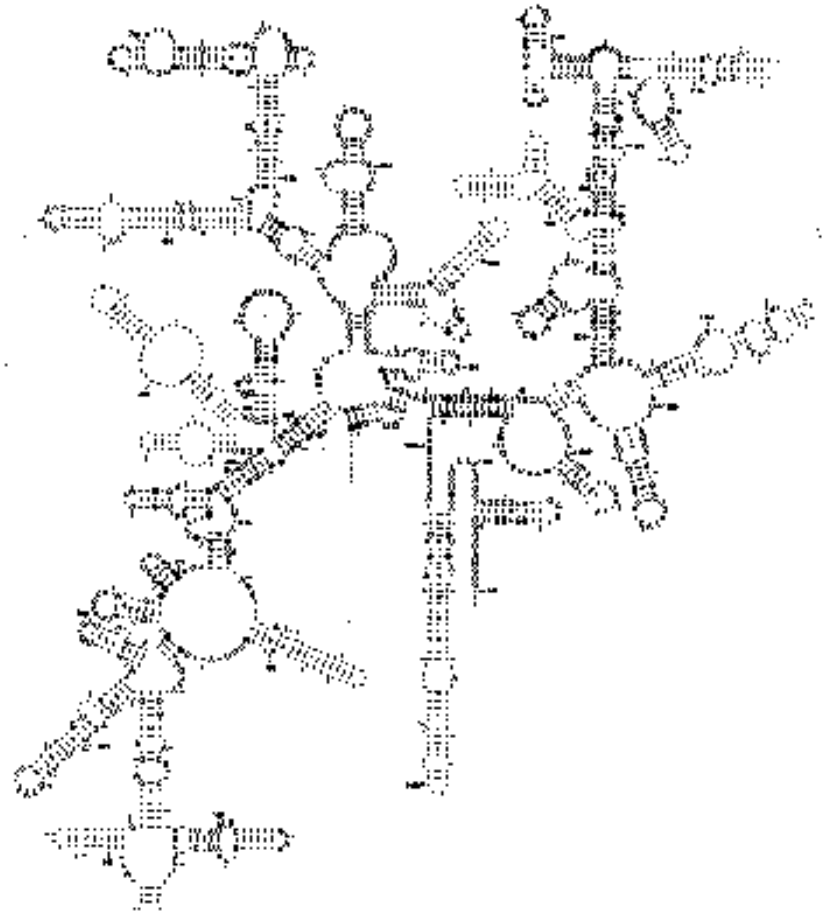
Figure from Daniel (2005) *Nature Rev. Microbiol*. **3**, 470-478.



In modern, NGS-based metagenomics there is no longer a need for generating cloning libraries.

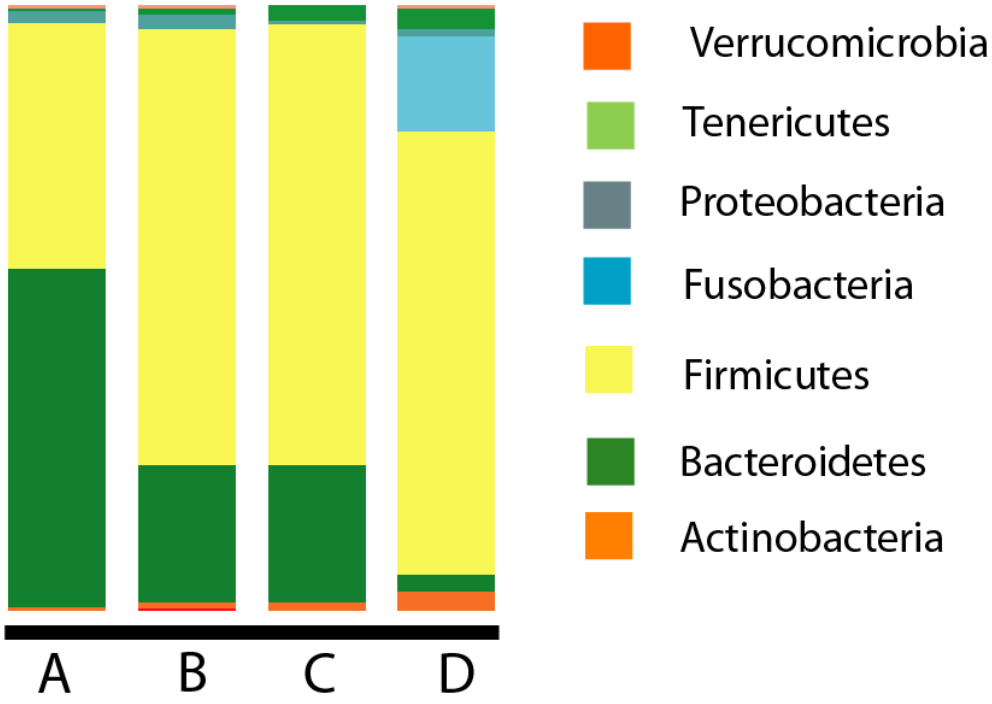
Figure from Loman et al. (2012) *Nature Rev. Microbiol*. **10**, 599-606.

- We can either sequence the entire metagenome (*shotgun metagenomics*) or a target sequence such as 16S rDNA (*tag sequencing*)
- The 16S rRNA gene is the most commonly used target because:
  - it is present in all organisms Til staðar í öllum lífverum
  - contains highly conserved domains (and well defined, less well conserved domains)
  - it is apparently only very rarely passed horizontally



2° structure of 16S rRNA

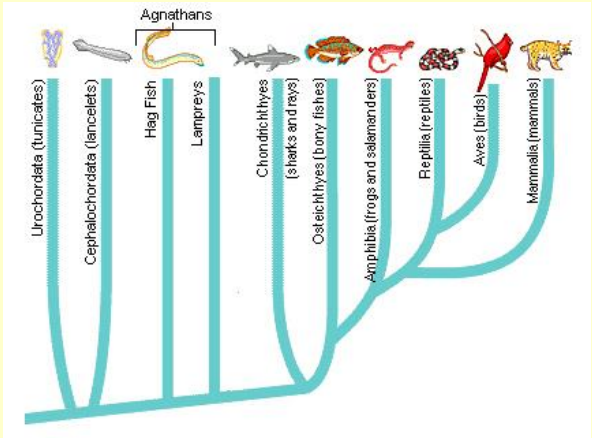
Comparative metagenomes yield differences in microbiome composition



