L07a

Microbial Ecology

- Study of the **interactions** of microorganisms with their **environment (including organic matter)**, **each other (microrganisms)**, and plant and animal species (**other organisms**) —> symbioses, biogeochemical cycles, climate change

Methods

- Quantification of microbial abundance

1. Cultivation-Based Methods

- **Purpose:** Isolate and characterize microbes under controlled lab conditions.
- **Tools/Examples:**
  - Agar plates, enrichment cultures, selective media
  - Pure culture techniques
- **Pros:** Allows physiological and biochemical studies
- **Limitations:** Only ~1% of environmental microbes are culturable ("*great plate count anomaly*")

2. Microscopy and Imaging

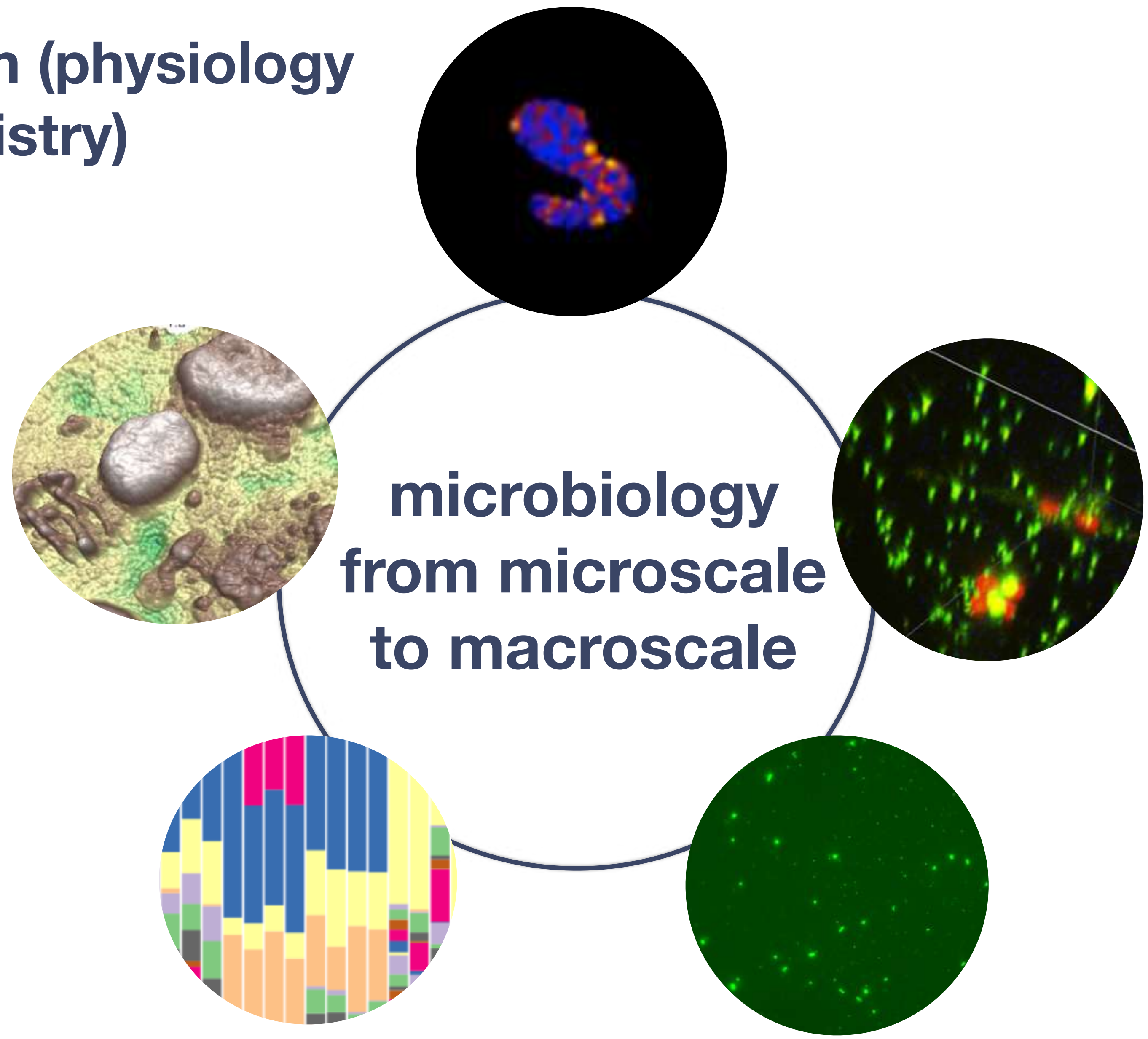
- **Purpose:** Visualize microbial cells and structures in situ.
- **Techniques:**
  - Light microscopy, fluorescence microscopy
  - Confocal laser scanning microscopy (CLSM)
  - Scanning/transmission electron microscopy (SEM/TEM)
  - Fluorescence in situ hybridization (FISH) — species-specific probes

Mechanistic integrative approach

Metabolism (physiology & biochemistry)

Structure

Function



Phylogeny & Diversity

Interaction

- Identification of microbes—>microbial community diversity and structure

3. Molecular Methods (Culture-Independent)

a. DNA-Based Techniques

- **16S/18S rRNA Gene Sequencing**: Identify and classify microbial taxa
- **Metagenomics**: Analyze all DNA in a sample (whole-community structure + functional genes)
- **qPCR / ddPCR**: Quantify specific genes or organisms

b. RNA-Based Techniques

- **Metatranscriptomics**: Study active genes (gene expression) in microbial communities
- Reveals what microbes are doing under certain conditions

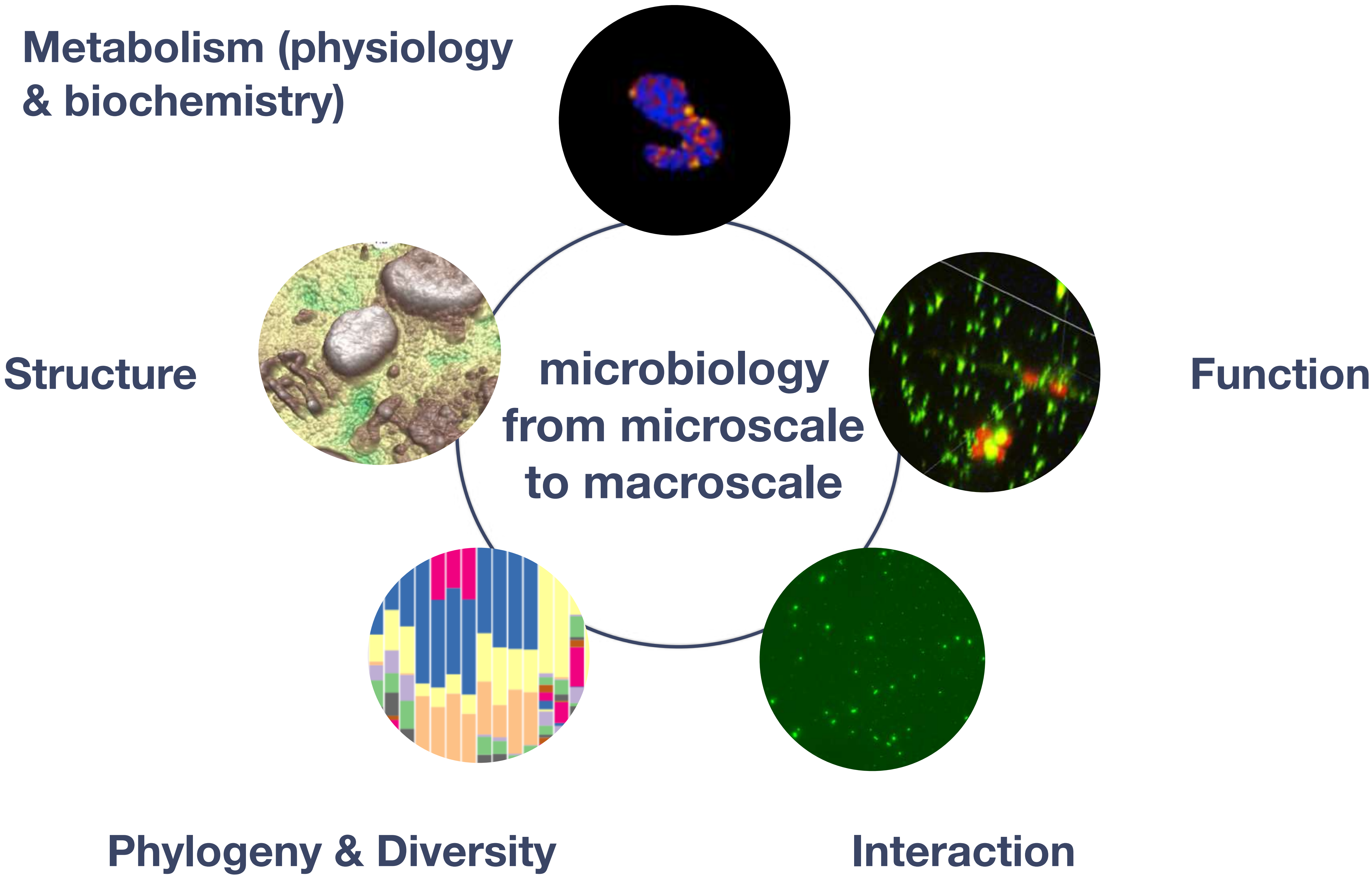
c. Other ‘Omics Approaches

- **Metaproteomics**: Identify and quantify community proteins (functions in action)
- **Metabolomics**: Measure small molecules/metabolites produced by microbes

4. Bioinformatics and Computational Tools

- **Purpose:** Analyze massive sequencing datasets from omics approaches
- **Common Tools:**
  - QIIME2, DADA2 (amplicon data analysis)
  - MG-RAST, MetaPhlAn, Kraken (metagenomic profiling)
  - R and Python for statistics, visualization, and network analysis

Mechanistic integrative approach



• Measuring activity rates

5. Radioactive Isotope assay and Stable Isotope Probing (SIP)

- **Purpose:** Link microbial identity with function
- **Method:** Feed microbes substrates labeled with heavy isotopes (e.g., ^{13}C-3H 14C molecule) and track incorporation into DNA/RNA/proteins
- Helps identify active populations involved in specific processes (e.g., methane oxidation)

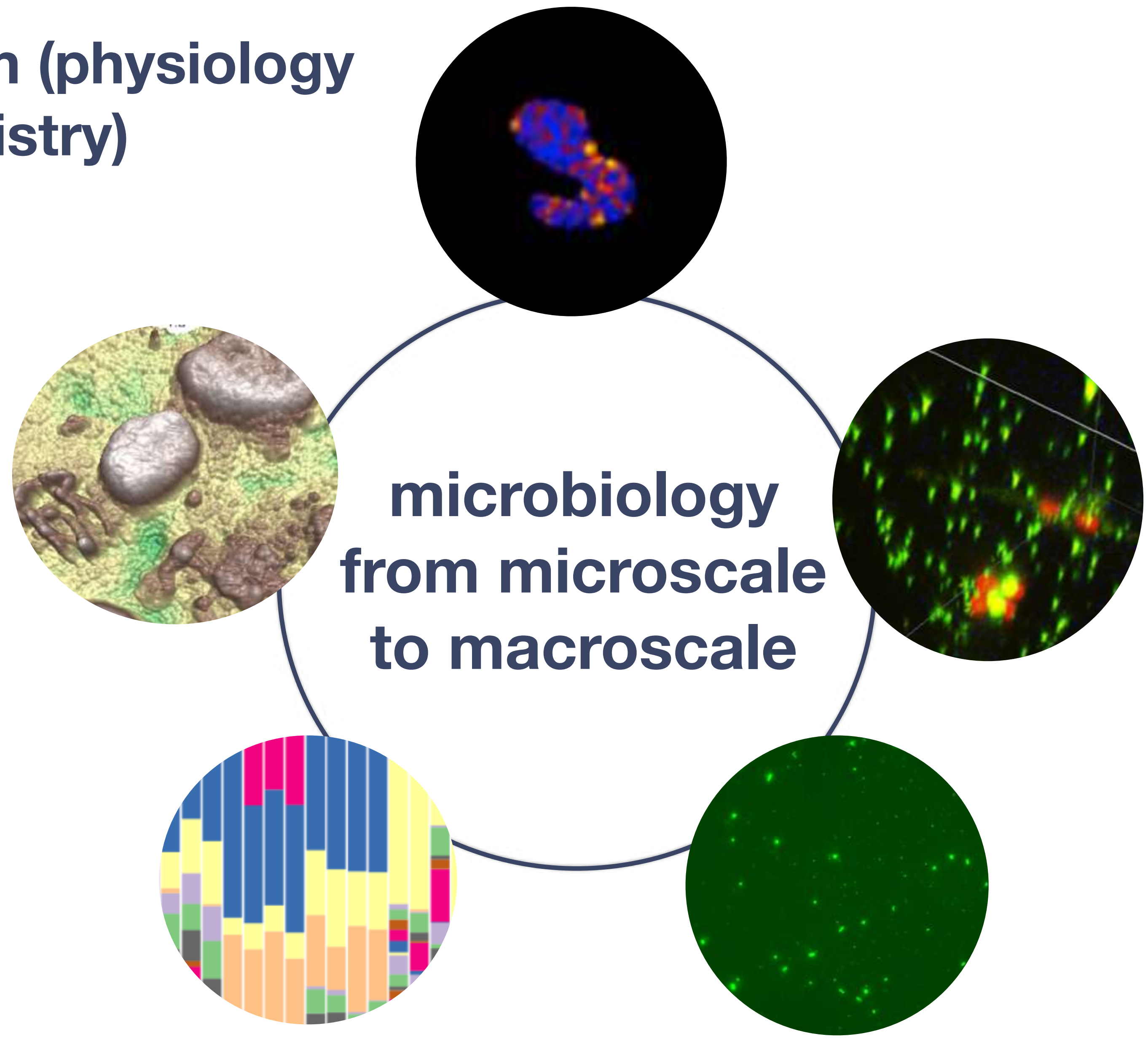
6. Fluorogenic substrate for hydrolysis rate

- **Purpose:** Quantifying enzymatic activites (organic matter degradation)
- **Method:** Add to microbes fluorogenic substrates that have specific bound, once substrate is cleaved, the free fluorophore increases its fluorescent signal
- Helps understanding organic matter degradative activities (e.g., protease, lipase, phosphatase, galactosidase, chitinase, etc..)

Mechanistic integrative approach

Metabolism (physiology & biochemistry)

Structure



Function

Phylogeny & Diversity

Interaction

- Linking small scale to ecosystem scale microbial action

6. Environmental and Biogeochemical Measurements

- Collect metadata: pH, temperature, nutrients, salinity, oxygen, etc.
- Correlate microbial patterns with abiotic factors
- Use in multivariate statistical analyses

7. Experimental Microcosms & Mesocosms

- **Microcosms:** Small-scale controlled lab experiments
- **Mesocosms:** Intermediate-scale controlled environments mimicking natural systems
- Allow for controlled manipulation (e.g., temperature, pollutants, community composition)

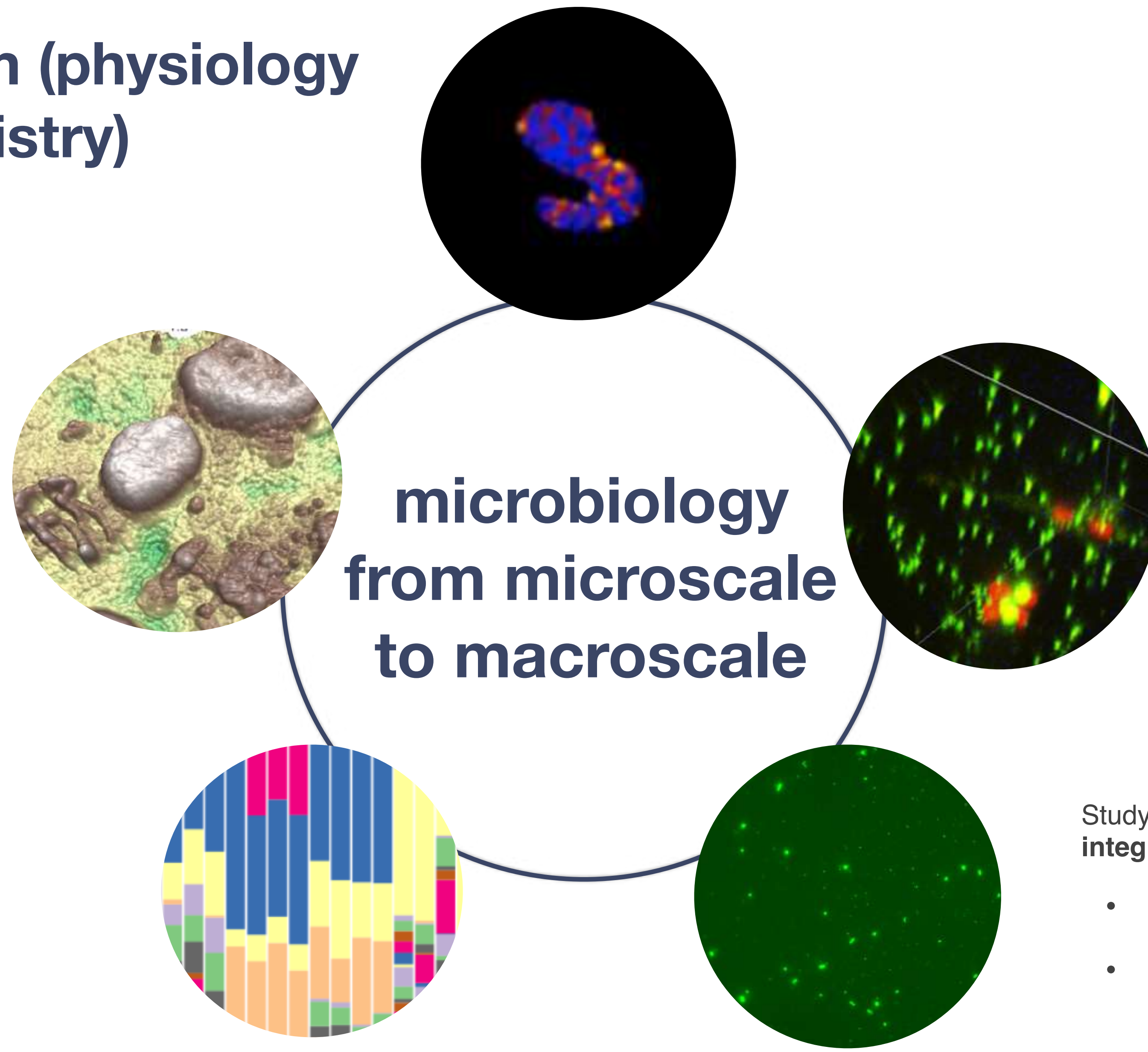
8. Network and Systems Ecology Approaches

- Construct microbial interaction networks (e.g., co-occurrence)
- Identify keystone species or functional hubs
- Integrate multi-omics with environmental data

Mechanistic integrative approach

Metabolism (physiology & biochemistry)

Structure



Function

Phylogeny & Diversity

Interaction

Studying microbial ecology today involves an **integrative approach**:

- Combine **classical microbiology** with **modern -omics** and **computational methods**
- Always interpret microbial data in the context of their **environment and function**

Microbial Ecology

- Microbial Evolution
- Microbial Species
- Niche
- Microbial Diversity-Metabolic Diversity
- Ecosystem
- Carrying capacity
- Bottom-up and Top-down control
- Microbial roles in ecosystem functioning

Microbial Evolution, I

- **Microbial evolution** refers to the **heritable genetic changes** that a microbe accumulates during its life time, which can arise from **adaptations in response to environmental changes** (thus including the immune response of the host)
- Because of their **short generation times and large population sizes**, microbes **can evolve rapidly**
- **Allele: sequence variance of a gene**
- *Evolution is defined as a **change in allele frequencies** (= change in a sequence variance of a gene) in a population of organisms over time resulting in descent with modification*

Microbial Evolution, II

- **New alleles** are created through the processes of **mutation and recombination**
- Mutations occur at **random** and most mutations are neutral or deleterious, but some are beneficial
- **Natural selection and genetic drift** are two mechanisms that cause **allele frequencies to change in a population over time**
- ***Evolution occurs by four fundamental processes: mutation, recombination, natural selection, and genetic drift*** *(results in a <u>change in allele frequencies in a population as a result of random changes</u> in # of offsprings from each individual over time)*

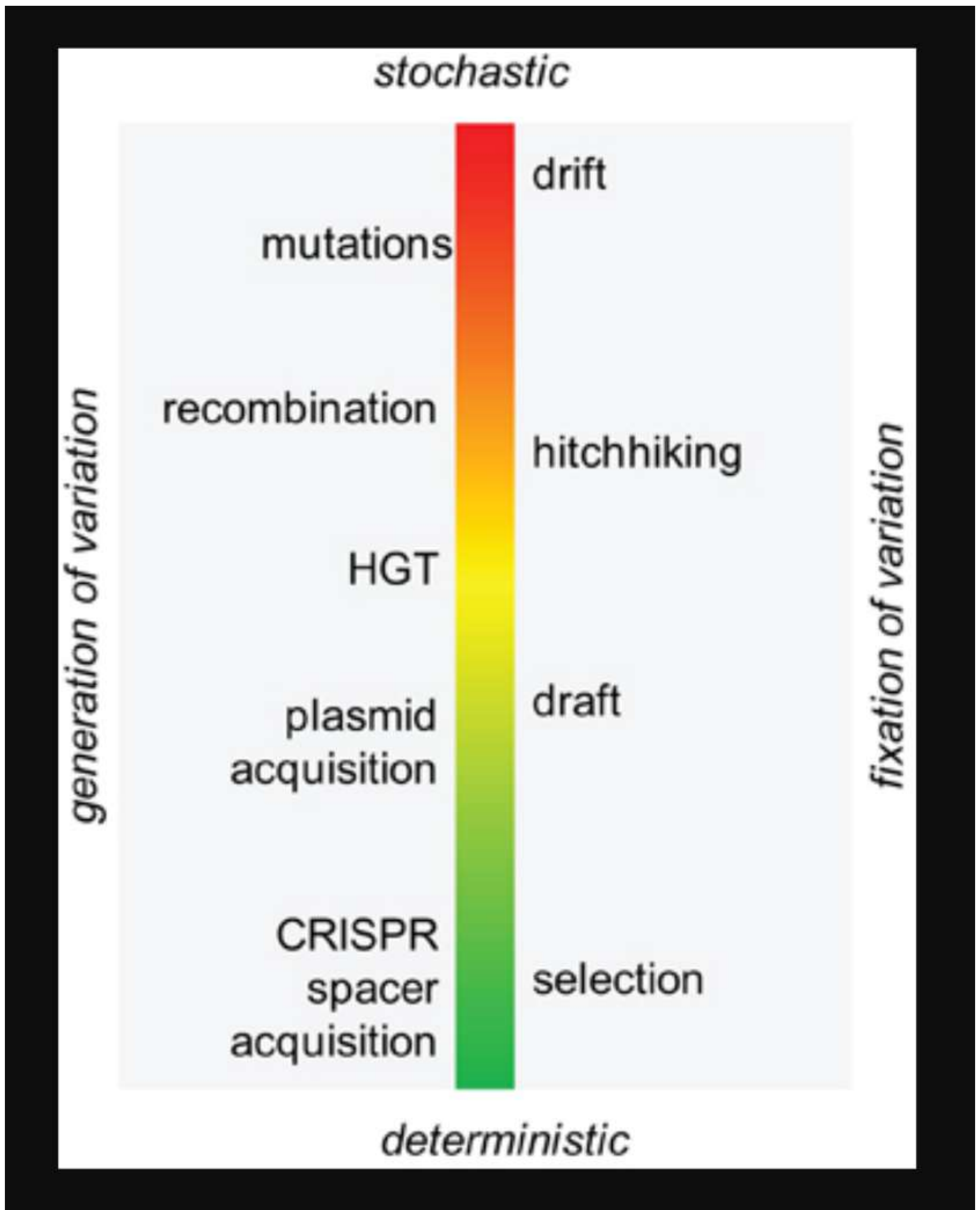
Microbial Evolution, III

- **Mutation, recombination** (gene flow, interspecific hybridization, and horizontal gene transfer are special forms of recombination. The first describes the movement of genes across a **spatial landscape**; the second and third involve genes **moving between species and microbial lineages**, respectively) produce **genetic variation**
- Natural selection, and genetic drift **govern the fate of variants**

Microbial Evolution, IV

- **Mutation, recombination and genetic drift are stochastic** in the sense that the specific variants produced or lost in a given generation are (or appear to be) **a matter of chance** (**whether any specific event happens is unknowable** or, at the least, impossible to incorporate into a mathematically efficient and useful theory of evolution)
- **Natural selection is a deterministic process** that reflects systematic differences in the propensity of alternative genotypes to survive and reproduce, **depending on their fit to the environment**

The continuum of evolutionary processes, from stochasticity to determinism

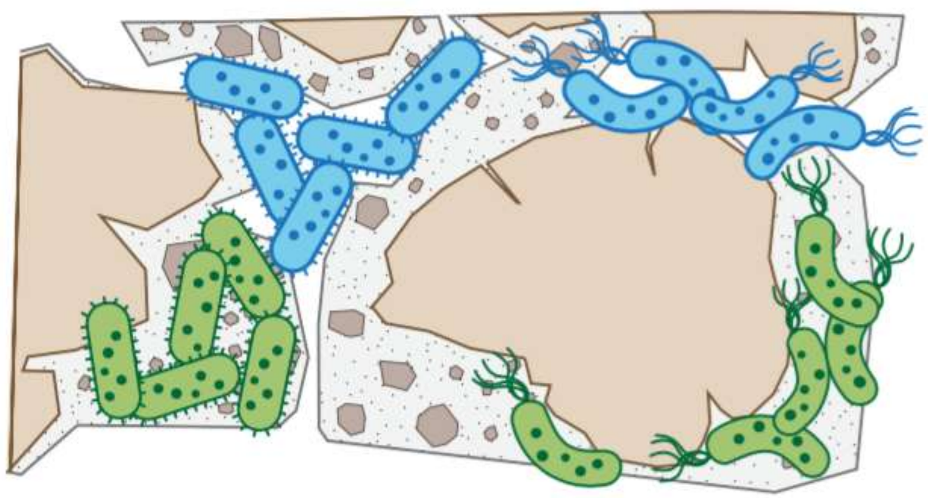


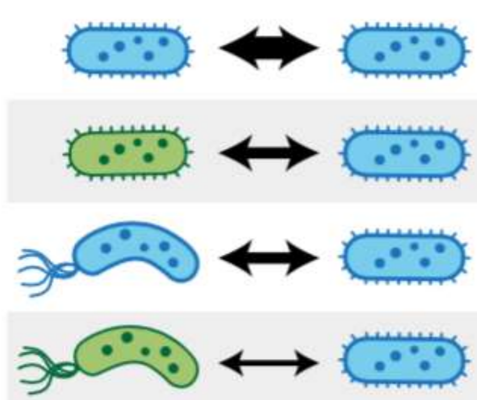
Kooning & Wolf, 2012

The **Modern Synthesis** of evolutionary biology emphasizes the **randomness of mutations** that provide the **starting material for selection** which engenders **survival of the fittest under the given conditions** and hence constitutes the adaptive, deterministic component of evolution

- **Lamarck**
- **CRISPR-Cas immune system responds to an environmental cue**
- **HGT depends on gene present in environment**
- **Stress-induced mutagenesis (error prone repair, SOS system) depends on environmental conditions**

Microscale environment where the gene flow and speciation take place





	genetic similarity	ecological overlap	gene flow
	+++	+++	+++
	+++	+	++
	+	+++	++
	+	+	+

The magnitude of **gene flow between microbial populations** is shaped predominantly by the **genetic similarity and ecological overlap** of the individual strains that make up those populations

While the **efficiency of homologous recombination decreases exponentially with sequence divergence**, the **likelihood of transfer increases** with greater **physical contact** between strains that occupy similar physical niches

VanInsberghe et al., 2020

How to work with a prokaryotic species/taxon/organism ?

Only 1 - 10% microbes are cultivable on Earth

—>DNA only

Woese

16S ribosomal RNA gene

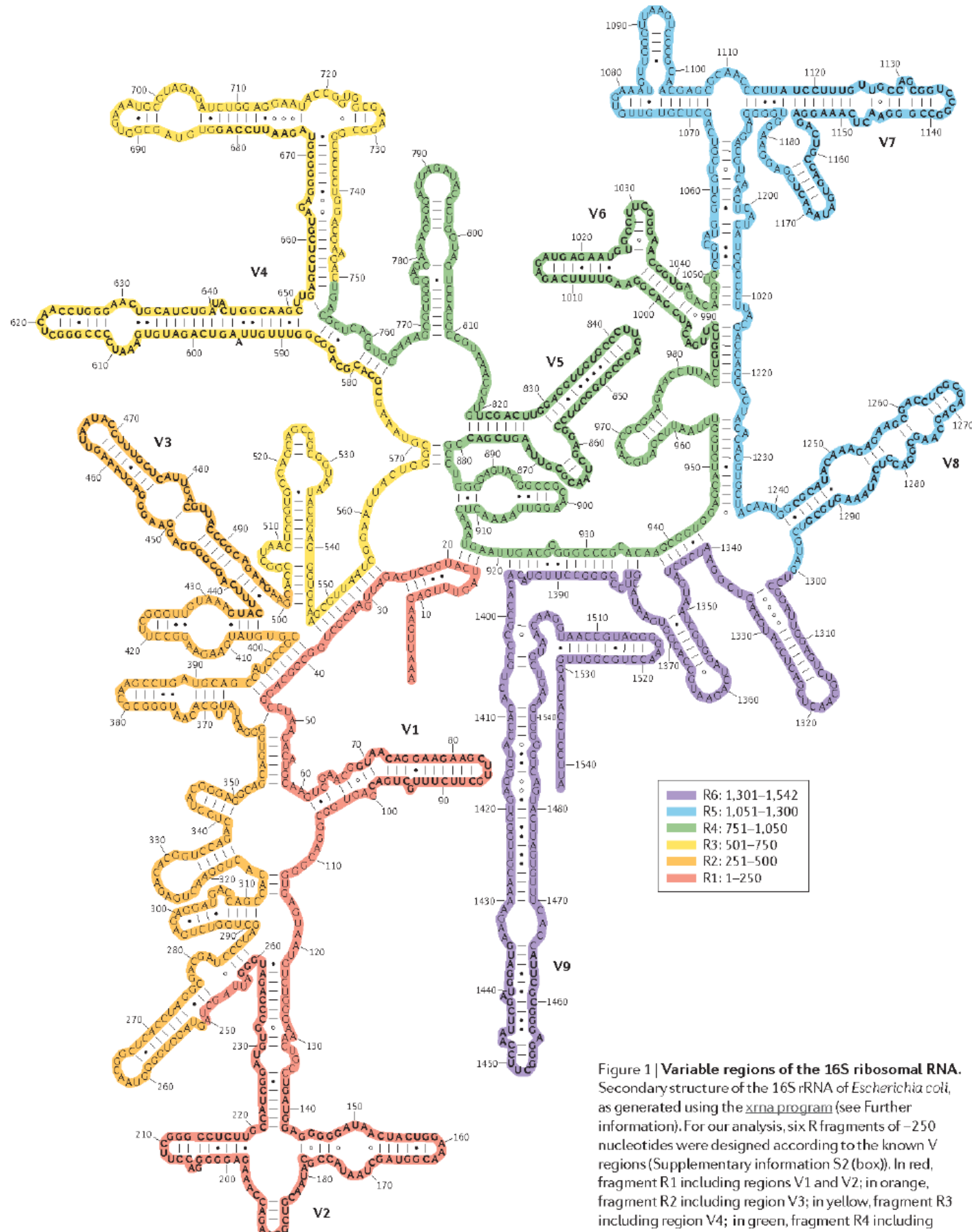


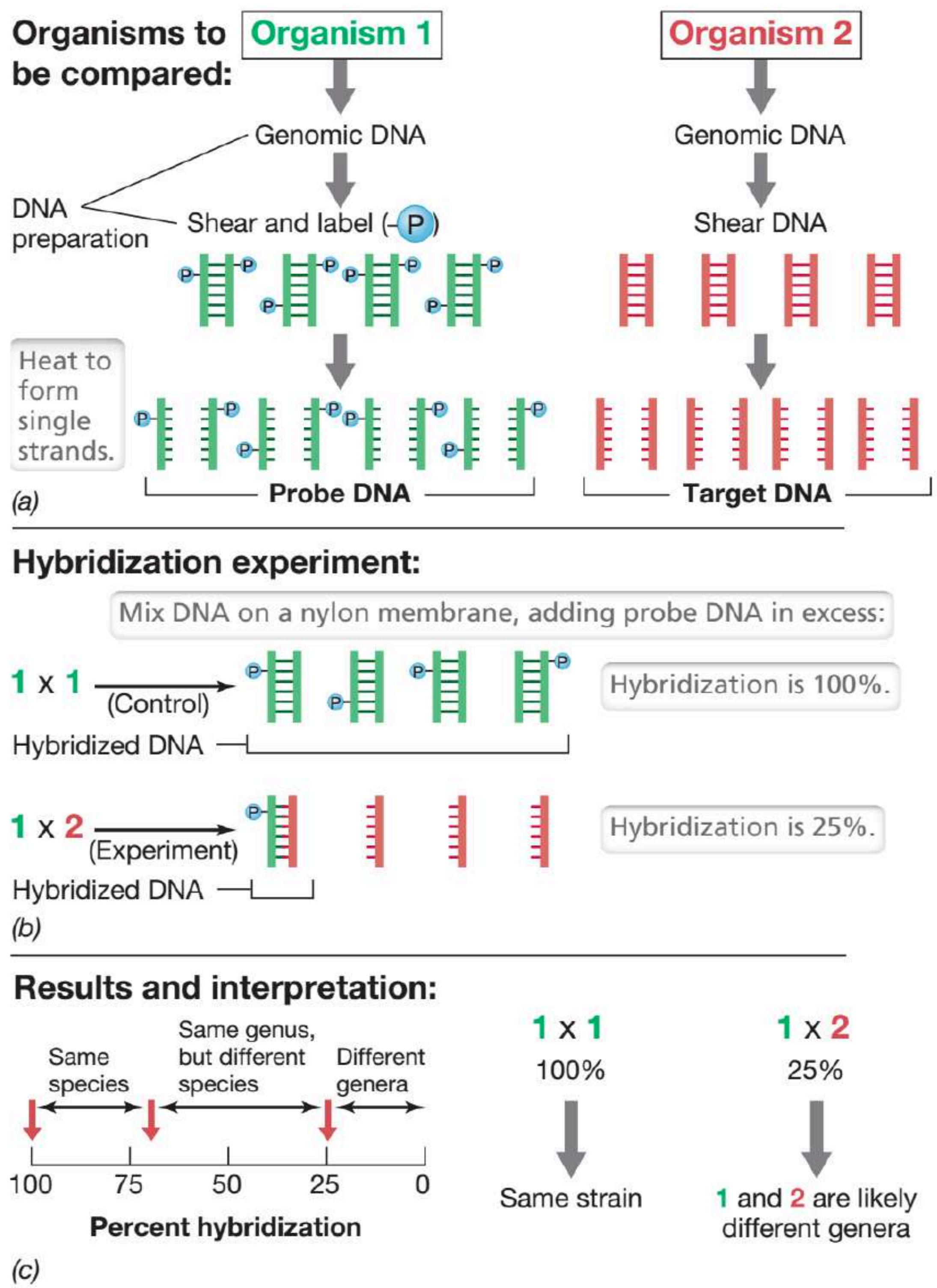
Figure 1 | **Variable regions of the 16S ribosomal RNA.** Secondary structure of the 16S rRNA of *Escherichia coli*, as generated using the xrna program (see Further information). For our analysis, six R fragments of ~250 nucleotides were designed according to the known V regions (Supplementary information S2 (box)). In red, fragment R1 including regions V1 and V2; in orange, fragment R2 including region V3; in yellow, fragment R3 including region V4; in green, fragment R4 including regions V5 and V6; in blue, fragment R5 including regions

Ribosomal RNAs are components of ribosomes, the structures that synthesize new proteins in the process of translation.

