



**Ciências
ULisboa**

Faculdade
de Ciências
da Universidade
de Lisboa



MICROBIOLOGIA E ENGENHARIA DE ECOSSISTEMAS

Microbiologia na engenharia dos sistemas aquáticos

13th April 2026

Cristina Cruz



Table of Contents

13th April 2026

01 Ecossistemas microbianos – Ecossistemas aquáticos

02 Distribuição e funções ecológicas.

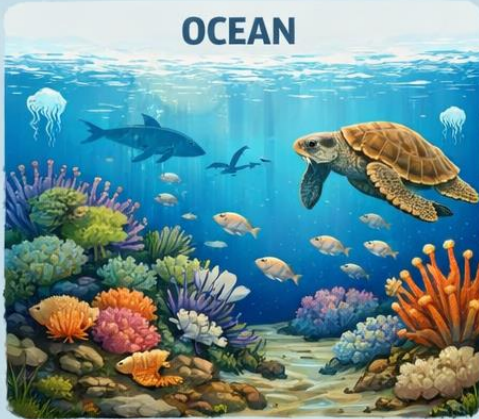
03 Poluição aquática e tratamento biológico:

Papel dos microrganismos na degradação de poluentes orgânicos e inorgânicos em sistemas de tratamento de água.



TYPES OF WATER ECOSYSTEMS

OCEAN



COASTAL WETLANDS



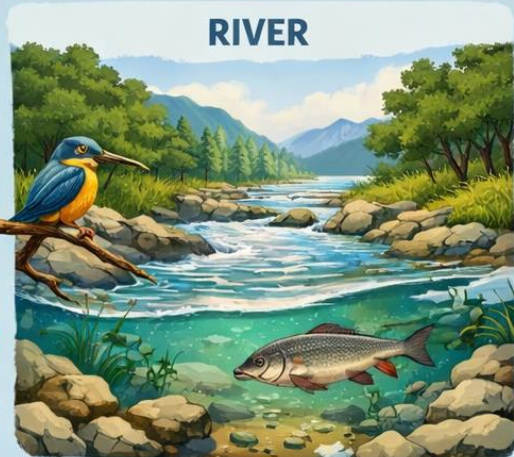
CORAL REEF



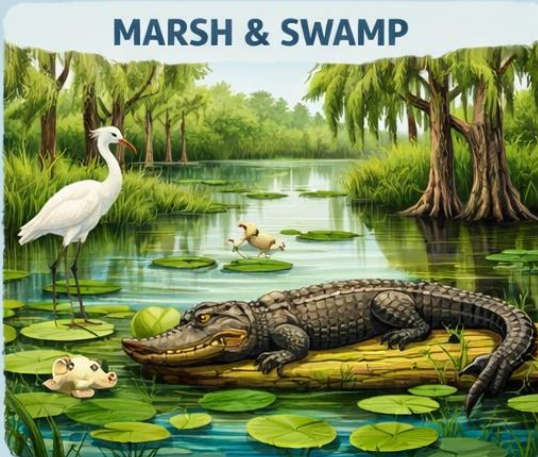
LAKE



RIVER



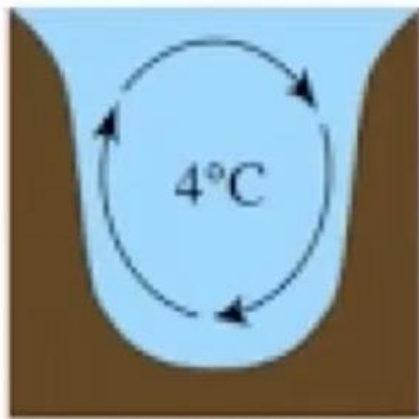
MARSH & SWAMP



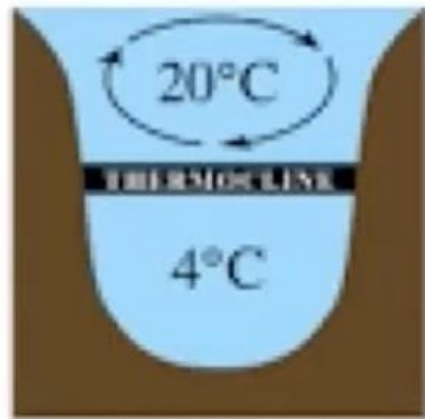


Lake stratification

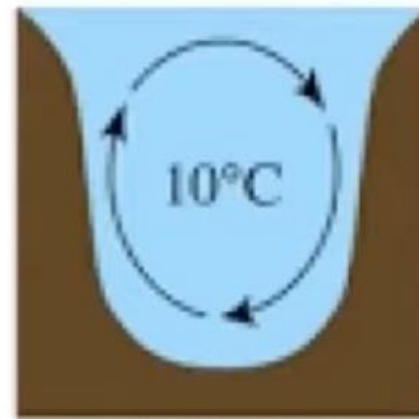
Dimictic large lakes



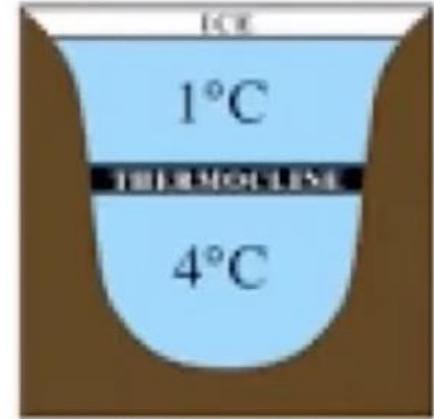
SPRING



SUMMER



FALL

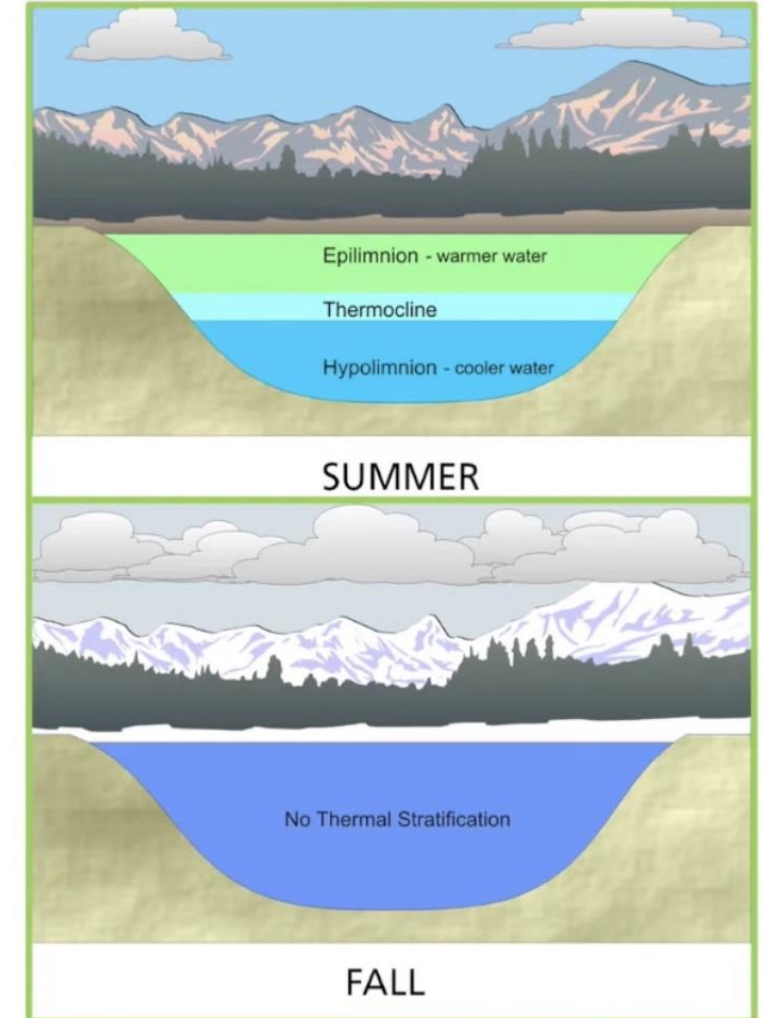


WINTER



Zonation Based on Temperature (Thermal Stratification)

- in summer water column in lakes becomes stratified
- separated into layers with different physical and chemical characteristics that constitute a stratified water column
- Epilimnion- warmer and less dense surface layers
- Thermocline- is the transition zone from epilimnion to hypolimnion, **prevents mixing of water** throughout the year, mixing only occurs during fall and spring as the water either cool or warms, respectively, and the thermocline disappears
- Hypolimnion- colder and denser bottom layers, anaerobic conditions prevail

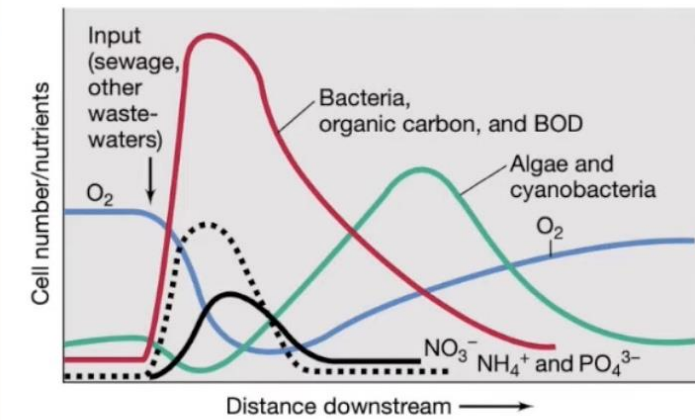




Biochemical Oxygen Demand

- stream systems both produces and consumes O_2
- the microbial oxygen-consuming capacity of a body of water is called its **biochemical oxygen demand (BOD)**
- BOD determination gives a measure of the **amount of organic material** in the water that can be **oxidized by the microbes** present in the water
- **higher BOD** indicates more O_2 is required, less O_2 is available for higher organisms, and signifies **low water quality**, aquatic organisms suffocate and die

The BOD of water, such as that from a temperate lake, is determined by taking a sample, aerating it well to saturate the water with dissolved O_2 , placing it in a sealed bottle, incubating it in the dark (to prevent photosynthetic oxygen production) for 5 days at 20 °C, and determining the residual oxygen in the water at the end of incubation. A BOD determination gives a measure of the amount of organic material in the water that can be oxidized by the microbes present in the water.



(a)



(b)

T. D. Brock



total microbial cell numbers are typically about 10-fold lower in the oceans than in freshwater environments ($10^6>ml$ versus $10^7>ml$, respectively).

- What is a primary producer? In a freshwater lake, would primary producers such as cyanobacteria more likely reside in the epilimnion or the hypolimnion, and why?
- Will addition of organic matter to a water sample increase or decrease its BOD?
- What factor(s) might account for the prokaryotic diversity of freshwater lakes?



1. Extract lake microbial community DNA
2. Metagenomic analysis of microbial community 16S rRNA genes

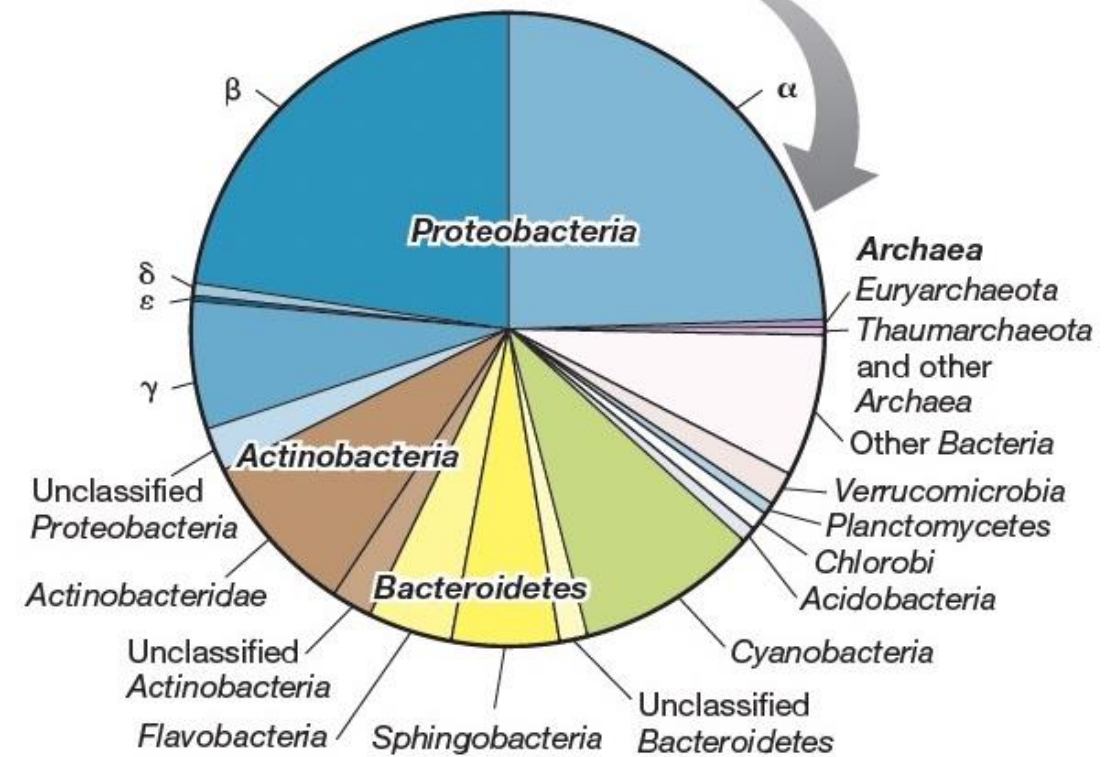
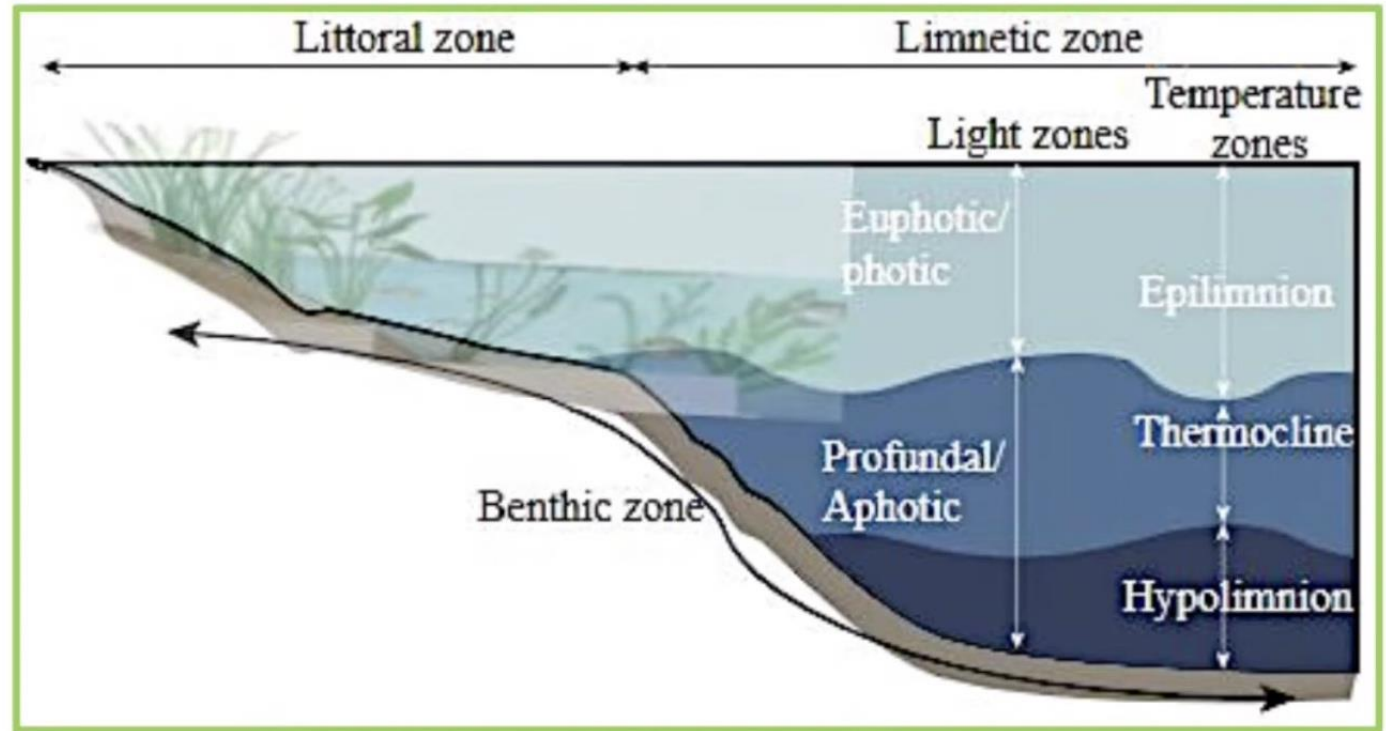


Figure 20.20 Freshwater lake bacterial and archaeal diversity. Distribution of 16S ribosomal RNA gene sequences by phylum determined from analysis of a collective dataset of 16S genes collected from the epilimnion of several freshwater lakes. Data assembled and analyzed by Nicolas Pinel.