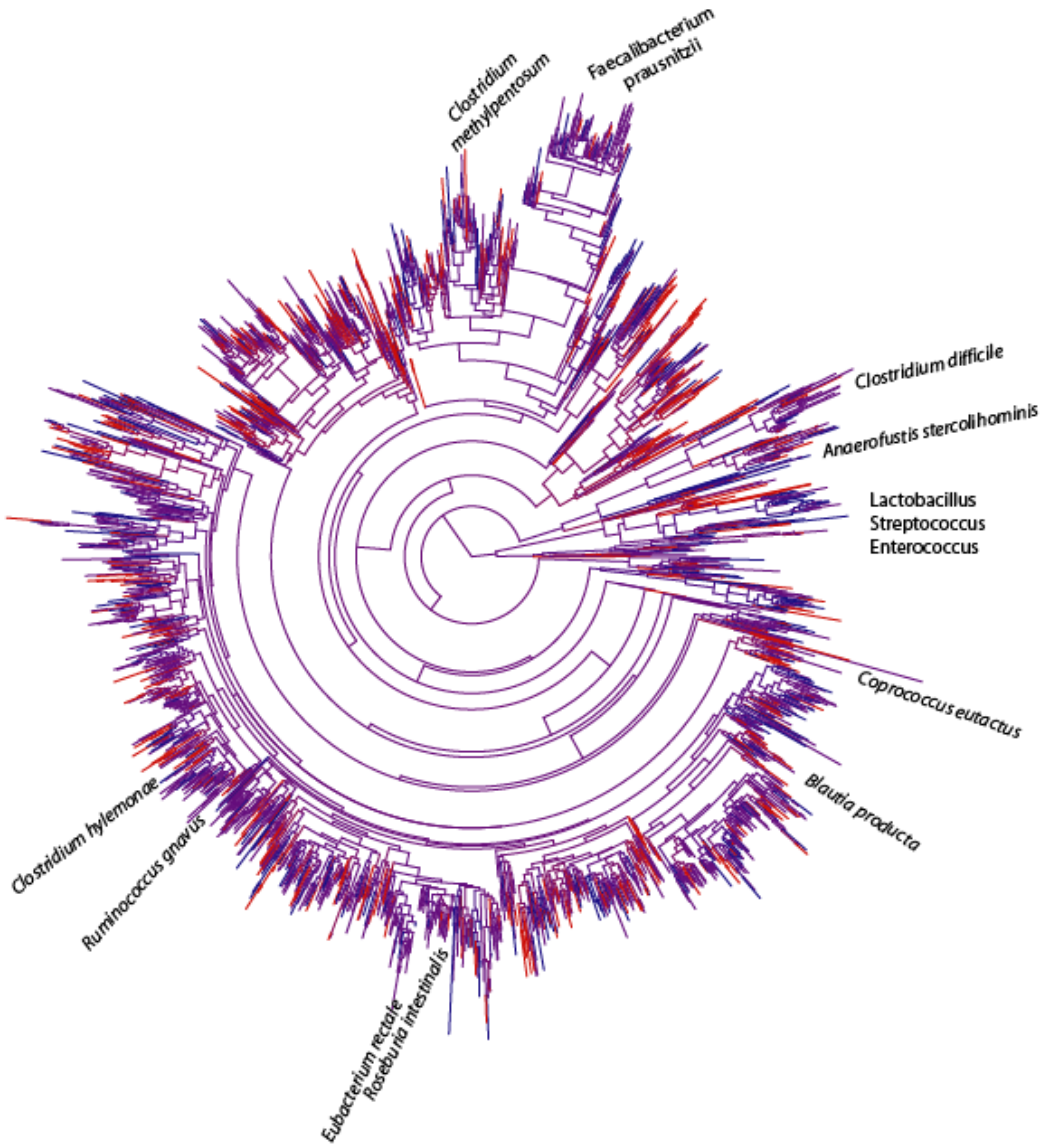
Human gut microbiota at the phylum level.

Figure from Yatsunenko *et. al.* 2012. Nature.

The phylum is a very coarse unit: Humans, birds, fish and tunicates all belong to the same phylum (Chordata)



Firmicutes in 2 healthy human individuals



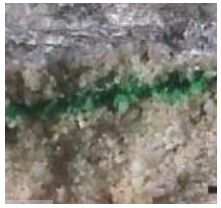
Individual variation is considerable

=> Robust descriptive and inferential statistics are required before conclusions can be safely drawn.

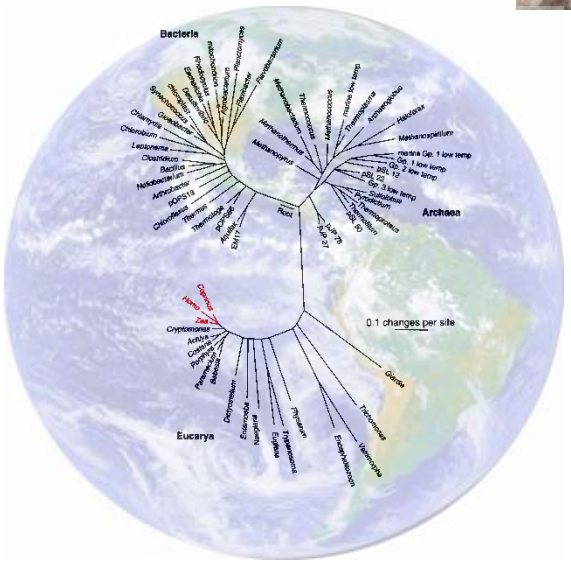
Temperature

0-100°C

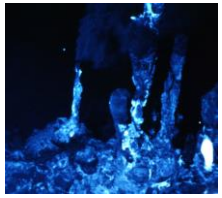
pH



1-12



Pressure



1-200 atm

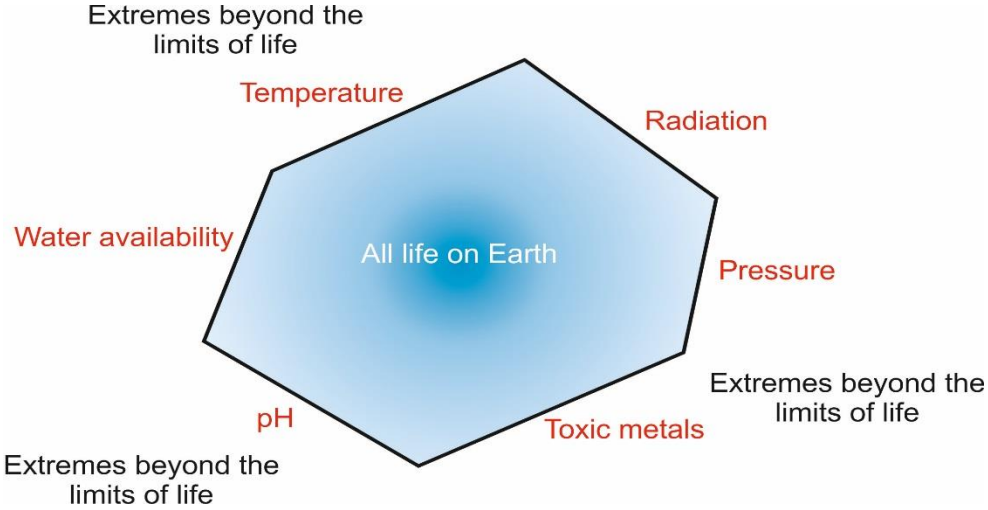
Trophy

Oligotrophic vs. eutrophic

Lozupone CA & Knight R (2007) Global patterns in bacterial diversity. *Proc Natl Acad Sci U S A* **104**: 11436-11440.

The biospace

One way to conceptualise the biosphere is to consider its tolerances to physical and chemical extremes. One can view all organisms to be within an enormous biological zoo surrounded by a fence, where the fence is a set of physical and chemical extremes beyond which life cannot adapt – a fence that separates life from death.



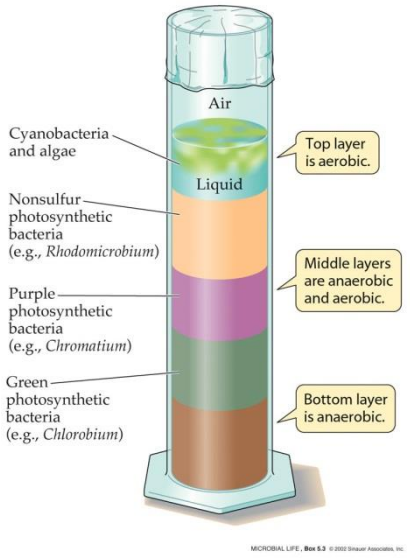
A simplified depiction of the biospace, the space defined by physical and chemical extremes within which life can exist. There are many more extremes that define the limits of life than those shown here. These limits could be defined for survival, metabolic activity (growth) or reproduction.

Image from: Cockell (2016) *Astrobiology. Understanding Life in the Universe*. Wiley.

