

University of Naples Federico II

Environmental Metagenomic

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CORE CONCEPTS IN MICROBIAL ECOLOGY

Donato Giovannelli



Microbial species concept. The species concept is complex when applied to microbiology. In biology species are defined primarily based on their sexual segregation, either by genetic, behavioral or geographical barrier (and even this can be sometimes challenging, as all human-imposed categories).

For microbes the **absence of sexual reproduction, horizontal gene transfer** and a **high genome plasticity** make the classic species concept difficult to apply.

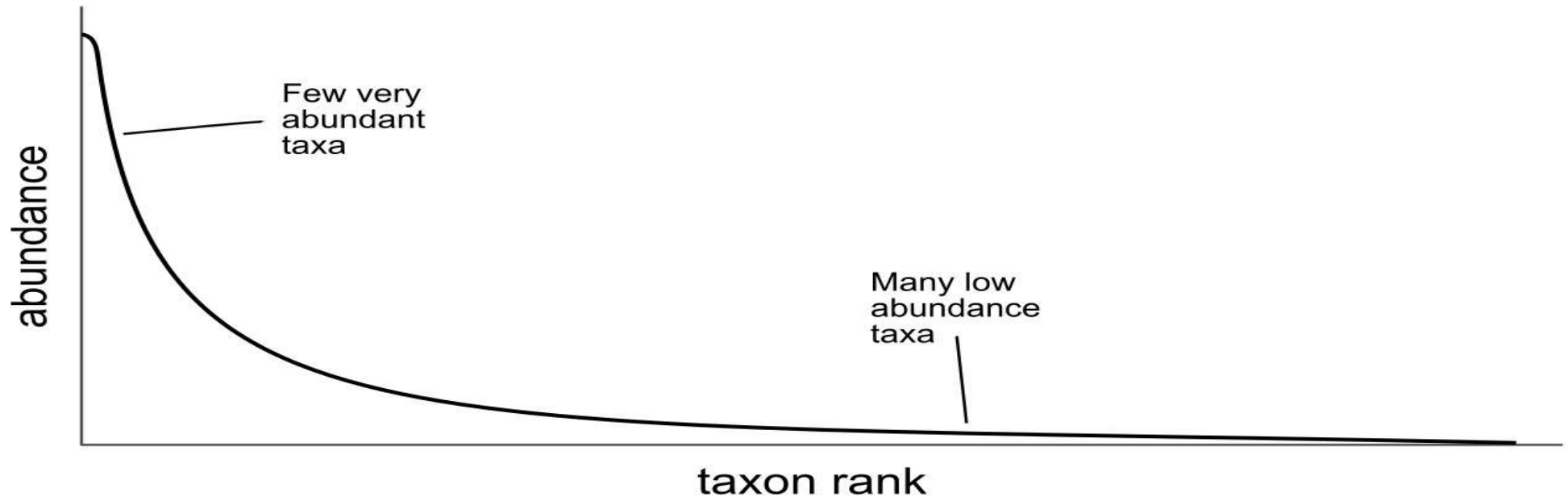
The current golden standard for defining a species is called a **polyphasic approach**, combining **phylogenetics, physiology and chemotaxonomy**.

In more simple terms, microbial species are defined based on their genetic properties, specifically the similarity of the **16S rRNA gene** (since it is a conserved universal marker not subject to HGT) or the **whole genome similarity** (also called ANI, Average Nucleotide Identity).

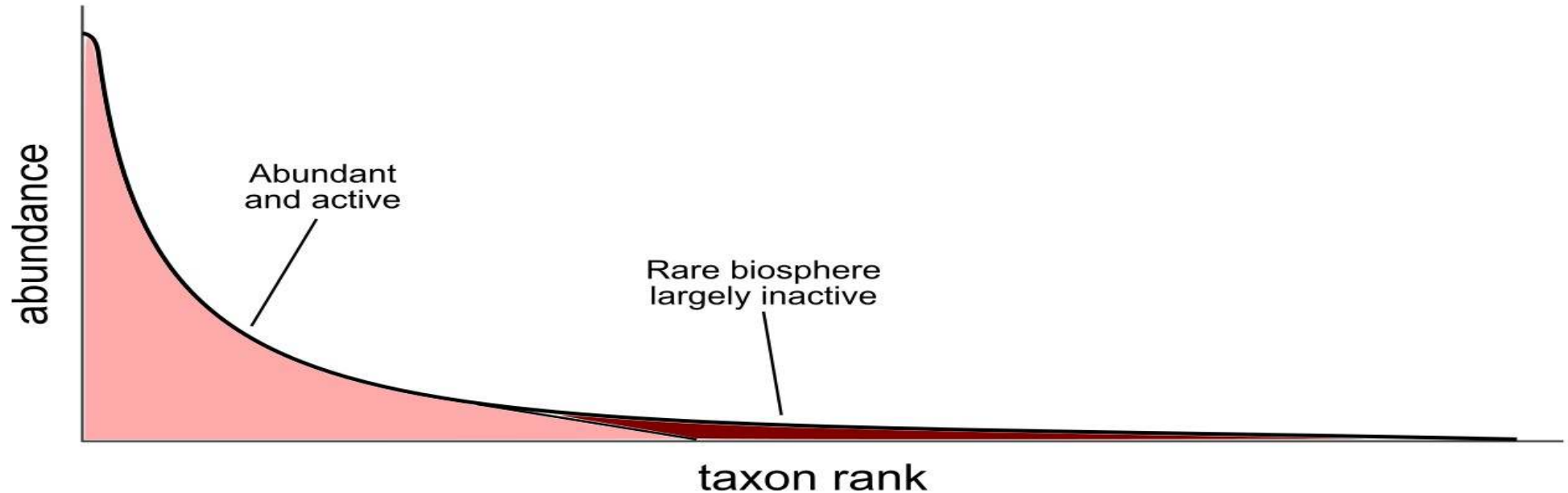
Current standard are **16S rRNA similarity >97%** for the same species and an **whole genome ANI >70%**.

Structuring of microbial communities

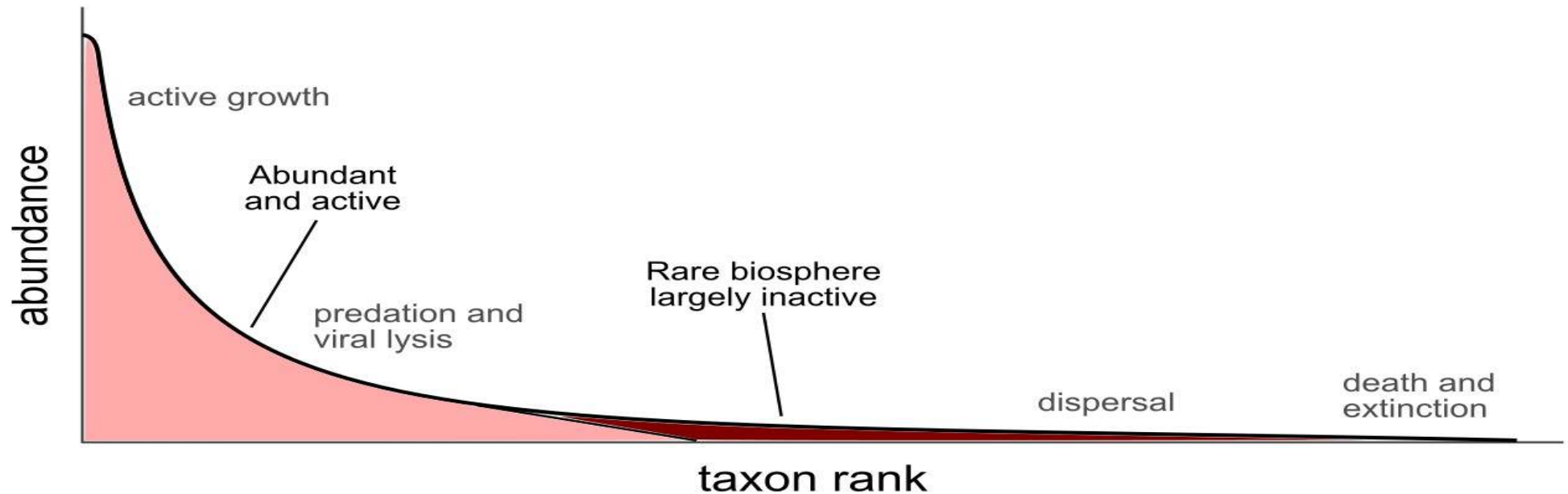
Microbial seed-banks theory. Relatively few microbial taxa are abundant and active, while the large majority of the diversity is constituted by a large number of low abundance taxa, often in a dormancy state. Dormancy is a bet-hedging strategy. Dormant microorganisms generate a seed bank capable of being resuscitated following environmental change



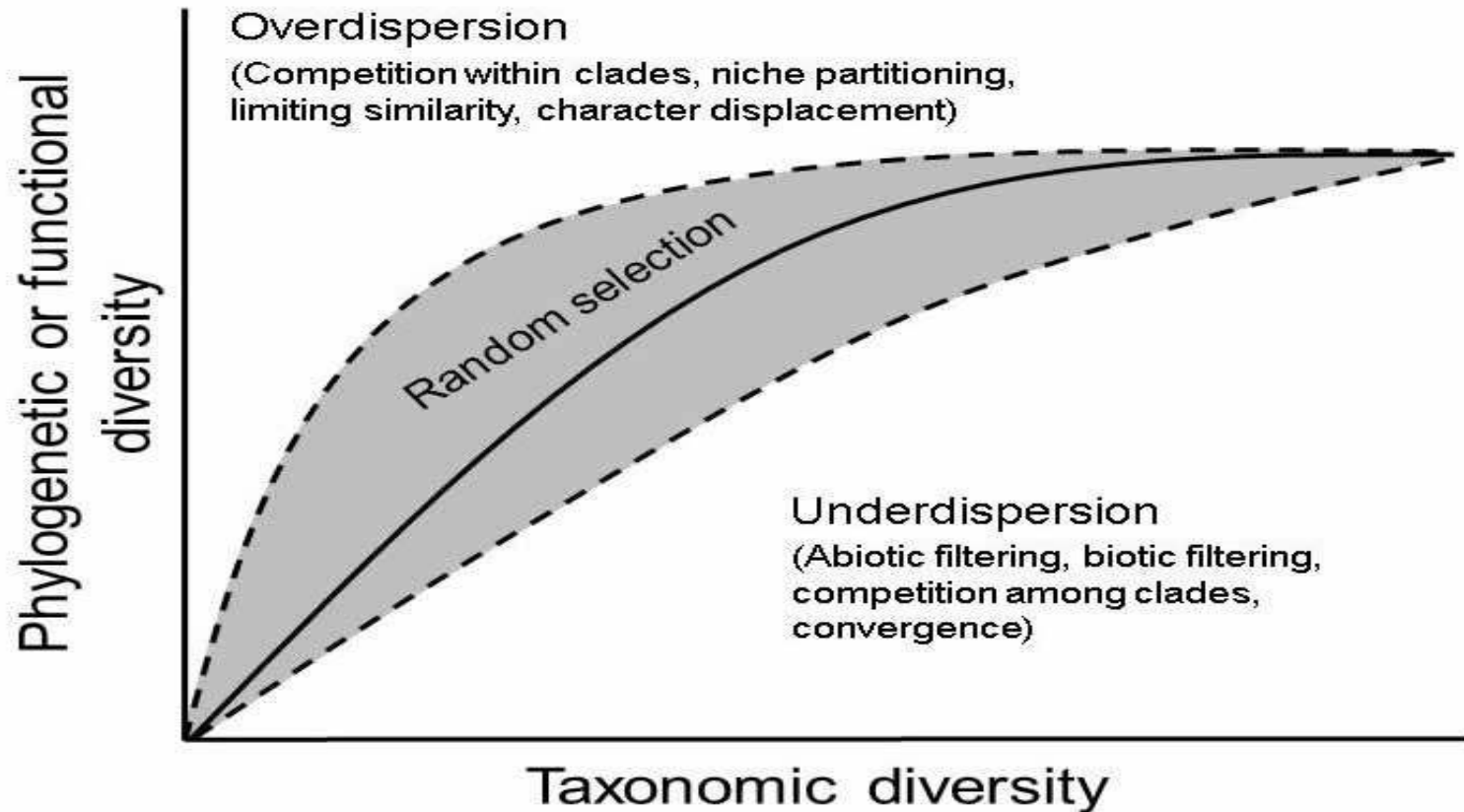
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Functional redundancy



Proc Natl Acad Sci U S A. 2002 Dec 24;99(26):17020-4. Epub 2002 Dec 5.

Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*.

Welch RA¹, Burland V, Plunkett G 3rd, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HL, Donnenberg MS, Blattner FR.

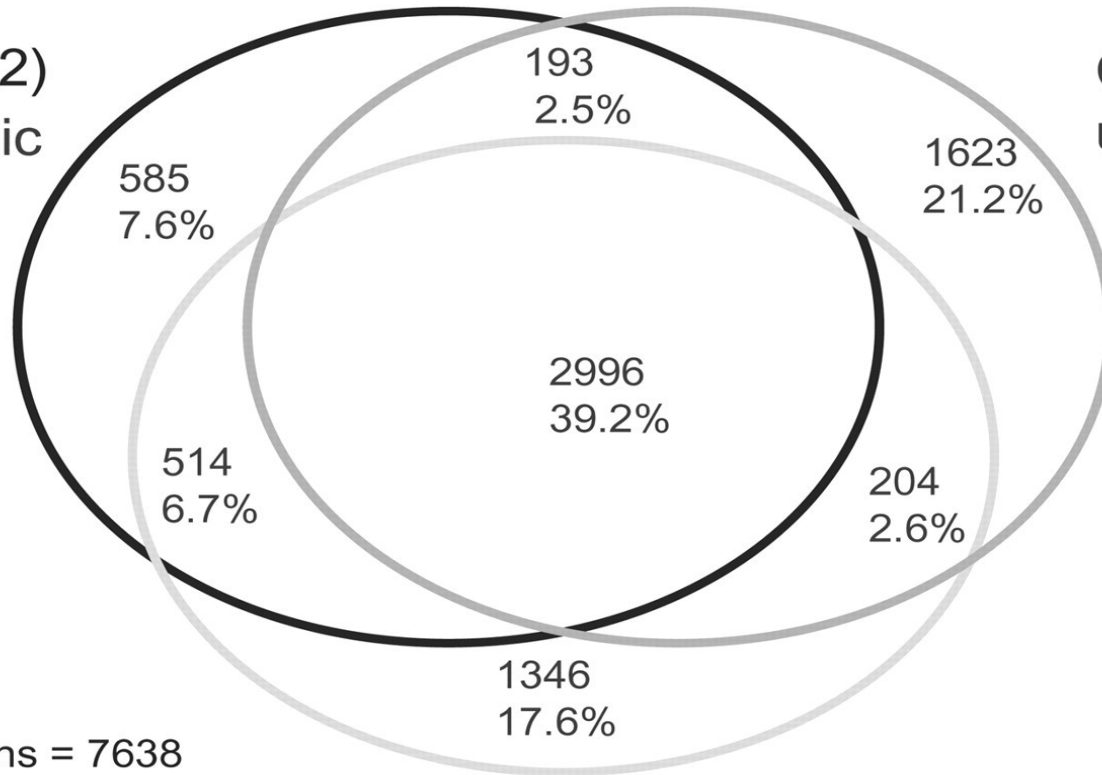
⊕ Author information

Abstract

We present the complete genome sequence of uropathogenic *Escherichia coli*, strain CFT073. A three-way genome comparison of the CFT073, enterohemorrhagic *E. coli* EDL933, and laboratory strain MG1655 reveals that, amazingly, only 39.2% of their combined (nonredundant) set of proteins actually are common to all three strains. The pathogen genomes are as different from each other as each pathogen is from the benign strain. The difference in disease potential between O157:H7 and CFT073 is reflected in the absence of genes for type III secretion system or phage- and plasmid-encoded toxins found in some classes of diarrheagenic *E. coli*. The CFT073 genome is particularly rich in genes that encode potential fimbrial adhesins, autotransporters, iron-sequestration systems, and phase-switch recombinases. Striking differences exist between the large pathogenicity islands of CFT073 and two other well-studied uropathogenic *E. coli* strains, J96 and 536. Comparisons indicate that extraintestinal pathogenic *E. coli* arose independently from multiple clonal lineages. The different *E. coli* pathotypes have maintained a remarkable synteny of common, vertically evolved genes, whereas many islands interrupting this common backbone have been acquired by different horizontal transfer events in each strain.

MG1655 (K-12)
non-pathogenic

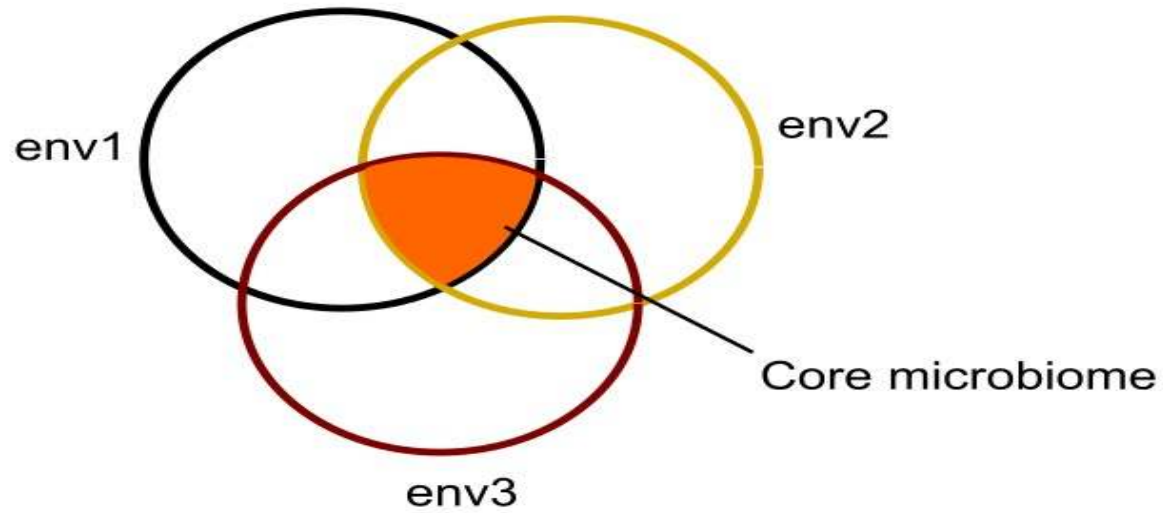
CFT073
uropathogenic



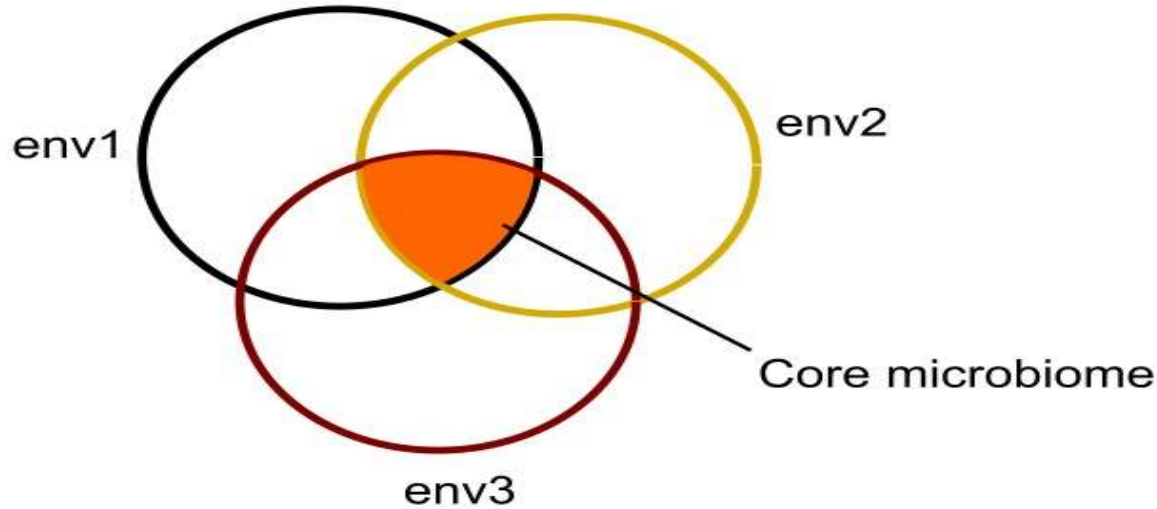
Total proteins = 7638
2996 (39.2%) in all 3
911 (11.9%) in 2 out of 3
3554 (46.5%) in 1 out of 3

EDL933 (O157:H7)
enterohaemorrhagic

Core microbiome

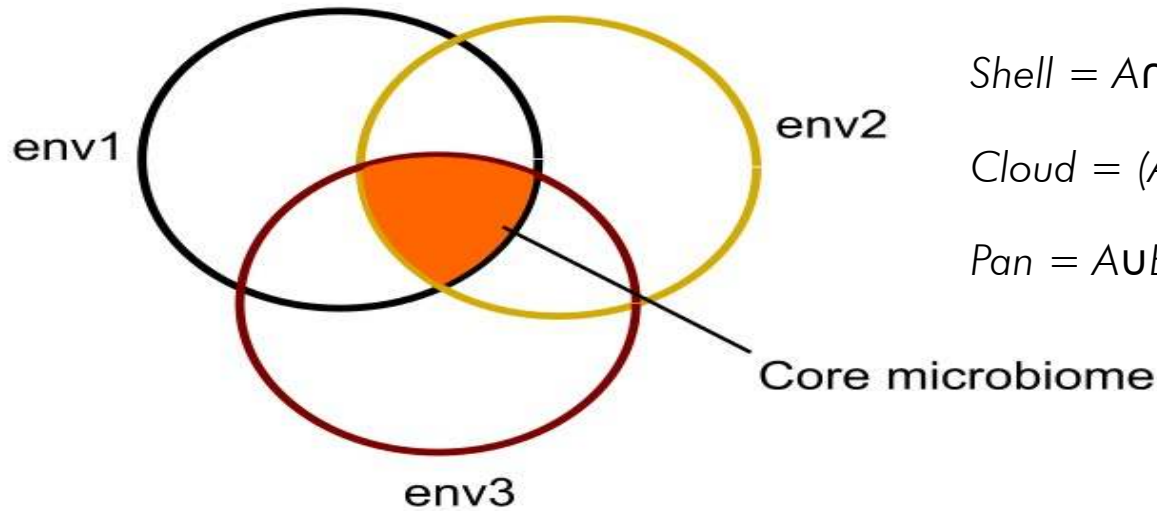


Core microbiome



The **core microbiome** (or microbiota) is the taxa present in all the observed samples. It can be defined **across an environment** (core microbiome in surface sediments), for a **selected area** (the core microbiome of the Antarctic waters) or for a **specific functions** (the core microbiome carrying out nitrogen fixation). A similar concept can be used for functional diversity (the core functions instead of taxa). It can be seen as the deterministic portion of the community.

Core microbiome



$$\text{Core} = A \cap B \cap C$$

$$\text{Shell} = A \cap B + A \cap C + B \cap C$$

$$\text{Cloud} = (A \cup B + A \cup C + B \cup C) - (A \cap B \cap C)$$

$$\text{Pan} = A \cup B \cup C$$

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The complementary portion of the core are the **shell microbiome** and the **cloud microbiome**. The union of all the taxa across the samples is called the **panmicrobiome**.