Yarza et al. 2014

10.1038/nrmicro3330

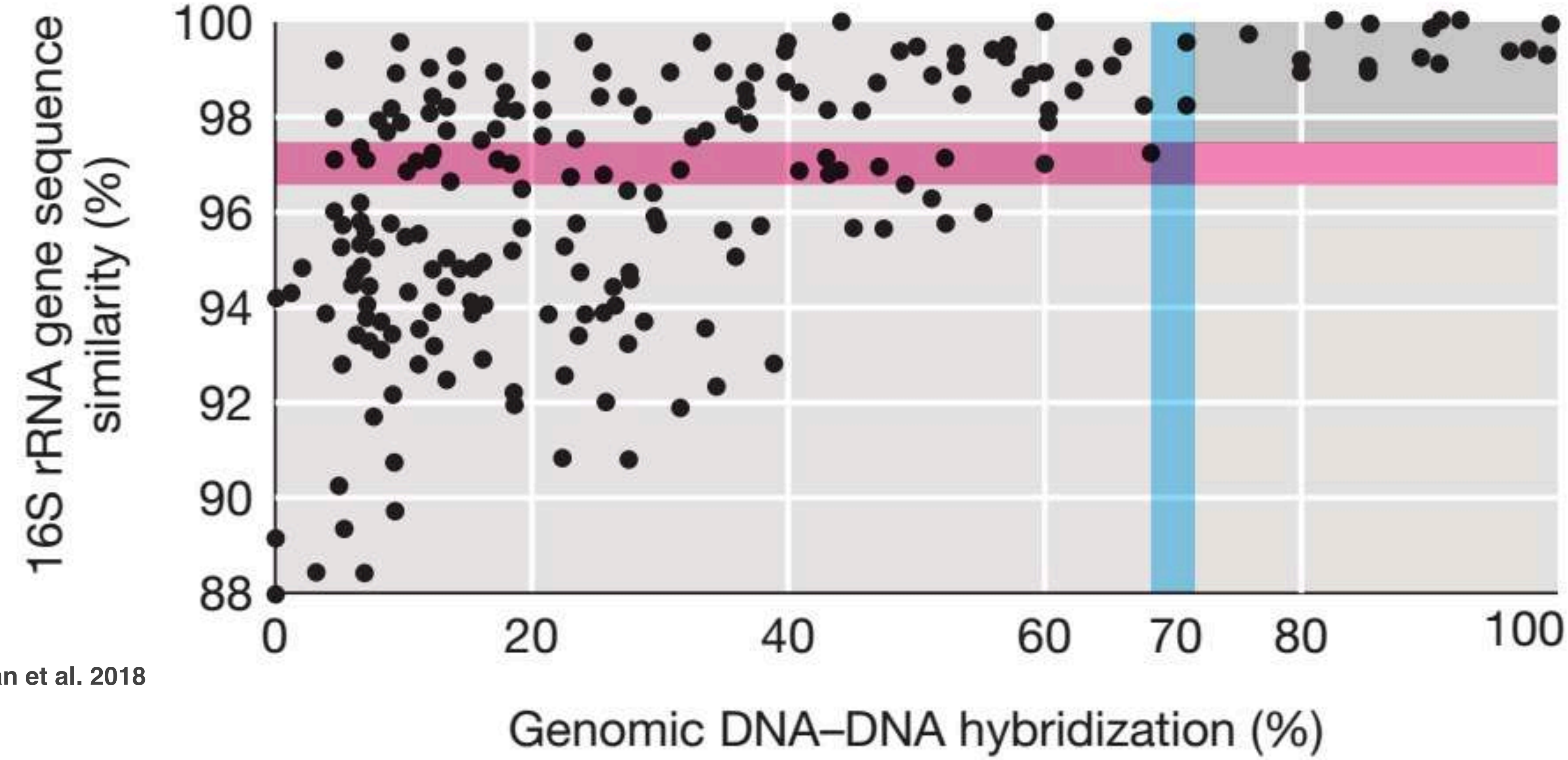
Microbial Species I



- Microbes are currently assigned to a **common species** if their **reciprocal, pairwise DNA re-association values are ≥70% in DNA–DNA hybridization** experiments under standardized conditions and their ΔTm (melting temperature) is ≤5°C
- All strains within a species must possess a **certain degree of phenotypic consistency**, and species **descriptions** should be based **on more than one type strain**
- **A species name is only assigned if its members can be distinguished from other species** by at least one diagnostic phenotypic trait

Madigan et al. 2018

Microbial Species II



Madigan et al. 2018

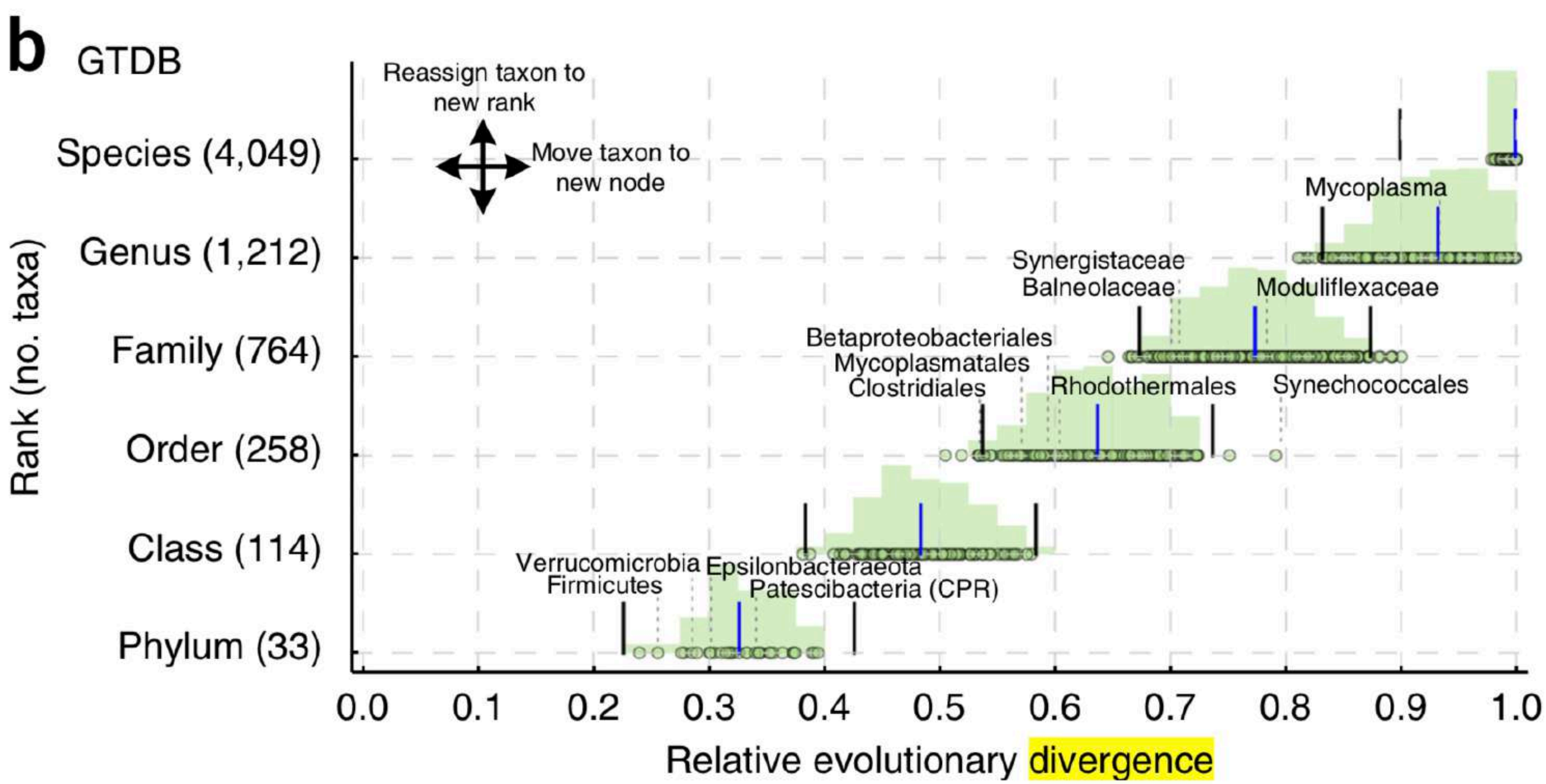
- Microbes with **16S ribosomal RNAs** (rRNAs) that are **≤98.7% identical** are always members of **different species**, because such strong differences in rRNA correlate with <70% DNA–DNA similarity
- Opposite is not necessarily true, and distinct species have been occasionally described with 16S rRNAs that are >98.7% identical

Microbial Species III

- **Most uncultured microbes** cannot be assigned to a classical species because we **do not know** their **phenotype**
- In some cases, uncultured microbes can be assigned a provisional '***Candidatus***' **designation if their 16S rRNA sequences are sufficiently different from those of recognized species**, if experimental in situ hybridization can be used to specifically detect them and if a basic description of their morphology and biology has been provided
- **OTU, operational taxonomic unit,** is a definition to classify groups of closely related individuals. It is based on an **empirical observation that 98% similarity threshold on the 16S ribosomal RNA gene** (database reference dependency, loss resolution)
- **ASV, amplicon sequence variance, unique, DNA sequences without clustering, highest degree of resolution** (independent from reference database)
- **Basis of the average nucleotide identity (ANI) of all orthologous genes** in **complete genome** sequences of pairs of strains —> whole genome comparison **(94-96% protein-genes**)

Relative evolutionary divergence

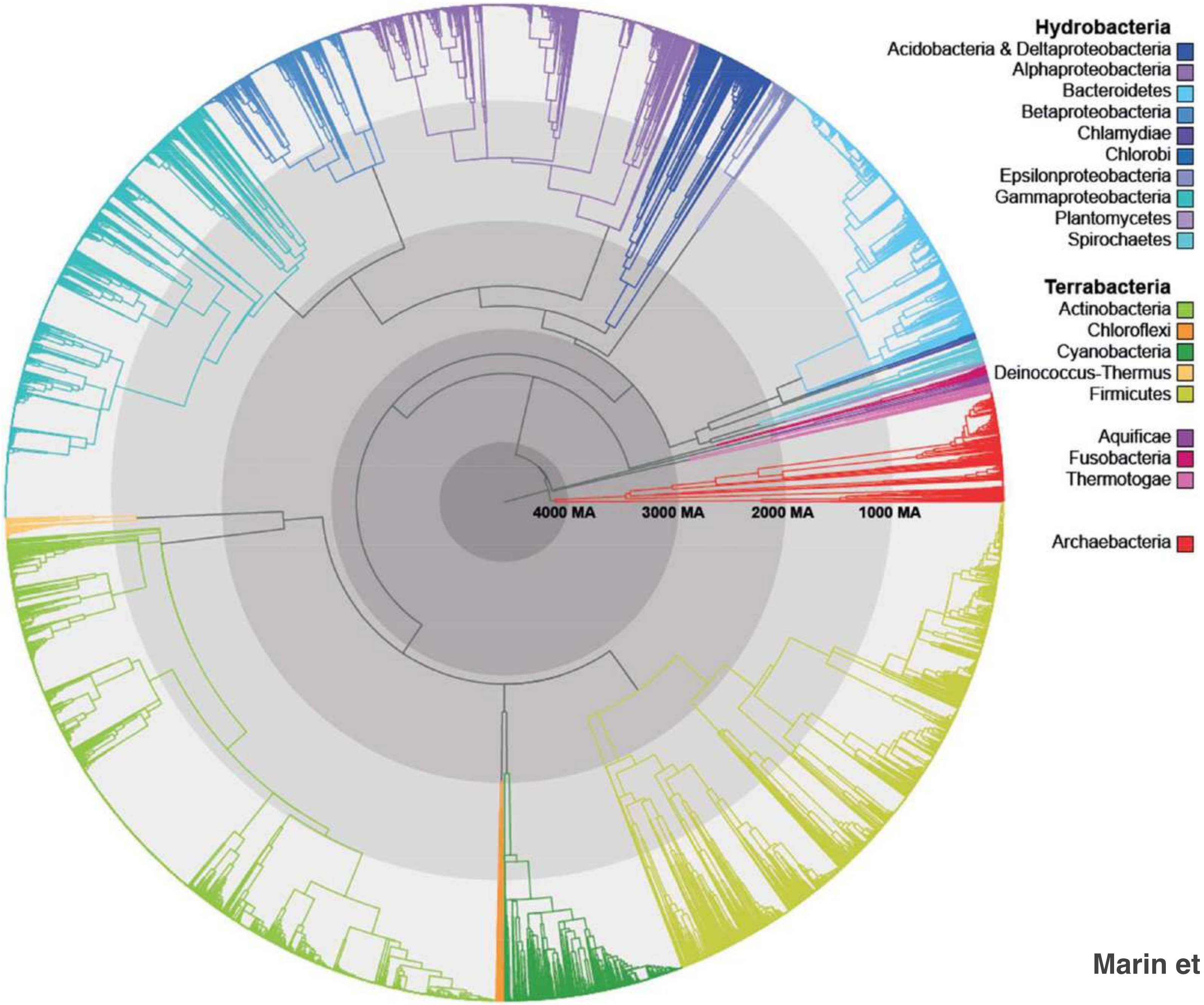
RED values provide an **operational approximation of relative time** with extant taxa existing in the present (RED=1), the last common ancestor occurring at a fixed time in the past (RED=0), and internal nodes being linearly interpolated between these values according to lineage-specific rates of evolution



Parks et al., 2018

Prokaryote timetree (PTT)

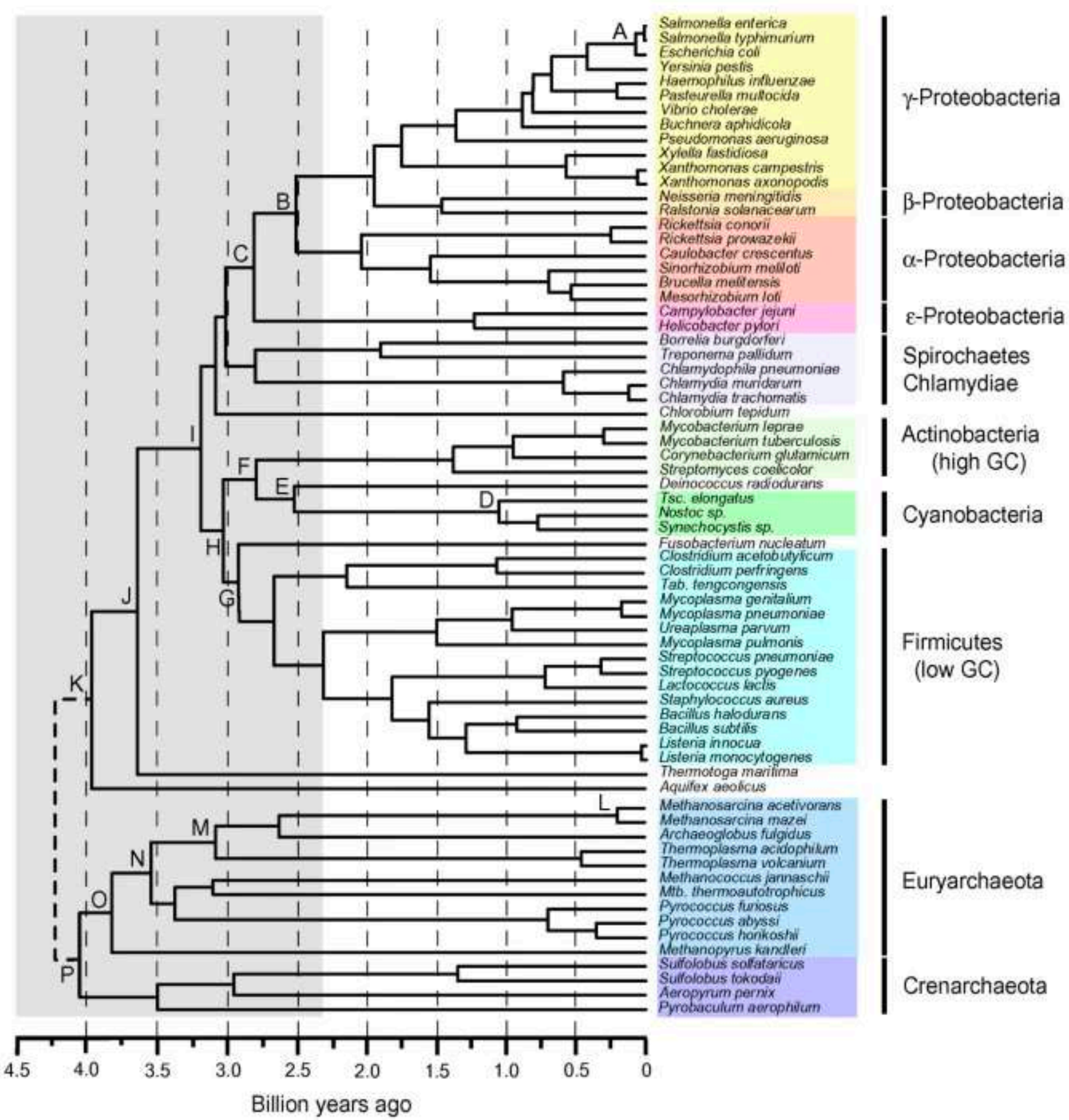
PTT (topology A; 11,784 species) based on the SSU rRNA genes
Divergence times



Marin et al., 2016

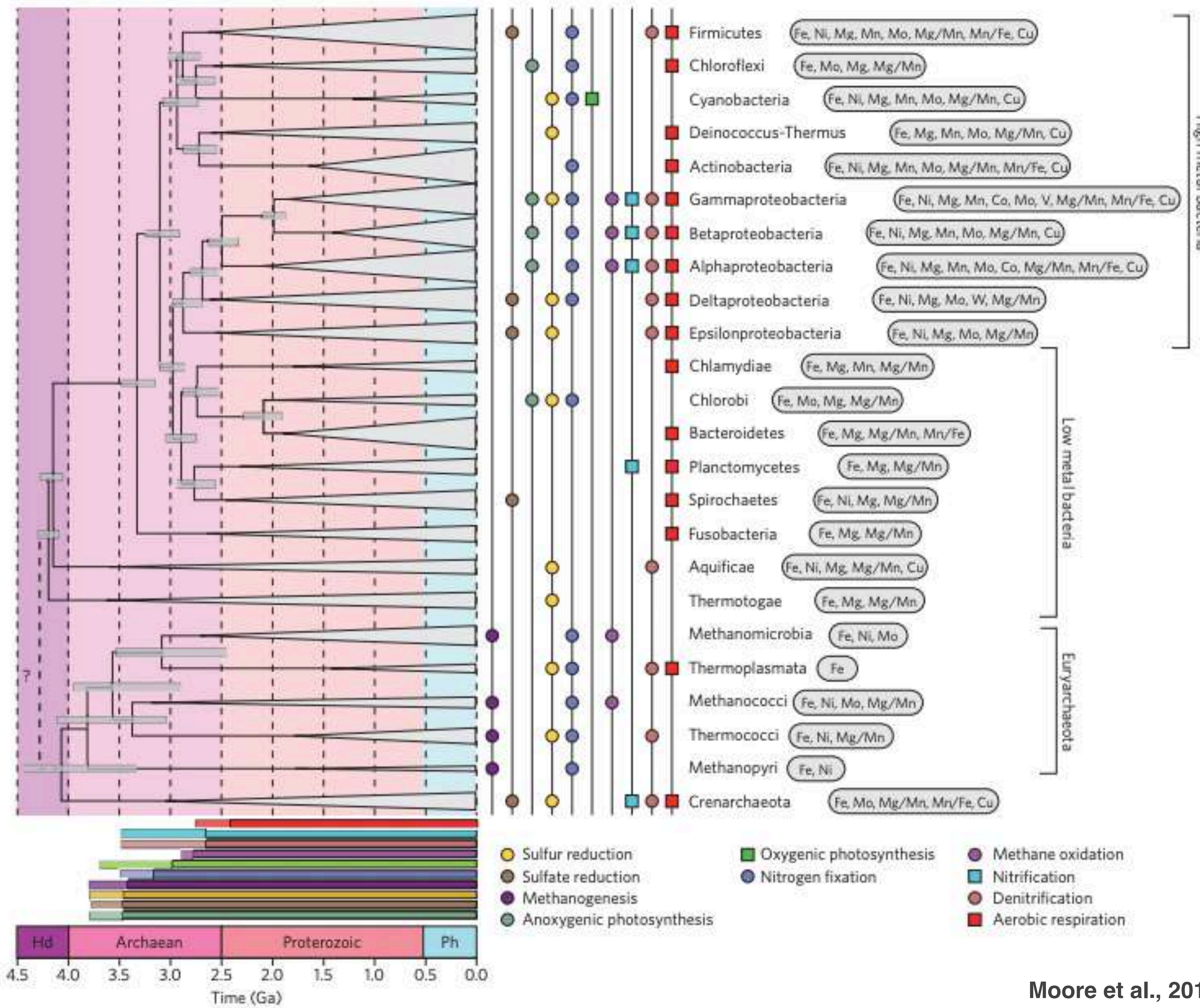
Mol Biol Evol, Volume 34, Issue 2, February 2017, Pages 437–446, https://doi.org/10.1093/molbev/msw245

A genomic timescale of prokaryote evolution



Battistuzzi et al., 2004

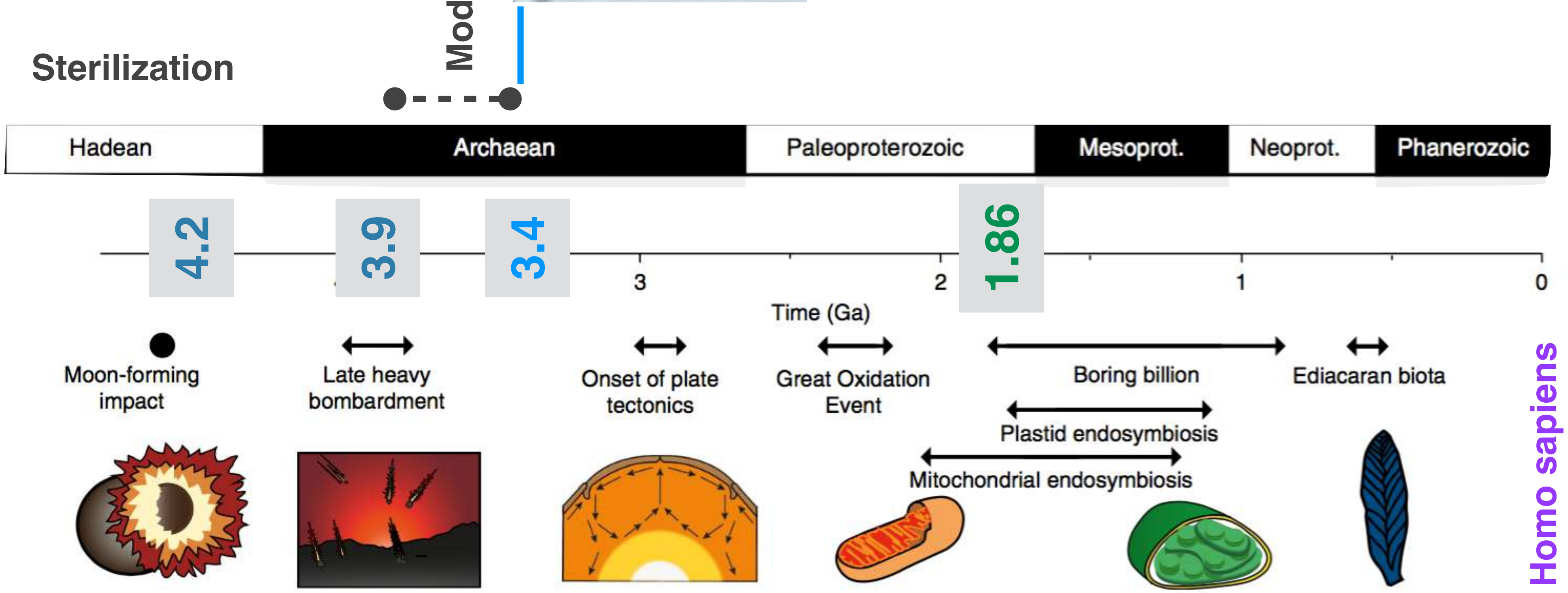
Phylogenetic tree of the main lineages of Bacteria and Archaea and their putative divergence times



Moore et al., 2017

Reconstruction the microbial “coral” of life

Strelley Pool Formation, stromatolites, Australia



Betts et al., 2018

Moody et al., 2024

LUCA: Last Universal Common Ancestor

LUCA

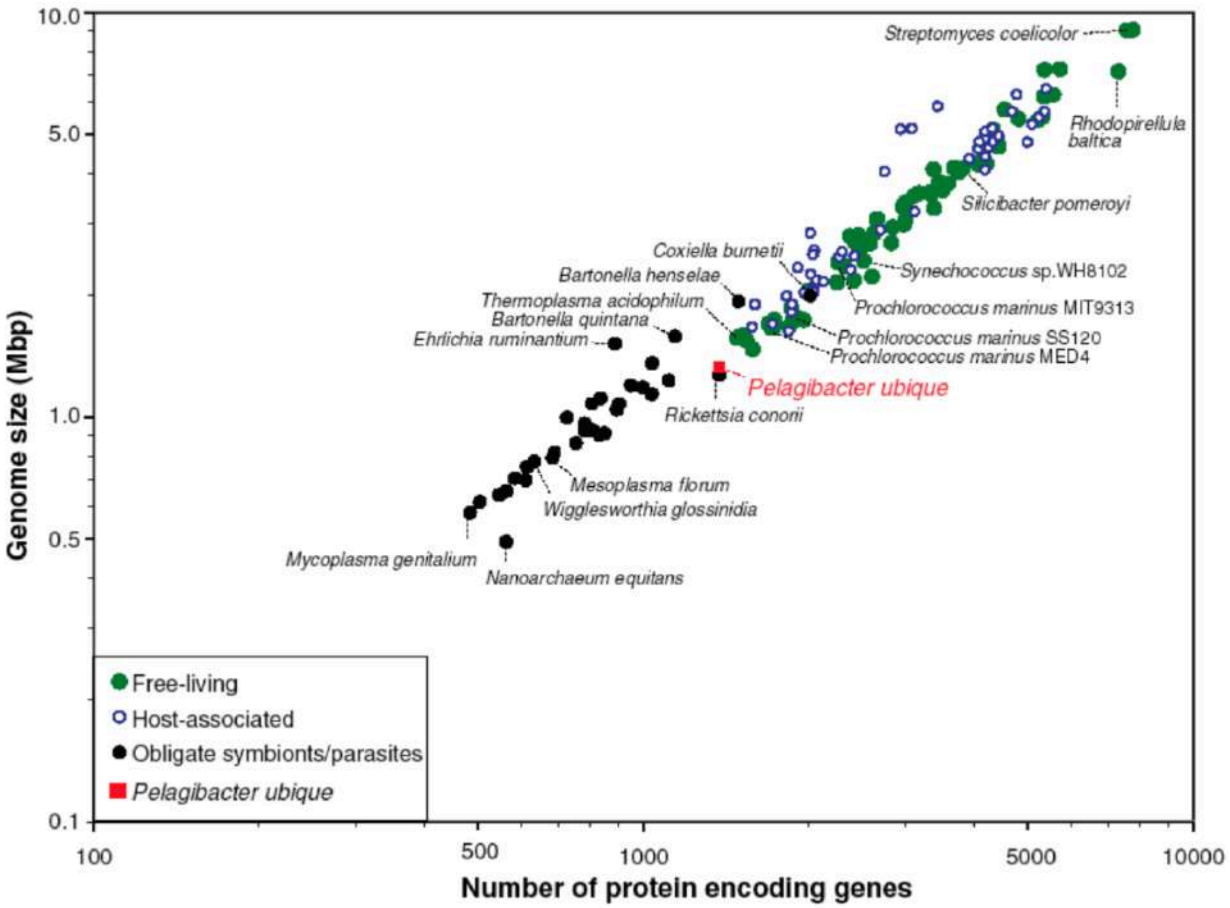
Bac & Arc

Euk

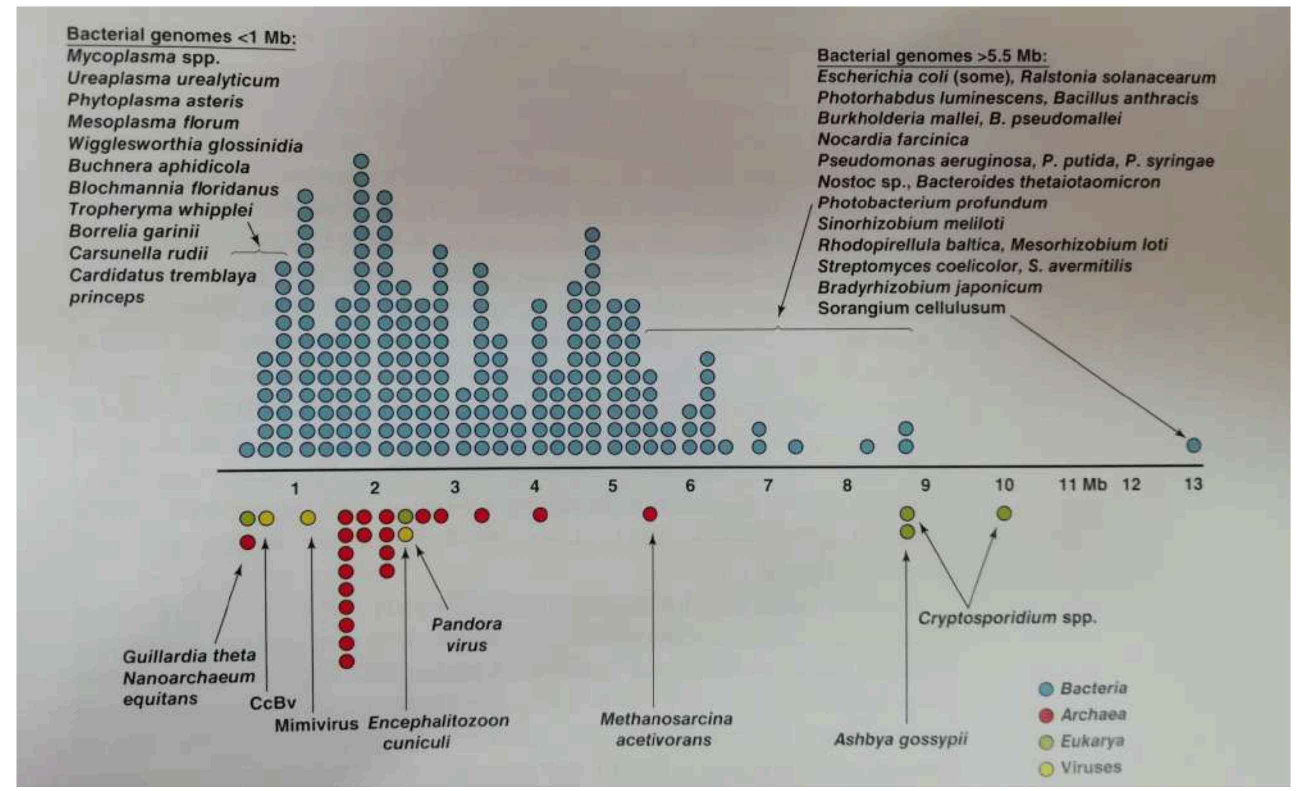
- About 1.4–1.9million extant bacterial lineages when lineages are defined by 99% similarity in the 16S ribosomal RNA gene, and that bacterial diversity has been continuously increasing over the past 1billion years (Gyr)
- Recent bacterial extinction rates are estimated at 0.03–0.05per lineage per million years (lineage–1 Myr–1), and are only slightly below estimated recent bacterial speciation rates
- Most bacterial lineages ever to have inhabited this planet are estimated to be extinct

Louca et al., 2018

Wide range of microbial genomes



Giovannoni et al., 2005



Madsen, 2016

Common multiples are:

- $1\ \text{kb} = 10^3$ bp
- $1\ \text{Mb} = 10^6$ bp
- $1\ \text{Gb} = 10^9$ bp

Bacterial genomes are typically expressed in Mb

- Genomes are constantly changes
- Genome size and genes related to life style

Cultivability and Phenotypic Analysis

- Phenotype: the physical and chemical characteristics of an organism that can be observed or measured

TABLE 13.1 Some phenotypic characteristics of taxonomic value

Category	*Characteristics*
Morphology	Colony morphology; Gram reaction; cell size and shape; pattern of flagellation; presence of spores, inclusion bodies (e.g., PHB,[a] glycogen, or polyphosphate granules, gas vesicles, magnetosomes); capsules, S-layers, or slime layers; stalks or appendages; fruiting body formation
Motility	Nonmotile; gliding motility; swimming (flagellar) motility; swarming; motile by gas vesicles
Metabolism	Mechanism of energy conservation (phototroph, chemoorganotroph, chemolithotroph); utilization of individual carbon, nitrogen, or sulfur compounds; fermentation of sugars; nitrogen fixation; growth factor requirements
Physiology	Temperature, pH, and salt ranges for growth; response to oxygen (aerobic, facultative, anaerobic); presence of catalase or oxidase; production of extracellular enzymes
Cell lipid chemistry	Fatty acids;[b] polar lipids; respiratory quinones
Cell wall chemistry	Presence or absence of peptidoglycan; amino acid composition of cross-links; presence or absence of cross-link interbridge
Other traits	Pigments; luminescence; antibiotic sensitivity; serotype; production of unique compounds, for example, antibiotics

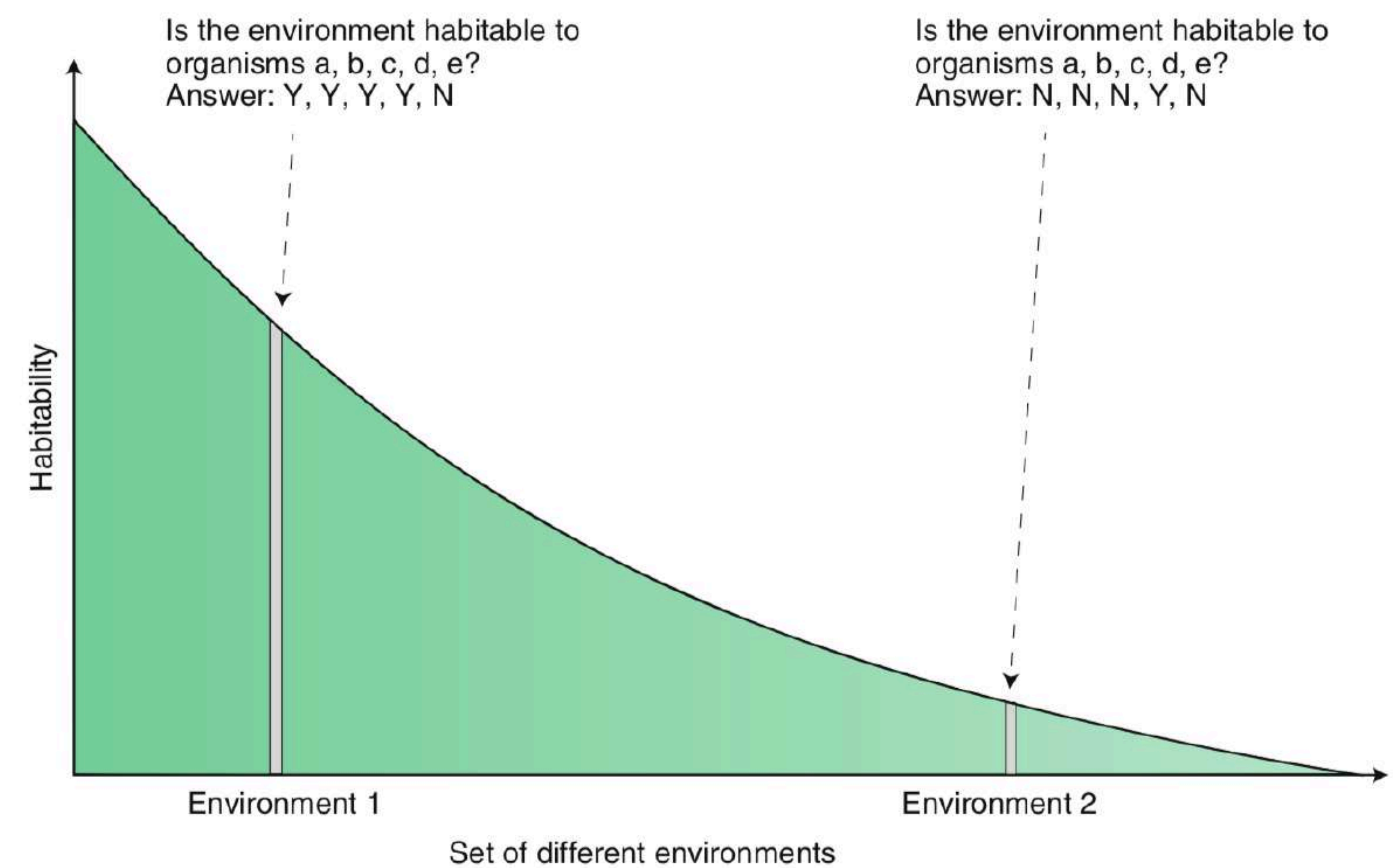
[a]PHB, poly-β-hydroxybutyric acid (Section 2.8).
[b]Figure 13.28.