Selective isolation

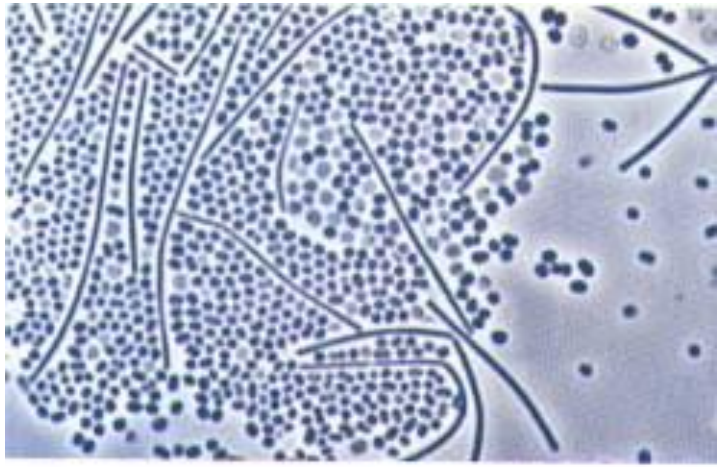
- So, with all this diversity in a single gram of soil, how do we isolate individual species and/or determine their roles in the environment?
- *Mesocosms*: simulations of natural habitats.
- ***Enrichment cultures***: cultures where bacteria possessing specific traits have higher fitness than the other inhabitants of the inoculation and are, therefore, enriched.
- In situ *analysis*: bacteria can be stained *in situ* and tractable samples analysed chemically and monitored over time.
- *Genomics/metagenomics*: analysing the genes present in the sample allows us to infer function of specific players.

Pioneers of microbial ecology: Martinus Beijerinck (1851-1931) developed the concept of **enrichment cultures** and Sergey Winogradsky (1856-1953) invented the **Winogradsky column** (a simple stagnant water mesocosm).



Symbiosis and syntrophy

- **Parasitism** – the parasite benefits while the host duffers damage.
- **Commensalism** – the guest benefits, the host does not, but neither does it suffer damage.
- **Mutualism** – all participants benefit.
- **Syntrophy** – Not symbiosis *per se*, but cooperative utilization of resources within a habitat. For example: oxidation of ethanol to acetate is thermodynamically unfavourable unless the resulting hydrogen is continously removed for methane production. (The methane can then be further processed by methanotrophs, creating biomass from which ethanol can be formed again, completing the cycle)



A methane-producing guild of three microbial species, one of which (dark cocci) needs the others for survival.

Source: http://www.aist.go.jp/NIBH

Ethanol fermentation

$2\ CH_3CH_2OH + 2\ H_2O \rightarrow 4\ H_2 + 2\ CH_3COO^- + 2\ H^+$

Ethanol — Acetate $\Delta G^{0\prime} = +19.4$ kJ/reaction

Methanogenesis

$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$

Methane $\Delta G^{0\prime} = -130.7$ kJ/reaction

Syntrophic, coupled reaction

$2\ CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2\ CH_3COO^- + 2\ H^+$

$\Delta G^{0\prime} = -111.3$ kJ/reaction

Two or more organisms require a resource in limited supply

⇨ Competition

The Verhulst model of population dynamics:

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right)$$

r-strategists

- High growth rate – utilize the resource before the competitors can
- Respond rapidly when conditions become favourable
- Become inactive (e.g. spores) when conditions become unfavourable
- Example: Algal bloom

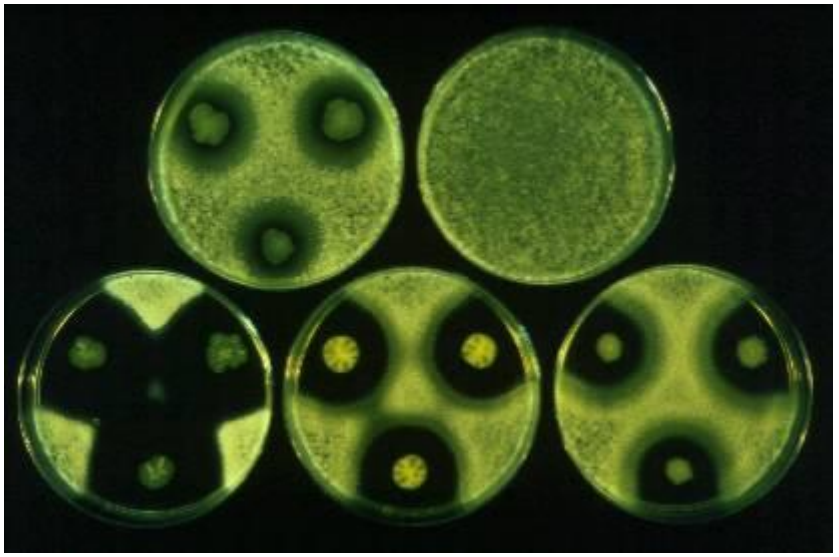
K-strategists

- Comparatively low growth rates
- Adapt to the environment: Assign comparatively more energy to adaptive processes
- Withstand disruptions to the environment (stress tolerant)
- Example: Euryhaline bacteria

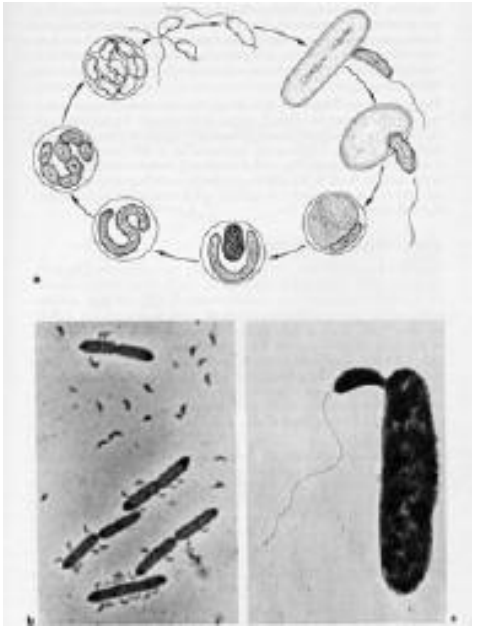
Antagonism

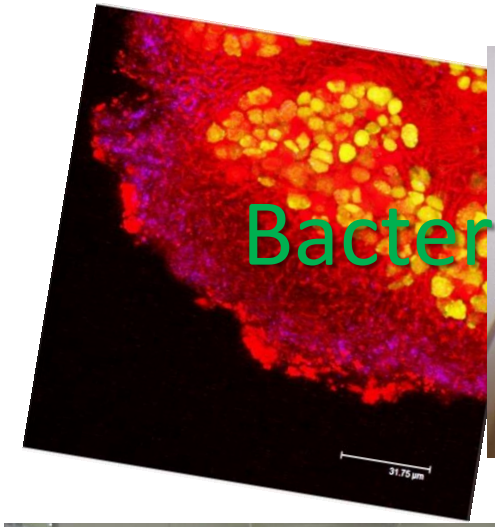
A bacterium has several ways to defend its „territory“:

- **Competition**
- **General antagonism** – the microbe produces by-products (t.d. peroxide, acids, ...) that are inhibitory to growth of other microbes
- **Specific antagonism** – the microbe produces bacteriocins or antibiotics, specifically in order to kill off particular competitors
- **Predation** – some bacteria are capable of killing and „eating“ others, e.g. myxococci and *Bdellovibrio*.



Antagonism of several *Pseudomonas* strains against the Dutch elm disease fungus.





Bacteria - more than just pathogens!

Found in all habitats.

In glacial ice, boiling hot springs, in deep-sea trenches, in bedrock, inside other organisms, ...

At various levels of temperature, pressure, acididty, oxidative potential, radiation, humidity, ...