Ecosystem Functions (microbial)

Ecosystem Functions

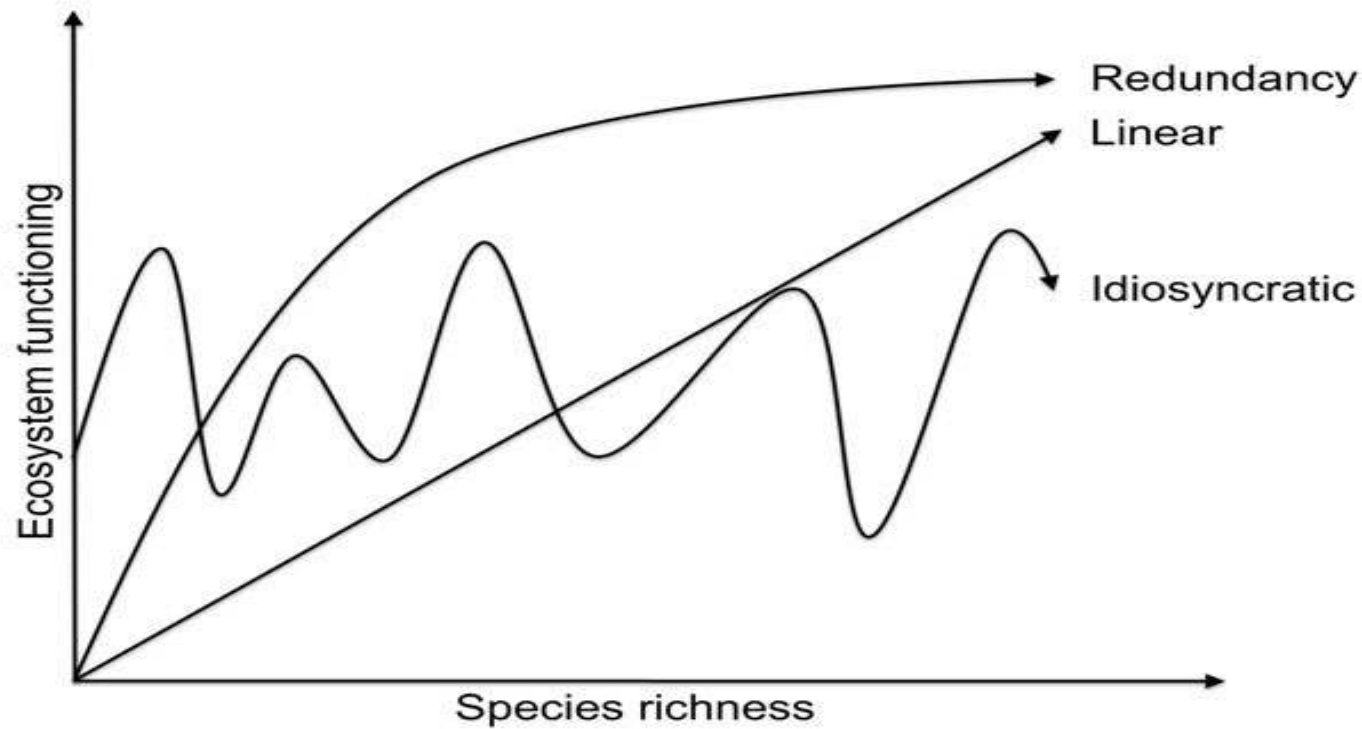
'Ecosystem function' is the technical term used in the framework to define the biological, geochemical and physical processes and components that take place or occur within an ecosystem

Or more simply put, ecosystem functions relate to the structural components of an ecosystem (e.g. vegetation, water, soil, atmosphere and biota) and how they interact with each other, within ecosystems and across ecosystems

Sometimes, ecosystem functions are called ecological processes. Different ecosystem functions provide ecosystem services to humans and the environment alike.

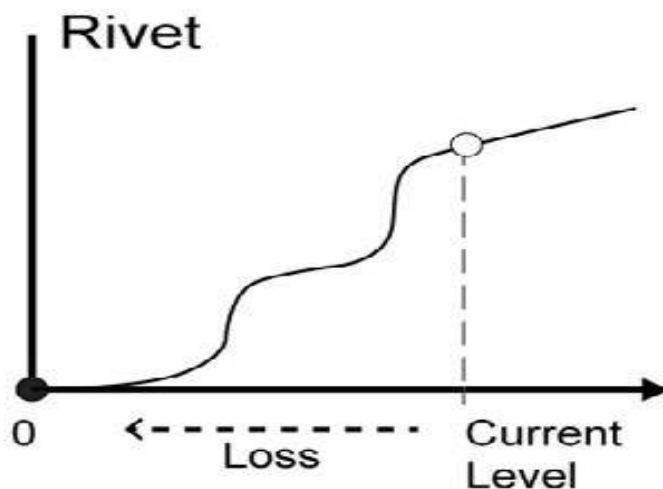
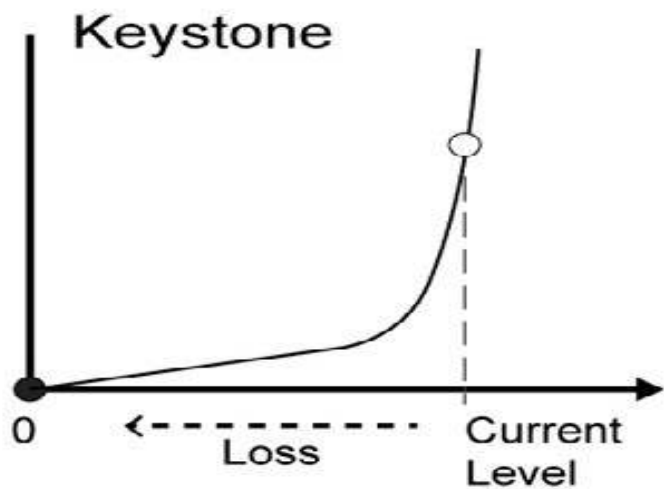
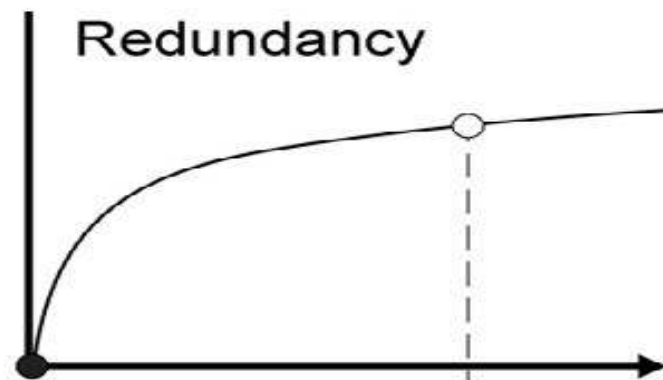
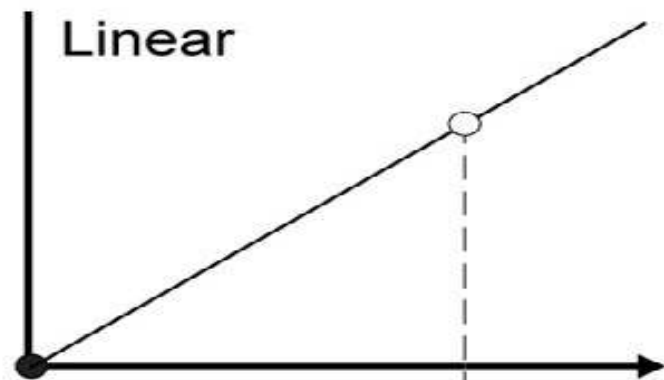
Given the key role of microorganisms in element and nutrient cycling the ecosystem functions provided by the microbial community (and by specific groups of microbes) are fundamental for the functioning of the ecosystem.

Biodiversity-ecosystem function relationship



The relationship between **biodiversity and ecosystem functioning** (called **BEF** relation) are widely studied to understand the role of different taxa and the overall functioning and vulnerability of an ecosystem