Madigan et al. 2018

Niche

Habitability

- **Habitability is a binary concept** at a fundamental level
- Consider an environment with **respect to one microorganism then integrate** the answers for all microbes —> derive of a continuum
- Assessment of habitability is circumscribed by the state of biological knowledge and it is always open to improvement



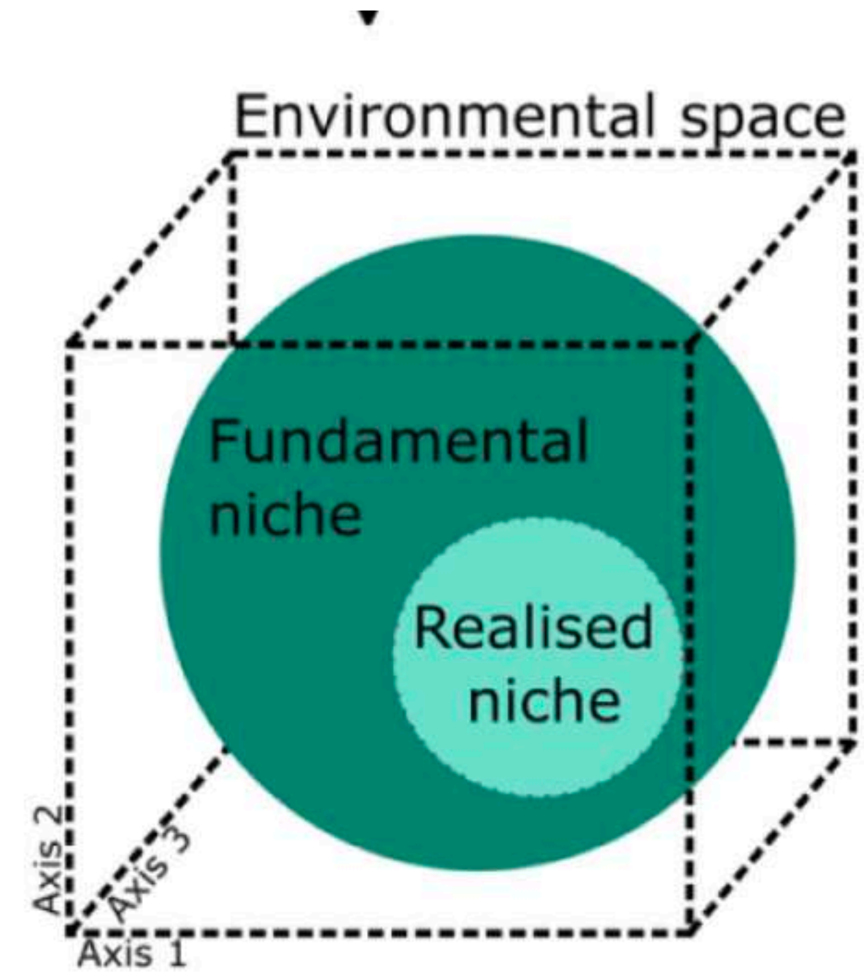
Cockell et al. 2019

Hutchinson's definitions:

1. **Fundamental environmental niche:** the set of environmental conditions in which a species can theoretically (i.e., physiologically) live and reproduce in (e.g., as defined experimentally)

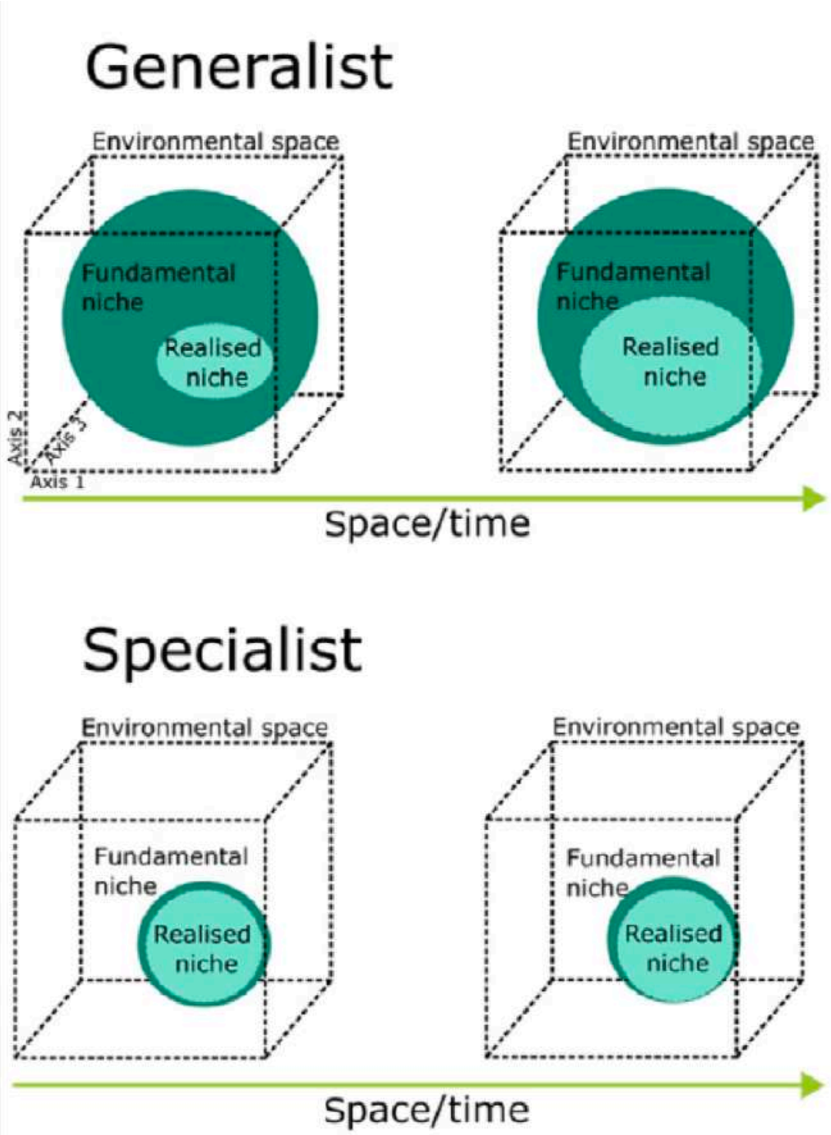
2. **Realised environmental niche:** the restricted set of conditions a species actually occupies in situ when accounting for biological interactions (e.g., competition, predation), thus a subset of the fundamental niche

Niches as **'n-dimensional hypervolumes'**, where the dimensions are the set of abiotic conditions that define the requirements of an individual or a species for its population to persist, constrained or not by biotic factors



Malard & Guisan 2023

Sales, L.P. et al. (2021) What do you mean by 'niche'? Modern ecological theories are not coherent on rhetoric about the niche concept. Acta Oecol. 110, 103701



Malard & Guisan 2023

- **Generalist species can thrive in a variety of habitats or situations**
- **Specialists are restricted to a smaller set of conditions**

GENES

Metabolic plasticity is the capacity to alter a physiological response to environmental conditions

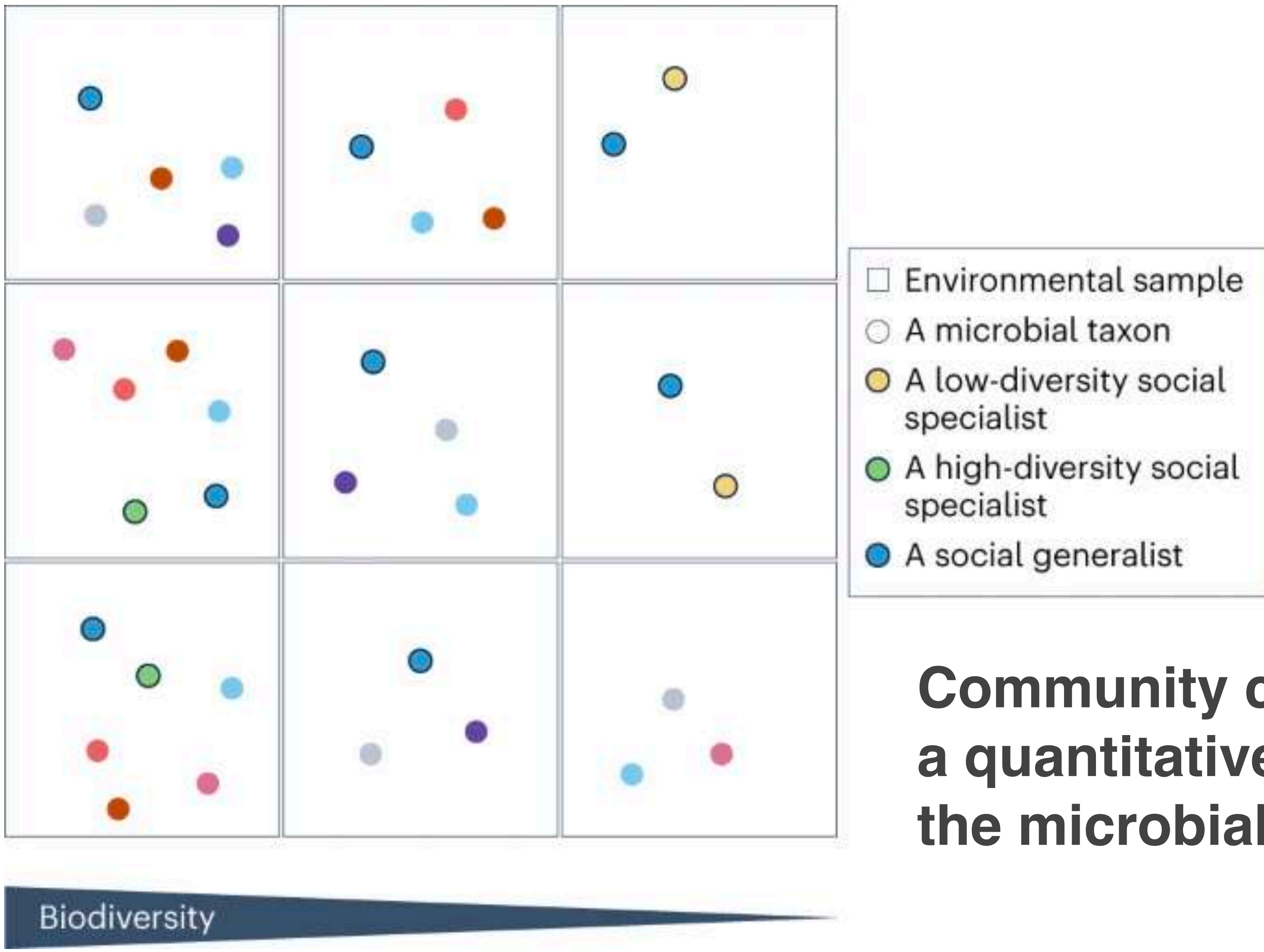
Generalist —> a large fundamental metabolic niche with high metabolic plasticity, occupying different fractions of the fundamental niche as a function of the environmental conditions and potentially changing in time and/or space

Specialist with a restricted distribution likely has a small fundamental metabolic niche because it may **lack many of the genes required to adapt to other environmental conditions** —> the fundamental metabolic niche is likely small with limited metabolic plasticity and, as a result, a specialist will always occupy the same fraction of the fundamental niche, performing the limited number of functions encoded in its genome

The social niche

‘**Social niche**’, which reflects the degree of **constraint** in the community of **other microbes** with which the species is observed in environmental samples

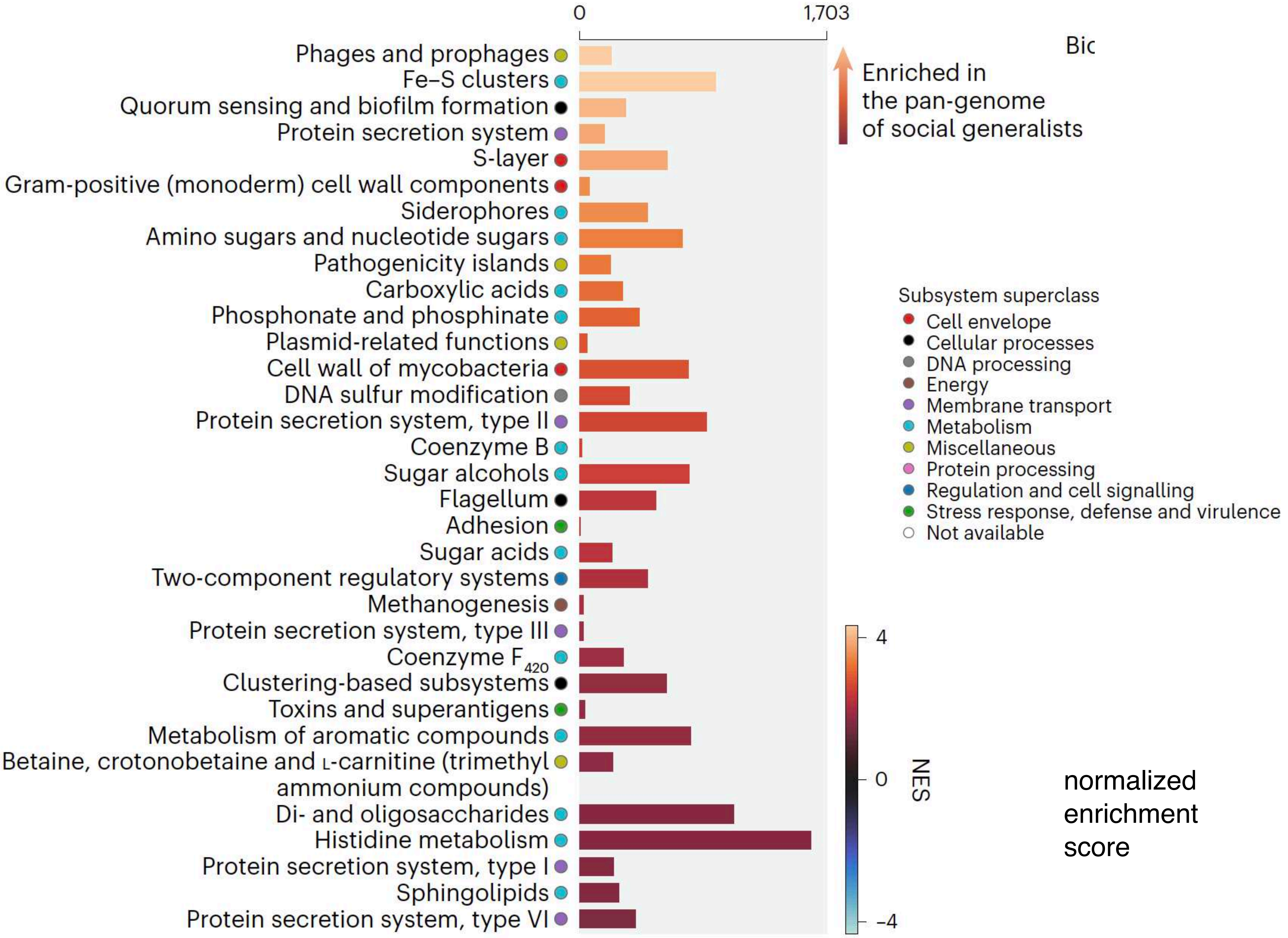
Social niche gradient at the microscale



Community composition similarity as a quantitative ecological feature of the microbial social niche

- The squares represent independent environmental samples comprising several microbial taxa, as coloured circles
- The dark blue microorganism occurs in communities that are compositionally very dissimilar across the different samples: social generalist
- The yellow microorganism always occurs with the same dark blue taxon: a social specialist
- **Social specialists can be found in either low-diversity samples (as with the yellow microorganism) or in high-diversity samples (as with the green microorganism, which is always found in samples with the same composition)**

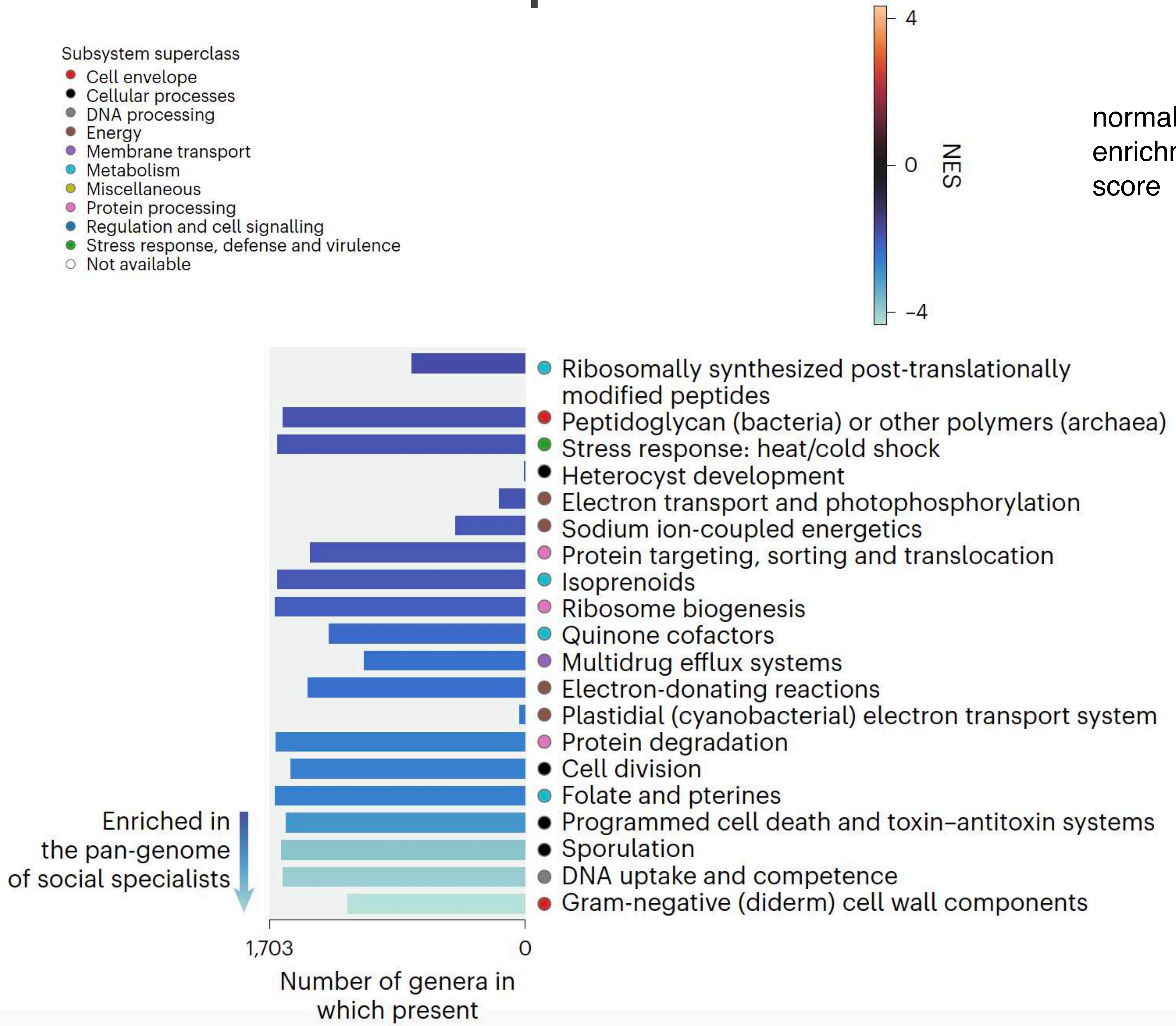
Social niche breadth: Social Generalists



normalized enrichment score

von Meijenfeldt et al., 2023

Social niche breadth: Social Specialists

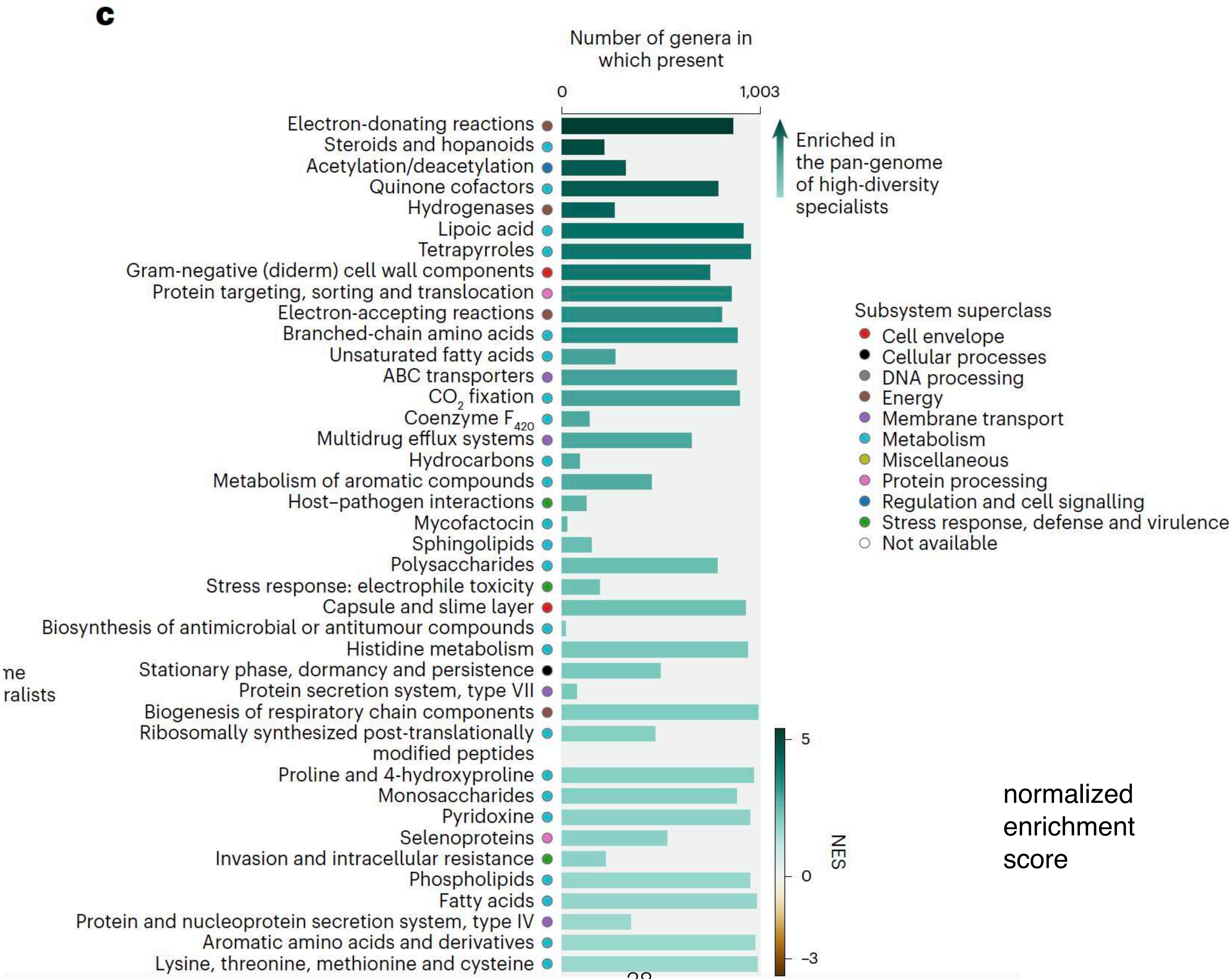


normalized enrichment score

von Meijenfeldt et al., 2023

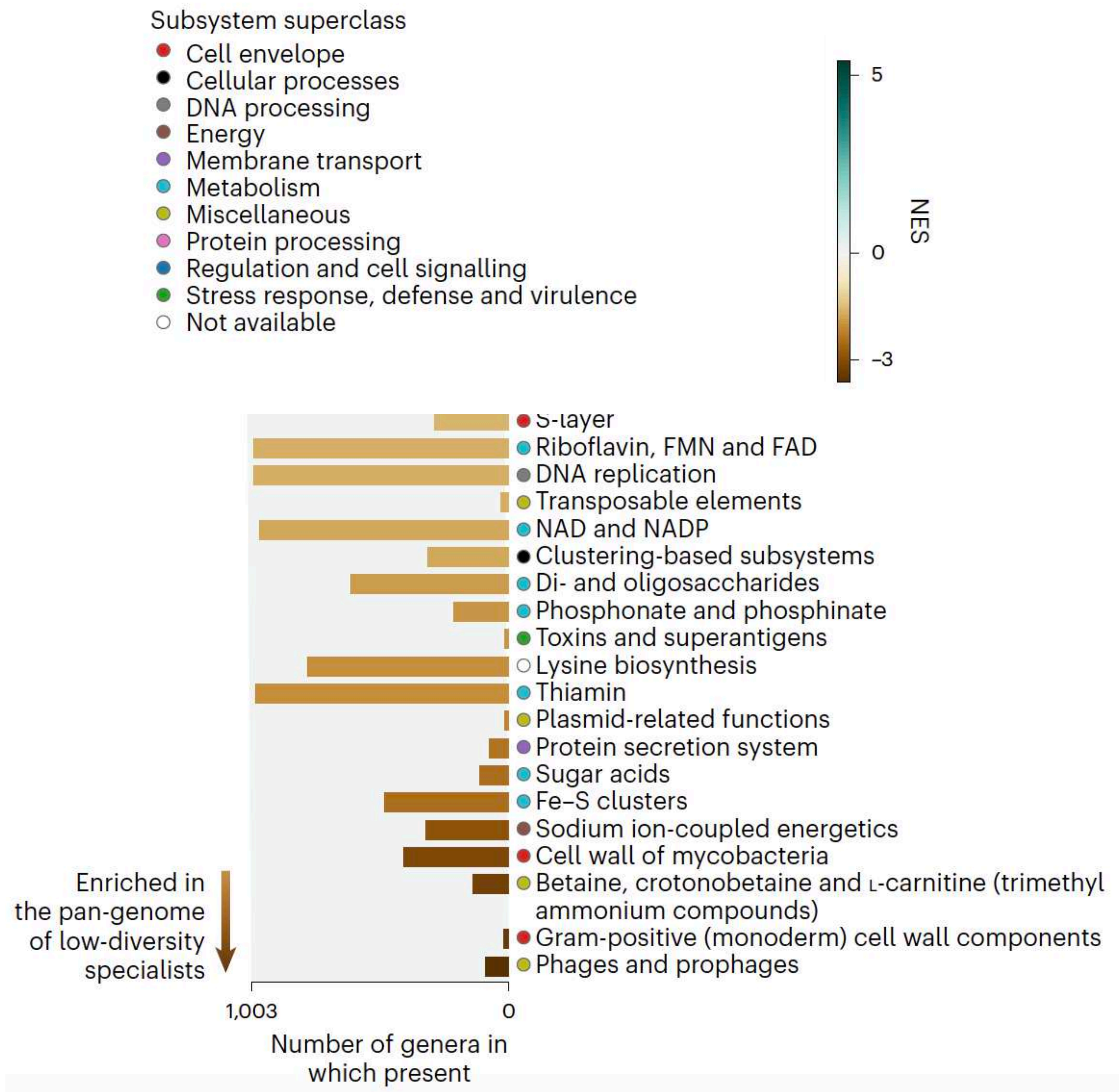
Social niche breadth: High-diversity specialists

von Meijenfeldt et al., 2023



normalized enrichment score

Social niche breadth: Low-diversity specialists



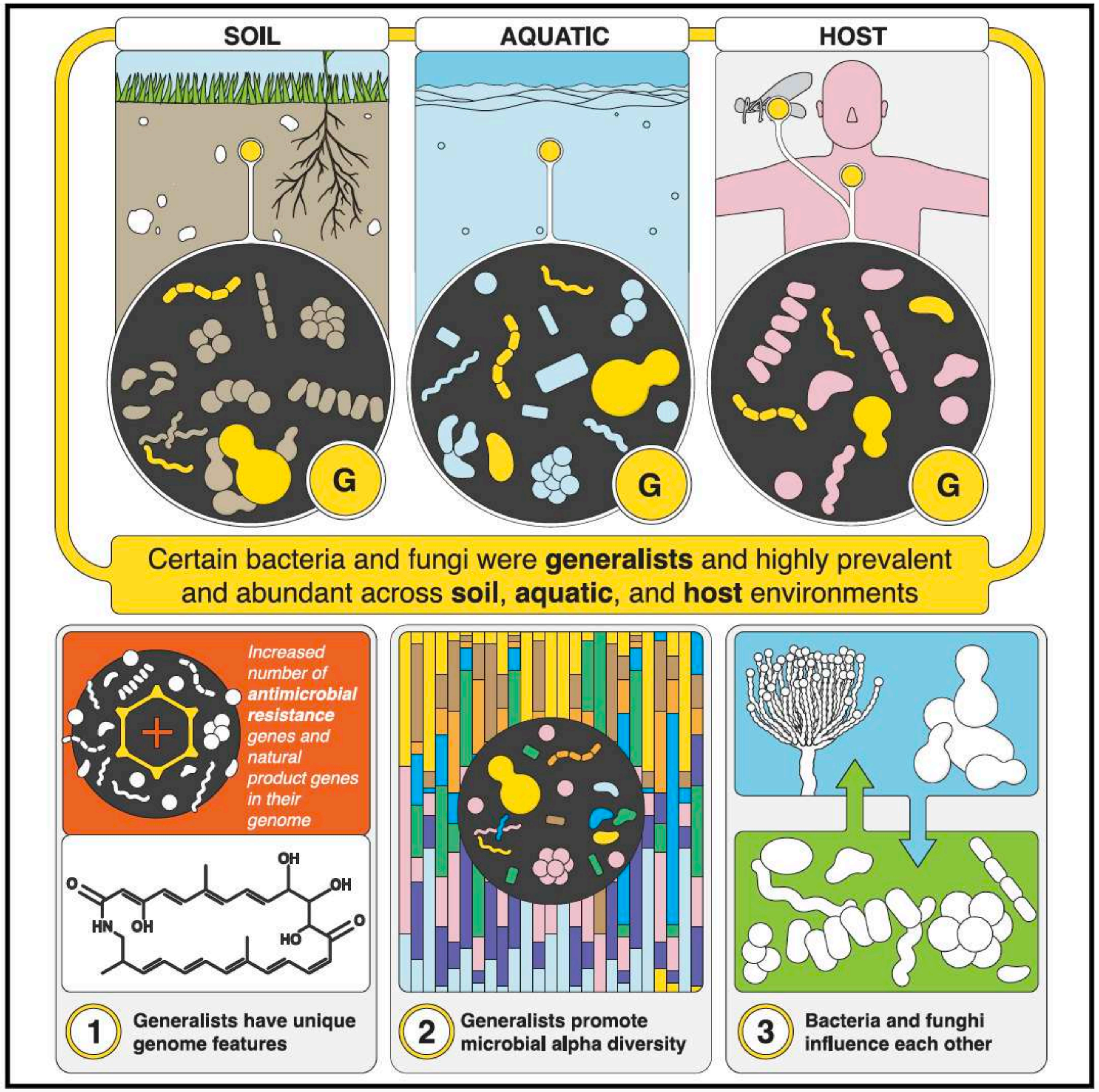
normalized enrichment score

von Meijenfeldt et al., 2023

Generalists *vs.* Specialists

- Bacterial and fungal **generalists** are **widely distributed** in aquatic, host, and soil biomes
- Generalists have **larger genomes** with more **secondary metabolites** and **AMR genes**
- Samples containing generalists show **higher alpha diversity**
- Generalists **underpin cross-kingdom** community structure

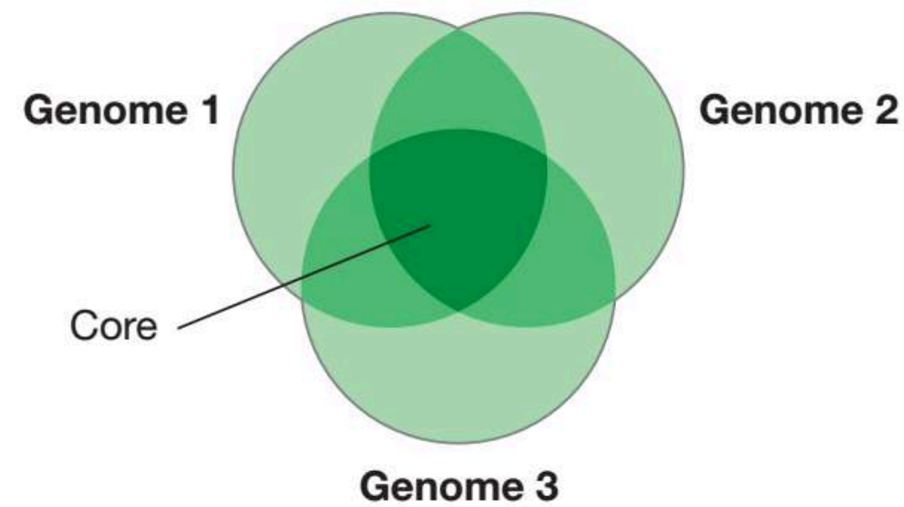
Ecological theory predicts that *generalists, or organisms that are fit across a wider range of conditions, will be more resilient to changing environmental conditions*



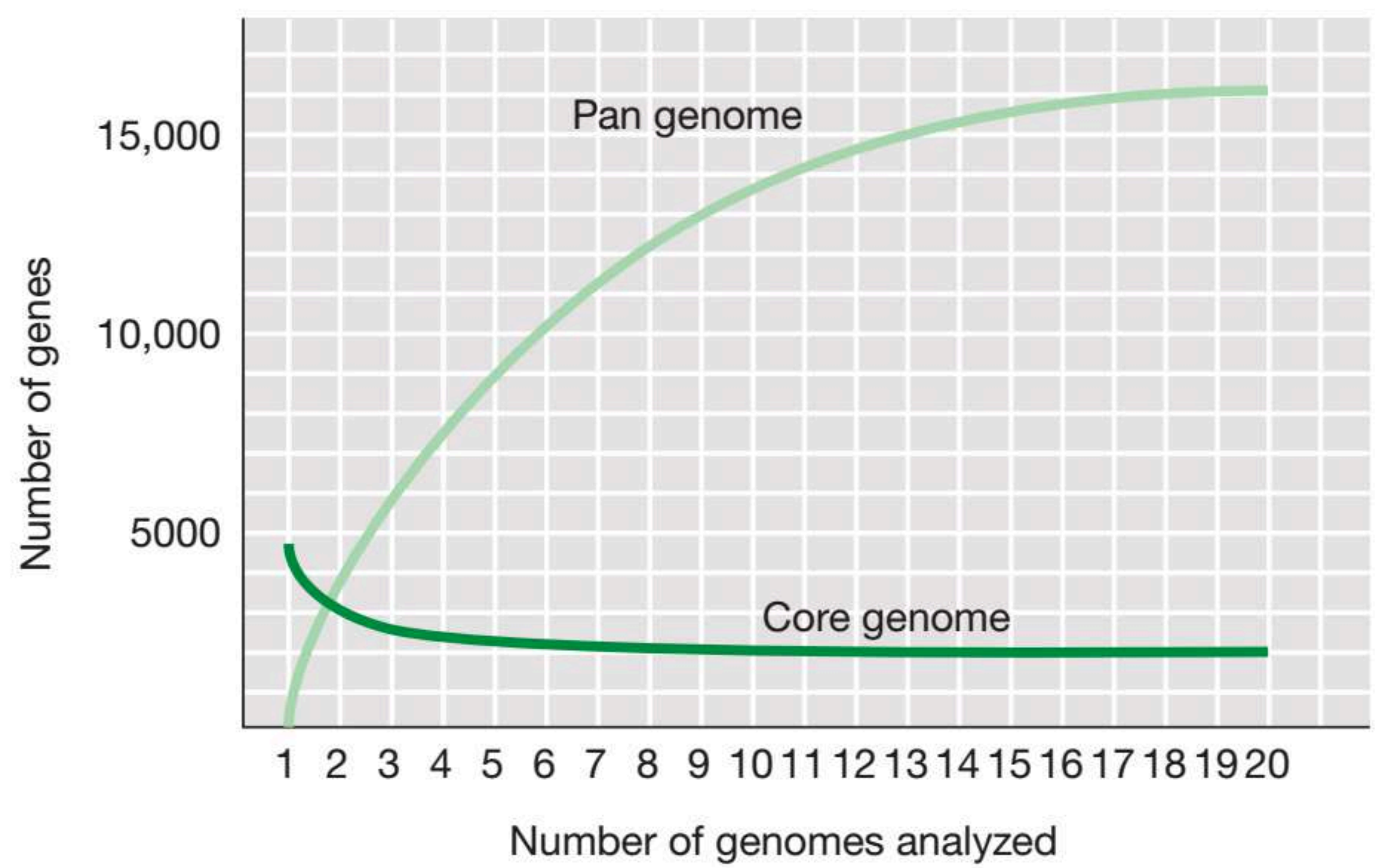
Loos et al., 2024

Microbial genome

- Microbial genomes are **dynamic**: genome size and gene content can vary considerably between strains of a species
- **Core genome** is defined as the **set of all genes shared** by a species
- **Pan genome** is defined as the core genome plus **genes whose presence varies among strains of a species**