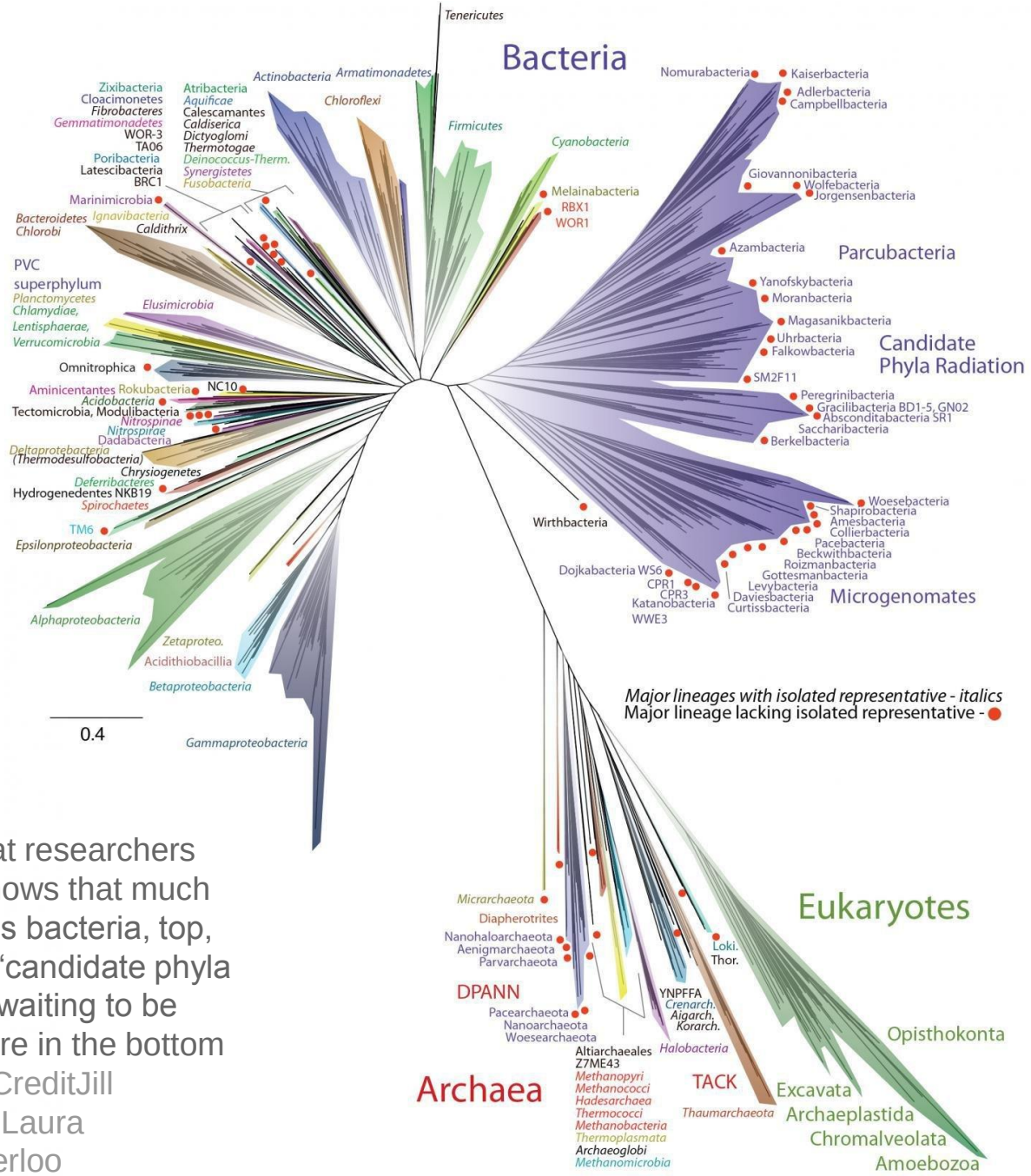
Amazing diversity (a billion species?).

The World is host to 300,000 plant species, 60,000 vertebrate species, 1½ million invertebrate species.

Huge biomass (500 billion tonnes).

A thousandfold the biomass of *Homo sapiens.*

One thousand billion billion billion (10^{30}) individuals.



The new tree of life that researchers published in April. It shows that much of Earth's biodiversity is bacteria, top, half of which includes "candidate phyla radiation" that are still waiting to be discovered. Humans are in the bottom branch of eukaryotes. CreditJill Banfield/UC Berkeley, Laura Hug/University of Waterloo

Bacterial phyla

- At present, there are 30 validly named bacterial phyla.
- In addition, there are known to exist several phyla known only from environmental DNA sequences.
- The vast majority of culturable environmental bacteria belong to the “classical” phyla (*Proteobacteria, Firmicutes, Actinobacteria, Cyanobacteria, Bacteroidetes, Spirochaetes*)

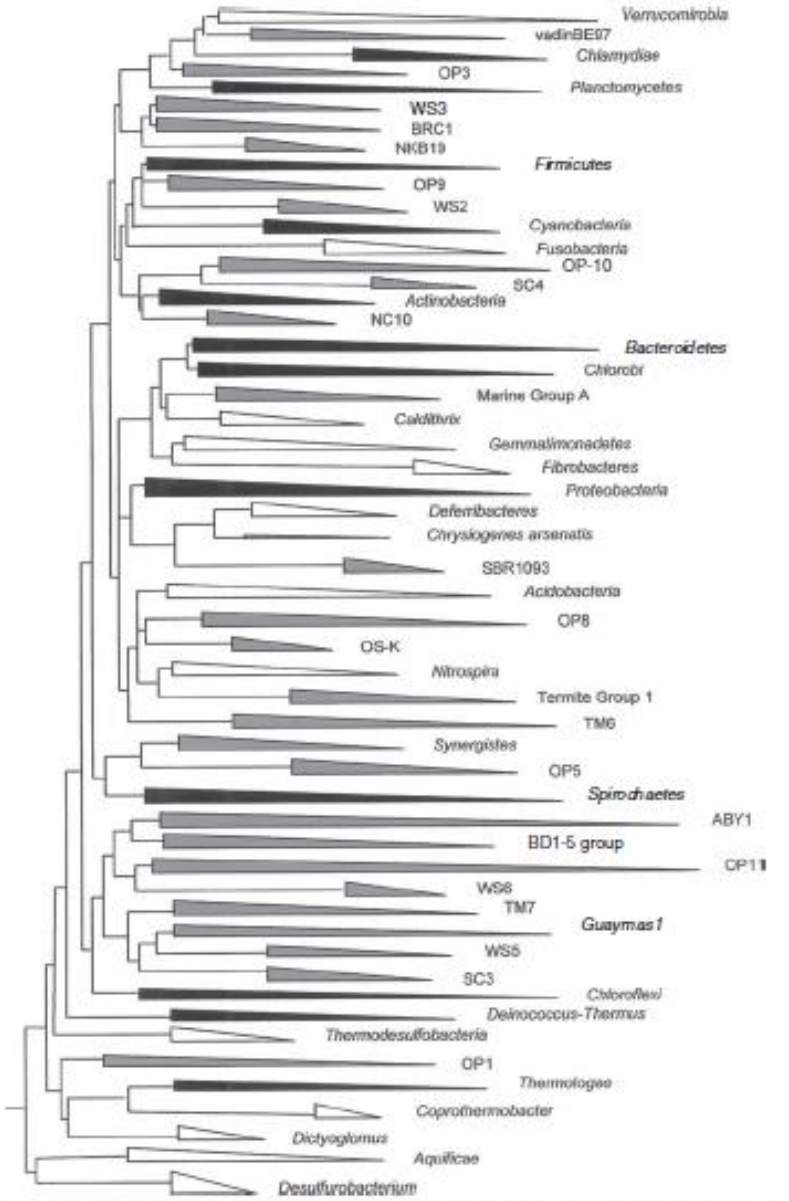


Figure 2.5. Fifty-two bacterial phyla depicted in Rappé and Giovannoni (2003). Black blocks represent the original 11 phyla (one of which was split into two phyla); white blocks represent phyla with at least one cultivated member; gray blocks represent phyla with no cultivated members.