Ecosystem process

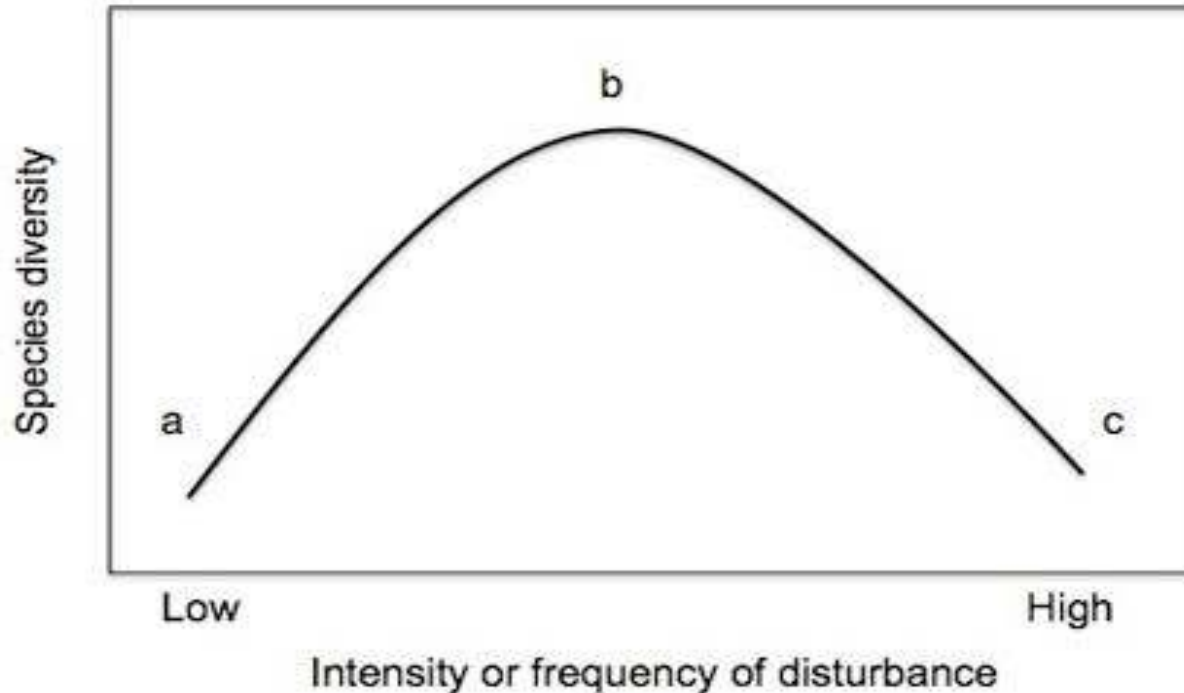


Biodiversity

Disturbance theory and ecosystem recovery

Intermediate disturbance hypothesis

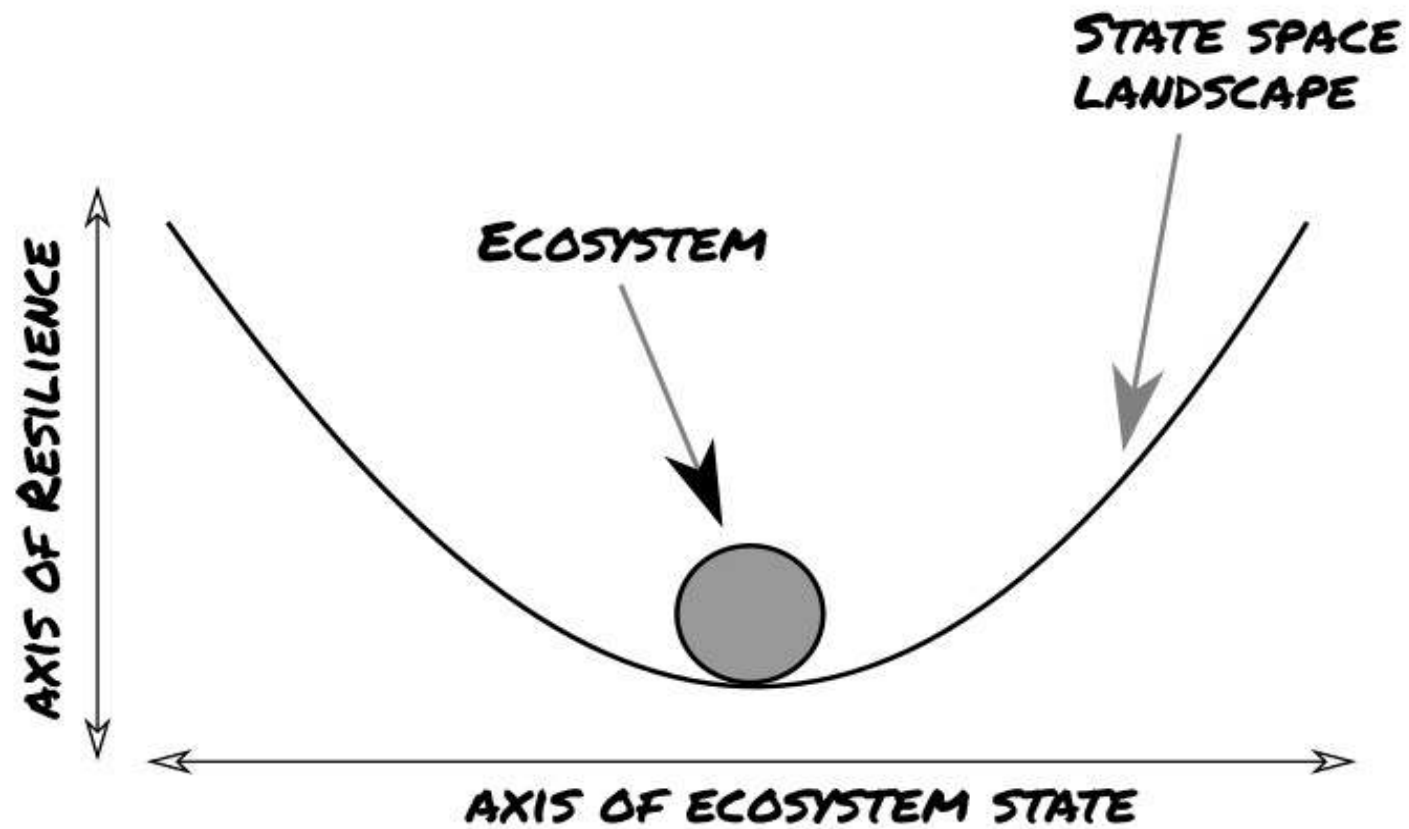
The intermediate disturbance hypothesis (IDH) suggests that local species diversity is maximized when ecological disturbance is neither too rare nor too frequent

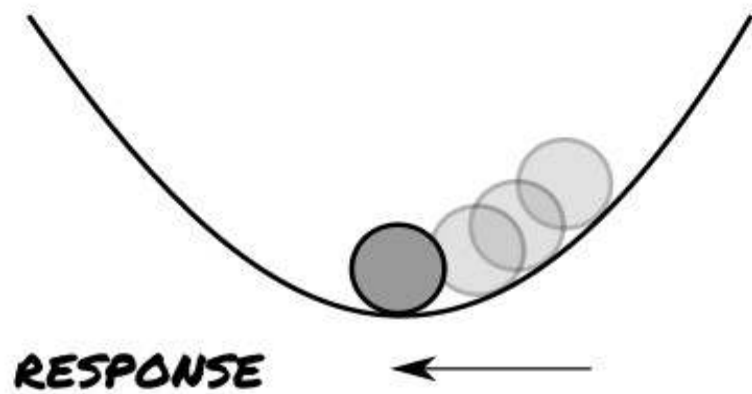
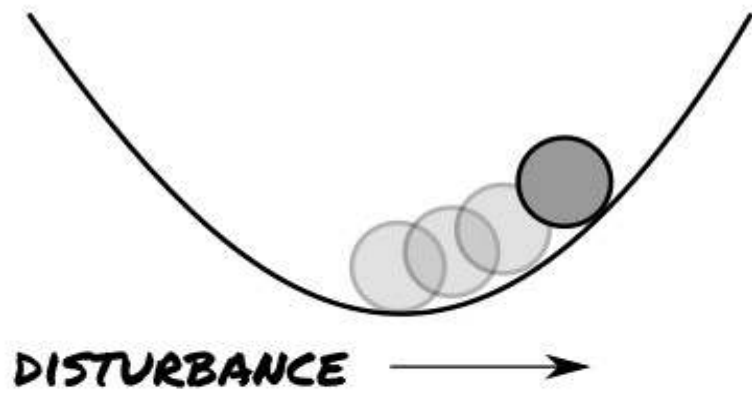


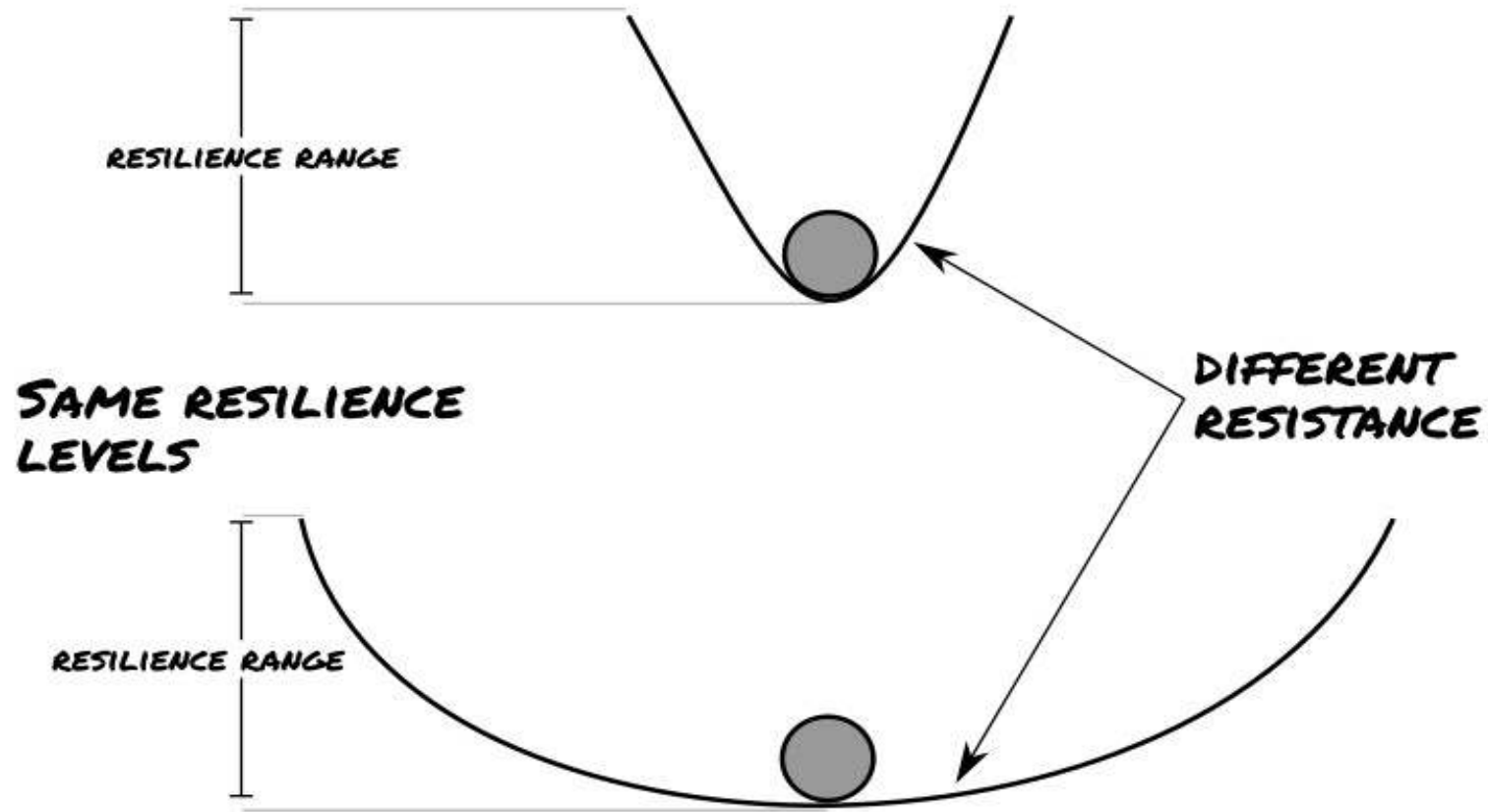
For each ecosystem we can define two basic properties that describe how an ecosystem responds to forcing factors (i.e. change):

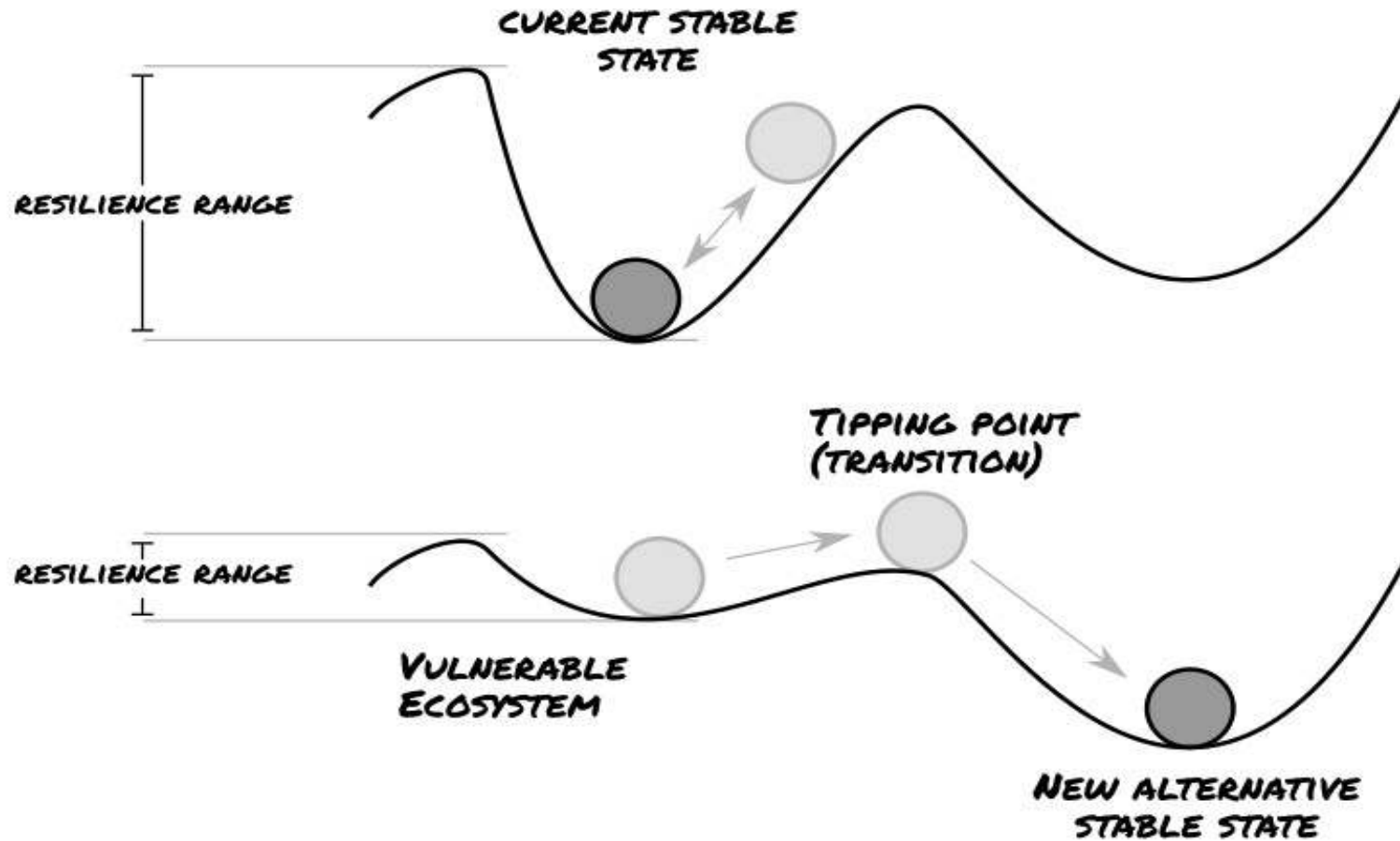
Resistance is the ability for an ecosystem to remain unchanged when being subjected to a disturbance or disturbances. Some ecosystems are better at resisting change than others, and therefore have high resistance