Zonation Based on Depth and Distance

- **Littoral zone**
 - adjoins the shore, home to rooted aquatic plants, phytoplankton
 - extends to the light compensation level
- **Limnetic zone**
 - open water beyond the littoral zone
 - down the light compensation level
 - composed of planktons, nektons, and neustons
 - derives oxygen from phytoplankton
- **Euphotic Zone**
 - upper surface of limnetic zone from the open water contacting the surface oxygen to the region where the sunlight could penetrate
- **Pro-fundal zone**
 - the bottom and deep-water area
 - beyond the depth of effective light penetration
 - lacks oxygen, low fish population
- **Benthic zone**
 - lowest level of a body of water
 - includes sediment surface
 - harbors bacteria, mostly decomposers

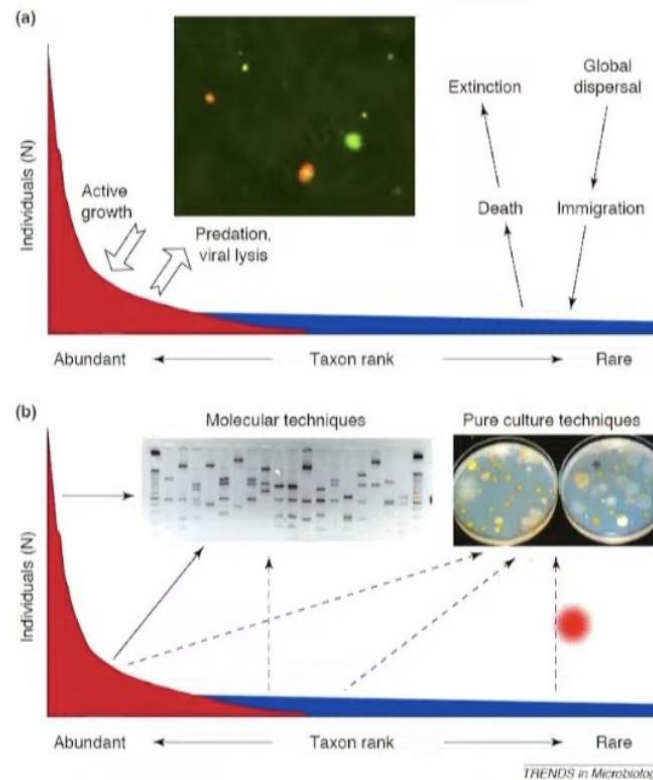




The rare biosphere concept

Abundant taxa:
Main contribution to
biogeochemical cycles

Rare biosphere:
Role of genetic diversity "seed bank"

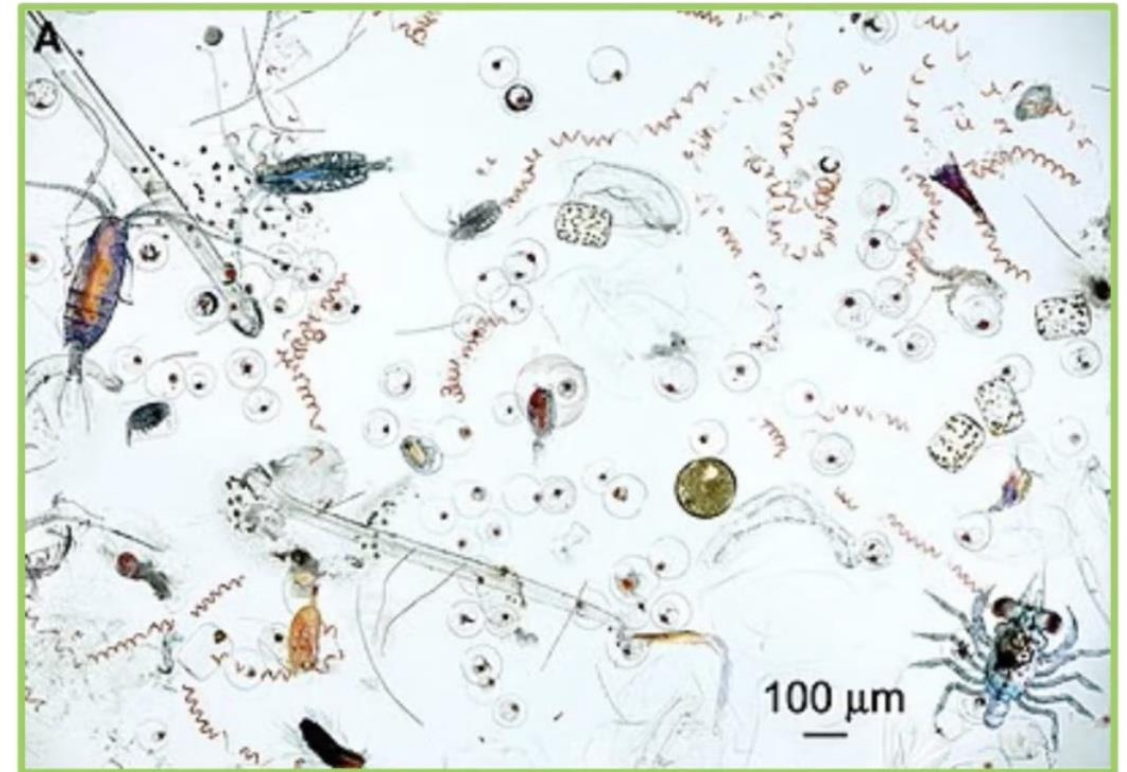


Pedrós-Alió, 2006, Trends in Microbiology



Freshwater Microbial Habitats

- Two types of freshwater environments
 - Lentic environments- contains still water (lakes, wetlands)
 - Lotic environments- contains flowing water (rivers, streams)
- microorganisms in aquatic environment
 - planktons- aggregation of floating and drifting microbes on the surface of the water, composed of cyanobacteria, phytoplankton (algae, diatoms) and zooplanktons (protozoa)
 - benthic microorganisms- inhabit the bottom region of the body of water
- oxygenic phototrophs, which obtain their energy from light and use water as an electron donor to reduce CO_2 to organic matter, are the main primary producers in freshwater aquatic ecosystems
- oxygenic phototrophs include the algae and cyanobacteria



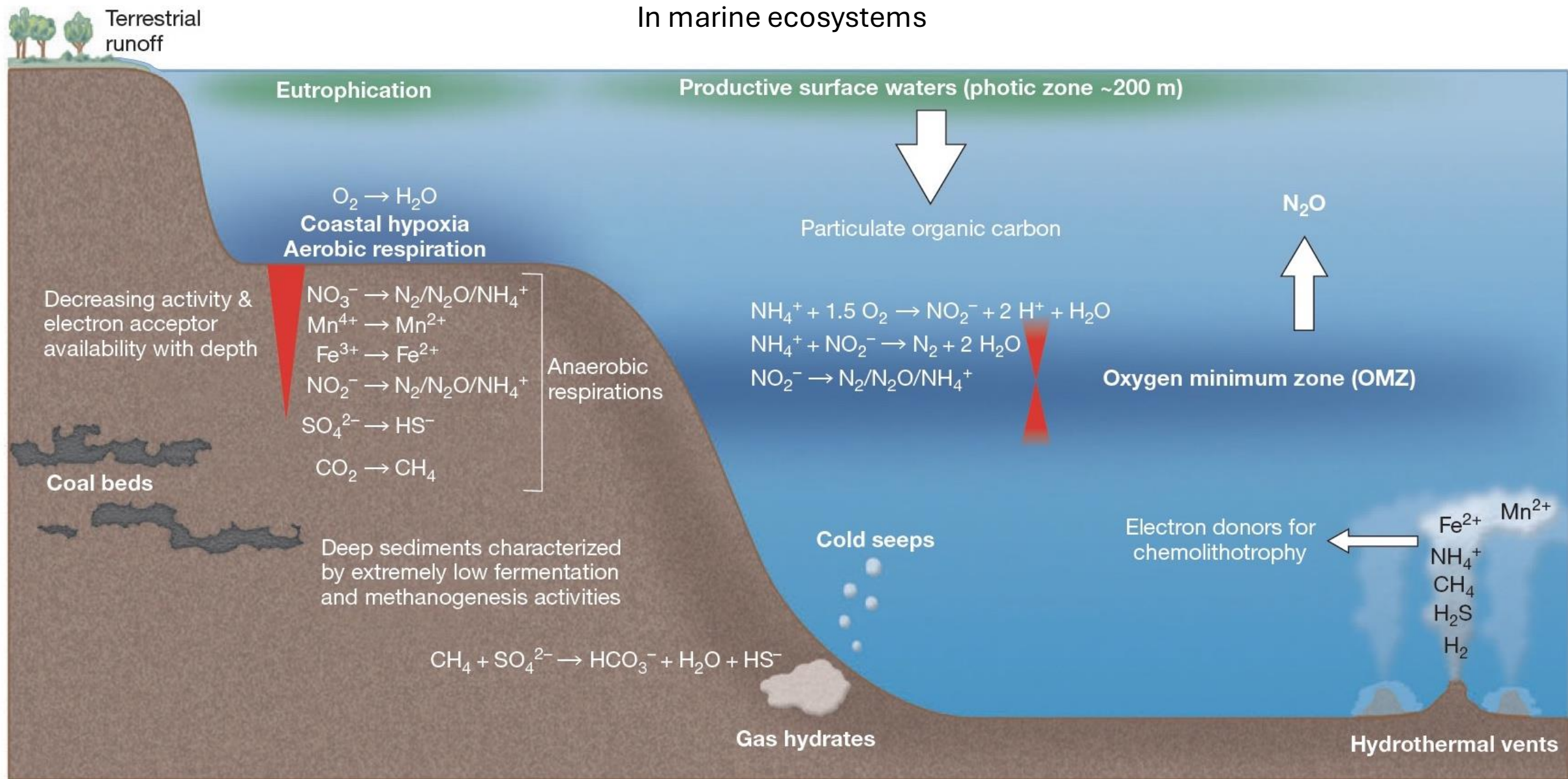
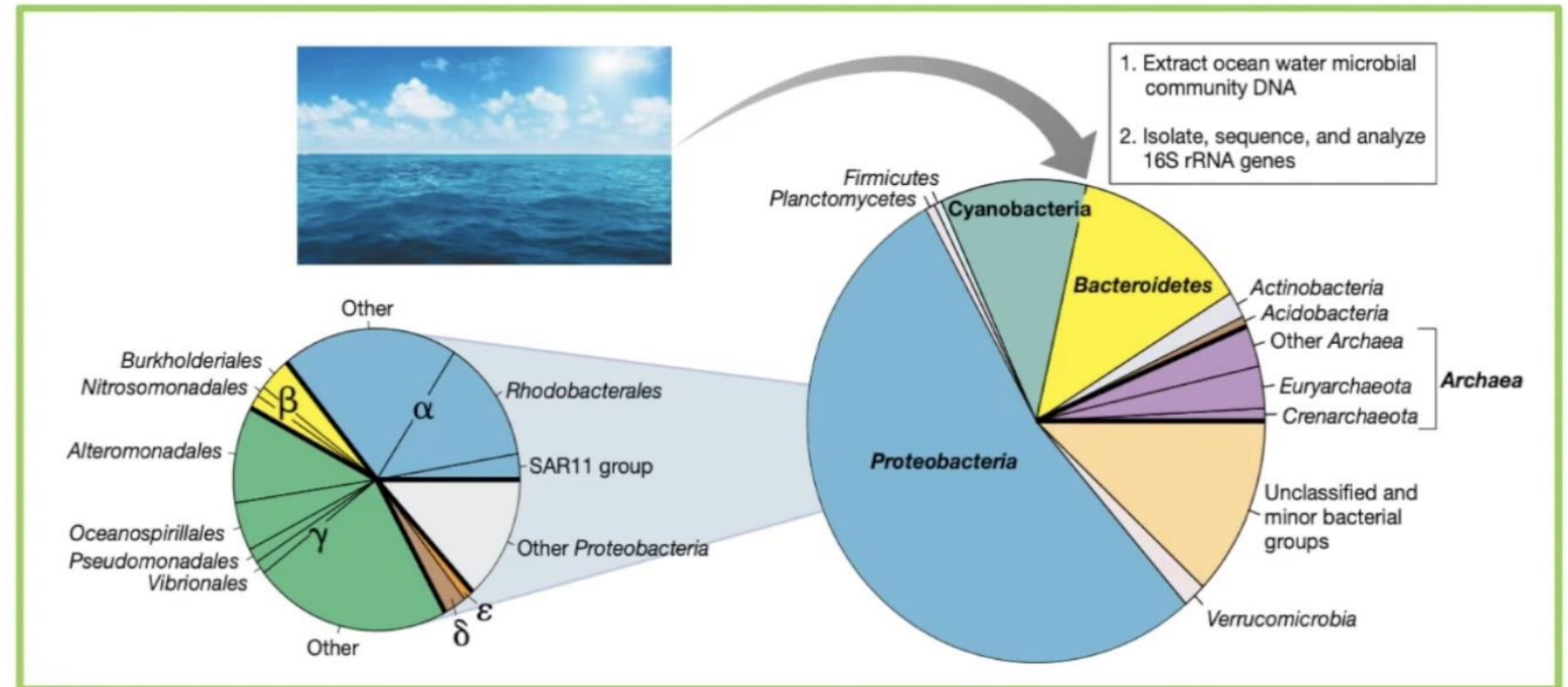


Figure 20.22 Diversity of marine systems and associated microbial metabolic processes. Decreasing electron acceptor availability with depth into the sediment or with increasing distance into an oxygen minimum zone is indicated by red wedges. Sulfate becomes limiting only at greater depths in marine sediments. The indicated metabolic diversity is described in Chapter 14.



Marine Bacterial and Archaeal Diversity

- **Bacteria:** *Alpha-* and *Gammaproteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Betaproteobacteria* and *Actinobacteria*; *Firmicutes*
- **Archaea:** *Euryarchaeota*, *Crenarchaeota*, and *Thaumarchaeota*, most of which have not yet been brought into laboratory culture





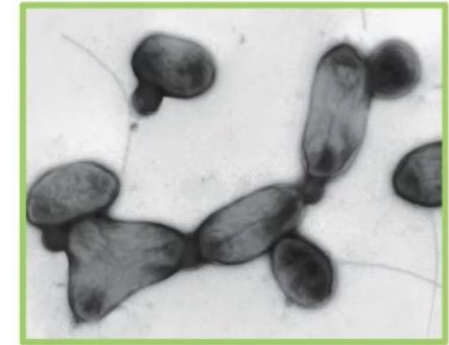
Marine Environment

- with the exception of oxygen, nutrient levels in the open ocean (the *pelagic zone*) are often very low compared with many freshwater environments
- this is especially true of key inorganic nutrients for phototrophic organisms, such as nitrogen, phosphorus, and iron
- in addition, water temperatures in the oceans are cooler and more constant seasonally than those of most freshwater lakes
- the activity of marine phototrophs is limited by these factors, and thus total microbial cell numbers are typically about 10-fold lower in the oceans than in freshwater environments
- most microbes in marine waters have very **small cells**, a typical characteristic of organisms living in nutrient-poor environments
- **smallness** is an adaptive feature for nutrient-limited microorganisms in that it requires less energy for cellular maintenance
- the trade-off is that a greater number of transport enzymes relative to cell volume are needed for organisms to acquire nutrients from very dilute (**oligotrophic**) than from nutrient-rich (**eutrophic**) aquatic environments

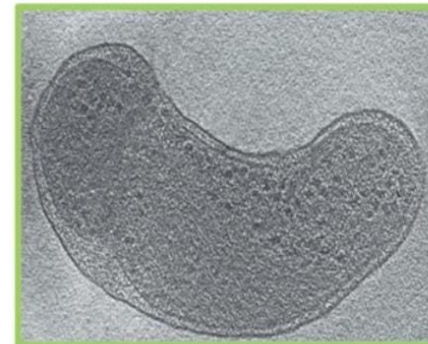
Prochlorococcus



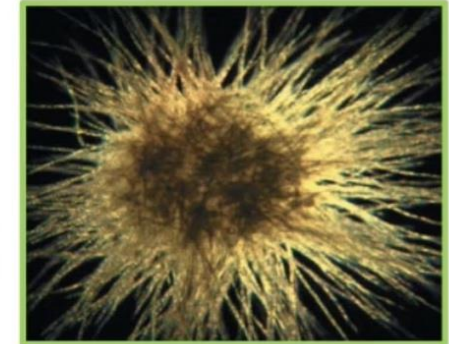
Citromicrobium



Pelagibacter



Trichodesmium

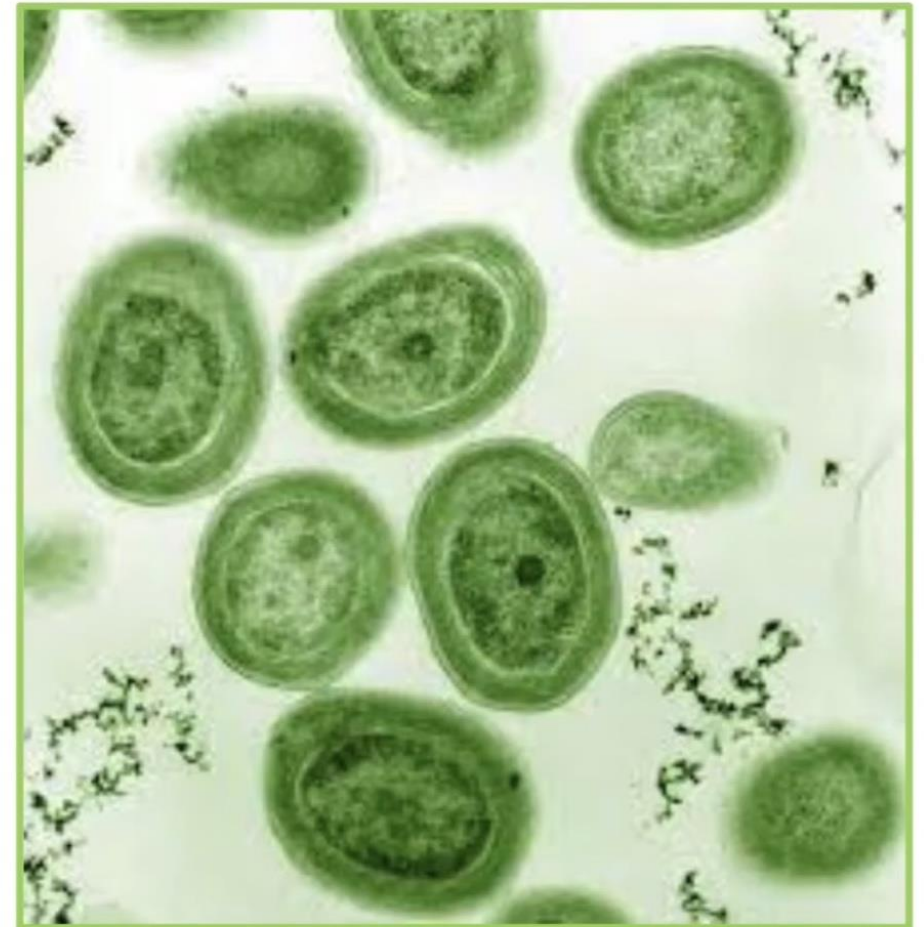




Major Marine Phototrophs

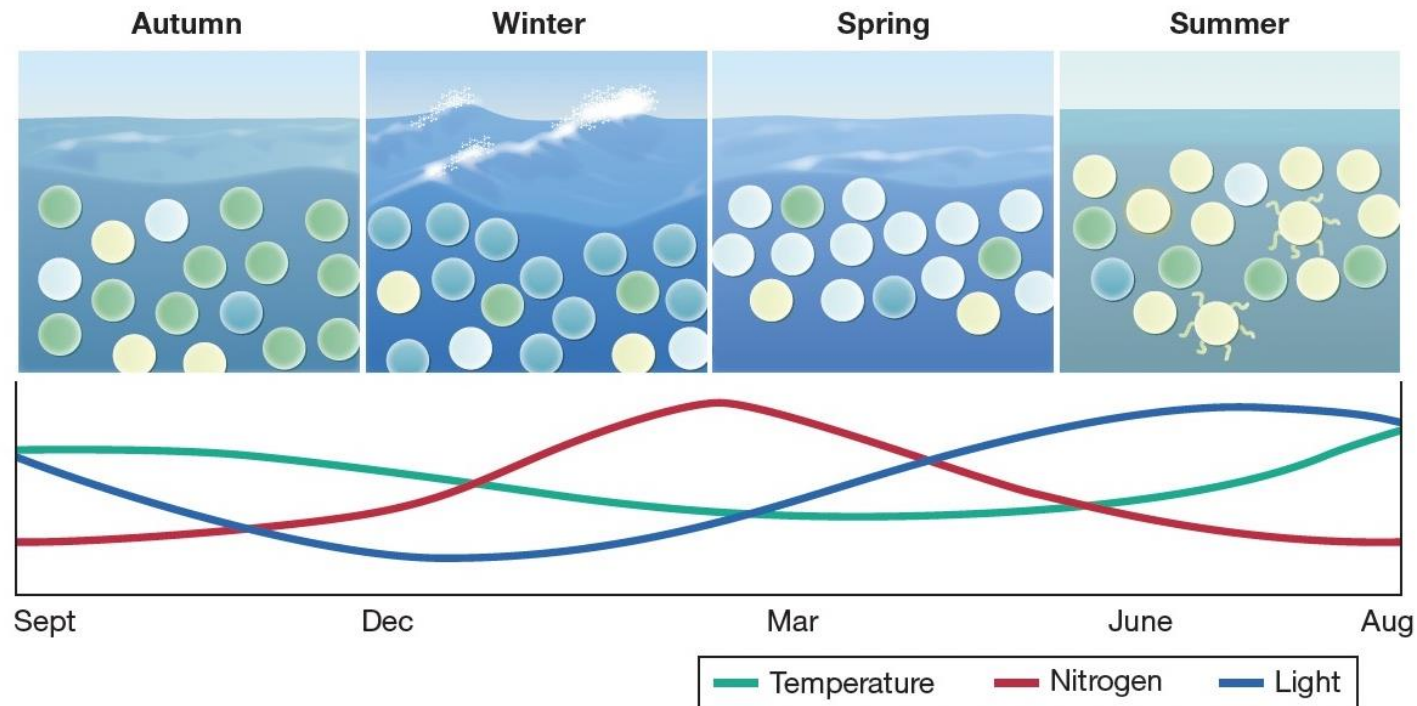
- much of the primary productivity in the open oceans, even at significant depths, comes from photosynthesis by **prochlorophytes**
- **prochlorophytes** contain chlorophylls *a* and *b* but do not contain phycobilins
- the organism *Prochlorococcus* is a particularly important primary producer in the marine environment
- *Prochlorococcus* lacks **phycobilins**, the accessory pigments of the cyanobacteria, dense suspensions of *Prochlorococcus* cells are olive green (as are green algae) rather than the blue-green color of cyanobacteria