(a)



(b)

Madigan et al. 2018

Generalist *vs* Specialist genome structure

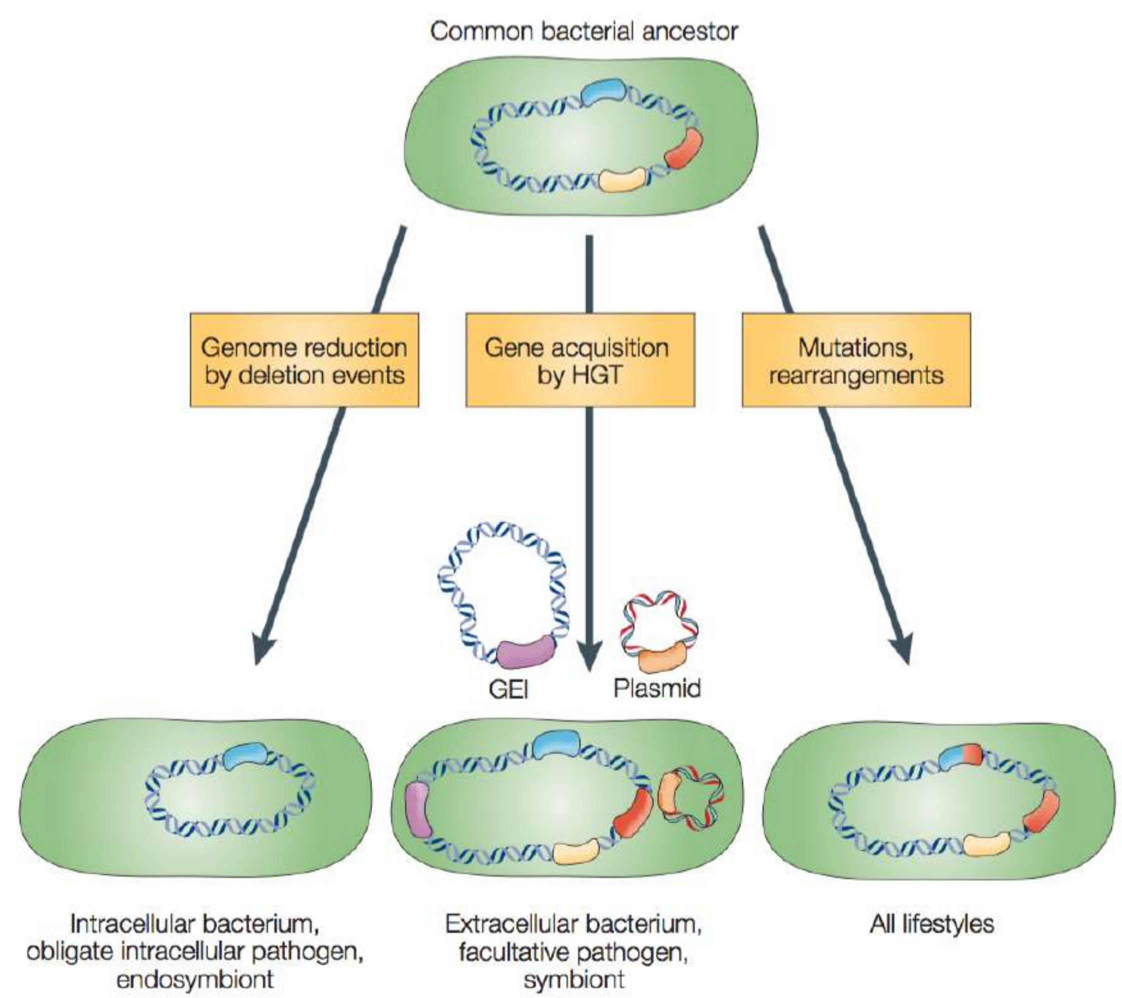
Genome structure reflects microbial lifestyle

Genome reduction is common in **intracellular bacteria** (obligate intracellular pathogens, endosymbionts) **contributes to the evolution of strictly host-dependent bacterial variants** — as microbes rely on the host cell to compensate for the gene functions that are lost

Gene acquisition by horizontal transfer between different species is common in **extracellular bacteria** (facultative pathogens, symbionts), which involves **mobile genetic elements** (plasmids, genomic islands, GEIs, and bacteriophages), **increases** the **versatility and adaptability** of the recipient — allows microbes to **adapt** to a new or changing environment

Point mutations and genetic rearrangements constantly contribute to evolution of new gene variants in **all types** of bacteria

Generalist genera are older than specialist genera and have large and open pan-genomes with which they have adapted to different habitats



Dobrindt et al., 2004

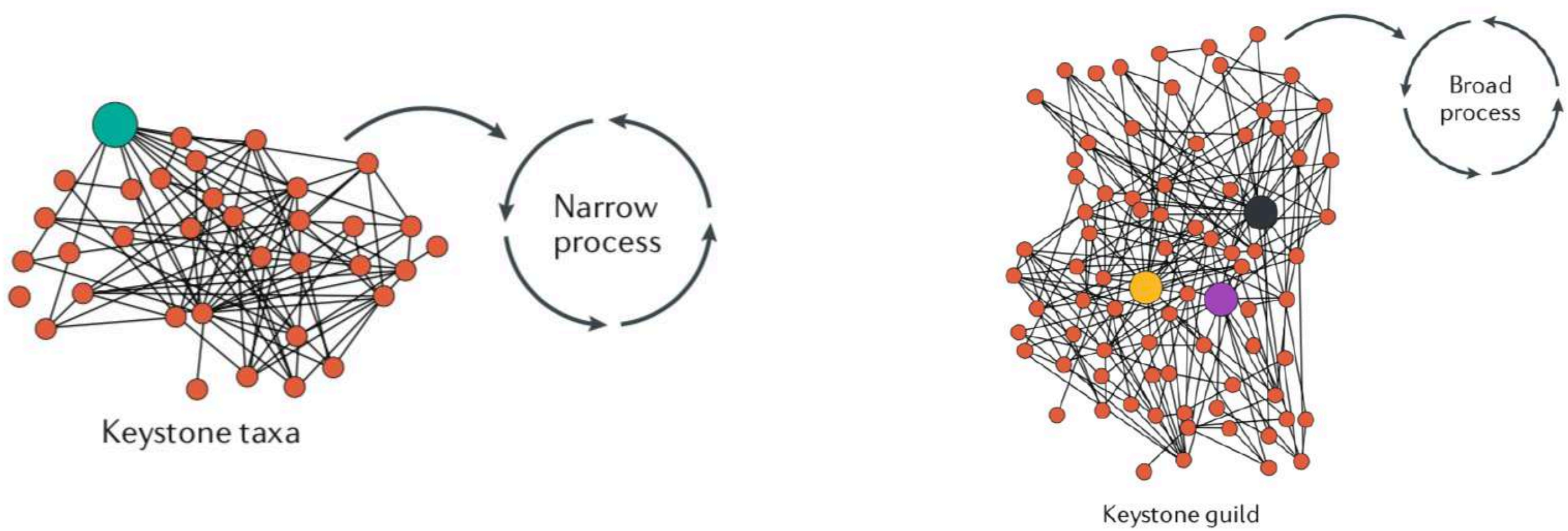
Keystone Species
Microbial Guilds
Functional Hubs

Feature	Keystone Species	Microbial Guild	Functional Hub
Defined by	Impact on community	Shared ecological function	Network connectivity
Abundance	Often low	Variable	Variable
Role	Critical for stability	Redundant contributors	Connector or coordinator
Identified by	Experimental removal, impact	Functional assays	Co-occurrence network analysis

Keystone taxa, I

- **Microbial keystone taxa** are **highly connected** taxa that individually or in a guild (**groups of keystone taxa with similar functioning**) exert a **considerable influence** on microbiome structure and functioning irrespective of their abundance across space and time
- Microbial keystone taxa have a unique and crucial role in microbial communities, and their removal can cause a **dramatic shift** in microbiome structure and functioning
- **Keystone taxa are driver of microbiome structure and functioning**

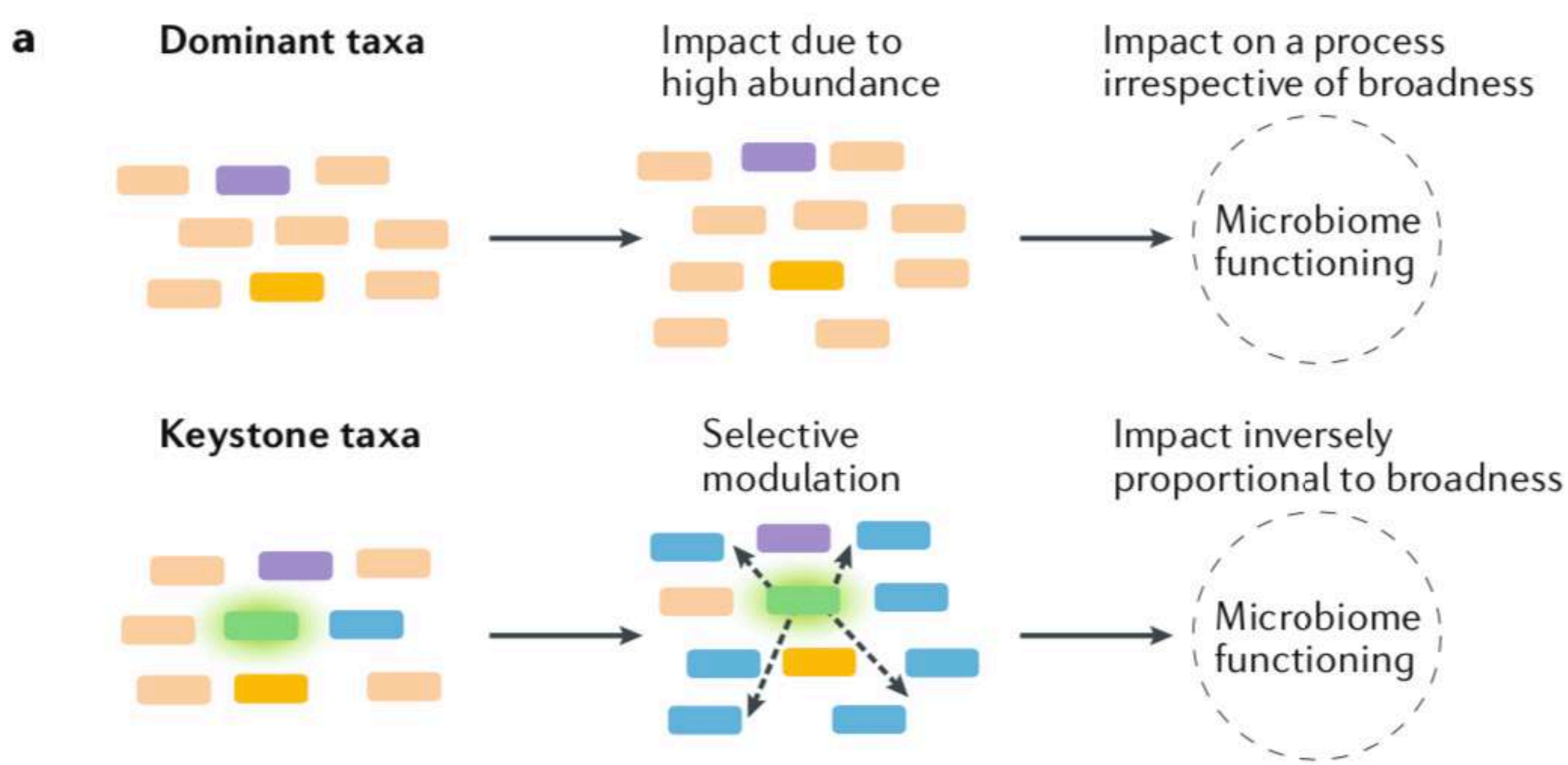
c



Banerjee et al., 2018

Keystone taxa, II

- Keystone taxa (green) **exert their influence irrespective of their abundance**
- **Broadness implies** that a **particular process consists of many steps** and involves **diverse** microbial groups
- Keystone taxa exert their **influence by selectively modulating accessory microorganisms**, and thus, they might have a greater influence on **narrow processes (the processes that consist of a single step or a few steps and involve a select group of microorganisms)**
- Accessory microorganisms whose abundance is selectively promoted by keystone taxa are shown in blue, whereas other community members are shown in dark orange and purple



Banerjee et al., 2018

Computational *vs* Empirical

Ecosystem or habitat	Keystone taxa[a]	Refs
Computational inference		
Grasslands	• Burkholderiales • Sphingobacteriales • Clostridiales • Actinomycetales • Acidobacteria GP4	34–36
Forest or woodlands	• Actinomycetales • Acidobacteria GP4 • Rhizobiales • Burkholderiales • Clostridiales • Sphingobacteriales • Rhodobacterales • Verrucomicrobia	8,35,37,38,61
Agricultural lands	• *Gemmatimonas* • Acidobacteria GP17 • Xanthomonadales • Rhizobiales • Burkholderiales • Solirubrobacterales • Verrucomicrobia	35,40,42,43
Arctic and Antarctic ecosystems	• Rhizobiales • Burkholderiales • Actinobacteria • Alphaproteobacteria	25,26,44,46
Contaminated soil	• Rhizobiales • *Nitrospira* • Pseudomonadales • Actinobacteria	47,48
Plant-associated microbiota	• Acidobacteria GP1, GP3 and GP6 • Rhizobiales • Burkholderiales • Pseudomonadales • Bacteroidetes • Frankiales	40,49,50
Aquatic ecosystems	• *Pelagibacter* • Oceanospirillales • Flavobacteriaceae • *Nitrospira* • Rhodobacteradaceae • Alteromonadaceae • *Chromatium* • Rhizobiales • Burkholderiales • *Chlorobium* • Verrucomicrobia • *Chloracidobacterium* • Chloroflexi • *Candidatus* OP3	24,51–55,72

Empirical evidence		
Agricultural lands[b]	• *Gemmatimonas* • Acidobacteria	39,41
Phyllosphere	• *Albugo* • *Dioszegia*	20
Human oral microbiome	*Porphyromonas gingivalis*	64,74
Human gut microbiome	• *Helicobacter pylori* • *Methanobrevibacter smithii* • Actinobacteria • *Bacteroides fragilis* • *Bacteroides stercoris* • *Bacteroides thetaiotaomicron* • *Ruminococcus bromii* • *Klebsiella pneumoniae* • *Proteus mirabilis*	22,23,56–60,76

- **Keystone species** are microbes that, despite their low abundance, have a **disproportionately large impact** on the ecosystem (e.g., methanotrophs controlling methane emissions)
- Blended strategies to tap into diversity and functioning in ecosystems
- Understanding keystone taxa is essential in order **to predict microbial response to natural and anthropogenic-induced changes**

Banerjee et al., 2018

Microbial Guilds

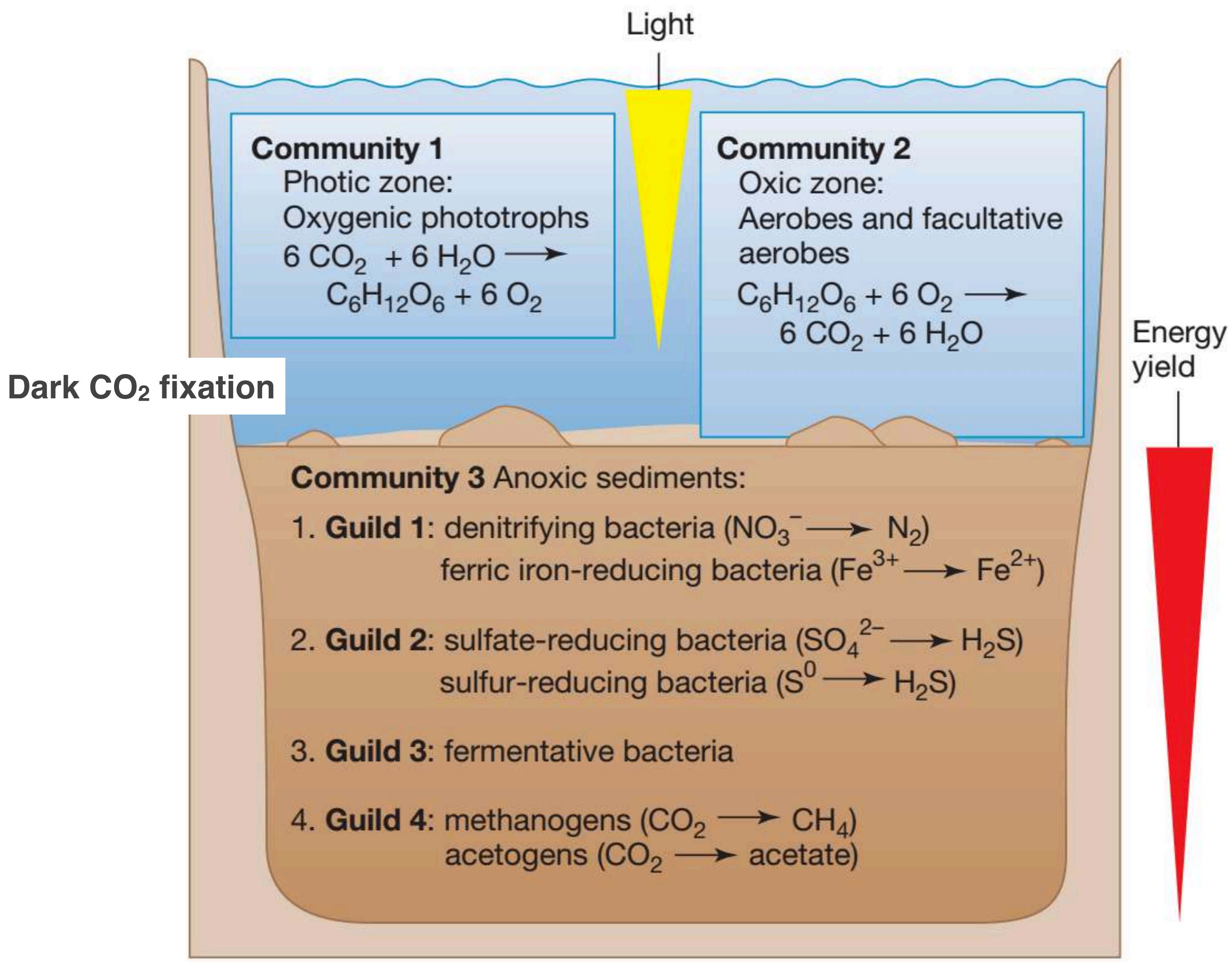


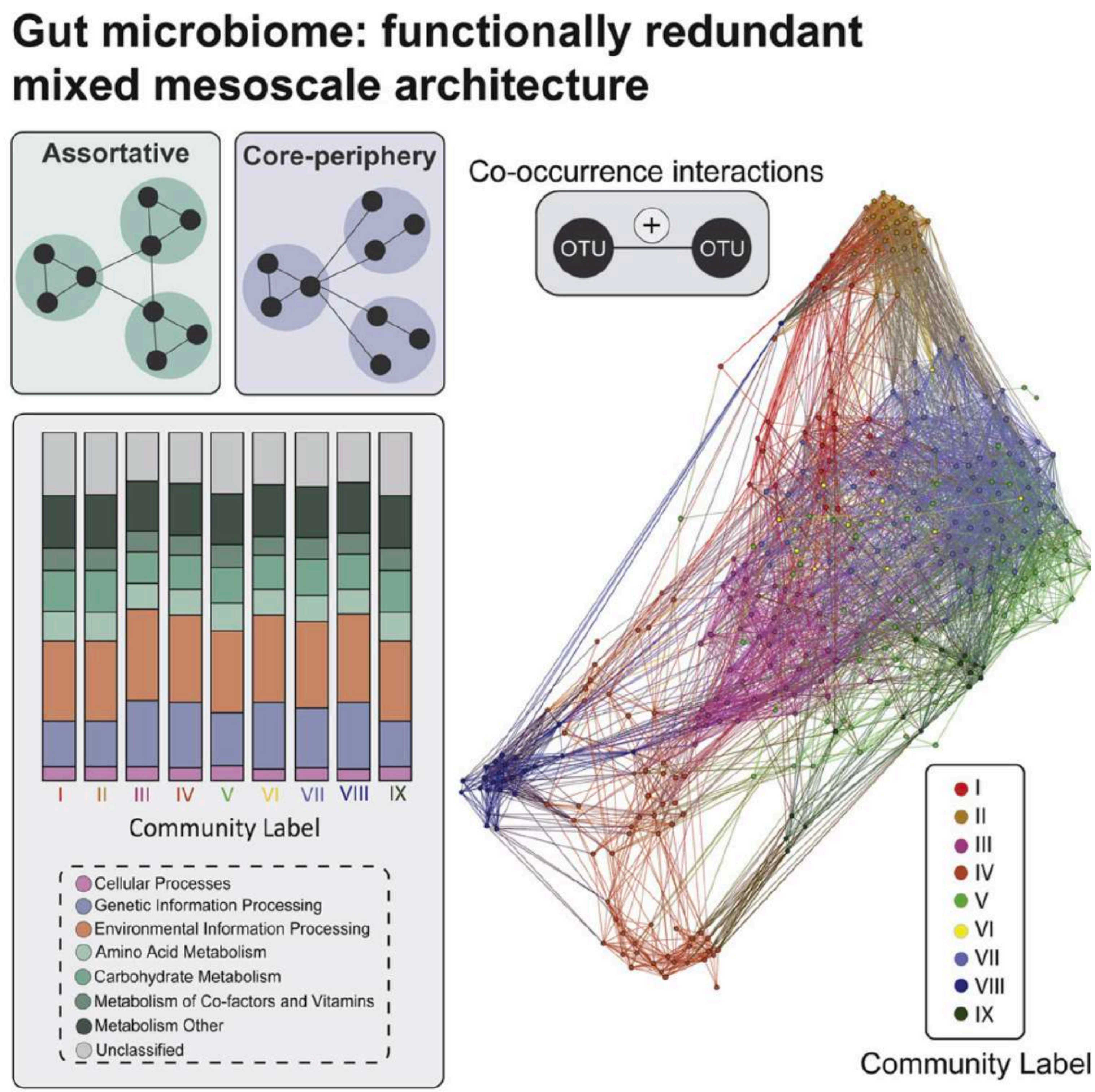
Figure 20.2 Populations, guilds, and communities. Microbial communities consist of populations of cells of different species. A freshwater lake ecosystem would likely have the communities shown here. The reduction of NO_3^-, Fe^{3+}, SO_4^{2-}, S^0, and CO_2 are examples of anaerobic respirations. The region of greatest activity for each of the different respiratory processes would differ with depth in the sediment. As more energetically favorable electron acceptors are depleted by microbial activity near the surface, less favorable reactions occur deeper in the sediment.

- **Microbial guilds** are groups of microbes that perform the same **functional role** in the ecosystem, regardless of their taxonomic identity
- Keystone species and microbial guilds concepts help us understand function-based relationships rather than just who is present

Madigan et al. 2018

Functional Hubs

- **Definition:** Species (or taxa) that are **highly connected** in microbial interaction networks — they interact with many others and **coordinate or stabilize** community function.
- **Key Traits:**
  - Identified via **network analysis**
  - May overlap with keystone species
  - Can be connectors, mediators, or “communication centers”



Hall et al., 2019

Distinct ecological roles

1. Keystone Species

- **Definition:** A species that has a **disproportionately large impact** on the structure or function of an ecosystem, **relative to its abundance**.
- **Key Traits:**
  - Not necessarily abundant
  - Removal causes a **cascade of changes** in community composition or function
- **Microbial Example:**
  - *Nitrosopumilus maritimus* (an ammonia-oxidizing archaeon) plays a critical role in marine nitrogen cycling, even though it may be rare.
- **Analogy:** Like a key stone in an arch — remove it, and the whole system collapses.

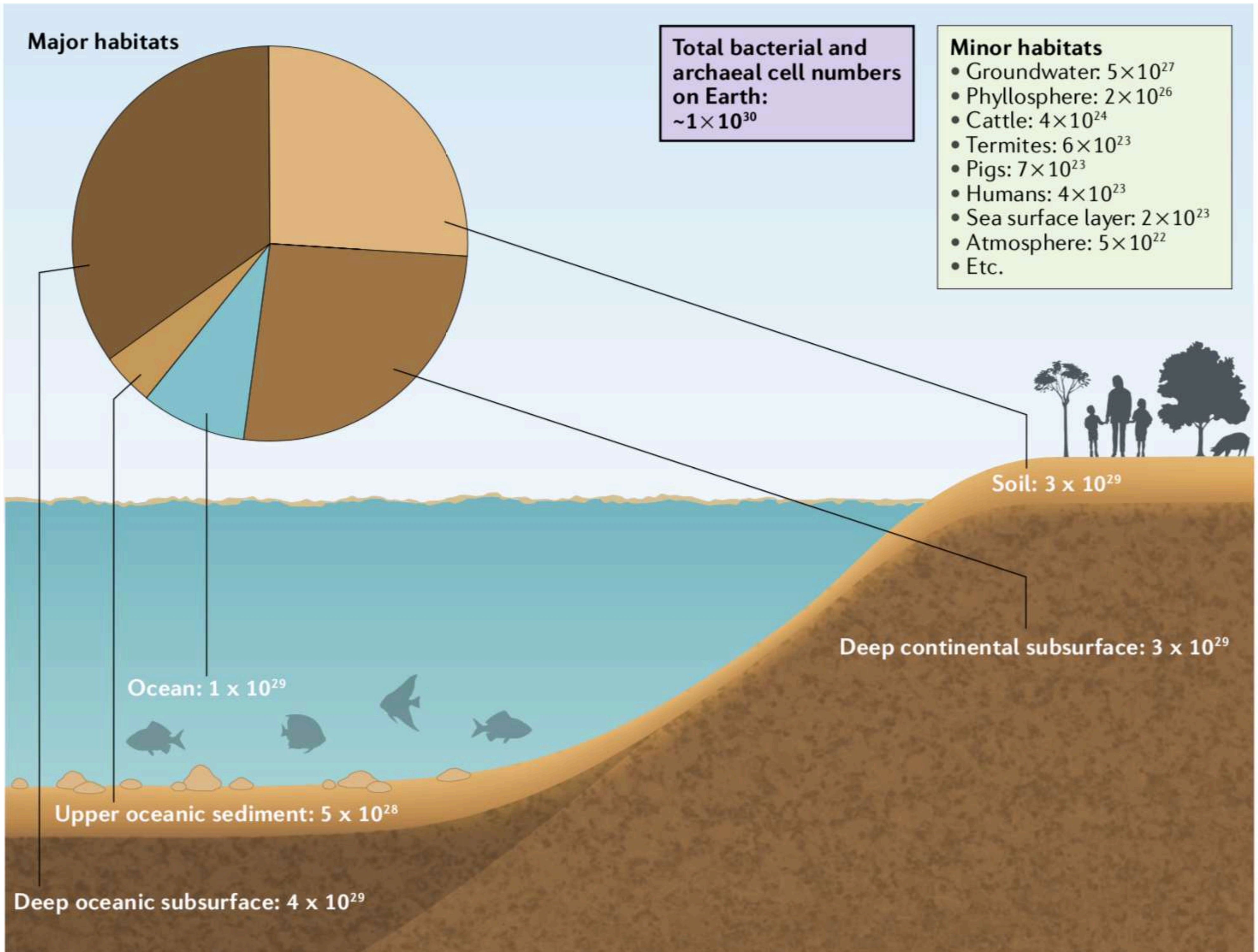
2. Microbial Guilds

- **Definition:** A group of **taxonomically different microbes** that perform the **same ecological function**.
- **Key Traits:**
  - Defined by **function**, not species identity
  - Often found across different environments
- **Microbial Example:**
  - **Denitrifiers** (e.g., *Pseudomonas*, *Paracoccus*, *Bacillus*) — all reduce nitrate to nitrogen gas
- **Analogy:** Like different brands of workers doing the same job — plumbers from different companies all fixing pipes.

3. Functional Hubs

- **Definition:** Species (or taxa) that are **highly connected** in microbial interaction networks — they interact with many others and **coordinate or stabilize** community function.
- **Key Traits:**
  - Identified via **network analysis**
  - May overlap with keystone species
  - Can be connectors, mediators, or “communication centers”
- **Microbial Example:**
  - A bacterium that maintains connections between nitrogen cyclers, carbon degraders, and methanogens in a soil network.
- **Analogy:** Like an airport hub — even if it’s not the biggest city, it connects many routes and keeps the system flowing.

Microbial abundance



Atmo

Bio

Aqua

Geo

Spheres

Flemming & Wuertz, 2019

Microbial Diversity

—> 16S rRNA gene
—> Whole genome

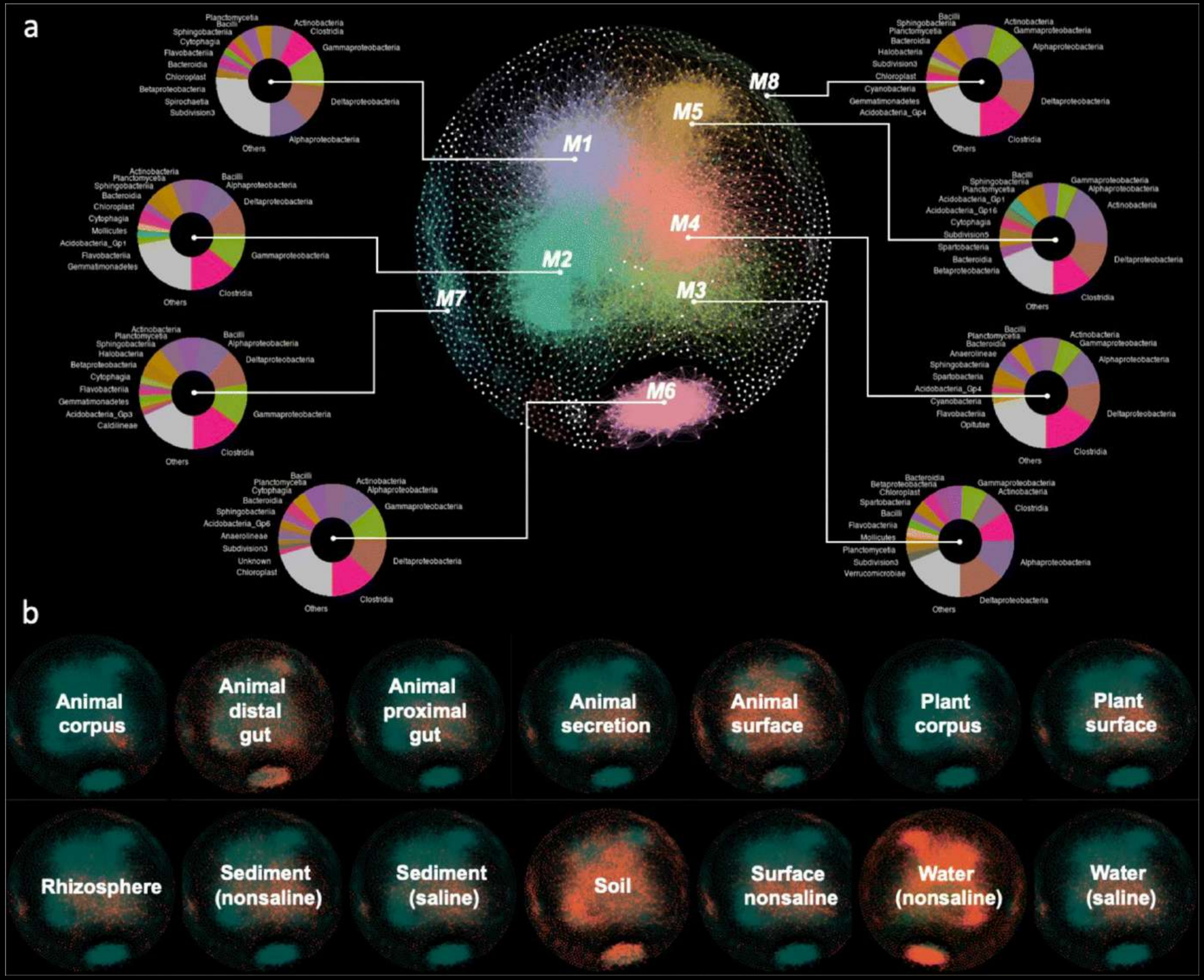
ALPHA DIVERSITY:

Diversity within a sample—-> number of different microbes (richness), but how evenly distributes in terms of total abundance (evenness)

BETA DIVERSITY:

Diversity between sample diversity

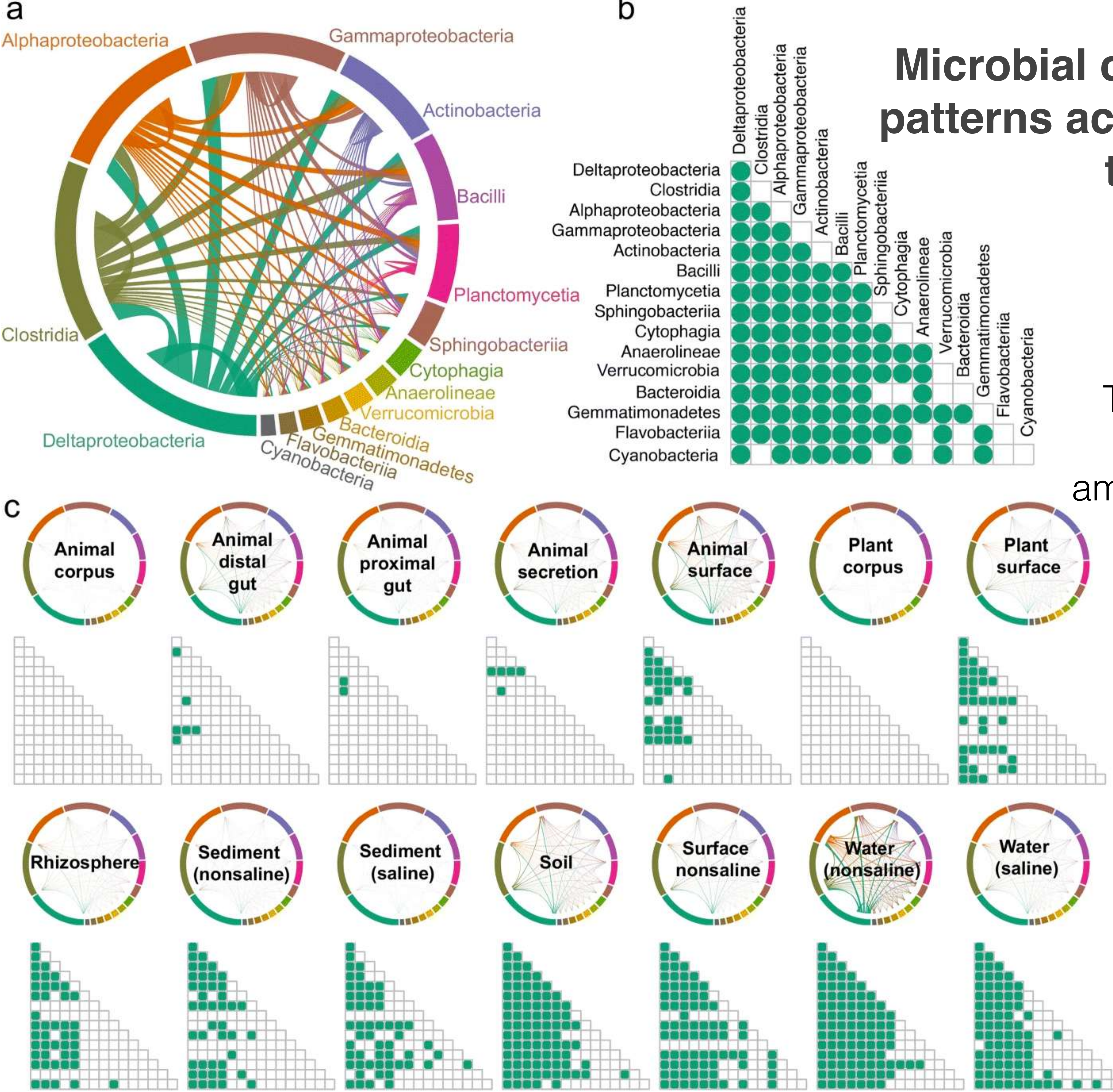
Earth microbial co-occurrence network reveals interconnection pattern across microbiomes



Ma et al., 2020

8 taxonomy distinct modules linked with different environments, which featured environment specific microbial co-occurrence relationships