Resilience is the ability and rate of an ecosystem to recover from a disturbance and return to its pre-disturbed state. Some ecosystems can shift greatly from their previous state and still return to pre-disturbance conditions. The measure for how far an ecosystem can be shifted from its previous state and still return to normal is called its amplitude









Most ecosystem state are metastable, and can be pushed to a number of alternative (meta)stable states that depend on the nature of the the ecosystem itself

alternative stable states



Risk factors for *C. difficile* colonization

- Previous hospitalization
- Exposure to antibiotics
- Chemotherapy treatment
- Corticosteroid use
- Haemodialysis / renal disease

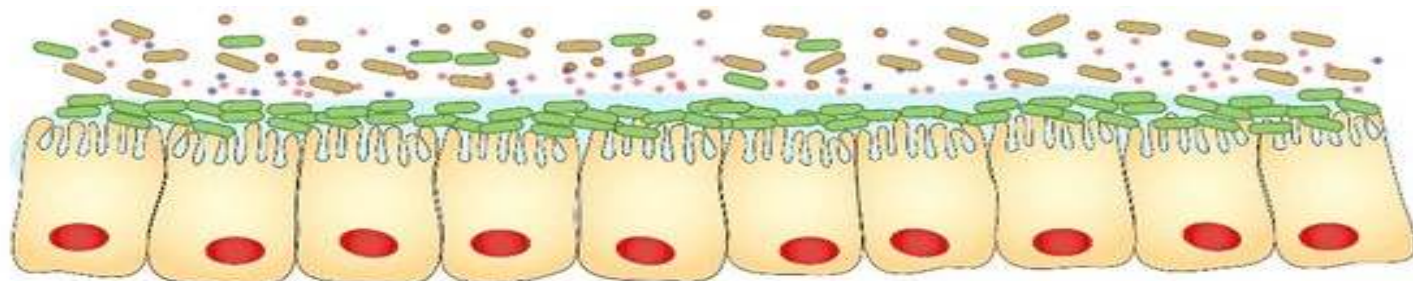


Exposure to *C. difficile* spores leading to asymptomatic *C. difficile* colonization

Exposure to *C. difficile* spores leading directly to symptomatic CDI

Risk factors for symptomatic CDI

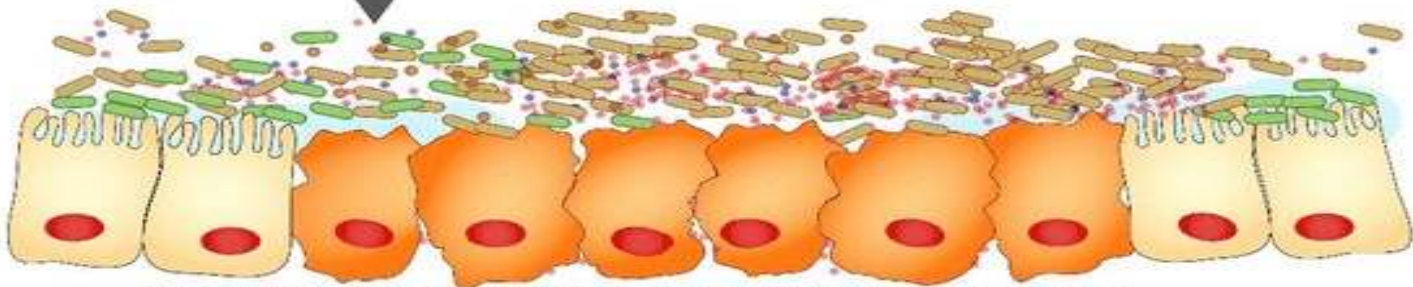
- Increased age
- Exposure and duration of antibiotics
- Presence of nasogastric tube
- Severe underlying disease
- Prolonged hospital admission
- Exposure to drugs that reduce stomach pH



Healthy intestinal epithelial cells with intact microbiome in an individual with asymptomatic *C. difficile* colonization

Factors that protect against progression to symptomatic CDI

- Increased levels of IgG and IgA
- Intact indigenous microbiome
- Colonization by less virulent *C. difficile* strain



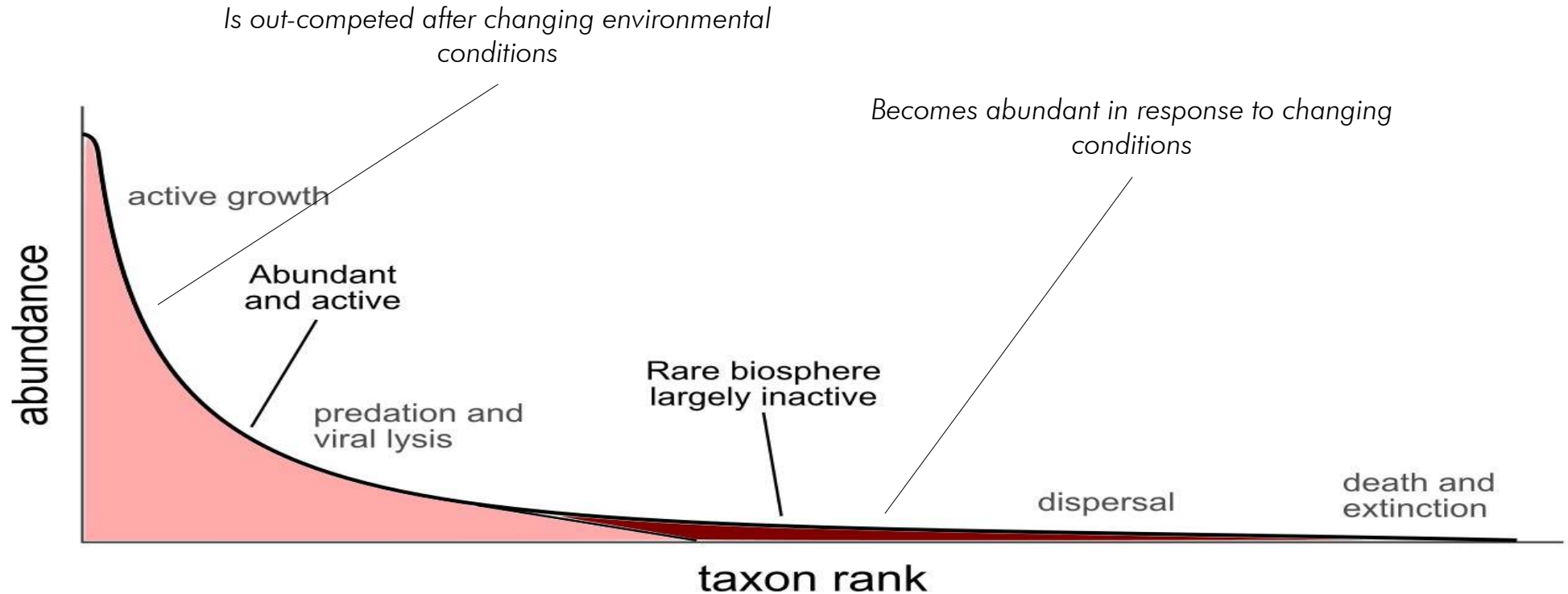
Damaged intestinal epithelial cells in an individual with symptomatic CDI

Legend

- Clostridium difficile* cell
- Normal colonic flora
- Clostridium difficile* spore
- Toxin A
- Toxin B



Role of rare and dormant species as a reservoir of functions and adaptations in response to environmental disturbance



Community Controls

Types of Control

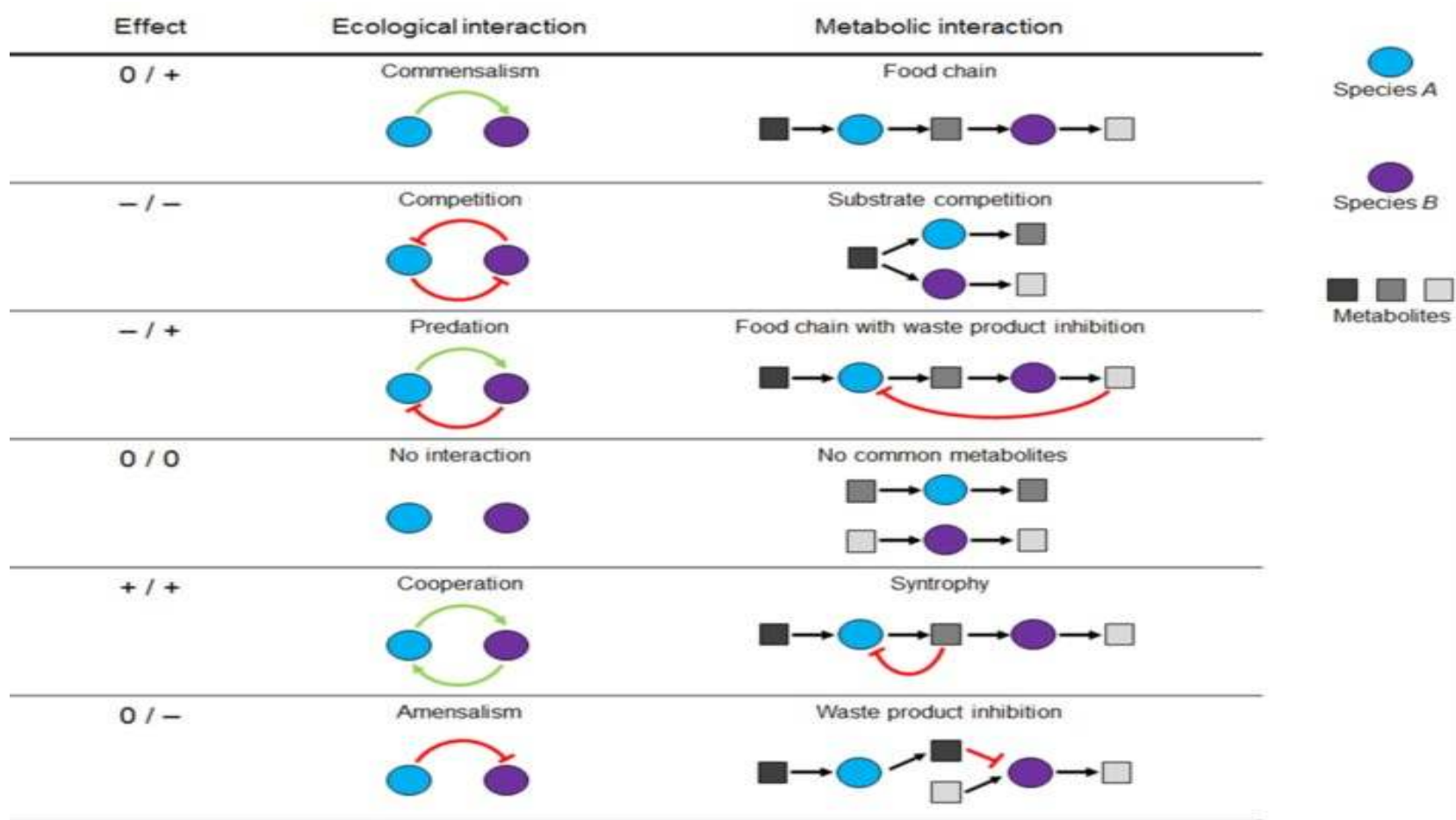
Resource or **bottom-up control** refers to the limitation of a prokaryotic community by **carbon and nutrients** derived from allocthonous inputs, primary production, and heterotrophic production

Top-down control refers to the **predatory limitation** of a prokaryotic community below levels supportable by resources alone. Top-down control refers to situations where the abundance, diversity or biomass of lower trophic levels depends on effects from consumers at higher trophic levels

A **trophic cascade** is a type of top-down interaction that describes the indirect effects of predators. In a trophic cascade, predators induce effects that cascade down food chain and affect biomass of organisms at least two links away

