Microbial Methods Course Logistics

Instructors:

- Joe Vallino, Starr 330, x7648, jvallino@mbl.edu
- Kristin Gribble, Lillie 305, x7194, kgribble@mbl.edu
- Office hours: Tuesdays after lab.

TA:

- Emma Daily, SES TA's office (?), email: emmacdaily@gmail.com

Schedule

- Class meets Tuesdays and Thursdays, 10:15 - 12:00, ***Except week 8.5 – 10 (Nov 4 – Nov 11)***

Requirements

- Courses in first year chemistry, biology, and biochemistry (suggested).
- Readings. No required text. Recommended text: Madigan, Martinko, Dunlap and Clark (2009) *Brock Biology of Microorganisms*, 12th ed. Copies of *Brock* are in the lab. Can be purchased through Amazon.com (search ISBN: 0-132-32460-1)
- Problem Sets: Set of questions on readings and lab, *due Thursdays at beginning of class*. *Email solutions in Word or PDF format* to Joe Vallino (jvallino@mbl.edu). Pictures of calculations work fine.
- Lab participation.

Organization

- Combination of lectures and labs.
- 8 focus topics over 10 weeks.
- Lectures, readings and problem sets, available at: **http://ecosystems.mbl.edu/MicrobialMethods**

Grading

- 95% Problem Sets. Five (5) points are deducted with each day past due date. **Contact me if you need extension.**
- 5% Participation and laboratory technique (**Asking Questions**, **use of lab notebook, etc.**)
- Final contingent on individuality of problem sets and class participation.

Course Objectives

- Introduction to microbial methods applicable to the study of ecosystems
  - State variables (i.e., concentration, abundance)
  - Processes (i.e., rates, fluxes)
- Introduction to microbial systems (or systems biology)
  - Diverse communities of microorganisms exhibiting some homeostasis.
- Introduction to microbial biogeochemistry
  - Chemistry that results from both biotic and abiotic processes.

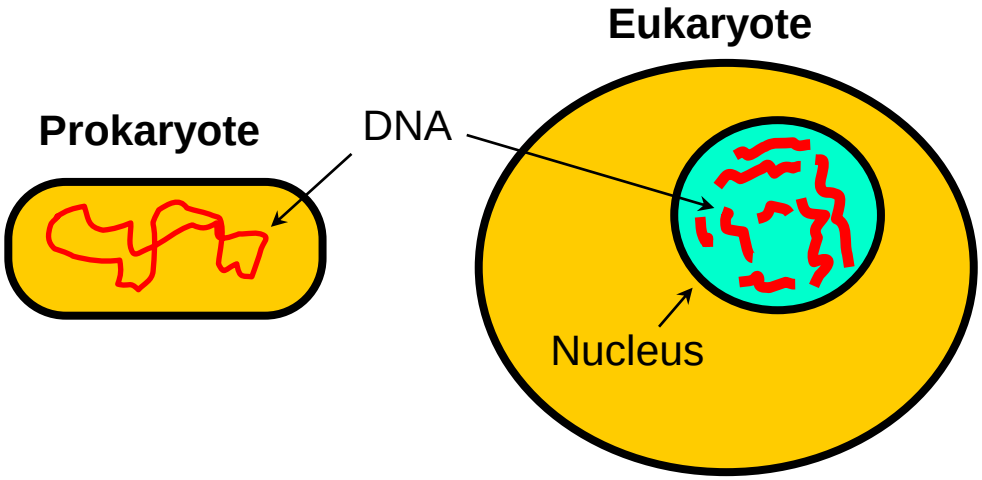
<u>My Background and Research:</u>

- Chemical and Biochemical Engineering (metabolic engineering, long ago).
- Modeling microbial dynamics
- Understanding and modeling of *whole system* biogeochemistry
- Living systems and nonequilibrium thermodynamics (“Systems Biology”)
- Transport modeling

Prokaryotes and Eukaryotes

Two general classes of cells:

- **Prokaryotes**: Most primitive cell. Lack internal organelles and nucleus. DNA is single molecule (no chromosomes) aggregated in the *nucleoid*, although plasmids can also be present. Reproduction occurs by simple division. Prokaryotes is a synonym for bacteria.
- **Eukaryotes**: Typically, larger cells that contain a nucleus with DNA organized into chromosomes. Organelles, such as mitochondria or chloroplast, are usually present. Usually contain two copies of genes (diploid) and division occurs by mitosis. Example eukaryotes:
    - *Algae*: Phototrophic, single cells (or colonies).
    - *Fungi*: Heterotrophic single (yeast) or multicellular (molds, mushrooms) with cell wall.
    - *Protozoa*: Single cells lacking rigid cell wall, heterotrophic.
    - *Metazoans*: Plants (phototrophic) and animals (heterotrophic) (cell differentiation).



Five Kingdoms (old Phylogeny)

Animalia (Eukaryotes)
- Multicellular
- Organs
- No cell wall
- Ingests nutrition
- Nervous system
- Has locomotion

Plantae (Eukaryotes)
- Multicellular
- Cellulose cell walls
- Photosynthetic nutrition
- No nervous system
- No locomotion

Fungi (Eukaryotes)
- Most multicellular
- Chitin cell walls
- Absorbs food
- No nervous system
- No locomotion

Protista (Eukaryotes)
- Most unicellular or simple multicellular
- Some have cell walls
- Absorbs food, ingests or photosynthesizes
- No nervous system
- Locomotion in some

Monera (Prokaryotes)
- Most unicellular, some colonial
- Cell walls of polysaccharides
- Absorb food, chemosynthesis or photosynthesis
- No nervous system
- Locomotion in some

Microbiology (< 1 mm)

Phylogeny from DNA sequences

Methodology

- Compare DNA sequences of different organisms
- Closer DNA sequences are to each other, closer the evolutionary distance separating the organisms.
- Use DNA of ribosomal RNA (rRNA), since all organisms have rRNA and rRNA's are evolutionarily stable. Why is it important to use a conserved gene?

Example

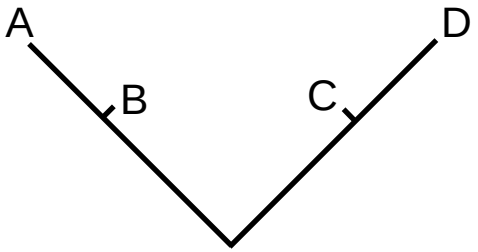
AACGTCGAAA (Organism A)

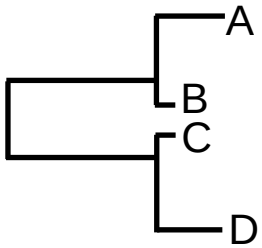
AACCTCGAAA (Organism B)

AGGCTAGAAA (Organism C)

AGGCTAGTAA (Organism D)

Evolutionary distance (base pairs)	
A → B	1
A → C	4
A → D	5
B → C	3
B → D	4
C → D	1



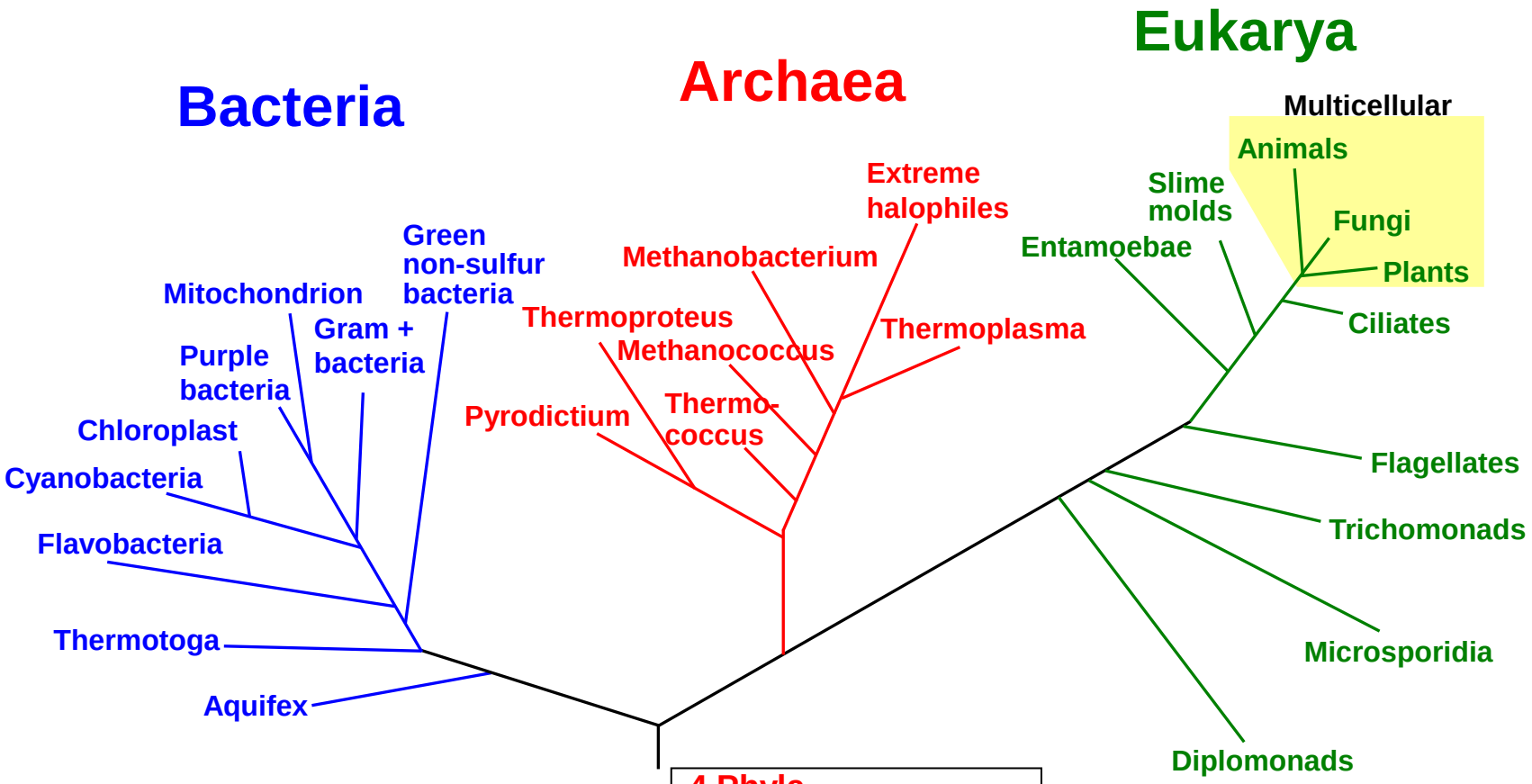


(Kristin Gribble will cover this in more detail in Module 7)