Microbial co-occurrence patterns across dominant taxa



The profiles of co-occurrence links among dominant taxa

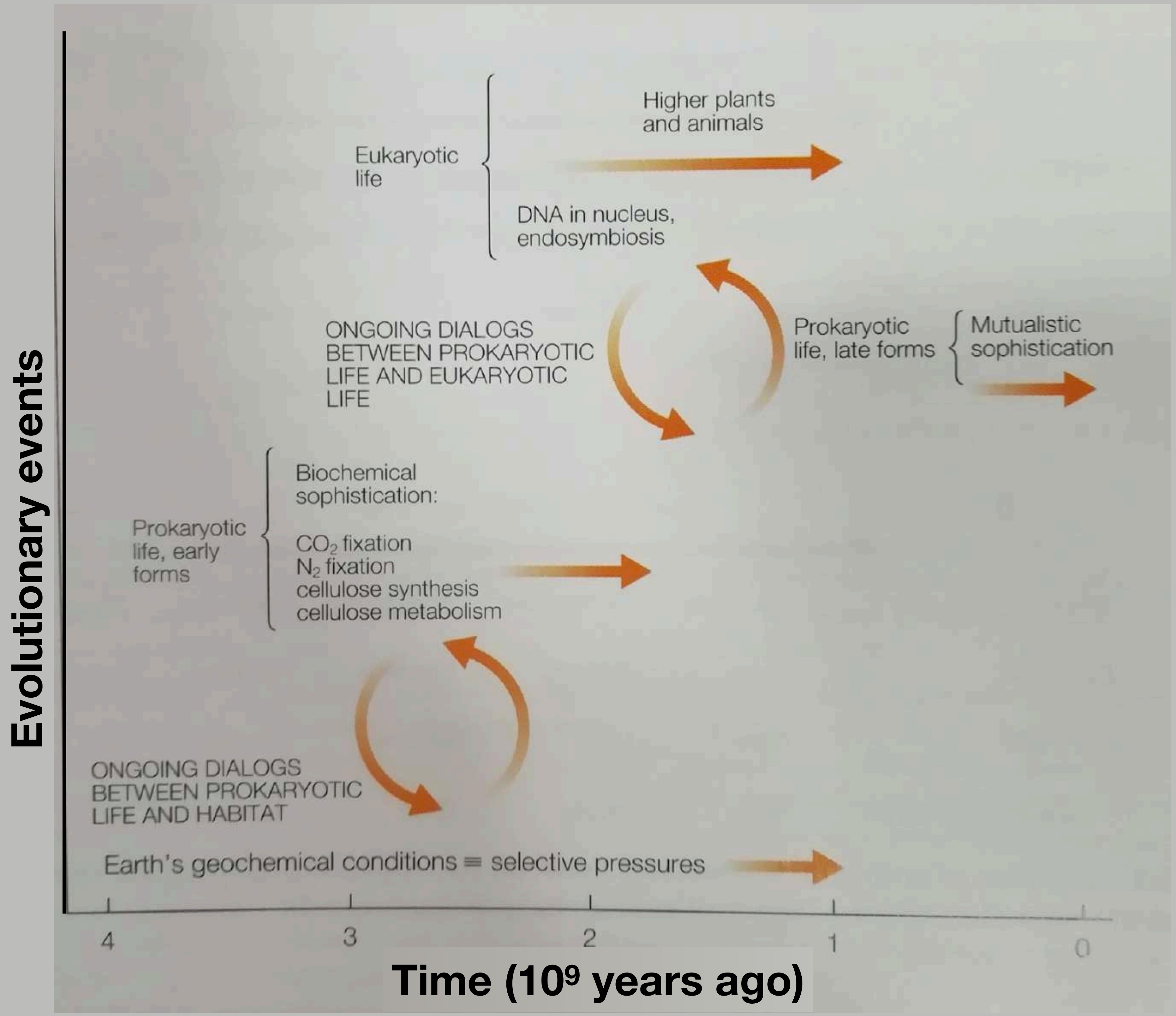
Ma et al., 2020

Ecosystem structure

- ***Primary producers***
- ***Consumers/Decomposers, Heterotrophic microbes****: a general term for microbes that cannot assimilate carbon from inorganic sources (such as carbon dioxide) and instead use organic carbon compounds for anabolism*
- Water cycle https://youtu.be/oaDkph9yQBs
- Carbon biogeochemical cycle in soil/sediment and ocean/freshwater
- Nutrient biogeochemical cycles in soil/sediment and ocean/freshwater

Continuum of microbial interactions

- **Evolution at the species level**
- Evolution of **interactions and behaviours**
- *Microbial interactions link microbial diversity with metabolic diversity*
- *Microbial interactions has structured the environment*



Madsen, 2016

Microbial Diversity-Metabolic Diversity

- **Coupling of microbial diversity and metabolic diversity keep the ecosystem functioning**
- Microbes influence habitability
- Habitability influence microbes
- Habitability is a binary continuum

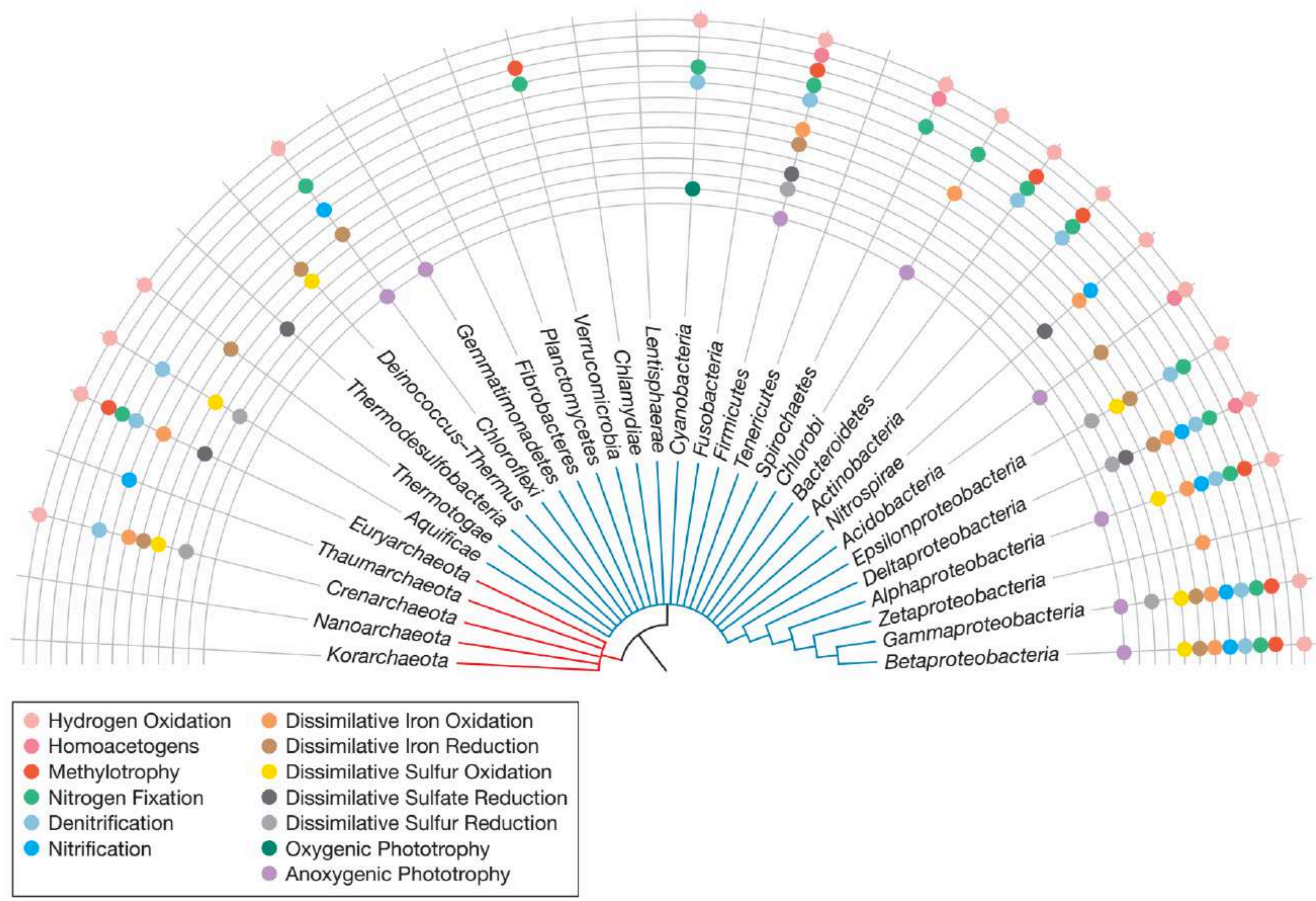
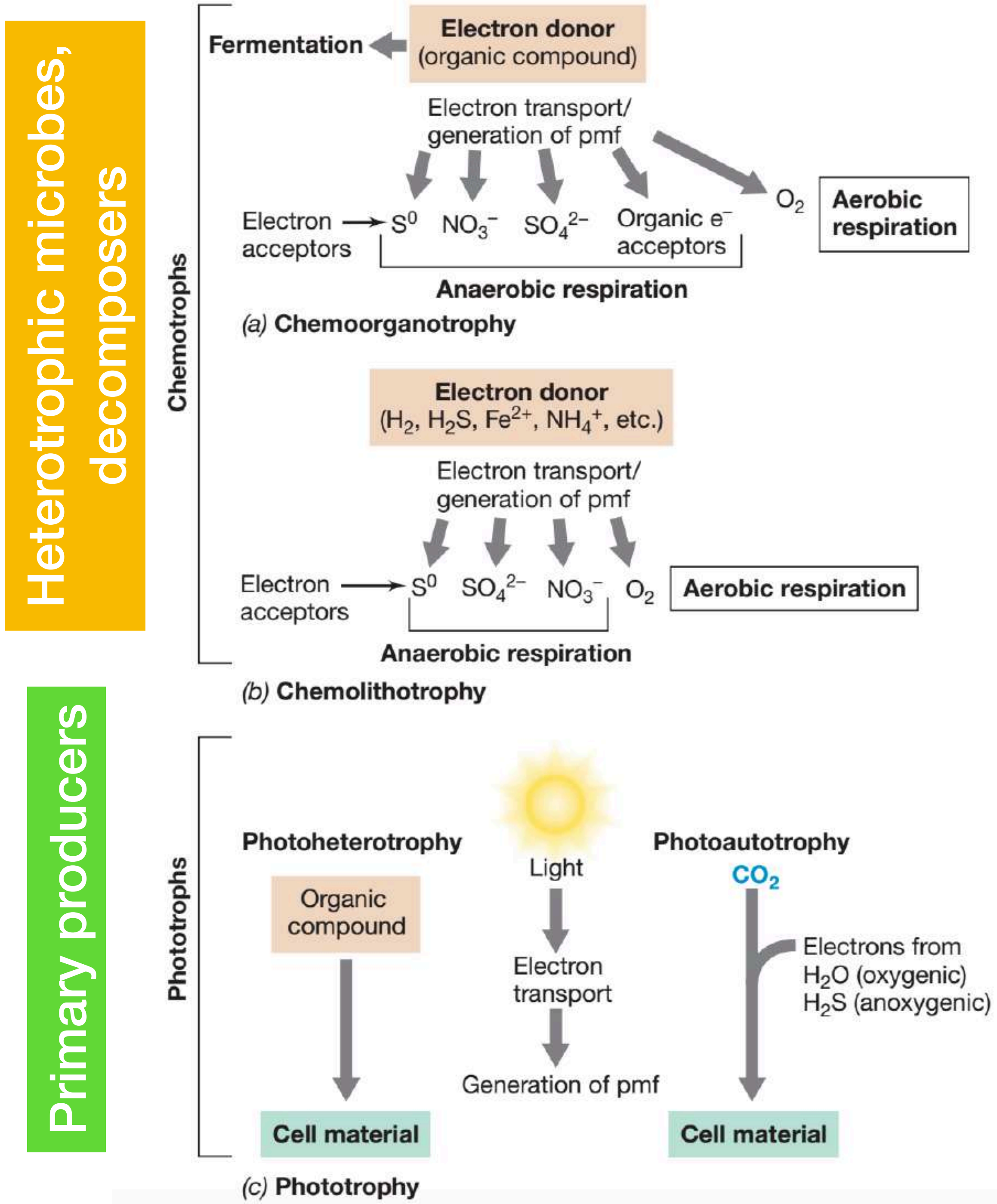


Figure 15.1 Major functional traits mapped across major phyla of *Bacteria* and *Archaea*.
The dendrogram shows relationships between microbial phyla as inferred by analysis of 16S ribosomal RNA gene sequences. Blue branches are used to denote phyla of *Bacteria* and red branches phyla of *Archaea*. Colored circles indicate phyla that contain at least one species with a functional trait indicated in the color key.

Madigan et al. 2018

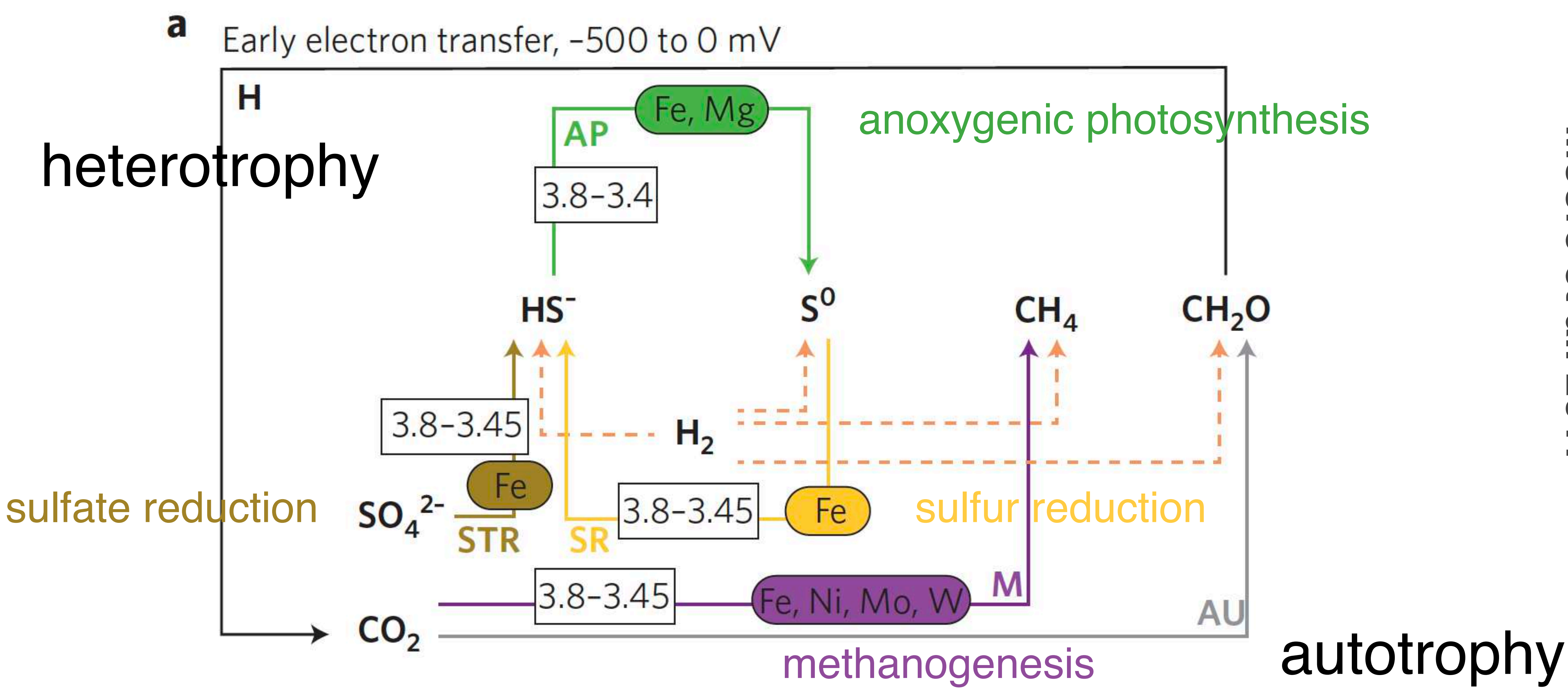
Main microbial players

- Diverse energy sources
- Using inorganic nutrients
- Using organic nutrients
- **Primary production is coupled with decomposition in every ecosystem**



Madigan et al. 2018

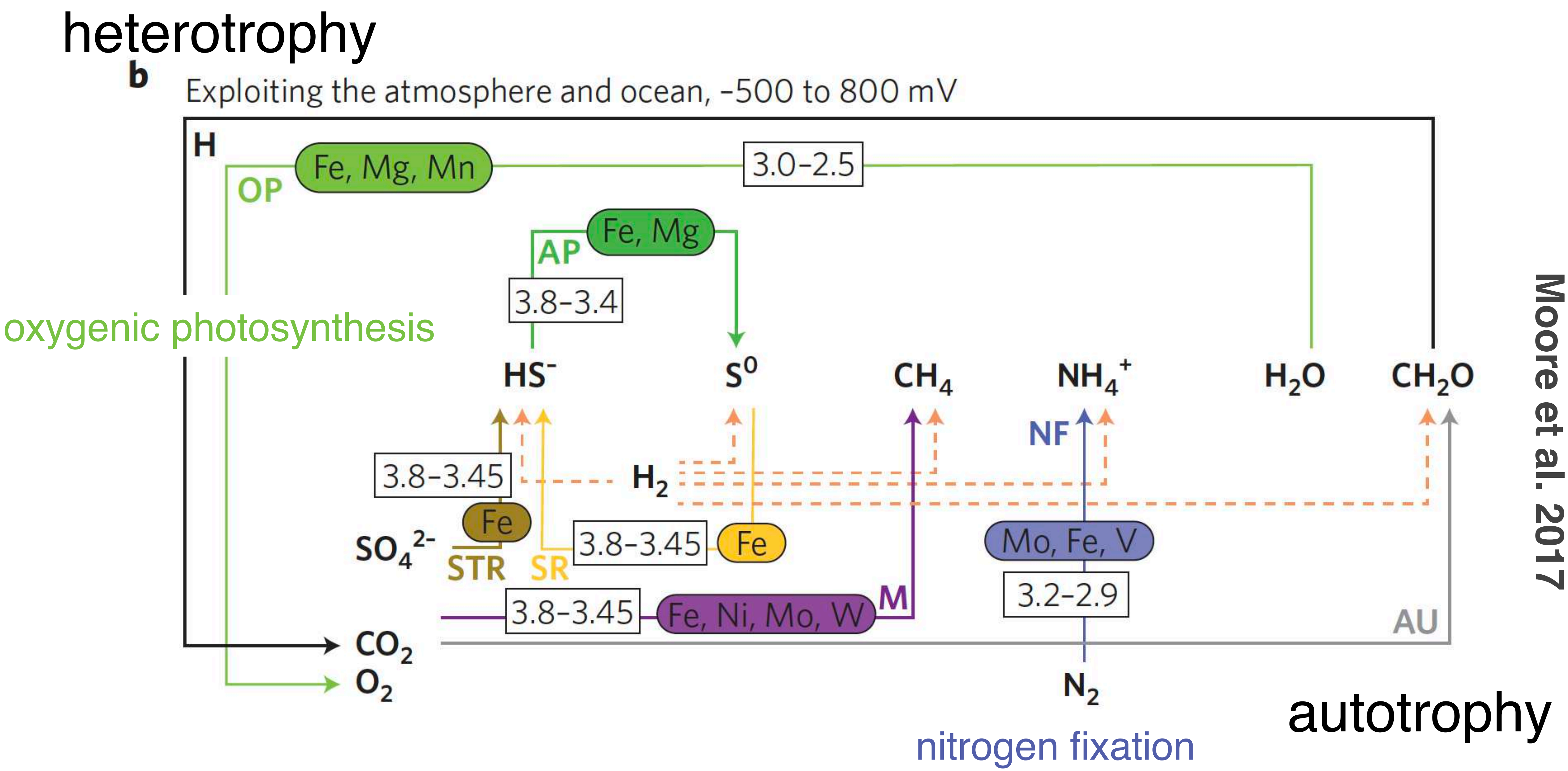
Microbial metabolic pathways during Earth history: *anoxygenic world*



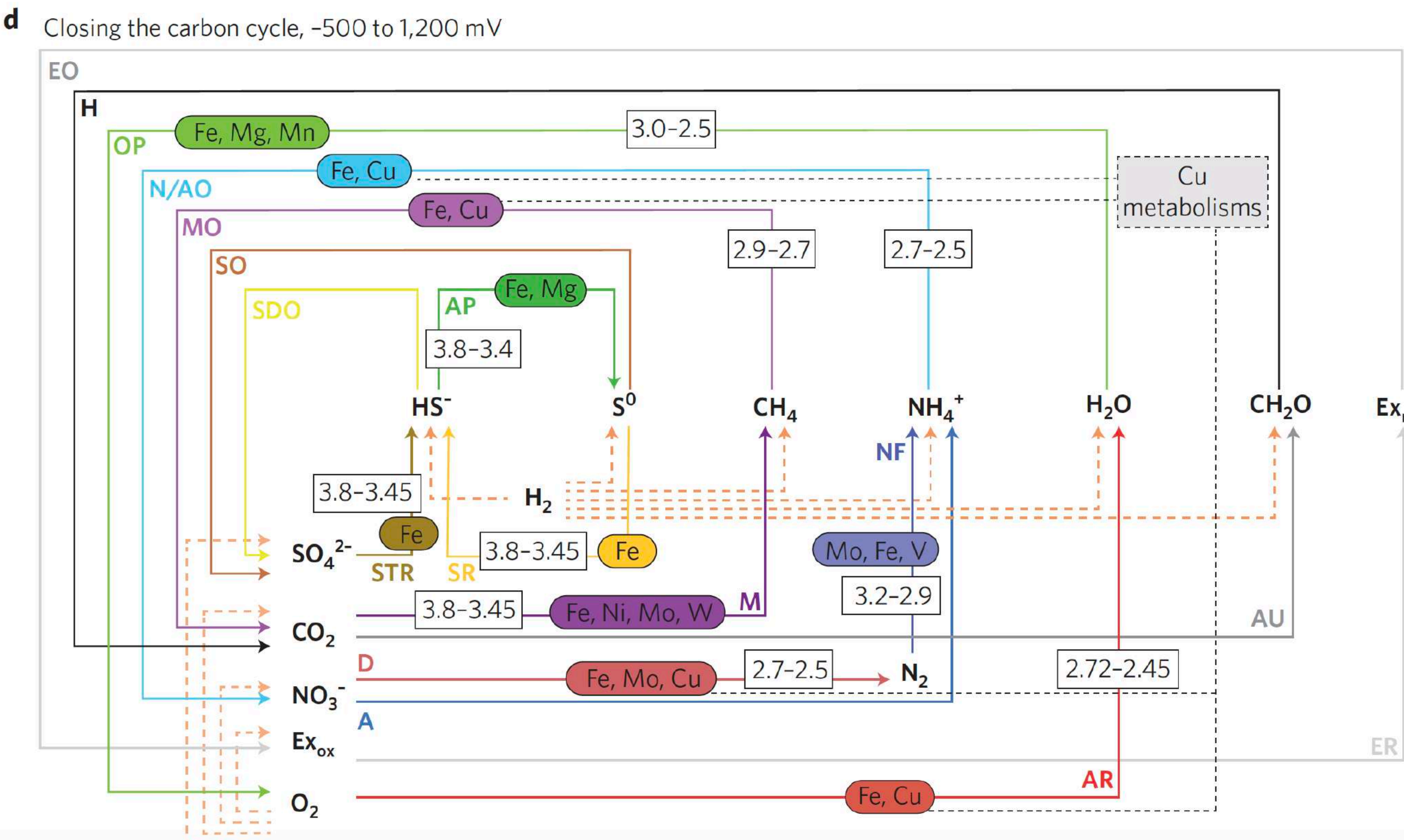
Moore et al. 2017

Molecules listed horizontally (HS–, S0, CH4, NH4+, H2O, CH2O, Exred) are reduced substrates, molecules listed vertically (SO42–, CO2, NO3–, O2, Exox) are oxidized substrates, and N2 is an intermediate and atmospheric reservoir of unreactive nitrogen

Microbial metabolic pathways during Earth history: *oxygenic world*



Present microbial metabolic pathways

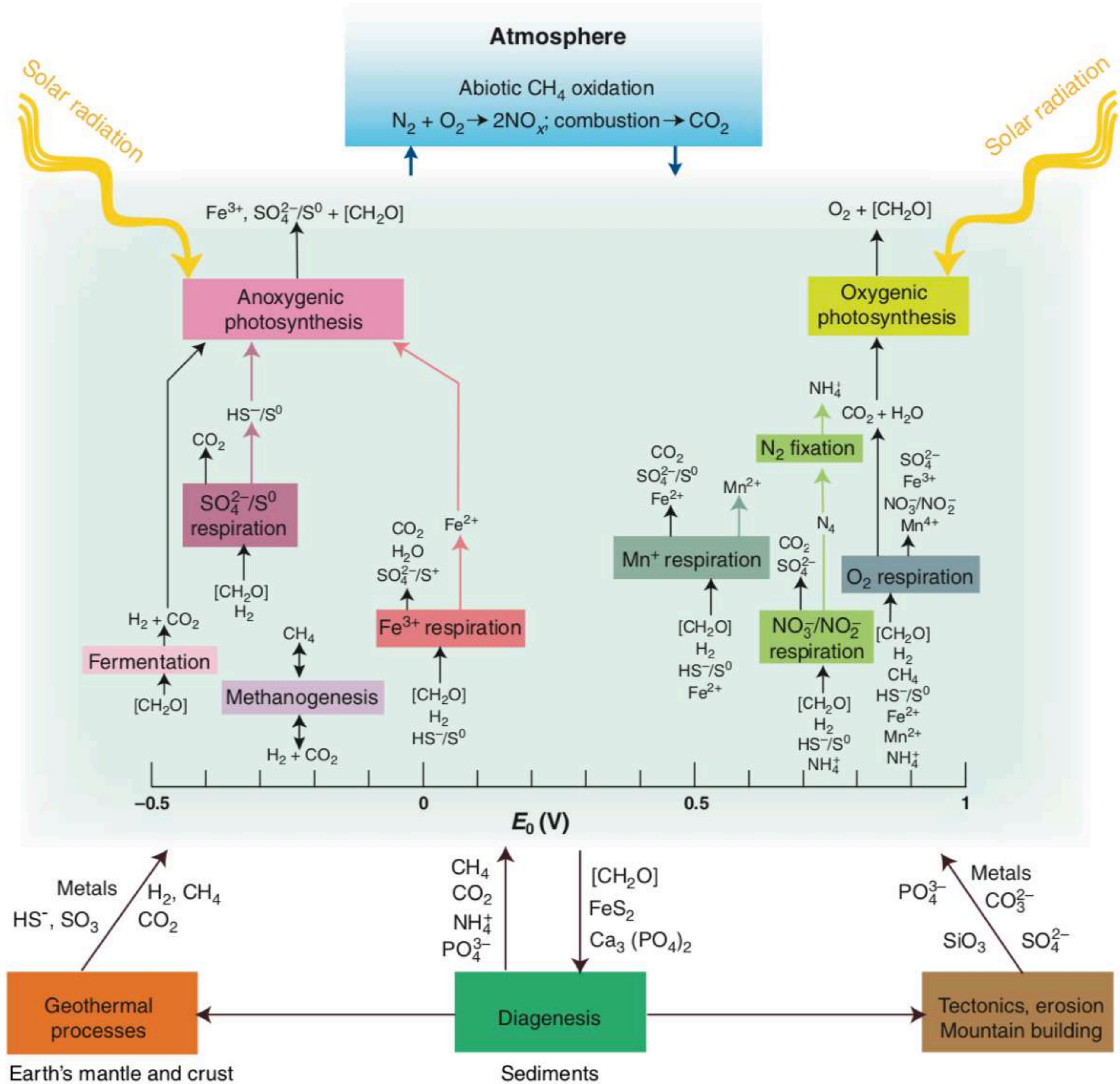


Moore et al. 2017

A, ammonification; AP, anoxygenic photosynthesis; AR, aerobic respiration; AU, autotrophy; D, denitrification; EO, other elements oxidation; ER, other elements reduction (EO and ER include Fe and Mn oxidation and reduction); H, heterotrophy; M, methanogenesis; MO, methane oxidation/methanotrophy; N/AO, nitrification/ammonia oxidation; NF, nitrogen fixation; OP, oxygenic photosynthesis; SDO, sulfide oxidation; SO, sulfur oxidation; SR, sulfur reduction; STR, sulfate reduction

Moore et al. 2017

Microbial microscale actions structure planet-scale functioning

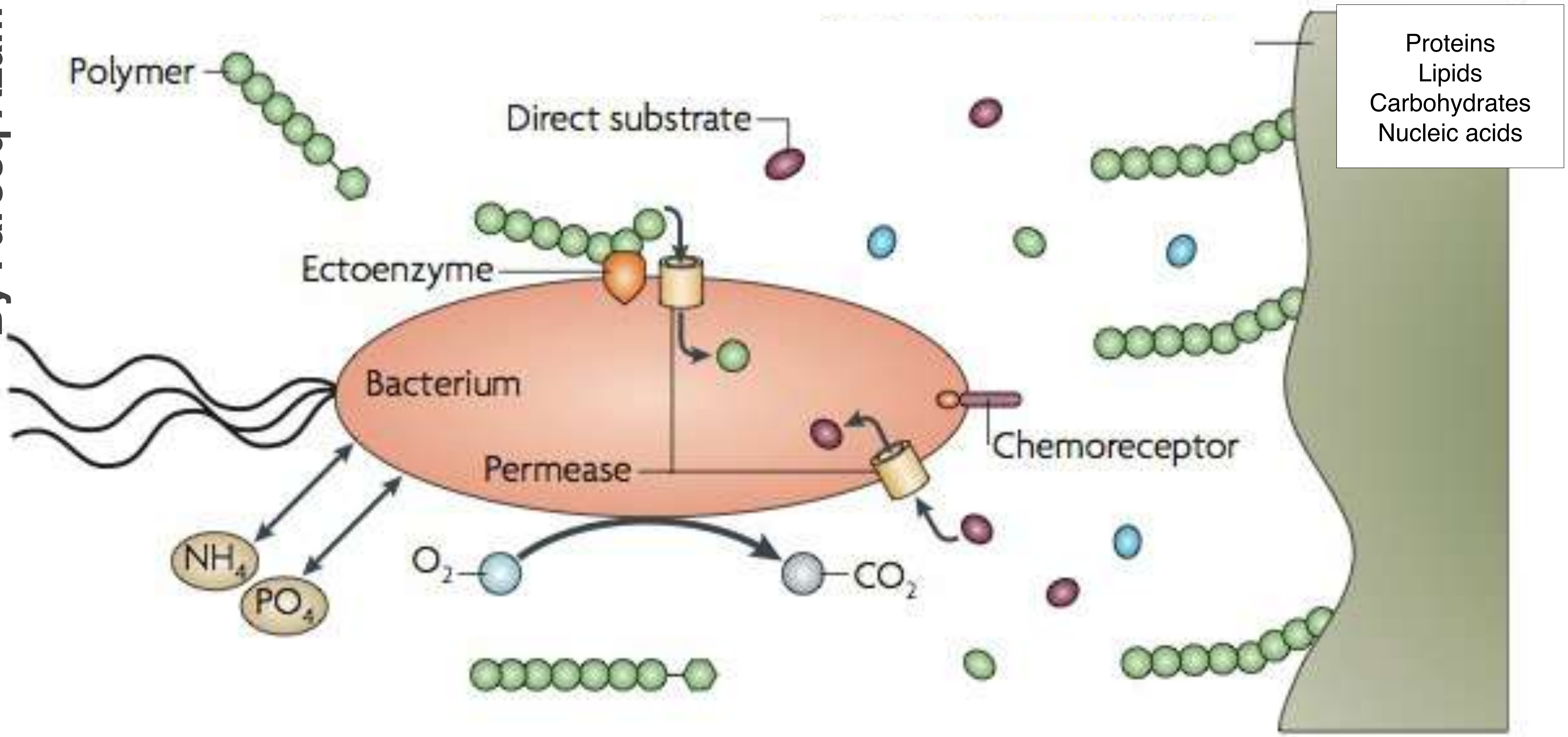


Falkowski et al., 2008

Biosphere model of energy fluxes and elemental cycles

Microbial adaptive strategies at the microscale

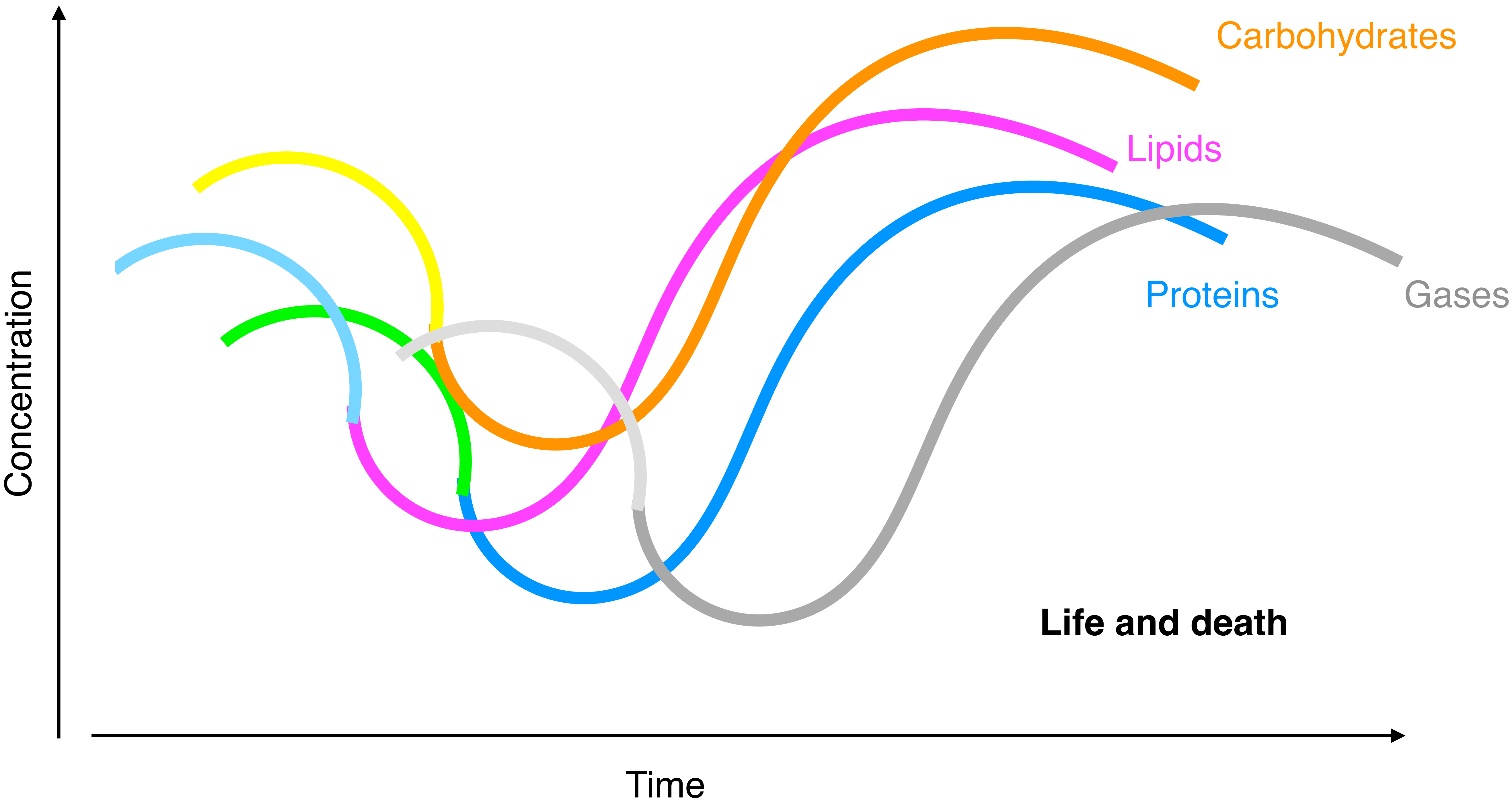
By Farooq Azam



Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

- Heterotrophic microbes, decomposers
- Interaction with the continuum of organic matter
- Significance of spatial coupling hydrolysis-uptake (permease) on the cell
- Cell surface hydrolases; 10^2-10^4 x variability in cell-specific activity
- Degree of efficiency in hydrolysis-uptake coupling

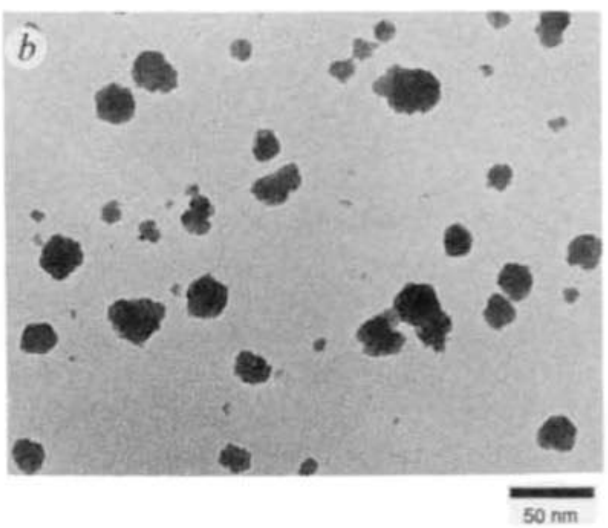
Biotransformations create chemically complex dynamics at the microscale



