Microbial Ecology

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Northeastern University
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Nicolai S. Panikov

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Abstract Ecology is the science that specifically examines the relationship between microorganisms and their biotic and abiotic environment. Like plant, animal and human ecology, the microbial ecology applies the general ecological principles to explain life functions of microorganisms in situ, i.e., directly in their natural environment rather than simulated under artificial laboratory conditions ex situ or in vitro. In this chapter "Microbial Ecology," we will focus on specific aspects of this extensive scientific discipline, which seem to be essential for biotechnological developments. Assuming that the reader is not a professional ecologist, in the first part of the chapter we summarize the major theoretical concepts and "laws" of macroecology needed to understand the language in this esoteric area. The second part deals with the modern instruments and tools of microbial ecology. The final and third part of the chapter surveys the major types of the Earth's ecosystems with special emphasis on quantitative analysis of the diversity of natural environments and microbial inhabitants as well as biotechnological applications associated with the respective natural ecosystems.

Key Words Ecology • microorganisms • natural ecosystems.

1. INTRODUCTION

The word *ecology* was coined by the German zoologist Ernst Haeckel, who applied the term *oekologie* to the "relation of the animal both to its organic as well as its inorganic environment." The word comes from the Greek *oikos*, meaning "household, home, or place

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to live." Thus, ecology deals with the organism and its environment. The word *environment* includes both other organisms and physical surroundings. It involves relationships between individuals within a population and between individuals of different populations.

Ecology draws upon numerous fields, including climatology, oceanography, soil science, chemistry, geology, animal behavior, taxonomy, and mathematics. Ecology is often confused with environmental science. It contributes to the study of environmental problems, but it is a distinct scientific discipline (note that environmental science is broader and combines the power of ecology with many other natural and social sciences for a better understanding and management of the local and global environment).

Some definitions stress the point that ecology, as a part of life science, studies living matter at levels above an organism: populations, communities, ecosystems, and biosphere.

Microbial ecology is the science that specifically examines the relationship between microorganisms and their biotic and abiotic environment. Like plant, animal, and human ecology, microbial ecology applies the general ecological principles to explain life functions of microorganisms in situ, i.e., directly in their natural environment rather than simulated under artificial laboratory conditions ex situ or in vitro. Although the in situ microbial processes are the ultimate goal in the majority of ecological studies, it does not exclude laboratory experiments and mathematical modeling as efficient research tools at intermediate stages aimed at the elucidation of underlying mechanisms and testing hypothesis.

The biotechnological importance of microbial ecology is obvious first of all for the development of *environmental biotechnologies* aimed at in situ activation or release of the beneficial microbial populations such as ice-nucleation bacteria, producers of plant hormones, nitrogen-fixing bacteria, antagonists of soil pathogens, pollutant's degraders etc into the environment. Cleaning of soils and ocean from pollutants, waste water treatment, pest control and many other modern environmental technologies do require understanding of microbial ecology. However, even conventional branches of biotechnology distinct from environmental science can greatly benefit from the close cross-link with microbial ecology. The reasons are that:

- The natural environment is the ongoing source of new microorganisms, which carry novel functions to be exploited in various technological applications. Microbial ecology provides the guiding principles and helps to optimize the search of new organisms with desirable technological qualities.
- The natural microbial community has been evolved for billions of years and shaped by "merciless" natural selection. That is the way in which natural communities could be often considered as optimally designed systems, having remarkably high efficiency and parsimony and therefore desirable for modern biotechnology. The knowledge of nature's optimal design can efficiently help in optimizing the man-made technological systems.
- The natural systems are not only older, but also more complicated, e.g., have higher number of links with other systems. Sometimes, the behavior of such systems can be counter-intuitive with sudden twists and unpredicted sideeffects. From this point of view, microbial and general ecology is a valuable source of instructive examples teaching the art of balance and wisdom in any kind of biotechnological development.

In this chapter, we focus on specific aspects of this extensive scientific discipline, which seem to be essential for biotechnological development. The first part of the chapter summarizes the

major theoretical concepts and "laws" of macroecology needed to understand the language in this esoteric area. The second part deals with the modern instruments and tools of microbial ecology. The final part of the chapter surveys Earth's major ecosystems with a special emphasis on a quantitative analysis of the diversity of natural environments and microbial inhabitants as well as biotechnological applications associated with the respective natural ecosystems.

2. THE MAJOR TERMS, PRINCIPLES, AND CONCEPTS OF GENERAL AND MICROBIAL ECOLOGY

Most ecological principles and "laws" do not belong to the category of experimentally confirmed facts or mathematically derived statements, as is the case in physics, chemistry, or molecular biology. Rather, they are reasonable assumptions or empirical generalization based on numerous observations on how plants and animals establish themselves in various natural environments. No doubt, general ecology was developed mainly from the studies of higher forms of life, the microbial world being mostly neglected. The major advantage of macro- vs. microorganisms in ecological studies stems from the fact that plant and animal communities are much better visualized, enumerated, and identified, and with greater precision and less cost. The "golden age" in microbial taxonomy has started only recently because of the remarkably quick progress in molecular biology and molecular ecology. In the last decade, we have found a way to bring an order to bacterial taxonomy and develop reliable methods of assessing microbial diversity on the basis of phospholipid analysis and nucleic acids sequencing. On the other hand, the great advantages of microbial ecology over ecology of macroorganisms are: (a) essentially deeper understanding of molecular, chemical, and physical mechanisms behind life functions in situ, (b) much quicker development of microbial communities (e.g., days and months vs. years and centuries for plants communities), and (c) wider possibilities for experimental simulation, and testing of theoretical hypotheses. Therefore, in microbial ecology we are closer to realizing the full understanding, prediction, and control of the natural systems on the basis of solid quantitative knowledge rather than wealth of practical/empirical experience. Probably in the nearest future, the conceptual framework of general ecology will be experimentally tested and improved on the basis of studies of microbial populations in situ and their interactions with macroorganisms. In the following line, we give a short summary of the current concepts in general ecology and introduce the reader to the specific language in this area which often looks deceptively simple.

2.1. From Molecule to Biosphere: The Hierarchy of Organizational Levels in Biology

Figure 4.1 shows separate hierarchies for higher forms of life (plants-animals) and for microorganisms. The complexity and multitude of internal links increases in the following order: molecules < macromolecular complexes < cell organelles < cell < tissue < whole organism < population < community < ecosystem < biosphere. Ecology focuses only on the top levels, starting from *organism* and *population* level up to *ecosystem* and *biosphere* levels. Note that although the majority of microbes (bacteria, archaea and yeasts) formally belong to the category of *unicellular organisms,* the functional analog of macroorganism is

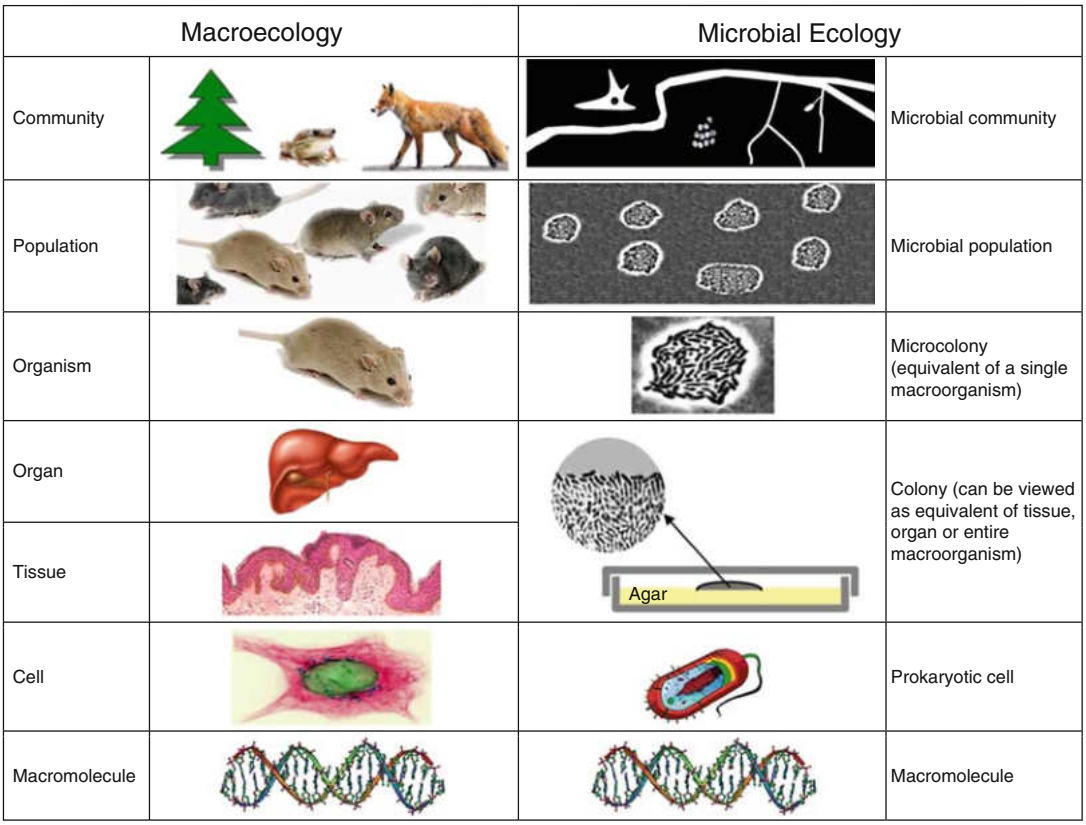


Fig. 4.1. Levels of biological organization. The ecosystem level incorporates the interactions among organisms and their abiotic environment. The left column shows the conventional definitions accepted in general ecology (1), right column is modified to include microbial components.

not a single cell, but microbial colony, flock, biofilm and other cell congeries. In spite of morphological simplicity and uniformity, the bacterial cells within a colony are differentiated in a way similar to the cells and tissue of plants and animals (2). The morphologically differentiated microbial prokaryotes and eukaryotes, such as *Mixococcus* and *Dictyostelium* as well as numerous spore- and rhizome forming fungi, produce structures similar to tissues of plants and animals and are called *pseudotissues*. Finally, prokaryotes have signal metabolites resembling primitive endocrine system of animals: some cells in the bacterial population produce hormone-like compounds which are delivered to other members of cell population

and "order" them to turn on or off several essential life functions: dimorphic transition "cells-mycelium," attachment to or detachment from solid surface, biofilm formation, transition to virulent state or sporulation. Thus, modern molecular data indicate that unicellular organisms are not as primitive as we believed 10–20 years ago. The hierarchy structure for microbial prokaryotes and eukaryotes should be appended with "tissue" and "multicellular organism" levels similar (although not identical) to plants and animals.

The term *population* refers to a group of individual organisms that belong to one species or one functional type and occurs in a specified habitat. In microbial ecology, we can speak of, for example, the population of *Arthrobacter globiformis* in tundra soil or populations of free-living and symbiotrophic N_2-fixing prokaryotes in the soil under a clover field. There could be many other categories of microbial populations, taxonomically homogeneous or mixed, but combined by identical physiological function: denitrifying, nitrifying, photosynthetic, methanogenic, sulfate-reducing, H_2-oxidizing, PCB-degrading microorganisms, etc.

Community (sometimes called *biotic community*) includes all populations occupying the given habitat. As a rule we speak of *microbial community* occupying sediment, lake or soil which includes all the diverse microbial world of specified habitat. However, the full term *community* includes all biotic components: microorganisms, plants and animals which are found within the boundary of the habitat and interact with each other in various degrees (see discussion below). The community interacts also with *abiotic* environment; they tightly couple together to form the *ecosystem:*

$$\text{Ecosystem} = \text{Biotic community} + \text{Abiotic environment}$$

Many European and especially Russian ecologists use the terms "biogeocenosis" and *beocenosis* instead of : "ecosystem" and "community" respectively. Although there are some subtle differences in the content of these terms, it is advisable to take them as full equivalents and use the terms ecosystem/community as a preferential and shorter option. All terrestrial and aquatic (freshwater and marine) ecosystems are combined into a *biosphere* or *ecosphere*, which includes all organisms on Earth interacting with abiotic components supporting life.

None of the known ecosystem is devoid of the microbial component. At the same time, some ecosystems are fully microbial: hyperthermal, ultra cold (permafrost), hypersaline and other ecosystems of the so-called extreme type, which is discussed below.

2.2. The Ecosystem Concept

The ecosystem concept, introduced by Arthur Tansley in 1935, is central to modern ecology; it provides a framework for understanding the flows of energy and elements between organisms and their abiotic surroundings. The concept of *food chains* (introduced in the 1920s by Charles Elton) specifies the direction of energy flows between several trophic levels (Fig. 4.2).

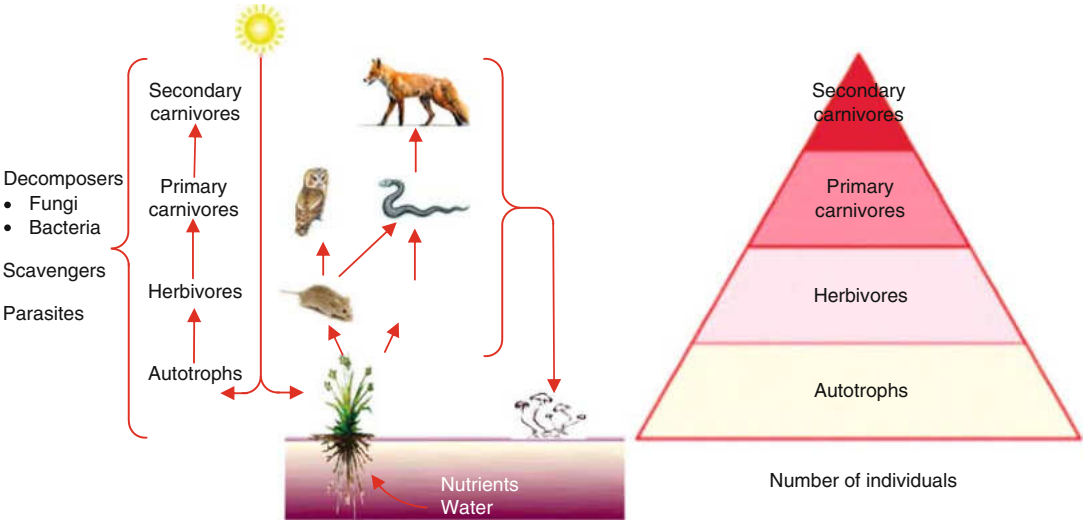


Fig. 4.2. (**a**) A generalized diagram of an ecosystem showing trophic interactions. (**b**) Charles Elton's pyramid of numbers. The number of individuals in each trophic level is represented by the size of the bar. Both of Elton's findings are evident in this figure: The number of individuals decreases moving up the food chain, and food chains are rarely longer than four to five levels. With permission from Wiley, Nature Encyclopedia of Life Sciences.

All organisms are grouped into several discrete categories:

1. *Producers*, the autotrophic organisms (photosynthetic plants as well as photo- and chemosynthetic bacteria) constructing their bodies from CO_2 and other inorganic compounds. These organisms form the base of the food chain.
2. *Herbivores* are animals that consume plants.
3. *Primary carnivores* are meat-eating animals that consume herbivores.
4. S*econdary carnivores* that consume other animals (in some ecosystems we can find also *tertiary carnivores* feeding on the secondary ones).
5. *Decomposers*. The majority of microorganisms (bacteria, archaea, and fungi) as well as small animals utilize the dead organic matter (plant litter and animals residues) as a source of energy and building blocks for their bodies. As a result of decomposition, they release (mobilize) inorganic elements from dead bodies and make them available for plants to keep the primary production going.

Groups 2–5 are also called *heterotrophs;* contrary to *autotrophs* they require organic compounds as nutrients. Herbivores and carnivores belong to the *consumers* category *(holozoic* type of nutrition characteristic for all animals using jaws and tooth or equivalents for intake of food), while in Group 5, decomposers are organisms with *osmotrophic* type of nutrition (transporting soluble nutrients through cellular membrane). Insoluble substrates (e.g., lignocellulose and other insoluble organic matter, oil and sulfur droplets, etc) should be converted to soluble forms with extracellular enzymes, surfactants or chelating agents. We can subdivide organisms also as *biophages* (eating other living organisms) and *saprophages* or *detritophages* (consuming dead organic matter). Microbial biophages include (a) parasites (*Bdellovibrio*)

which invade host cells and multiply inside causing cell lysis, (b) predators attacking other cell with extracellular lytic enzymes (mixobacteria, nematode-trapping fungi), and (c) symbiotic heterotrophic microorganisms closely associated with autotrophic macroscopic partners (mycorrhiza, rhizobia, mycobiont in lichen, etc). The majority of soil and aquatic microbes belong to the category of saprophages or saprotrophs using dead organic matter as a source of nutrient and energy.

Generally, food chains are rarely longer than four to five trophic levels, and lower trophic levels contain more individuals (higher number of species and bigger biomass) than higher trophic levels. The latter pattern came to be known as Elton's "pyramid of numbers" (Fig. 4.2). The progressive reduction in the size of each trophic level is explained by the fact that only approximately 10% of the total energy in a trophic level is passed along to the next trophic level, with the rest being lost as indigestible material and heat from metabolic respiration. Purely microbial food chain is generally more efficient, e.g., grazing of bacterial prey by protozoa can be characterized by conversion of at least 20–40% of consumed bacterial mass into cell mass of protozoa. An even higher efficiency of conversion is observed for decomposers growing on easily available organic substrates.

2.2.1. Food Chain and Metabolic Network

Microbial populations either in situ or ex situ (in laboratory culture) produce a significant amount of extracellular metabolites. In natural habitats, these compounds form a pool of C-compounds which encourage both competition for common substrates and cooperation through the so-called *metabiotic interactions*, in which the product of one species is utilized by other species. Several simple compounds often participate in such *interspecific exchange* of mass and energy that are called *central metabolites* or *centrobolite*. Examples include molecular hydrogen, acetate, methane, etc. For instance, H_2 is produced by cyanobacteria and by microbes with active nitrogenase as well as by fermenting bacteria and fungi; it is consumed by methanogens, acetogens, sulfate-reducers and aerobic H_2-oxidizing bacteria. Removal of H_2 by methanogens is essential to sustain anaerobic degradation of plant residues; otherwise, equilibrium is shifted toward the formation of toxic fatty acids:

$$CH_3CH_2COOH + H_2O \overset{\text{Syntrophic bacteria}}{\longleftrightarrow} CH_3COOH + CO_2 + H_2 \uparrow$$

Interestingly, the functional group of *synthrophic bacteria* can catalyze this reaction in both directions depending on the activity of complementing microbial population, e.g., methanogens or acetogens (the synthrophy stands for the cross-feeding that occurs when two organisms mutually complement each other in terms of nutritional factors or catabolic enzymes related to substrate utilization).

The metabolic network (see example in Fig. 4.3) and food chain have one common feature: both provide flows of energy and matter between organisms and abiotic environment. The difference is that metabolic interspecific exchange occurs within the same trophic level of osmotrophic organisms, while the food chain or food web (the highly branched chain) assumes the flow of energy between different trophic levels. The efficiency of energy conversion by osmotrophic organisms is analyzed by a scientific discipline called growth stoichiometry.

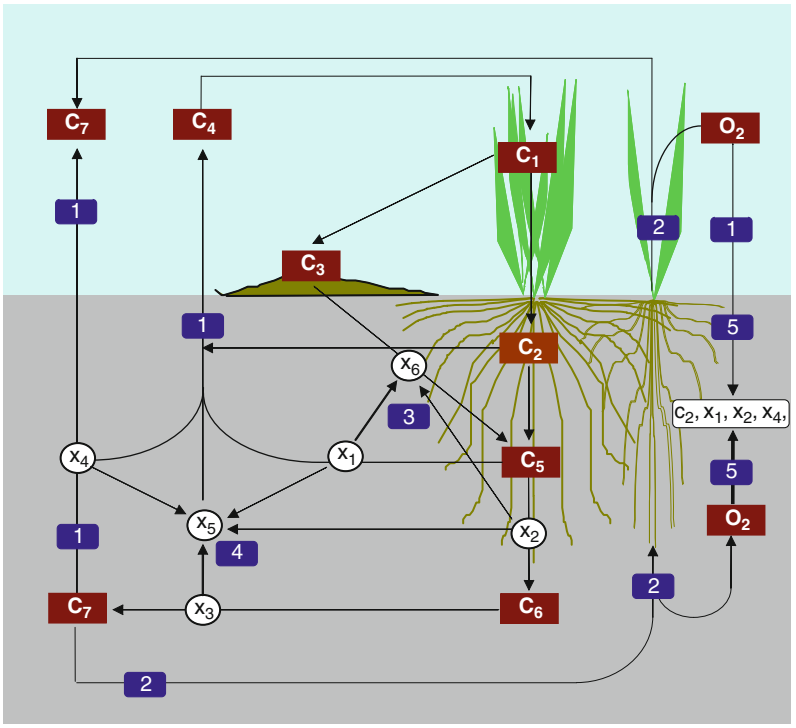


Fig. 4.3. Example of metabolic network functioning in submerged soils and wetlands (3). *Carbon*-reservoirs**:** c_1, green phytomass, c_2, below-ground phytomass (roots and rhizoid), c_3, plant litter, c_4, CO_2, c_5, low molecular weight C-compounds, c_6, volatile fatty acids, c_7, CH_4. *Biocatalysts:* x_1, aerobic soil microorganisms, x_2, fermenting microorganisms, x_3, methanogens, x_4, methanotrophs, x_5, protozoa (microscopic animals feeding on microbial cells), x_6, hydrolytic enzymes. The *arrows* indicate the following basic processes: (*Plant*-mediated) $c_4 \rightarrow c_1$, plant photosynthesis, $c_1 \rightarrow c_2$, transport of C-compounds (photosynthates) from leaves to roots, $c_1 \rightarrow c_3$, plant litter formation, $c_2 \rightarrow c_5$, root exudation, $c_2 \rightarrow c_4$, root respiration. (*Microbial*) $c_3 \rightarrow c_5$, depolymerization of plant litter, $c_5 \rightarrow c_6$, fermentation (anaerobic conversion of sugars to acetate and other volatile compounds), $c_6 \rightarrow c_7$, CH_4 formation, $c_5 \rightarrow c_4$, total microbial respiration, $c_7 \rightarrow c_4$, CH_4 consumption/oxidation. (*General*) 1 gas molecular diffusion, 2 gas vascular transport, 3 biosynthesis of hydrolytic enzymes, 4 protozoan grazing, 5 oxygen uptake for respiration.