Bacterial taxonomy resources on the web

- LPSN (*List of Prokaryotic Names with Standing in Nomenclature*): http://www.bacterio.cict.fr/
- The NCBI Taxonomy Browser: http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Root
- GBIF (*Global Biodiversity Information Facility*): http://www.gbif.org/
- Bacterial systematics and nomenclature at DSMZ (*Deutsche Sammlung für Mikroorganismen und Zellkulturen*): http://www.dsmz.de/bacterial-diversity.html

C-flow in the ecosystem

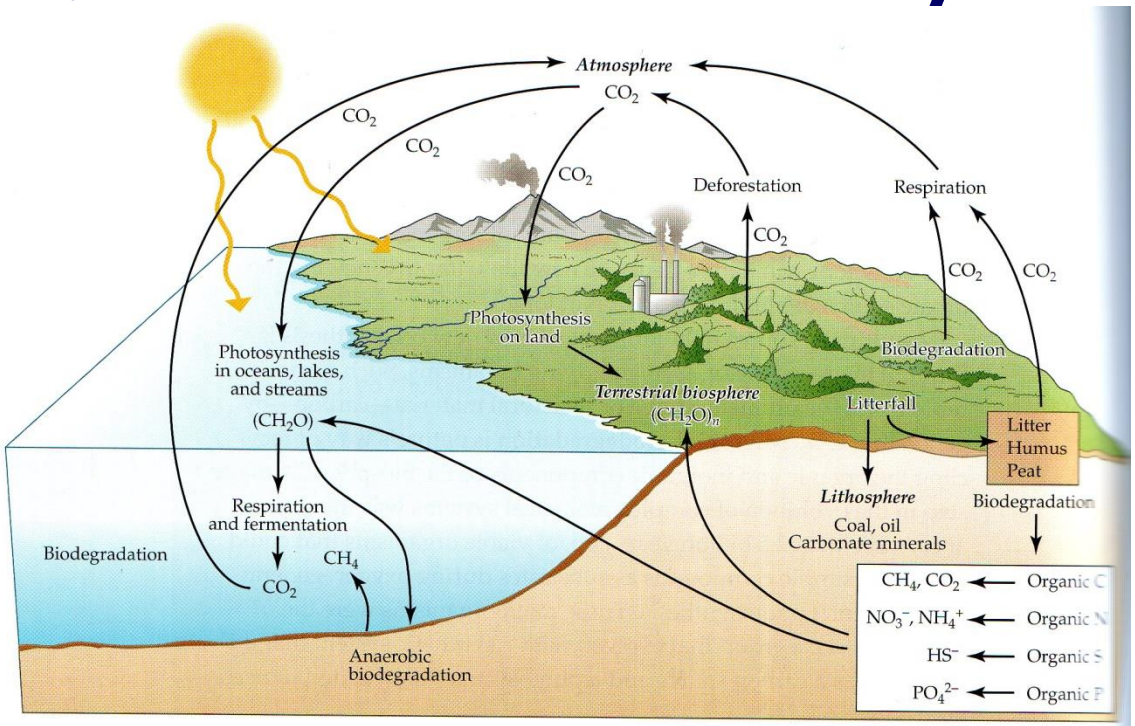


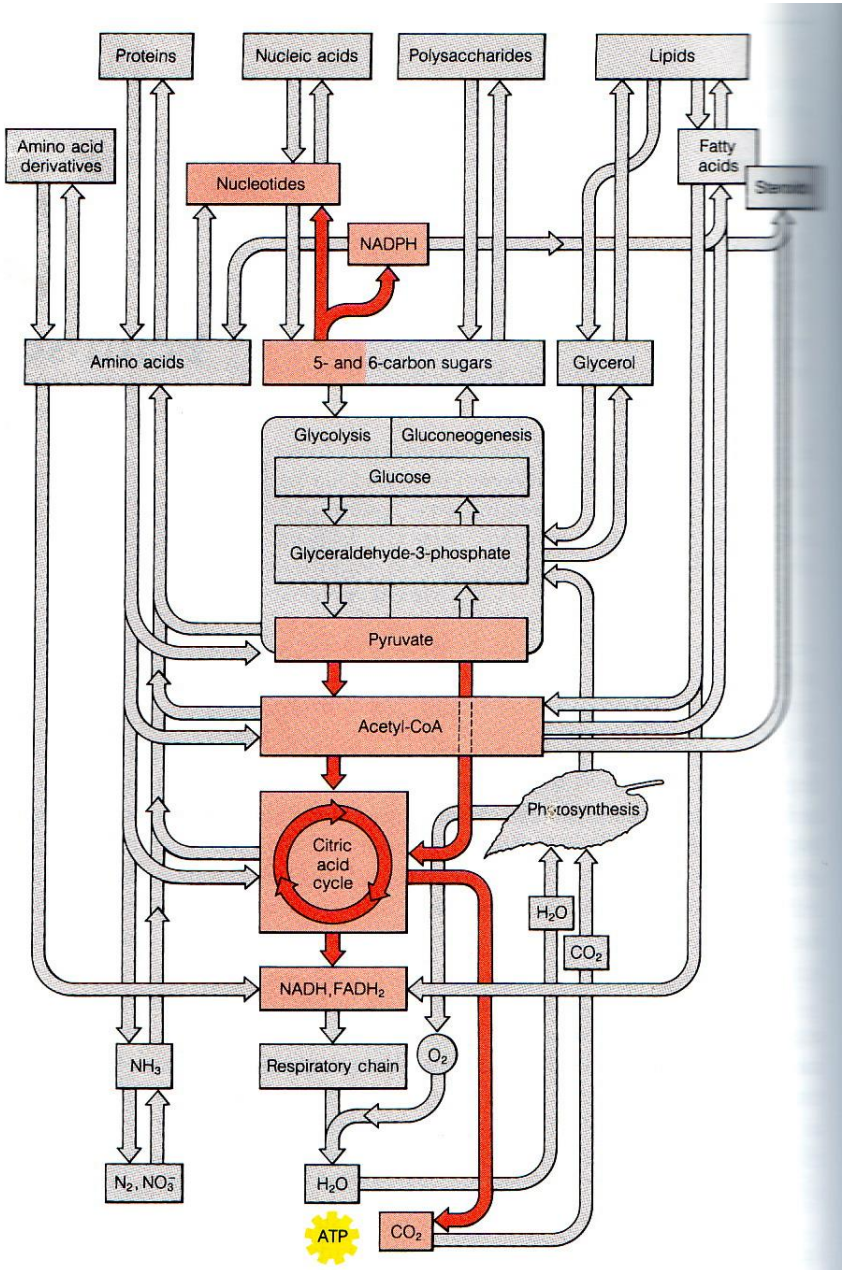
Figure 12.1 Carbon cycle
Photosynthesis uses light energy and fixes carbon dioxide into organic material. The organic material is an energy and carbon source for other microorganisms and animals. Organisms recycle carbon dioxide by respiration and decomposition of organic materials. (See text for details.)

- Primary producers (autotrophs) remove CO_2 from the atmosphere and produce biomass.
- Why isn't the Earth overgrown with biomass?

- Other organisms degrade the biomass back to CO_2 and water by means of
  1. **Respiration (aerobic degradation)**
  2. **Fermentation (anaerobic degradation)**

Cornelis B. van Niel (1897-1985) was a pioneer in microbial biochemistry.

- **C. B. van Niels' laws:**
  1. For every organic component or product of a living cell there exists a microbe capable of utilizing it as a source of carbon or energy
  2. Microbes are present in all habitats



Basic metabolic pathways and their connections.