Phylogenetic Tree (Three Domains)

Based on 16s or 18s rRNA sequences

Carl R. Woese (1977)



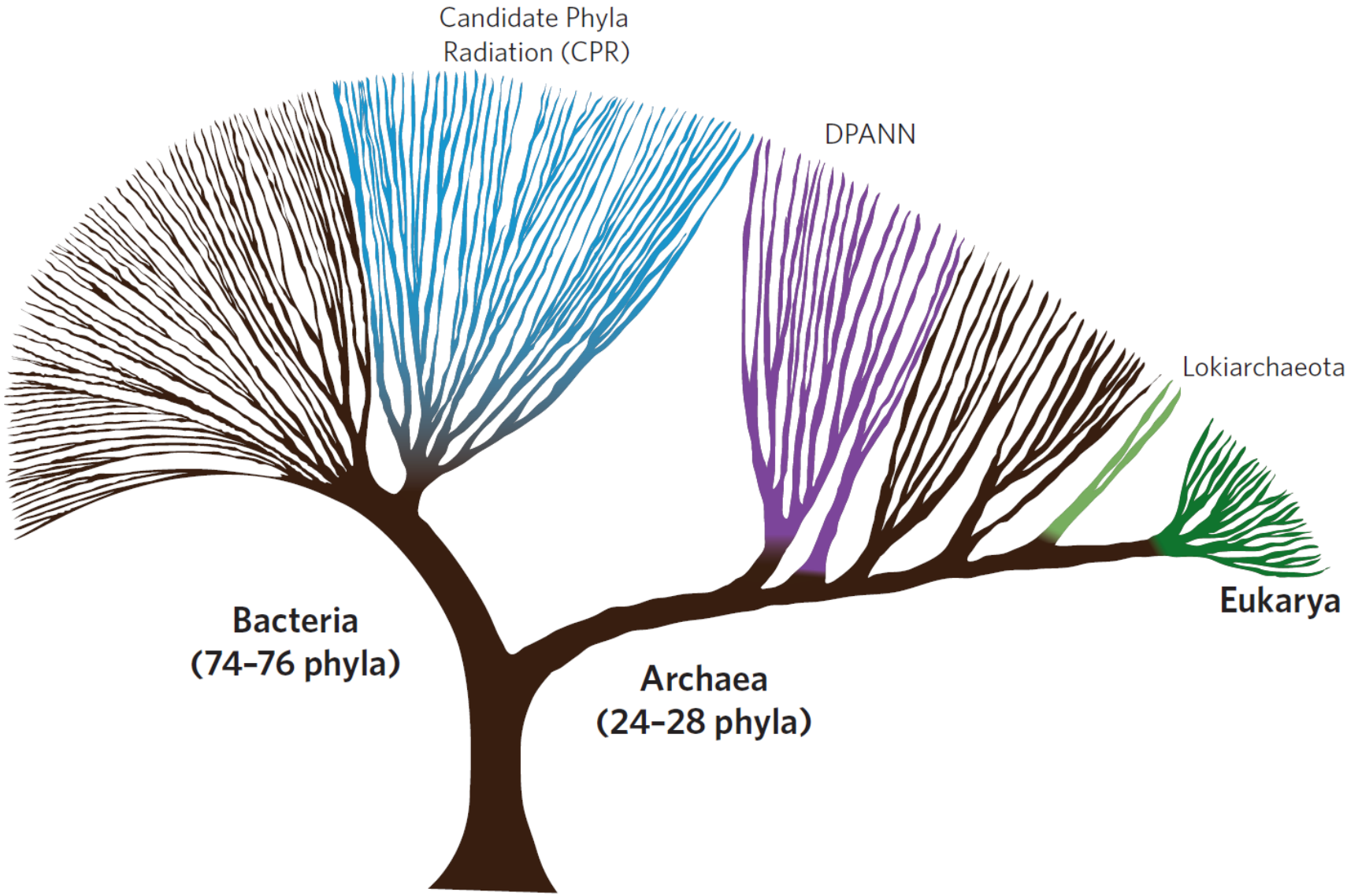
- 18 Phyla

4 Phyla
- Euryachaeota
- Crenarchaeota
- Korarchaeota
- Nanoarchaeota

- Protists
- Fungi
- Plants
- Animals

How about Viruses?

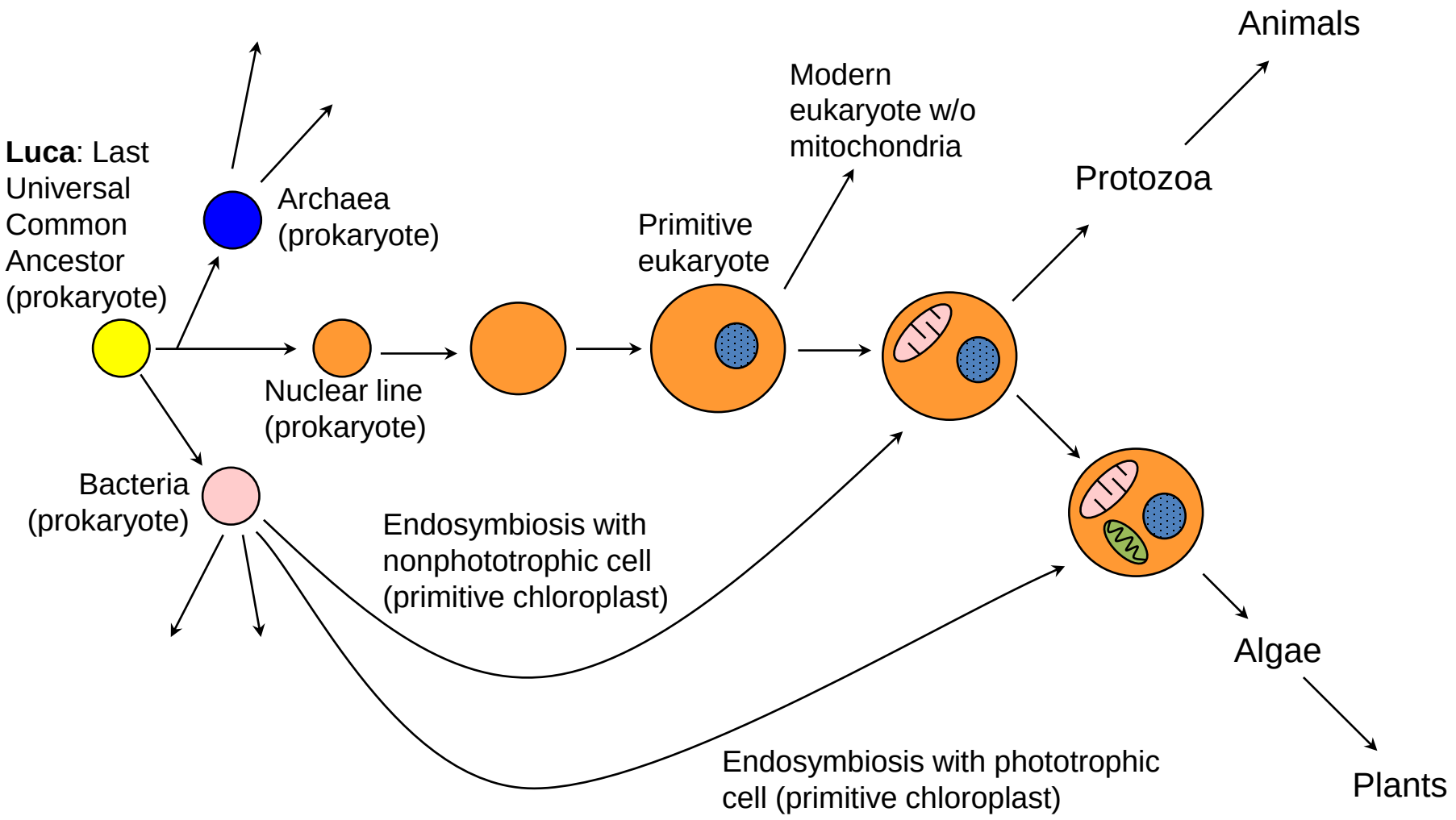
Just Two domains?



Spang A, Ettema TJG. 2016. Microbial diversity: The tree of life comes of age. Nature Microbiology 1: 16056
Also see: Parks, D. H., et al. (2018). "A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life." Nature Biotechnology: 1-14.