2.2.2. The Basics of Microbial Stoichiometry

Two groups of chemical species serve as substrates for microbial growth both in situ and ex situ: (a) *catabolic substrates,* which are sources of energy, and (b) *anabolic or conserved substrates,* which are sources of biogenic elements forming cellular material. Examples of catabolic substrates are H_2 for lithotrophic hydrogen bacteria, NH_4^+ and NO_2^- for nitrifying bacteria, oxidizable or fermentable organic substances for heterotrophic species, etc. Their consumption is accompanied by oxidation and dissipation of chemical substances into waste products which are no longer reusable as an energy source (H_2O, NO_3^-, SO_4^{2-}, CO_2, etc.).

Fermentation products (acetate, ethanol, butyrate, H_2 etc.) seem to be the exception as they do contain reusable oxidation potential, but reutilization can take place only by other organisms or after dramatic changes in environmental conditions, e.g., after transition from anaerobic conditions supervising fermentation to aerobic conditions switching to respiratory catabolism.

The anabolic substrates after uptake are incorporated into de novo synthesized cell components, which are conserved in biomass (that is why they are called sometimes *conserved substrates*). Unlike catabolic substrates, they can be reabsorbed after excretion or cell lysis. The conserved substrates include nearly all the noncarbon sources of biogenic elements (N, P, K, Mg, Fe, and trace elements), CO_2 for autotrophs, as well as the indispensable amino acids and growth factors.

Historically, microbial ecologists dealing with the marine environment focused mainly on conserved substrates that seem to limit growth of phytoplankton (Fe, Co, P, vitamin B12), while terrestrial studies focused on energy sources (available organic compounds in soil solution, CH_4, NH_4^+, etc).

The stoichiometric parameters *growth yield* is defined as:

$$Y = -\mathrm{d}s/\mathrm{d}t \sim -\Delta x/\Delta s \tag{1}$$

Where, Δx is the increase in microbial biomass consequent on utilization of the amount Δs of substrate. Dividing both parts of Eq. (1) by $x\mathrm{d}t$, gives the relationship between growth rate and substrate consumption:

$$Y = -\frac{\mathrm{d}x}{\mathrm{d}s} = \frac{\mathrm{d}x}{x\mathrm{d}t} : \frac{\mathrm{d}s}{x\mathrm{d}t} = -\frac{\mu}{q} \tag{2}$$

where μ is specific growth rate and q is specific rate of substrate consumption.

The reason for Y variation is different for catabolic and anabolic substrates. In the case of energy sources, some fraction of the total substrate flux is diverted from growth per se to meet the so-called *maintenance functions* including:

- Resynthesis of self-degrading cell proteins, nucleic acids, and other macromolecules
- Osmotic work to keep the concentration gradient between cell interior and environment
- Cell motility

$$\begin{array}{c} \text{total energy source uptake} = \text{consumption for growth} + \text{consumption for maintenance} \\ q \qquad\qquad\qquad\qquad \mu/Y^{\max} \qquad\qquad\qquad\qquad m \end{array} \tag{3}$$

where m is the maintenance coefficient, the specific rate of catabolic substrate consumption by non-growing cells (i.e., $m = q$ when $\mu = 0$).

With some rare exceptions (fungal exospores and bacterial cysts), microbial cells are not stable at $\mu = 0$ and either grow ($\mu > 0$) or lyse ($\mu < 0$). Therefore, the maintenance coefficient is found by linear extrapolation of a series of $q(\mu)$-measurements to the point where μ is zero. Under chronic starvation, the maintenance coefficient m decreases as compared with intensive growth; as a result, when $\mu \to 0$, the growth yield Y tends to some low limit $Y^{\min} > 0$ rather than to zero.

There is also *wasteful oxidation* of substrate under at least three specific circumstances: (a) when growth is nutrient-limited and energy-sufficient, (b) when starving cells are brought to rich nutrient medium (famine-to-feast transition) and (c) under effect of some uncoupling inhibitors. In all listed cases, the cell catabolic machinery produces more energy that can be used for ATP generation. Such wasteful catabolism frequently occurs in natural environment under transition from one trophic regime to another (e.g., spring bloom after winter starvation) as well as ex situ when ecologists try to cultivate natural microbial populations on rich artificial media (famine-to-feast transition occurring with conventional plating). The wasteful catabolism should be differentiated from the maintenance per se.

Cell yield on anabolic substrates varies mainly as a result of alterations in biomass chemical composition expressed by parameter σ_s, the intracellular content of deficient element or *cell quota*. The variation in N content in bacteria from 5 to 15% gives the σ_s diapason 0.05–0.15 g N (g cell mass)$^{-1}$. For most known cases, the quota σ_s increases parallel to growth acceleration because the higher growth rate requires higher *intracellular content* of proteins and RNA (contain N, P, S) as well as K^+, Mg^{2+} and vitamins participating in all primary metabolic reactions. The yield and cell quota are inversely related to each other, e.g., the low N-content $\sigma_N = 0.05$ g N/g corresponds to the high cell yield $Y_N = 1/\sigma_N = 20$ g cell/g N utilized. The high N-content in rapidly growing cells can be attained only with low cell yield $Y_N = 1/0.15 = 6.67$ g cell/g N.

2.2.3. Microbial Loop

The concept of a microbial loop was first introduced in marine ecology (4). In essence, it postulates that part of the primary production reaches grazers as soluble organic matter (SOM) instead of being channeled directly to them. The concentration of SOM is very low and only bacteria are able to absorb SOM for their growth. Finally, the particulate bacterial cell mass which is essentially more concentrated food than SOM is grazed by protozoa and other animals. Similar microbial loop functions in terrestrial habitats (Fig. 4.4): plants produce not only phytomass per se, but also significant amount of root and shoot exudates (at least up to 30% of gross photosynthesis) providing C-source for microbes in *rhizosphere* and *phyllosphere* respectively (see below Sect. 3). The microbial loop in soil and water greatly accelerates the cycling of carbon and other elements, mainly due to the fact that exudation products of plants and other phototrophic organisms are much more available than dead organic matter in marine or terrestrial detritus.

Usually in general ecology, the autotrophic and heterotrophic processes are considered spatially separated, and food chains are believed to vary between two extremes called *pastoral* and *detrital* food chains: in the pastoral type, plants are directly consumed/grazed by phytophages, while in the second type, there is significant accumulation of dead organic matter (detritus). The microbial loop uniformly and widely spread across most of known ecosystems should form the third type of food chain.

2.2.4. Homeostasis

Ecosystems possess the remarkable ability for *homeostatic* self-regulation; they are able to resist perturbations and preserve stability in a changeable environment. The homeostatic

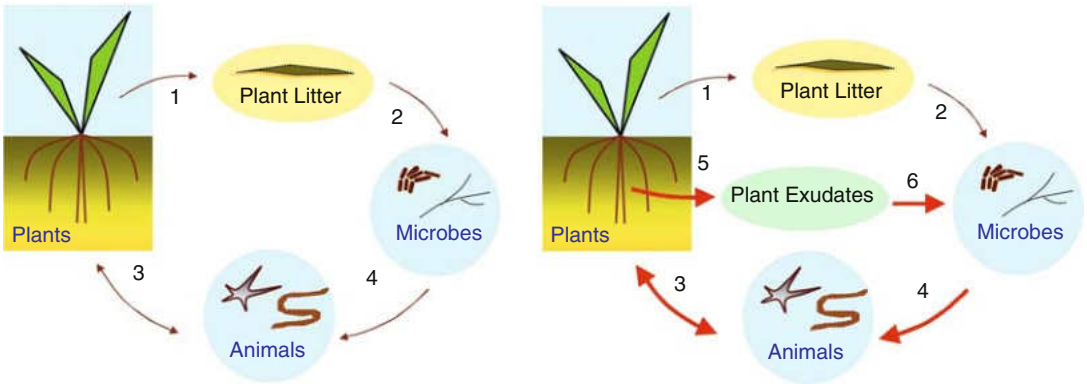


Fig. 4.4. Simplified illustration of microbial loop concept as applied to soil community. Left: soil C-cycle without microbial loop. Right: C-cycle with microbial loop initiated by root exudation (red arros).

mechanisms include various *negative feedbacks* that result when a perturbation induces a response from a biotic component of the ecosystem, decreasing the size of perturbation. The *positive feedbacks* generally play a destabilizing role (although they are needed for development of organisms). One example of destabilizing positive feedbacks is the greenhouse effect exerted by radiative gases CO_2 and CH_4: their accumulation in atmosphere causes warming, while soil warming activates more production than consumption of these gases via methanogenesis and aerobic decomposition of dead organic matter.

2.2.5. Ecosystem Productivity

The primary productivity of ecosystem shows the rate of photosynthetic production, the conversion of the solar energy into phytomass. Gross primary production (GPP) is the sum of net primary production (NPP) and plant respiration (R), which is the reverse process of photosynthesis, the oxidation of phytomass to CO_2. The secondary productivity (SP) of ecosystem is the rate of biomass formation by heterotrophic components of ecosystem, consumers and decomposers. All terms of ecosystem's energy balance are *rates*, and should not be confused with instant biomass of producers, consumers and decomposers which is characterized as a *standing crop*. If we draw an analogy with terms of chemical and microbiological kinetics, then we will see that a standing crop is equivalent to concentration (current or instant concentration) of microbial cell mass (x), e.g., mg cell/L or g cell mass/m^2. The secondary microbial productivity is equivalent to microbial growth rate, which is a product of $\mu \cdot x$ of the true specific growth rate μ [see Eq. (2)] and cell mass x with dimension g cell mass/day/m^2. Finally, the seasonal production Δx is integral:

$$\Delta x = \int_0^{150} \mu(t)x(t)\mathrm{d}t$$

where 150 is a typical mid-latitude duration of season in days; note that both μ and x are time dependent variables. It is very important to distinguish true (μ) and apparent (μ_{app}) growth rates:

$$\mu_{app} = \mu - a$$

where the term "a" is an integral measure of elimination (washout, grazing, lysis, etc). If we observe the dynamics of $x(t)$, then time-derivative dx/dt gives us only an apparent value of the growth rate, the true value being hidden by cell mass elimination (see sections below for a review of experimental approaches to assess the true growth rate of microbial populations in situ).

2.3. *Environmental Factors*

In this section, we will only touch on the effects of environmental factors on natural microbial populations. Interested readers can find detailed descriptions of specific factors (temperature, pressure, nutrient concentration, pH, tonicity, radiation, toxic compound and inhibitors, aeration etc) in comprehensive survey and books on microbial ecology (5–7); here, we will consider only the most general approaches.

2.3.1. Liebig's "Law of Minimum"

Justus von Liebig in 1842 came to the conclusion that the growth of crop plants was held in check by the most limiting mineral nutrient. Later, Cambridge botanist Blackman (8) gave a mathematical formulation to this law:

$$\mu = \min\{k_i s_i\} \tag{4}$$

where $s_1, s_2, \ldots, s_n$ are quantitative expressions for various environmental factors affecting growth of plants or other organisms and k_i is respective first order kinetic constant. Therefore, only one factor from many potential environmental variables happens to be limiting and controls the activity and growth of given population. For example, phytoplankton in the ocean are most likely to be controlled by availability of Fe (9), while heterotrophic bacteria in most of aquatic and terrestrial habitats are tended to be limited by organic substrates.

In precise laboratory experiments with chemostat (Fig. 4.5), Liebig's Law of Minimum was shown to stop working in the domain of so-called dual or multiple growth limitations where not one, but several factors (e.g., two nutrients) simultaneously affect activity of population. Thus, Liebig's law is no more than an approximation to the reality if we neglect the interaction between several nutritional factors. Another common failure of Liebig's law is observed when community is not stable but moving from one steady state to another; in this case, the effects exerted by various environmental (external) and metabolic (internal) factors can transiently change in a rather complicated way, which does not fit into a simplistic Liebig formula. For example, a transient process can start from microbial population limited with C-source by an abrupt increase in its availability; the next most probable bottleneck should be intracellular concentration of ribosomal particles (the biggest metabolic inertia) and after growth acceleration, the availability of oxygen can be the most probable limiting factor in the case of aerobic population. Finally, one should remember that Liebig's law is applicable only

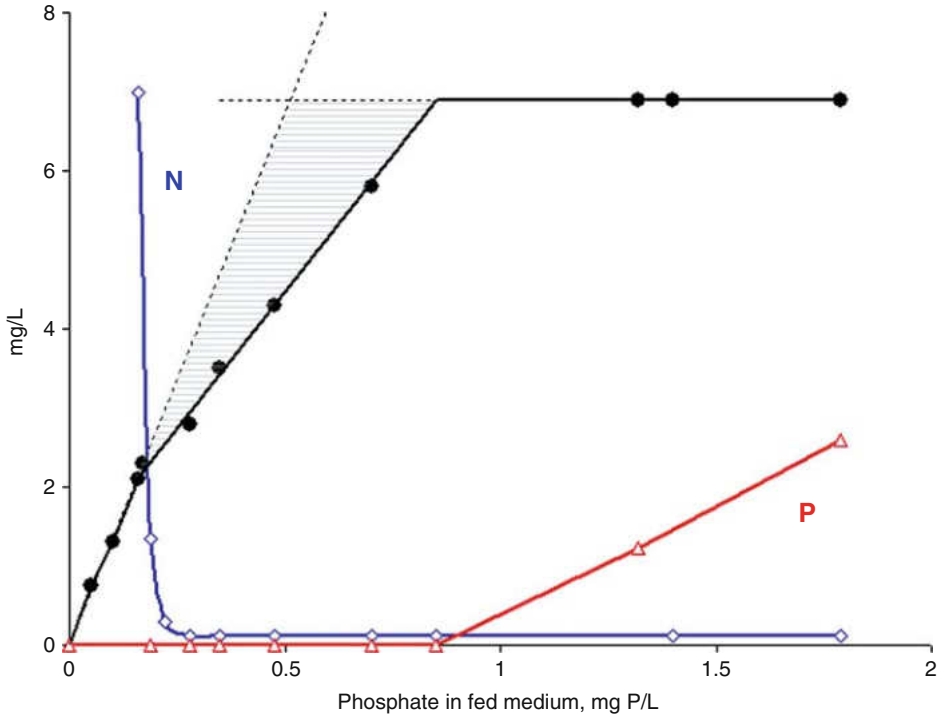


Fig. 4.5. Violation of the Liebig's Law: control of microbial growth by two factors simultaneously (10).

to such environmental factors which belong to the category of resources (e.g., concentration of nutrients or dissolved O_2, water content, etc), while other factors characterizing the state of environment (temperature, pH, Eh, soil texture, etc) do not follow this law and are to be considered within the "tolerance" law, which is discussed below.

2.3.2. Shelford's Tolerance "Law"

The lack of possibility to exist and flourish in natural environment for a particular species is determined by both deficiency and excess in the expression of any environmental factors. This law is much more universal and can be applied to practically all abiotic factors: nutrients (at high concentration any nutrient can be toxic), temperature, pH, light, etc. In each case, the effect of environmental factor on life function appears as bell-shape curve (it can be symmetrical or asymmetrical) between ecological minimum and maximum. Several factors can interact, shifting the tolerance range to either direction, for example, with an ample supply of nutrients, microbes can remain active under colder and hotter climates than starving populations. On the other hand, starving nongrowing and half-dormant microorganisms display better survival capability as compared with actively growing cells.

Several conclusions derived from Shelford's law are as follows:

1. Organisms can have wide tolerance to one factor and narrow one for another factor.
2. Organisms with wider tolerance to many factors are generally ubiquitous.

3. Under unfavorable conditions in respect to one environmental factor, the tolerance to other factors also can be significantly reduced.
4. Under natural conditions, most organisms occur far from the environmental optimum found in laboratory or field experiments due to competition with other populations.

The tolerance range for microbial populations can be determined by two major approaches: (a) varying the factor intensity in laboratory or field experiments and follow the respective response of a studied population (growth rate, metabolic activity); and (b) long-term observation of population abundance in situ with simultaneous recording of environmental factor in question with subsequent use of statistical (e.g., linear or nonlinear regression) analysis).

Both approaches are subject to errors due to: competition with other populations (decline in response can be caused by competitive exclusion rather than inadequacy of environment), effects of other environmental factors (error especially high with second approach), restricted size of population in question (in laboratory experiments we can use isolates with a lower tolerance range as compared with community in situ).

Ecotone is a transitional zone between two communities containing the characteristic species of each, e.g., tundra-forest, meadow-forest, or soft–hard ground transition in marine ecosystems. There is a trend to increase the populational density and species diversity at ecotones, this phenomenon is called the *border effect.*

The gradient of environmental (ecotopic) factors is often observed in nature as progressive continuous changes from one level of pH, light intensity, salinity, dissolved oxygen, redox potential, nutrient content, temperature, and other characteristics. Various organisms having different tolerance limits occupy their own unique position along the gradient minimizing competition for life resources (Fig. 4.6). The ecological minimum, L defines the low boundary of habitat colonization below which life is no longer supported (we will use this notion below to describe specialized life strategy of extremophiles).

2.4. Population Dynamics, Succession and Life Strategy Concept

In this section, we will summarize studies on dynamics and evolution of (microbial) ecosystems. The major challenge of such research is to attain such a level of understanding of the particular ecosystem which allows us predict its dynamics including species abundance (population dynamics) and the replacement of one set of populations by another (succession).

2.4.1. Population Dynamics and Fluctuations

Usually, population density is expressed as a number of organisms per unit area or per unit volume of habitat (N). The rate of changes in N is determined by the relationship between birth rate (r) and mortality rate (a), which is described by the empirical logistic equation:

$$\mathrm{d}N / \mathrm{d}t = rN - aN^2 = rN(1 - N/K), \quad K = r/a \tag{5}$$

If growth is started at some low values of $N << K$, then growth is almost exponential ($\mathrm{d}N/\mathrm{d}t \sim rN$). Afterward, the growth rate progressively declines because the birth is proportional to N, while mortality is proportional to N^2. As soon as the term rN is larger than aN^2, the derivative $\mathrm{d}N/\mathrm{d}t > 0$ and population grows, approaching the upper asymptotic value K, called the carrying capacity of respective ecosystem. The logistic equation is fully

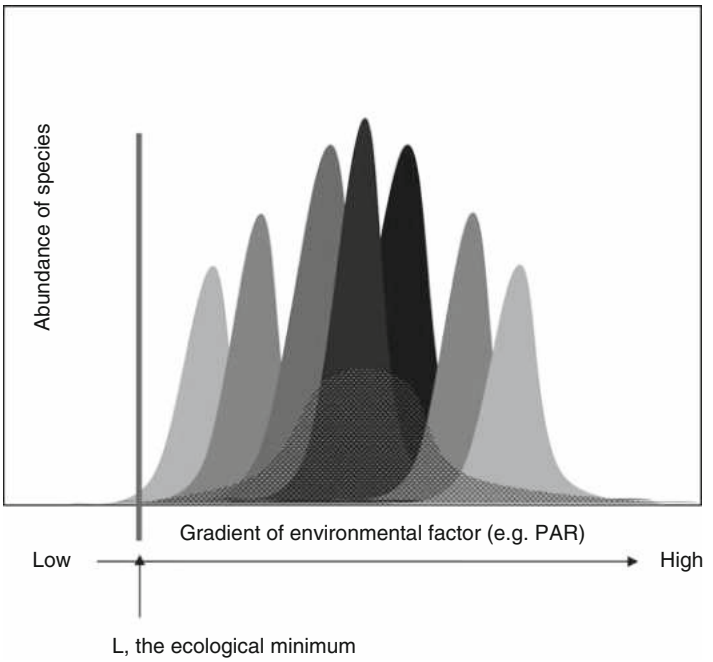


Fig. 4.6. Species continuum across environmental gradient.

empirical, but has a surprisingly wide area of application for the numerous observation data on population/community transient dynamics. Typically, these data are the time series of population dynamics after some kind of perturbation of the natural steady state ecosystem, e.g., forest fire, volcano eruption, soil tillage or fumigation, irrigation, drainage, etc. (see below section on succession). In all known cases, we have one common phenomenon, the temporal relief from competition between various populations for common limiting substrate and temporal excess of free nutrient reserves which allows the population to grow with the rate close to r-value of the logistic equation. As soon as the population density approaches the carrying capacity K, the environmental space is getting fully occupied with organisms, competition increases.