*Prochlorococcus accounts for up to half of the photosynthetic biomass and primary production in the tropical and subtropical regions of the world's oceans, reaching cell densities of 10^5 > ml. A number of strains of *Prochlorococcus* have now been identified in culture, and each inhabits its own depth range in pelagic waters. The different *Prochlorococcus* strains are considered distinct ecotypes, genetic variants of a species that differ physiologically and therefore occupy slightly different niches.*

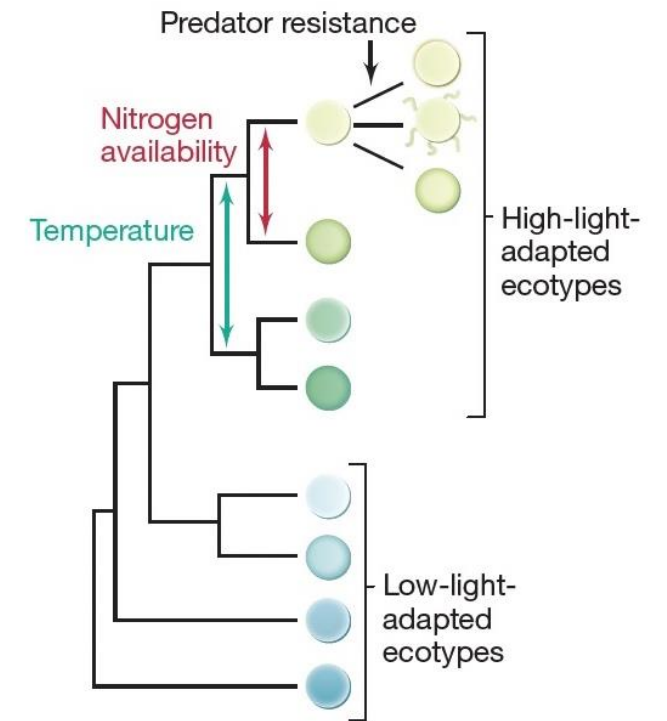




<https://www.youtube.com/watch?v=ylOlZz7s52Q>



(a)

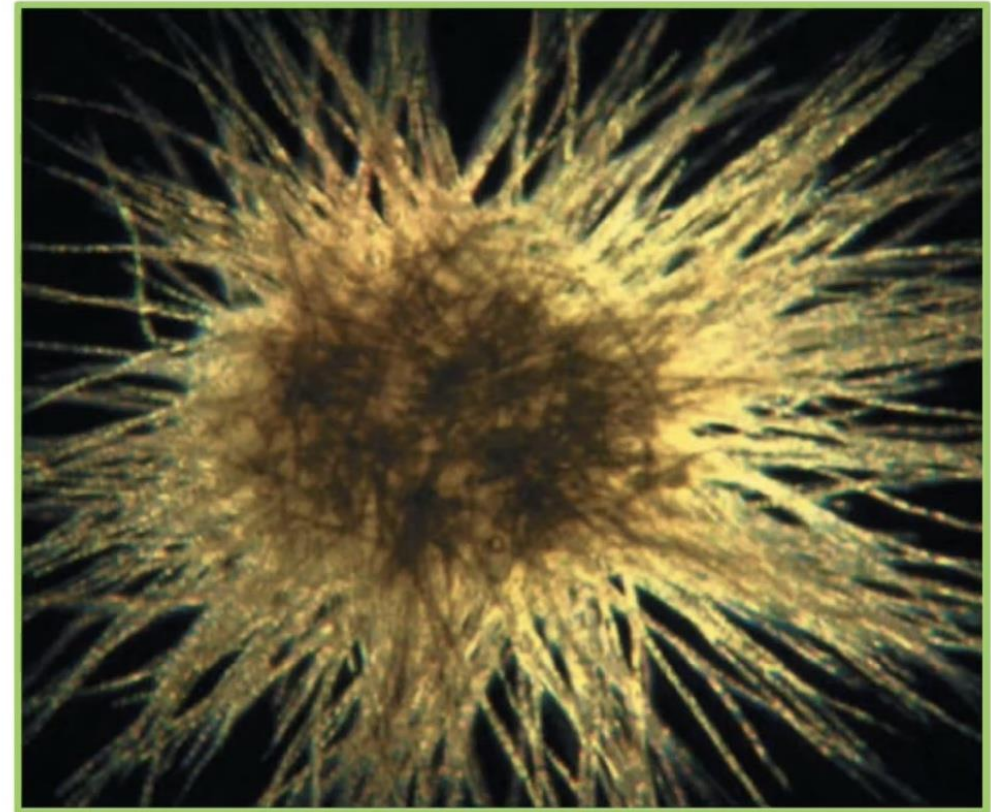


(b)

Figure 20.26 Seasonal variation of *Prochlorococcus* ecotypes in marine surface waters. (a) Single-cell genome sequencing (Section 10.11) has shown that the abundance of different genotypes, presumptive ecotypes (represented by cell color and surface features), correlate with seasonal changes in temperature, light, nutrients, and predators (grazers and bacteriophages). (b) Adaptations to different physical conditions (light and temperature) partition at higher taxonomic levels than do adaptations to different nutrient conditions (e.g., nitrogen availability) and resistance to specific predators, which show closer genetic relationships among ecotypes (see also Figure 19.24).



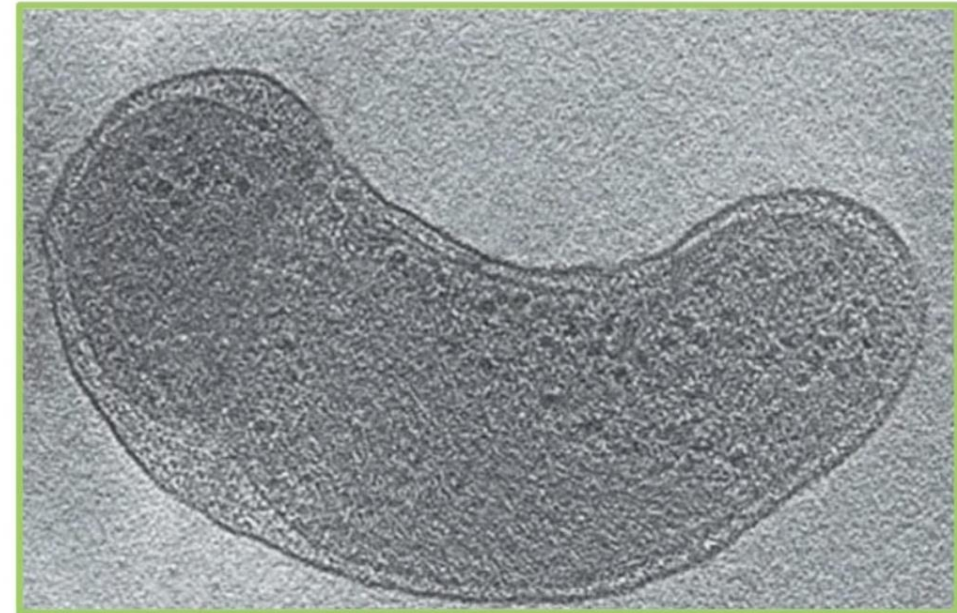
- *Trichodesmium*- planktonic filamentous marine cyanobacterium
- cells of *Trichodesmium* form puffs (colonies) of filaments, each puff can contain many hundreds of individual filaments, each filament composed of 20–200 cells
- *Trichodesmium* is a **nitrogen- fixing cyanobacterium**, and the production of fixed nitrogen by this organism is thought to be an important link in the marine nitrogen cycle
- *Trichodesmium* contains **phycobilins** (light-harvesting pigments found in cyanobacteria)





Pelagibacter: The Most Abundant Bacterium

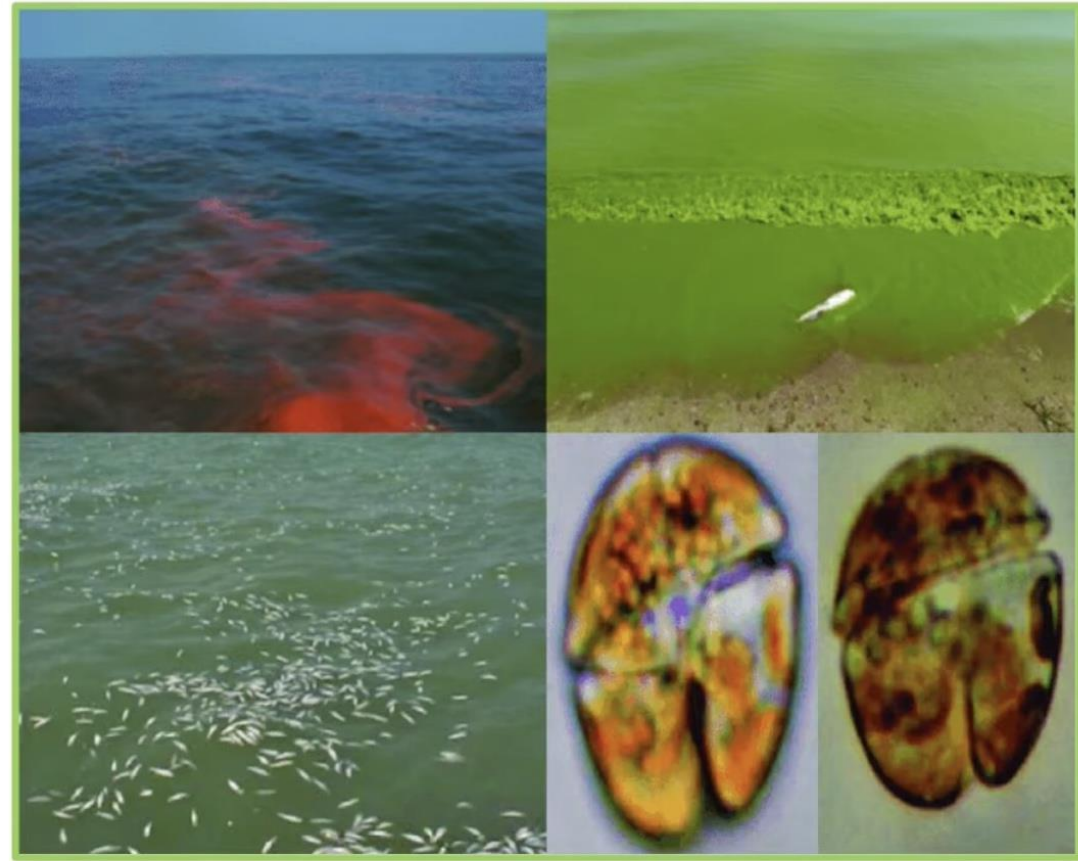
- *Pelagibacter* is an **oligotroph**, an organism that grows best at very low concentrations of nutrients
- cells are small rods with a diameter of only 0.2–0.5 μm
- the resulting **high surface-to-volume ratio** facilitates nutrient transport, increasing substrate concentration and processing rates within the cell
- high abundance of **periplasmic substrate-binding proteins** for soluble nutrients such as phosphate, amino acids, and sugars
- genome also contains genes encoding a form of the visual pigment **rhodopsin** that can convert light energy into ATP
- **bacteriorhodopsin**, a light-activated protein complex present in the extreme halophile *Halobacterium* (Archaea); bacteriorhodopsin functions in ATP synthesis as a simple light-driven proton pump
- the form of rhodopsin in *Pelagibacter* and other pelagic *Bacteria* is structurally similar to bacteriorhodopsin and has been called **proteorhodopsin** (“proteo” referring to *Proteobacteria*)
- the different variants of proteorhodopsins in marine microbes have absorption properties that reflect changing spectral properties of light at increasing depths in the water column, with **near-surface variants absorbing green light** and those at **greater depths absorbing blue light**





Harmful Algal Blooms or HABs (Red Tide)

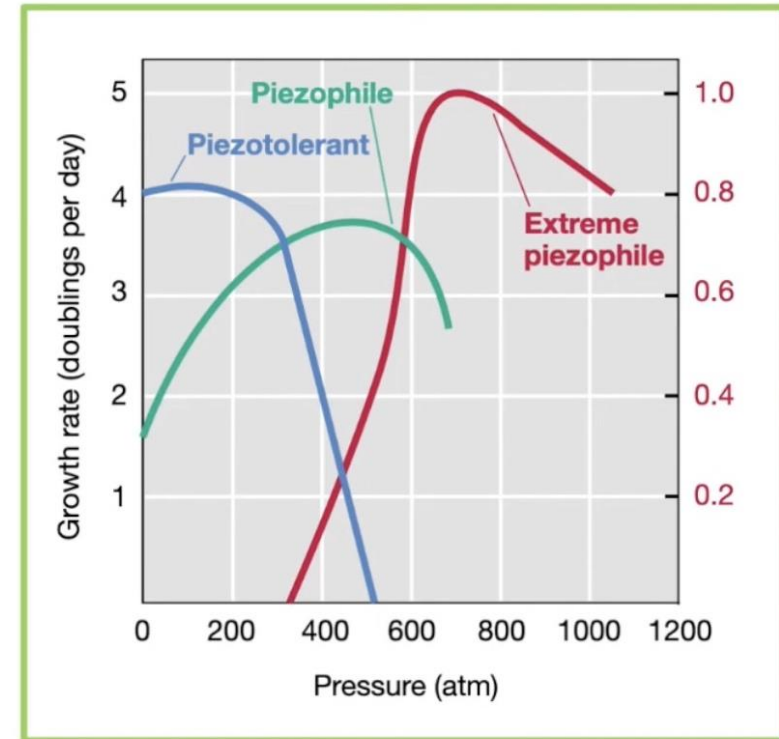
- **eutrophication** is overabundance of nutrients (nitrogen, phosphorus) fertilizers run-off into nearby water can cause harmful algal blooms or red tide, anoxic events, and fish kills
- colonies of algae feed on the nutrients and grow out of control while producing toxic or harmful effects on people, fish, shellfish, marine mammals, and birds
- Diatom *Pseudo-nitzschia* spp. in shellfish produce Domoic acid can cause short-term memory loss, vomiting, cramps
- Dinoflagellate *Karenia* spp. in shellfish produce Brevetoxin can cause nausea, diarrhea, eye irritation, respiratory distress, death
- *Cyanobacteria* in water produce Microcystins are potent liver toxins and possible human carcinogen





The Deep Sea

- light penetrates no farther than about 300 m in pelagic waters
- this illuminated region is called the **photic zone**
- beneath the photic zone, down to a depth of about 1000 m, there is still considerable biological activity
- water at depths greater than 1000 m is, by comparison, much less biologically active and is known as the **deep sea**
- greater than **75% of all ocean water is deep-sea water**, lying primarily at depths between 1000 and 6000 m
- organisms that inhabit the deep sea face three major environmental extremes:
 - low temperature
 - high pressure
 - low nutrient levels
- in addition, deep-sea waters are completely dark such that photosynthesis is impossible, thus, microbes that inhabit the deep sea must be **chemotrophic** and able to grow under high pressure and **oligotrophic** conditions in the cold.
- bacteria isolated from marine waters below 100 m are **psychrophilic** (cold-loving) or at least psychrotolerant





Deep-Sea Sediments

- archaeal and bacterial populations at depths greater than 2000 m
- **depletion of electron acceptors and organic matter with increasing depth** constrains energy available to the deep subsurface microbial communities, accounting for the major decrease in cell numbers with depth
- deep core samples have clearly established that novel **Archaea** make up a large fraction of the archaeal diversity and that bacterial diversity in both deep and shallow marine sediments is dominated by *Proteobacteria*
- **sulfate reduction** is the major form of anaerobic respiration in marine sediment
- employ many of the strategies similar with marine pelagic microbes including **small cell size** and **small, compact genomes**



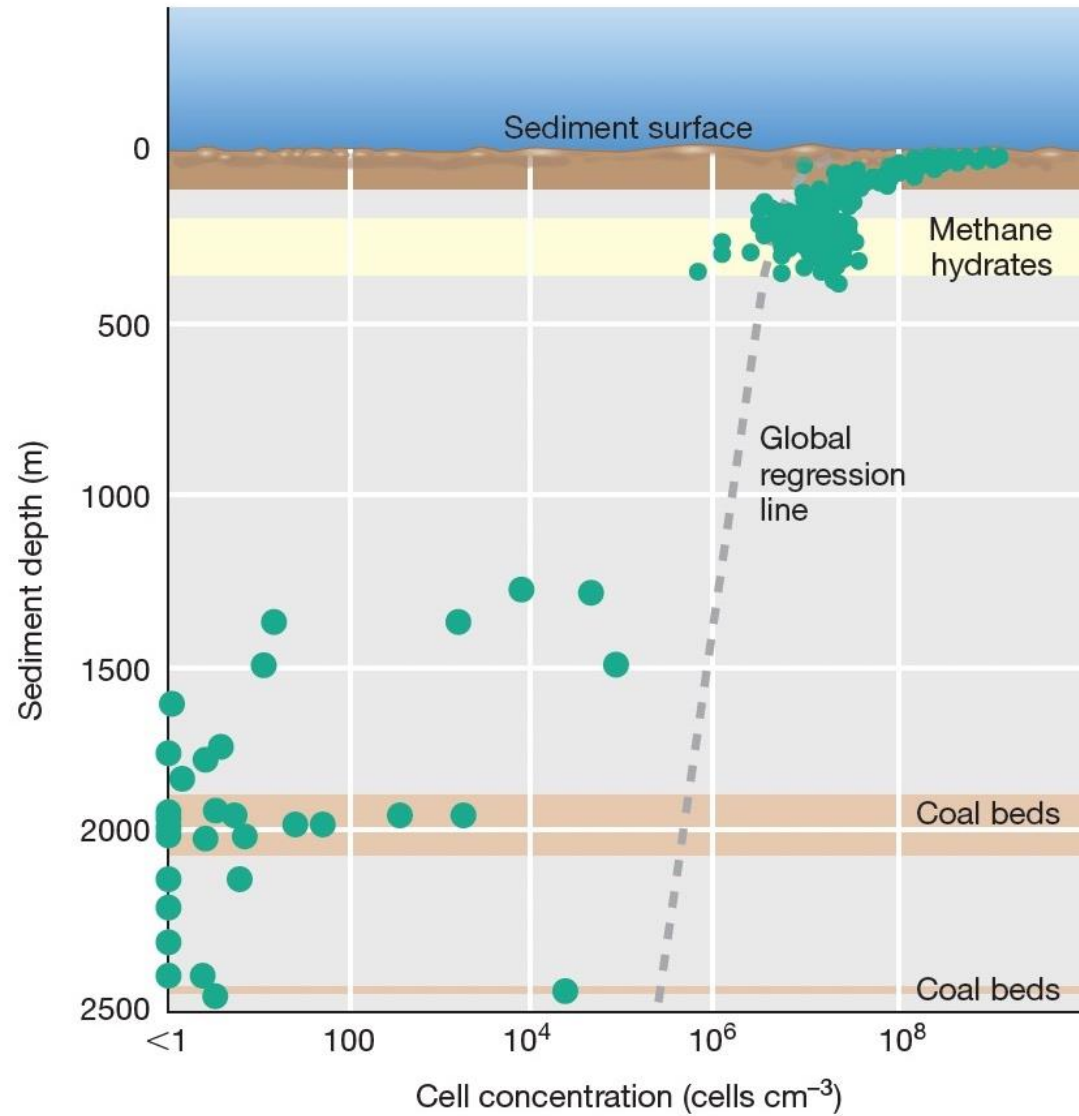


Figure 20.41 Microbial cell numbers in deep-sea sediments. Depth-related cell abundance from the analysis of one coastal sediment (filled circles) and the global average for all sampled ocean sediments (dashed regression line) based on cell count data.