Mathews & van Holde (1990) *Biochemistry*. Benjamin/Cummings. Redwood City.

- A great many catabolic and anabolic processes pass through, or are connected with, the classic glycolysis/Krebs-cycle/respiratory chain metabolism
- The basic biochemistry of the most distantly related microbes is quite similar

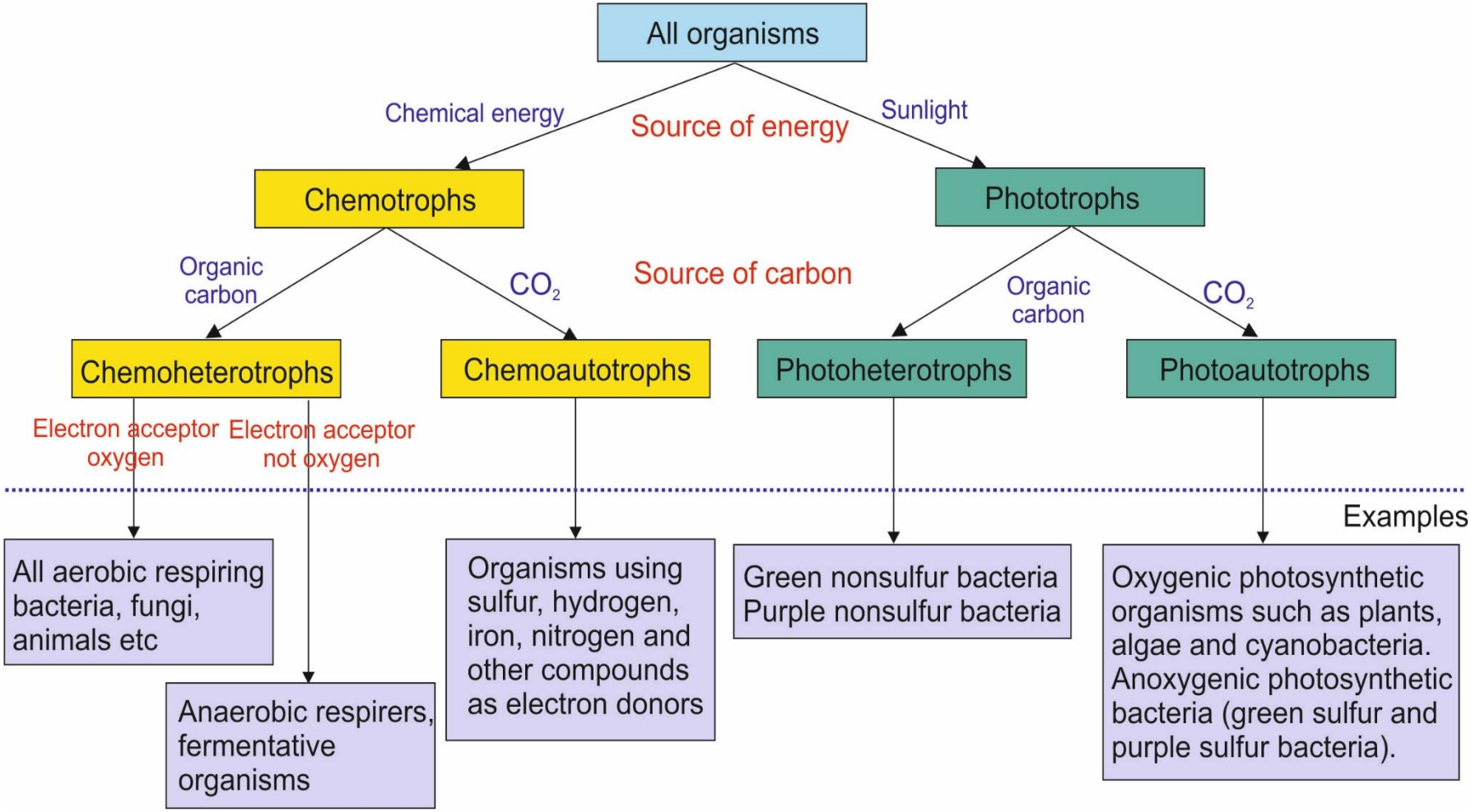
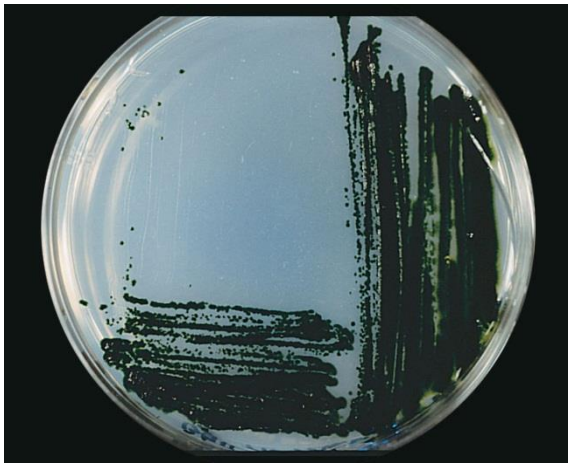


Image from: Cockell (2016) *Astrobiology. Understanding Life in the Universe*. Wiley.

Metabolic diversity



Anabaena viriabilis is a photoautotroph

Micrococcus luteus is a *chemoheterotroph*

- *Photoautotrophs*: light is the energy source, CO_2 the C-gjafa. Obligate photoautotrophs can only grow in the presence of light and CO_2. Use inorganic e^- -donors (H_2O, H_2S, H_2)
- *Photoheterotrophs*: can use light as an energy source (often supplementary), but need an organic source of C.
- *Chemolithoautotrophs*: obtain energy by oxidation of inorganic compounds (H_2, NH_3, NO_2^-, H_2S, Fe^{2+}). Carbon source: CO_2
- *Mixotrophs*: chemolithotrophs that require organic carbon as a carbon source.
- *Chemoheterotrophs*: need an organic source of C and energy.