Population at a density of about K as a rule displays *fluctuations* and *cyclic oscillations*. It is important to distinguish (a) seasonal fluctuations which are controlled mainly by environmental factors such as temperature, radiation and precipitation, and (b) changes which have both longer and shorter than one year characteristic time and generally are related to some internal controlling factor at genetic or phenotypic levels. A classical example of the latter cyclic oscillations is 9–10 years of oscillations in populations of lynx and white hare in Hudson Bay (11) or 5–7 days of oscillations in numbers of soil bacteria and microbial activity (12). It is not known for certain what is the main inducer of the observed oscillations: genetic program, cosmic factors such as periodic changes in the nature of solar radiation, or mobile signal metabolites (H_2, ethylene oxide) playing the role of "community hormone."

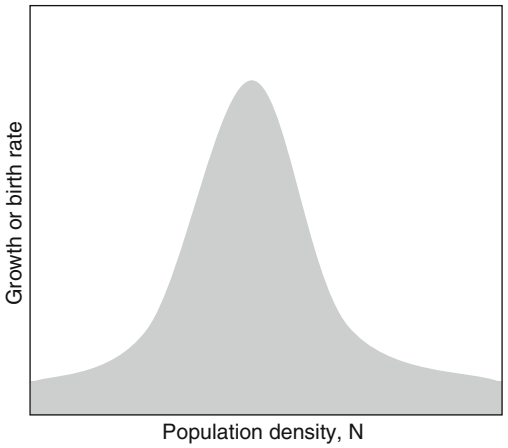


Fig. 4.7. Illustration of the Alle-principle.

Less mysterious is the so-called "Alle principle," which states that overcrowding of environment as well as a too low density tends to restrict population growth: the plot of growth rate versus N is usually bell-shaped with a maximum at "optimal" density of individual organisms (Fig. 4.7). That is why sparse populations resist being evenly distributed and instead aggregate into colonies of various sizes and shapes. The molecular mechanisms of both positive and negative interactions between individual organisms within single population are combined now under general term "quorum sensing."

Bacteria and other unicellular organisms show group behavior: for example, in living biofilms, individual cells at different locations in the biofilm may have different activities. The molecular mechanism of quorum sensing is used to monitor the bacterial population density. This process relies on the production of a low-molecular-mass signal molecule (often called "autoinducer" or recently quormon), the extracellular concentration of which is related to the population density of the producing organism. Cells can sense the signal molecule allowing the whole population to initiate a concerted action once a critical concentration (corresponding to a particular population density) has been reached. Gram-negative and gram-positive bacteria use different signal molecules to measure their population density (Fig. 4.8). Gram-negative bacteria have the cell–cell communication based on *N*-acyl-homoserine lactone (AHL) signals. The first example and the paradigm of gram-negative quorum signaling is the luxI–luxR quorum sensing system of *Vibrio fischeri*, involved in population density-dependent regulation of bioluminescence. *V. fischeri* is a free-living marine bacterium that also occupies the light organ of the squid *Euprymna scolopes*. The high population density required for bioluminescence is reached only in the microenvironment of the light organ.

The AHL signaling system of *V. fischeri* involves two major components: luxI is the AHL synthase gene that is part of the bioluminescence operon luxICDABEG and luxR codes for the transcriptional activator. At low population density, the transcription of luxICDABEG is weak. The AHL quorum sensing signal molecule produced by LuxI at a basal level, 3O,C6-HSL

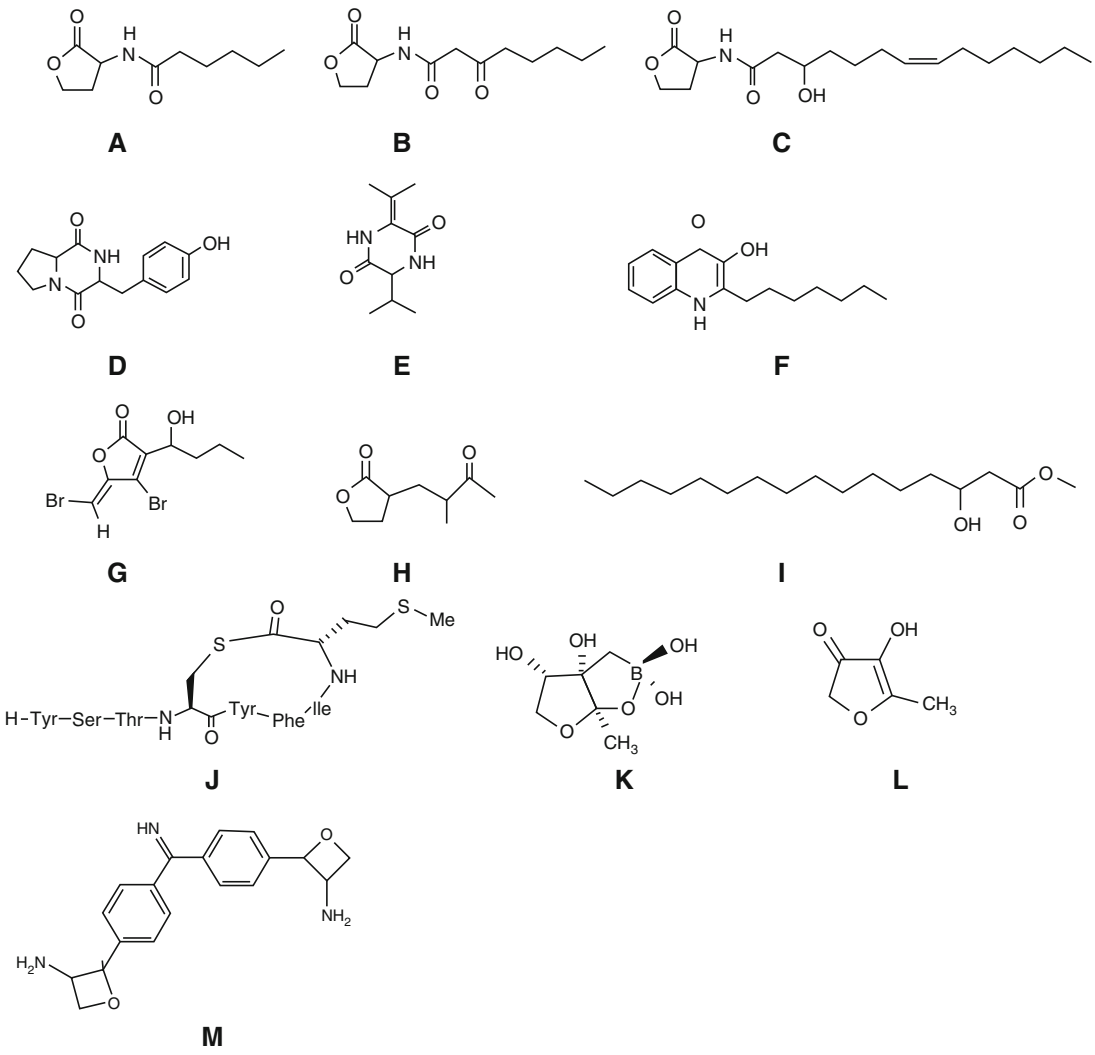


Fig. 4.8. Different quorum sensing signal molecules (13). (**A–C**) Examples of microbial AHLs without substitution on the C3, or with an oxy or hydroxyl group. (**A**) *N*-hexanoyl-L-homoserine lactone or C6-HSL. (**B**) *N*-(3-oxooctanoyl)-L-homoserine lactone or 3O,C8-HSL. (**C**) *N*-(3R-hydroxy-7-cis-tetradecenoyl)-L-homoserine lactone or 3OH,C14:1-HSL. (**D, E**) Microbial diketopiperazines. (**D**) cyclo(L-Pro-L-Tyr). (**E**) cyclo(D-Ala-L-Val). (**F**) 2-Heptyl-3-hydroxy-4-quinolone (PQS) produced by *Pseudomonas aeruginosa*. (**G**) 4-Bromo-5- (bromomethylene)-3-(10-hydroxybutyl)-2(5H)-furanone of *D. pulchra*. (**H**) c-butyrolactone produced by *Xanthomonas campestris*. (**I**) 3-Hydroxypalmitic acid methyl ester of *Ralstonia solanacearum*. (**J**) Group IV cyclic thiolactone from *Staphylococcus aureus*. (**K**) Putative structure for Vibrio harveyi AI-2. (**L**) It is also possible that this compound and 4-hydroxy-5-methyl-3(2H)furanone (MHF) are interconvertable. (**M**) bradyoxetin, a four-membered oxetane ring, from *Bradyrhizobium japonicum*. (With permission from Elsevier).

Table 4.1
Effect of ecological interactions on population growth

Type of interaction	Effect on population A	Effect on population B
Neutralism	0	0
Amensalism	0	−
Commensalism	0	+
Mutualism	+	+
Predation/parasitism	+	−
Competition	−	−

(see below), diffuses through the membrane. The LuxR transcriptional activator is inactive at this moment. With increasing population density, the AHL concentration increases. When a threshold concentration is reached, the signal molecule binds to the LuxR transcriptional activator. This complex is active and binds to the promoter region of the bioluminescence operon luxICDABEG. This leads to a rapid amplification of the AHL signal 3O,C6-HSL and consequently induces bioluminescence.

Types of interactions between organisms are summarized in Table 4.1. There are many examples of each of the listed types of interactions (5). Only competitive interactions have been studied in a precise experiment with two and more populations of protozoa cultured in the same flask (14). One of the competing species was always completely eliminated. On the basis of such experiments, the principle of competitive exclusion was formulated, which states that a particular ecological niche can be occupied by only one species. Below, we will clarify why natural habitats always contain coexisting species.

2.4.2. Development and Evolution of Ecosystems

The development of ecosystems is usually called *ecological succession.* Questions related to the notions of ecological succession or evolution of ecosystems include: What are the limits for community stability after perturbation/disturbance of environment? What are the driving forces for community dynamics? Can we predict it based on environmental data? Is there relationship between composition of biotic community and ecosystem's functions?

The English word "succession" and scientific term "ecological succession" are not identical. The second term is defined in many ways, starting from the simplistic version "the replacement of populations by other populations better adapted to fill the ecological niche" (5) to a descriptive inclusive one: "The *gradual and orderly process* of ecosystem development brought about by changes in community composition and the production of a climax characteristic of a particular geographic region" (15). We can observe changes in community composition in seasonal or multiyear dynamics because of fluctuation. But contrary to fluctuations and seasonal dynamics which are *cyclic or random*, the ecological succession proceeds as an *orderly, unidirectional and irreversible* process. Succession is usually initiated by dramatic changes in the state of abiotic environment: climatic warming or cooling, flooding or desertification, fire, volcano eruption with lava-stream, etc. We can distinguish between

autotrophic and heterotrophic succession. The former assumes the development of plants or other autotrophic community on the initially bare land (e.g., on the magma rocks). Heterotrophic succession takes place after heavy deposition of organic matter, e.g., amendment of poor soil with manure. Succession is called *primary* if the development of ecosystem starts from zero: on the suddenly released rocks, lava-stream or sand dune. The *secondary* succession is much quicker and takes place, say, as reforestation of abandoned arable field or after forest fire or clear cutting.

Succession in microbial community takes place concurrently with the evolution of an entire ecosystem because the gradual and orderly replacement of plant and animal species affects microbial microenvironment. We can also observe purely microbial succession in the laboratory or field experiments with microcosms (microecosystems). Figure 4.9 shows the growth dynamics of consecutive replacement of one microbial group by another after soil amendment with glucose or cellulose.

The mechanisms of succession are viewed entirely differently by ecologists supporting one of the two competing paradigms: holistic or meristic.

According to the holistic concept (syn: *organisms*), the biotic component of ecosystem is a kind of superorganisms. It has stable structure and strong deterministic interactions based on differentiation of econiches similar to interactions between specialized tissues and cells within multicellular organism. Succession is analogous to ontogenetic development of individual differentiated organisms. It can be accurately predicted and is driven by changes in the physical state of habitat caused by community: the early populations modify the physical state of habitat providing better growth conditions for the next stage organisms; such replacement continues until the equilibrium is attained between the biotic and abiotic components in climax community.

The meristic approach (syn: continualism) assumes that various species have a relatively high degree of freedom. Although there are some biotic interactions between species, they can enter and leave a community through immigration and emigration. The replacement of species during succession is also not strictly deterministic and has clearly expressed stochastic nature. The replacement occurs mainly as a result of competition between organisms occupying the same econiche. One cannot accurately predict the temporal profile of the transient community (i.e., the list of species and schedule of replacement) due to significant effects of chance, local conditions and past history. However, there is a well-expressed trend in consecutive changes in the community structure from predominantly r-selected to predominantly K-selected species.

2.4.3. The Concept of Life Strategy

The most essential element of the second approach is the concept of *life strategy* and *continuum.* Life strategy is defined as "a combination of adaptive reactions which provides the possibility for a given population to coexist with other organisms and occupy some part of niche hyperspace" (16). Usually, the strategy is characterized by the so-called "survival triad": (a) the ability to compete with other populations, (b) to recover after perturbations, and (c) to survive stresses. In this manner, one may distinguish three types of natural selection:

1. K-selection operates in climax ecosystems under stable and predictable conditions without frequent perturbation and stresses. The habitats of this type are overcrowded, thus the main

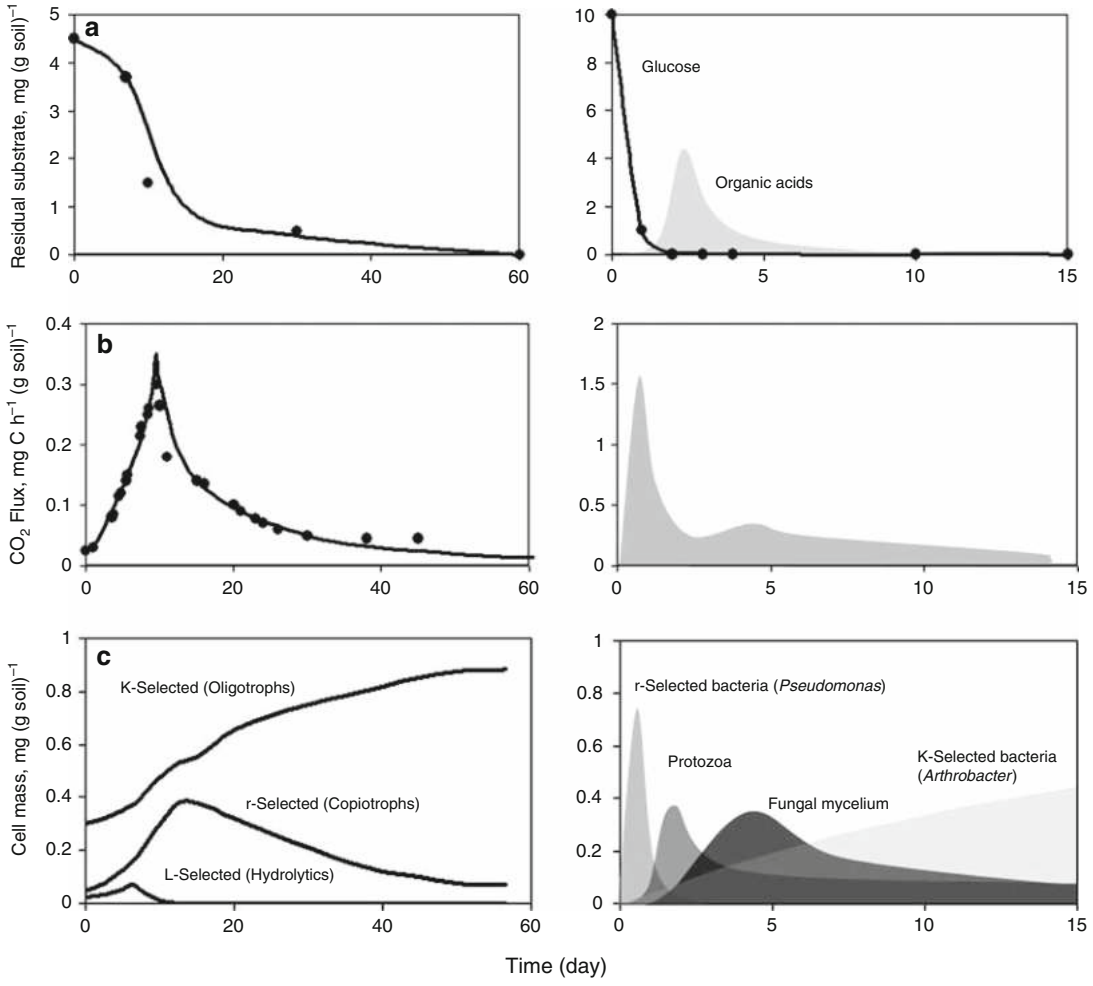


Fig. 4.9. Examples of microbial successions induced by soil amendment with cellulose (*left column*) and glucose (*right column*); after (7). Decomposition was recorded as dynamics of residual substrate (**a**) and CO_2 evolution rate (**b**). Abundance of various microbial groups (**c**) was evaluated on the basis of microscopic observations with UV microscope and simulation with SCM.

feature of *K*-selected species should be a high competitive ability ("lions" type of strategy). Their generation time is relatively long and they have few progeny, but nevertheless these species maintain high population densities.

2. *r*-Selection operates on the pioneer stages of succession initiated by some perturbation of a climax ecosystem i.e., a sudden change of environmental conditions (not necessarily adverse), a flash of nutrients, a cataclysmic elimination of competitors. The main result of perturbation is temporary relief from the pressure of severe competition for nutrient resources. *r*-Selected species survive in ephemeral, unpredictable habitats because of mobility and high reproduction rates (opportunistic

or a "jackal" type of strategy). They are not good competitors and are always ready to leave the resources once they become depleted or overcrowded.

3. *L*-selection operates under adverse environmental conditions caused by various stresses. Stress factors could be abiotic (nonoptimal salt concentration, temperature, pH, water content, etc.) or biotic (antagonism, starvation caused by the depletion of substrate by more successful competitors). The products of *L*-selection are the patient species resistant to a particular stress factor ("camel" type of strategy).

The *r* and *K* notations is derived from logistic equation: *K* stands for carrying capacity and state of community close to climax with maximal competition, while *r* is the maximal growth/birth rate observed at the origin of logistic curve and corresponding to pioneer stages of succession. *L* stands for ecological minimum on the environmental gradient or the minimal density of population under unfavorable environmental conditions allowing positive birth rate (Figs. 4.6 and 4.7).

The concept of *rKL*-selection is not absolute, being meaningful only in the comparison of several organisms. The best way to identify the life strategy of some studied organisms would be to locate them in one common *rKL-continuum*. The more prosperous a particular species is under the conditions (1), (2) or (3), the closer it is placed to the *K*-, *r*- or *L*-pole of this continuum. An example of such an ordination is shown in Fig. 4.10.

The differences between two competitive paradigms are summarized in Table 4.2 and flowchart diagram. The first concept of a superorganism tends to overemphasize the strength of biotic and in particular symbiotic interactions and underestimates the competition between

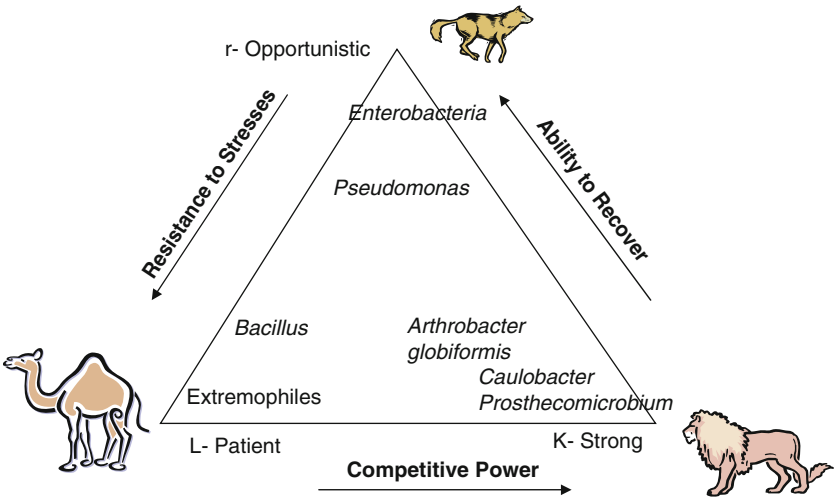
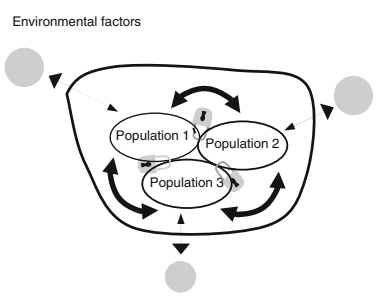
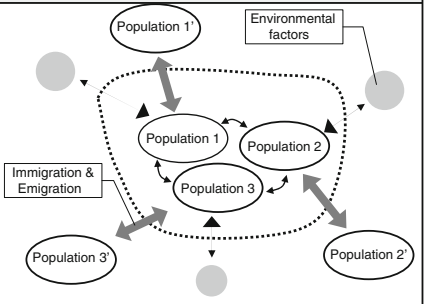


Fig. 4.10. The illustration of the concept of life strategies. All natural microorganisms are located along three axes characterizing survival triad: the ability to compete for resources (K-axis), recover after stresses (r-axis) and resist unfavorable environment (L-axis). Respectively, one can distinguish the following three types of natural selection which correspond to three types of life strategy.