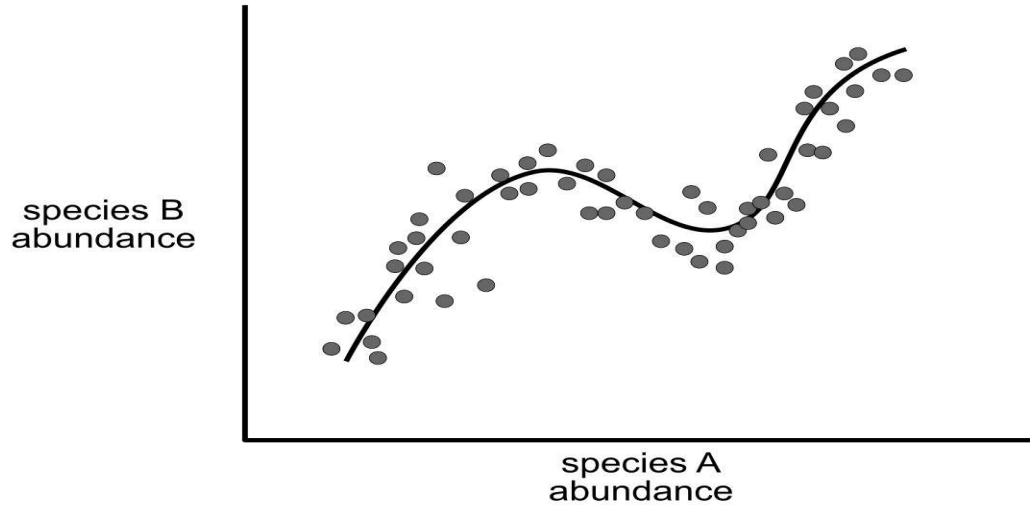
Sideways control refers to within community interaction, such as **competition and chemical warfare**

Community interactions



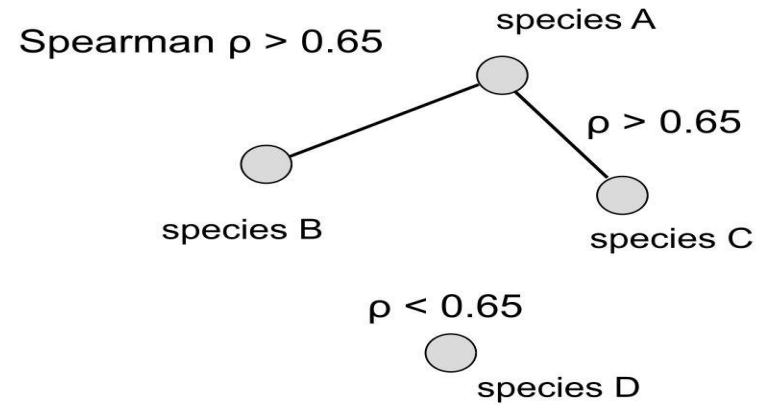
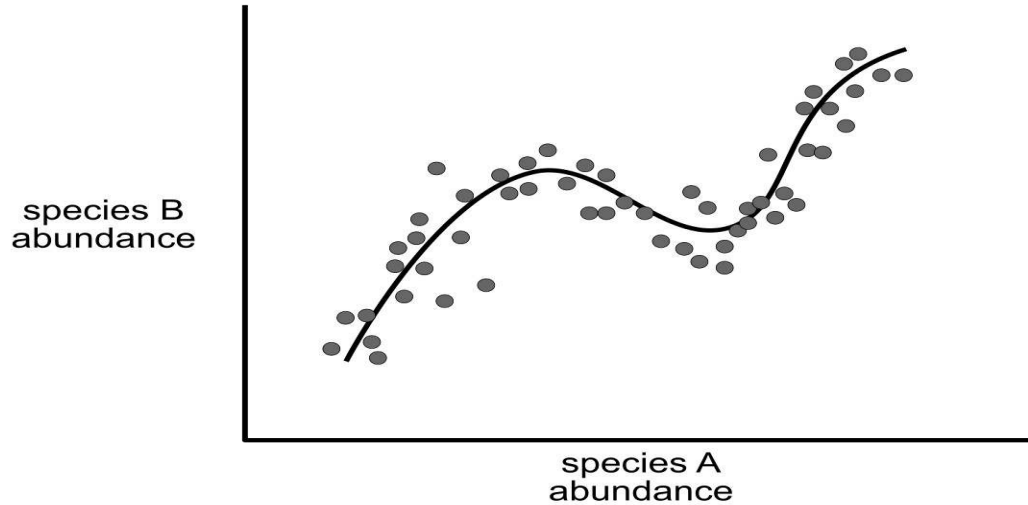
Core microbiome

Co-occurrence networks



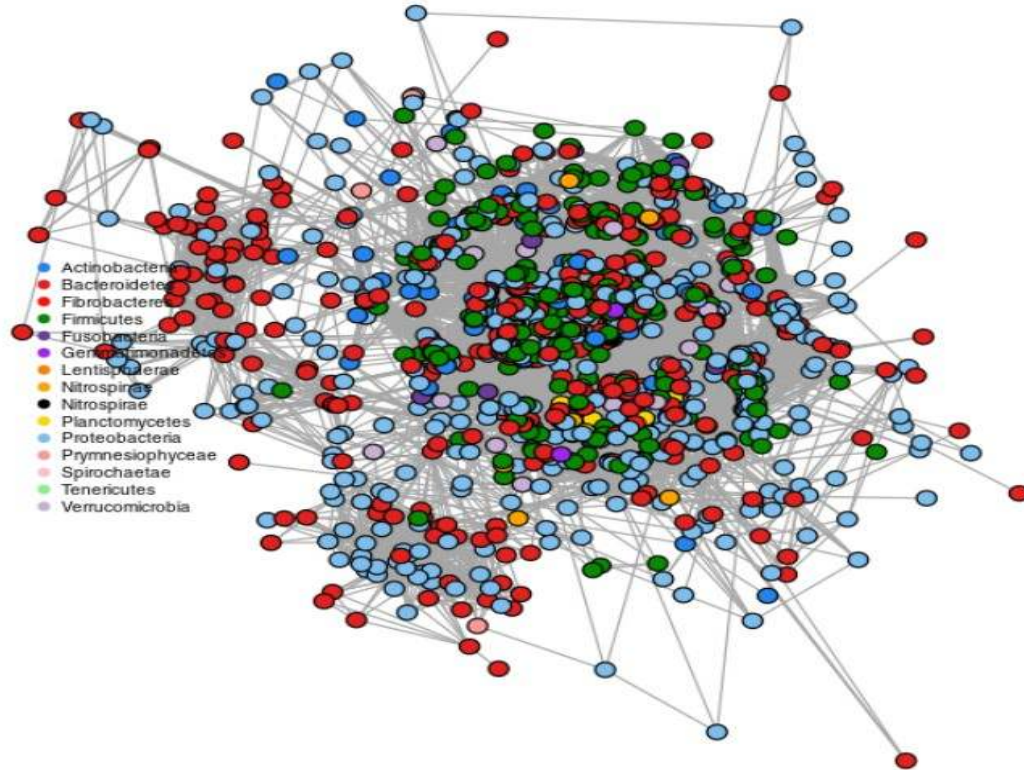
Core microbiome

Co-occurrence networks

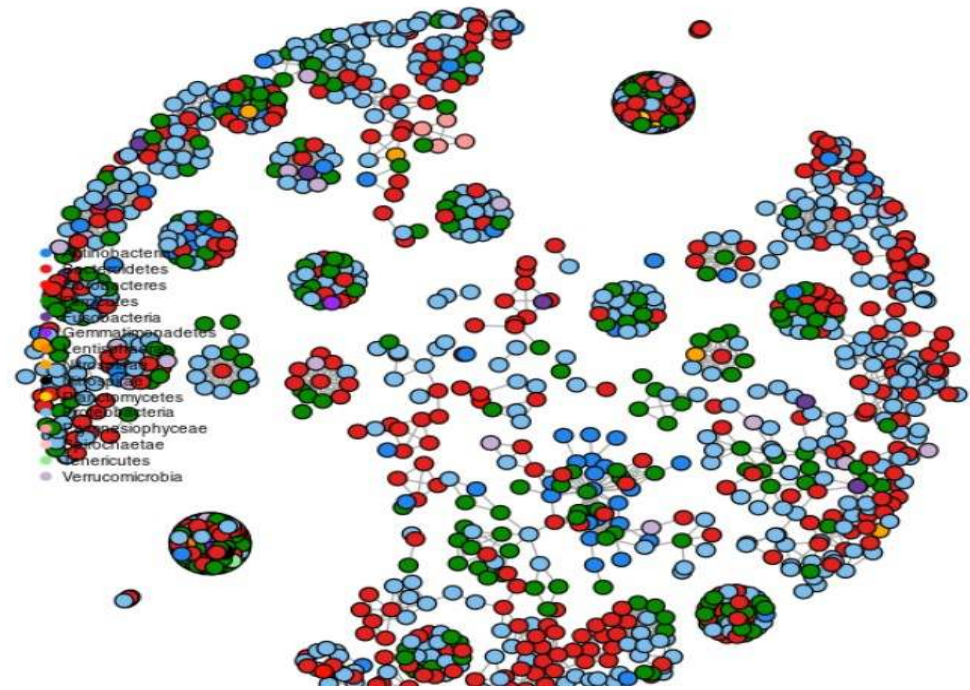


Core microbiome

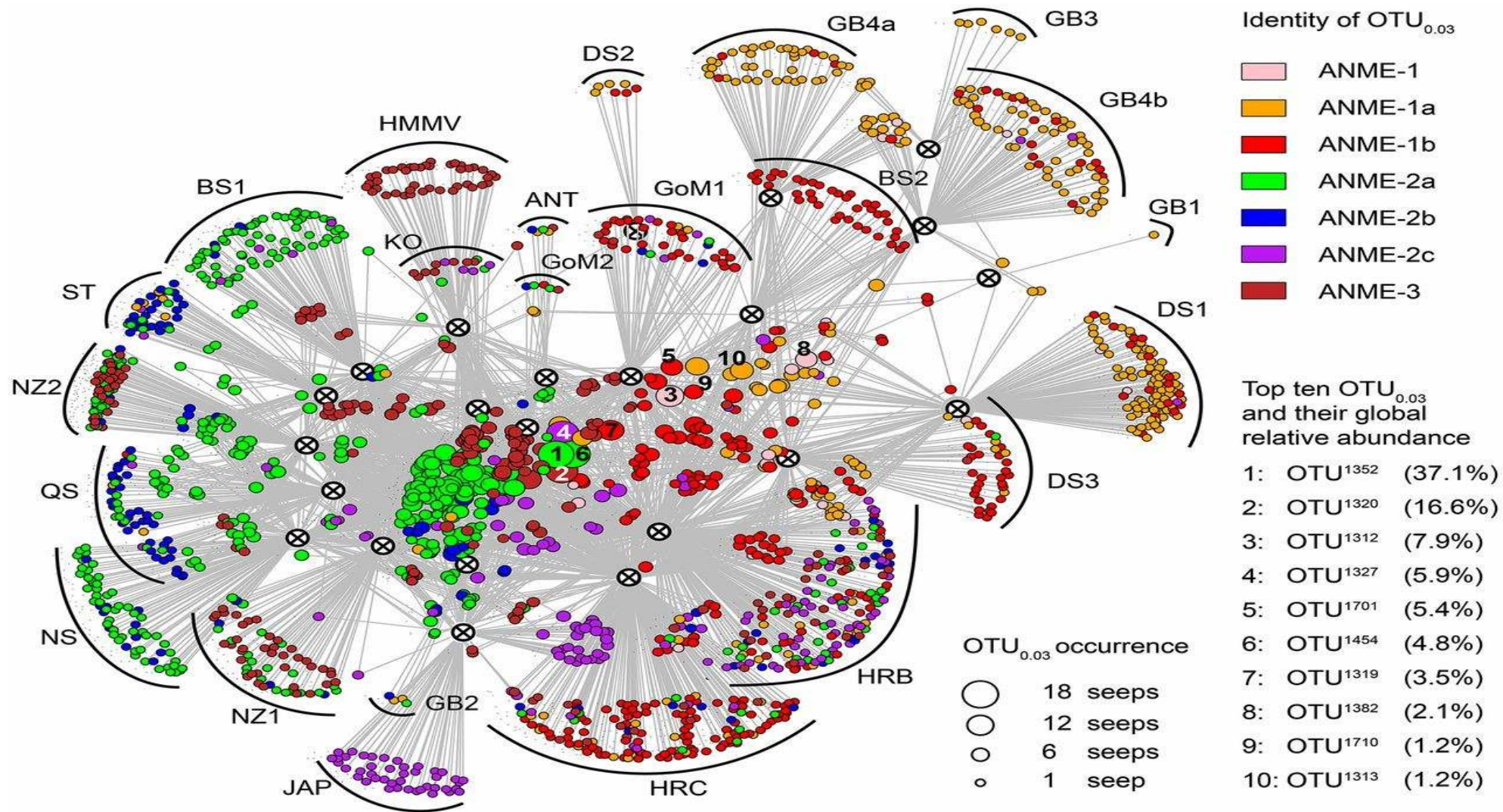
Co-occurrence networks



$$\rho^2 > 0.65$$



$$\rho^2 > 0.90$$



Temporal and Spatial Variation

All biological systems are dynamic on one or more scales. The term '**dynamics**' specifically refers to **changes over time**, resulting from **growth and death, import and export of organisms and intraspecies evolution**.