Physical continuum of organic matter: in “Aquasphere”

By Farooq Azam

Colloids (10^{8} mL^{1})



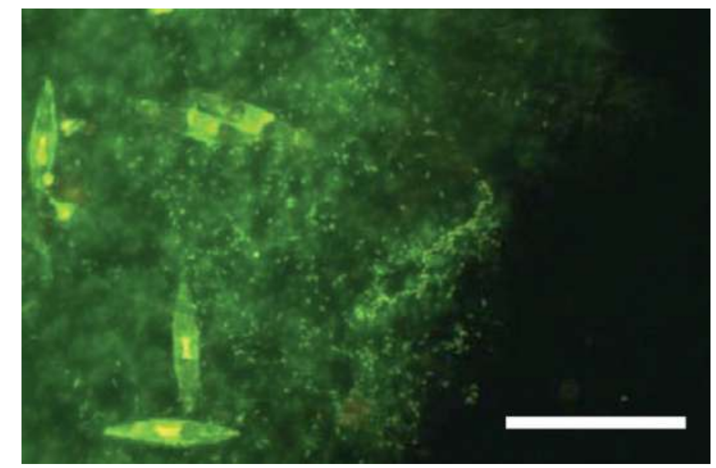
Wells & Goldberg, 1991

Koike et al., 1990

Coomassie Stained Particles (CSP)

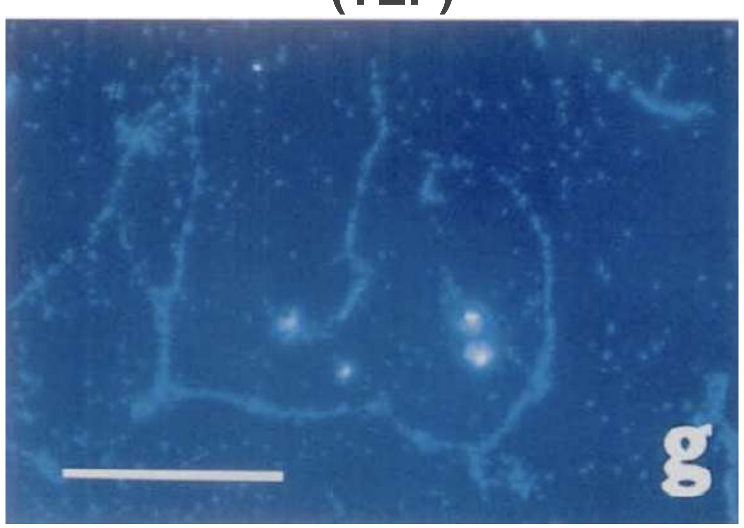
Long & Azam, 1996

Filter Fluorescing Particles (FFP)



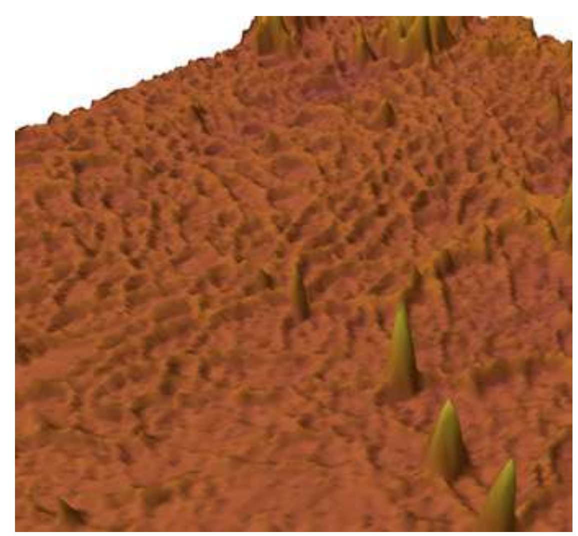
Samo, Malfatti & Azam 2008

Transparent Exopolymeric Particles (TEP)



Alledrege et al., 1993

Gel network from Adriatic Sea



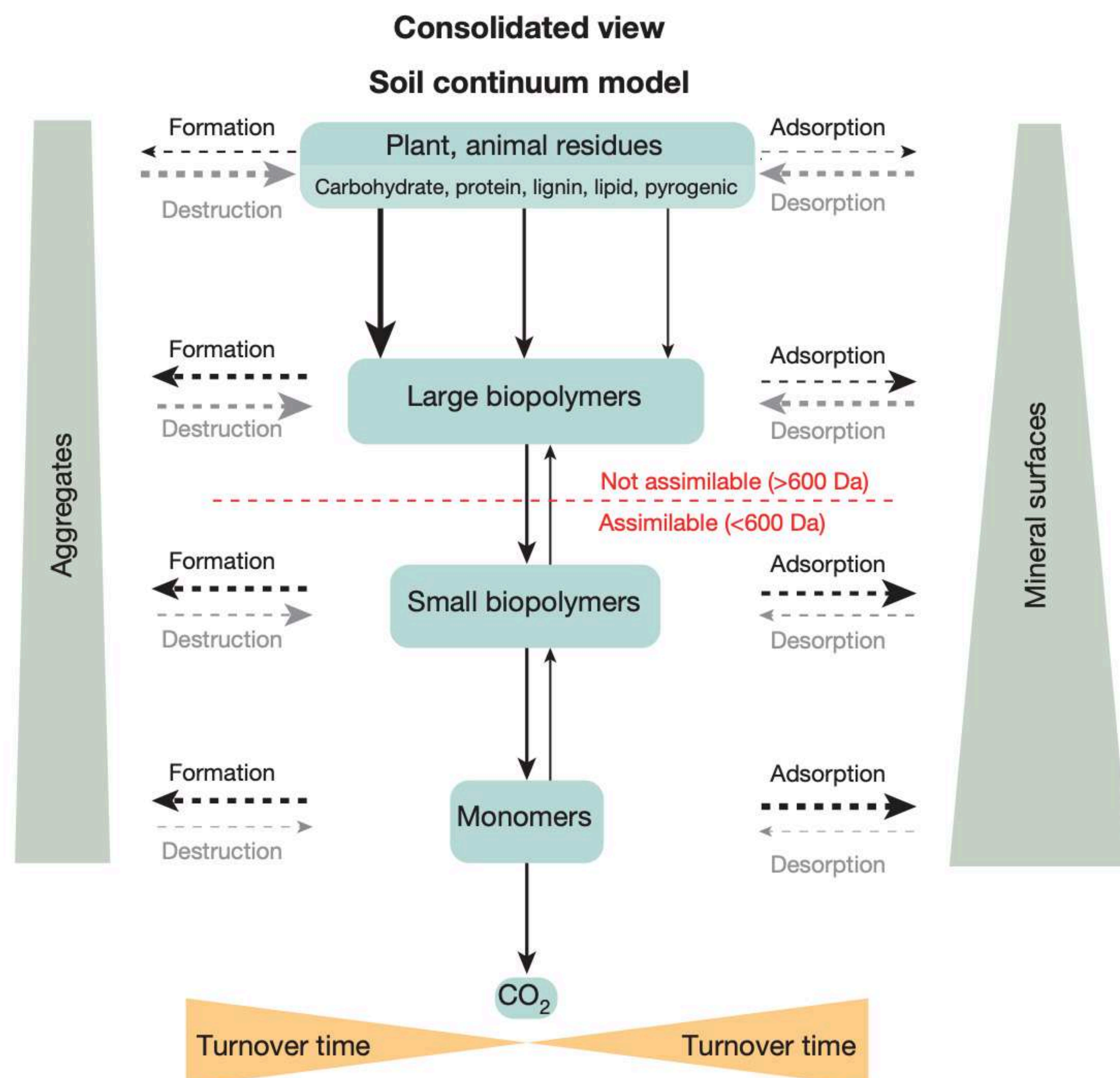
Malfatti - AFM- unpub.

Nino Caressa

- Heterogeneous and patchy size continuum
- Chemically diverse and diverse reactivity
- Labile-recalcitrant continuum (=microbial utilization)

Physical continuum of organic matter: in "Soil-Geosphere"

- At any time within a living soil, a continuum exists of many **different organic compounds at various stages of decay**, <u>moving down a thermodynamic gradient from large and energy rich compounds to smaller energy-poor compounds</u>



Lehmann & Kleber, 2015

Natural control of microbial growth

TABLE 20.1 Resources and conditions that govern microbial growth in nature

Resources
Carbon (organic, CO_2)
Nitrogen (organic, inorganic)
Other macronutrients (S, P, K, Mg)
Micronutrients (Fe, Mn, Co, Cu, Zn, Mn, Ni)
O_2 and other electron acceptors (NO_3^-, SO_4^{2-}, Fe^{3+})
Inorganic electron donors (H_2, H_2S, Fe^{2+}, NH_4^+, NO_2^-)
Conditions
Temperature: cold → warm → hot
Water potential: dry → moist → wet
pH: 0 → 7 → 14
O_2: oxic → microoxic → anoxic
Light: bright light → dim light → dark
Osmotic conditions: freshwater → marine → hypersaline

Madigan et al. 2018

Microbial metabolic pathways shaping Earth ecosystem

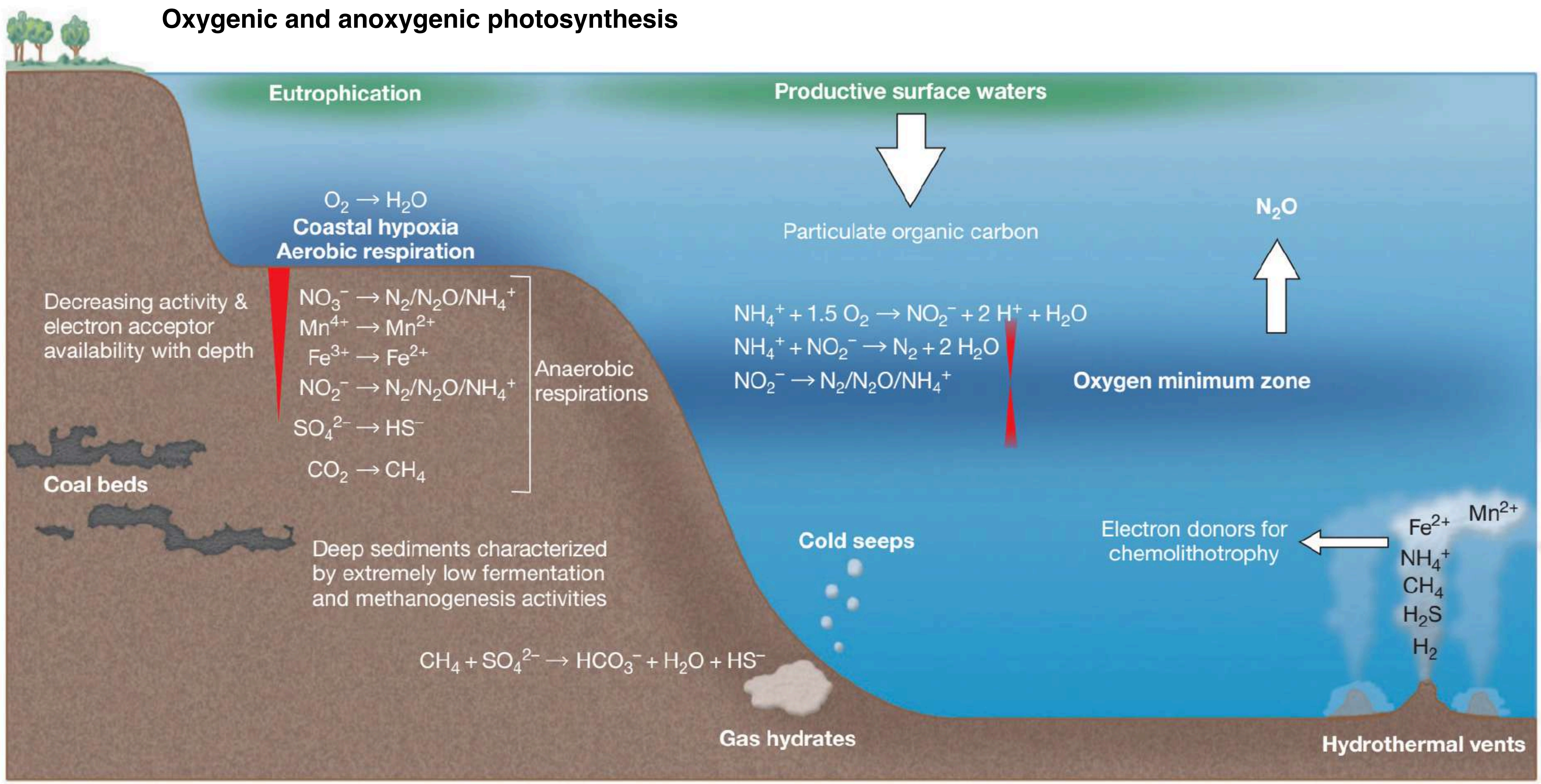


Figure 20.20 Diversity of marine systems and associated microbial metabolic processes. Decreasing electron acceptor availability with depth into the sediment or with increasing distance into an oxygen minimum zone is indicated by red wedges. Sulfate becomes limiting only at greater depths in marine sediments. The indicated metabolic diversity is covered in Chapter 14.

Madigan et al. 2018

Community-coupling in the ocean

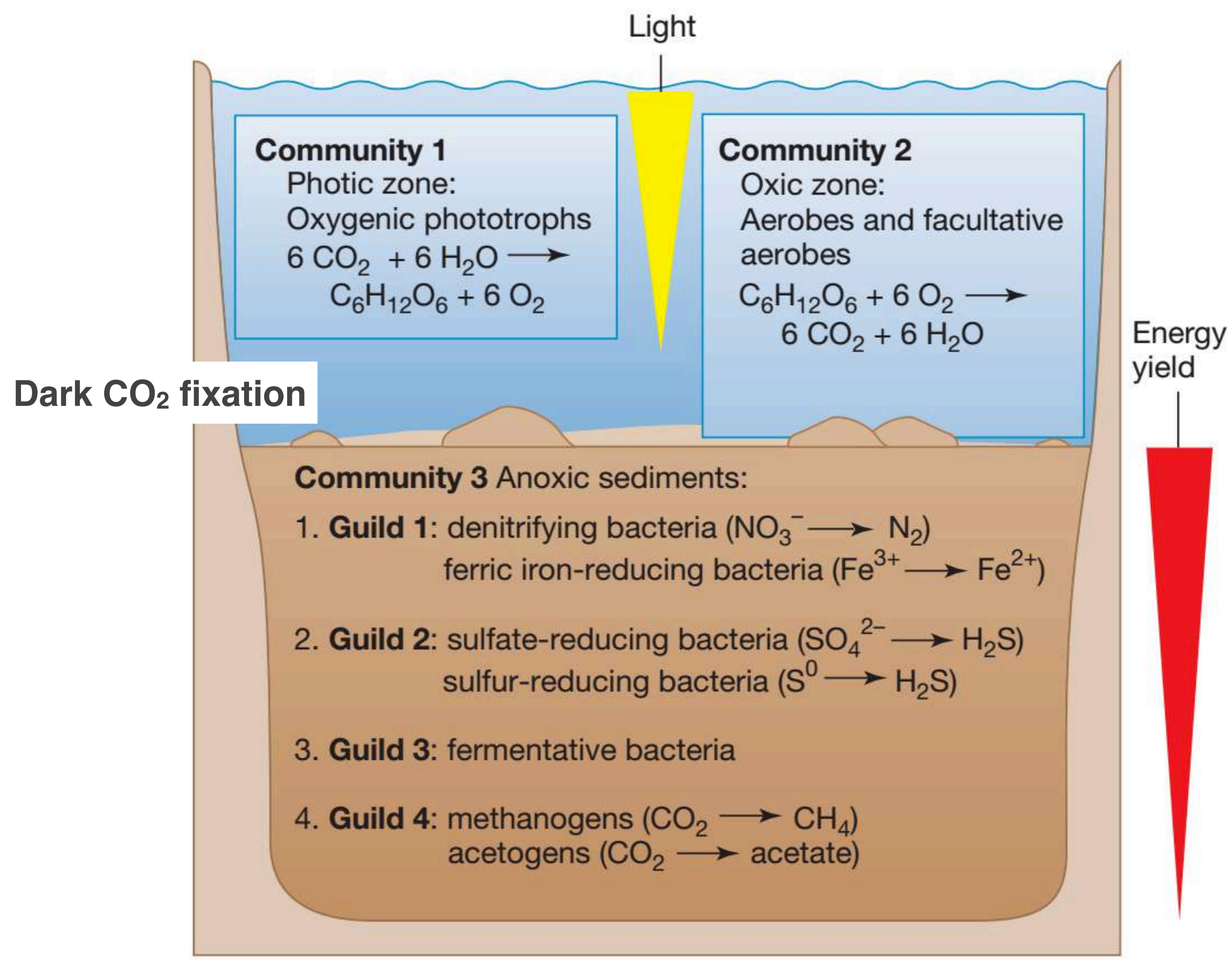


Figure 20.2 Populations, guilds, and communities. Microbial communities consist of populations of cells of different species. A freshwater lake ecosystem would likely have the communities shown here. The reduction of NO_3^-, Fe^{3+}, SO_4^{2-}, S^0, and CO_2 are examples of anaerobic respirations. The region of greatest activity for each of the different respiratory processes would differ with depth in the sediment. As more energetically favorable electron acceptors are depleted by microbial activity near the surface, less favorable reactions occur deeper in the sediment.

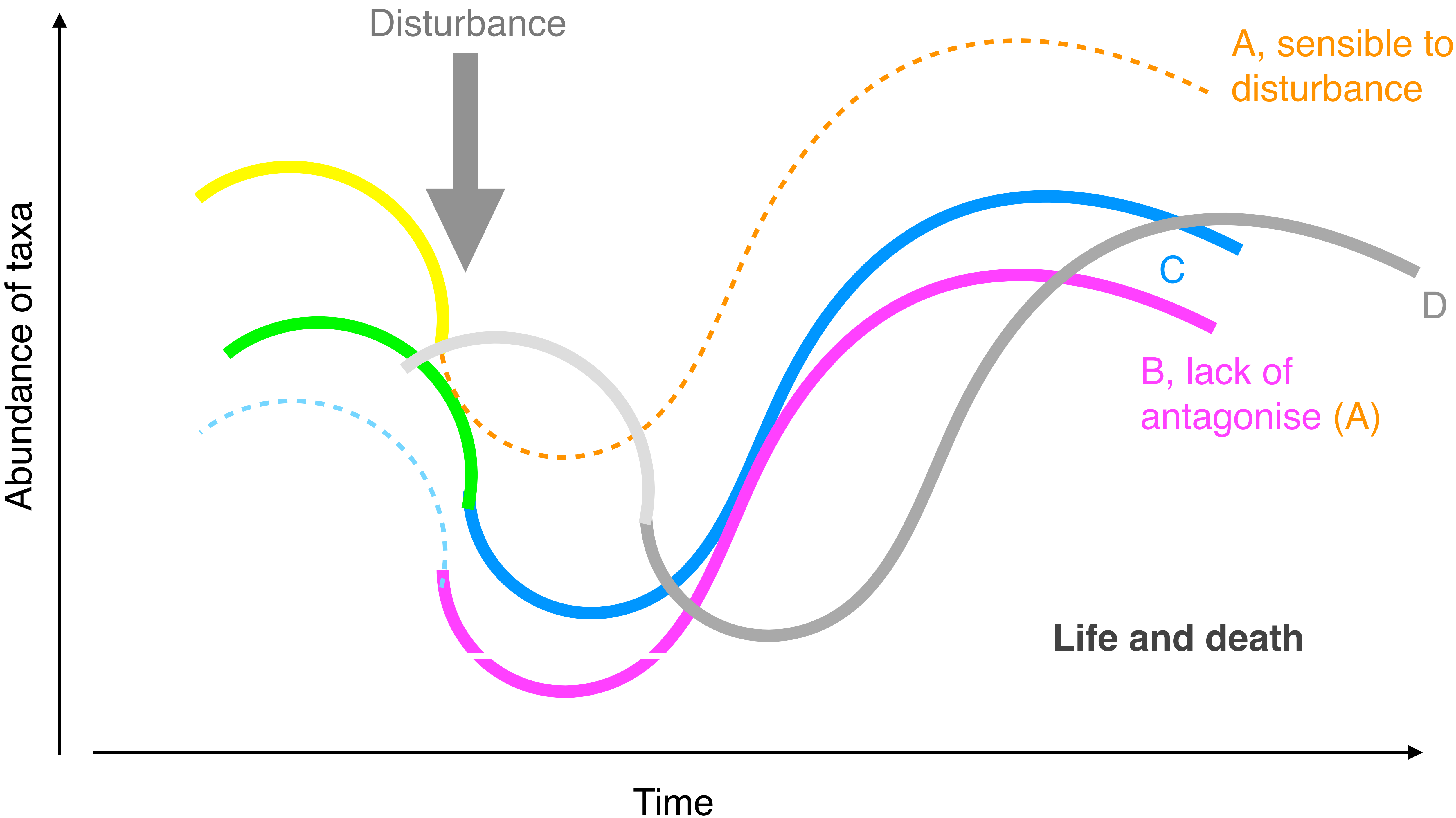
Upper ocean primary production and deep ocean chemo-lithoauthotrophy (aka: dark CO_2 fixation) are fueling organic matter degradation communities

Madigan et al. 2018

Community Structure and Succession

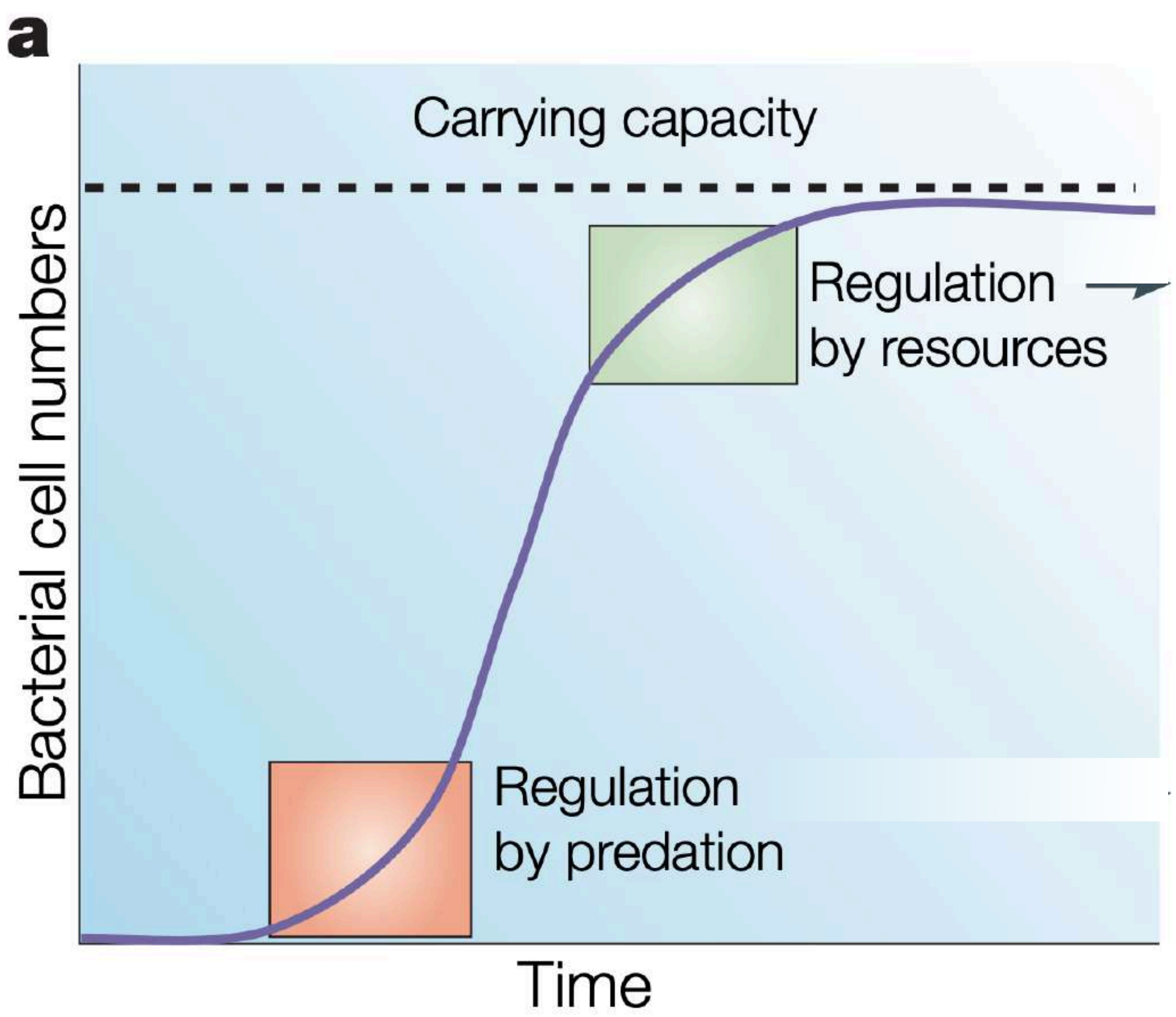
- **Community structure** refers to the **composition** (which microbes are present), **abundance**, and **diversity** of microbial species in a given environment
- **Succession** is the natural **progression or change** in microbial community structure over time, often **following a disturbance** (*e.g.*, oil spill, seasonal shift, land use change)
- Microbial succession can occur rapidly and is driven by resource availability, environmental conditions, and species interactions
- **Example:** In compost, early colonizers (fast-growing bacteria) are replaced by thermophiles as temperature rises during decomposition

Microbial succession



Carrying capacity

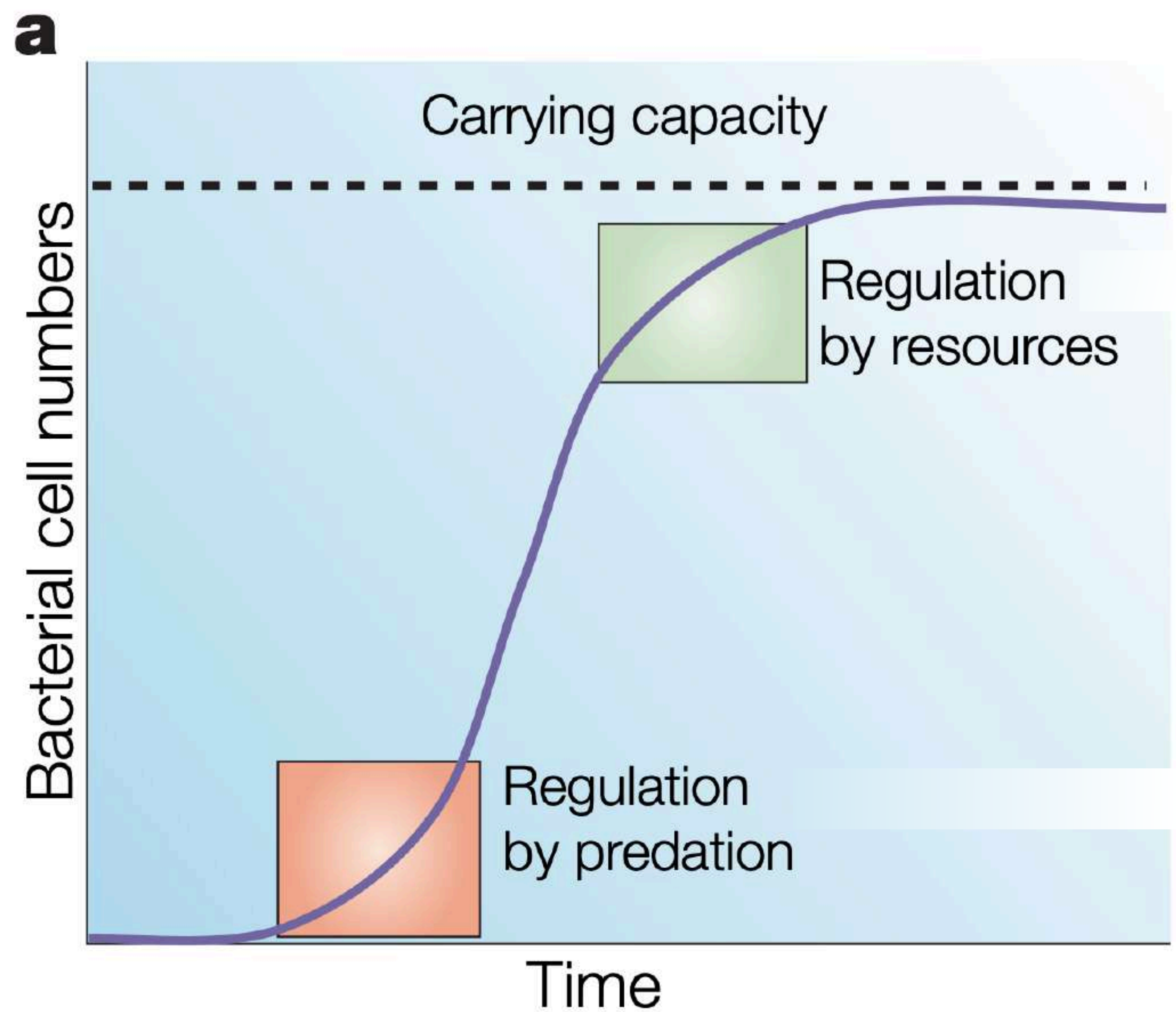
- Carrying capacity, the average population density (cell numbers) or population size of a species below which its numbers tend to increase and above which its numbers tend to decrease because of shortages of resources
- The carrying capacity is different for each species in a habitat because of that species' particular food, shelter, and social requirements
- The **carrying capacity** of a biological species in a particular habitat refers to the maximum number of individuals (of that species) that the environment can carry and sustain, considering its geography or physical features



Pernthaler, 2005

Top-down control

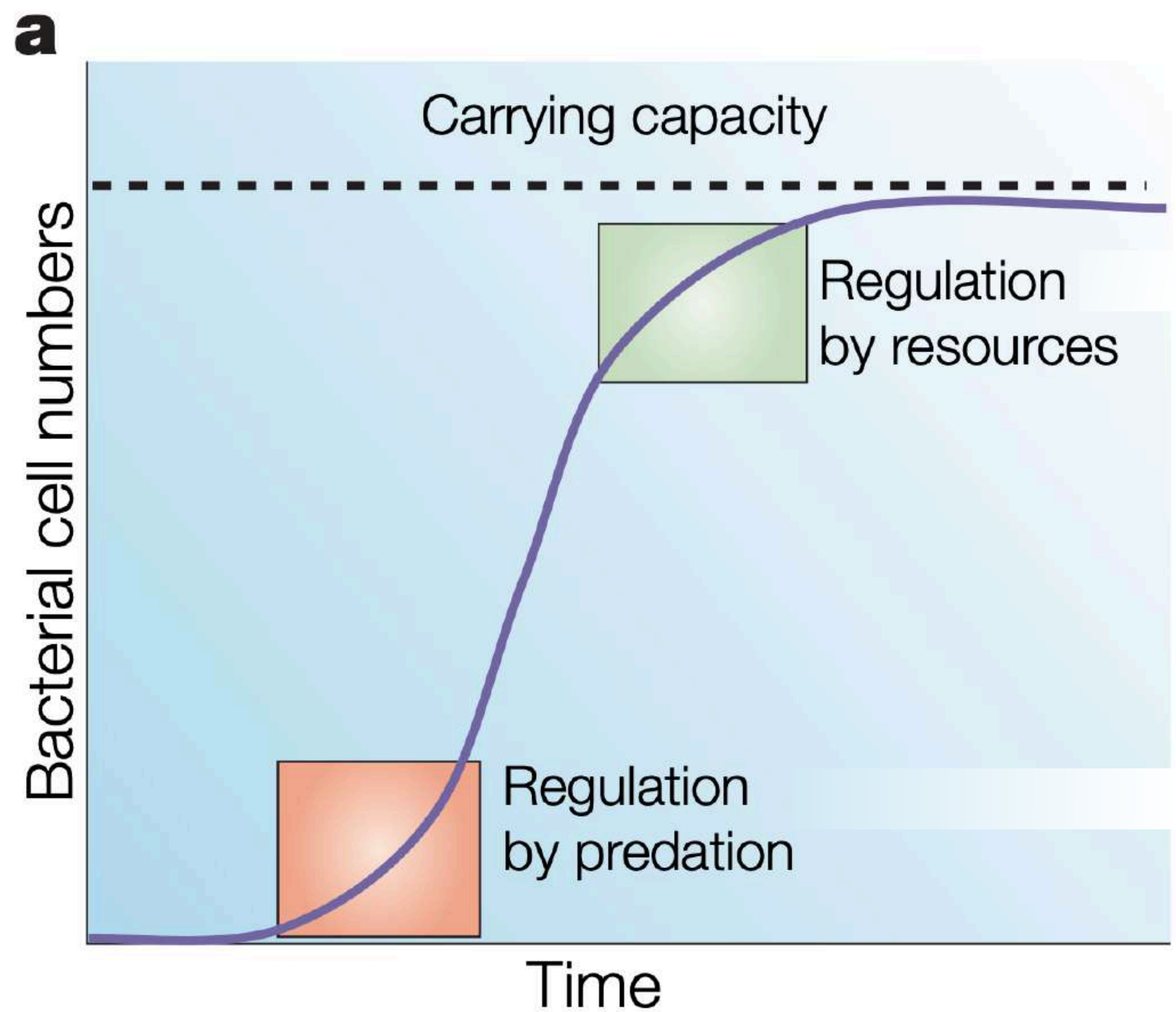
- **Top-down**: Ecological scenario in which the abundance or biomass of organisms is mainly determined **by mortality owing to predation**
- **Viruses and protists (less studied antagonistic reactions and bacterial predators)** can directly impact bacterial communities either through their **host specific lysis and size selective grazing respectively** or indirectly **through the alteration of organic pools by mortality processes**



Pernthaler, 2005

Bottom-up control

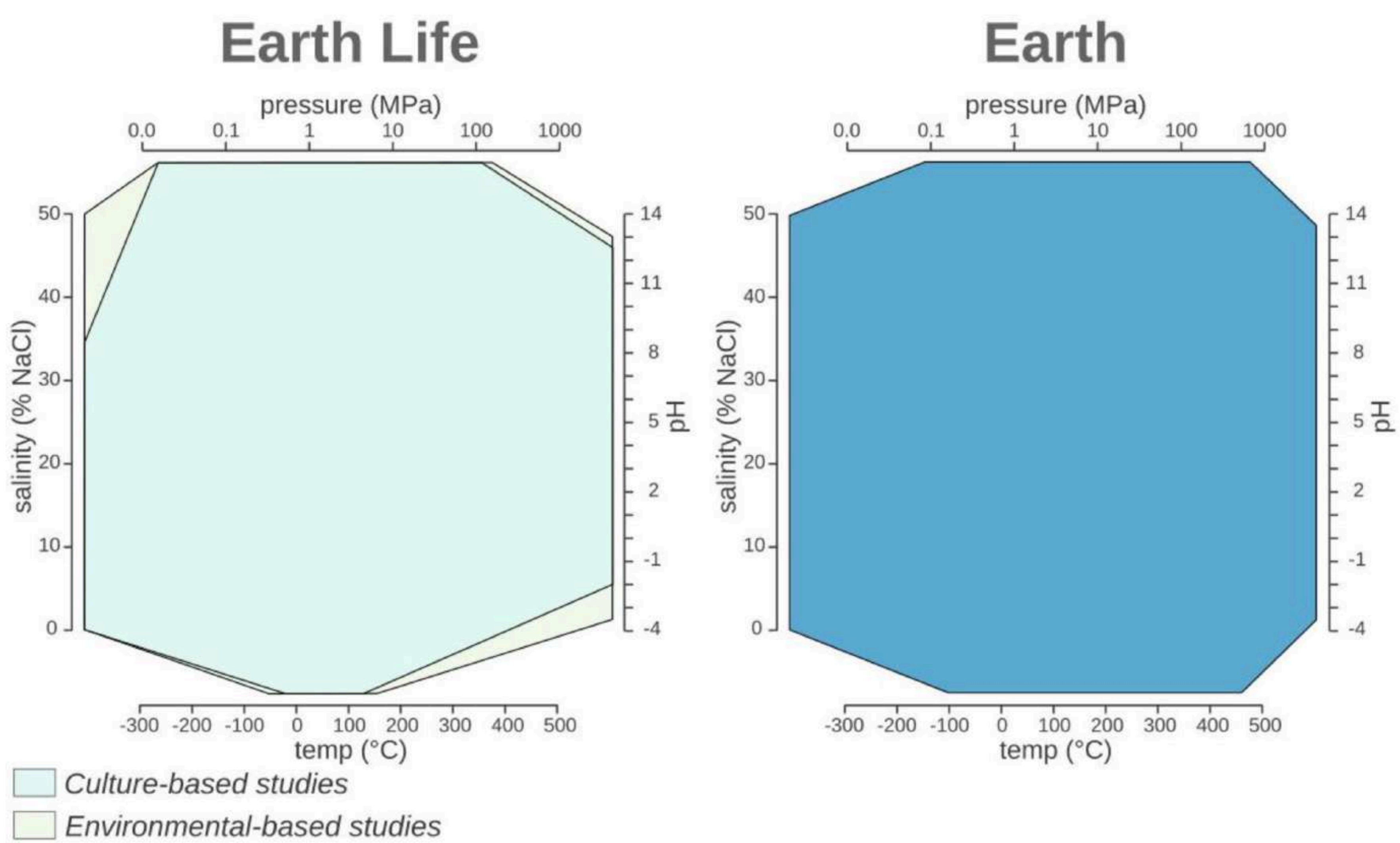
- **Bottom-up**: Ecological scenario in which the abundance or biomass of organisms is mainly determined **by a lack of resources and mortality owing to starvation**
- Bottom-up (**nutrients, organic matter** and also **energy, salinity, pH**) influence the growth and the physiological state of the microbial community