Table 4.2
Comparison of holistic and meristic paradigms explaining the driving forces of succession and evolution of community

Holistic (superorganism) paradigm	Meristic (continuum) paradigm
	
Community structure	
Constant, precisely determined by strong interspecific links	Flexible, depends on immigration & emigration, past history and local conditions
Boundary of ecosystem	
Clearly expressed	Not clearly expressed, peripheral gradient
Prediction possibility	
High	Low
Pioneer stages of succession	
Abiotic environment is not appropriate for life. The first colonizers are stress-resistant species which improve environment for other organisms	Ecological vacuum: environment is not saturated by organisms and the first colonizers are opportunistic species, no competition, no sever stress
Transient community: effects of organisms on abiotic environment	
Abiotic environment is getting better and better due to 'edification'; the productivity of community increases. The reverse effect of environment on organisms is usually not emphasized although not rejected.	Strong selective effects of environment on organisms, progressive increase of competition for resources which are getting more and more limited. The 'edification' effect of organisms on environment is not emphasized although not rejected.
Climax community	
Stabilized community with maximal symbiotic interactions, biomass and information content per unit of available energy flux	Stabilized community with maximum of competitive interactions between biotic components occupying similar econiches

biotic components. The second paradigm appears more realistic (stochastic nature of ecosystem's evolution, importance of competition and selection pressure from environment), but probably underestimates the significance of gradual modification of environment by organisms (such as soil forming processes) as essential component of long-term succession.

In microbial ecology, the superorganismal paradigm is intuitively more attractive for ecologists focusing on metabolic networks within microbial community (Fig. 4.3). Such networking assumes the existence of strong interactions between different members of community and is more naturally associated with deterministic approach and the holistic view of community as a superorganism. At least, the organismal paradigm is appropriate at initial theoretical studies aimed at characterization of the most essential key functional features of the studied natural ecosystem. The following comparison of diverse ecosystems and tracing their evolution probably would greatly benefit from the second more realistic continuum paradigm and concept of life strategy. However, before we can discuss the microbiological interpretation of a life strategy concept, we must touch on the basics of microbial growth kinetics.

2.4.4. Growth Kinetics of Microorganisms with Different Life Strategy

Under favorable growth conditions (temporary excess of nutrient substrates, absence of inhibition), the bacterial growth rate should be proportional to the instant cell mass, x, the quotient μ remaining constant:

$$\mathrm{d}x/\mathrm{d}t = \mu x \tag{6}$$

The integration of Eq. (6) at initial condition, $x = x_0$ at time $t = 0$, gives the exponential equation:

$$x = x_0 e^{\mu t} \text{ or } \ln x = \ln x_0 + \mu t \tag{7}$$

However, the specific growth rate μ remains constant only for limited time and narrow environmental conditions. According to the popular Monod model (17), the μ value is controlled by concentration of *limiting substrate* and the biomass formation is linked to substrate uptake by mass-conservation condition [Eq. (2)], then:

$$\begin{aligned} \frac{\mathrm{d}x}{\mathrm{d}t} &= \mu(s)x; \quad \mu(s) = \mu_\mathrm{m}\frac{s}{K_\mathrm{s}+s} - a \\ \frac{\mathrm{d}s}{\mathrm{d}t} &= -\frac{1}{Y}\frac{\mathrm{d}x}{\mathrm{d}t} \end{aligned} \tag{8}$$

Equation set (8) contains four parameters: yield Y, maximal specific growth rate μ_m, saturation constant K_s (substrate concentration at which $\mu = 0.5\,\mu_\mathrm{m}$), and specific maintenance rate which is related to maintenance coefficient $a = Y^{\max}m$ [see Eq. (2.2.2)]. The set of these four parameters can be thought of as "ID" for particular organisms and used to predict their growth dynamics. Remarkably, this model was used to develop a *chemostat theory* before actual experiments with continuous culture were undertaken – a very rare event in the history of mathematical biology! The model predicts a number of counter-intuitive features of chemostat, e.g., that specific growth rate μ can be set up by experimentalist by changing

the medium flow at any values between 0 and μ_m (before exponential growth was believed to occur only at $\mu = \mu_m$) and that μ-values do not depend on the feed-substrate concentration and is governed solely by the *residual* substrate concentration in the culture.

However, the Monod model fails to explain a number of essential growth phenomena observed experimentally: lag-phase, death of starving cells, product formation and any kind of adaptive changes in microbial population, such as induction-repression of enzymes, yield variation, changes in the cell RNA content etc. These gaps were filled in by so-called structured models.

Structured models explicitly describe variations in cell composition. They usually include mass balance equations not only for *external* substrate(s), but also several *intracellular* components, C_1, C_2, ..., C_n. For each variable C_i, a differential equation is written which takes into account all sources, r_+, and sinks, r_-, as well as its dilution due to cell mass expansion (growth),

$$\frac{dC_i}{dt} = r_+(s, C_1, \ldots, C_n) - r_-(s, C_1, \ldots, C_n) - \mu C_i \tag{9}$$

The earliest structured models accounted for no more than three to five cell constituents, e.g., the total cell proteins, RNA and DNA, reserved polysaccharides, ATP-pool, etc. The modern meticulous models contain up to hundreds and even thousands of internal variables borrowed directly from available genomic data bases. The recent challenge was to develop a *virtual cell,* to construct a biological system in silico without essential reductionistic compromise. However, the predictive capability of these intricate models are rather modest: they are still a "caricature parody" of the real cell, but already too complex to be studied mathematically (stability analysis, parameters identification, etc.) or to improve understanding of the biosystem. The best choice of a mathematical model lies, apparently, midway between unstructured and highly structured models outlined here. One of the best known examples is *synthetic chemostat model* (SCM).

According to SCM (7), the microbial growth occurs as a conversion of exosubstrate S into a number of cell macromolecules X′ via a pool of intermediates L part of which are respired to CO_2 (Fig. 4.11):

Macromolecular cell components are susceptible to degradation (turnover), and intermediates L can leak out. The array $\mathbf{X}'$, the cell composition is not fixed and varies in response

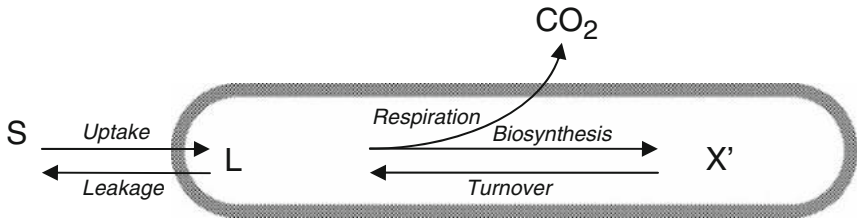


Fig. 4.11. Chart-flow diagram describing cell growth according to SCM.

to a changing environment. The heart of the SCM is the solution of the problem; how to characterize these variations without going to extreme intricacy.

For this purpose, all the macromolecular cell constituents are divided into two groups:

1. **P**rimary cell constituents necessary for intensive growth (P-components).
2. Components needed for cell s**U**rvival under any kind of growth restriction (U-components).

The content of P-components (ribosomes and all enzymes of the primary metabolic pathways) increases parallel to growth acceleration. The contribution of U-components under good growth conditions decreases (to comply with conservation conditions $\mathbf{P} + \mathbf{U} = \text{const}$), and attains the maximum under chronic environmental stress to improve cell resistance. The typical U-components are enzymes of the secondary metabolism, protective pigments, reserved substances, transport systems of high affinity. An interdependent variation of individual P- and U-components is approximated by a linear function of some *master variable* r^*:

$$\mathbf{P} + \mathbf{U} = \frac{P_1}{P_n} + \frac{U_1}{U_m} = \frac{P_1^{\min}}{P_n^{\min}} + \frac{U_1^{\min}}{U_m^{\min}} + r^* \frac{P_1^{\max} - P_1^{\min}}{P_n^{\max} - P_n^{\min}} + (1 - r^*) \frac{U_1^{\max} - U_1^{\min}}{U_m^{\max} - U_m^{\min}} \quad (10)$$

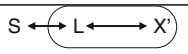
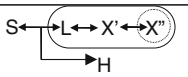
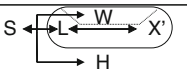
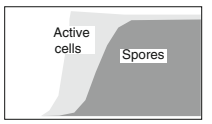
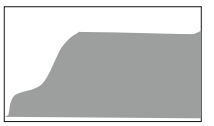
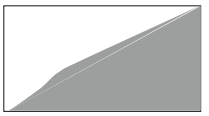
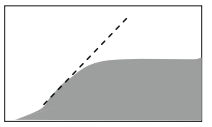
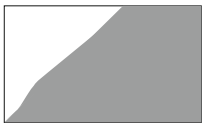
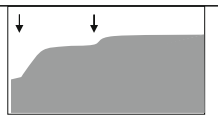
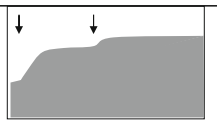
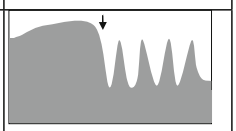
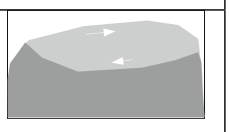
Where, $P^{\max}$, $U^{\max}$ and $P^{\min}$, $U^{\min}$ are respectively upper and low boundaries for **P** and **U** adaptive variations, and r is the *scalar* function, not *array!* The r^*-value depends directly on environmental factors, e.g., on the limiting substrate concentration and r^*-variable is participating in all kinetic expressions (q_s, m, μ) to simulate the combined effects of the *current* environmental factor(s) and cellular physiological state determined by the growth conditions *in the past*.

The simulative capabilities of structured models like SCM are high enough to mimic and explain the majority of available experimental data on various microbial cultures (steady-state and transient, continuous and batch from lag- to decline phases). What is important for ecological applications, the SCM realistically describes and predicts not only growth per se, but also many other dynamic phenomena: survival dynamics under starvation, formation of dwarf cells under growth restriction, the adaptive adjustment in cell maintenance requirements, variation of growth potential and affinity to substrate, utilization of substrate mixture, etc.

Going back to the concept of life strategy, we can now use kinetic data to describe quantitatively, why the variation in the pressure of natural selection (K-, r- and L-types) resulted in diversity of dynamic growth patterns of various microbes isolated from natural habitats. Table 4.3 summarizes the results of kinetic studies of the typical microbial r-, K- and L-selected species chosen on the basis of field observations (how frequently respective microbial species were found in climax or pioneer communities or in unfavorable habitats) as well as on complementary laboratory experiments with cultivation under conditions simulating respective natural environment (7).

Enterobacteria, pseudomonas, baker and fodder yeast are mainly products of r-selection. Their dynamic behavior is erroneously considered to be typical for *all* microbes: rapid and balanced growth, short lag-periods and smooth transitory processes. They dominate in those natural habitats which are frequently "rejuvenated" to the pioneer succession stage: hot spots of substrate amendment, animals gut and feces, rhizosphere with diurnal fluctuations in exudation rate and perpetual changes in "addresses" of exudation loci due to apical extension

Table 4.3
Diversity of growth patterns in soil bacteria stemming from difference in the life strategies (18)

Type	*Pseudomonas-Enterobacteria*	*Bacillus-Streptomyces*	*Arthrobacter-Caulobacter*
			
Scheme of metabolic flow			
Batch: cell growth and starvation decline			
Dialysis culture: cell mass dynamics			
Chemostat transients: dynamics of cell mass after abrupt change in D			
C-limited chemostat culture: steady-state biomass versus D			

Diversity of growth patterns displayed by soil bacteria of various life strategies. Upper row shows typical morphology of selected microbial groups, next row demonstrates cell growth chart and the main state variables of the respective modification of SCM used for simulation (X″ is prospore compartment, H is autoinhibitor and W is poly-β-oxybutyrate (reserved compounds), S, L and X′ and explained in the text. Black arrows indicate the time of switching from one dilution rate, D, to another in chemostat culture, white arrow shows direction of sequential D-changes, either from low D to high or reverse, in chemostat culture displaying multistability.

of root hair. Petri dish with reach medium like LB or yeasts extract is good simulation of such hot spots; that is why *r*-selected species are easily isolated from soil.

K-selected bacterial species are much less amenable to isolation and cultivation. Probably, most unculturable microbial species belong to this type of life strategy. When cultivated

under artificial laboratory conditions, they are fastidious and unpredictable. The best option for their cultivation is continuous culture with cell retention: fed-batch, dialysis culture or batch culture with C-substrate delivered via gas phase (volatile C-substrates, such as ethanol or VFA). Under these cultivation conditions, the K-selected species display high yield and almost 100% viability even at extremely slow cell division (generation time up to months). In batch culture, their growth is slow with the "false diauxie," biphasic growth on the single substrate. In chemostat and turbidostat, they display oscillations and multiple steady states. To simulate the described abnormal behavior of *Arthrobacter* and other oligotrophic species, the SCM was elaborated to include intermediates with autoinhibition functions (e.g., peroxides as respiratory by-products) and the possibility of direct incorporation of deficient C-substrate to pool of reserved compounds (Table 4.3). The main feature of the growth control in oligotrophic species is the relative independence of transport, catabolic and anabolic reactions which does not allow rapid balanced growth, but gives great advantage in consumption of highly dilute substrates and survival prolonged starvation.

Many *L*-selected microorganisms (bacilli, actinomycetes, some fungi) share the following common features: spore formation, production of antibiotics, and synthesis of hydrolytic enzymes. All these features help them survive even if they fail in direct combat with competitors for deficient nutrients. Kinetic studies allow us to understand why they are weak competitors. The most striking feature was observed in *Bacillus* dialysis culture: the bacteria stopped growth after 2–3 weeks when the residual glucose level dropped below the threshold value of 20–50 μg/L. In rich environments even with intermittent supply of nutrients (feast-to-famine transitions simulated in batch culture), these bacteria perform well. They rapidly deplete the available substrates, which triggers sporulation and transition to a dormant state, preserving the bacteria from extinction. However, the chronic starvation typical for most oligotrophic environments is the "trap" for bacilli; they are provoked to sporulate but are not able to finish it in a normal way. The slow feed via the dialysis membrane provides glucose levels which are too high to allow termination of normal sporulation and too low for growth because of the the uncoupling action of metabolite H and acceleration of turnover rate (see Table 4.3).

3. METHODS OF MICROBIAL ECOLOGY

As in any other biospheric and ecological sciences, there are three main approaches in microbial ecology:

1. In situ (field) observations with minimal disturbance of the studied processes and communities
2. Laboratory and field experiments with deliberate modification of the natural object aimed at revealing of unknown functional relationships
3. Mathematical and conceptual modeling aimed at generation of new theoretical knowledge, testing hypotheses and comparison of theoretical concepts

Microbial ecology has its own "sore spot": a relatively weak development of theoretical concept (mathematical modeling is not as popular and appreciated as in other ecological disciplines) is associated with the ongoing problem of inadequate laboratory surrogates for natural populations. Contrary to macroecology, microbial ecology has long been developing

as an experimental science with a doubtful and elusive research subject. Just imagine the frustration of an animal ecologist who is confined in his supposedly comprehensive study with only a domesticated cow, goat and donkey! In microbial ecology, we have had to deal with a limited range of cultivated microbes for a detailed study of their possible functions in situ. This is because many subtle features of microbial behavior can be only disclosed in accurate laboratory studies with pure cultures. This leads the researcher to ask: Is such extrapolation really justified? Are axenic laboratory cultures sufficiently representative of their natural progenitors?

3.1. Natural Microbial Populations and "Laboratory Artifacts"

Different opinions have been expressed in respect of this ongoing problem. On the one hand, "pure cultures could certainly be regarded as a physiological artifact" (Kluyver) and so "a clear demarcation line should be drawn between data obtained under abnormal experimental conditions, which invoke microorganisms to reveal some new features, and data from observations on ecological factors in nature" (19, pp. 25–47). On the other hand, "many properties of pure laboratory cultures are also exhibited by microbial populations under natural conditions" (20), and "unless there are indications to the contrary, it is justifiable, and operationally necessary, to assume that in most characteristics pertinent to the habitat, pure cultures do resemble their progenitors in nature" (21, pp. 100–101).

Properties of microbes in pure laboratory cultures may differ from those of their ancestors in natural habitats because of the following factors: (a) the lack of metabolic interaction with other organisms normally present in situ; (b) autoselection of mutants in the long-term course of isolation, purification, and maintenance of cell culture; (c) phenotypic changes in the physiological state of microbial cells in response to a changed environment (different with respect to the availability and spectrum of substrates and modifiers, temperature, humidity, etc.). It was primarily the third factor that Winogradsky was referring to when he wrote of "invoking" laboratory forms to grow abnormally. We also regard this as a fundamental factor. The first factor is not decisive since, in soil, subsoils and sediments, microbial growth is confined to microsites where practically pure cell clones develop. In homogeneous natural habitats, such as waters, there are negative and positive interactions via metabolites. However, antagonists are not able to sustain co-existence and, in the case of positive cooperative effects, it is in fact, microbial associations that are isolated from the natural habitat rather than pure cultures. Factors (b) and (c) are almost indistinguishable in practical terms. They are also fairly similar in principle because both the selection and phenotypic variations are not random, but tend toward a better adjustment of the population to the given growth conditions.

In terms of quantitative microbiology, factor (c) may be interpreted as a difference in the vector of physiological state of a laboratory culture as compared with a population in situ. At Winogradsky's time, two major cultivation techniques were available, plating on solid agar media and liquid batch culture with nutrient broth. Microbial cells grown under such conditions do have a peculiar physiological state which is indeed dissimilar from that of in situ soil microbes. Today, we have a much wider assortment of cultivation techniques. Consequently, we have the improved ability to control the physiological state of a laboratory culture and may intentionally shape it by cultivation conditions. Particular challenging is to

use (a) continuously starving batch culture with spend/exhausted nutrients and dialysis culture to maintain deeply limited and very slowly growing cell populations; (b) nonsteady state cultures with deliberate fluctuation of cultivation conditions simulating natural rhythms; (c) careful design and selection of chemical composition of nutrient media resembling the most essential features of the natural habitat; special efficient approach is to use dialysis membrane separating cultivation chamber with almost intact natural community producing the whole spectrum of metabolic products needed for growth of indigenous populations. With these approaches, microbial ecologists have made significant progress in their attempts to increase the number of cultured microorganisms.

3.2. "Great Plate Count Anomaly"

It was discovered as early as the nineteenth century that plating on nutrient agar and serial dilutions fails to encourage growth of the most abundant in situ microbial species. The first explanation to this phenomenon was given by S. Winogradsky and only recently via environmental gene retrieval (extraction of total soil DNA, amplification of, say, 16S rRNA gene and following sequencing) it was confirmed explicitly that cultured forms are only minor components of the entire natural community. This inability to recover the most numerous organisms from natural habitats by using cultural approaches has been called the "enumeration anomaly" or the "Great Plate Count Anomaly" (22). For example, Hugenholz et al. (23) reported the discovery of 36 major phylogenetic groups of eubacteria in natural communities, which is about triple the number of those that have been cultivated in pure culture. The relative proportion of uncultured forms varies in different habitats. Sometimes, environmental gene retrieval and plating give identical results indicating that ALL microbes can grow on artificial laboratory media. For instance, the plating and MPN enumeration of psychrophilic bacteria in summertime Arctic pack ice from the Chukchi Sea gave up to 62% of culturability as compared with direct microscopy (24). However, most of the complex natural habitats have as low as 0.1–1% of the total amount of phylotypes able to grow on artificial media. A similar proportion is normally reported by comparison of plate count with direct microscopy: the last one gives $\sim 1,000$ times higher number than the first one.

What is the reason for discrepancy between plate count and direct microscopy? Let us consider the following equation:

$$\text{Direct Count} = M_1 + X_1 + A(\text{CFU} + X_2 + X_3 + X_4 + X_5) \tag{11}$$

In this equation, Direct Count stands for the total amount of cells seen under microscope, M_1 is the number of microscopic errors, i.e., spherical or rod-shaped abiotic particles erroneously taken as cells.

CFU is the number of Colony Forming Units, the actual result of plating shows the number of cultured cells. A is the average number of cells in aggregates in the droplet of suspension added to the plate. The higher A is, the more significant is the underestimation of the real number of culturable cells in natural habitat. The A-value is higher for soils and sediments than aquatic habitats (large amount of solids catalyze aggregation) and for filamentous and slimy cells as compared with small cells without capsule.

X_1 is the number of cells/aggregates which do not grow on selected media; it is obvious that there are no universal media adequate for all physiological groups of microorganisms, there are no common cultivation conditions, say temperature, pH, Eh, CO_2 and O_2 partial pressure in the head space to satisfy all the multitude of the growth requirements (for example, methanogenic bacteria cannot be grown on an aerobically incubated Petri dish with yeast extract agar).

X_2 is the number of cells or cell aggregates attached to a pipette during the preparation of serial dilution. We can minimize this number by using hydrophobic plastic tips, but can never completely eliminate this error.

X_3 is the number of stressed or viable-and-unculturable cells/cell aggregates. The reason for stress is not fully understood, but we have several experimental methods to reproduce such metabolic stress as "substrate-accelerated death" by prolonged incubation of the "normal" soil bacteria on media with excess of catabolites (e.g., glucose) and deficient in nitrogen (7). It was also found that hormone-like signal metabolites were inhibiting cell division.

X_4 is the number of microcolonies which stopped their development because of any kind of competition (lack of available space on the agar plate, inhibition by antibiotics produced by other colonies, etc); contribution of this factor is especially high when analyzed microbial suspension is too dense giving more than 50 CFU already the first 2 days.