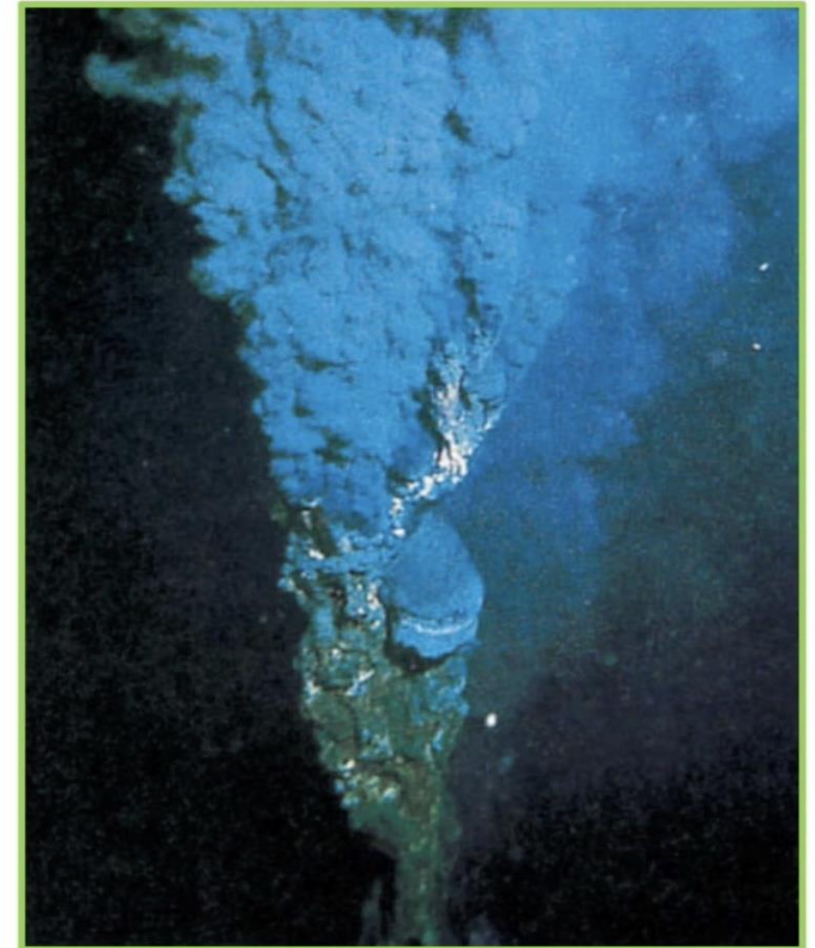


Figure 20.42 Marine sediment bacterial and archaeal diversity. The results are pooled analyses of 13,360 16S ribosomal RNA gene sequences from several studies of shallow and deep marine sediments. Many of the groups indicated are covered in Chapters 15 and 16 (*Bacteria*) or 17 (*Archaea*). For *Proteobacteria*, major subgroups are indicated. Note the high proportion of archaeal sequences and of *Gamma*-, *Delta*-, and *Epsilonproteobacteria*. Data assembled and analyzed by Nicolas Pinel. Compare the prokaryotic diversity of marine sediments with that of open ocean water shown in Figure 20.31.



Hydrothermal vents

- **hydrothermal vents** are underwater hot springs that are located at depths from less than 1000 m to greater than 4000 m in regions of the seafloor where volcanic magma and hot rock have caused the floor to rift apart at crustal spreading centers
- hot vents, called **black smokers**, form upright sulfide edifices called **chimneys** that can be less than 1 m to over 30 m in height
- walls of smoker chimneys are teeming with **hyperthermophiles** such as *Methanopyrus*, a species of *Archaea* that oxidizes H_2 and makes CH_4
- the most thermophilic of all known **sulfur-reducing microbes**, species of *Pyrolobus* and *Pyrodictium*, were isolated from black smoker chimney walls
- **methanogens** of the genus *Methanosarcina* and are nourished by the H_2 -rich fluids that permeate the porous chimney walls



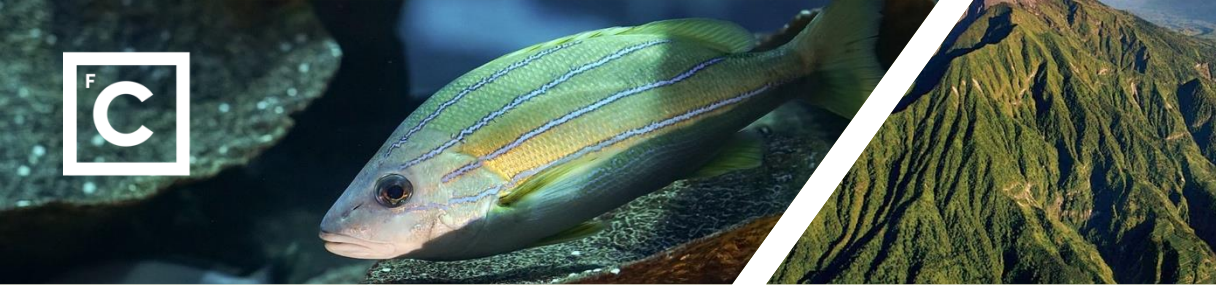
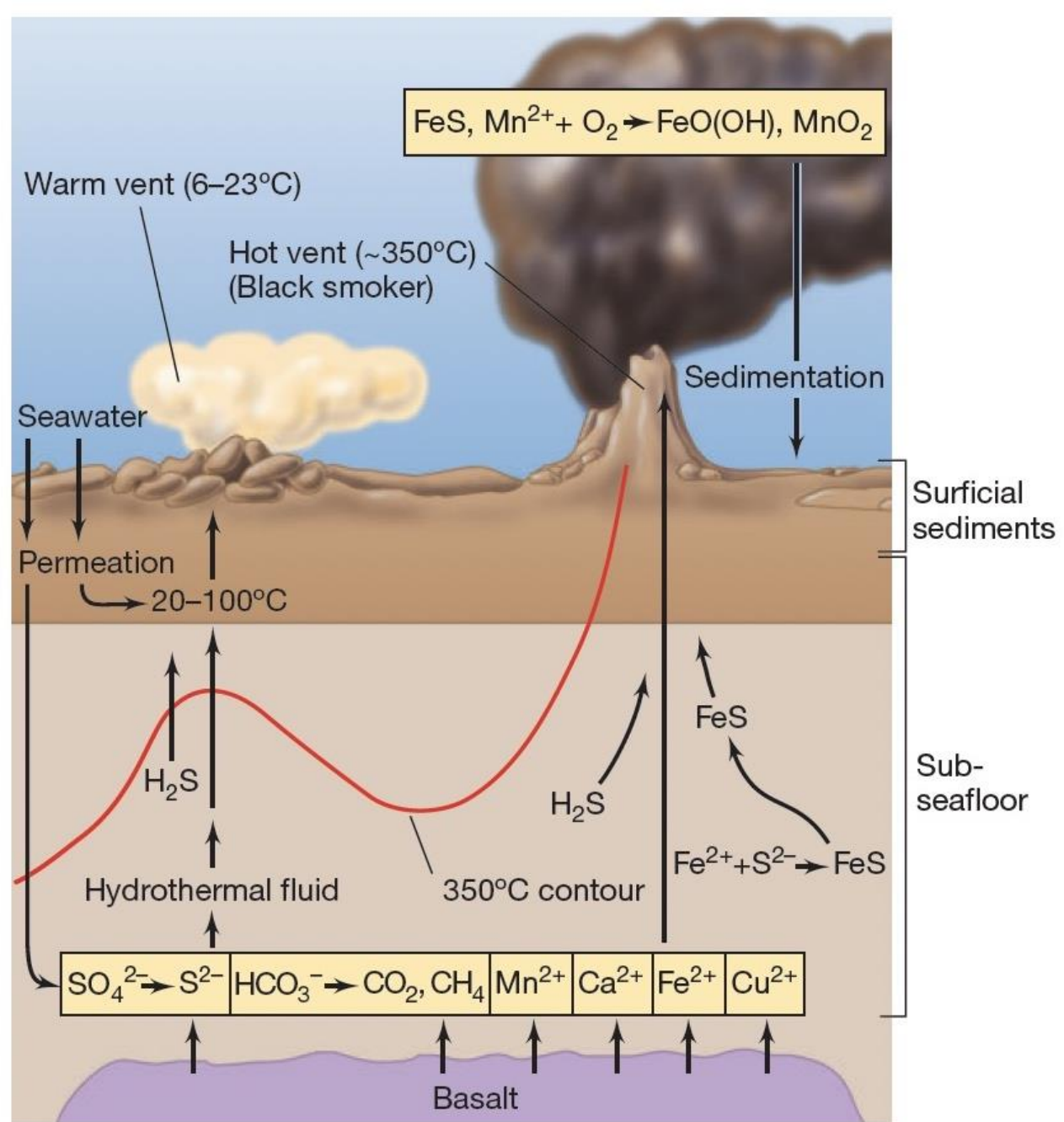


Figure 20.43 Hydrothermal vents. Schematic showing geological formations and major inorganic chemicals and minerals that are emitted from warm vents and black smokers. In warm vents, the hot hydrothermal fluid is cooled by cold 2–3°C seawater permeating the sediments. In black smokers, hot hydrothermal fluid near 350°C reaches the seafloor directly. The term “surficial” is a geological term meaning surface, in this case, the top layers of sediment.



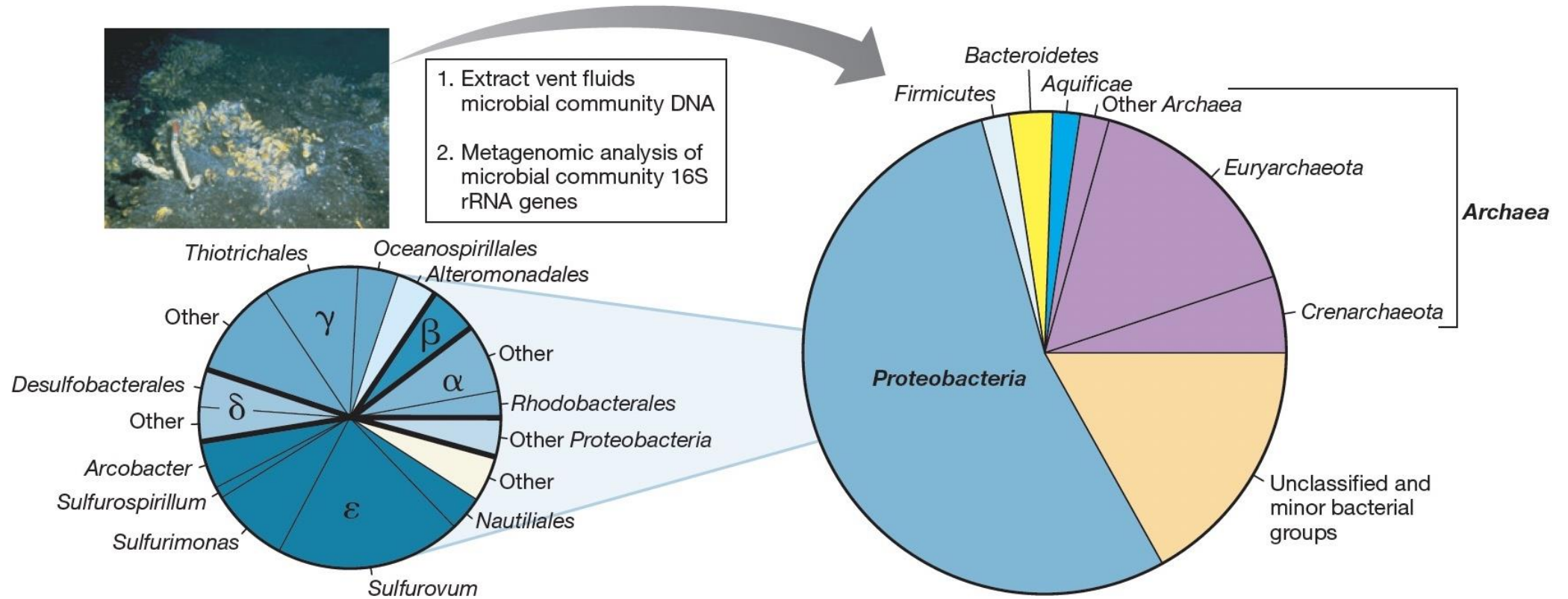


Figure 20.47 Hydrothermal vent bacterial and archaeal diversity. The results are pooled analyses from several studies of the 16S rRNA gene content of warm and hot hydrothermal vents. Many of these groups are covered in Chapters 15 and 16 (*Bacteria*) or 17 (*Archaea*). For *Proteobacteria*, major subgroups are indicated. Note the high proportion of *Archaea* and of *Epsilonproteobacteria*. The physiology of many of these organisms is summarized in Table 20.2. Most of the chemolithotrophs listed in Table 20.2 grow with CO₂ as carbon source (autotrophic growth). Data assembled and analyzed by Nicolas Pinel.



Diversity of inland waters

Surface Size
Depth

Stability of
environmental
conditions

Chemistry

Nutrients

Connectivity



Freshwater
Saline

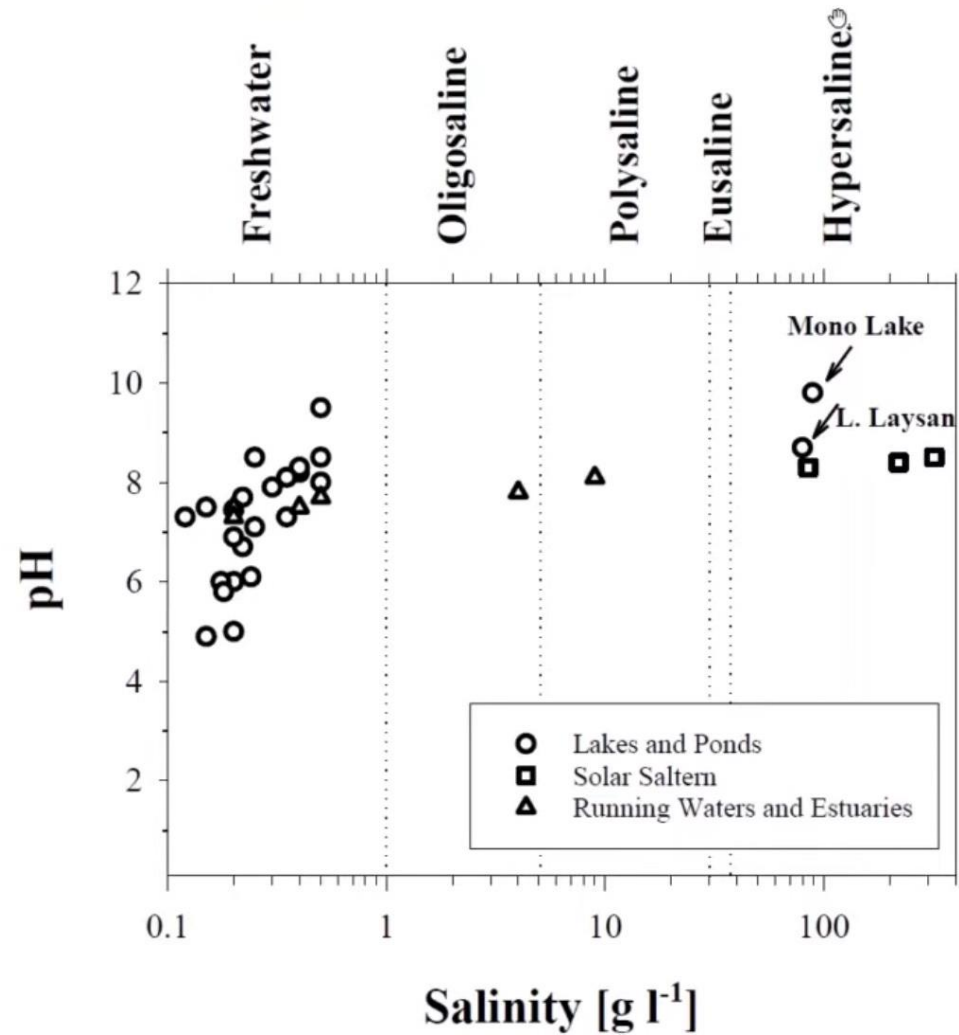
Standing waters
Running waters

Lakes
Ponds
Puddles

Permanent
Temporary

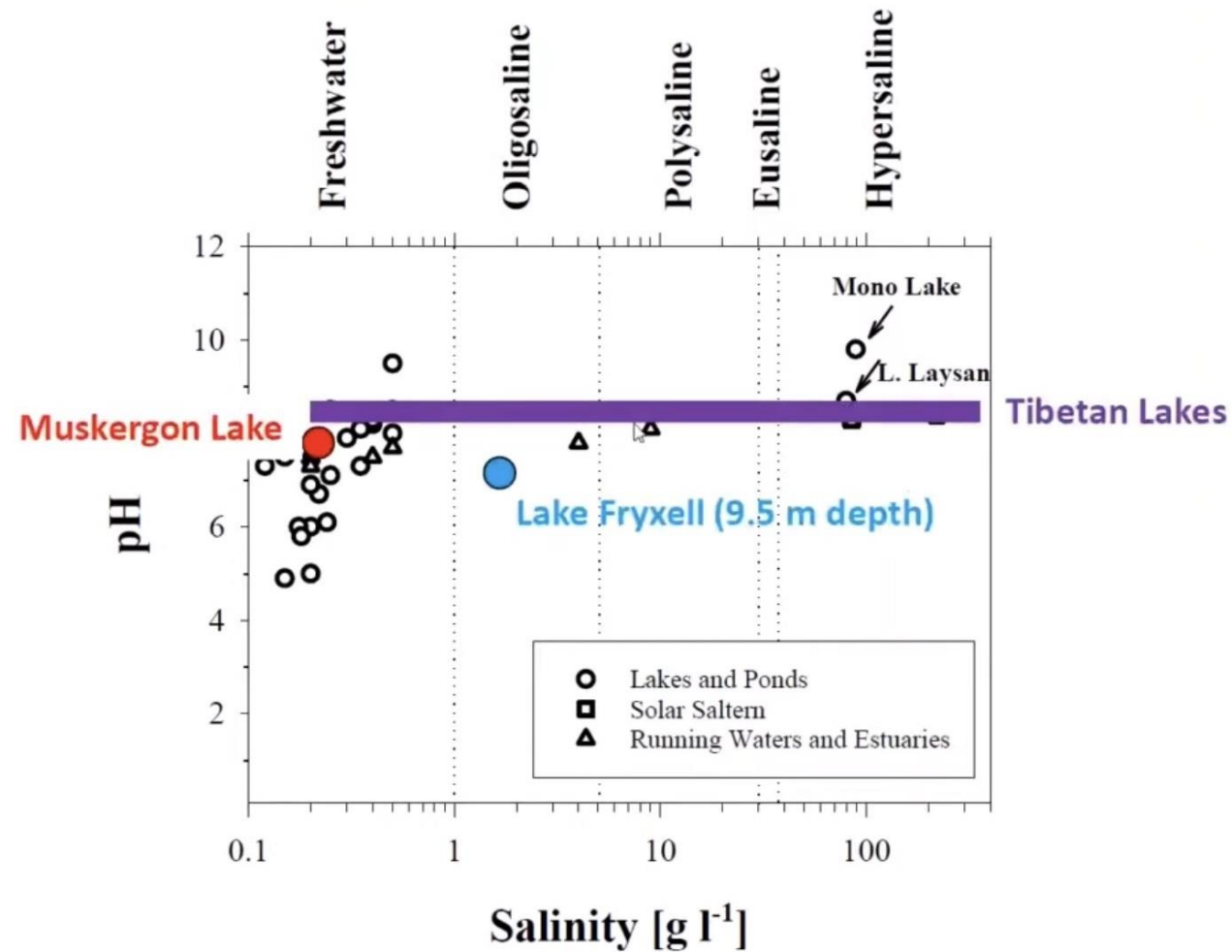


Salinity and pH are major factors controlling microbial community composition





Salinity and pH are major factors controlling microbial community composition





Inland water systems are chemically highly diverse

- pH
- Salinity
- Major ions
- DOC (concentration, quality)
- Concentration of nutrients -> productivity, growth limitations