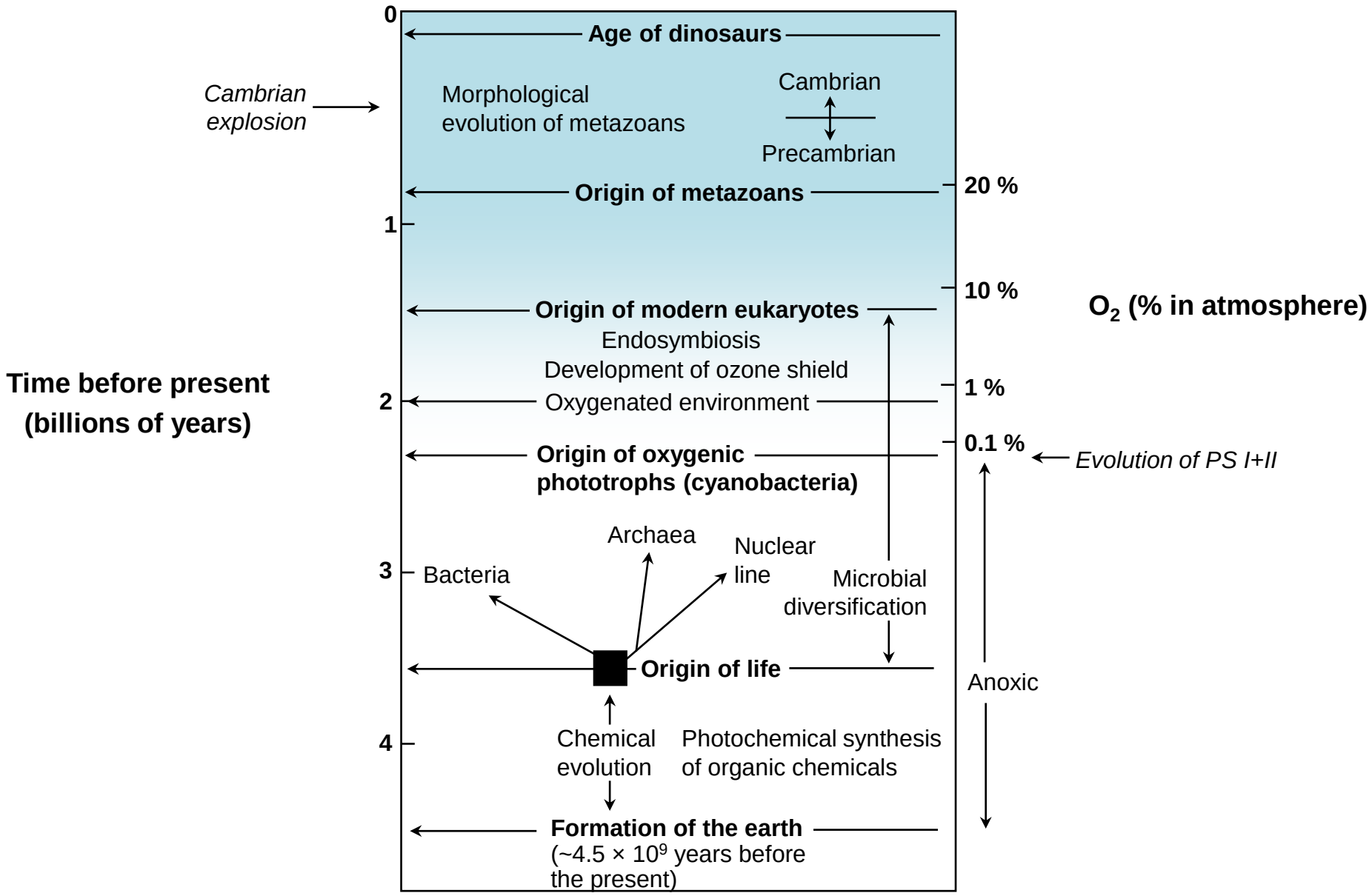
Endosymbiosis

Evolution of modern eukaryotic cells.



Lynn Margulis was the champion of this paradigm, which has gained wide acceptance

Evolution of life and atmospheric O_2



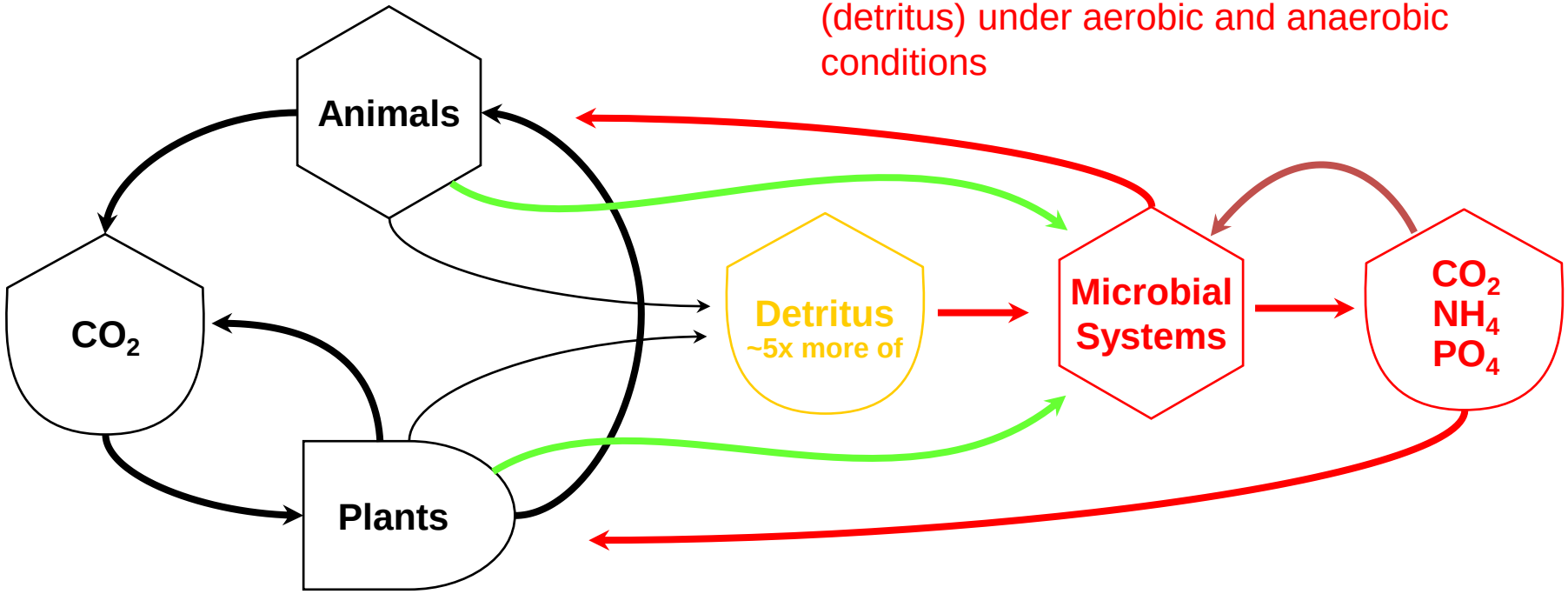
From *Brock Biology of Microorganisms*

The importance of microorganisms in Ecosystems

You could probably remove all higher organisms without significantly altering the Earth's biogeochemistry or homeostatic properties.

Higher Trophic Levels Metabolically Simple
"*Charismatic mega-fauna*"

Microbial Systems responsible for decomposition of dead organic material (detritus) under aerobic and anaerobic conditions



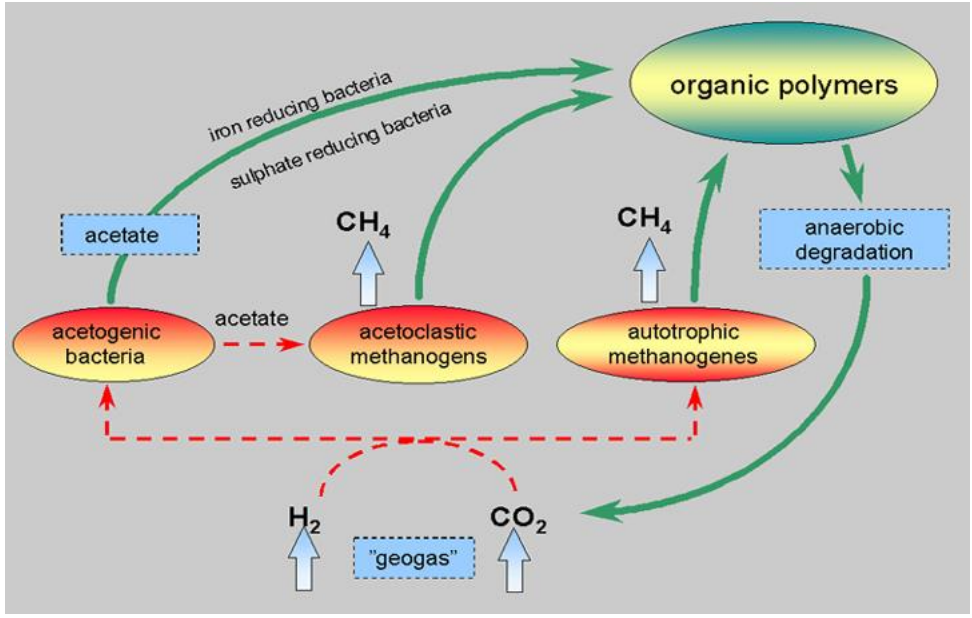
As well as being consumed by higher trophic levels, microbial systems also recycle many inorganic nutrients: N, P, S, Trace metals.

William B. Whitman, David C. Coleman, and William J. Wiebe. Prokaryotes: The unseen majority. PNAS 95 (12):6578-6583, 1998.

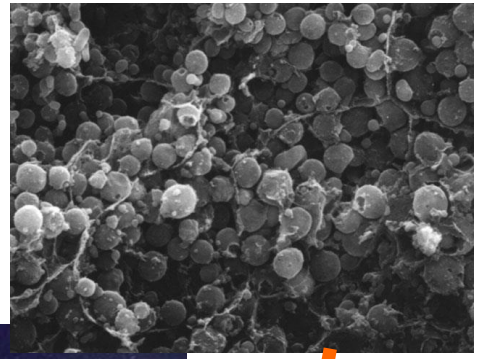
Deep Biosphere

Strong evidence now supports the presence of a biosphere living up to 1 km below the earth's surface. Bacteria in this deep biosphere have been found in even crystalline basalt rocks below marine sediments, and their biomass is on the order of that above the surface. This biosphere is driven by geogasses, and is similar to deep ocean vent ecosystems. Such communities may also exist on other planets, e.g., Mars.