Metabolic diversity – anaerobic respiration

Electron Tower

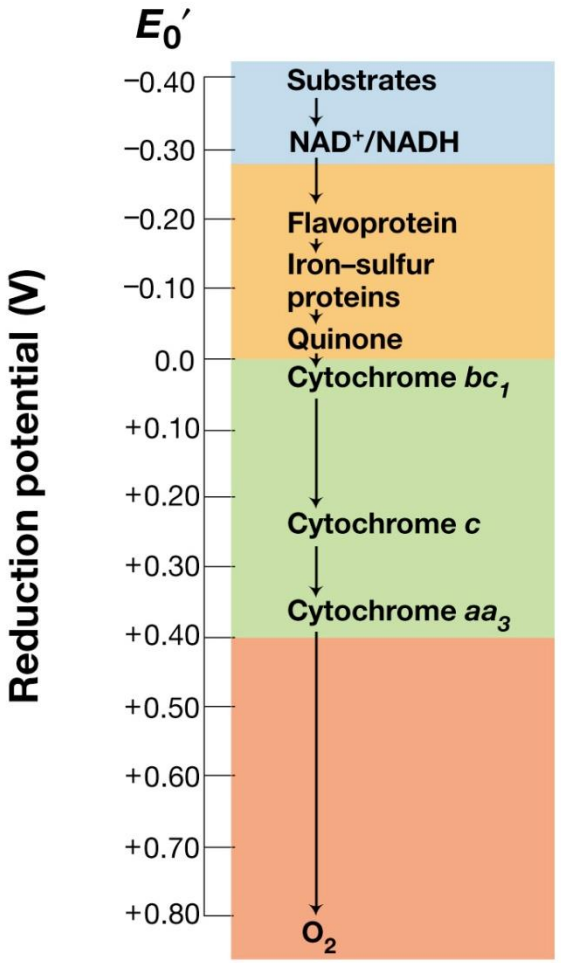
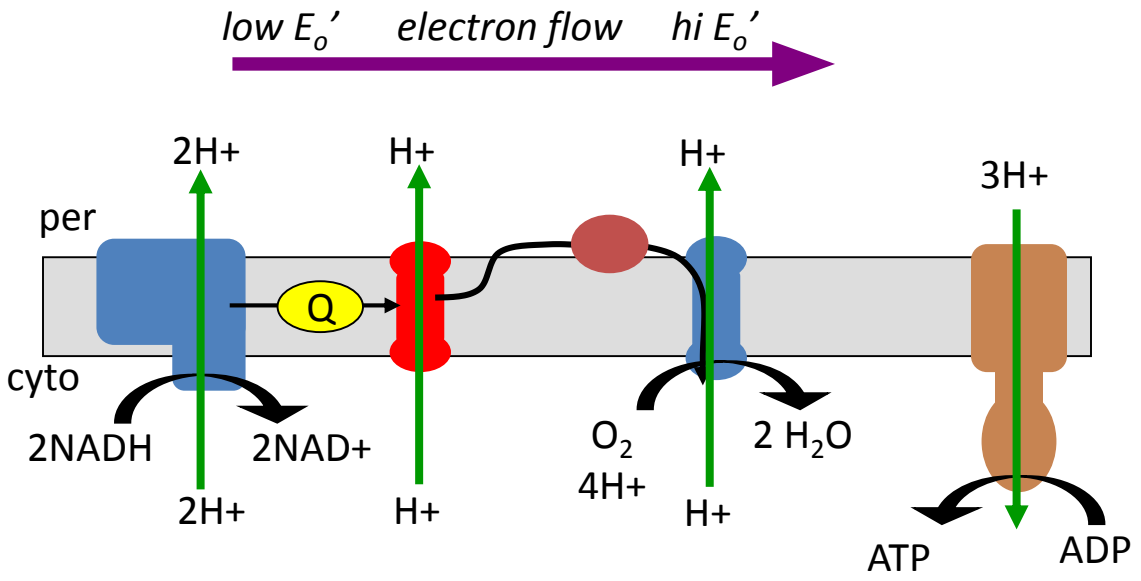


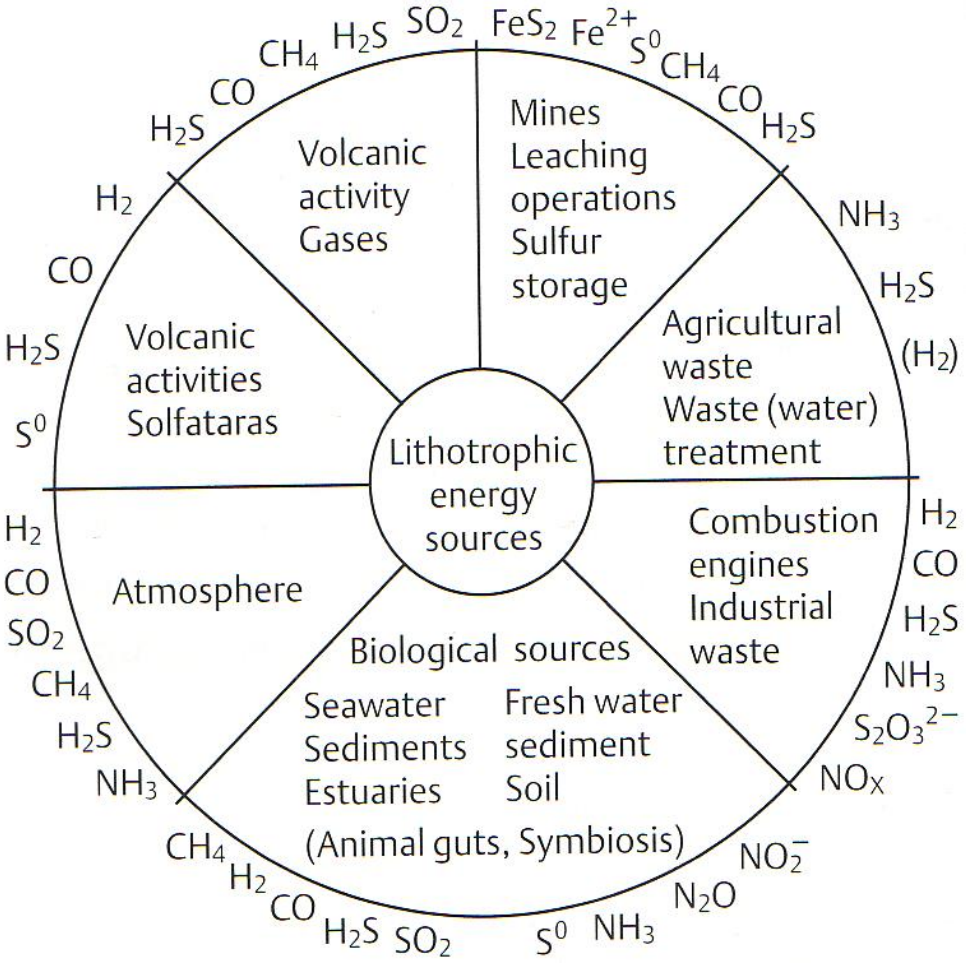
Table 20.1 Energy yields from the oxidation of various inorganic electron donors[a]

Electron donor	Chemolithotrophic reaction	Group of chemolithotrophs	E_0' of couple (V)	$\Delta G^{0\prime}$ (kJ/reaction)	Number of electrons/ reaction	$\Delta G^{0\prime}$ (kJ/2e$^-$)
Phosphite[b]	$4\,HPO_3^{2-} + SO_4^{2-} + H^+ \rightarrow 4\,HPO_4^{2-} + HS^-$	Phosphite bacteria	−0.69	−91	2	−91
Hydrogen	$H_2 + \frac{1}{2}O_2 \rightarrow H_2O$	Hydrogen bacteria	−0.42	−237.2	2	−237.2
Sulfide	$HS^- + H^+ + \frac{1}{2}O_2 \rightarrow S^0 + H_2O$	Sulfur bacteria	−0.27	−209.4	2	−209.4
Sulfur	$S^0 + 1\frac{1}{2}O_2 + H_2O \rightarrow SO_4^{2-} + 2\,H^+$	Sulfur bacteria	−0.20	−587.1	6	−195.7
Ammonium[c]	$NH_4^+ + 1\frac{1}{2}O_2 \rightarrow NO_2^- + 2\,H^+ + H_2O$	Nitrifying bacteria	+0.34	−274.7	6	−91.6
Nitrite	$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^-$	Nitrifying bacteria	+0.43	−74.1	2	−74.1
Ferrous iron	$Fe^{2+} + H^+ + \frac{1}{4}O_2 \rightarrow Fe^{3+} + \frac{1}{2}H_2O$	Iron bacteria	+0.77	−32.9	1	−65.8

[a]Data calculated from E_0' values in Appendix 1; values for Fe^{2+} are for pH 2, and others are for pH 7. At pH 7 the value for the Fe^{3+}/Fe^{2+} couple is about +0.2 V.
[b]Except for phosphite, all reactions are shown coupled to O_2 as electron acceptor. The only known phosphite oxidizer couples to SO_4^{2-} as electron acceptor.
[c]Ammonium can also be oxidized with NO_2^- as electron acceptor by anammox organisms (Section 20.13).