Confluence of Rio Negro and Amazon River

Inhabited by distinct fish communities

Rio Negro blackwater

pH 3-4
conductivity $< 10 \mu\text{S}/\text{cm}$
rich in humic substances
high water transparency

Amazon River (Rio Solimões)

whitewater
pH 6.5 – 7.5
Conductivity $> 200 \mu\text{S}/\text{cm}$
rich in inorganic substances
low water transparency



Photo: European Space Agency



Oxygen Relationships in Freshwater Lakes

The biological and nutrient structure of lakes is greatly influenced by seasonal changes in physical gradients of temperature and salinity. In many lakes in temperate climates, the water column becomes

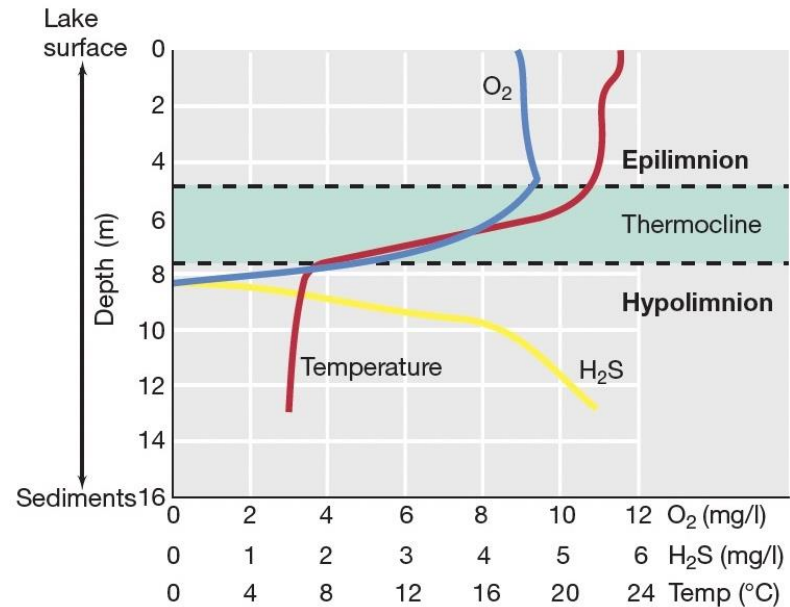
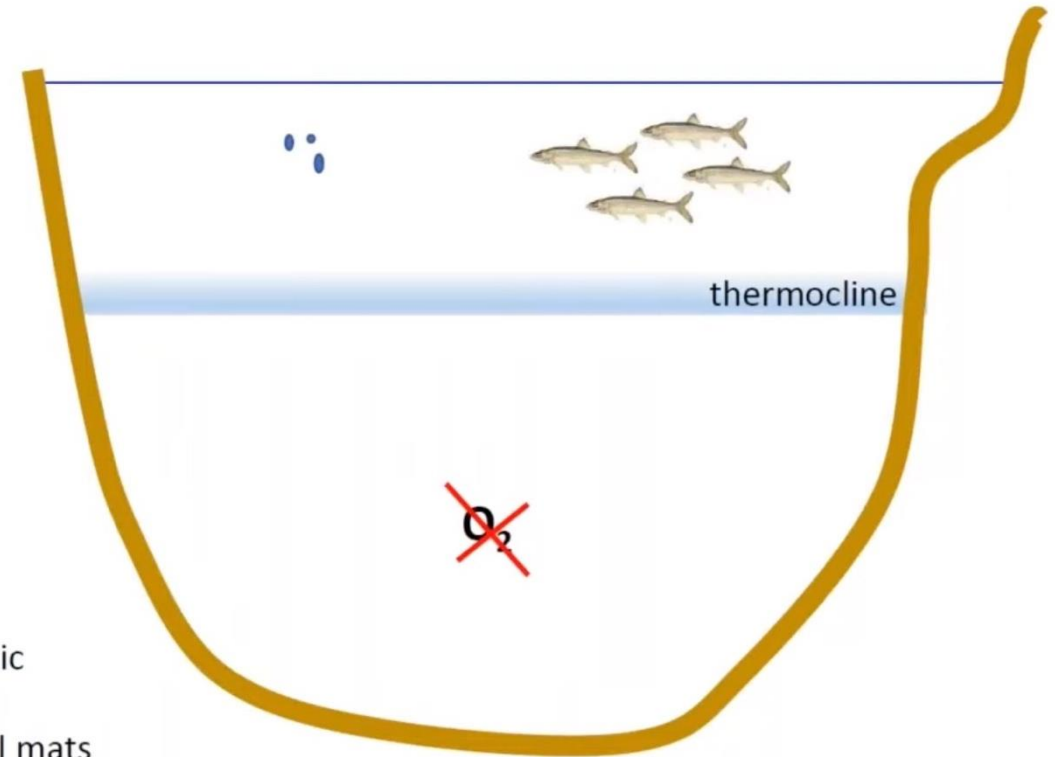


Figure 20.18 Development of anoxic conditions in waters of a temperate lake due to summer stratification. The colder bottom waters are more dense and contain H₂S from bacterial sulfate reduction. The thermocline is the zone of rapid temperature transition. As surface waters cool in the fall and early winter, they reach the temperature and density of hypolimnetic waters and sink, displacing bottom waters and effecting lake turnover. Data are from a small freshwater lake in northern Wisconsin (USA).

Microhabitats



- planktonic
- attached
- microbial mats
- host associated
- sediment surface
- etc.



Challenge for “Freshwater” Microbial Ecology

- Microbial community composition/assembly
- Diversity
- Ecosystem services
- Etc.

Find universal mechanisms valid for all types of inland waters

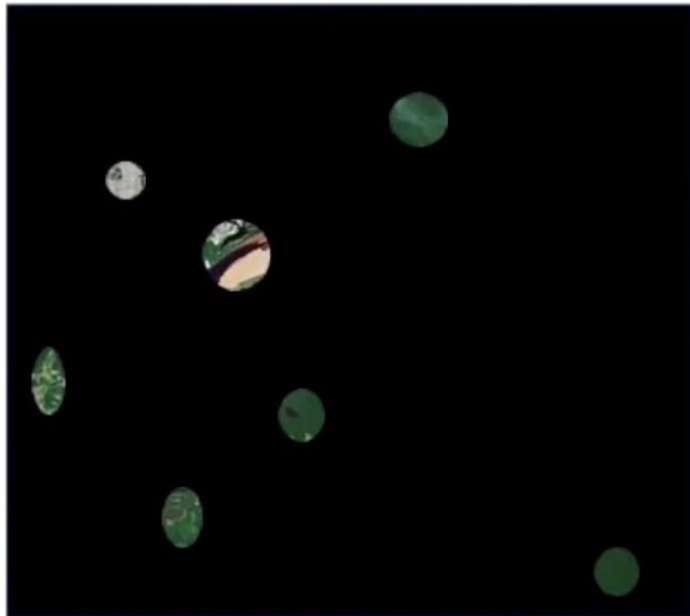
or

identify groups of systems/habitat types/microhabitats sharing mechanisms



How far have we come?

universal mechanisms
versus
non-universal mechanisms





FEMS Microbiology Ecology, 96, 2020, fiae029

doi: [10.1093/femsec/fiae029](https://doi.org/10.1093/femsec/fiae029)

Advance Access Publication Date: 27 February 2020

Research Article

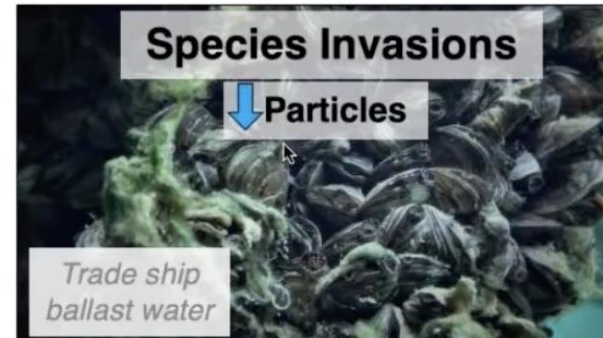
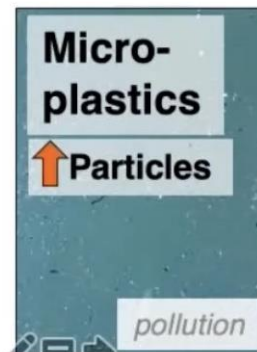
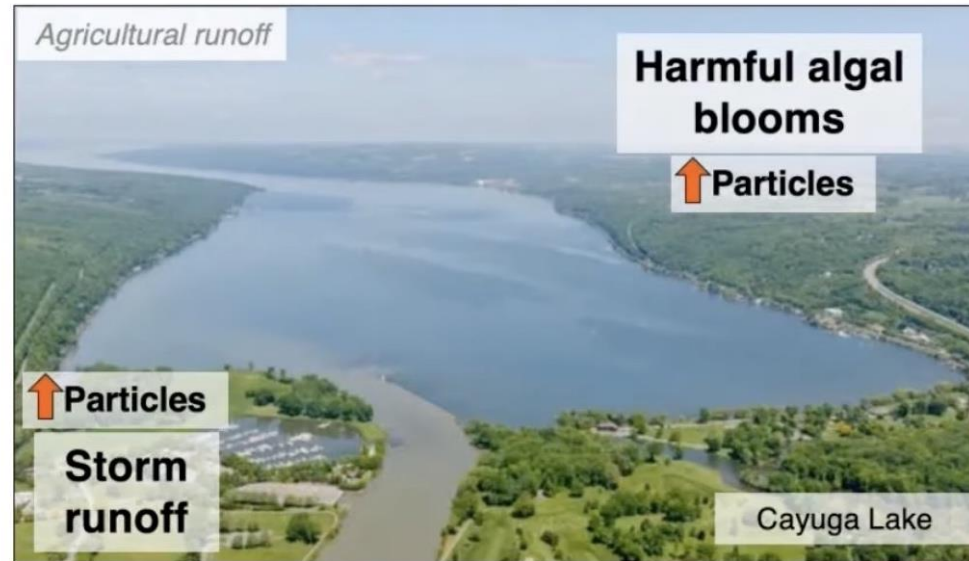
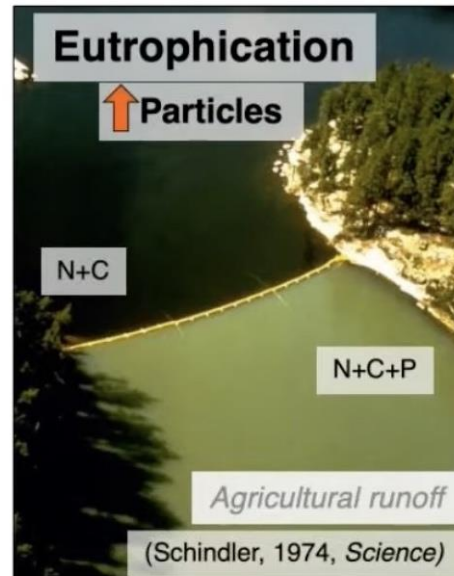
RESEARCH ARTICLE

Microhabitats are associated with diversity–productivity relationships in freshwater bacterial communities

Marian L. Schmidt^{1,2,*†}, Bopaiah A. Biddanda³, Anthony D. Weinke³,
Edna Chiang^{1,4}, Fallon Januska³, Ruben Props^{1,5,§} and Vincent J. Denef^{1,‡}



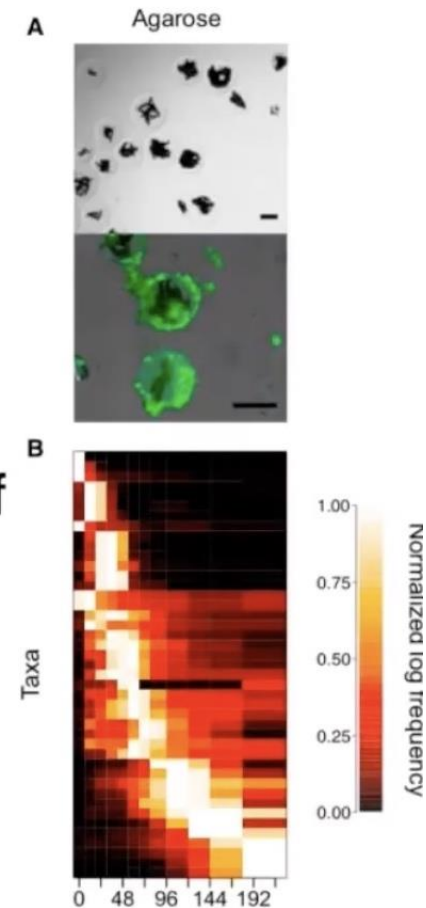
Freshwater ecosystems are changing... and so will the type and amount of particles





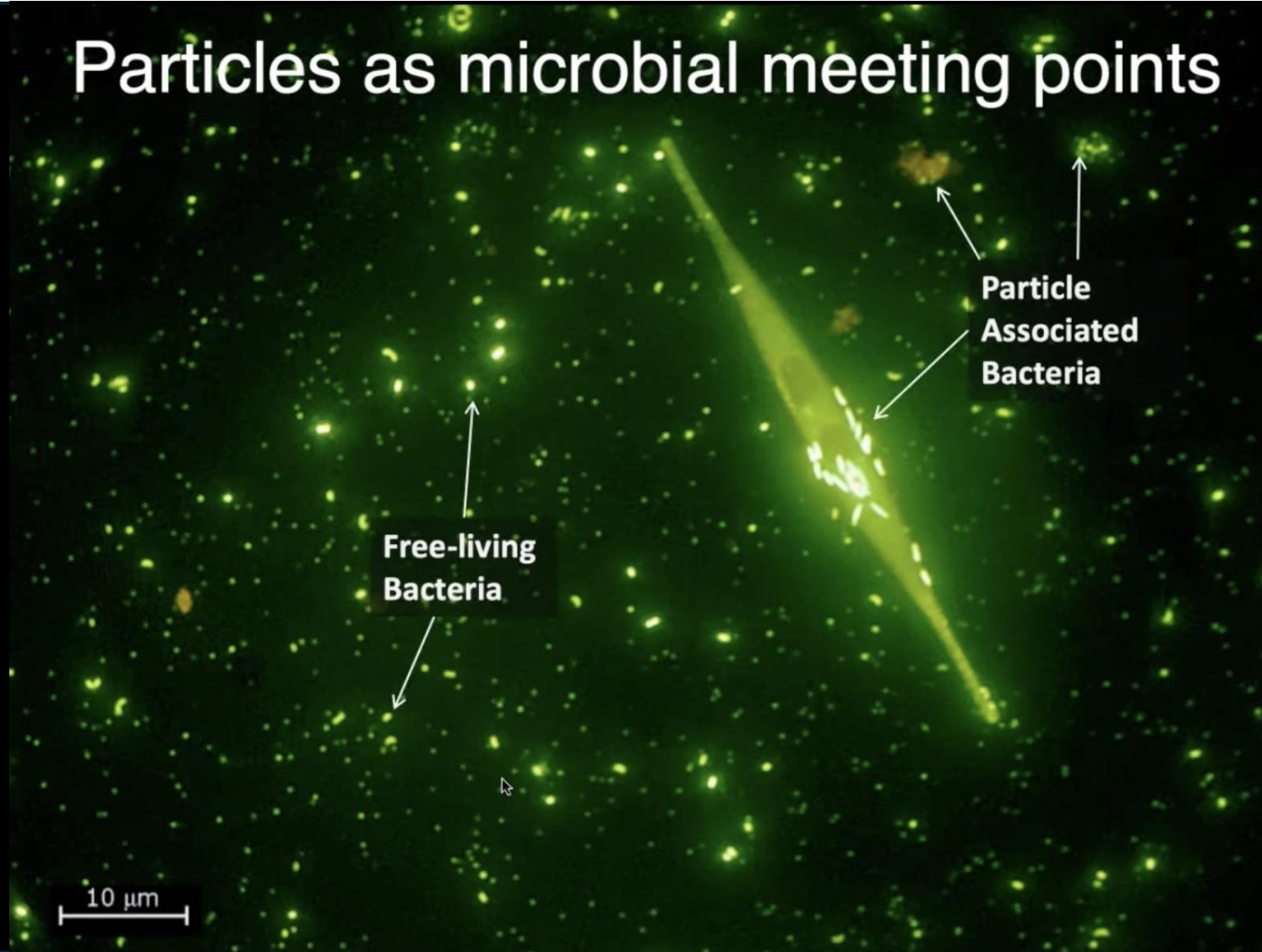
Particles have important ecological contributions

- While the particulate organic carbon pool is 10X smaller, it's more bioavailable. (Grossart, 2010, *Environ Micro*)
- Nitrogen cycle: Nitrate reduction to nitrite only takes place in particles (Ganesh et al., 2014; 2015 *ISME J*)
- Microbes in particles have a wider spectrum of polysaccharide hydrolase activities (Balmonte et al., 2018, *Environ Micro*)
- Model particles undergo rapid species turnover (Datta et al., 2016, *Nat Comm*; Enke et al., 2019, *Curr Bio*)





Particles as microbial meeting points





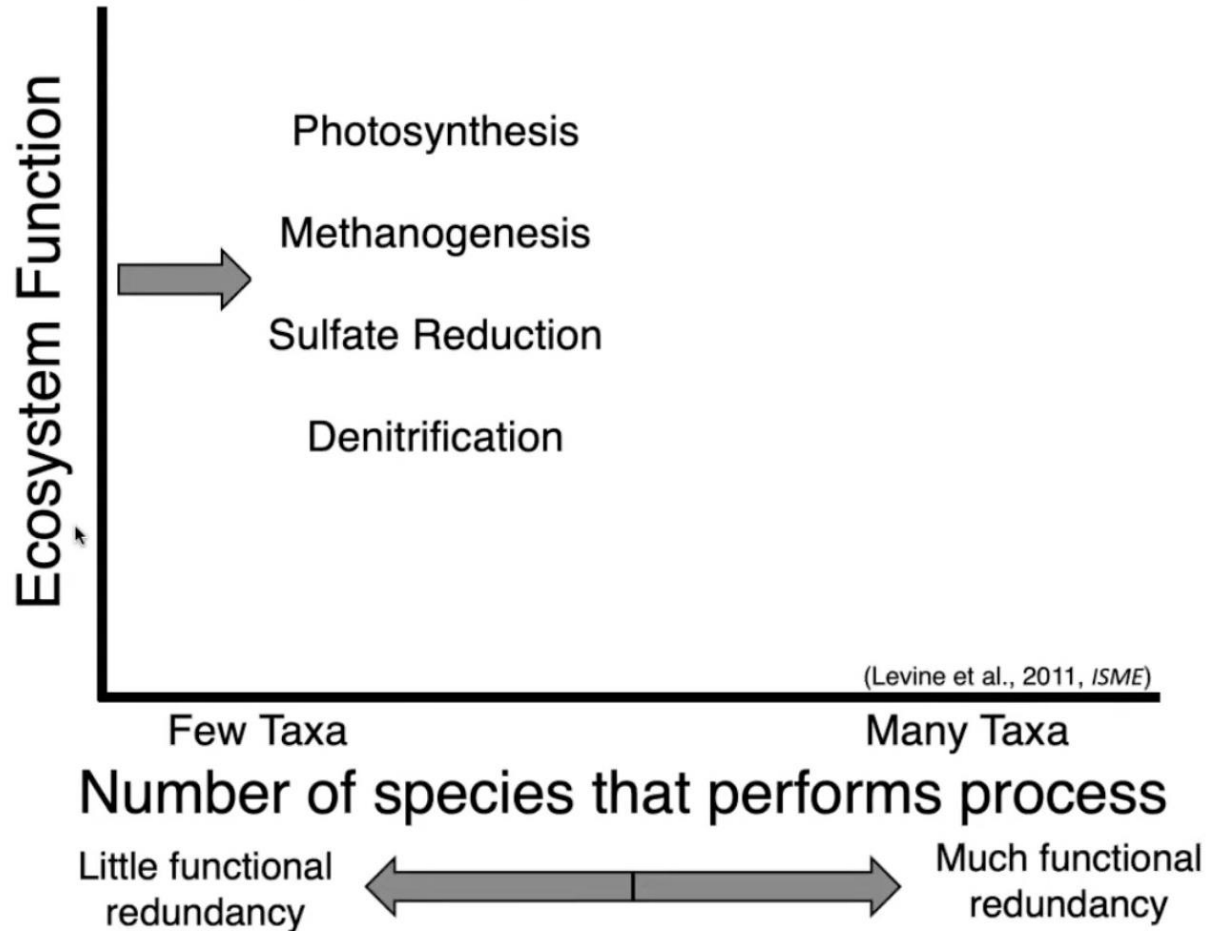
Testing microbial ecology in lakes

1. Do particle microhabitats support more diverse communities than the free water?
2. How does bacterial heterotrophic production vary between microhabitats?
3. Are there biodiversity-ecosystem function relationships in lake microhabitats?