Finally, X_5 are those K-selected microbes which grow too slowly. We know from in situ measurements that a generation time of more than one month is quite a probable event, implying up to one year period for development of a visible colony. However, 1–3 weeks is too long to await plating results; also, agar layers tend to be dried or contaminated. Fortunately, we can estimate roughly the number of slow-growing microbes by occasionally recording the dynamics of CFU on a single Petri dish during several months, for example. The plot of CFU versus time usually gives several waves (25), each of which can be approximated by the first-order rate equation (Fig. 4.12):

$$N = \sum_{i=1}^{n} N_i^{\infty}(1 - e^{-ki(t-t_i)}), \quad i = 1, 2, \ldots, n \tag{12}$$

where n is the number of waves (usually $1 < n < 4$), and $N^{\infty}{}_i$, k_i, and t_i are empiric constants.

Now, let us assign to all terms numerical values which we have in some "typical" top soil: "Direct Count"$= 5 \times 10^9$ cell/g, CFU $= 0.5 \times 10^7$ CFU/g (3 days aerobic incubation, YEA – yeast extract agar), M_1 was assumed to be 20% of the total count (fair assumption even for experienced microscopist!), A $= 10$, $X_2 = X_4 = 10^5$, $X_3 = 0.2 \times$ CFU $= 10^6$, and $X_5 =$ $10 \times$ CFU. To comply with mass balance, the main unknown variable X_1 should be equal to approximately 85%, which seems to be a reasonable estimate. Thus, the main reason for lack of agreement between direct microscopy and plating is the immense metabolic diversity of the majority of natural habitats.

Thus, about 15% of the total unicellular objects revealed by direct microscopy should be considered known and potentially culturable aerobically on standard YEA medium if all technical errors of plating are eliminated (cell aggregation and adhesion, stress, nonoptimized

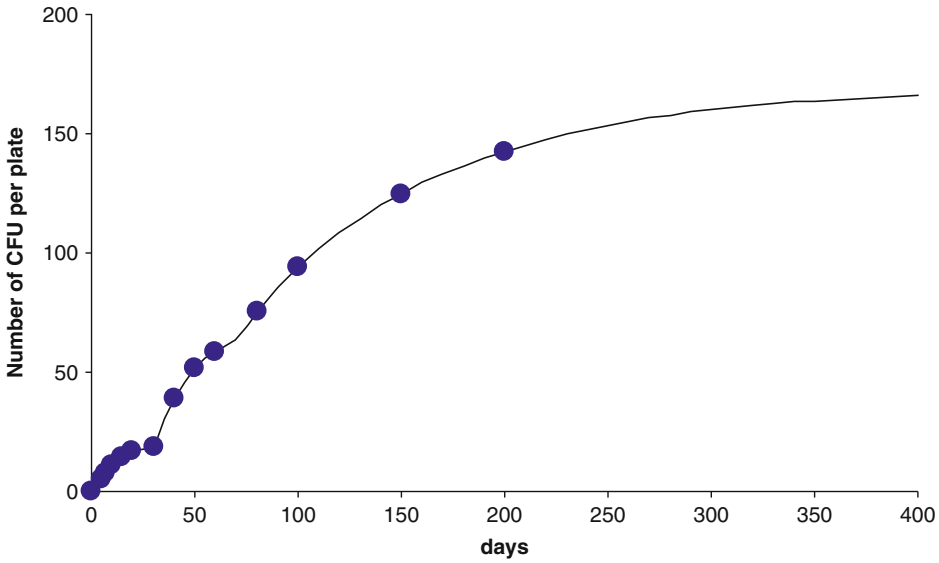


Fig. 4.12. Dynamics of colonies formation on Petri dish with YE agar. The continuous curve calculated from Eq. (12) to find out an amount of slowly growing microbes.

dilution and too short incubation). The remaining 85% of the soil community is missed because they are not aerobes or do not like yeast extract. Application of the full range of available cultivation techniques would at least double the number of culturable organisms, implying that we should know more than 30% of the entire soil community.

The molecular techniques based on sequencing of SSU rRNA usually give less optimistic results: no more than 10% of known phylotypes. The reassociation kinetics of the total soil DNA (26) displays a high degree of heterogeneity of microbial DNA in the majority of habitats. Calculations based on empirical relationships between reassociation kinetic constants and DNA heterogeneity expressed as conditional genome numbers reveals also a huge gap between known and total microbial diversity.

3.3. Estimation of the Microbial Numbers and Biomass in Soils and Water

There are five classes of analytical techniques suitable for determining microbial biomass:

1. Ex situ germs enumeration (plating and MPN),
2. Direct microscopy,
3. Kinetic methods (biomass of specific microbial group is calculated from kinetic data on instant response of natural samples to added substrate).
4. Biochemical methods (detection of specific microbial metabolites – ATP, DNA, muramic acid, chitin, phospholipids profile or fumigation flux)
5. Methods based on DNA sequencing (FISH – Fluorescence In Situ Hybridization)

Table 4.4 outlines the specific advantages and shortcomings of these approaches (for details, see discussion in a numerous experimental papers and reviews, e.g., (27–30)). Obviously, the

Table 4.4
Comparison of different method for soil biomass determination

Methods	What is measured	Conversion factor	Shortcomings	Advantages
Plating and MPN	Number of CFU (colony forming units) or highest positive dilution	Absent or extremely unreliable	Low recovery resulted from cell adhesion and clumping, and unculturability; variability of CFU sizes	Isolation and identification of microbes, differentiation of physiological and taxonomic groups, detection of individual populations carrying genetic markers
Direct microscopy	Bacterial number and mycelia length	Derived from measured cells biovolume and assumed dry matter content and cells bulk density. Not reliable	Subjective procedure, unreliable differentiation of cells from soil particles and plant debris	Characterization of bio-morphological structure of community across space; detection of individual populations in combination with DNA probes and immunofluorescence
Biochemical	Content of unique cell constituents: ATP, DNA, membrane lipids, or OM release after fumigation	Estimated from (1) analysis of isolates, (2) mass balance in lab incubations, (3) comparison with direct microscopy	Occurrence of analyzed chemical species in plant roots, animals and in extracellular milieu, wide variation of conversion factor	High precision, open to standardization and automation, compatibility of results obtained in different laboratories, effective in combination with isotope technique to label microbial cells in situ
Kinetic	Rates of specific metabolic reactions estimated in incubation experiments with amended soil samples	Found as parameters of mathematical model describing microbial growth on added substrate. Precise and objective procedure	Underestimates the resting forms of natural microbial populations	All technical advantage of biochemical methods (see above), exact calibration, differentiates microbial biomass into functional groups, assessment of the physiological state of microorganisms in situ

choice of method depends on the targets of a particular study, and there can be no absolute preference for one unique methodological tool. It is sufficient to mention that even plating and MPN techniques (which have been subjected to most severe criticism last decades) remain to be valuable and indispensable in some specific fields of research.

In the last decade, growing interest was paid to nontraditional approaches based on biochemical determinations (class 3, Table 4.4). New generations of these methods gave strong impetus to the development of quantitative microbial ecology. However, these methods have at least two drawbacks: (a) they use doubtful conversion factors from measured chemical index (ATP, DNA, or chitin content, fumigation flush, etc) to real biomass, and (b) they generally neglect diversity of soil microbial community. The most popular method today remains direct UV microscopy with new "functional" staining methods, analysis of phospholipids profile and various techniques based on extraction and sequencing of the total community DNA: wide range of techniques starting from specific oligonucleotide probes (FISH) to metagenome analysis.

3.4. Estimating Microbial Growth Rates In Situ

The majority of biologists who are unfamiliar with microbial ecology naively believe that bacteria multiply very fast. This is a gross misconception. First, we should acknowledge that some bacteria do grow very fast, but there are plenty of slow growing *K*-selected microorganisms with multiplication speeds essentially lower than, say, rats or herring. Second, even opportunistic bacteria displaying explosive growth rate with generation time down to 15–20 min on specially designed laboratory media fail to grow fast in situ, the main restrictive factor being amount and quality of nutrients. In this section, we briefly survey the available literature on techniques used to measure the actual growth rate of microbial populations in situ. This issue is especially important for the development of environmental biotechnologies for a very simple reason: microbial growth rate in situ is an integral parameter related to the actual activity of microbial populations in their technological performance. Additionally, the actual growth rate of indigenous and released to natural environment populations must be known to predict their fate after termination of biotechnological processes.

3.4.1. Microscopy In Situ

Direct microscopic observations of this type are normally done only in aquatic habitats with the use of a submerged-slide technique. At regular intervals, glasses with microbes attached are removed from water for microscopy and afterward are returned back. Instead of standard glass slides, microcapillaries may be used (31). An alternative approach, which has only been used by the most courageous ecologists, is to immerse a microscope directly into the pond and to carry out a diurnal observation of individual cells attached to glass surface (32). Obviously, this technique requires the discrimination between true growth of attached bacteria and their immigration from surrounding waters. The cell settlement or detachment could be accounted for by a microscopic count of UV-sterilized control slides. The generation time of aquatic bacteria was found to vary from 2 to 30 h.

3.4.2. Methods Based on the Analysis of the Cell-Division Cycle

In eukaryotes, the cell cycle consists of four phases: mitosis, G_1, S, and G_2. Mitosis can be recognized morphologically. In many cell types, the time of mitosis (t_m) represents a constant fraction of the total cell-division cycle. If t_m is known, then the generation time, g, can be found from the relationship $t_m/g = 1.44\,R$, where R is the fraction of cells in mitosis. This method was used initially to measure the growth rate of the protozoa *Entodinium* in the rumen. The division frequency at night was higher than during the day, and the average generation time was about 15 h.

Hagström et al. (33) suggested that the growth rate of Gram-negative bacteria could be estimated from the frequency of occurrence of dividing cells. The division event (formation of septa and subsequent separation of the two daughter cells) is known to occupy a more or less constant time within the bacterial cell cycle. So, the higher the growth rate, the higher the probability of finding a cell at this stage. The calibration of the method was achieved using a mixed chemostat culture of marine bacteria. In the coastal region of the Baltic Sea, the frequency of dividing cells was below 5%, and the estimated mean generation time varied seasonally from 10 to 100 h.

3.4.3. Genetic Methods

Meynell (34) devised an elegant method to measure microbial growth rate using bacteria with a nonreplicating genetic marker. At each cell division, the fraction of the population which contains the label is halved. Once the dynamics of total and labeled populations are determined, the doubling time can then be calculated from the rate of marker dilution. Meynell studied the growth of pathogenic enterobacteria in the gut and blood circulation system of laboratory animals. The genetic markers were various superinfecting mutants of phages, which enter the bacterial cells, but do not replicate. It was observed that after intravenous inoculation into a mouse, virulent *Salmonella typhimurium* cells became lodged in the spleen. Their viable count doubled every 24 h, whereas the true doubling time as determined from the rate of marker dilution was 8 to 10 h. The same strain grew 20 times faster on nutrient broth ($g = 0.5$ h).

3.4.4. Techniques Stemming from Chemostat Theory

Many natural habitats are open systems, with a continuous supply of nutrients and the simultaneous elimination of cells. Under such conditions, the growth rate μ is eventually adjusted to the elimination rate, D (similar to the dilution rate in the chemostat). Now, the value of D is often easier to measure than μ. For example, in the case of bacteria growing in an animal's intestines, D is measured by feeding the animal food tagged with some inert label (lignin, silica-gel, dyes, etc). The time of 50% reduction in the output label concentration is expressed as $t_{0.5} = \ln 2/D$. The steady-state (or quasi steady-state) cell concentration, is measured in the gut of sacrificed animals. Using this method, enterobacteria in laboratory rodents (mice, rats, hamsters) were shown to yield between one and six generations per day.

Another ingenious technique was developed by Brock for measuring the growth rate of thermophilic algae in hot-spring drainways (35). The technique involved measuring the algal wash-out rate after growth was prevented by darkening the system. The spring was sheltered

from ambient light by a screen and the exponential decrease in cell concentration in the effluent stream was monitored. The value of D, which is equal numerically to μ under normal day-night cycles, was found to be 0.4/day ($g = 40$ h).

In static aquatic environments such as lakes and ponds, the main factor responsible for microbial cell elimination is no longer wash-out, but their predation by protozoa and probably other small animals. The growth of cells and their grazing rate are about to be balanced. If under in situ experiments, predation is completely suppressed by passing the water sample through filters retaining large protozoan cells, then the μ value may be measured from the recorded increase in the bacterial population (7). However, this approach should be used with care. Suppose we are to measure the value of μ in a chemostat culture from the x dynamics after stopping the flow. It is obvious that by halting the pump operation, we terminate not only cell washout but also the substrate input with fresh medium. Therefore, the use of this method is restricted to only nonlimited microbial growth. These conditions are fulfilled in the chemostat only at $s \gg K_s$ (i.e., at high s_0 and subcritical D), so that reliable application of the method is limited to natural eutrophic habitats.

3.4.5. Isotope Techniques

The high sensitivity of radio-isotope techniques allows for the measurement of the rates of consumption of labeled substrates added to water at nearly background concentration. The main problems include: (a) how to estimate the ratio between added labeled and natural nonlabeled compounds, and (b) how to derive the rate of microbial growth from the measured rate of label consumption. Several examples are provided below.

Dark $^{14}CO_2$ fixation rate as a measure of total heterotrophic bacterial production was originally suggested by Romanenko (36). Heterotrophic CO_2 fixation is an anaplerotic metabolic reaction, serving to regenerate those metabolic intermediates which are "lost" from the TCA cycle for the synthesis of macromolecules. Hence, the measured fixation rate is expected to be tightly coupled to the overall cell growth, through metabolic control. However, the experimentally observed ratio of carbon fixed from CO_2 to total carbon assimilated has been found to vary in a wide range, from 0.01 to 0.12. In view of this fact, it was suggested (37) that measurements of CO_2 fixation should be accompanied by a determination of the activity of PEP-carboxylase, the principal anaplerotic enzyme. This would allow for more rigorous conclusions about the stoichiometry involved.

The primary productivity of phytoplankton is determined by the measurement of the rate of $^{14}CO_2$ photoassimilation. In recent modifications of the technique, it was suggested that the label incorporation be determined in the fraction of chlorophyll *a* rather than in whole particulate matter. This allows the estimation of phytoplankton biomass and avoids possible underestimations caused by label transfer from algae to bacteria and zooplankton via excretion and grazing respectively (38).

Nowadays, the most promising technique for the evaluation of secondary productivity (microbial growth rate) in waters is considered to be the measurement of the uptake of labeled precursors of nucleic acids biosynthesis, thymidine, uridine and adenine (39). The use of isotopes with high specific activity guarantees minimal alterations of in situ growth conditions. At the same time, the amount of added nucleoside should be large enough to suppress their

synthesis de novo from endogenous cell compounds. The main deficiency of this routine is the ambiguity of conversion factors from a nucleoside uptake rate to a microbial growth rate.

3.4.6. Assessment of Productivity from Fluctuation Frequency of Microbial Biomass

The first systematic studies of bacterial production in soil were undertaken by Aristovskaya (40). The work involved daily measurements of the number and size of bacterial cells by direct microscopy of soil smears. Bacterial production was evaluated from the shape of the dynamic curve $x(t)$. This curve was always characterized by a seesaw pattern. Every 3–8 days increases in x were observed followed by declines down to background level. Fluctuations did not depend immediately on environmental factors and occurred even under stable hydrothermal conditions. This type of fluctuating dynamics was first observed as early as the beginning of the century and was explained by two mechanisms: (a) by a predator-prey interaction of soil bacteria with microfauna (mainly with amoebae), which usually gives rise to oscillations in the population densities of both prey and predator (41); and (b) by the accumulation in soil of self-inhibitory metabolic products (H_2, ethylene oxide, a hypothetical compound "periodine," etc.), which are susceptible to spontaneous autoxidation, decomposition or dispersion (40).

For calculating productivity, Aristovskaya assumed that bacterial growth is periodically interrupted by toxin accumulation while grazing of microbes was executed continuously. From this, the overall production of "seesaw" bacterial growth was calculated as the following sum: apparent x increase (measured during intervals where $dx/dt > 0$)+bacterial biomass elimination (estimated as x decreases at time intervals when $dx/dt < 0$). This calculation algorithm may underestimate as well as overestimate the true bacterial productivity. The generation time was found to vary in the seasonal dynamics from 7 to 100 h, with a seasonal bacterial production of 1–6 tons of dry weight per hectare. When compared with natural waters, the microbial growth rate in soils was roughly the same, whereas the seasonal productivity was higher by an order of magnitude. For example, the net bacterial production over one season in the Rybinsk water reservoir was as low as 200 kg/ha, while in podzolic soil of the same bio-climatic zone it was 1,500 kg/ha.

3.4.7. Estimation of Productivity from C-Balance

A simple relationship exists between the respiration rate of aerobic chemoorganotrophs v_{resp}, their biomass (x) and specific growth rate (μ):

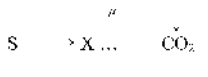
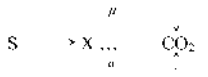
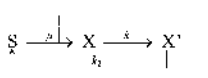
$$v_{resp} = Y_{p/x}\mu x \tag{13}$$

Although the biomass yield, $Y_{p/x}$, depends on numerous factors, it can be measured as the net average value in calibration experiments for the entire microbial community of a particular soil. The main advantage offered by this method is the possibility for continuous and exact recording of in situ metabolic rates by CO_2 analysis. Of course, one must be able to distinguish microbial and plant roots activity to the overall soil respiration, but this is basically feasible. A rough estimation based on soil respiration data (42), revealed lower productivity of soil microorganisms as compared with previous calculations, but systematically this approach has not been implemented.

Table 4.5a
Estimation of soil microbial growth from mass balance of entire ecosystem

Ecosystem, ref	Microbial biomass, kg/ha	Warm period, day/year	Input of plant litter, kg/ha/year	Mean generation time (day) according to equation			Microbial production, t/ha/year
				Eq. (14)	Eq. (15)	Eq. (16)	
Subarctic bog (43)	310	100	1,560	34.4	$\mu < 0$	20.7	1.0
Mixed forest (44)	148.4	150	12,000	3.2	3.6	1.9	8.1
Coniferous forest (45)	192	150	4,920	10.1	15.6	6.1	3.3
Soil under wheat (46)	400	100	7,080	9.8	14.8	5.9	6.8
Soil under continuous wheat (28)	1,140	100	2,400	82.3	$\mu < 0$	49.4	1.6
Virgin steppe (47)	800	190	23,900	11.0	17.8	6.6	16.0

Table 4.5b
Mathematical expressions used for the calculation of microbial productivity in soils

Model	Graph	Equation of mass balance	Production term	
Simple chemostat-type model		$\dot{s} = F - \mu x / Y$ $\dot{x} = \mu x - kx$	$\mu x = YF$	(14)
Account of maintenance		$\dot{s} = F - \mu x / Y^{max} - ax / Y^{max}$ $\dot{x} = \mu x - kx$	$\mu x = Y^{max}F - ax$	(15)
Account of microbial biomass reutilization		$\dot{s} = F - \mu x / Y + k_2 x'$ $\dot{x} = \mu x - k_1 x$ $\dot{x}' = k_1 x - k_2 x'$	$\mu x = \frac{FY}{1 - Y}$	(16)

Symbols: S organic substrate, X viable biomass, X' necromass or microbial detritus, F input of OM, μ is specific growth rates of microorganisms, Y and Y^{max} are yield coefficients, k_1, k_2 and a are specific death or decay rates.

The second type of mass balance evaluations of microbial production in soil is more common and involves the estimation of C input to soil from plant litter and root deposition. These values are supposed to be equal to C-substrate consumption by the heterotrophic microbial community.

Tables 4.5a and 4.5b present the available experimental data on C-budget of several terrestrial ecosystems, which we used to calculate the rates of microbial growth by different methods. Equations (14) and (16) give, respectively, an upper and lower boundary of the microbial growth rate. Calculated generation times vary within a range of 5 to 50 days, corresponding to 3–25 generations per season. (The only exception is soil 5, where microbial

biomass is definitely overestimated; as a result, average generation time increased here up to 3 months). The seasonal production of microbes is of the order of tons dry weight per hectare and exhibits a steady increase parallel to the primary production of an ecosystem from 1 ton/ha for tundra to 16 tons/ha for chernozem under virgin steppe. When compared with the "seesaw" method, the mass balance calculation yields smaller microbial growth rates, but the difference is small when more realistic models [like Eq. (16)] are used. Thus, in four out of six soils, the generation time is less than one week, which corresponds to the oscillation period found in most observations. Calculations with the use of incongruous kinetic models [like Eq. (15)] yield longer generation times, the difference being up to several orders of magnitude. Sometimes, such models "predict" that microbial communities in particular soils are not sufficiently supplied by energy even to maintain their viability. Ironically, it was this type of incongruous kinetic model which shaped the dominating concepts on the physiological state of soil microorganisms.

4. DIVERSITY OF MICROBIAL HABITATS IN NATURE

Traditionally, the *biosphere* (ecosphere) is divided into atmo-, hydro-, and litho-ecospheres to describe the portions of the global expanse inhabited by living things in air, water, and soil environments, respectively. Here, we will survey the major types of microbial habitats to underpin the origin of their diversity and importance for various biotechnological applications.