Dynamics are relatively straightforward in closed, artificial systems. In marine systems, dynamics are complicated by **water mixing**, advection, currents and eddies, dispersal, etc...

Current microbial oceanography data suggest that microbial community composition in the **water column** remains relatively constant in the order of 2–20 km in horizontal extent. At fronts, where conditions such as temperature or chlorophyll levels (or other microbially important parameters) change abruptly, sharper gradients in microbial composition are expected.

In **marine sediments** and other ecosystems controlled by sharp geochemical gradient community changes on much shorter scales (μm to meters).

Temporal Scales

Hours

The **physiological response** of microorganisms to changing conditions can occur **rapidly**, but compositional changes observed at subdaily timescales can only occur in communities in which **organisms replicate relatively rapidly**.

A **diel periodicity** of whole-community bacterial productivity and abundance is sometimes observed in the marine environment. Cyanobacteria have a diel pattern of abundance, which is due to the synchronization of cell division with the photoperiod

Unpredictable short-term changes include those induced by **storm or wind events** (which induce Upwelling and deposit Allochthonous material) and those that are consequences of biological interactions, such as **competition, grazing, sloppy feeding, viral infection and lysis, and cross-feeding**

Daily to weekly

In surface oceans estimated whole-community **biomass turnover times** range from less than a day to about a week. The forces that vary substantially on this scale include weather, mesoscale oceanographic processes, interactions with larger organisms (from protists to fish), food-web cascade effects and microbial interactions involving all types of viruses, bacteria, archaea and protists

Monthly to seasonal

Forcing functions that vary at this scale include **changes in solar angle** (including associated changes in light intensity and penetration), **seasonal weather patterns**, seasonal upwelling and the associated changes in nutrient availability, and stratification. Other forcing factors include changes in **temperature and day length**; seasonal variation in land runoff and atmospheric deposition. **They are highly dependent on geographic location**

Interannual

Forcing functions that are most variable at this scale include phenomena like the **El Niño Southern Oscillation**, the **Pacific Decadal Oscillation**, **stochastic year-to-year weather** and/or climate variability, directional climate change (e.g., anthropogenic global warming and ocean acidification), other **anthropogenic regional environmental changes** (such as hypoxia and anoxia caused by eutrophication).

There are even longer-term climatological variations at time scales much longer than decades, which emerge owing to geological and astronomical processes that are associated with changes in microbial assemblages (e.g., foraminiferal changing over scales of tens to thousands of years and diatom and dinoflagellate populations have trended towards species with smaller cell sizes over the past 50 million years).

Long-term time series

Long-term time series in microbial oceanography are limited to the surface ocean. There are no long term time series for the deep-sea or extreme environments, so the annual and interannual variability in those ecosystem are unknown

Bermuda. The Bermuda Atlantic Time-series Study (BATS) samples the subtropical waters ($\sim 33^\circ$ N) of the northern Sargasso Sea

Hawaii. The Hawaii Ocean Time-series (HOT) is located in the North Pacific Gyre, 100 km north of Oahu, at the northern boundary of the tropics ($\sim 22^\circ$ N) 4Southern

California. The San Pedro Ocean Time-series (SPOT) is in the San Pedro Channel, which is ~ 20 km off the coast of Southern California. Similarly to BATS, it has a subtropical latitude ($\sim 33^\circ$ N)

Western English Channel. Station L4 in the western English Channel is located 10 km off the southwest coast of England, over a shallow continental shelf, and has a depth of 55m

Northwestern Mediterranean Sea. There are two microbial observatories in the Mediterranean Sea, located just over 100km from each other, in the temperate zone at $\sim 42^\circ$ N. The Banyuls observatory is ~ 4 km off the coast of Banyuls, France, and the Blane Bay Microbial Observatory (Spain) is to the west and ~ 1 km offshore

Biofilms

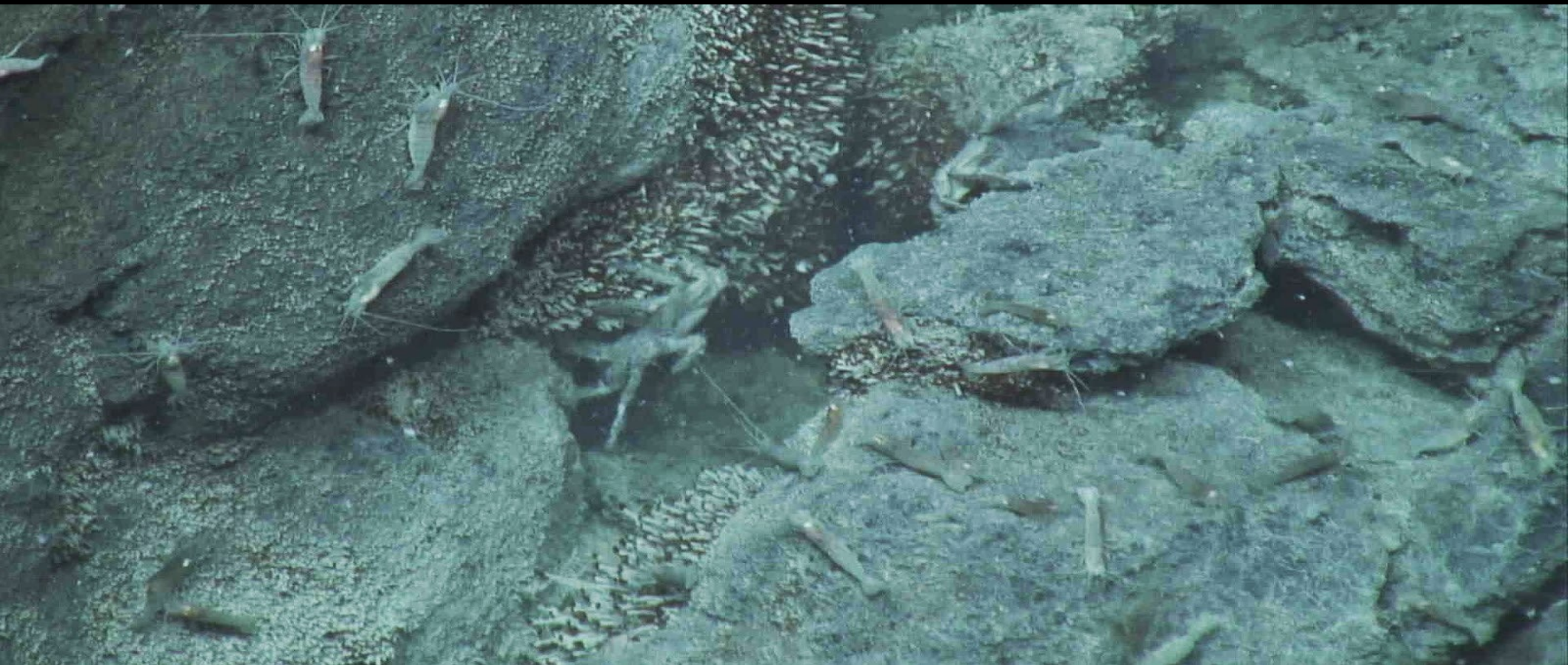


Why a Biofilm?

Biofilm assure the **proximity to nutritional source** (electron donor and/or acceptor, carbon source, appropriate pH/Eh conditions)

Biofilms increase the opportunity for **gene transfer** between/among bacteria. Gene transfer can convert a previous non virulent commensals organism into a highly virulent pathogen, confer antibiotic resistance and new metabolic traits. Formation of biofilm is crucial for the establishment of several pathogens

Microbial biofilms are pervasive in marine ecosystems and represent a widely used ecological strategy by many marine microbial taxa