Chemistry of the Deep Biosphere



Hydrothermal Vent Tubeworms and bacterial symbionts

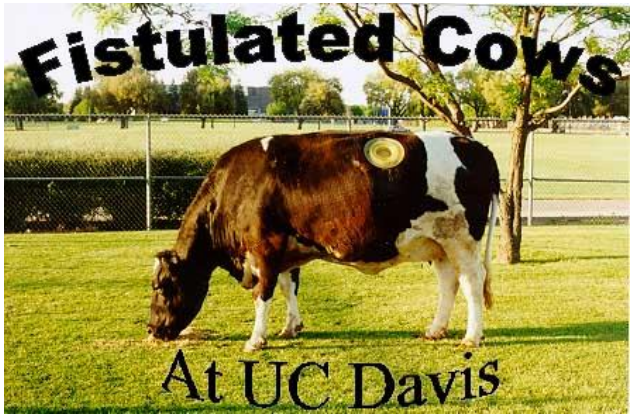


Kotelnikova, S. and Pedersen, K. 1998. Distribution and activity of methanogens and homoacetogens in deep granitic aquifers at Äspö Hard Rock Laboratory, Sweden. FEMS Microbiology Ecology, Vol. 26, 121-134.

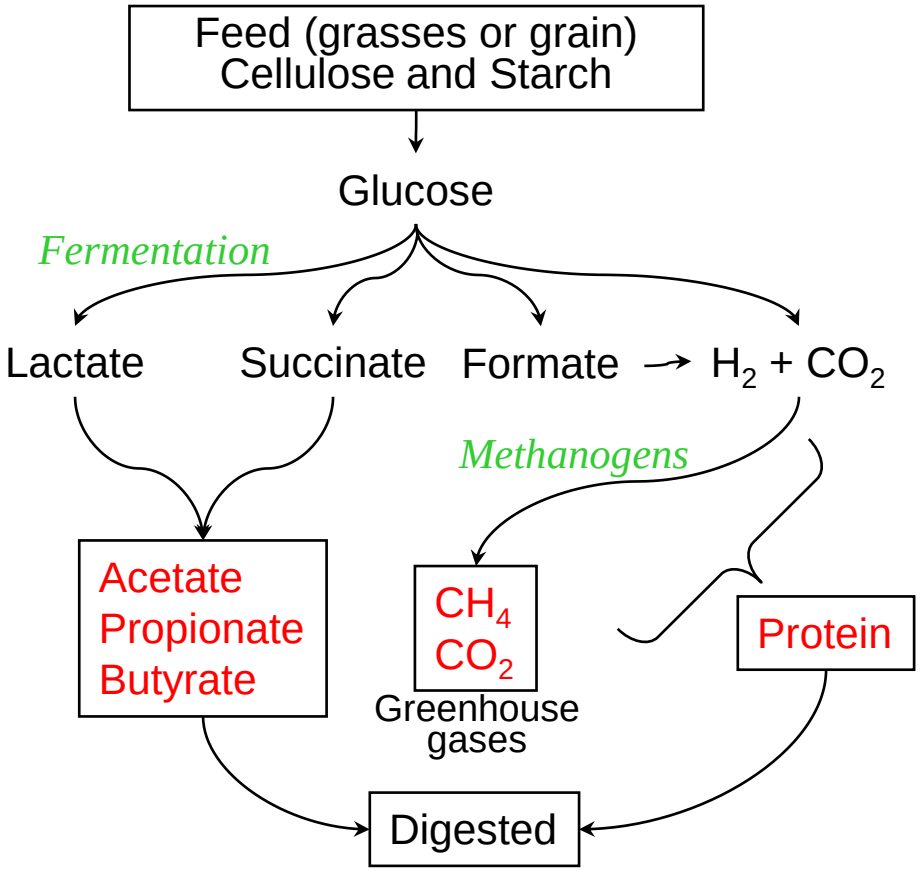
N. R. Pace. A molecular view of microbial diversity and the biosphere. Science 276 (5313):734-740, 1997.
Roussel, E.G. et al. Extending the Sub-Sea-Floor Biosphere. Science 320 (5879): 1046, 2008.

Rumen Microbiome

Complex *anaerobic* microbial system found in the rumen



Similar systems found in termites



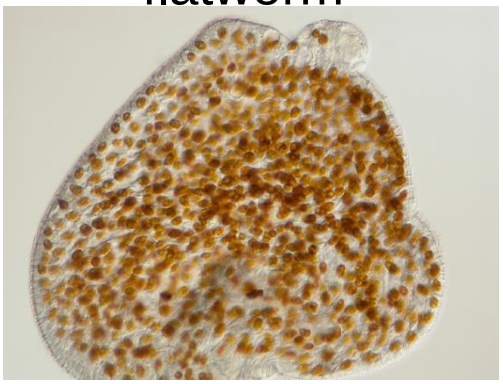
Reactions mediated by dozens of bacterial species, including protozoan grazers such as ciliates.

Microbes and higher organizms

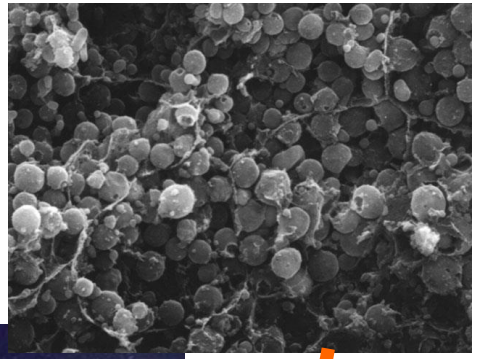
Symbiosis and Endosymbiosis

Lichen: Fungi+Algae

Dinoflagellates in flatworm



Sulfur bacteria in *Riftia*

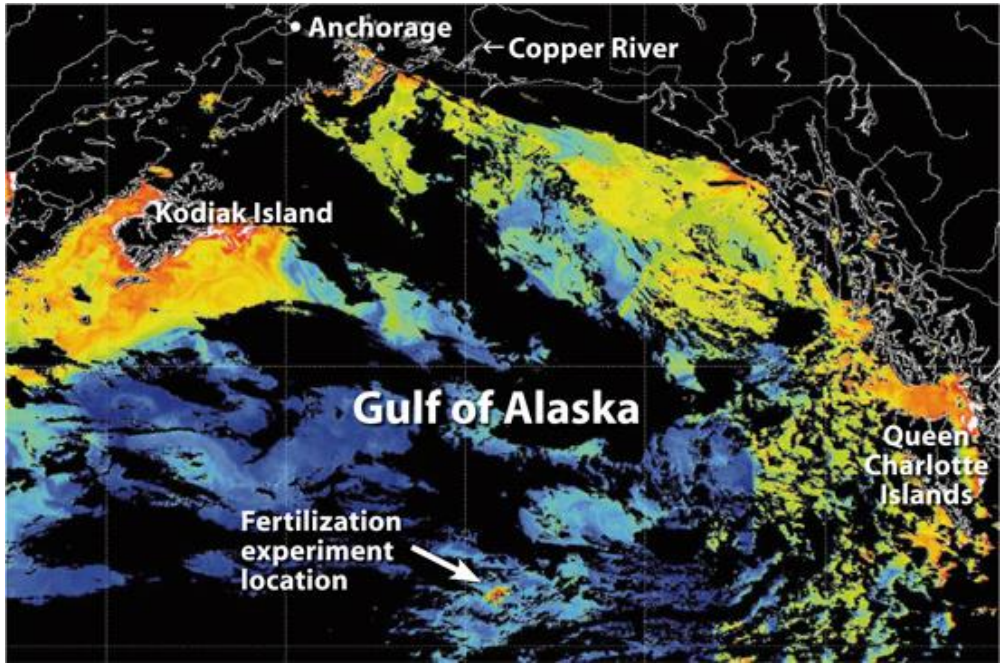


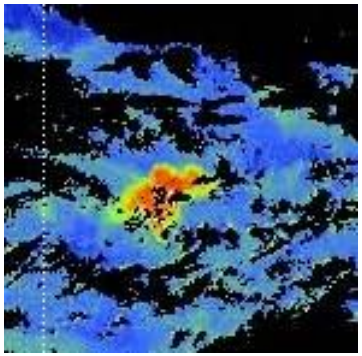
Mycorrhizae

Caldwell et al. 1997: Biological systems develop with multiple levels of organization and multiple levels of proliferation.

Biogeoengineering and Biofuels

Iron Fertilization?