4.1. Terms and General Principles (How to Classify Habitats)

Each habitat has a set of physical, chemical, and biological parameters that determine the microbial populations that may thrive there. As a result of natural selection forces, characteristic communities develop within each habitat. In some cases, particularly in extreme habitats such as salt lakes and thermal springs, the indigenous microbial populations exhibit adaptations to their physical and chemical surroundings that permit their survival. In other habitats, intense competition dictates which populations survive and become the autochthonous members of the communities living there.

A *habitat* is the physical location where an organism is found. The term *ecological niche* includes also "the profession" of a respective population, i.e., what organisms do there. The niche is the functional role of an organism within an ecosystem.

Some microorganisms are *autochthonous* or indigenous within a given habitat. They occupy the available environmental niches and are able to escape predation and compete successfully with the other members of the microbial community. Other so-called *allochthonous* microorganisms are grown elsewhere and transported into a given habitat to be there a transient member of community. They do not occupy the functional niches and typically they are weak competitors (although temporary could be abundant).

Although the definitions of autochthonous and allochthonous microorganisms are mutually exclusive, it is often difficult to determine whether a microorganism found in a particular ecosystem is indeed autochthonous or allochthonous. The truly autochthonous organisms can temporarily slow down growth and activity in situ, while allochthonous microorganisms that

have arrived into a new habitat may be able to survive, grow, and carry out active metabolism and perhaps to become autochthonous microorganisms.

For higher organisms, such as animals that range over wide territories, the habitat may be on the scale of a landscape. By contrast, the habitat for microorganisms often occurs on a microscale. Hence, for microorganisms, one must consider not only the overall characteristics of the general habitat but also the fine features of the microhabitats in which the microorganisms live.

Most natural habitats (soil, subsoil, sediments, and wetlands) are heterogeneous on a microscale. They form mosaics of relatively independent microsites, each of which can be drastically dissimilar in the amount and nature of growth substrates, aeration level, texture and moisture, etc. However, there are several very general features which are the most essential for determining the fate and functioning of microorganisms occupying respective macrohabitat or microsite:

1. *The way of delivery of nutrient substrate(s) to habitat.* The quality and quantity of microbial substrates vary widely. If we consider only the limiting and preferential/available substrates, then the most essential factor controlling microbial growth in situ is the dynamic pattern of substrate input, which can be a continuous (*cont*) and a discontinuous (*Dis*), single-term delivery of respective chemical species to particular loci.
2. *The elimination of growing cells.* Bacteria or fungi growing in situ can be retained within a microhabitat (*Ret*) or removed/eliminated (*Rem*). Examples of elimination include: cell washout, predation (consumption of microbial cells by protozoa, nematodes, microarthropods) or lysis by parasites, active migration due to taxis (motile bacteria) and tropism (vectorized apical growth of hyphal organisms). With low or zero elimination and continuous supply of nutrients, biofilms or microbial mats are formed, which are visible multilayer cell accumulation on various inert solid surfaces. In soil and waters, such accumulated immobile cells perform an important function of *geochemical barrier* to key elements used as nutrient substrates: the element's concentration in water or air drops by several orders of magnitude after passing through the microbial layer.
3. *The spatial organization of habitat.* Habitats can be homogeneous (*Hom*) and heterogeneous (*Het*) or spatially organized. In the first type of system, we have an even or at least random distribution of cells, substrate, and metabolic products across the space; in the second type of habitat, we can see regular spatial gradients. Typically, most aquatic habitats (ponds, lakes, sea) are rather homogeneous and well mixed at micro- to mesoscale (from 10^{-6} to 1 m) although there is distinct vertical stratification at the higher scale (see below). Solid habitats (soils, subsoils, sediments) are generally much more heterogeneous and mosaic; however, there are many situations when the soil environment can be safely considered homogeneous, e.g., strong homogenization is done by soil tillage and by fossorial animals. Homogeneous soil microenvironments are also formed when the growth substrates are mobile, i.e., gases and volatiles.
4. *Extreme and favorable habitats.* Favorable physico-chemical conditions are vaguely defined as those which are close to a "physiological optimum" of the majority of organisms: pH $\sim$ 7, mild hydrothermal conditions, absence of toxic compounds and any other stressful factors (moderate salinity, pressure, radiation level, etc). Under these favorable conditions, competition is very strong and plays the most essential role in community dynamics. Extremely unfavorable ecotopic conditions imply that one or more environmental factors are outside of the tolerance limits for most of known organisms: too cold or too hot, strongly acidic or alkaline, dried, irradiated, intoxicated, etc. Biological competition between *extremophiles* occupying a given habitat is minimal; the main selection factor is resistance to the key unfavorable factor.

Table 4.6
Matrix of growth patterns in situ and ex situ for favorable habitats (after (7))

Spatial organization	Substrate input			
	Continuous		**Dis**continuous	
	Cell **Rem**oved	Cell **Ret**ained	Cell **Rem**oved	Cell **Ret**ained
Homogeneous	*Con-Rem-Hom*	*Con-Ret-Hom*	*Dis-Rem-Hom*	*Dis-Ret-Hom*
Heterogeneous	*Con-Rem-Het*	*Con-Ret-Het*	Forbidden combination	Forbidden combination

The first three independent characteristics of ecotopic conditions produce $2^3 = 8$ potentially possible combinations (Table 4.6), some of them logically disallowed, e.g., any heterogeneous habitats are not compatible with the single-term delivery of substrates because even in the simplest case, molecules of substrates are *continuously* delivered to microbial cells across the concentration gradient.

Having reviewed the general principles, we now proceed to a short survey of the major types of microbial habitats in the biosphere. The main focus is an assessment of the quality of respective ecosystem as microbial habitat, discussion of the degree of understanding of the key mechanism controlling growth, elimination, competition and functioning of microbial inhabitants. Such information seems to be the most essential for the development of biotechnological and bioengineering applications based on an understanding of the functional mechanisms rather than on an empirical trial-and-error approach.

4.2. Atmosphere

The atmosphere consists of 79% nitrogen, nearly 21% oxygen, 0.038% carbon dioxide, and trace amounts of some other gases.

4.2.1. Atmosphere as Extreme Habitat

The atmosphere is saturated with water vapor to varying degrees, and it may contain water droplets, ice crystals, and dust particles. The atmosphere is divided into regions (Fig. 4.13), the troposphere interfacing with both the hydrosphere and the lithosphere. Above the troposphere is the stratosphere, and above this lies the ionosphere.

For the most part, the chemical and physical parameters of the atmosphere do not allow microbial growth and even survival: low temperatures (from -43°C to -83°C), lack of substrates, low moisture content and intensive UV radiation. Therefore, we should classify the atmosphere as the largest of the Earth's *extreme* microbial environments. The supply of substrates which could potentially support heterotrophic, methanotrophic or autotrophic growth (respectively volatile organic compounds like VFA, alcohols, aldehydes, methane and CO_2) is continuous via turbulent and diffusive flux, but the ambient concentration is so low (ppb range) that it could be kinetically manageable only if air is pumped through a microbial cell layer retained on some immobile support (types *Con-Ret-Hom* or *Con-Ret-Het,* in the Table 4.6). However, the retention of cells in air is fully excluded and formally

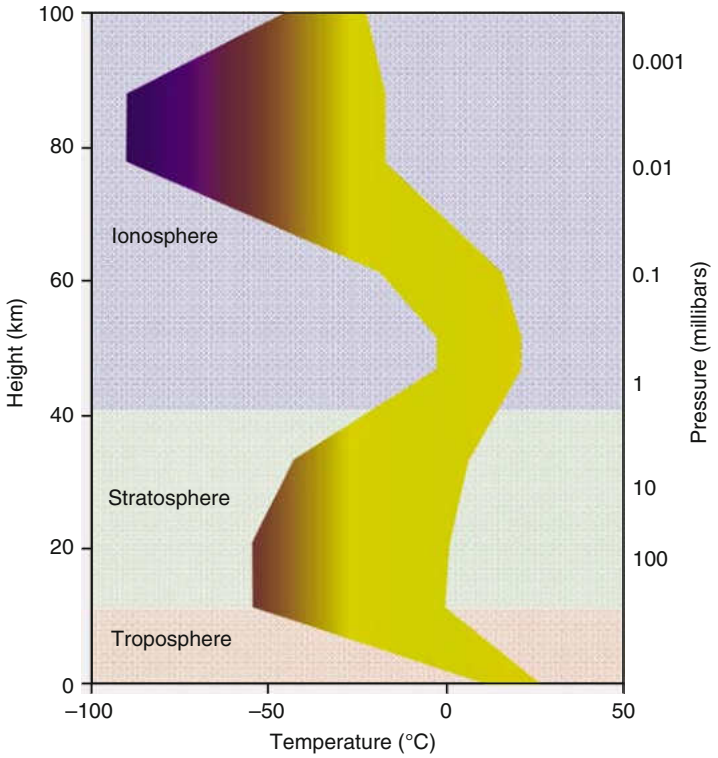


Fig. 4.13. Atmosphere as microbial habitat. Divisions of the atmosphere showing temperature and pressure gradients. The two lines indicate seasonal shifts in temperature. After (5), with permission from Pearson Education Publisher.

this type of habitats belongs to the category *Con-Rem-Hom*. Exposure to UV is probably the most powerful elimination factor; as the atmosphere thins at increased heights and offers less shielding from UV radiation, it causes lethal mutations and the death of microorganisms.

The stratosphere contains a layer of high ozone concentration, which acts to absorb UV light, protecting the Earth's surface from excessive UV radiation (48). There is a justified concern today that certain human activities, such as the flying of supersonic military and commercial jets, excessive use of fluorocarbons, and increased use of fertilizers (which results in increased release of N_2O from microbial denitrification), will decrease concentrations of ozone in the stratosphere, thus allowing increased amounts of UV light to reach the Earth's surface. The seasonal development of an Antarctic ozone hole is a clear symptom of the lessening atmospheric concentration of ozone.

4.2.2. Organisms

The stratosphere represents a barrier to the transport of living microorganisms to or from the troposphere and is characterized by a slow mixing of gases. Organisms in the stratosphere are thus transported slowly and are exposed for prolonged periods to the prevailing concentrations of ozone and high UV light intensities. Only microorganisms shielded from these conditions in the stratosphere – as perhaps within a spacecraft – could survive passage out of the Earth's atmosphere. For all practical purposes, the atmoecosphere does not extend above the troposphere (5).

Even though the atmosphere is a hostile environment for microorganisms, there are substantial numbers of microorganisms in the lower troposphere, where, because of thermal gradients, there is a rapid mixing of air (6). Some microorganisms have evolved specialized adaptations that favor their survival in and dispersal by the atmosphere. Several viral, bacterial, and fungal diseases are spread through the atmosphere; outbreaks of disease from such microorganisms often follow prevailing winds.

Temporary locations in the troposphere may provide habitats for microorganisms. Clouds possess concentrations of water that permit growth of microorganisms. Light intensities and carbon dioxide concentrations in cloud layers are sufficient to support growth of photoautotrophic microorganisms, and condensation nuclei may supply some mineral nutrients. In industrial areas, there may even be sufficient concentrations of organic chemicals in the atmosphere to permit growth of some heterotrophs. Nevertheless, such "life in the sky" is only a fascinating possibility; conclusive proof is lacking, and the practical importance of such life appears to be negligible (5).

Although many microorganisms that grow in the hydrosphere or lithosphere can become airborne, there are no known autochthonous atmospheric microorganisms. During dispersal, aquatic and soil microorganisms may enter and pass through the atmosphere before reaching other favorable aquatic or terrestrial ecosystems.

4.2.3. Significance for Environmental Engineering

The most important bioengineering task today is the development of monitoring of the atmosphere for potential biohazardous organisms, first of all pathogenic bacteria, fungi and viruses. Airborne pathogens are especially dangerous because of their direct invasion into the respiratory tract; on the other hand, bacterial aerosols could be easily detected (with higher speed and better sensitivity and precision) as compared with bacterial populations in soils and waters. The most promising procedure for scanning bacterial aerosols seems to be laser-based IR spectroscopy. A new and highly intriguing direction of environmental biotechnology is to regulate the physical state of the atmosphere by introducing bacterial aerosols of ice-nucleation bacteria which affect snowfall, freeze-resistance of plants, cloud formation, etc.

4.3. Aquatic Ecosystems

The hydrosphere is divided into freshwater (lakes, ponds, springs, swamps, streams and rivers) and marine habitats (seas, oceans and estuaries). All these habitats are interconnected to each other and terrestrial systems (Fig. 4.14). The world's oceans occupies 71% of the Earth's

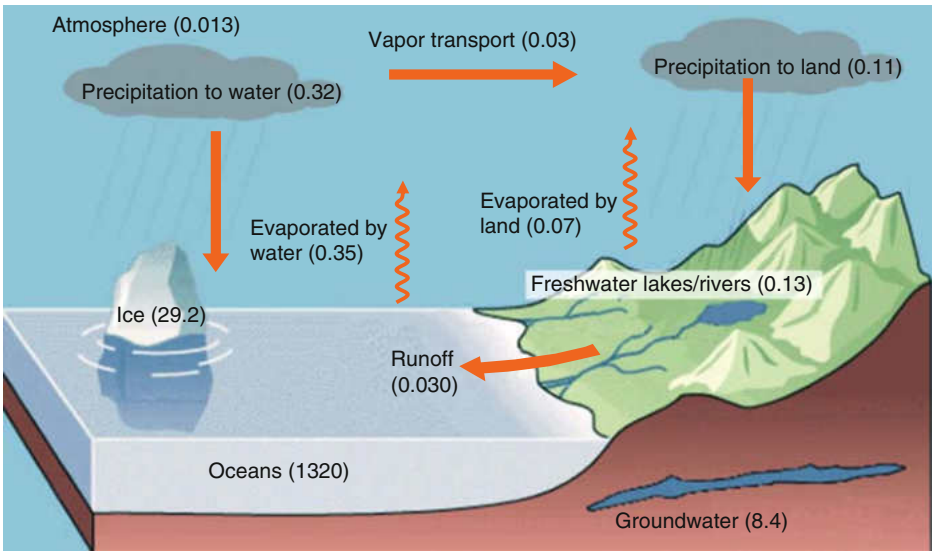


Fig. 4.14. The present-day surface hydrologic cycle. The numbers in parentheses refer to volumes of water in millions of cubic kilometers, and the fluxes adjacent to the arrows are in millions of cubic kilometers of water per year. After (49).

surface. Its huge water masses have an important buffering effect on the global climate, serving as the ultimate reservoir and receptacle of the global water and energy cycle. About 50% of the incident solar energy is consumed in the evaporation of water. Water vapor eventually precipitates as rain or snow, releasing the stored energy. The precipitation returns water to ocean directly or after passing over/through land as runoff. Therefore, the ocean is the ultimate basin for all water-soluble minerals and soluble recalcitrant organic matter derived from the terrestrial environment.

Freshwater habitats are classified based on their physical and chemical properties. Those with standing water (lakes, ponds) are called *lentic habitats*; those with running water are *lotic habitats* (rivers, streams, and brooks).

4.3.1. Lakes

Lakes are divided into several zones based on the penetration of light. In the upper *euphotic zone,* light is available to support photosynthesis. The deeper *profundal zone* is practically dark and does not support photosynthesis; two zones separated at a so-called *compensation depth* where photosynthesis is equal to respiration (usually here the photon flux is $\sim$ 1% of the full sunlight intensity). The *littoral zone* is the region of a lake where light penetrates to the bottom (Fig. 4.15). The *limnetic zone* refers to open waters inhabited by plankton. The bottom of the lake, or *benthos,* is the interface between water (hydrosphere) and solid sediments (part of lithosphere). Particulate nutrients (dead cells and cell aggregates of phototrophic organisms) are deposited by gravitational forces and concentrate on the surface of the benthic sediments.

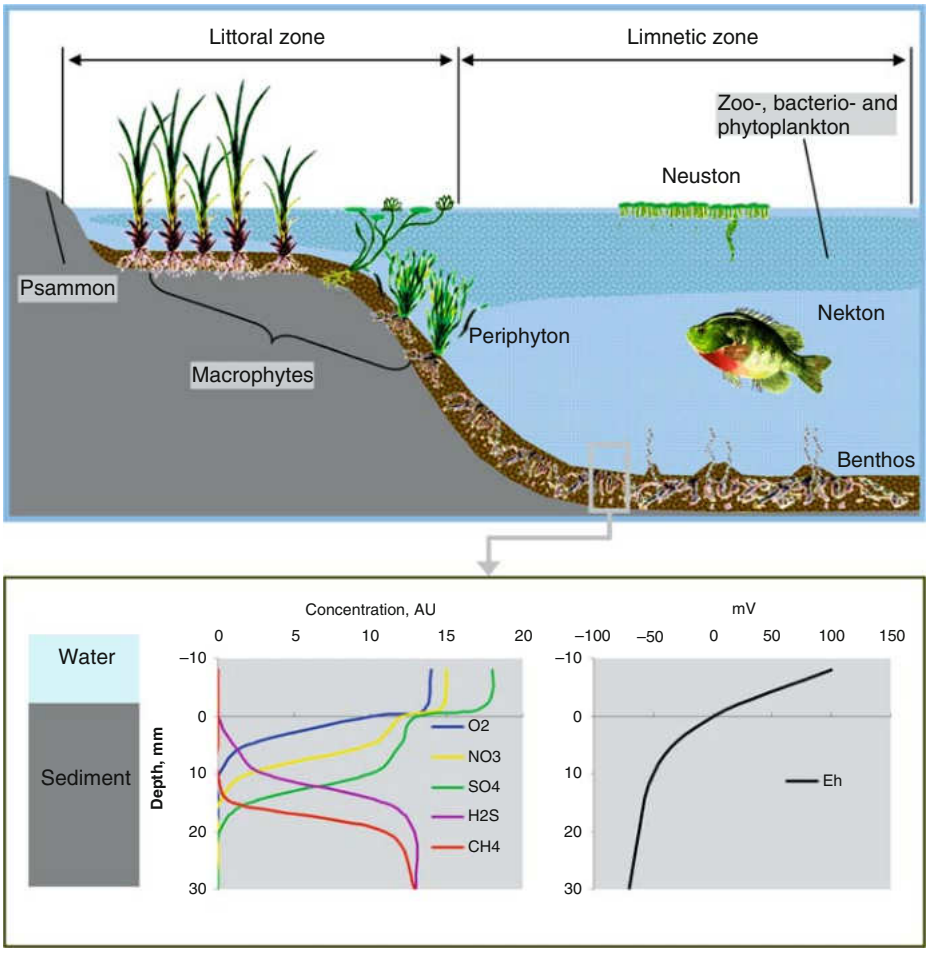


Fig. 4.15. The vertical profile of the typical lake. Insert shows vertical gradient of hydrochemical characteristics; note the sequential order of switching from one predominant electron acceptor to another with sediment depth.

The oxygen diffusion from the water to underlying sediment is rather low, thus only first several mm of sediment are aerated. Deeper layers accommodate anaerobic microorganisms which sequentially use alternative electron acceptors in the order of decrease of respective redox potential: $O_2 > NO_3^- > SO_4^{2-} > H_2O$. Generally, stratification of silt material may be found at the scale of micrometers into aerobic layers, denitrifiction, Fe-reduction, sulfate reduction and methanogenesis.