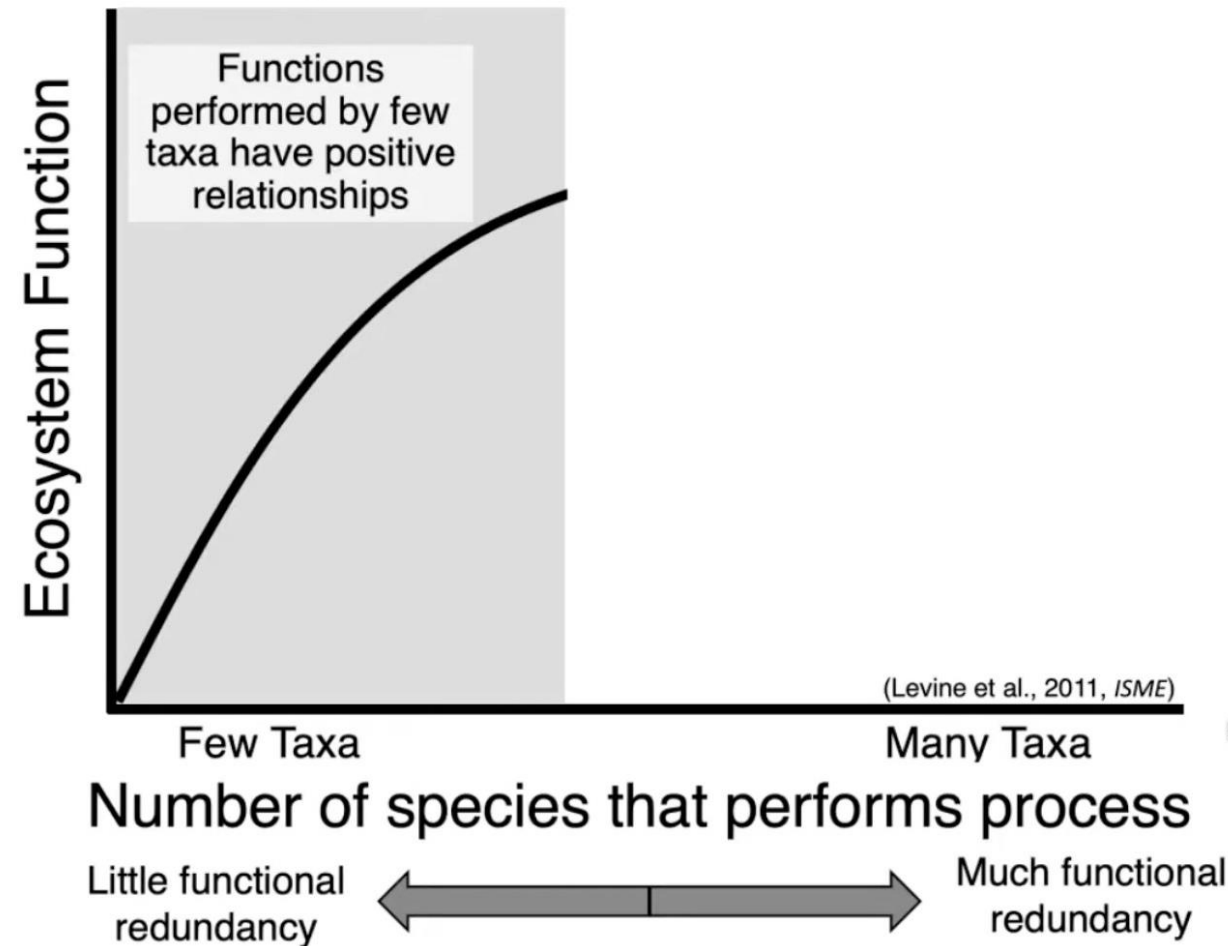


Diversity-function relationships depend on how many taxa perform the function



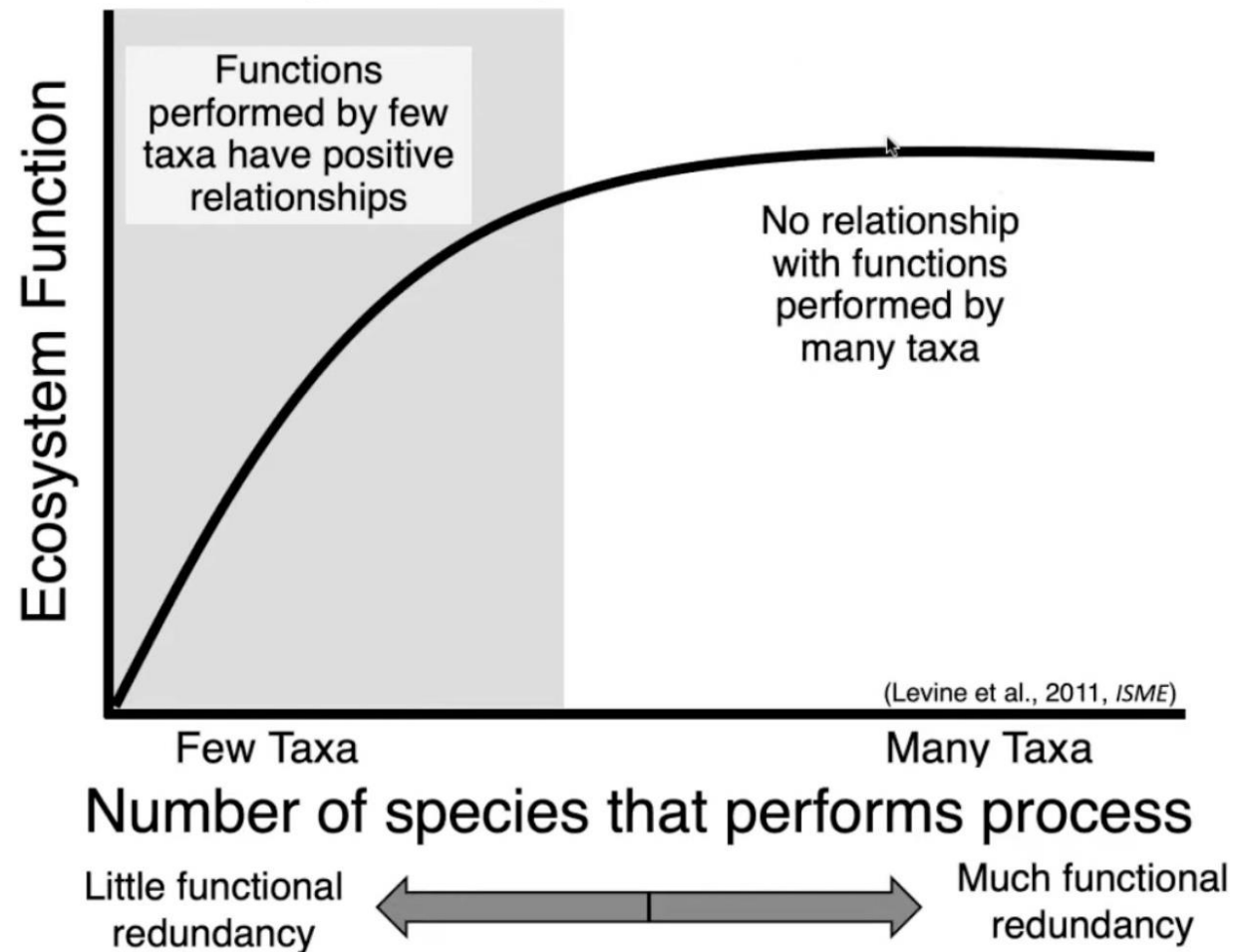


Diversity-function relationships depend on how many taxa perform the function



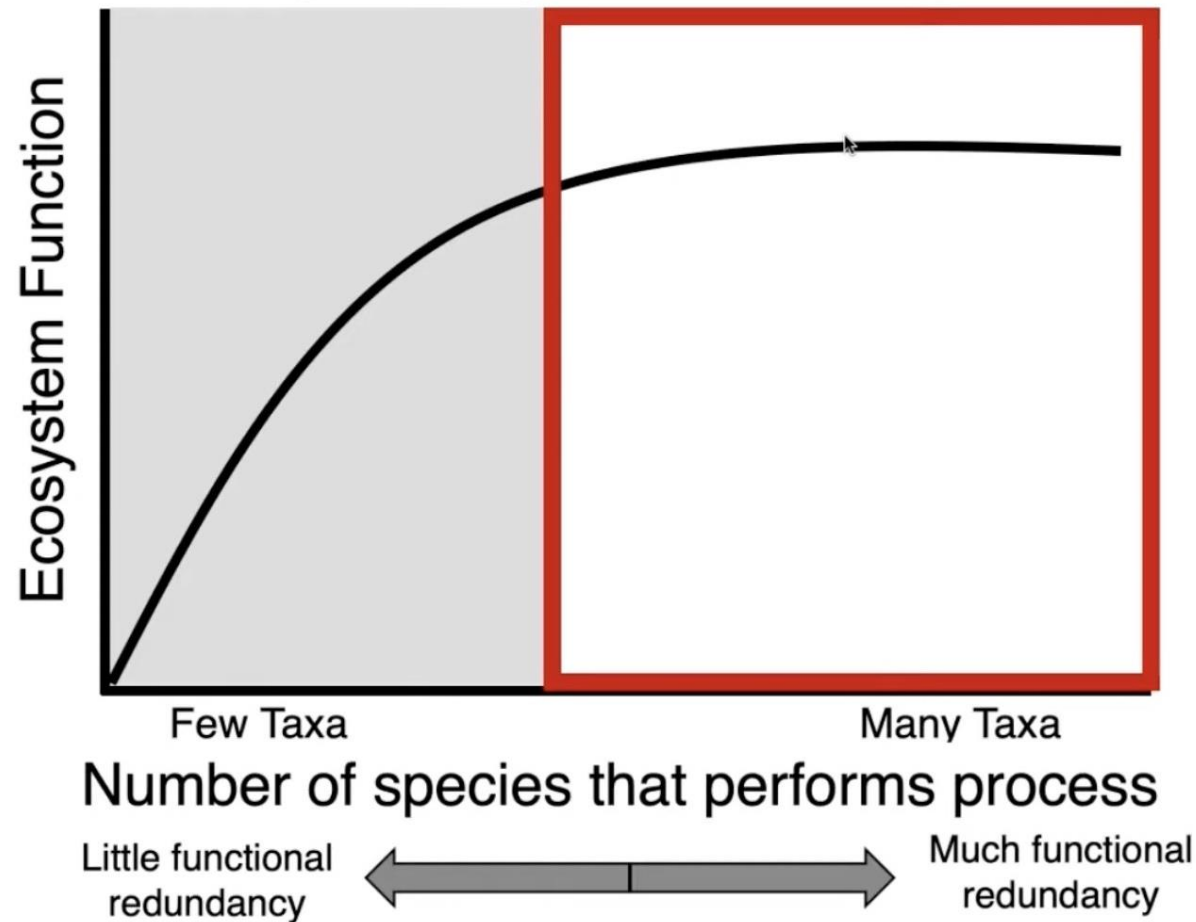


Diversity-function relationships depend on how many taxa perform the function



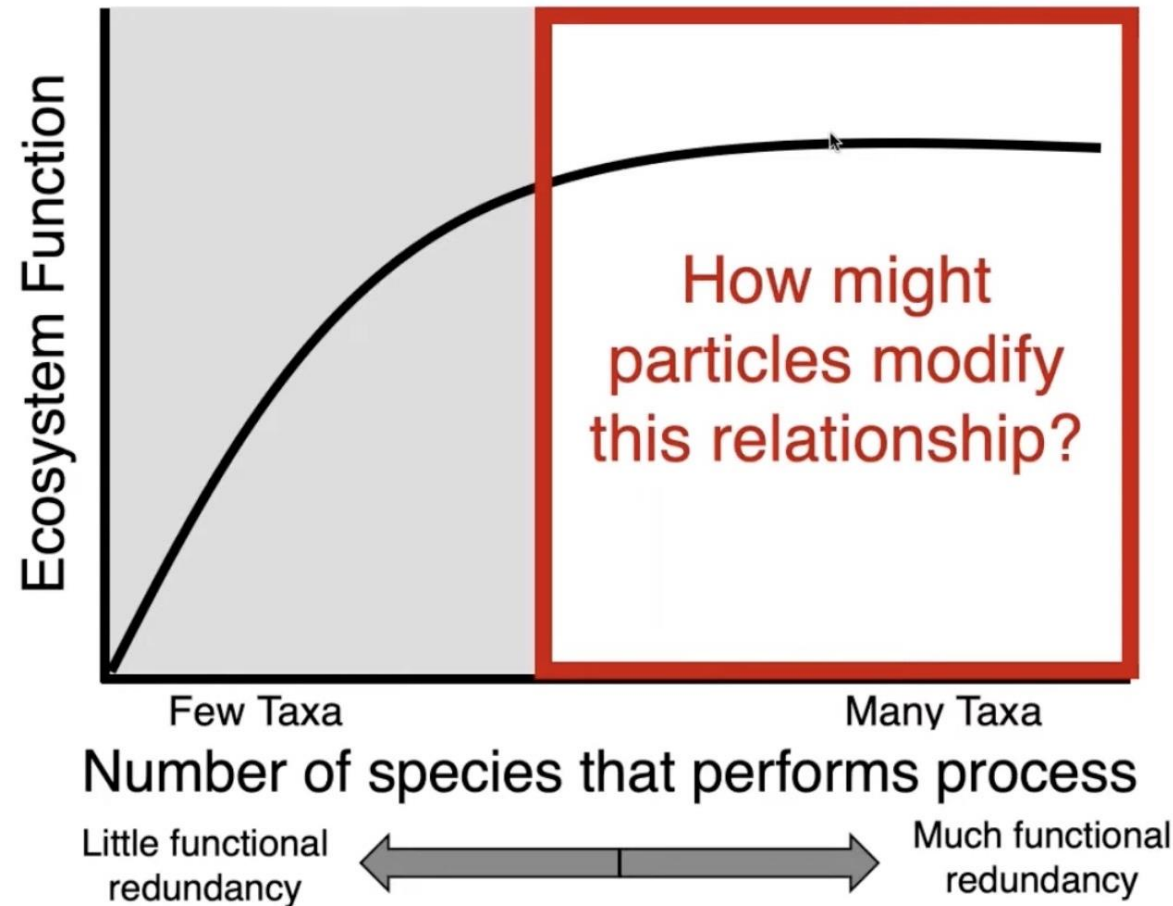


Diversity-function relationships depend on
how many taxa perform the function



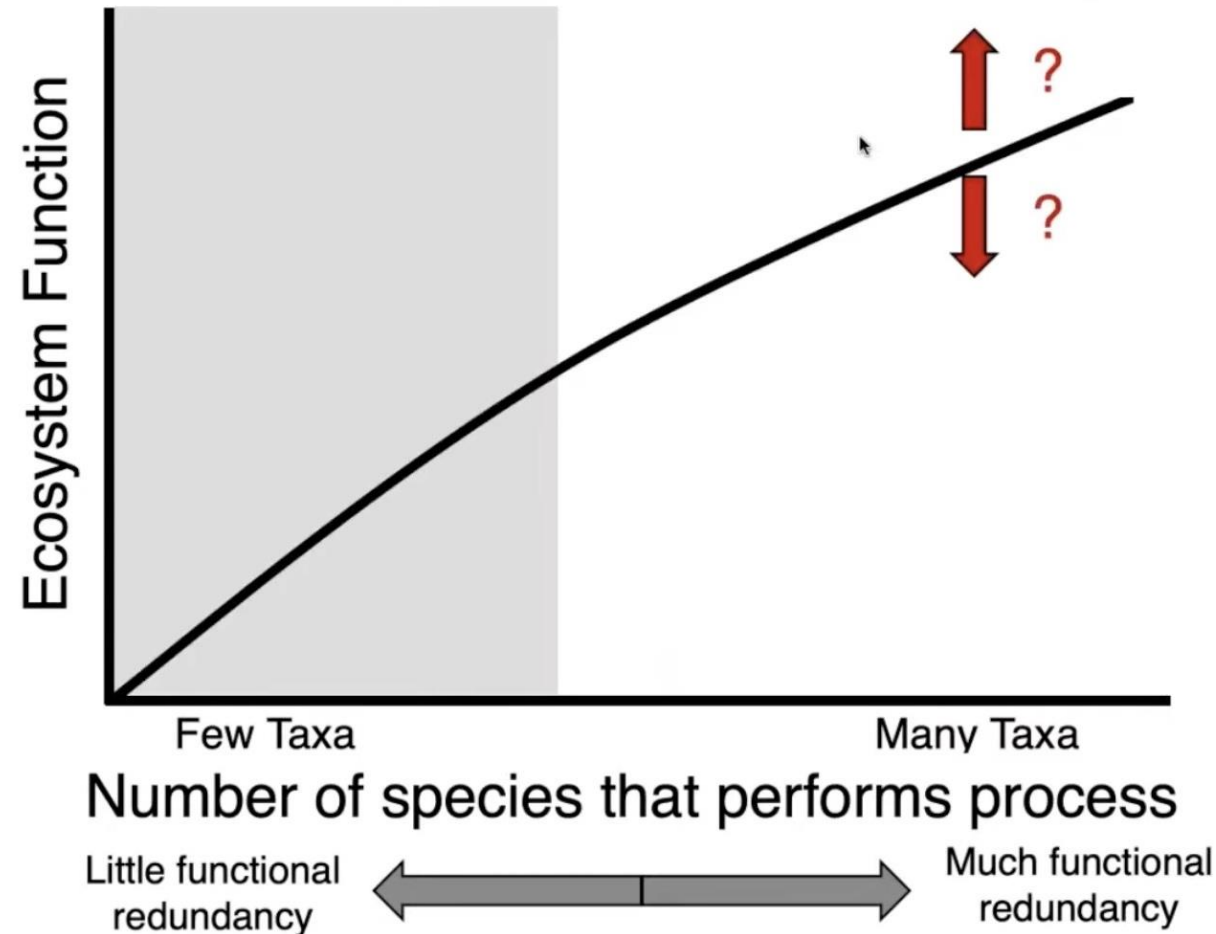


Diversity-function relationships depend on
how many taxa perform the function



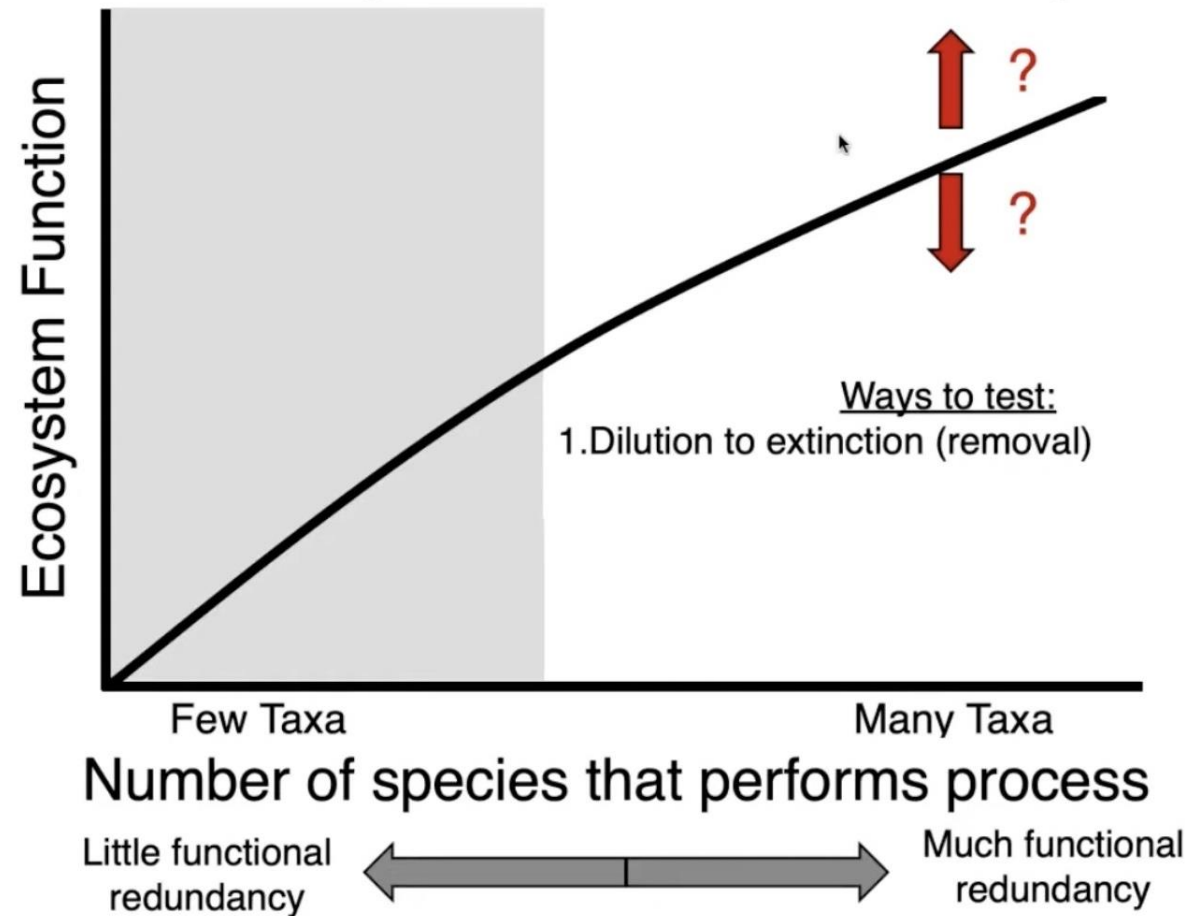


Hypothesis: **Particles** influence the strength of diversity-function relationships