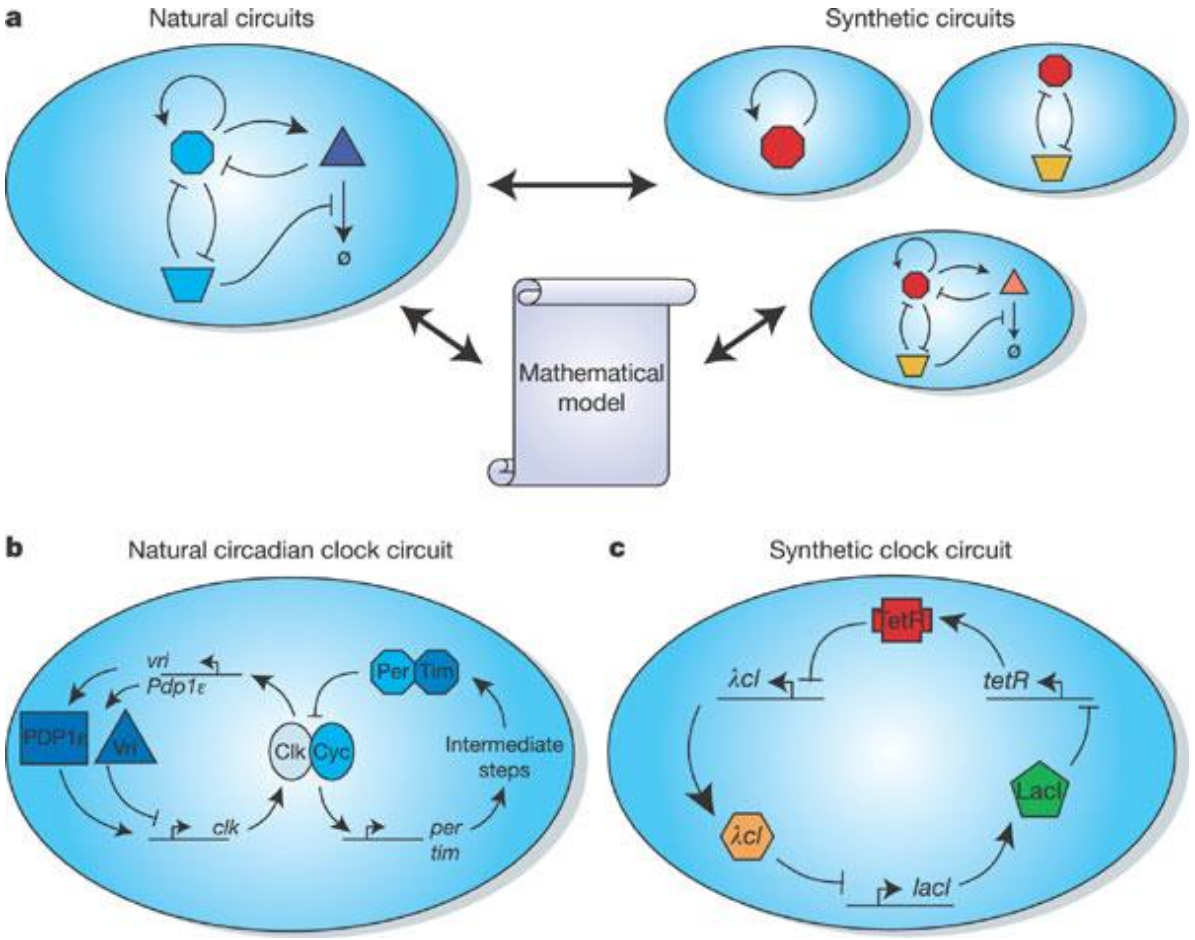




Oceanus, 46 (1) 2008
http://www.whoi.edu/oceanus/

Biodiesel from Algae

Synthetic Biology



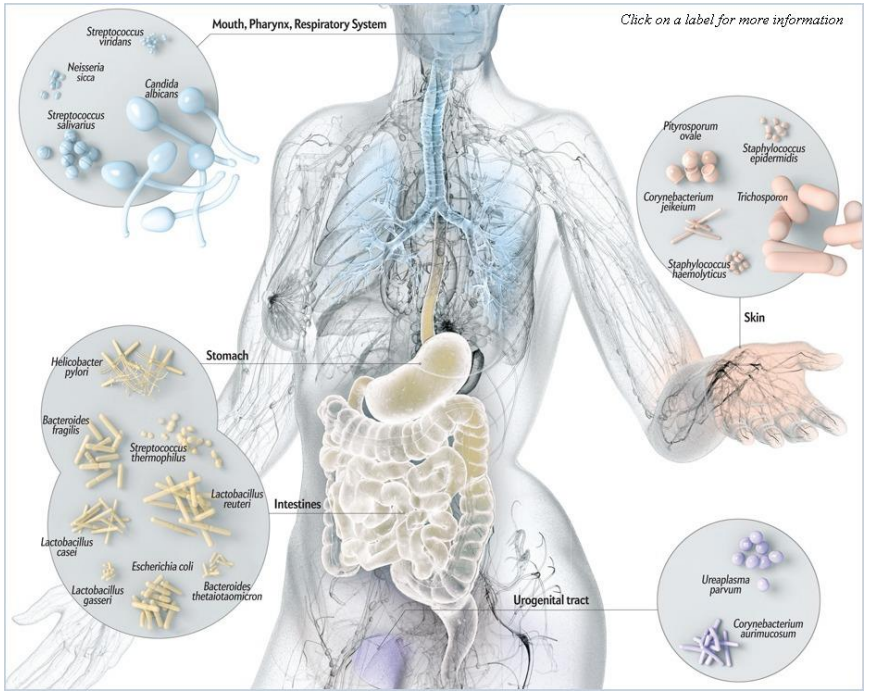
David Sprinzak and Michael B. Elowitz
Nature 438, 443-448 (24 November 2005)

See: Smanski, M. J., et al. (2016). Synthetic biology to access and expand nature's chemical diversity. *Nature Reviews Microbiology* 14: 135.
http://www.sciencemag.org/site/special/syntheticbio
http://biobricks.org

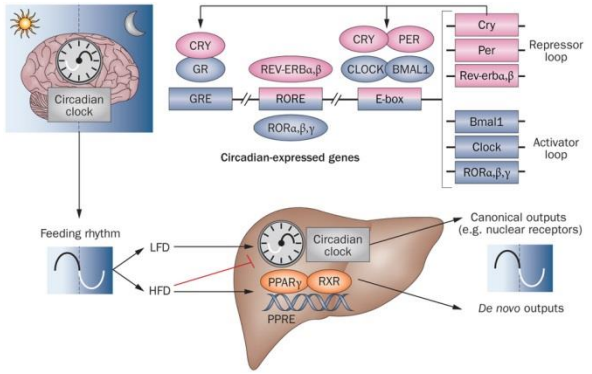
Human Microbiome

See: Turnbaugh et al., *Nature* 449 (7164):804-810, 2007.

http://commonfund.nih.gov/hmp/index



Circadian Rhythm, fat and microbiota



Leone, V., et al. 2014.. The FASEB Journal ***28*** (1)

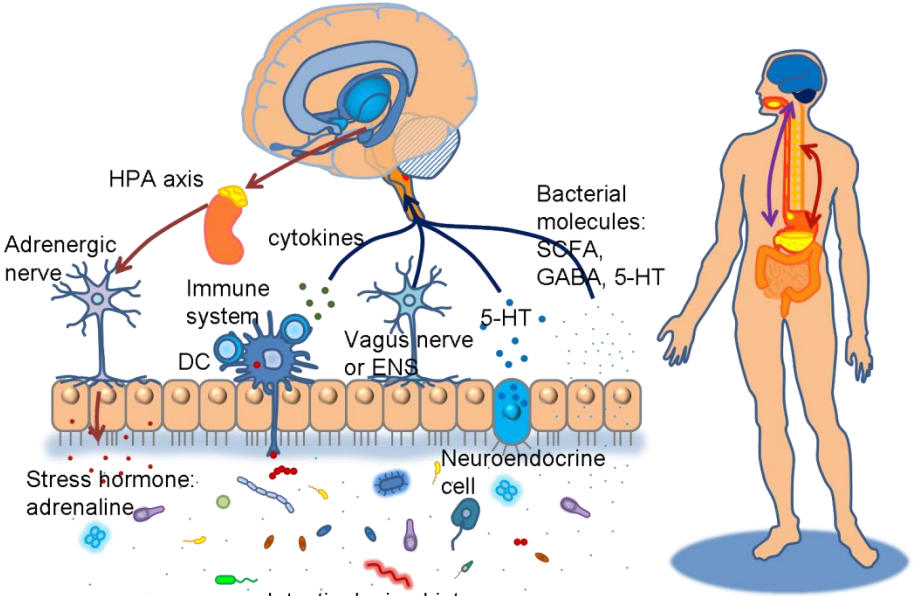
Human:

- Somatic+germ Cells: ~10 Trillion
- Genome: 20,000 protein encoding genes

Intestinal microbiota:

- Cells: ~100 Trillion
- Metagenome: ~100x human

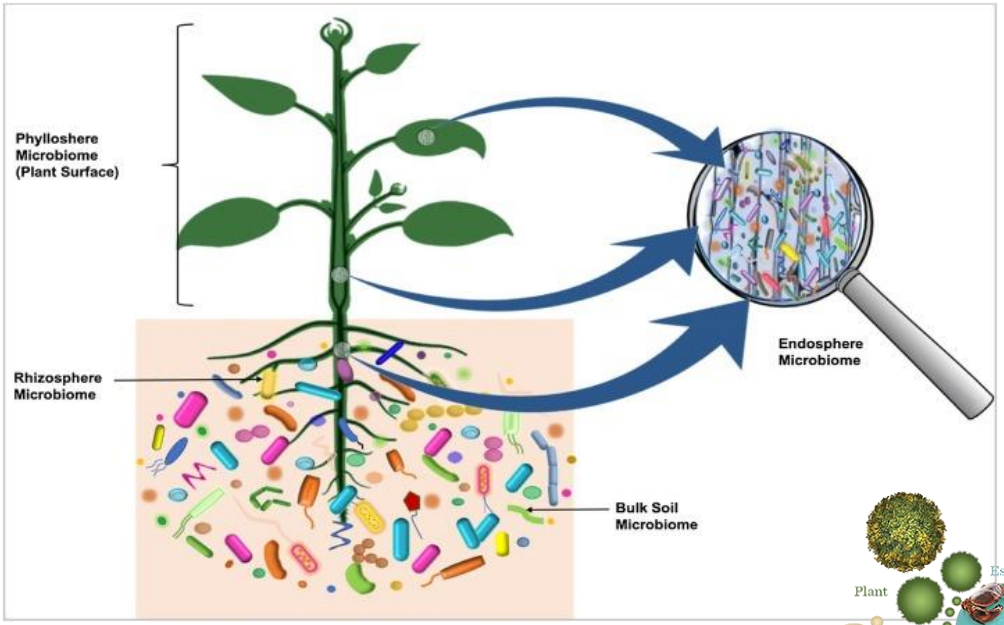
(Gill et al. Science 312 (5778):1355-1359, 2006.)