In addition, the lower portion of the water column in most freshwater lakes becomes seasonally anoxic. The mechanism is explained and illustrated by Fig. 4.16. The starting point is the fact that the maximal density of water corresponds to the temperature $+4^\circ$C and both warming and cooling decrease water density. In the spring, as the sun warms the water, a

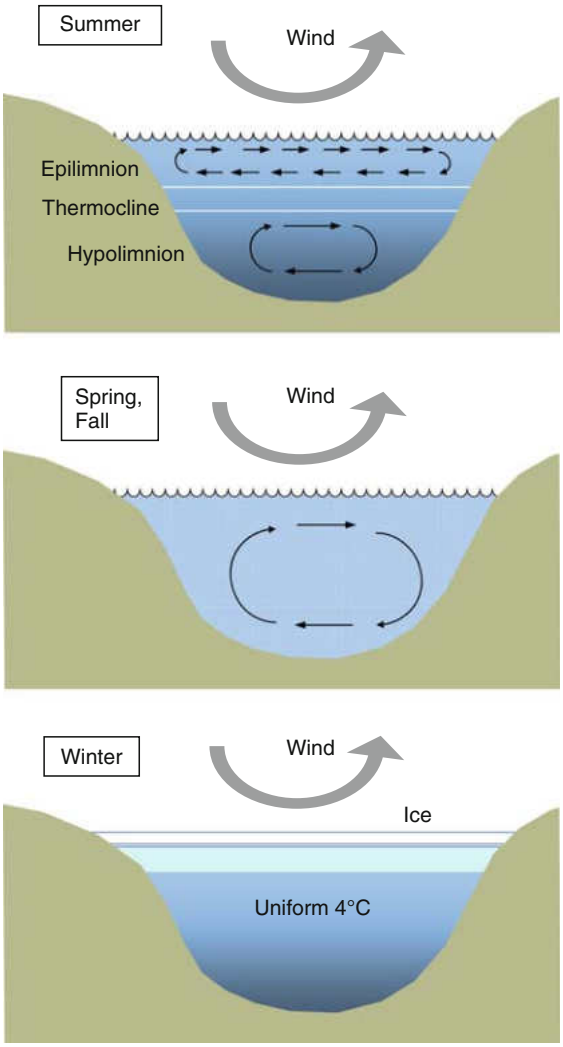


Fig. 4.16. Annual circulation patterns in a dimictic lake. The typical dimictic lake undergoes stratification in the summer and complete overturn in the autumn and spring. During winter, surface ice prevents further mixing by the wind. Small differences in density and temperature exist, with cooler water (0°C) staying near the surface and warmer, more dense water (4°C) extending to the bottom.

warm surface layer called the *epilimnion,* is formed. This warm, lightweight water ceases to mix with the lower, colder and denser layer (*hypolimnion*). The boundary between these layers is the *metalimnion* or *thermocline*, a zone of rapid temperature change. With the onset of autumn, the epilimnion cools and the water becomes denser, sinking and mixing with the hypolimnion. The work required to mix the two layers is provided by wind, and the lake

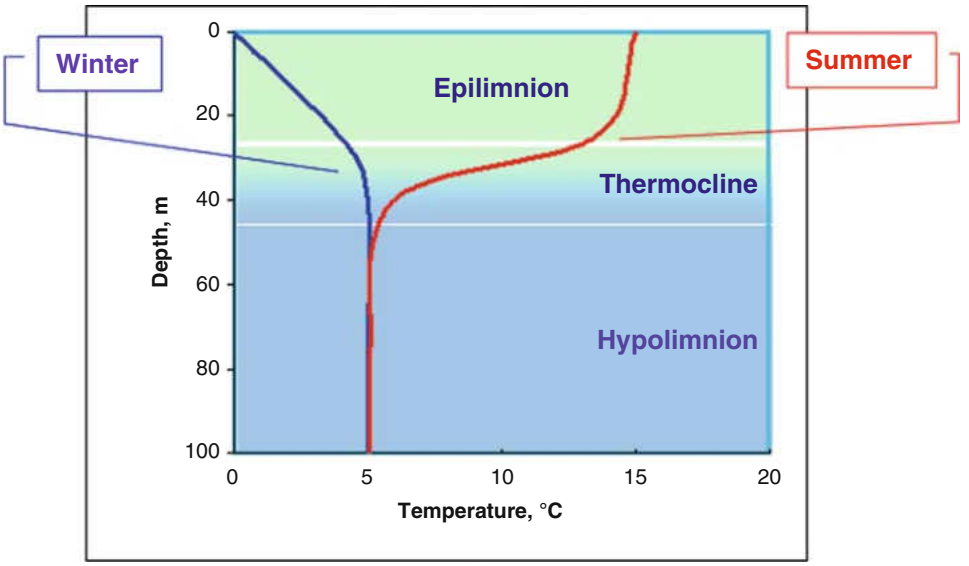


Fig. 4.17. Temperature-driven stratification of lake in summer and winter.

circulates, or overturns, completely. Circulation continues until the surface ice protects the lake from further wind action. The lake overturns again in spring after surface ice melts, and by summer it is stratified once again (Fig. 4.17).

Thermal stratification has a strong impact on the nutrient status of habitats. The epilimnion is not only warm, but also oxygen rich; the vigorous growth of phototrophic organisms tends to deplete the mineral nutrients. The cold and dark hypolimnion does not support high biological activity, the phototrophic organisms being suppressed more than the heterotrophic ones, therefore oxygen is partly depleted while mineral nutrients tend to be relatively abundant. In the fall, the thermocline breaks down, resulting in complete mixing of the lake.

In deep, freshwater lakes, the primary producers (plants) are found either at the shallow edges of the lake (emergent, submerged, or floating macrophytes) or free-floating within its upper layers (microscopic algae, cyanobacteria, and photosynthetic bacteria of the plankton community) (Fig. 4.15). Plants are found only in the photic zone. Animals and decomposers are found in both the photic and aphotic zones.

Other major biological components include:

- *Plankton*, which contains tiny floating plants (*phytoplankton*) and animals (*zooplankton*) as well as microbes (bacterioplankton);
- *Benthos* (bottom-dwelling organisms);
- *Nekton* (free-swimming forms in the water column);
- *Periphyton* (microscopic biota on submerged objects);
- *Psammon* (biota buried in sediments); and
- *Neuston* (biota associated with surface film).

The population density of microorganisms is significantly higher in so-called *eutrophic lakes* (high primary productivity and nutrient content, partial diurnal anoxia, usually shallow with large epilimnion and small or zero hypolimnion) as compared with *oligotrophic* lakes (low primary productivity and low nutrient content, high oxygen content, usually deep and clean with large hypolimnion). A more detailed classification in quantitative terms (Tables 4.1 and 4.2) gives several distinct categories of habitats:

- *Limnetic zone – a* homogeneous habitat with a continuous supply of organic substrates for heterotrophic microorganisms (release of exometabolic products by photosynthetic organisms, products of their cell lysis) and elimination of bacteria via protozoan grazing; photosynthetic microorganisms probably are limited by inorganic compounds whose availability significantly increases by inter-season mixing, formally it corresponds to a discontinuous supply of limiting substrate.
- *Littoral zone* should be characterized as a more eutrophic habitat with a continuous supply of mineral nutrients from terrestrial source; probably, most heterotrophic bacteria are directly associated with macrophytes (see below rhizosphere and phyllopshere sections).
- *Sediments* are split into at least two different categories. The first is the aerobic interface of sediment with water, the kinetic analog of the top soil layer with aerobic conditions and a continuous supply of C-substrates as deposition of particulate necromass of plankton; elimination due to grazing or washing should be very low. The second type of habitat is deeper and preferentially anaerobic layers of accumulated silt material, the major C-substrates are products of depolymerization and fermentation of necromass; these products are delivered slowly and continuously across the concentration gradient.
- Digestive tract of aquatic animals (see below special section).

4.3.2. Rivers

Contrary to lakes, rivers are characterized by flowing waters (Fig. 4.18). They have zones of rapid water movement and pools with reduced currents. The first type of habitats occurs at shallow parts of river, while pools are associated with deep water column and intensive accumulation of silt similar to lakes sediments. Rivers do not form a high degree of thermal and chemical stratification due to the continuous mixing of water. The zones of rapid water movement contain the sessile (firmly attached to the rocky stony bottom of river) forms of

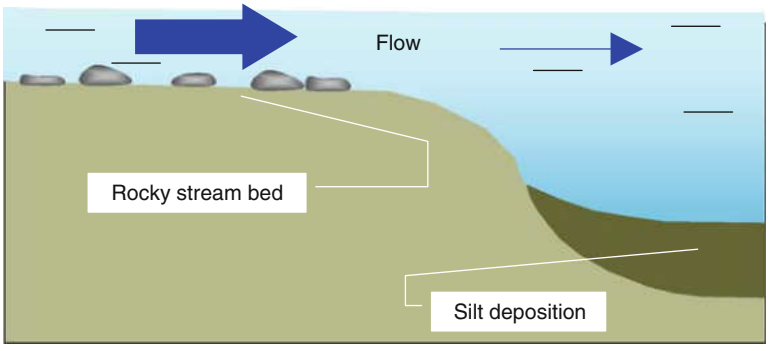


Fig. 4.18. Water flow in river provides different types of microbial habitats at shallow and deep parts.

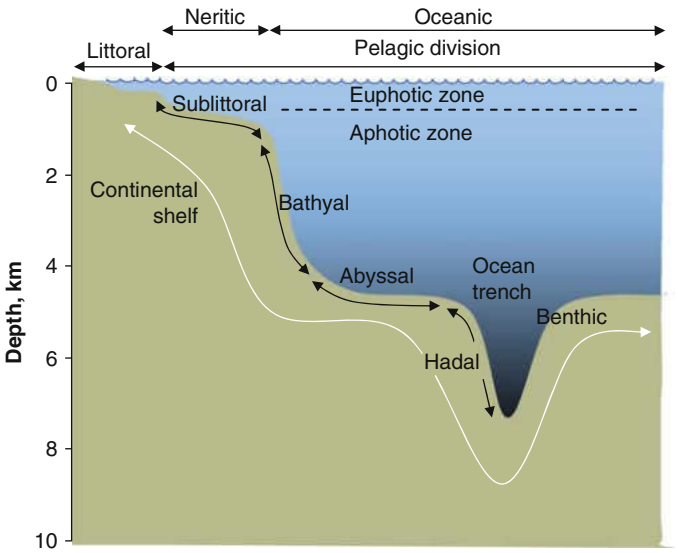


Fig. 4.19. Major vertical zones of an ocean profile.

life, such as macrophytes rooted to river bed and unicellular organisms forming biofilms. This attachment prevents the elimination of growing microorganisms by running water and provide a continuous supply of nutrients.

4.3.3. Marine Ecosystems

The availability of *light* is crucial for differentiating marine environment (49). The greater the depth of the water, the less light can penetrate until below a certain depth there is no light whatsoever. This area of inky darkness, which occupies the great bulk of the ocean, is called the *aphotic zone* (Fig. 4.19). The illuminated region above it is called the *photic zone*, within which are distinguished the euphotic (receives enough light for *photosynthesis* to occur) and *disphotic zones* (illuminated so poorly that rates of respiration exceed those of photosynthesis). Marine organisms are particularly abundant in the photic zone; however, many organisms inhabit the aphotic zone and migrate vertically to the photic zone every night.

Marine environments consist of water, or *pelagic, environment* and a bottom, or *benthic, environment* (Fig. 4.19). Within the pelagic environment, the waters are divided into the *neritic* province above the continental shelf, and the open oceanic waters. The neritic province is a much more eutrophic environment resulting from dissolved materials in riverine runoff. The pelagic water body is divided into several zones (epipelagic, mesopelagic, bathypelagic, and abyssalpelagic) according to depth. The intertidal, or *littoral, zone* ranges from the high-tide mark to the shallow, offshore waters. The sublittoral is the environment beyond the low-tide mark and is often used to refer to continental shelf (150–300 m). Sediments of the continental shelf that influence marine organisms generally originate from the land, particularly in the form of riverine runoff, and include clay, silt, and sand. Beyond the continental shelf is the

bathyal zone, which occurs at depths of 150 to 4,000 m and includes the descending continental slope and rise. The *abyssal zone* (between 4,000 and 6,000 m) represents a substantial portion of the oceans. The deepest region of the oceans (greater than 6,000 m) is the hadal zone of the deep-sea trenches. Sediments of the deep sea primarily originate from a rain of dead marine organisms and their wastes.

Summarizing the oceanographic data (Fig. 4.19), we can conclude that the trophic status of marine ecosystems depends on both the vertical and horizontal positions of a particular site. The supply of mineral substrates is smallest in a pelagic environment and grows while approaching coastal line (runoff). A significant increase in the nutrient level encourages circulation of oceanic waters and upwelling (Fig. 4.20). Probably, the majority of heterotrophic/saprotrophic marine organisms are limited by a supply of available organic substrates derived mainly from soluble exometabolites and the dead bodies of primary producers. The latter (photosynthetic bacteria such as *Prochlorococcus* and numerous algae) are limited mainly by mineral nutrients, among which are nitrogen, phosphorus and especially iron (9, 50). In most cases, a supply of substrates should be considered continuous with seasonal and diurnal fluctuations dependent on fluctuation of temperature and photone flux.

Marine protozoa have been shown to be important grazers of both prokaryotic secondary and microbial primary production. Enigmatic marine viruses, which proved extremely abundant in the sea, appear to be an important source of prokaryotic mortality, perhaps forming a smaller "viral" loop within the microbial loop.

Interactions between microorganisms in marine sediments differ from those in planktonic communities. Sediment assemblages are much more densely populated ($\sim 10^9$ cells/g vs. $\sim 10^6$ cells/mL in the water column), more diverse, with a rather slow turnover rate. Actually, the sediment community should be considered as being in mid-way between aquatic and terrestrial habitats (soils and subsoils). Sediment prokaryotes are probably limited by the

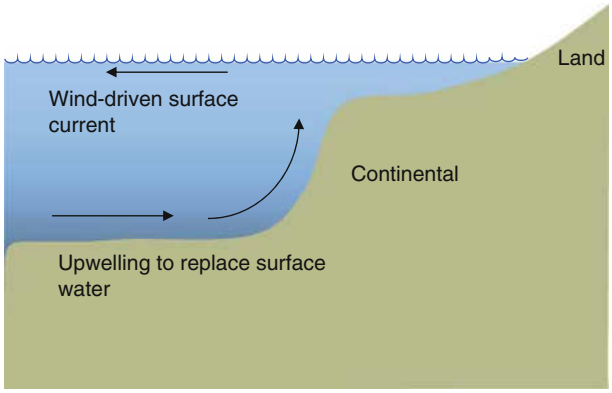


Fig. 4.20. Upwelling of deep ocean waters along continental slope to replace surface waters driven offshore by wind.

availability of electron donors and electron acceptors, which creates the fine vertical zonation described earlier.

4.3.4. Significance for Environmental Engineering

Most aquatic habitats are essentially less resistant to pollution than their terrestrial counterparts. In contrast to soil, which has a large active surface area and tremendous absorbtion capacity, aquatic habitats have a limited "buffering" capacity to resist pollutants. On the other hand, rivers, lakes and marine ecosystems are more homogenous and transparent, making them easier to monitor and control the course of remediation. Probably, the introduction of "beneficial" microbial cultures to waters is more efficient and feasible as compared to heterogeneous natural habitats. The composition and functions of the aquatic microbial community is better understood because it is less complicated (500–1,000 16 rRNA phylotypes as compared with 10^4–10^6 species in typical soils). Therefore, aquatic habitats are more probable candidates for development of the so called *ecosystem-based management* of these natural environments, probably first of all for controlled fishery (51). These include the fields of food processing and chemical production, medicine and bioactive materials production, cleaning of the oceans and of the air, and others.

4.4. Terrestrial Ecosystems

Within the terrestrial habitats, we will focus on arable and virgin soils, subsurface and continental wetlands as the most important natural objects.

4.4.1. Soil

Soil is typically known as the boundary upper layer of lithosphere supporting plant growth. There are several layers or horizons in the vertical soil profile which are morphologically distinguishable and associated with different quality of soil as microbial and plants habitats (Fig. 4.21). Apart from continuous soil horizons, there is a mosaic of rhizosphere soil which consists of soil particles firmly adhered to the plant roots and considerably affected by root exudates (low molecular weight compounds, mainly organic acids) as well as by *root sloughing*, the release into soil of polymeric polysaccharides and proteins from the surface of growing roots.

Soil differentiation into specific habitats is driven mainly by plants (roots development, supply of available organic matter, leaching of minerals with aggressive plant and microbial metabolites) and is dramatically different as compared with lakes and oceans: instead of continuous and regular vertical gradients of the key environmental parameters (temperature, light intensity, oxygen and nutrients), we can envisage various *microgradients* which form a mosaic of *microhabitats* or *microloci*. Figure 4.21 depicts several types of such habitats. The most spacious and the poorest/oligotrophic habitat is the *dispersion zone* (*Con-Ret-Hom*), the subsoil and the patches of bare top soil devoid for some reasons plant roots or fresh litter. Numerous microbial populations inhabiting these habitats grow very slowly on volatile or readily soluble compounds which continuously diffuse from other soil loci, where monomeric concentration is high due to intensive decomposition (plant litter) or excretory activity of plants (rhizosphere). The lack of elimination (no motility in majority of soil bacteria and no

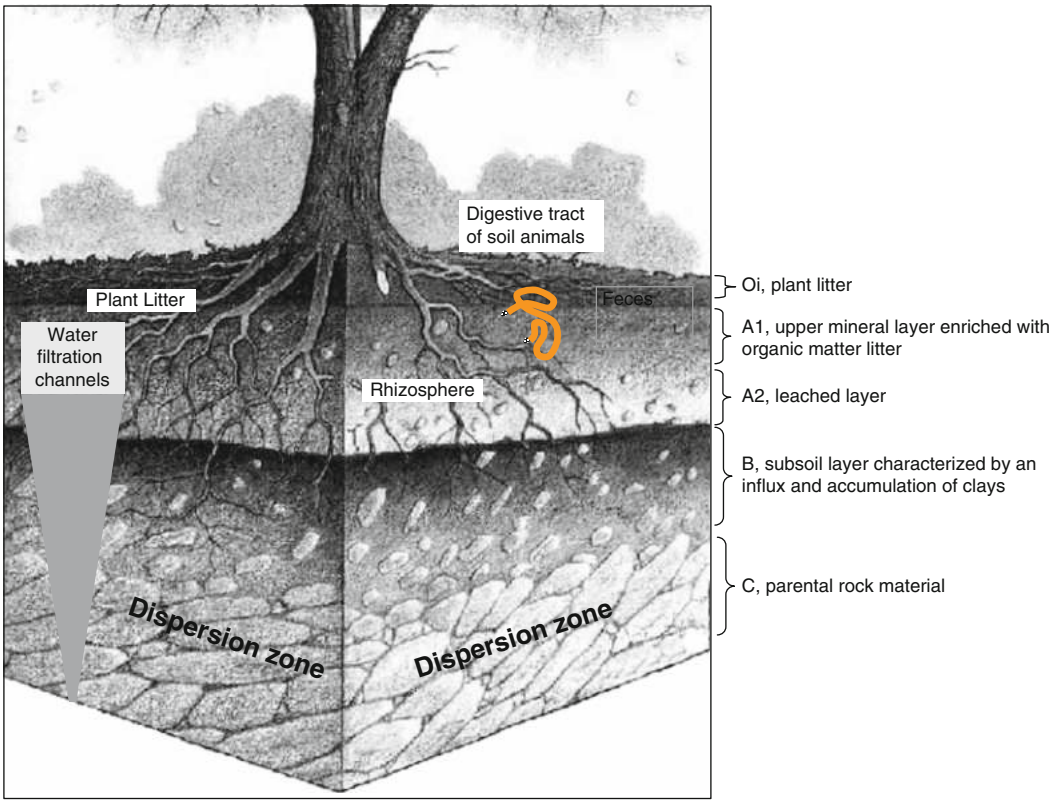


Fig. 4.21. Soil profile and major microbial habitats (see text and Table 4.6 for explanation). After (18) with permission of Elsevier.

predation due to low prey density) combined with slow but uninterrupted continuous growth results eventually in significant build-up of half-dormant cell mass.

The highest and continuous microbial activity is localized in the soil *litter layer, rhizosphere* and *digestive tract* of soil animals (habitats of the types *Con-Rem-Hom* and *Con-Rem-Het*. The C-substrates for microbial populations are the monomeric labile compounds (sugars and organic acids) derived from plants either as root exudation in the rhizosphere or released by extracellular hydrolytic enzymes from the lignocellulose and other polymeric material in plant litter. The litter layer on the soil surface is formed from the fall of aboveground plant remnants, while belowground plant senescence (root litter and root sloughing) provide microbial C-substrates in the rhizosphere. Root exudation is closely related to plant photosynthesis and displays diurnal dynamics, while hydrolytic release is monotonous. Spatially, all these habitats are rather heterogeneous; however, the random distribution of microloci combined with macroscopic sampling size allows us to use homogeneous kinetic models. Clear vertical special gradients are formed in the litter layer: from uncolonized fresh plant debris on the

top to highly decomposed sublayer at the interface with mineral soil. On a microscale, the spatial heterogeneity of microbial colonies (mainly fungi) is manifested in its differentiation into growing extension zone and nongrowing reproductive compartments (Fig. 4.22).

Within the rhizosphere, there are also several spatial gradients of different scales: (a) the vertical gradient of the root phytomass which reflects the spatial pattern of belowground allocation of photosynthate, (b) the horizontal gradient between distant trees or tussocks, and (c) the microscale gradient around root hair with a maximal concentration of microbial cells, microscopic grazers and substrates on the root surface (rhizoplane) and exponential decline outward (sometimes bacterial density declines in the vicinity of the plant surface due to excessive grazing or excretion of antibiotic compounds by plants).

The soil millipedes, isopods, some earthworms and other *primary decomposers* inhabit the litter layer and feed on plant debris. The ingested lignocellulose material is mechanically

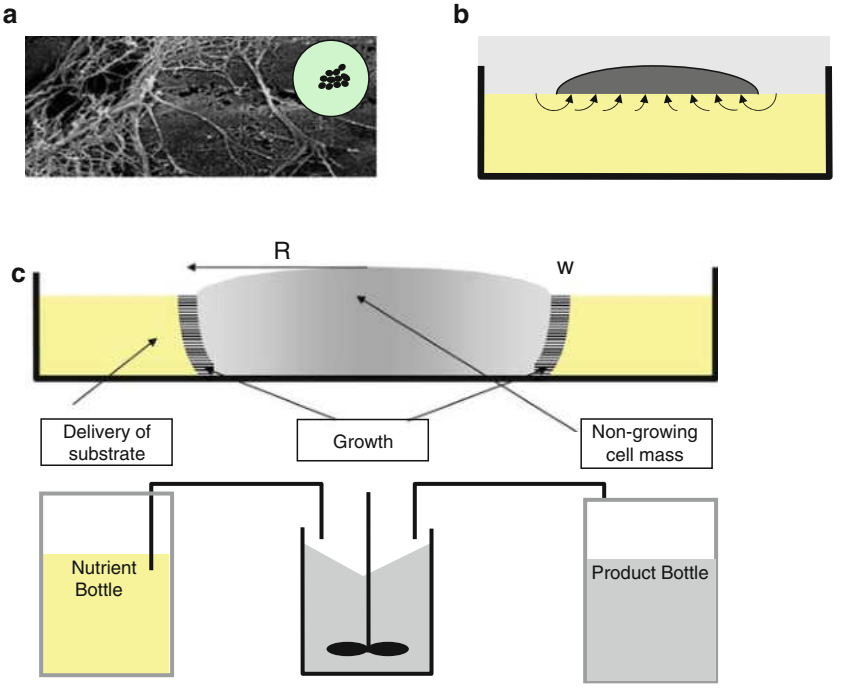


Fig. 4.22. The colony growth: (**a**) in the soil; (**b**) bacterial growth on agar plate; (**c**) fungal growth in nutrient agar. After (18) with permission of Elsevier. Note that bacteria grow only on the agar surface and the colony expansion is controlled by diffusion of substrates from outside of the colony. The fungi and actinomycetes are able to penetrate into the depth of the agar layer, so their mycelium expansion is not dependent on nutrient diffusion within agar layer. The fungal colony follows a chemostat-type growth pattern, being (**a**) continuous, (**b**) steady state, and (**c**) limited by substrate availability. The role of the fermentation vessel of the conventional chemostat is played by the peripheral zone of the colony; the product bottle is analogous to the central part of the colony, while the pump is substituted by the chemotropic movement of hyphae tips along the substrate concentration gradient.

disrupted by the decomposers' mandibles, moistened with saliva and then passed to mid- and hindgut. It is important that the digestive tract of various soil invertebrates harbor not only specific symbionts, but also the normal free-living microorganisms occurring in soil or plant litter, e.g., *Pseudomonas, Flavobacterium, Vibrio, Enterobacter, Streptomyces*. Acceleration of their growth in the hindgut is due to favorable conditions such as neutral pH, optimal moisture, elevated concentrations of nutrient and growth factors (amino acids, peptides, vitamins) as well as the continuous input of fresh substrate and concomitant removal of digestion products (glucose) to prevent the negative feedback (catabolic repression) on cellulase synthesis. Because of peristaltic motion, the content of the gut is mixed and homogeneous. The secondary decomposers (i.e., the earthworms *Allolobophora chlorotica*) feed on amorphous humus containing bacteria and fungal mycelium; they eliminate some microbial species and greatly stimulate the growth of others.