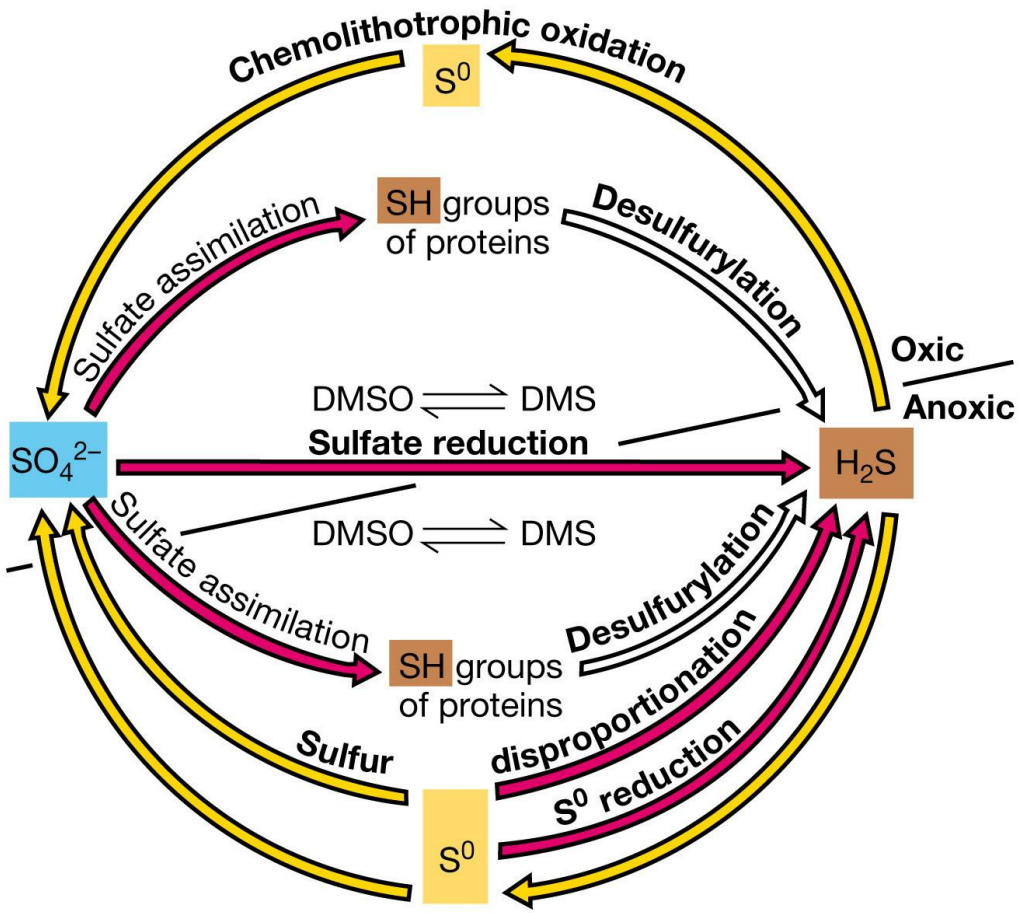
Geological, biological, and anthropogenic sources of reduced inorganic compounds supporting chemolithotrophs



<u>Typical habitats of chemolithotrohs:</u>

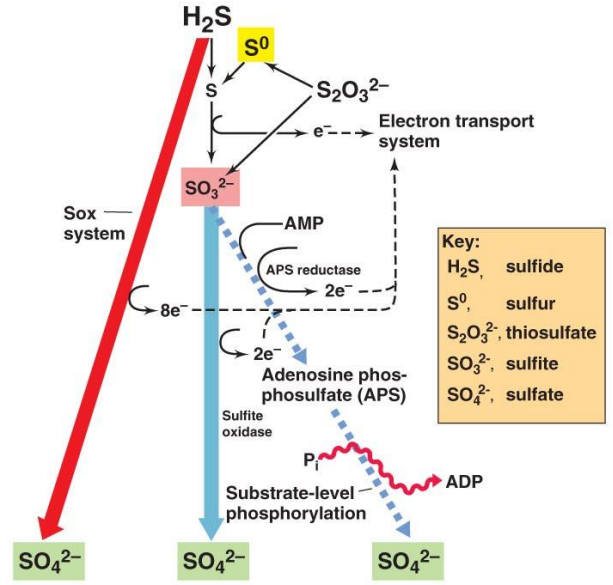
Near the interface of oxic/anoxic conditions

Biogeochemical cycling of sulfur.

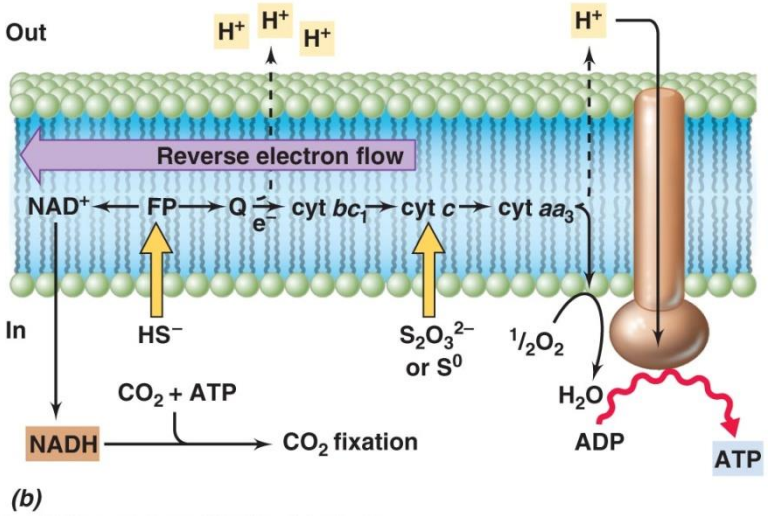


Oxidation of Reduced Sulfur Compounds

- Many reduced sulfur compounds are used as electron donors
- H_2S, S^0, $S_2O_3^-$ are commonly used
- One product of sulfur oxidation is H^+, which results in a lowering of the pH of its surroundings
- *Sox system* oxidizes reduced sulfur compounds directly to sulfate
- Usually aerobic, but some organisms can use nitrate as an electron acceptor



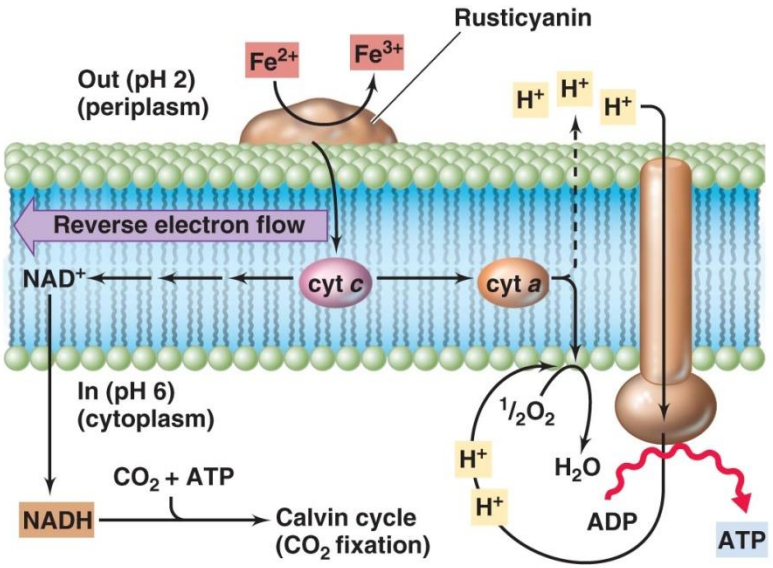
(a)



(b)

Iron Oxidation

- Ferrous iron (Fe^{2+}) oxidized to ferric iron (Fe^{3+})
- Ferric hydroxide precipitates in water
- Many Fe oxidizers can grow at pH <1
  - Often associated with acidic pollution from coal mining activities
- Some anoxygenic phototrophs can oxidize Fe^{2+} anaerobically using Fe^{2+} as an electron donor for CO_2 reduction



Iron oxide reducing bacteria

- Examples:
  - *Geobacter, Shewanella, Rhodoferax*