http://2012.igem.org/Team:NTU-Taida/Project/Background

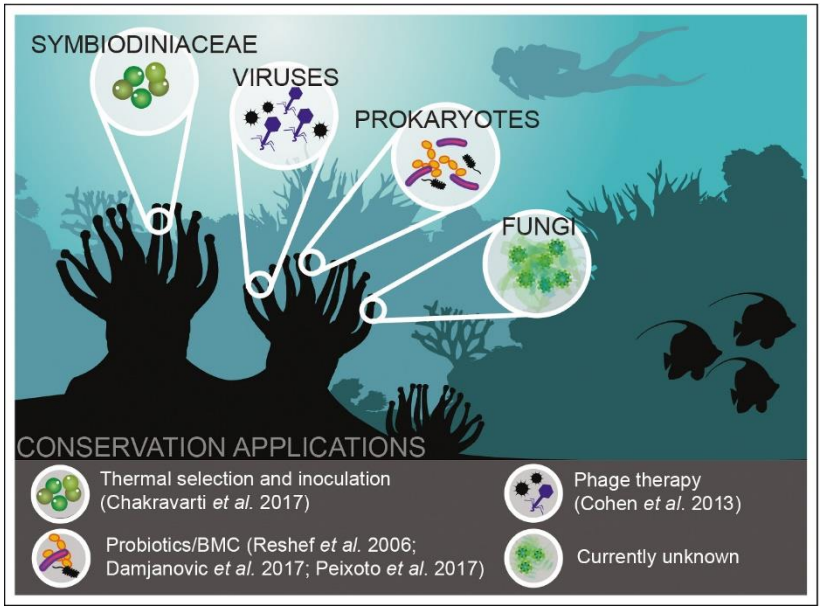
Microbiome: Ecosystems

Phyllosphere

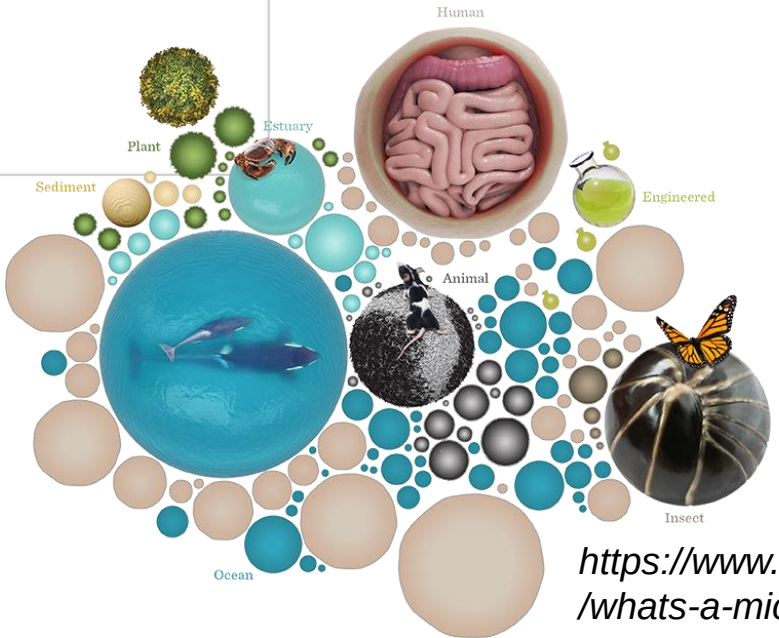


Agriculture
Carbon Sequestration

Coral Reefs



Epstein et al. (2019) https://doi.org/10.1002/fee.2001



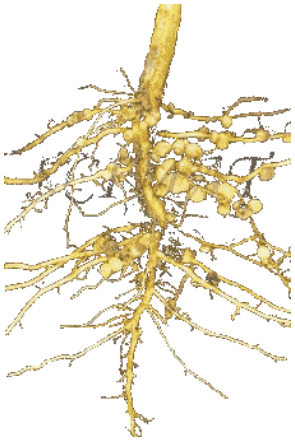
https://www.ebi.ac.uk/about/news/whats-a-microbiome

Other Important Microbial Processes

- Nutrient cycling under aerobic and *anaerobic* conditions.
- Removal of excess nitrogen via nitrification and denitrification in eutrophic systems.

  $NH_4 \rightarrow NO_3 \rightarrow N_2$
- Fixation of N_2 gas into organic N, especially in root nodules via symbiosis with bacteria, such as *Rhizobium*.

  $N_2 \rightarrow$ Amino Acids



- Remediation of toxic substances (bioremediation or natural attenuation).
- Almost all biomass and processes in the oceans are are dominated by microbes.
- Largest organism on Earth is a fungus (*Armillaria ostoyae*; honey mushroom).
- Major sources and sinks for atmospheric trace greenhouse gasses (CH_4, N_2O).
- Cycling of iron ($Fe^{3+} \Leftrightarrow Fe^{2+}$), Manganese ($Mn^{4+} \Leftrightarrow Mn^{2+}$) and other metals.
- Cause of many diseases, especially in 3rd world countries (such as recent Ebola outbreak).
- Very high “species” abundance, current estimate of 10^7 species in 10 g of soil.

Course Focus

Microbiology

Study of small, mostly single cell, living organisms

Public Health

- Pathogens
- Clinical
- Immunology
- Viruses

Laboratory Techniques

- Isolation
- Cultivation
- Identification

Function

- Biochemistry
- Molecular genetics
- Physiology

Microbiome

Applied Microbiology

- Biotechnology
- Industrial microbiology
- Food (beer, wine, cheese, soy sauce)
- Extraction of metals from ores
- Biofuels, Geoengineering

Origin of Life

- Diversity
- Phylogeny

Microbial Ecology

- In Natural Environments
- Biodiversity/Communities
- Organismal relationships
- Biogeochemistry

See American Society of Microbiology: www.asm.org

Microbial Ecology

To study the relations of organisms to one another and to their physical surroundings (i.e., environment).

Key Differences from clinical microbiology

- Communities of organisms, not isolated laboratory monocultures.
- Interaction with environment.

Challenges

- Must work with low concentrations of nutrients and organisms
- Most clinical laboratory techniques do not work in the field.

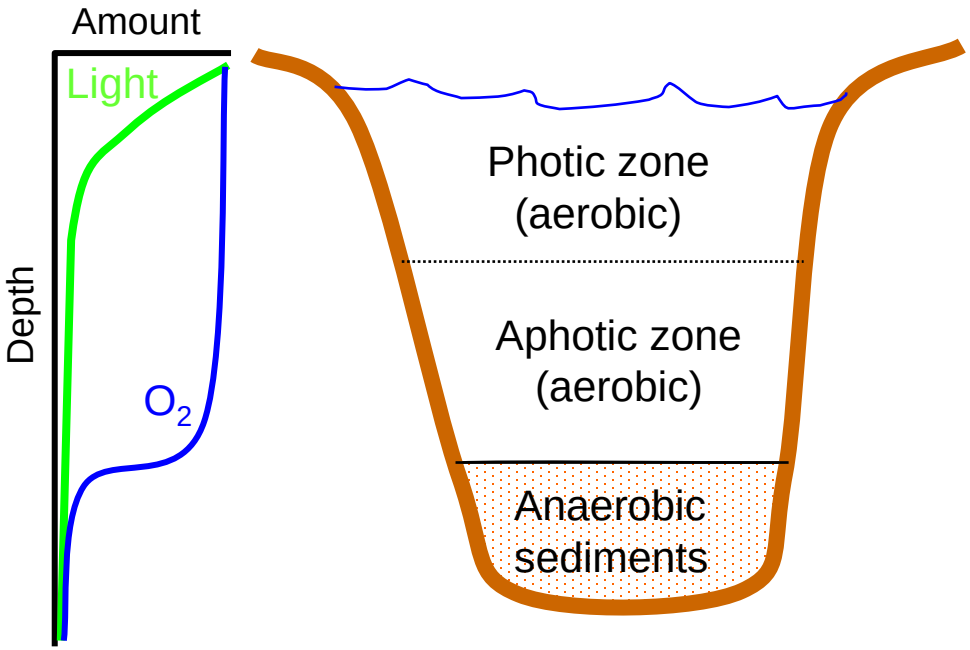
Objectives

- Identify organisms present (biodiversity) and their interactions: predator-prey, **syntrophy**, **symbiosis**, etc.
- Identify new pathways (e.g., SO_4^{2-} oxidation of CH_4)
- Identify processes and measure rates, such as primary production, nitrification, methanogenesis, etc.
- Develop models for understanding and prediction (both qualitative and quantitative).

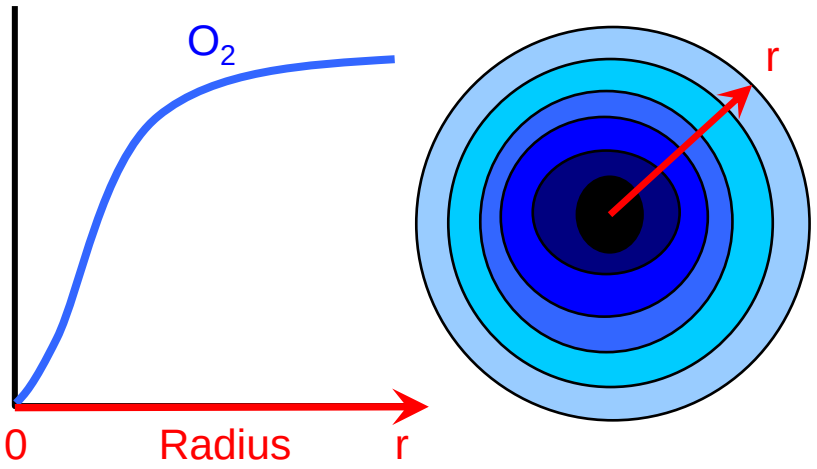
Habitats and biogeochemistry

The world is not “well-mixed”, so that sources and sinks of matter and energy produce gradients and microenvironments. Microbial communities develop as a function of resource availability.