Middle Atlantic Ridge Hydrothermal Vent Biofilm

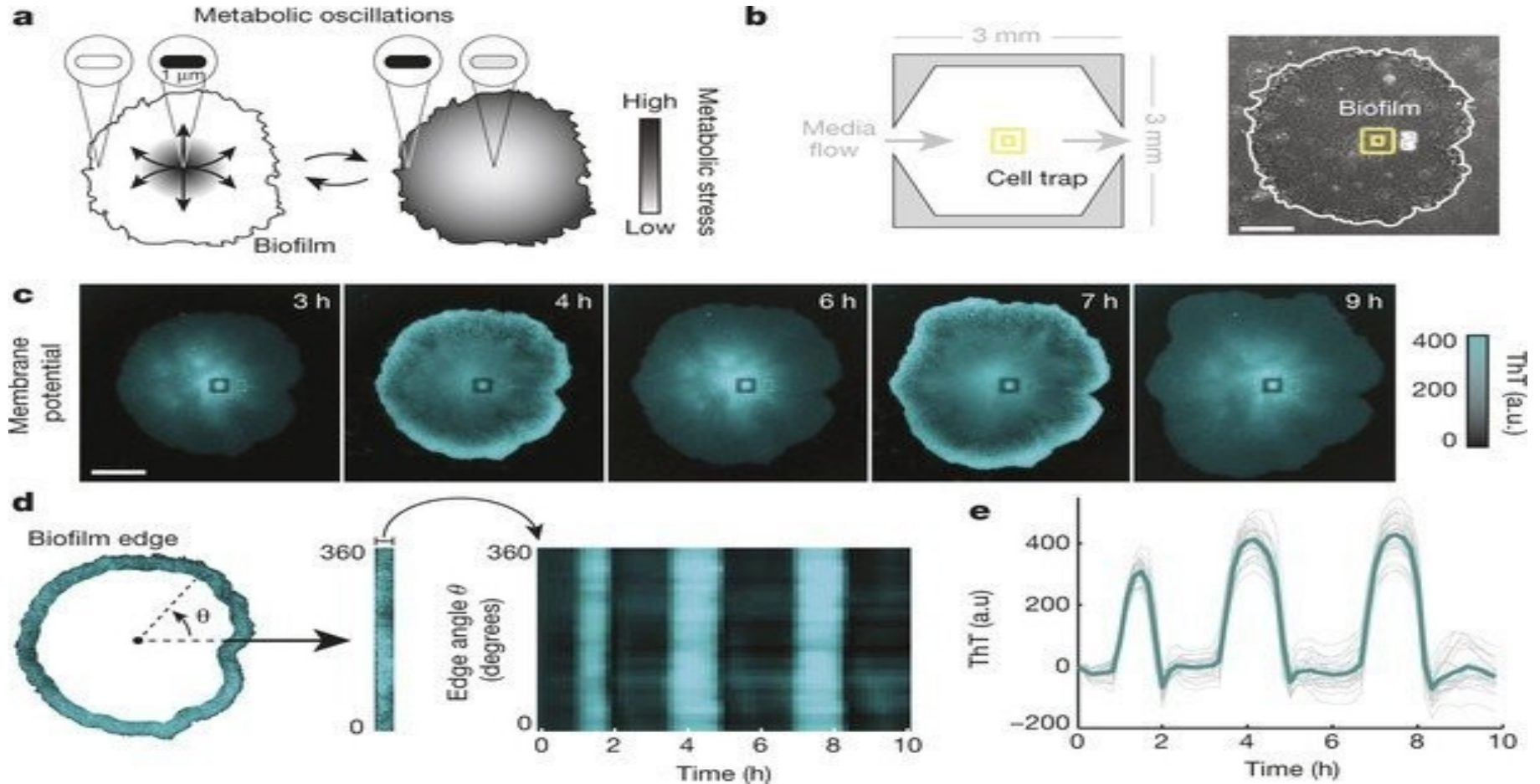


East Pacific Rise Hydrothermal Vent Biofilm



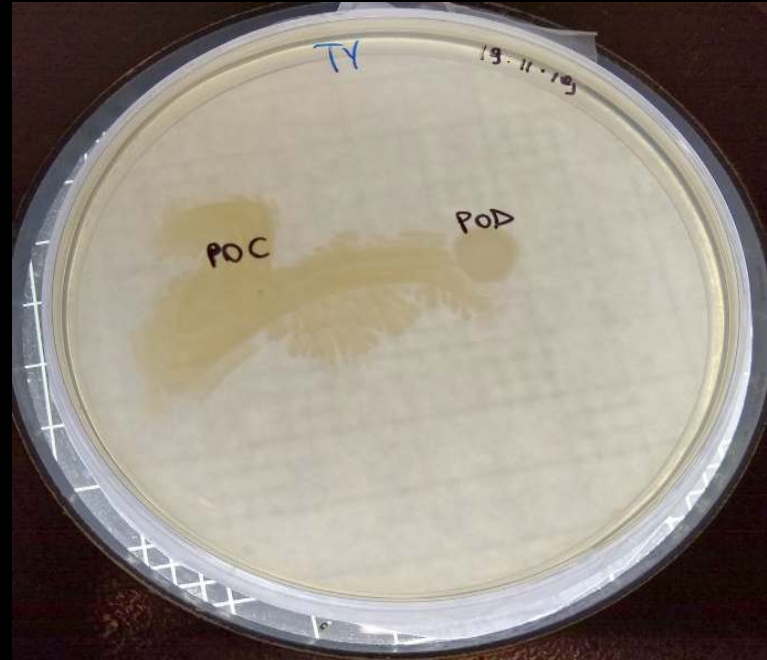
Tor Caldara Shallow Hydrothermal Vent Biofilm
photo credit C Vetraini (Rutgers U)

Biofilm Communication



*A type of side control:
'Chemical Warfare'*

Chemical Warfare



Different microbial isolates from a shallow-water hydrothermal vent interact on a plate

photo credit Martina Cascone / Giovannelli Lab

Chemical Warfare



Interactions between colonies of actinomycete bacteria. Streptomyces coelicolor (right) responds to an adjacent colony by initiating production of the red-pigmented antibiotics prodiginine and actinorhodin

Cornforth and Foster, 2015. PNAS

Antibiotics, Bacteriocines and others

Antibiotics are **natural compounds** (most of them), and are produced by microorganisms to **control their neighbor's growth**. They have been harvested and modified for human use in the last century. Antibiotics are **secondary metabolites**. Secondary metabolism (also called specialized metabolism) is a term for pathways and small molecule products of metabolism that are not absolutely required for the survival of the organism.

Bacteriocins are **ribosomally synthesized** (i.e., proteins) and produced during the primary phase of growth. Bacteriocins **restrict their activity to strains of related species** and particularly to strains of the same species. Antibiotics on the other hand have a wider activity spectrum and even if their activity is restricted this does not show any preferential effect on closely related strains.

The Microbial Loop

The microbial loop

The **microbial loop** describes a trophic pathway in the marine microbial food web where dissolved organic carbon (DOC) is returned to higher trophic levels via its incorporation into bacterial biomass, and then coupled with the classic food chain formed by phytoplankton-zooplankton-nekton.

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Published January 20

The Ecological Role of Water-Column Microbes in the Sea*

F. Azam¹, T. Fenchel², J. G. Field³, J. S. Gray⁴, L. A. Meyer-Reil⁵ and F. Thingstad⁶

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⁶ Institute for Microbiology, University of Bergen, Bergen, Norway

The microbial loop

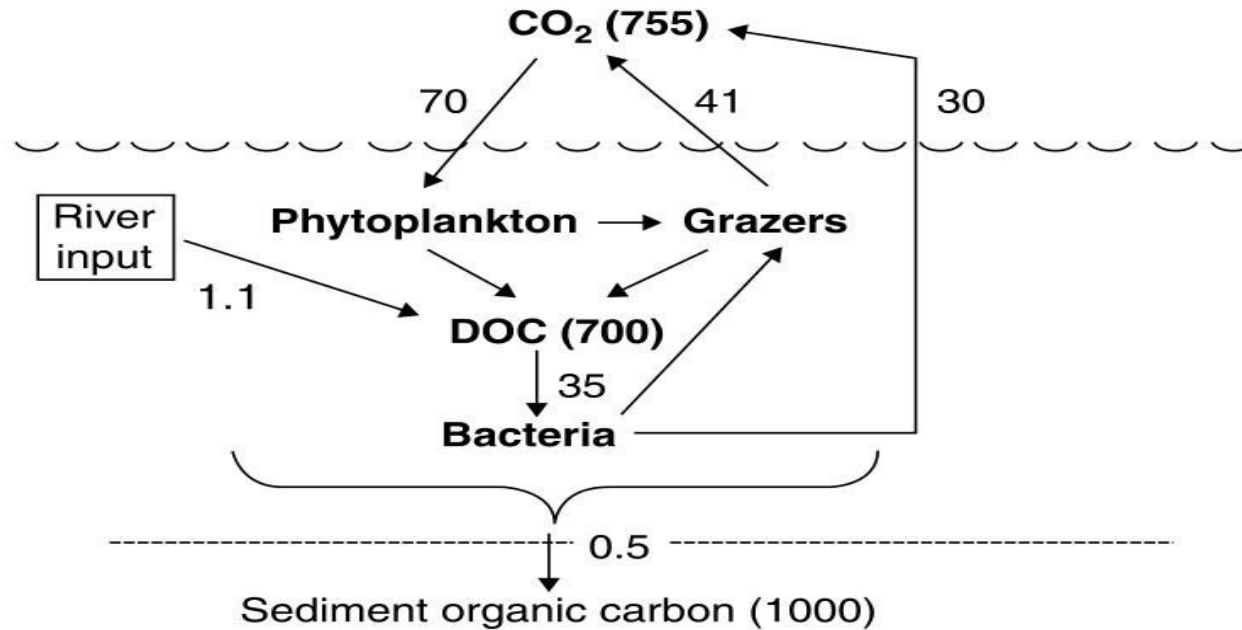


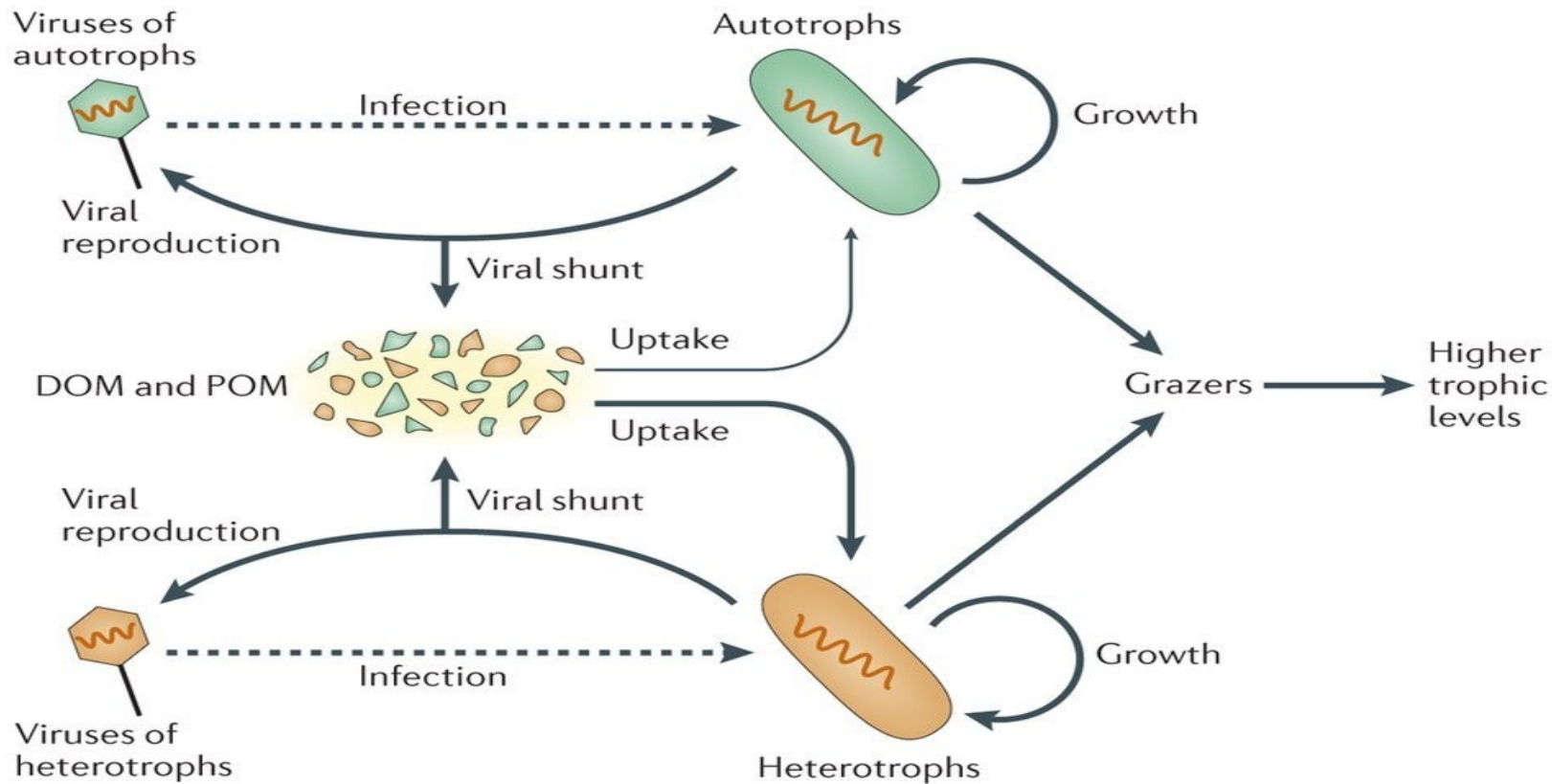
Figure 1.1 The role of microbes in the oceanic carbon cycle. The numbers in parentheses are standing stocks of carbon with units of pgC ($1 \text{ pgC} = 10^{15} \text{ gC}$). The other numbers are rates with units of pgC per year. The numbers are from Hedges and Oades (1997) and from estimates of the average fraction of primary production routed through dissolved organic carbon (DOC) to bacteria (Chapter 9), assuming a growth efficiency of 15 percent.



David Littschwager

seawater at 35x, photograph David Littschwager

The viral shunt



Nature Reviews | **Microbiology**

This week read

Barberán, A., Bates, S. T., Casamayor, E. O., and Fierer, N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* 6, 343–351. doi:10.1038/ismej.2011.119

Lozupone, C. A., and Knight, R. (2007). Global patterns in bacterial diversity. *PNAS* 104, 11436–11440. doi:10.1073/pnas.0611525104

Macalady, J. L., Hamilton, T. L., Grettenberger, C. L., Jones, D. S., Tsao, L. E., and Burgos, W. D. (2013). Energy, ecology and the distribution of microbial life. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 368, 20120383. doi:10.1098/rstb.2012.0383