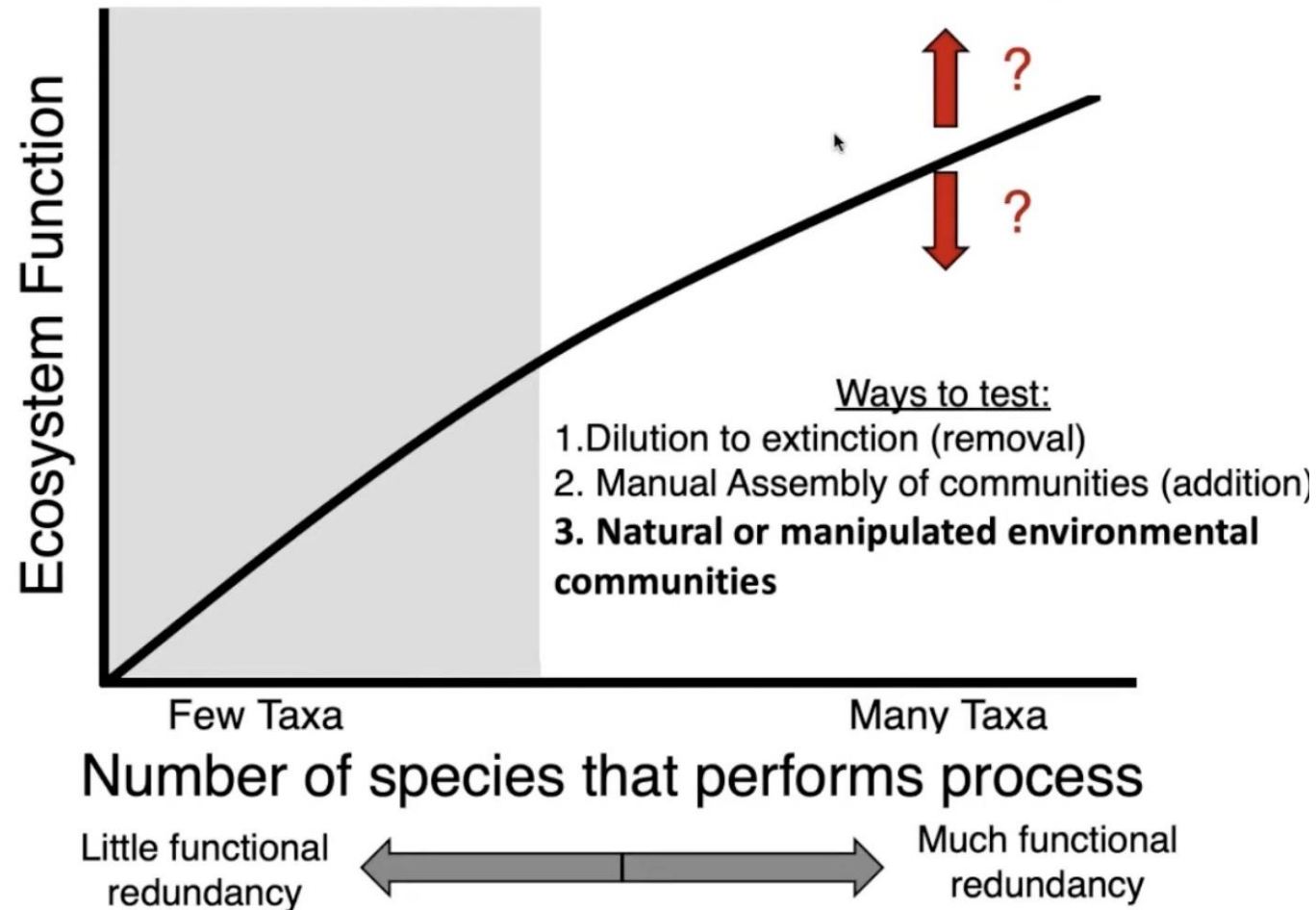


Hypothesis: **Particles** influence the strength of diversity-function relationships





Hypothesis: **Particles** influence the strength of diversity-function relationships





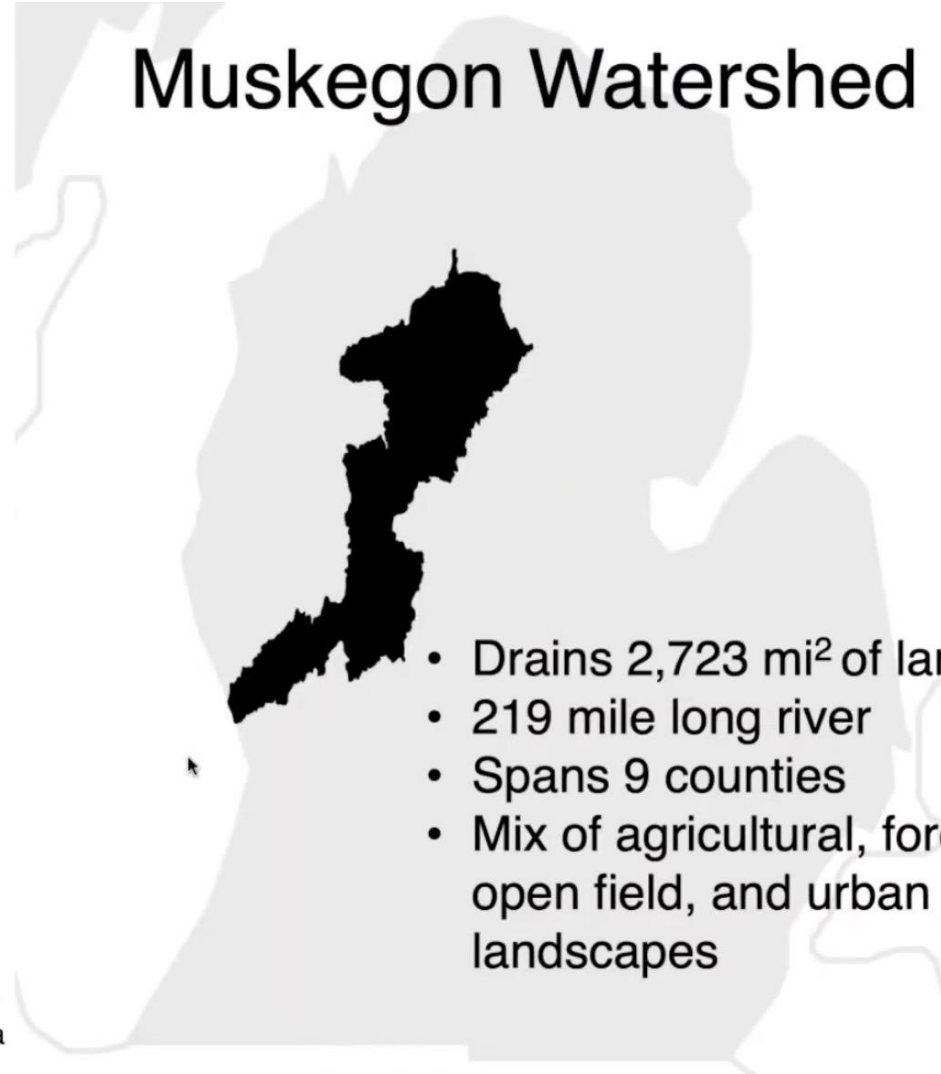
Muskegon Lake



Traditional
territories of the
Odawa & Peoria



Muskegon Watershed



- Drains 2,723 mi² of land
- 219 mile long river
- Spans 9 counties
- Mix of agricultural, forest, open field, and urban landscapes

Traditional
territories of the
Odawa & Peoria



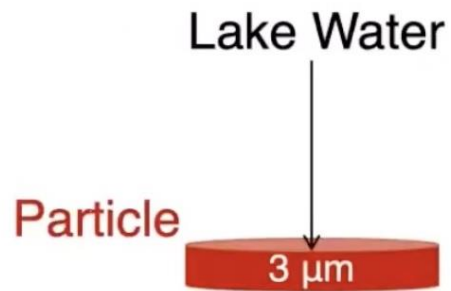


Spring, Summer, Fall
at 1m depth



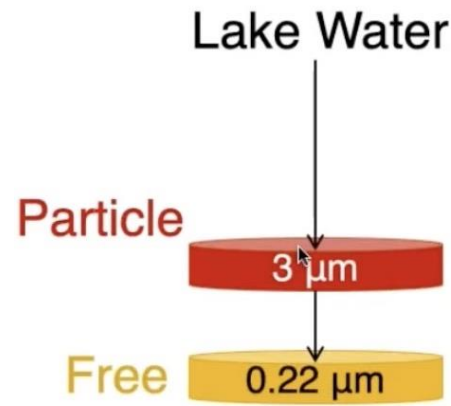


Sampling **particle** and **free-living** freshwater bacterial habitats





Sampling **particle** and **free-living** freshwater bacterial habitats



1. DNA extraction
2. 16S rRNA gene sequencing
3. Measure of diversity with iNEXT



How does one measure “diversity”?

Within-sample (alpha) Diversity: Hill Diversity Metrics

- Differentially weight species richness and relative abundances with effective number of species using the order q
- As q increases from 0 to 2, species abundances are weighted more, decreasing sensitive to sampling effort

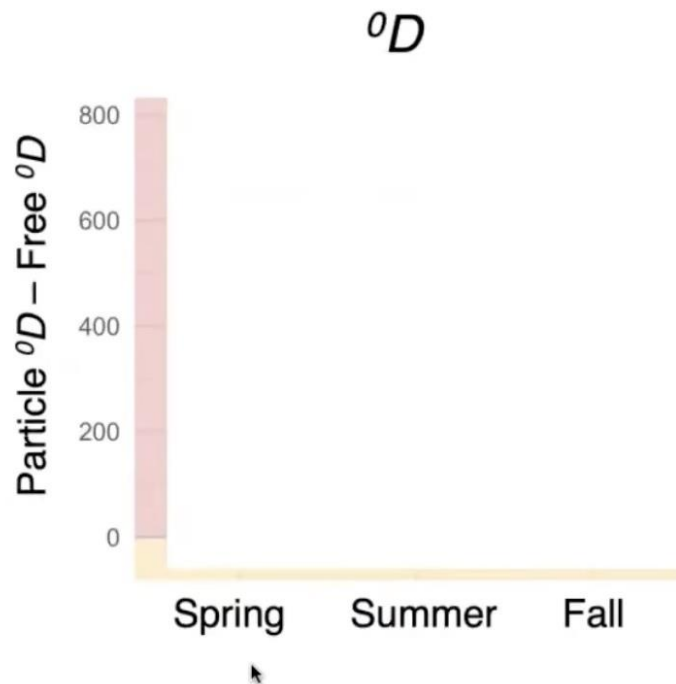
$${}^qD = \left(\sum_{i=1}^S p_i^q \right)^{\frac{1}{1-q}}$$

q = Hill order number: 0, 1, 2
 p_i = relative abundance of i th species
 S = number of species in sample

1. 0D : Effective number of observed species
2. 1D : Effective number of typical species = exp(Shannon)
3. 2D : Effective number of dominant species = Inverse Simpson



How does biodiversity differ between microhabitats through the seasons?

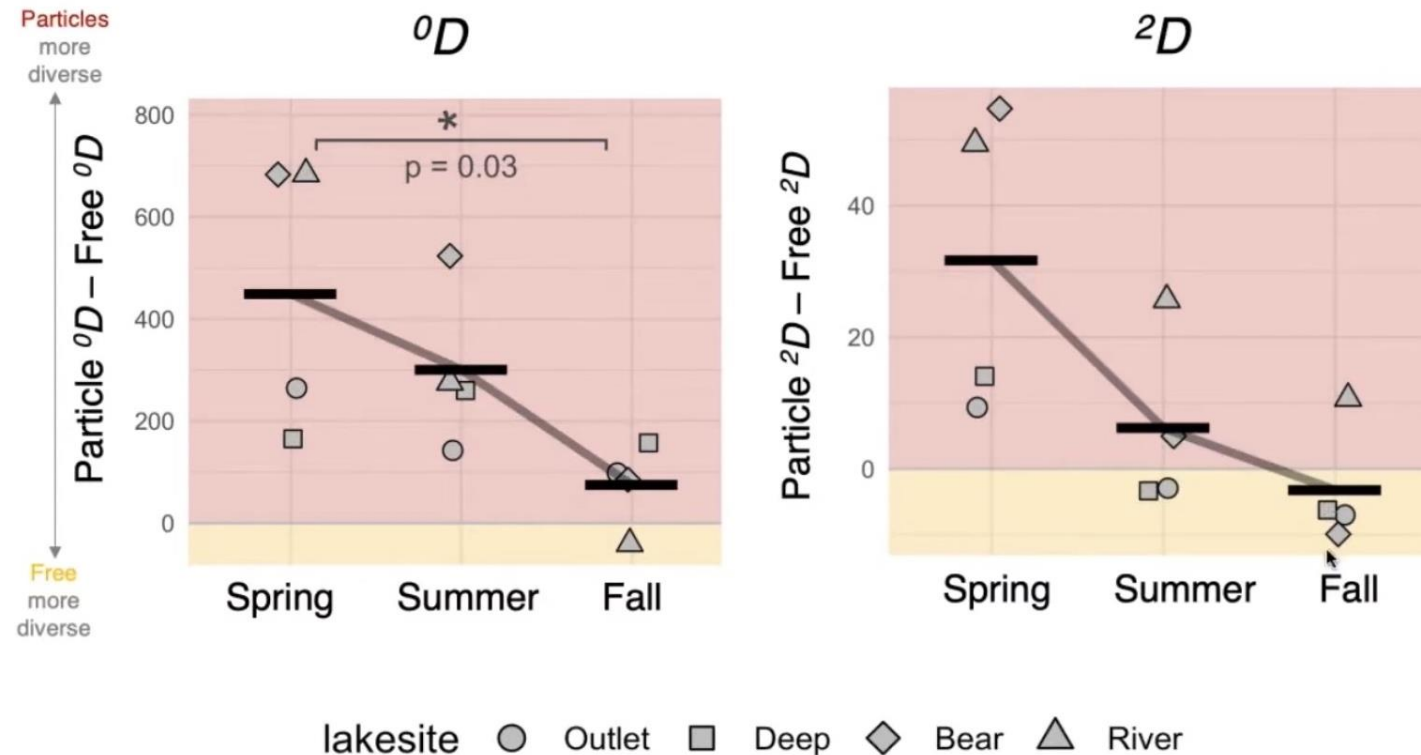




In spring, **particle** communities are more diverse than **free** but not for fall

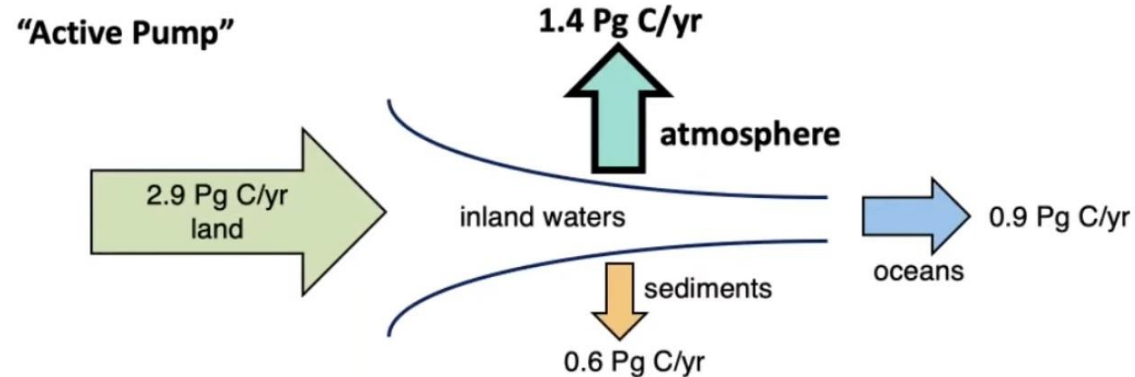
The River and Bear Lake inputs are especially diverse

Free has more dominant taxa in fall





Freshwater systems regulate the carbon cycle



~1.4 Pg C/yr respired by
heterotrophic bacteria annually!

1 petagram = 10^{15} grams = 1,000,000,000,000,000 g

Since 1 blue whale = 100,000,000 grams, that is...

14,000,000 blue whales worth of carbon/yr!

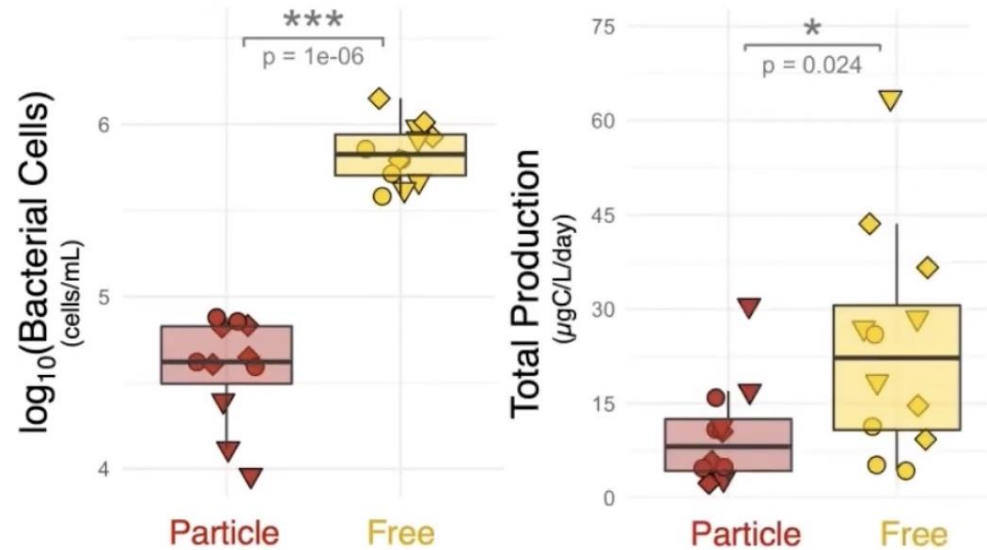
2 blue whales of carbon every 5 seconds!