Deep Lake



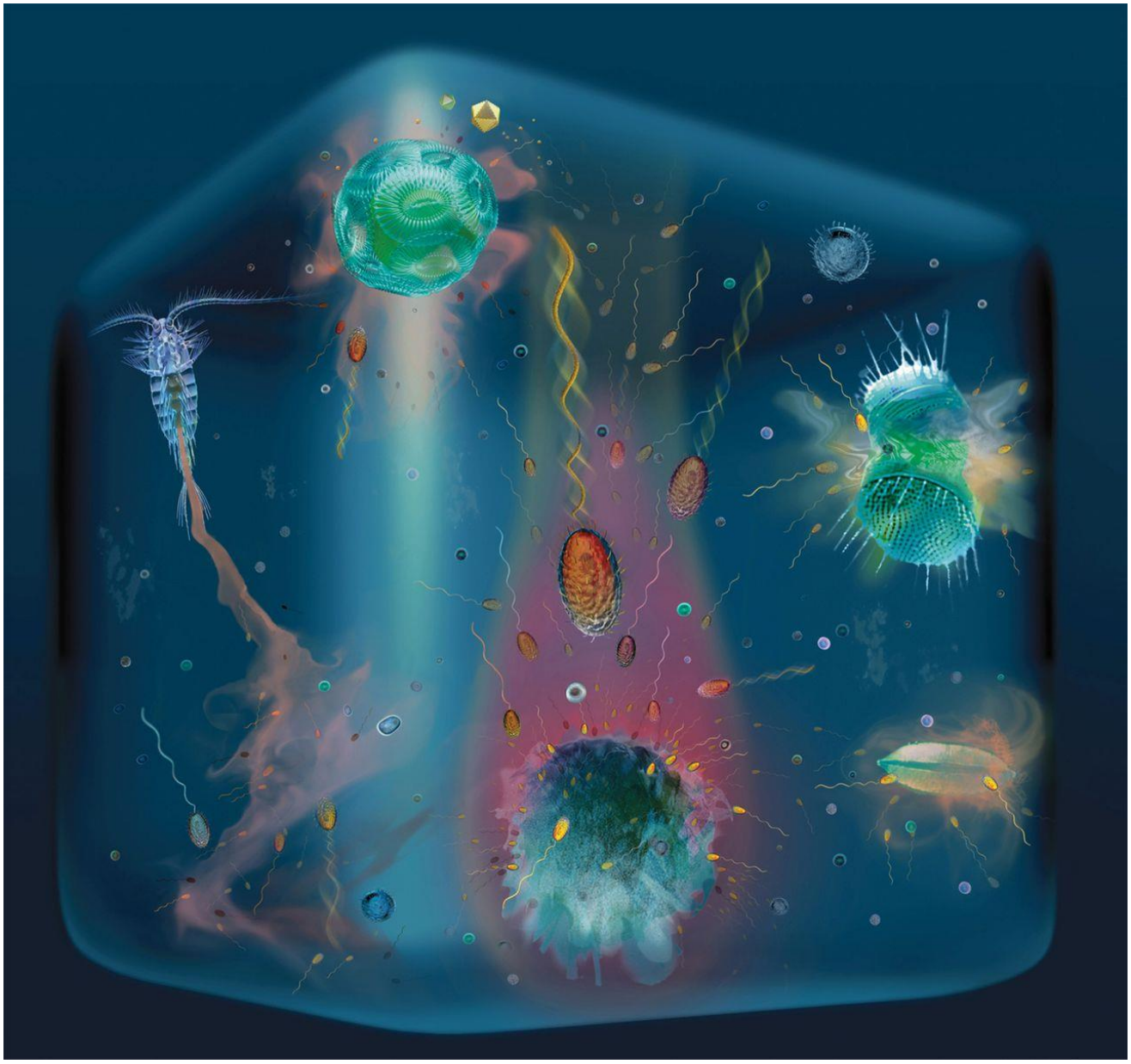
Gradients in Particles



These gradient dominated environments often can not be duplicated in the lab, so research must be conducted in the field.

Examples: Hot springs, Deep sea vents, hyper-saline, and anoxic environments.

Hypothetical marine microbial microenvironment
(far from homogeneous)

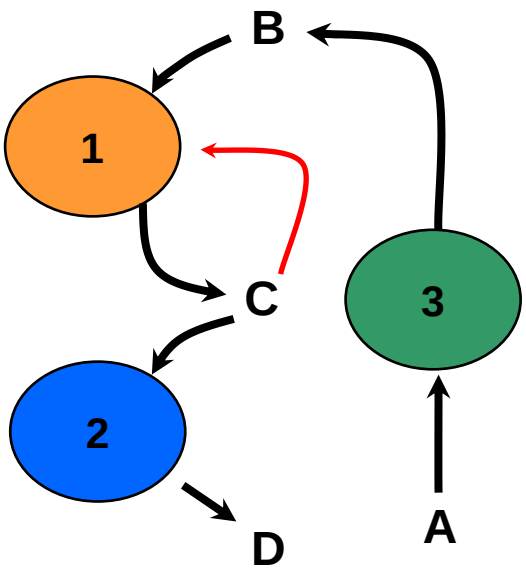


Stocker, *Science*
2012;338:628-633

Science
AAAS

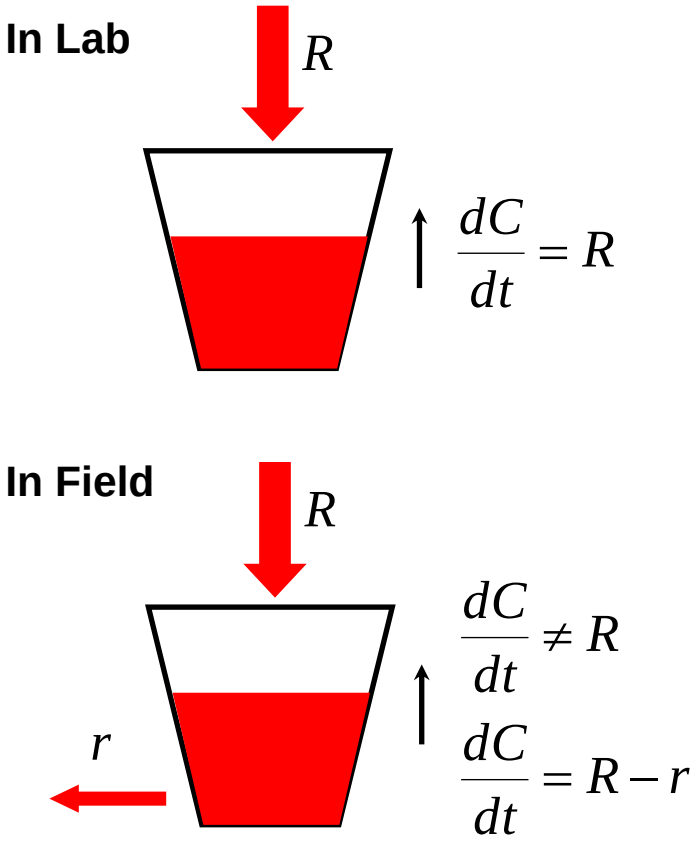
Works in the lab, but not in the field

Syntrophy
Nutrient cycling



Contained systems will often quickly diverge from true system.

Lab versus field measurements



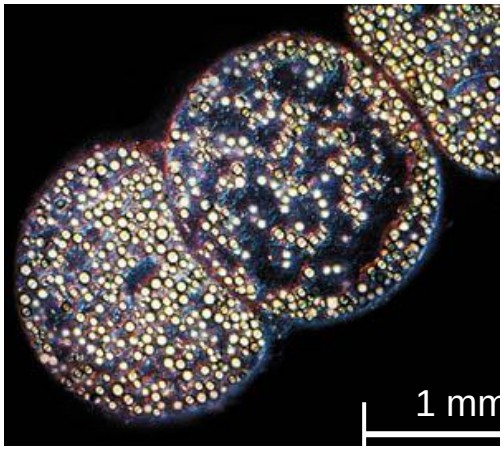
Finally, concentrations of compounds in natural systems are often at the limit of detection!

$\Rightarrow$ ***New methods often produce new understanding…Paradigm Shifts***

Chemical Potential Exploitation

H_2S oxidation by NO_3^-

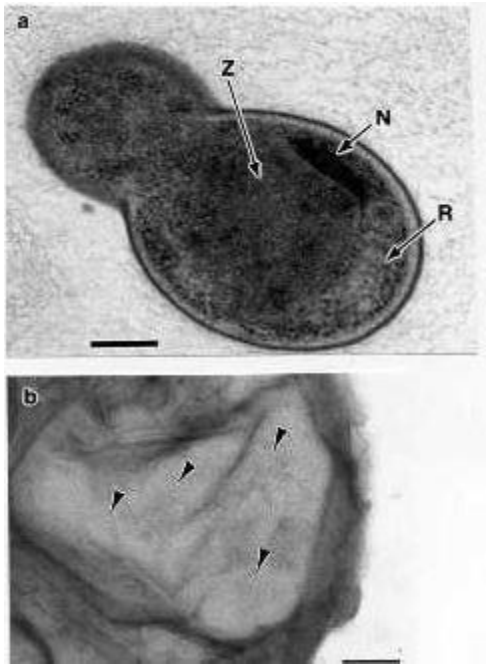
Schulz et al. 1999:
Thiomargarita namibiensis