Pernthaler, 2005

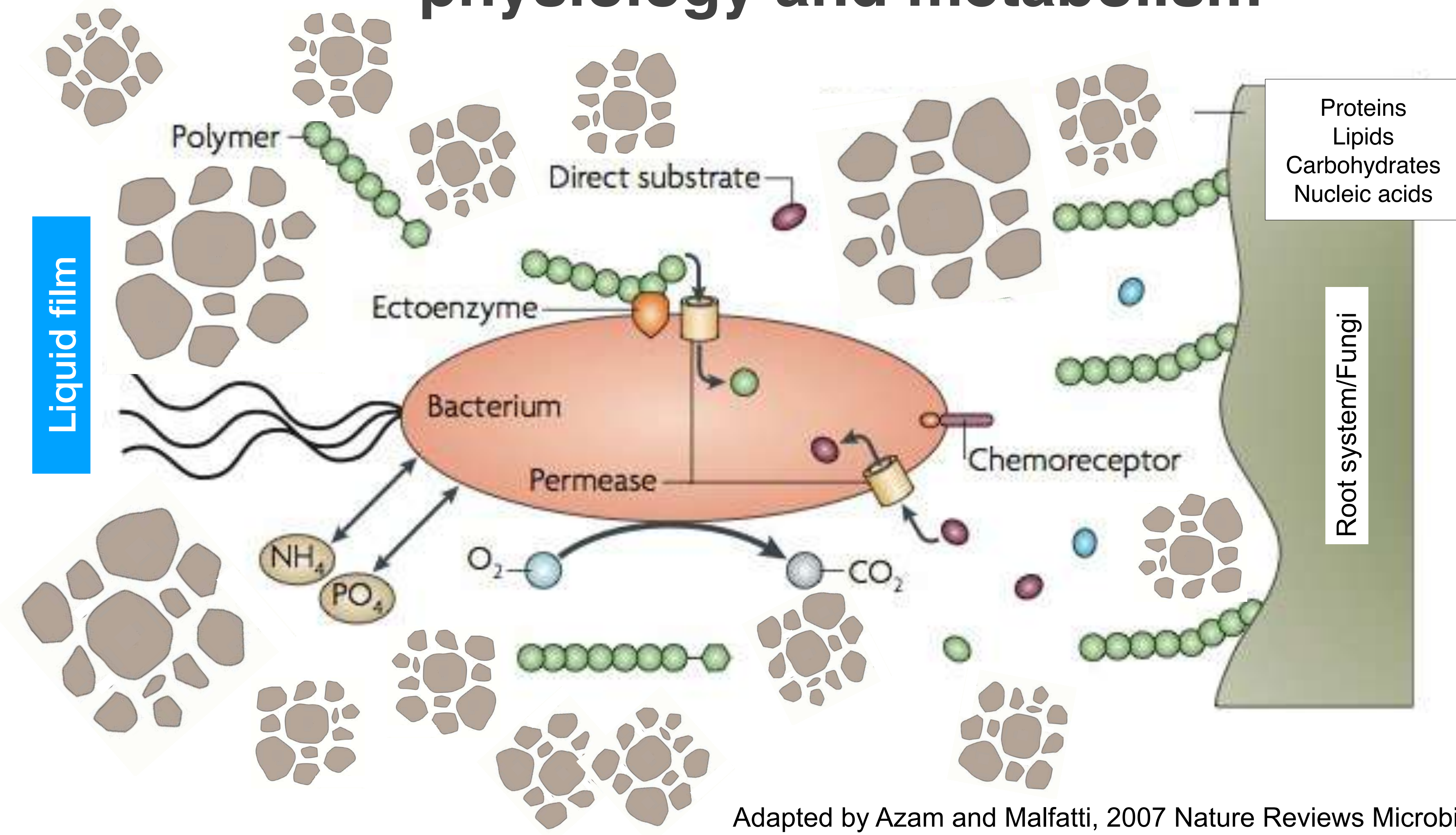
Microbial growth in the environment

- **Bottom-up (nutrient supply) and Top-down (protistan grazing, viral lysis, and antagonistic reaction and bacterial predators)** processes are known to influence and **control microbial community composition and diversity in time and space**



Merino et al. 2019

Microscale bottom-up control on microbial growth, physiology and metabolism

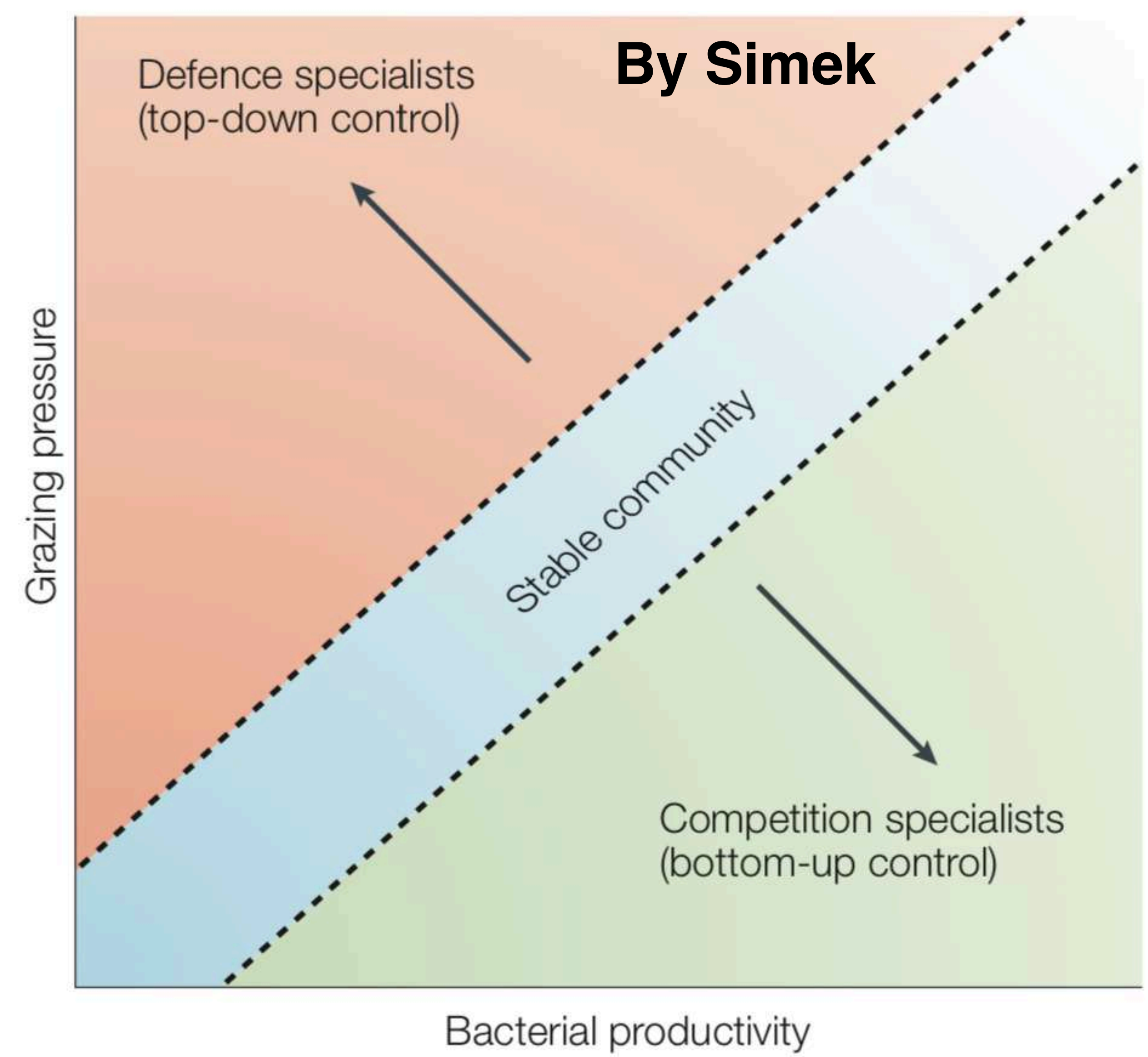


Adapted by Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

- **Cell machinery respond to stress**
- **Changing behavior (motility), enter into dormancy**
- **Changing expression for limited nutrient**

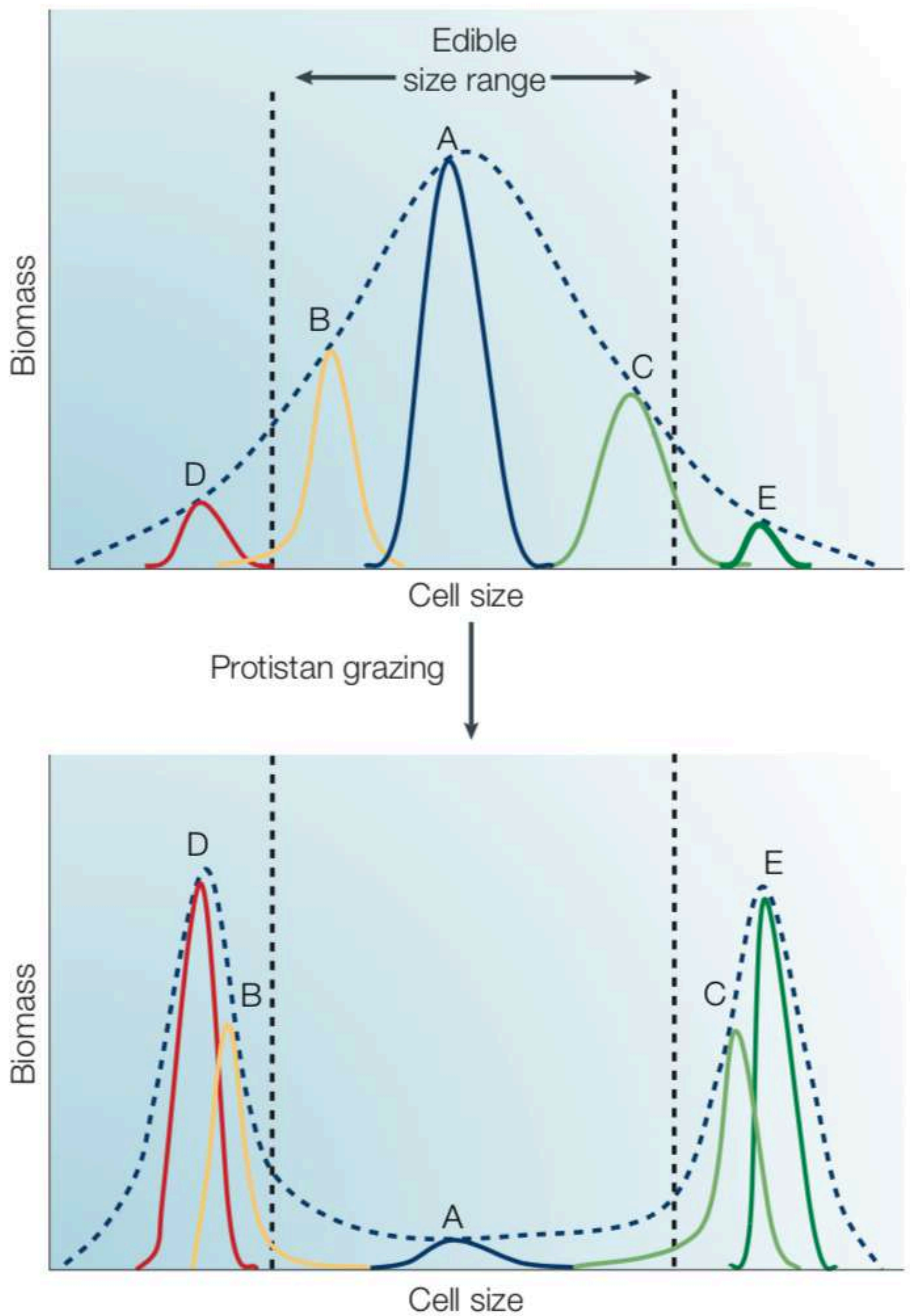
Stable microbial community

- **Stable communities** of microbial species will exist at **different levels of microbial productivity** if there is an approximate **balance** between **bacterial production and protistan bacterivory**
- **Changes** in species composition of the microbial assemblage are triggered by rapid shifts from ‘top-down’ to ‘bottom-up’ control
- Depending on the direction of such shifts, bacterial species are **favoured that are able either to minimize predation losses (‘defence specialists’) or to respond most rapidly to favourable changes in growth conditions (‘competition specialists’)**



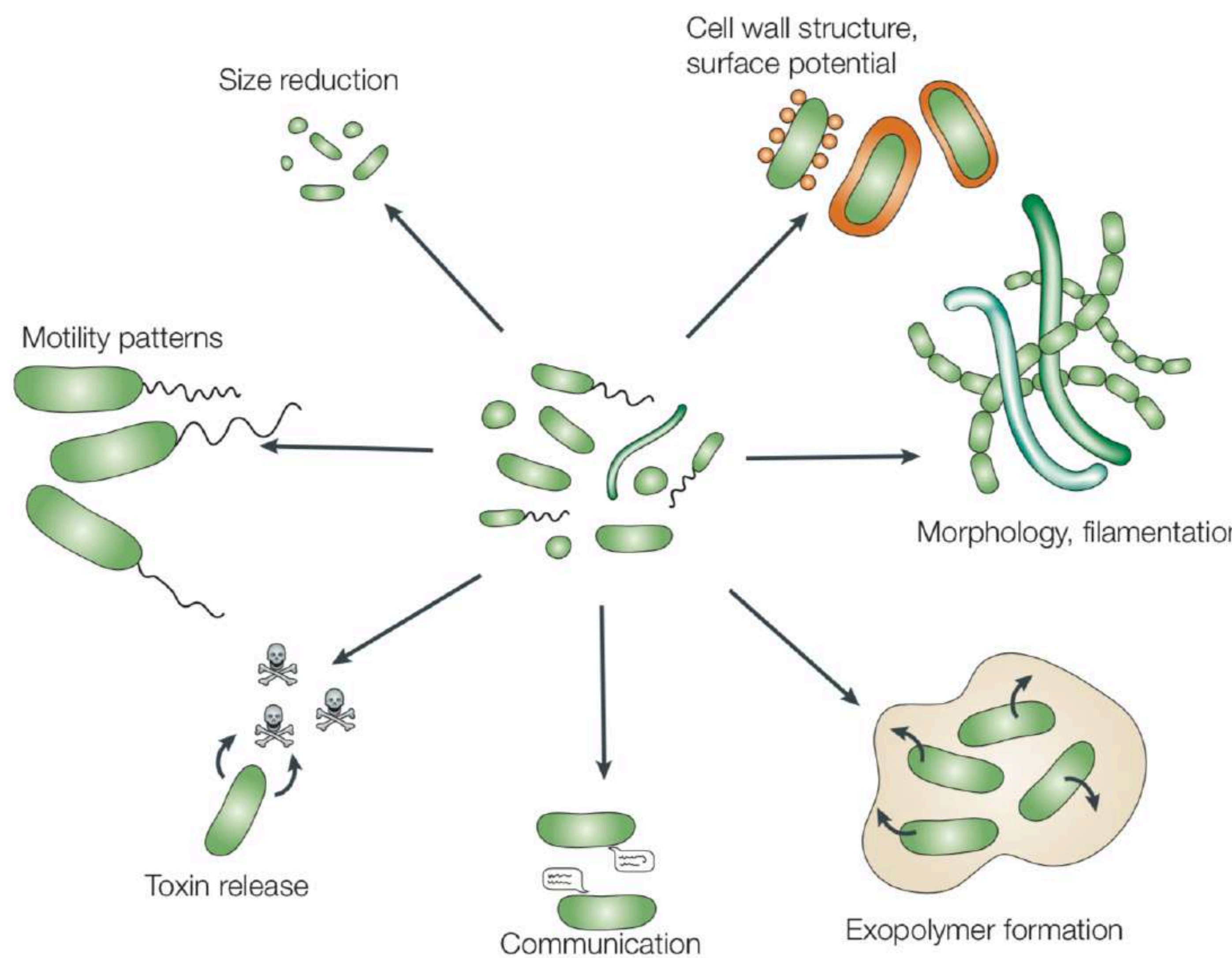
Pernthaler, 2005

Microbial strategies to resist to predators



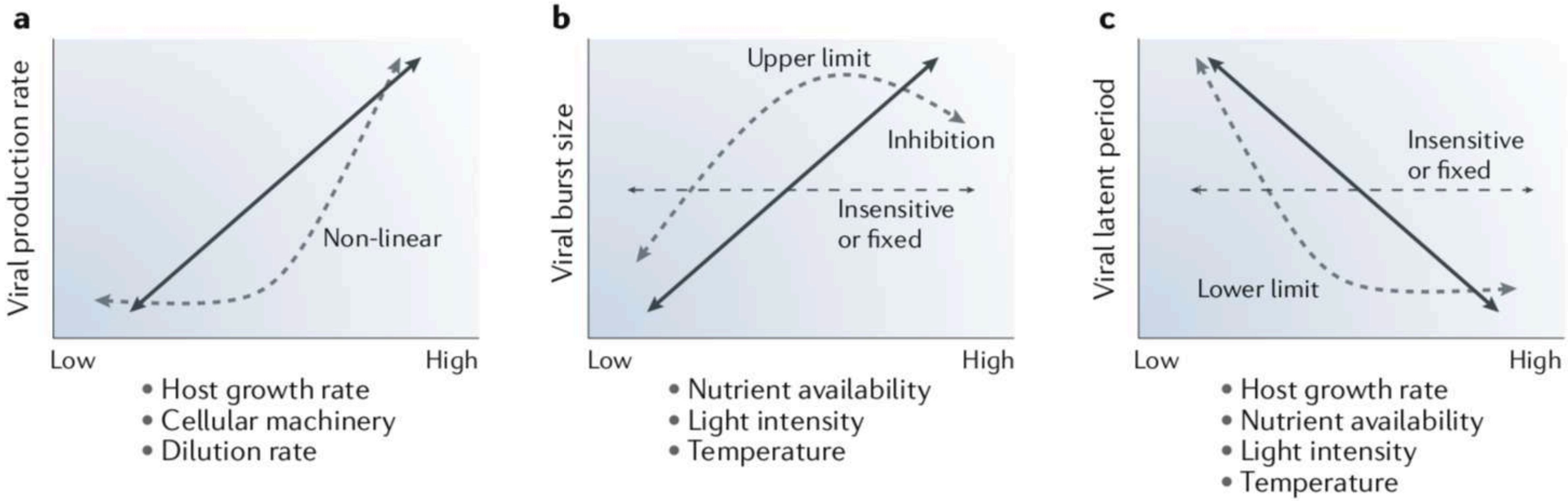
The effect of predation on microbial community structure effect on cell size

Cell surface and cell shape can influence size-dependent grazing



Pernthaler, 2005

Viral dynamics with host and nutrient status

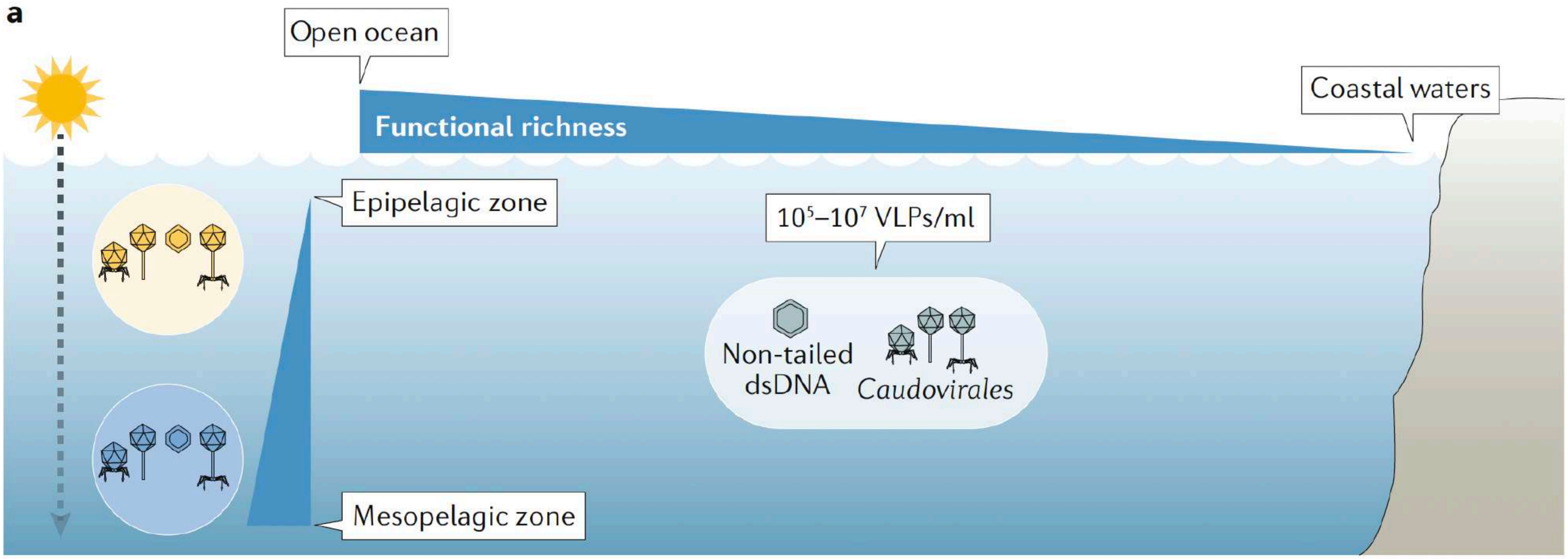


The **host growth rate and cellular machinery** (that is, ribosomes and enzymes) can be **manipulated by environmental variables** (for example, temperature, light intensity or nutrient availability)

Nutrient availability has the potential to alter viral production directly through limitation of substrates needed to build progeny virions or indirectly through the host growth rate, which in turn affects production yields or rates, respectively

Zimmerman et al., 2020

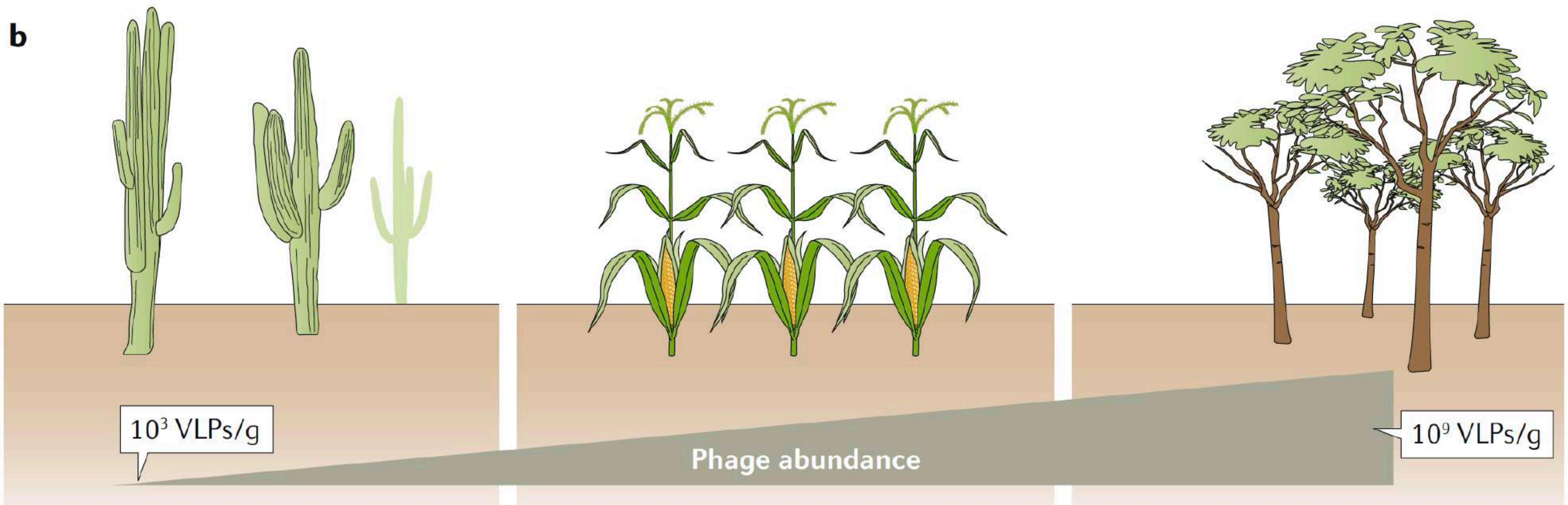
Phages in the marine environment



Dion et al., 2020

- Phages are extremely abundant, with a virus- to-bacteria ratio often ranging from 1:1 to 100:1
- Quantitative transmission electron microscopy of marine samples indicated that non- tailed phages are much more represented than tailed phages, which was also confirmed by metagenomic data
- Phages from the mesopelagic zone were distinct from phages isolated from the epipelagic zone in terms of gene content, life history traits and temporal persistence
- Functional richness was found to decrease from deep to surface water and with distance from the shore for surface water only

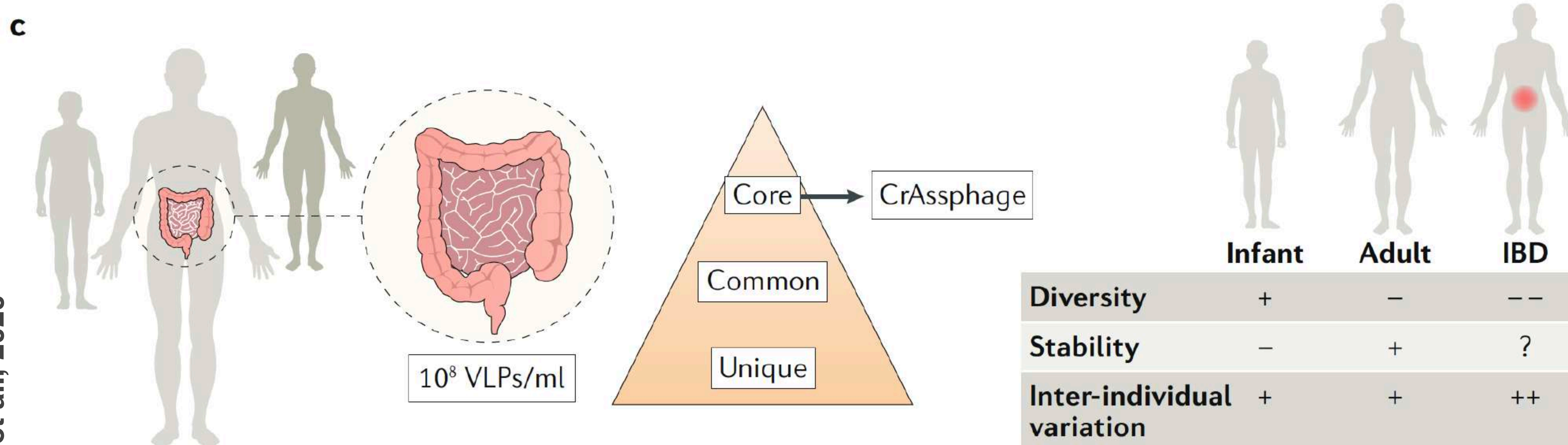
Phages in the soil environment



Dion et al., 2020

- Phage abundance in the soil is highly variable and correlates with biome type (for example, desert, agricultural or forest soils), pH and bacterial abundance
- Viral abundance is the lowest in hot deserts, intermediate in agricultural soils and the highest in forest and wetland soils
- Viral abundance also positively correlates with bacterial abundance in the soil and negatively correlates with pH, with phage counts decreasing at higher pH

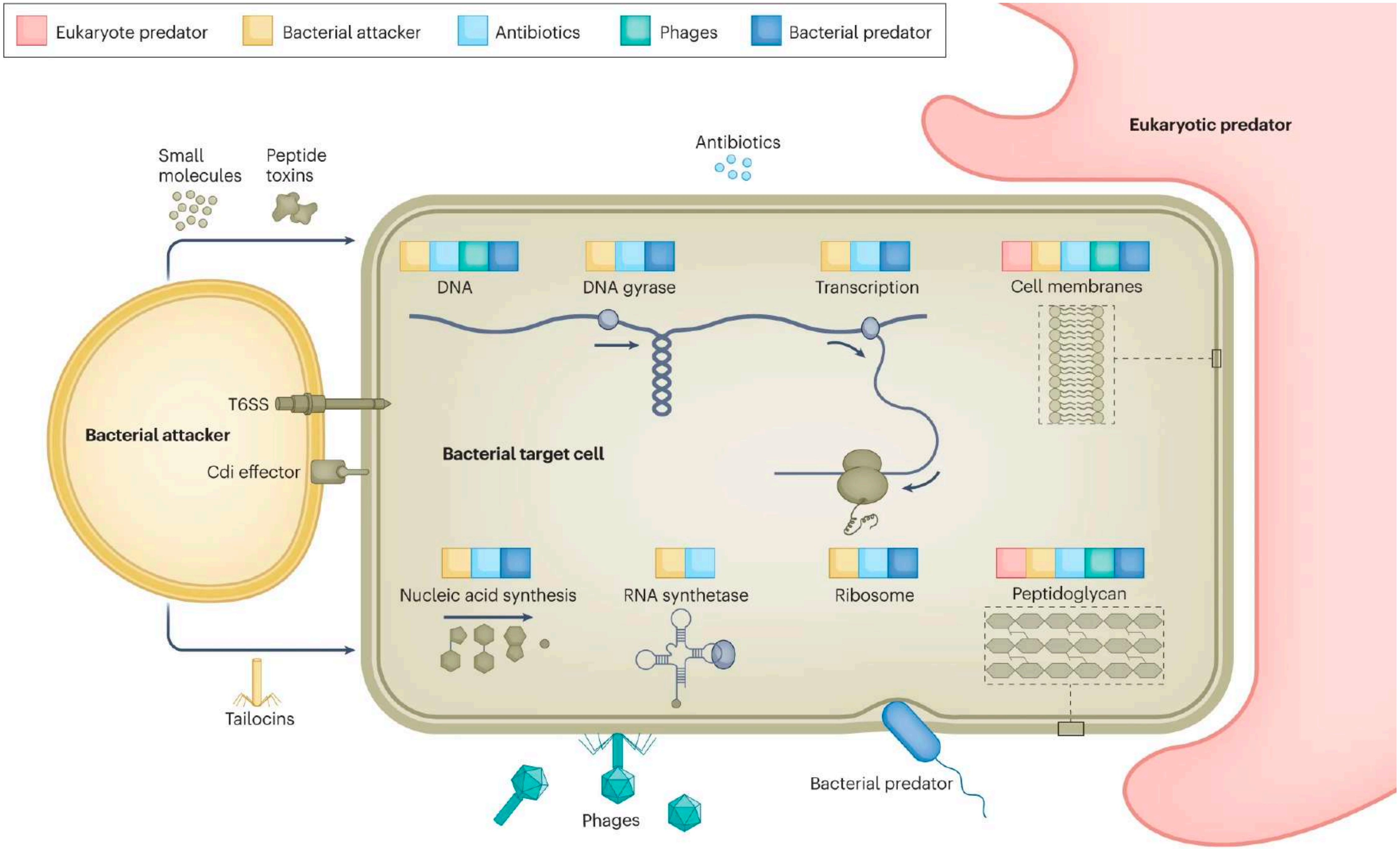
The human gut phage community



Dion et al., 2020

- The phage community in the human gut is mainly composed of members of the Caudovirales and Microviridae, and the majority of these phages remain unclassified
- Phage composition is unique to individuals, with global metagenomic analyses indicating that some phages are globally distributed
- The gut phage community is also stable over time, but rapid changes are observed in early life
- Changes in the diversity and composition of the human virome were also reported to be related to the gut health status, particularly in the case of inflammatory bowel disease (IBD)
- A set of widespread phages exists, named the core phage community , which includes crAssphage, likely the most prevalent human gut phage

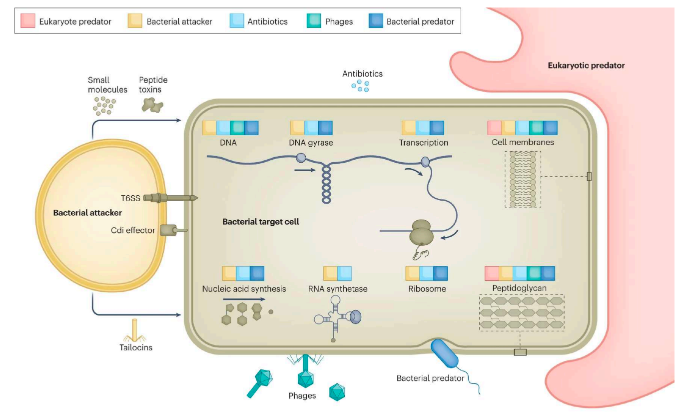
Microbes under attack



Smith et al. 2023

Most attacks target core cellular processes and functions of the bacterial cell. Coloured squares indicate whether a given threat type typically acts on a particular target.

Smith et al. 2023



Most attacks target core cellular processes and functions of the bacterial cell. Coloured squares indicate whether a given threat type typically acts on a particular target.

Bacterial competitors antagonize a target bacterium via diverse mechanisms, including both contact-dependent weaponry (the type VI secretion system (T6SS); Cdi effectors) and diffusible weaponry (small molecules, peptide toxins and tailocins).

The majority of clinical antibiotics are also derived from bacteria and other microorganisms.

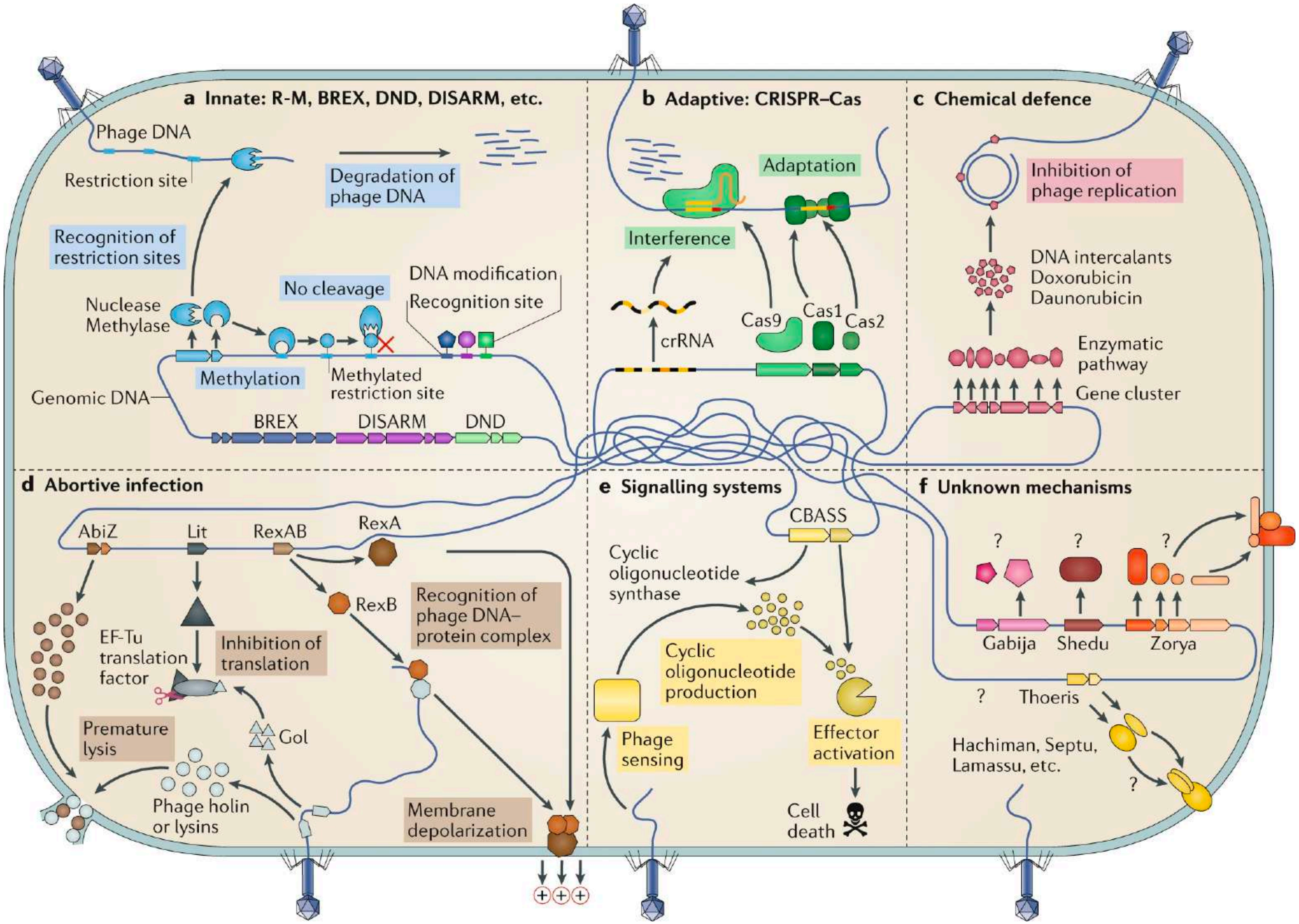
Following infection of a bacterial cell, phages attack cell walls and membranes to release their progeny via cell lysis.