Heterotrophic
bacterial production
measured
 ^3H -leucine
incorporation
analysis
a proxy for heterotrophic
bacterial biomass



More total production in **free**

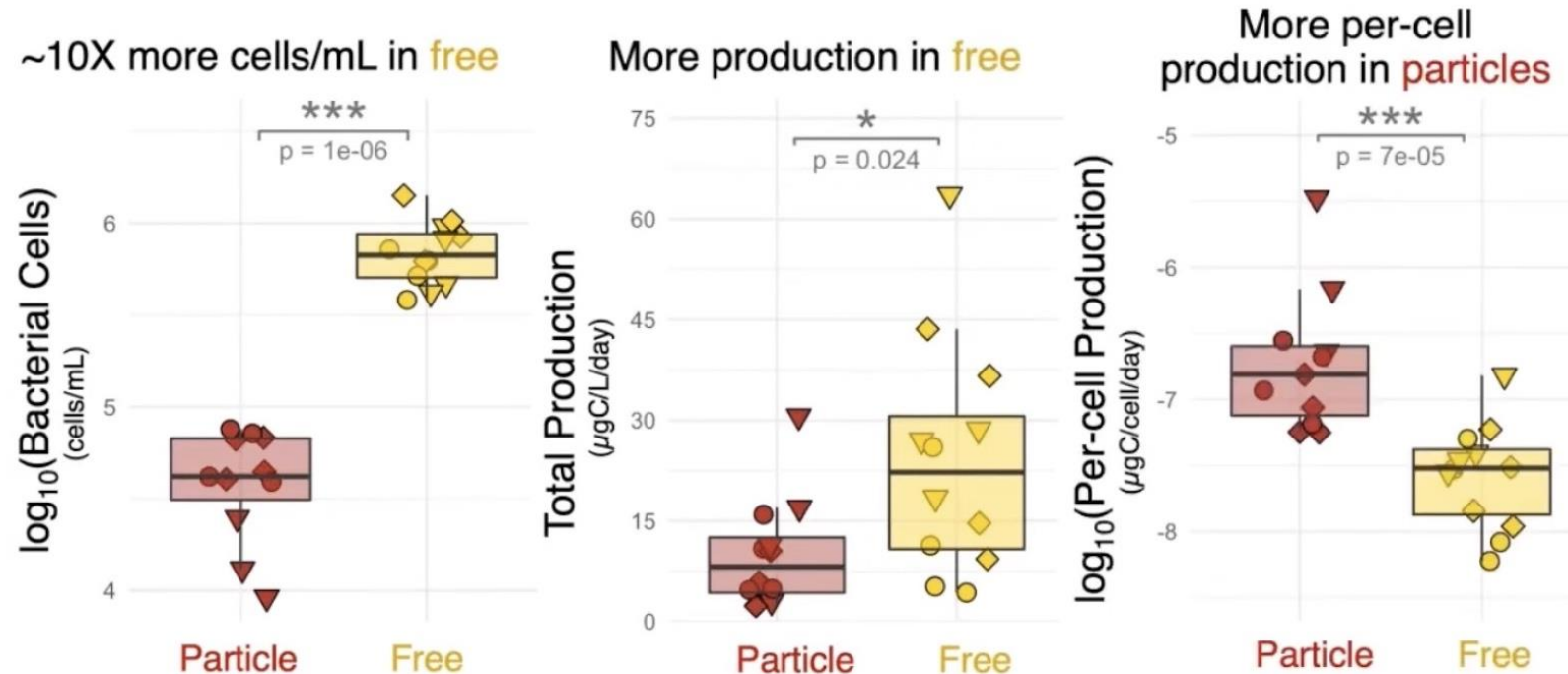
~10X more cells/mL in **free**



▽ Spring ◇ Summer ○ Fall ■ Particle ■ Free



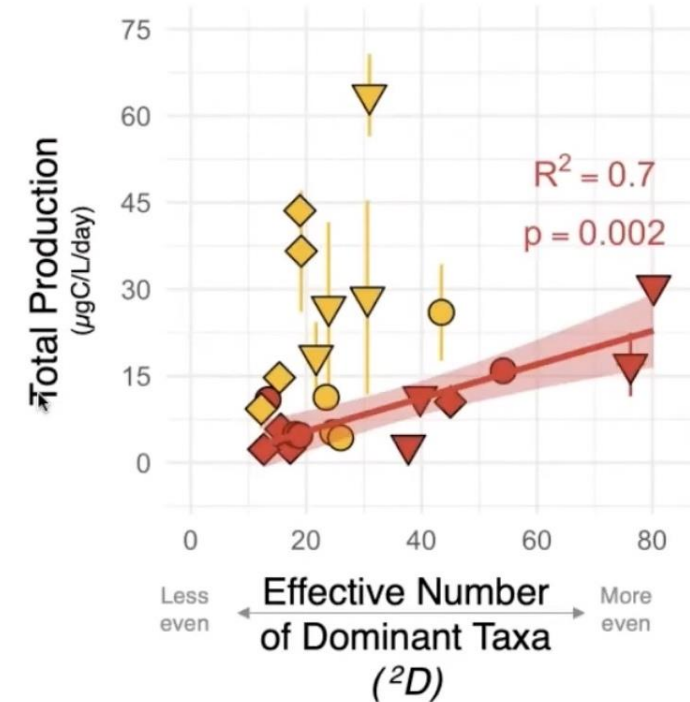
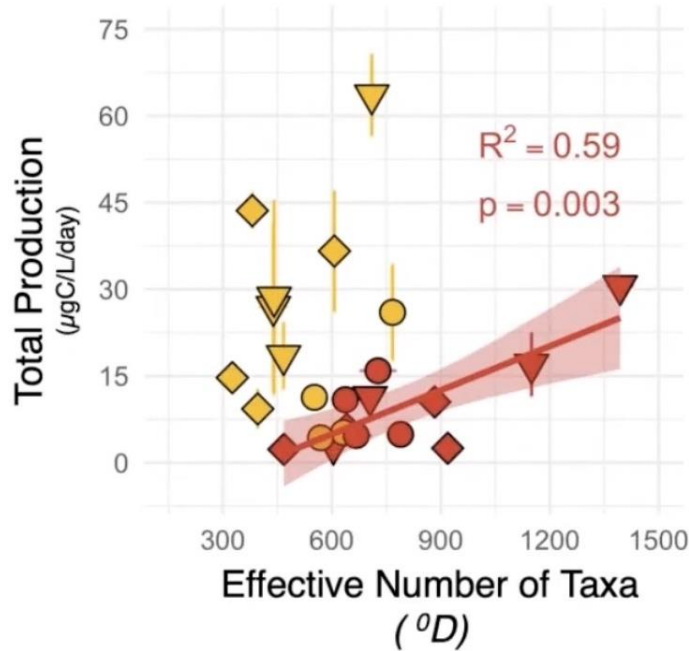
Particle cells are disproportionately active



▽ Spring ◇ Summer ○ Fall ■ Particle ■ Free



Diversity-productivity relationships only present in **particle** communities



Relationship strengthens with evenness

▽ Spring ◇ Summer ○ Fall ■ Particle ■ Free



What about environmental variables?

Does environment or biodiversity
better predict heterotrophic
production?



What about environmental variables?

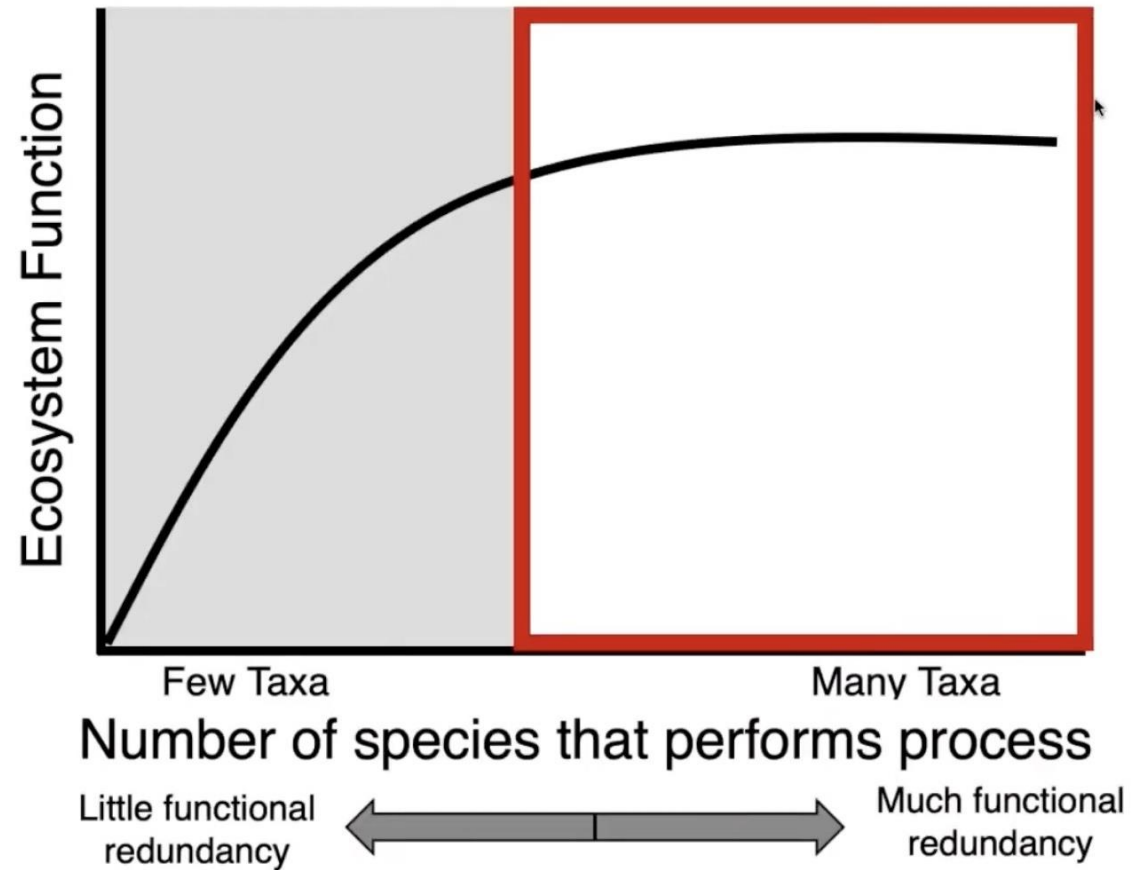
Does environment or biodiversity
better predict heterotrophic
production?

Particle = Biodiversity (^{0}D and ^{2}D)

Free = Environment (pH)

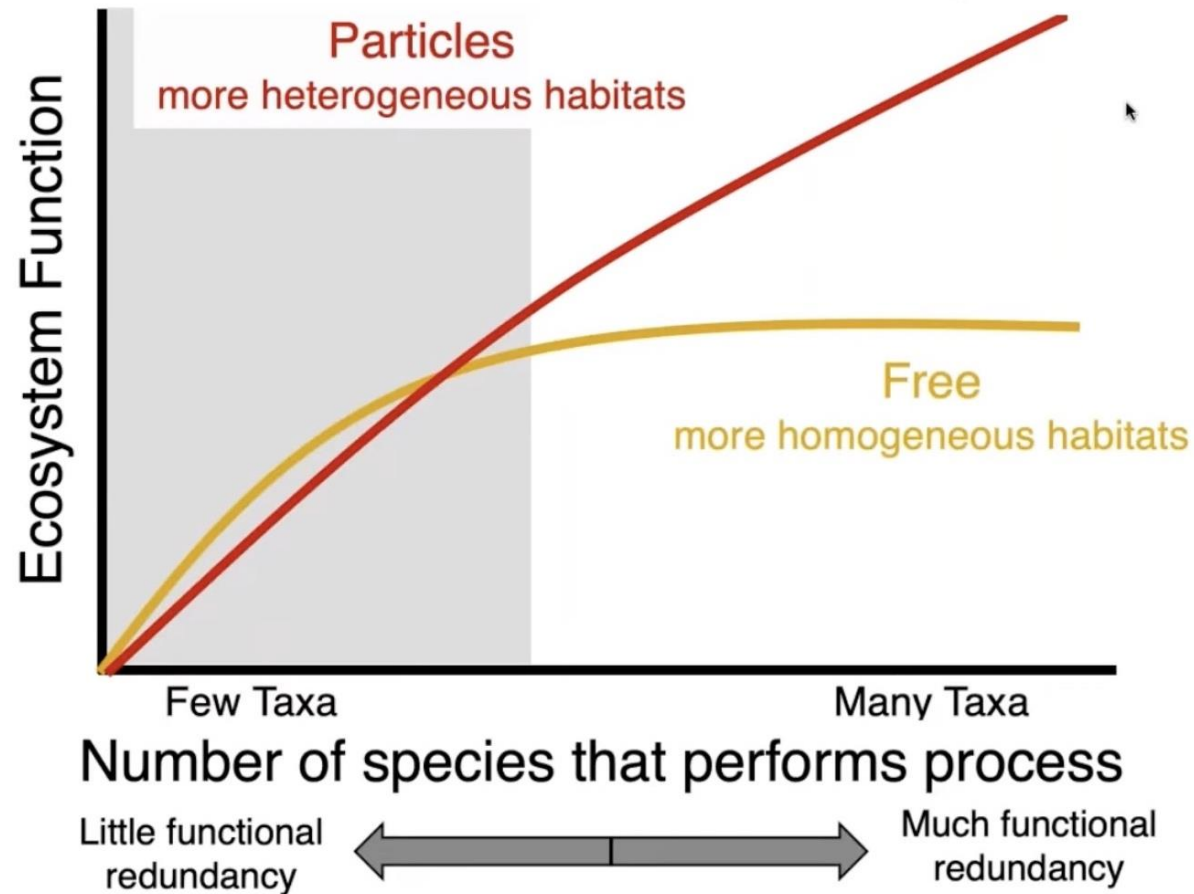


No relationship with functions performed by many taxa



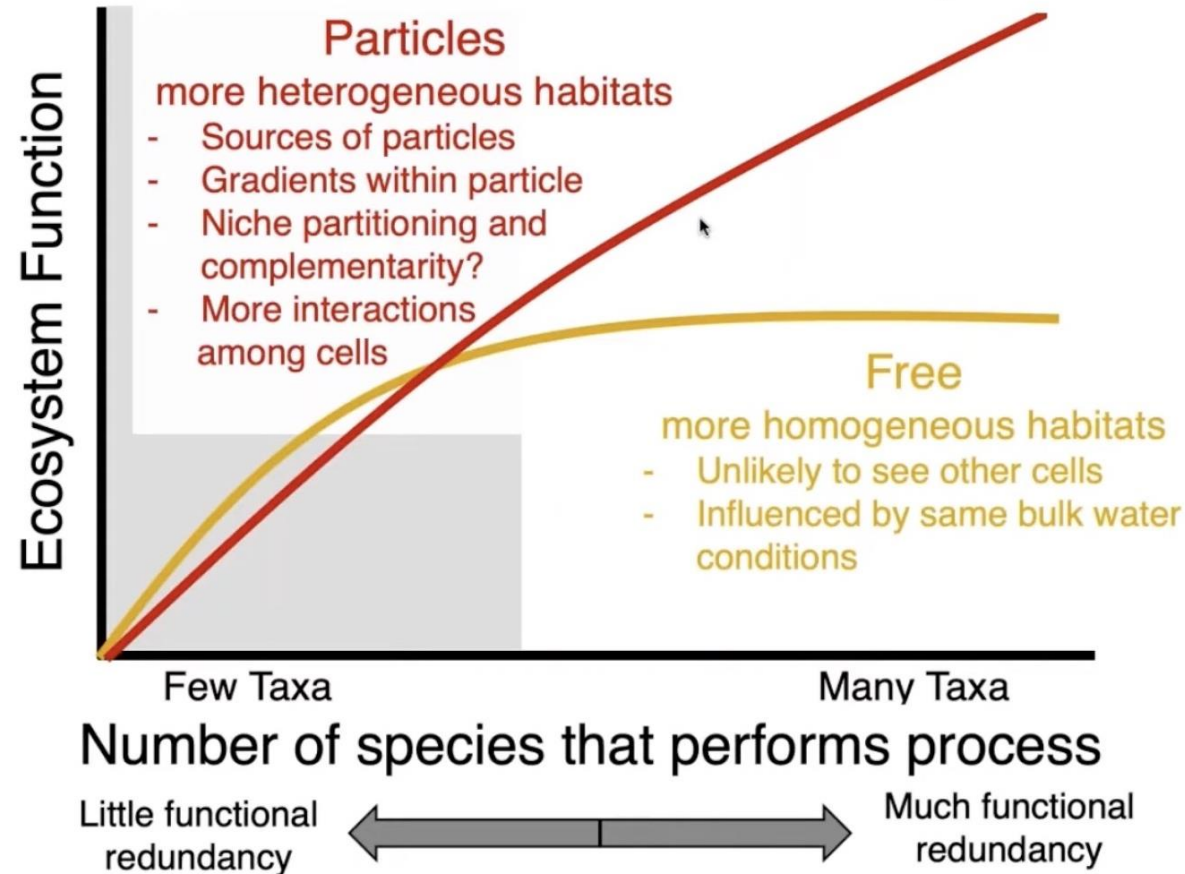


... maybe the number of taxa and habitat determine the relationship





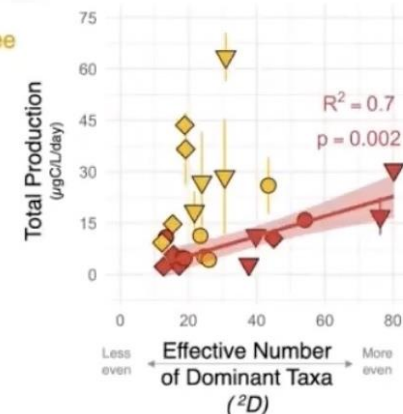
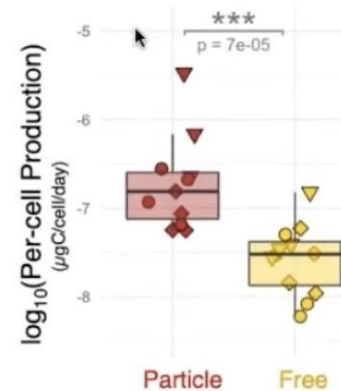
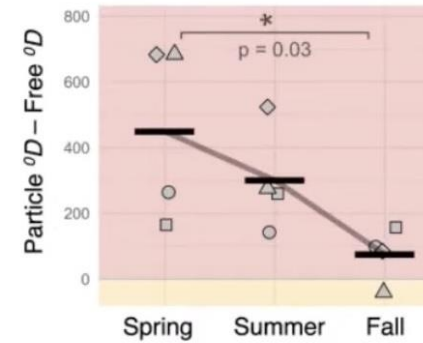
... maybe the number of taxa and habitat determine the relationship





Conclusions

1. Bacterial communities on **particles** are more diverse, especially in spring & near inputs to the lake
2. Bacterial cells on **particles** are more productive.
3. Biodiversity is the best predictor of production in **particles**, whereas pH was the best predictor of production in **free**.





**Ciências
ULisboa**

Faculdade
de Ciências
da Universidade
de Lisboa



MICROBIOLOGIA E ENGENHARIA DE ECOSSISTEMAS

...questions section...