Anammox

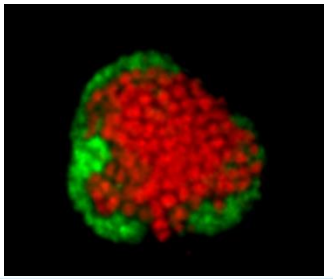
$NH_4^+ + NO_2^- = N_2 + 2H_2O$

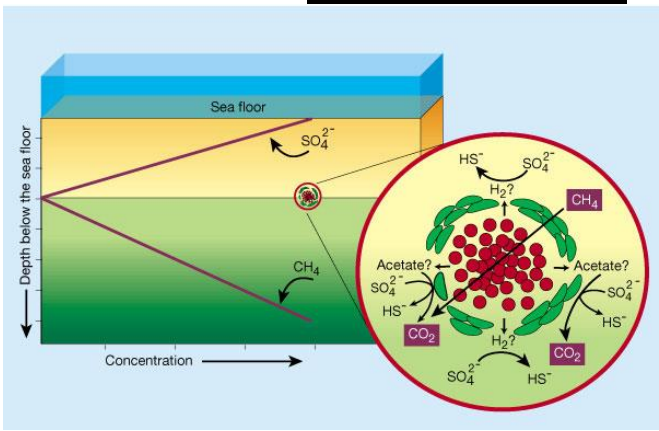
Strous et al. 1999:
Planctomycete



CH_4 oxidation by SO_4^{2-}

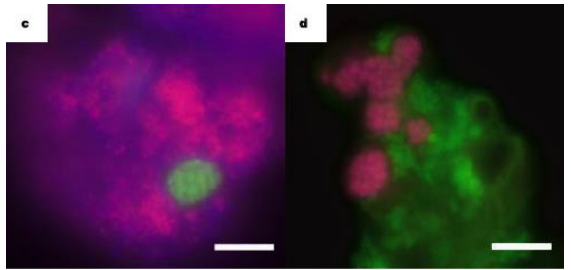
Boetius et al. 2000:



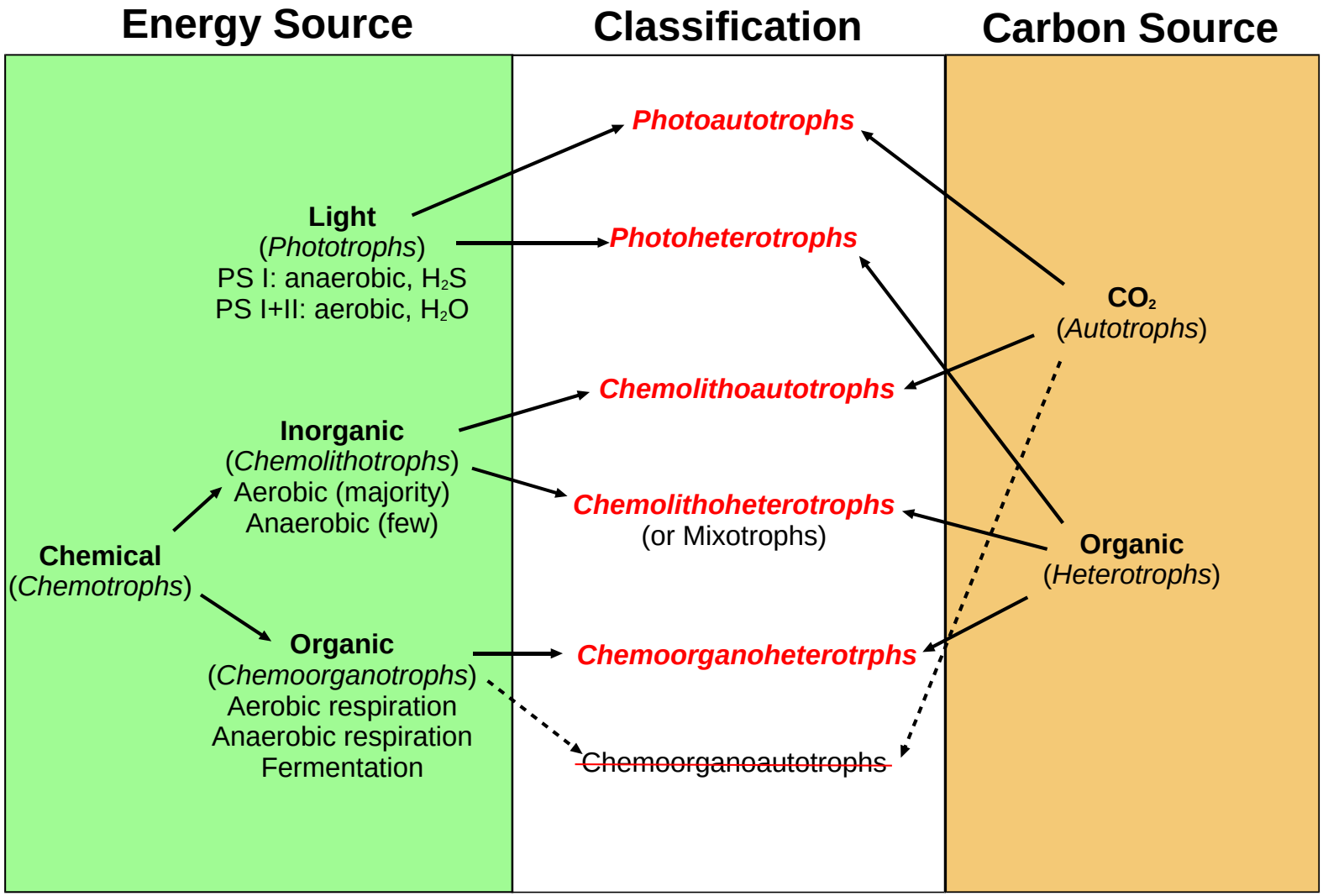


CH_4 oxidation by NO_3^- (Raghoebarsing et al. 2006)

$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O$



Metabolic Classification of Life

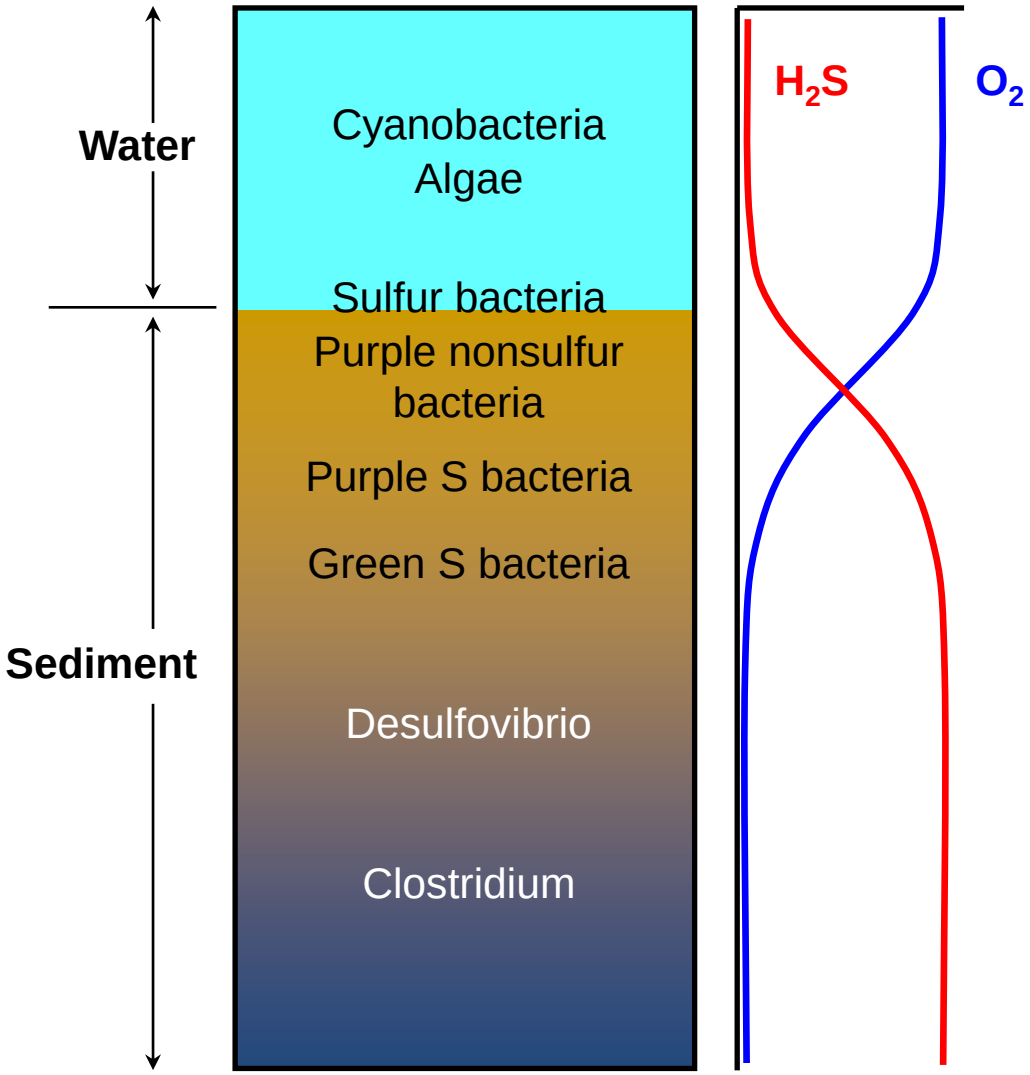


Course Overview

Lab and Lecture Topics:

1. Introduction	Background information and setup of Winogradsky columns.
2. Bacterial Abundance	Techniques to determine bacterial densities (plate and direct counts).
3. Bacterial Production	Measurement of bacterial growth rates using ^{14}C.
4. Enzyme Assays	Technique to measure activity of extracellular enzymes.
5. Chemolithotrophy	Use Winogradsky columns to study microbial biogeochemical diversity.
6. Bacterial Grazers	Microbial loop concept and determination of the bacterial consumption rate by predators.
7. Molecular Techniques	Polymerase chain reaction, etc. (Kristin Gribble).
8. Bacteria-Phyto. Comp.	Examine competition between bacteria and phytoplankton for inorganic nutrients.

Winogradsky Column



Sediment supplements:

- $CaCO_3$
- $CaSO_4$
- Carbon source

All five major metabolic groups will develop

- Sulfate reduction
- S oxidation
- Fermentation
- Photosynthesis PS II
- Photosynthesis PS I
- Methanogenesis
- Nitrification
- Denitrification?

Also see: https://www.hhmi.org/biointeractive/winogradsky-column-microbial-ecology-bottle

** Wear shoes (short pants too) that can get muddy for next Thursdays Lab **