Some bacterial predators, such as *Bdellovibrio* species and similar organisms, invade the host cell periplasm, injecting toxins that digest various cytoplasmic components.

Many eukaryotic predators engulf and digest target bacteria whole in phagosome compartments.

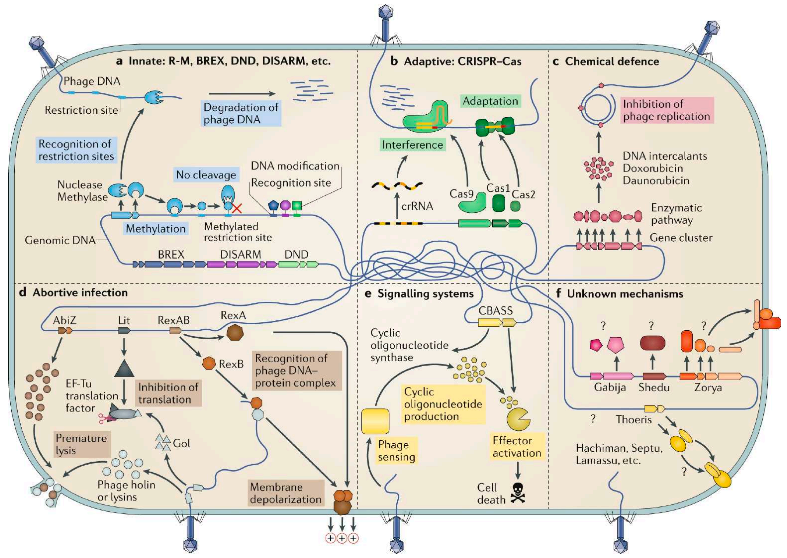
Bacterial antiviral strategies

Bernheim & Sorek, 2019



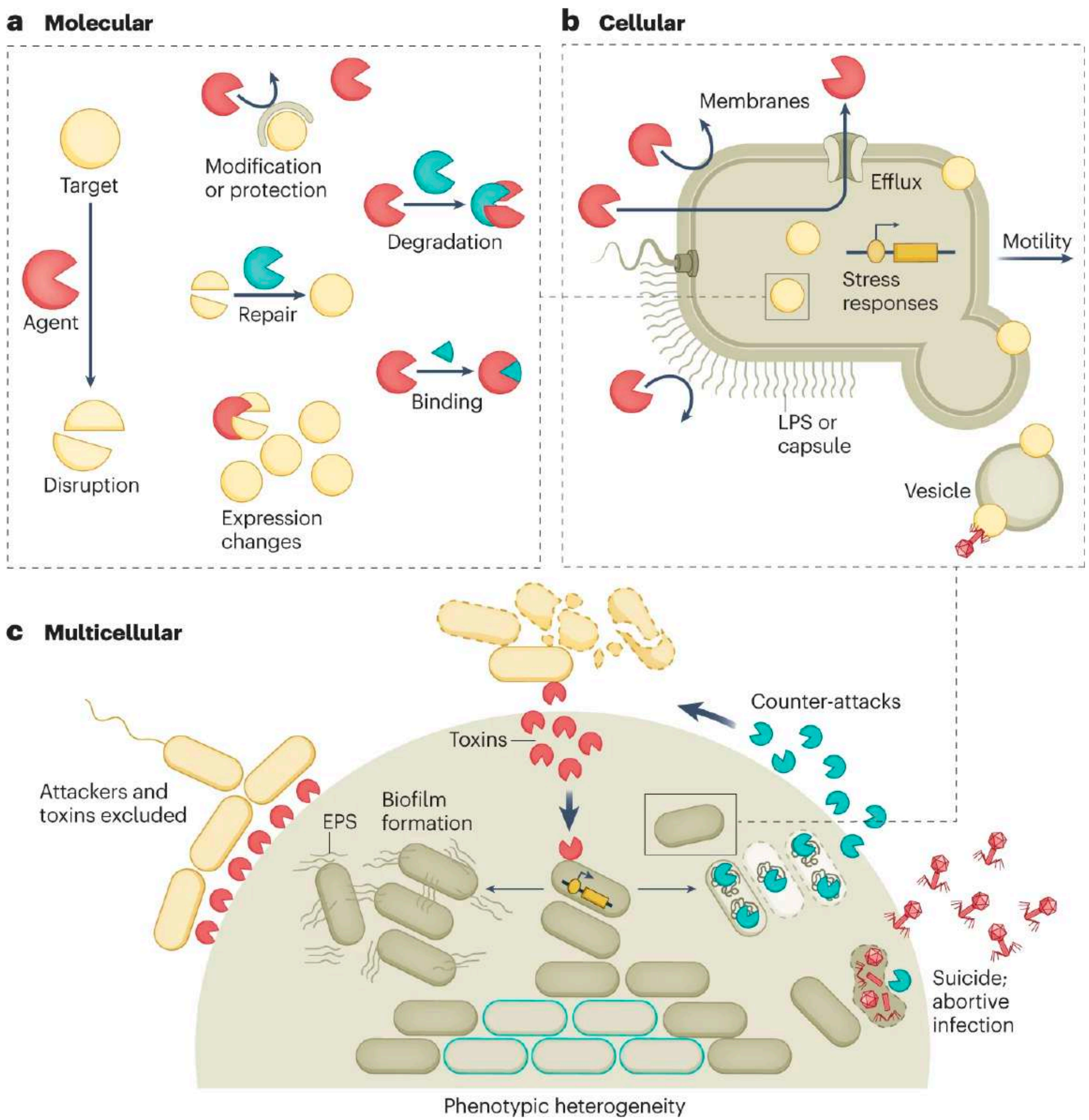
Bacterial defence systems that target nucleic acids encompass both innate and adaptive immunity and chemical defence and cell lysis or cell death induction

Bernheim & Sorek, 2019



Defence systems that target nucleic acids encompass both innate and adaptive immunity. **a** | Restriction-modification (R-M) and other related systems modify specific sequence motifs in the host genome and cleave or degrade unmodified foreign DNA. **b** | CRISPR–Cas systems work in two main phases: adaptation, where a complex of Cas proteins guides the acquisition of new bacteriophage (phage)-derived spacers; and interference, where Cas proteins in a complex with a spacer-derived CRISPR RNA (crRNA) target and degrade phage nucleic acids. **c** | Chemical defence has been described in *Streptomyces* spp., in which bacteria produce a small anti-phage molecule that intercalates into phage DNA and inhibits its replication. **d** | Abortive infection mechanisms are diverse. In concert with phage-encoded holins and lysins of phage Phi31, AbiZ from *Lactococcus lactis* accelerates lysis before phage assembly is completed. Upon expression of the T4 phage protein Gol, the *Escherichia coli* Lit protein inhibits translation through cleavage of the EF-Tu elongation factor. The *E. coli* protein RexA recognizes a specific DNA–protein complex formed by the λ phage, and activates RexB, an ion channel that depolarizes the membrane, leading to cell death. **e** | CBASS (cyclic oligonucleotide-based anti-phage signalling system) senses the presence of phage and generates a cyclic oligonucleotide small-molecule signal that activates an effector leading to cell death. **f** | Multiple systems have recently been demonstrated to have anti-phage roles, but their mechanisms remain unknown. Abi, abortive infection; BREX, bacteriophage exclusion; DISARM, defence islands system associated with R-M.

Bacterial multiple lines of defence against biotic threats

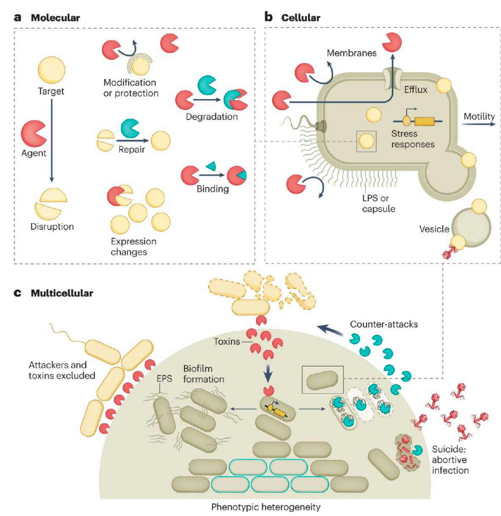


Smith et al. 2023

At both the individual and collective level, bacteria draw upon a plethora of defensive adaptations to escape harm

Defences are arranged according to the spatial scale at which they operate

Smith et al. 2023

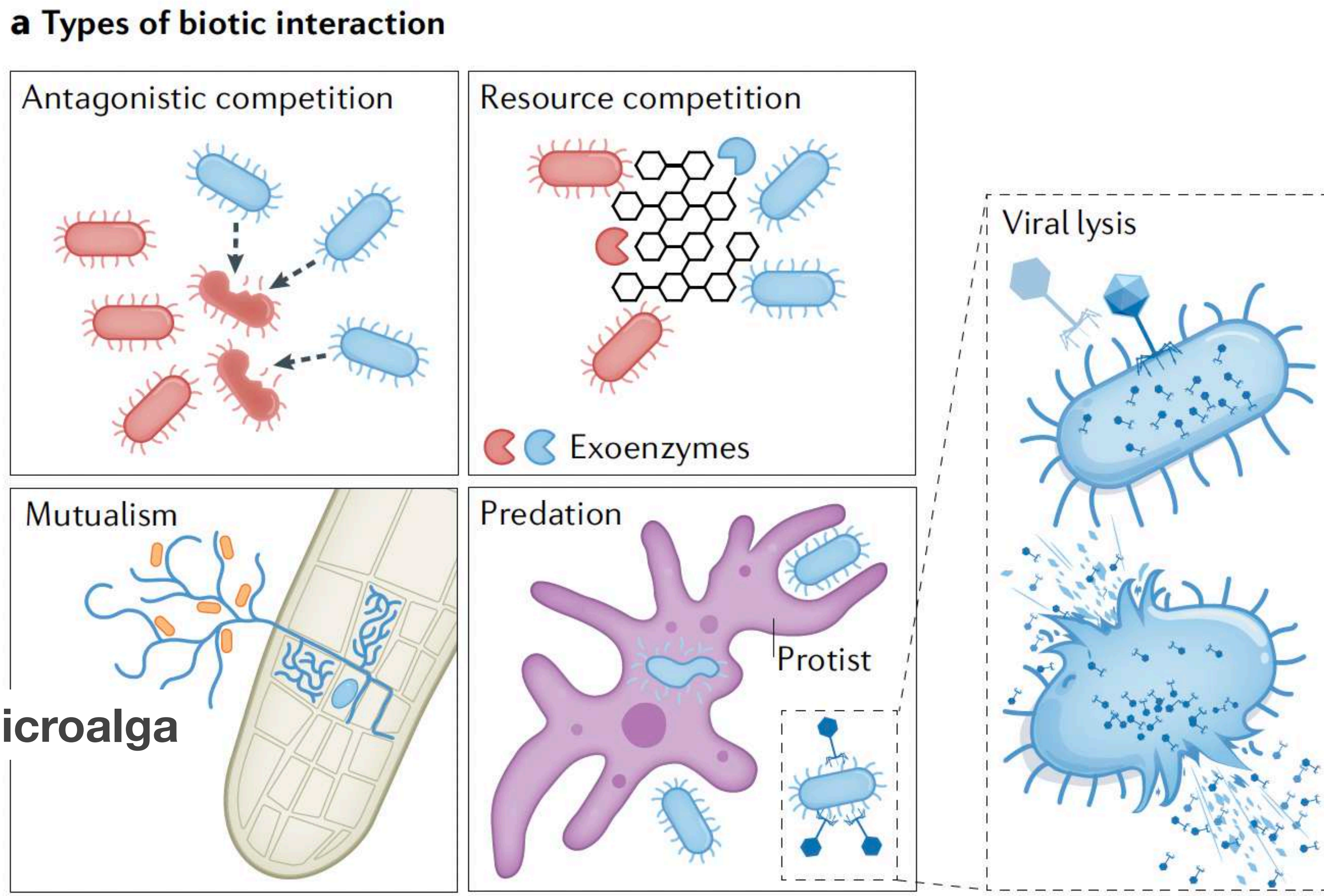


a, At the molecular level, attacks by competitors, phages and predators are mediated by harmful agents (for example, toxins, enzymes and genetic elements) that disrupt cellular functions by interacting with diverse targets. **Bacteria can mitigate disruption at a molecular level by altering the target or compensating for its disruption, or by destroying or binding to the harmful agent.**

b, At the cellular level, macromolecular barriers, including cell membranes, S-layers, lipopolysaccharide (LPS) or capsules, prevent harmful agents from entering a bacterial cell. **Efflux pumps remove harmful molecules that overcome barriers, and motile bacteria can escape harmful environments by repositioning themselves. Secreted membrane vesicles can bind and inactivate toxins and phages.**

c, At the multicellular level, bacteria **create collective barriers** (production of extracellular polymeric substances (EPSs); biofilm formation) that exclude attackers. Dense cell groups can limit toxin penetration via **reduced diffusion or collective degradation**. They may also contain resistant subpopulations (**phenotypic heterogeneity**), launch en masse counter-attacks and, in some circumstances (for example, abortive infection), **commit suicide to protect kin cells**. **Stress responses and other regulatory pathways enable these defences to be activated in response to specific or general threat cues.**

Biotic interactions in the soil /ocean microbiome, which shape microbial community structure and organic matter cycling —> interactions influence how organisms allocate carbon and can shape the chemical composition and flow of organic matter



With microalga

Sokol, 2022

Interactions include antagonistic competition (combative interactions for resources), exploitative competition (indirect competition for resources), mutualisms (for example, interactions between mycorrhizal fungi and plant roots) and predation (for example, protists consuming bacteria or viral lysis)

Microbial mortality in sum

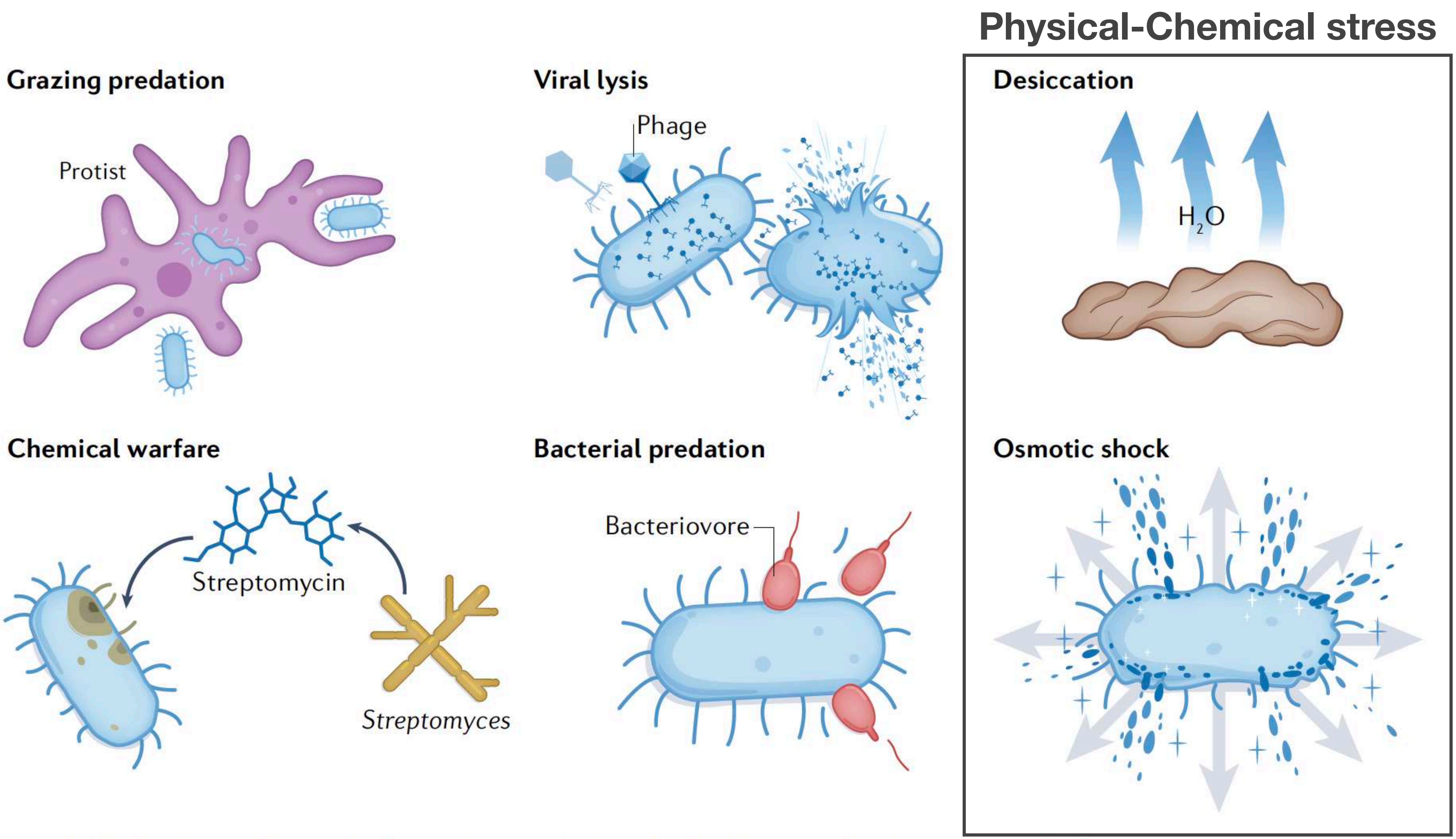


Fig. 4 | **Mechanisms of microbial mortality and theorized effects on the fate of microbial necromass.** There are different ways for a microorganism to die in soil, including grazing, bacterial predation, viral lysis, osmotic shock, desiccation and chemical warfare. The mechanism of death may affect the fate of its necromass, with direct consequences for organic matter cycling.

Sokol, 2022

Microbial roles in ecosystem functioning

Functional Redundancy and Resilience

- **Functional redundancy** means **multiple species can perform the same ecological function.** This ensures stability if some species are lost

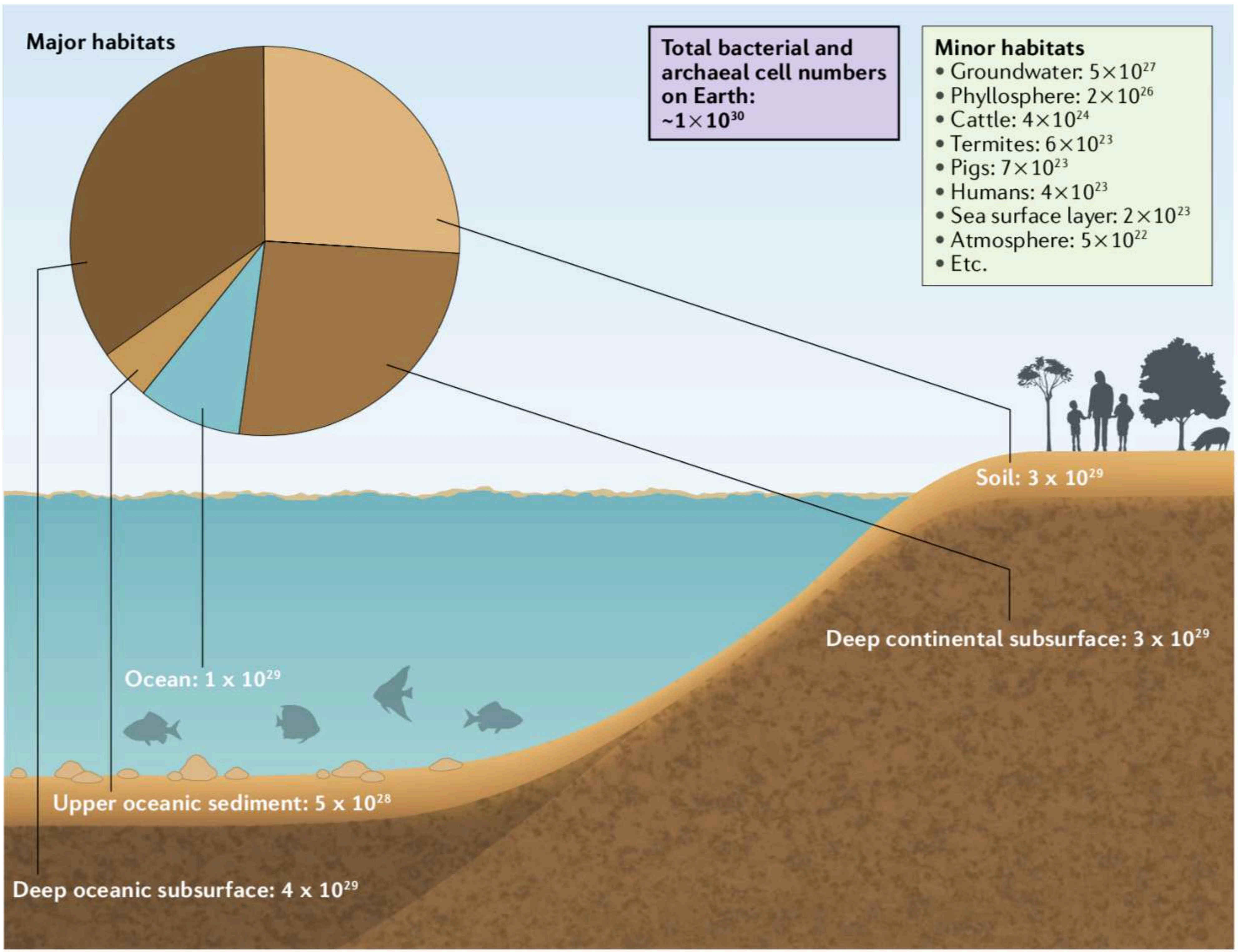
- **Resilience** is the ability of a microbial community to **resist or recover** from disturbances while maintaining its functions

- **Functional community profiling** — describing communities in terms of metabolic functions of interest suggest that certain **metabolic functions are strongly coupled to certain environmental factors** and can, in many cases, appear decoupled from the species assemblages associated with the mat a given place and time

- Systems with high redundancy tend to be more resilient and adaptable to stressors like pollution or climate change

- **Example:** In soil, many bacteria can fix nitrogen, so if one group is inhibited by drought, others may step in

Microbes are a pervasive force for Earth functioning



Atmo

Bio

Aqua

Geo

Spheres

Flemming & Wuertz, 2019

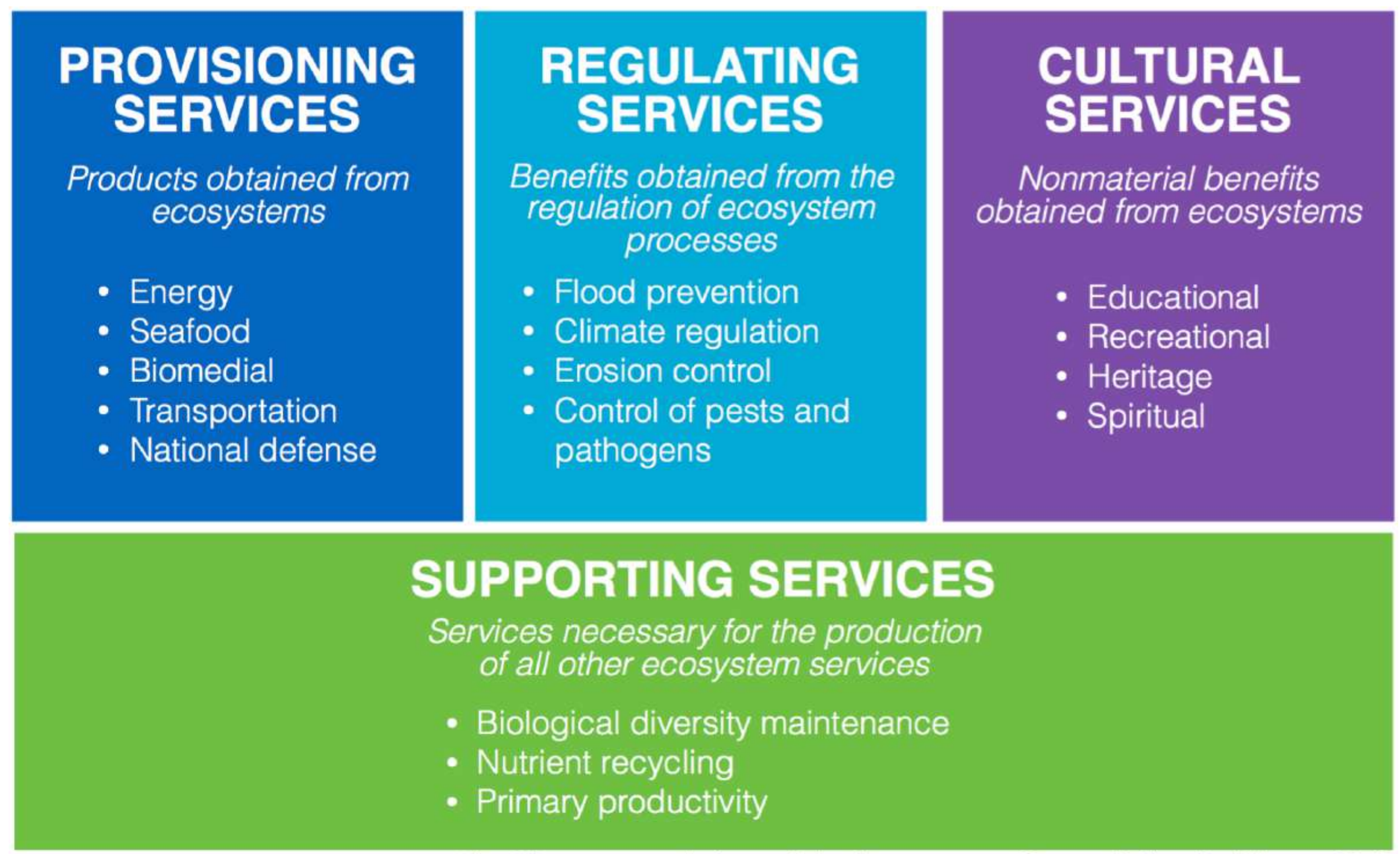
Ecosystem & Ecosystem services

Ecosystems consist of organisms, their environments, and all of the **interactions among the organisms and environments**

The organisms are members of populations and communities and are adapted to habitats —> species richness and abundance

Ecosystem services:
outputs, conditions, or processes of natural systems that directly or indirectly benefit humans or enhance social welfare

https://www.millenniumassessment.org/en/index.html



source: *Final Recommendations of the Interagency Ocean Policy Taskforce, 2010*

Microbes drive ecosystem services

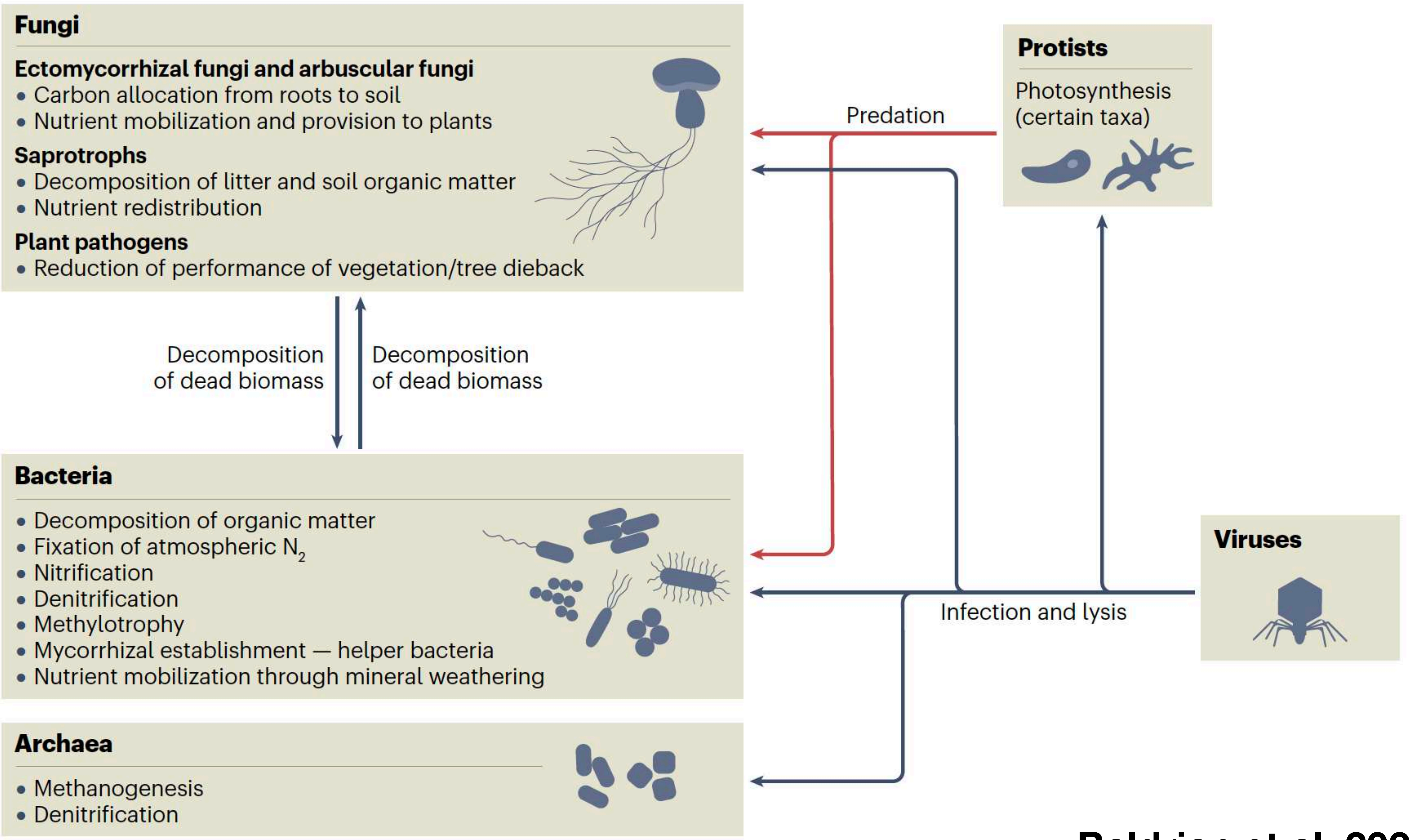
Table 1 | Major groups of microbes and ecosystem services they provide.

Microbial group	Process	Ecosystem service	Ecosystem service category
Heterotrophic bacteria/ archaea	Organic matter breakdown, mineralization	Decomposition, nutrient recycling, climate regulation, water purification	Supporting and regulating
Photoautotrophic bacteria	Photosynthesis	Primary production, carbon sequestration	Supporting and regulating
Chemo(litho)autotrophic	Specific elemental transformations (e.g., NH_4^+, S_2^-, Fe_2^+, CH_4 oxidation)	Nutrient recycling, climate regulation, water purification	Supporting and regulating
Unicellular phytoplankton	Photosynthesis	Primary production, carbon sequestration	Supporting and regulating
Archaea	Specific elemental transformation (e.g., metals, CH_4 formation, NH_4^+ oxidation), often in extreme habitats.	Nutrient recycling, climate regulation, carbon sequestration	Supporting and regulating
Protozoa	Mineralization of other microbes	Decomposition, nutrient recycling, soil formation	Supporting
Fungi	Organic matter breakdown and mineralization	Decomposition, nutrient recycling, soil formation, primary production (i.e., mycorrhizal fungi)	Supporting
Viruses	Lysis of hosts	Nutrient recycling	Supporting
All	Production of metabolites (e.g., antibiotics, polymers), degradation of xenobiotics, genetic transformation, and rearrangement	Production of precursors to industrial and pharmaceutical products	Provisional
All	Huge diversity, versatility, environmental and biotechnological applications	Educational purposes, getting students interested in science	Cultural

The last column depicts the ecosystem service category as was defined in the Millennium Ecosystem Assessment (2005). Modified from Ducklow, 2008.

Bodelier, 2011

Roles of the forest/soil/microbiome

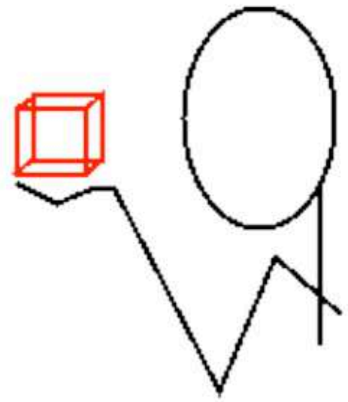
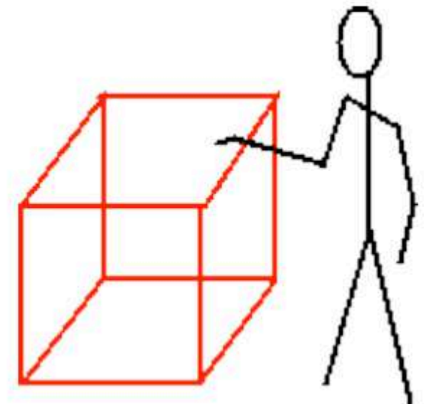
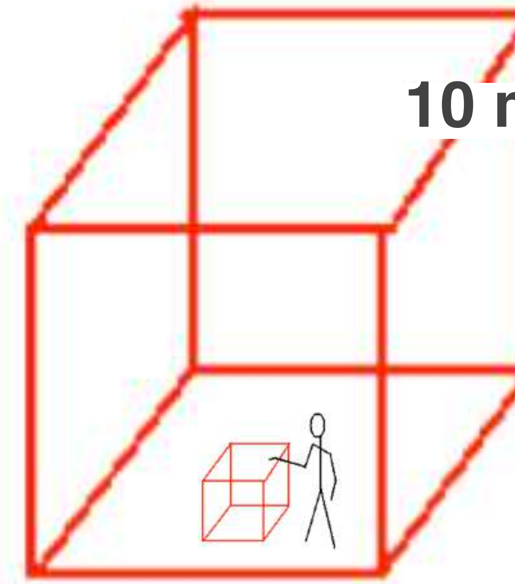


Baldrian et al. 2023

Microbial Contributions to Ecosystem Services

- Microbes play critical roles in **ecosystem services**, which are benefits that ecosystems provide to humans and nature:
  - **Nutrient cycling** (N, C, P, S)
  - **Decomposition and organic matter turnover**
  - **Water purification** through microbial breakdown of contaminants
  - **Climate regulation** via greenhouse gas flux (methane, CO_2, N_2O)
  - **Soil fertility** and plant health (e.g., rhizobia, mycorrhizae)
- Understanding microbial contributions is vital for managing agriculture, conservation, and restoration efforts

Feeling the microscale

1 mm	1 cm	10 cm	1 m=100 cm	10 m
				
10^{-6} m (1 micron)	10^{-5} m (10 microns)	10^{-4} m (100 microns)	10^{-3} m (1 mm)	10^{-2} m (1 cm)
bacteria	red blood cells	your hair	a grain of salt	a marble
				
		parasitic mite		
				

Microscale

Microbial life provides ecosystem services

Unique goal of microbial life: survival, maintenance, generation of ATP (energy storage), reducing power, growth of new cells

Decomposition, nutrient recycling, climate regulation, water purification

Primary production, carbon sequestration

Nutrient recycling, climate regulation, water purification

…… in an ecosystem context