Discontinuous explosive microbial growth occurs within hot spots initiated by a sudden increase in the available organic substrate/nutrient in the soil (*Dis-Rem-Hom – Dis-Ret-Hom*) coming from feces and carcasses of animals, rain washing of organic compounds from the plant foliage, drying-rewetting or freezing-thawing cycles, application of manure, soil fumigation, etc. Growth is usually accompanied by elimination in the form of grazing, myco- and bacteriolytic activity, as well as by the active migration of microbial cells.

4.4.2. Deep Subsurface

In the past decade, it was found that terrestrial microbial life was not limited to the dark-colored humus-containing upper soil layers: both plating and direct microscopy revealed up to 10^6–10^8/cells per cc of bacteria, yeasts and fungi in the subsoils going down to a thousand meters. The deepest samples that yielded bacteria were 3,900–4,200 m deep and contained thermophilic fermentative bacteria. Much of this research was supported in the USA and Europe to explore the consequences of the subsurface disposal of hazardous nuclear and chemical wastes (52). Obviously, microbial activity in subsurface geological formations could influence the fate and mobility of waste materials. The findings were surprising and had significance much beyond subsurface waste disposal.

At least in undisturbed formations, the age of geological layers increases with their depth. Some geological layers are water-permeable and constitute aquifers; others are water-impermeable. Aquifers separated from the surface by one or more water-impermeable layers are called "confined" aquifers (Fig. 4.23).

The obvious and still unresolved question is what food and energy resources do these subsurface bacteria survive on. Most of the bacteria appear to be heterotrophic and anaerobic species (methanogens, sulfate reducers, fermenting yeasts and bacteria). Photosynthetic production is impossible, and the leaching of undegraded but soluble organic matter to deep soil layers is very limited. There could be bacterial primary production based on chemosynthesis: oxidation of ammonium, sulfur and especially molecular hydrogen. The deposited organic carbon in sedimentary rock may become available at a slow rate to support heterotrophic activity, and mobile (gaseous and dissolved) organics may enter aquifers from fossil gas, oil, or lignite deposits. The population density of the deep subsurface microbes should be

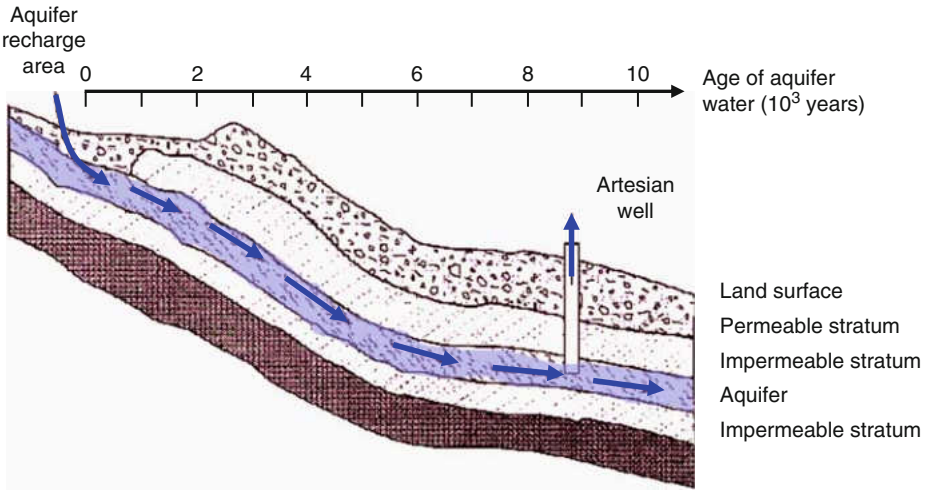


Fig. 4.23. Cross section of a geological formation with water-permeable and water-impermeable strata and a confined aquifer. Deep subsurface bacteria may be sampled in the water of artesian wells. The distance of the wells from the aquifer recharge area correlates with the time the water spends in the aquifer. Bacteria are present and sometimes abundant (10^6/mL) in aquifer waters from more than 1,000 m deep; these bacteria have spent several thousand years in the aquifer. After (5) with permission from Pearson Education Publisher.

essentially lower than that which supports intensive grazing by Protozoa (just imagine how efficient the feeding of wolves would be on mosquitoes!).

4.4.3. Wetlands

The term wetland implies at least two environmental qualities: water saturation and anoxia (lack of oxygen). Some wetlands are nonpeat-forming, such as intermittently flooded marshes, but most of them belong to category peatland or mires, ecosystems where the long-term production of organic material exceeds the rate of decomposition, leading to peat accumulation. Mires are usually classified as: a) bogs, which are fed by rainwater (ombrotrophic) and are therefore poor in dissolved nutrients; or b) fens, which are fed by ground water (minerotrophic) and therefore richer in mineral solutes (N, K, P, Mg...) from terrestrial sources (53, 54). In this section, we deal primarily with Sphagnum bogs, which also contain some other plants (sedges, ericads, and dwarf trees), but have in common such qualities as acidity (pH 3–5), and oligotrophy (low content of mineral compounds).

Sphagnum bogs are the predominant type of peatlands covering about 3% of the total land surface. Of the total mire area, 90% lies in the subarctic, boreal and temperate zones of the northern hemisphere, while the remaining 10% is found in the tropics. Geographic regions with an especially high density of peatlands include Alaska and Eastern Canada in North America as well as West Siberia and Northern Europe in Eurasia. Here, mires can cover as much as 10–30% of the land surface. The two largest continuous peatlands are those of the Hudson Bay lowland, Canada, covering $320,000\,\mathrm{km}^2$, and the Western Siberian Lowland,

covering 540, 000 km^2. Peatlands are estimated to contain about 450 Pg of carbon (55), which represents approximately 30% of all terrestrial carbon in biomass.

As a microbial habitat, the *Sphagnum* peat has several specific qualities: (a) an extremely low content of mineral nutrients delivered mainly through rain, (b) toxicity of Sphagnum metabolites, (c) low pH, (d) weak buffering capacity of soil solution, (e) predominantly low temperature, (f) anoxia, and (g) stagnation. There is a lack of consensus among wetland ecologists as to why microbial decomposition in mires is more restricted than plant growth. Three explanations are usually given: (a) the simultaneous action of all restrictive factors together (54), (b) "intrinsic" inability of microbes to degrade phenolic compounds under anaerobic conditions (56), and c) the severe limitation of microbial activity by mineral nutrients (7).

According to the last view, the peatland quality of having an extremely low nutrient content is the most essential, while low temperature and anoxia could not be restrictive thanks to the wide distribution of psychrophilic and anaerobic organisms. Other peatland qualities (b–d) are in fact not primary: they are derived from quality a (low nutrient content). Exudation of acidic exometabolites by Sphagnum results from C-overflow under limitation by N, P, and K and ample supply of CO_2 from air. The excreted organic acids remain mobile and aggressive because of the lack of free bases. These free organic acids are toxic at pH 3–5 near their pK's as a result of passive diffusion of uncharged molecules into the cell interior with subsequent ionization (pH $\sim$ 7 in the cell's interior) and discharge of transmembrane proton gradient. Finally, peatland quality g (stagnation) exacerbates self-poisoning due to extremely slow physical removal, leaching or volatilization of accumulated acids.

Contrary to the most familiar types of oligotrophic habitats (lakes, subsoils, sediments) which are C-limited and mineral-sufficient (57), the *Sphagnum* bog is mineral-limited and C-sufficient (practically unlimited supply of CO_2 from atmosphere to plants, and continuous flux of rhizodeposition from plants to soil microorganisms). We can hypothesize that indigenous microbes have evolved special metabolic mechanisms to live in an unbuffered, low-mineral, C-sufficient and toxic environment. That could be the main obstacle for microbial isolation because the conventional microbiological technique is a complete antithesis: C-limited buffered media with excess of mineral salts, agitation or exposure to fresh anaerobic mixture with removal of metabolic products.

4.4.4. Significance for Environmental Engineering

Terrestrial habitats are the major type of environment involved in agricultural production and remediation, the most essential branches of biotechnology and bioengineering. Resistance to pollution is very high due to these habitats' ability to absorb molecules and ions of pollutants by soil clays and humic polymers (all of them having acidogenic functional groups). On the other hand, accumulation of toxic compounds in the soil can be so severe that short-term remediation could be problematic. It makes the problem of early diagnosis of soil contamination especially important for environmental engineering to prevent irreversible chemical damage.

The main obstacle in many biotechnological developments remains the enormous complexity of the soil microbial community, the bulk of which is represented by unknown unculturable organisms with unknown metabolic features. Most are slowly growing microbes,

and restoration of the natural community after significant environmental perturbations can take years.

Subsoils are extremely important in relation to several industrial problems, such as prevention and prediction of subsurface contamination with heavy metals including radionuclides; pesticides and other inorganic and organic pollutants; as well as the development of bioremediation techniques aimed at cleaning and restoration of polluted subsurface environment. Other areas of environmental bioengineering as related to subsurface habitats include biometallurgy (use of microorganisms for leaching, separation and transformation of metals in ore deposits), enhancement of oil recovery (activation of microorganisms within the deep oil-carrying subsurface which allows to build-up the pressure and liquefies the oil forcing it up from the partly exhausted oilfields), deposit stabilization by microbial polysaccharides, etc. In all known examples, the positive effects are achieved either by deliberate stimulation of the indigenous microbial populations (adding growth substrates, aeration, amelioration) or by direct release of the specially prepared microbial biomass preliminary grown in fermentor.

Finally, the wetlands are now considered a major component of the global C-budget essential for controlling global warming, flooding and desertification. The reason is that peat accumulated in wetland's area concentrates significant resources of organic carbon and greenhouse gases (CO_2, CH_4, NO_x) affecting the Earth's thermal balance. Accelerated decomposition of the peat leads to CO_2 accumulation in the atmosphere, instability of the climate system expressed as frequent flooding, hurricanes, uneven distribution of water across terrestrial space (desertification of some areas combined with flooding in others).

Besides natural and arable soils and wetlands, the terrain is covered by numerous, man-made industrial objects that are becoming an essential part of the Earth's system. The concept of *industrial metabolism* was developed as a functional analog of the cellular metabolic network (58): the use of materials and energy by industry and the way these materials flow through industrial systems and are transformed and then dissipated as wastes. It is possible to trace the mass and energy flows and identify inefficient processes that result in accumulation of industrial waste and pollution. Further development of this concept combined with the attractive idea of making industrial systems emulate more efficient and sustainable natural systems, eventually led to the birth of a new branch of ecology called *industrial ecology* (59, 60). In an ideal industrial ecosystem, the waste produced by one company would be used as resources by another. No waste would leave the industrial system or negatively impact natural systems.

Industrial ecology relies on a *systems approach* which provides a holistic view of environmental problems, including the links between industrial activities and environmental processes, making them easier to model, identify and solve. A goal of industrial ecology is to change the linear nature of our industrial system "raw materials → products → wastes," to a cyclical system where the wastes are reused as energy or raw materials for another product or process.

NOMENCLATURE

s = (limiting) substrate concentration in external environment, mg/g soil, mL water
x = cell biomass concentration, mg/g soil, mL water
p = product concentration, mg/g soil, mL water
s_0, x_0, p_0 = the initial values (at time $t = 0$) of respectively s, x and p
N = cell population density or cell number, 10^6/g soil, mL water
C_i = intracellular content of the ith cell component (g/g cell mass)
Δx, Δs = changes in x and s respectively for a finite time interval Δt
dx, ds = respectively changes of x and s for infinitesimally small time interval dt
Y = stoichiometric parameter, the yield of cell mass per unit of consumed substrate, g cell mass per g substrate
$Y_{x/s}$, $Y_{p/x}$, $Y_{p/s}$. . . = yield of cell mass per unit of taken up substrate, yield of product per unit of cell mass produced, yield of product per unit of substrate consumed respectively, g/g
μ = specific growth rate, the gross cell growth rate (dx/dt) per unit of cell concentration x, h^{-1}
μ_m = maximal specific growth rate attained under ideal conditions $s >> K_s$, h^{-1}
K_s = saturation constant, parameter of Monod equation equal to such limiting substrate concentration which supports growth rate $\mu = 0.5\,\mu_m$, mg/g soil or mg/L
q = specific rate of substrate consumption, the gross uptake rate per unit of cell concentration x, g substrate/g cell mass per h
m = maintenance coefficient, the $q-$value at $\mu = 0$, g substrate/g cell mass per h
Y^m or Y^{max} = maximal biomass yield under idealized conditions $m = 0$, g cell mass per g of consumed substrate
Y^{min} = the minimal yield observed in the real microbial culture under progressive slowing down of growth rate ($Y \rightarrow$ when $\mu \rightarrow 0$)
σ_s = the intracellular content of deficient element S or *cell quota,* g element per g of cell mass
a = specific death rate (mortality rate), the gross rate of cell decline per unit of the current cell concentration x, h^{-1}
μ_{app} = apparent specific growth rates which is difference between true growth μ and death rate a, $\mu_{app} = \mu - a$, h^{-1}
r = the birth or reproduction rate of population, $r \equiv \mu_{app}$, h^{-1}
K = carrying capacity of ecosystem, the maximal population density supported by available resources, number per g soil or L of water of m_2 of surface area
P- and U-components = the terms of Synthetic Chemostat Model designating nonconstitutive (changeable) cell constituents, P-constituents are needed for intensive cell growth, while U-components provide cell survival under growth restrictive conditions, g component per g cell mass
r^* = the master variable of SCM which is generalized measure of the relative amount of P-components (star is introduced to avoid confusion with r parameter of logistic equation), dimensionless

Glossary

Acidophiles microorganisms that show a preference for growth at low pH, e.g., bacteria that grow only at very low pH values, ca. 2.0.

Actinomycetes members of an order of bacteria in which species are characterized by the formation of branching and/or true filaments.

Adhesins substances involved in the attachment or adherence of microorganisms to solid surfaces; factors that increase adsorption.

Adhesion factors substances involved in the attachment of microorganisms to solid surfaces; factors that increase adsorption.

Aerobes microorganisms whose growth requires the presence of air or free oxygen.

Aerobic having molecular oxygen present; growing in the presence of air.

Aerosol a fine suspension of particles or liquid droplets sprayed into the air.

Algae a heterogeneous group of eucaryotic, photosynthetic organisms, unicellular or multicellular, but lacking true tissue differentiation.

Allochthonous an organism or substance foreign to a given ecosystem.

Amensalism an interactive association between two populations that is detrimental to one and does not adversely affect the other.

Anaerobes organisms that grow in the absence of air or oxygen; organisms that do not use molecular oxygen in respiration.

Anaerobic the absence of oxygen; able to live or grow in the absence of free oxygen.

Anoxic absence of oxygen; anaerobic.

Antagonism the inhibition, injury, or killing of one species of microorganism by another; an interpopulation relationship in which one population has a deleterious (negative) effect on another.

Aquifer a geological formation containing water, such as subsurface water bodies that supply the water for wells and springs; a permeable layer of rock or soil that holds and transmits water.

Archaea (archaebacteria) prokaryotes with cell walls that lack murein, having ether bonds in their membrane phospholipids; analysis of rRNA indicates that the Archaea represent a primary biological domain distinct from both Bacteria and Eucarya.

Autecology branch of ecology that examines individual organisms in relation to their environment, emphasizing the "self-properties" of an organism's physiological attributes.

Autochthonous microorganisms and/or substances indigenous to a given ecosystem; the true inhabitants of an ecosystem; referring to trie common microbiota of the body or soil microorganisms that fend to remain constant despite fluctuations in the quantity of fermentable organic matter.

Autotrophs organisms whose growth and reproduction are independent of external sources of organic compounds, the required cellular carbon being supplied by the reduction of CO_2 and the needed cellular energy being supplied by the conversion of light energy to ATP or the oxidation of inorganic compounds to provide the free energy for the formation of ATP.

Bacteria members of a group of diverse and ubiquitous procaryotic, single-celled organisms; organisms with procaryotic cells, i.e., cells lacking a nucleus.

Bactericidal any physical or chemical agent able to kill some types of bacteria.

Bacteriophage a virus whose host is a bacterium; a virus that replicates within bacterial cells.

Bacteriostatic an agent that inhibits the growth and reproduction of some types of bacteria but need not kill the bacteria.

Barophiles organisms that grow best or grow only under conditions of high pressure, e.g., in the ocean's depths.

Barotolerant organisms that can grow under conditions of high pressure but do not exhibit a preference for growth under such conditions.

Benthos the bottom region of aquatic habitats; collective term for the organisms living at the bottom of oceans and lakes.

Biocide an agent that kills microorganisms.

Biodegradable a substance that can be broken down into smaller molecules by microorganisms.

Biodegradation the process of chemical breakdown of a substance to smaller molecules caused by microorganisms or their enzymes.

Biodeterioration the chemical or physical alteration of a product that decreases the usefulness of that product for its intended purpose.

Biofilm a microbial community occurring on a surface as a microlayer.

Biogenic element an element that is incorporated into the biomass of living organisms.

Biogeochemical cycling the biologically mediated transformations of elements that result in their global cycling, including transfer between the atmosphere, hydrosphere, and lithosphere.

Biological control the deliberate use of one species of organism to control or eliminate populations of other organisms; used in the control of pest populations.

Biomagnification an increase in the concentration of a chemical substance, such as a pesticide, as the substance is passed to higher members of a food chain.

Biomass the dry weight, volume, or other quantitative estimation of organisms; the total mass of living organisms in an ecosystem.

Bioremediation the use of biological agents to reclaim soils and waters polluted by substances hazardous to human health and/or the environment; it is an extension of biological treatment processes that have traditionally been used to treat wastes in which microorganisms typically are used to biodegrade environmental pollutants.

Biosphere the part of Earth in which life can exist; all living things together with their environment.

Carbon cycle the biogeochemical cycling of carbon through oxidized and reduced forms, primarily between organic compounds and inorganic carbon dioxide.

Carrying capacity the largest population that a habitat can support.

Chemoautotrophs microorganisms that obtain energy from the oxidation of inorganic compounds and carbon from inorganic carbon dioxide; organisms that obtain energy through chemical oxidation and use inorganic compounds as electron donors; also known as chemolithotrophs.

Chemocline a boundary layer in an aquatic habitat formed by a difference in chemical composition, such as a halocline formed in the oceans by differing salt concentrations.

Chemolithotrophs microorganisms that obtain energy through chemical oxidation and use inorganic compounds as electron donors and cellular carbon through the reduction of carbon dioxide; also known as chemoautotrophs.

Chemoorganotrophs organisms that obtain energy from the oxidation of organic compounds and cellular carbon from preformed organic compounds.

Chemostat an apparatus used for continuous-flow culture to maintain bacterial cultures in a selected phase of growth, based on maintaining a continuous supply of a solution containing a nutrient in limiting quantities that controls the growth rate of the culture.

Chemotaxis a locomotive response in which the stimulus is a chemical concentration gradient; movement of microorganisms toward or away from a chemical stimulus.

Chitin a polysaccharide composed of repeating A'-acetyl-glucosamine residues that is abundant in arthropod exoskeletons and fungal cell walls.

Circadian rhythms daily cyclical changes that occur in an organism even when it is isolated from the natural daily fluctuations of the environment.

Climax community the organisms present at the end-point of an ecological succession series.

Colonization the establishment of a site of microbial reproduction on a material, animal, or person without necessarily resulting in tissue invasion or damage.

Colony the macroscopically visible growth of microorganisms on a solid culture medium.

Colony-forming units (CPUs) number of microbes that can replicate to form colonies, as determined by the number of colonies that develop.

Colony hybridization hybridization that is combined with conventional plating procedures in which bacterial colonies or phage plaques are transferred directly onto hybridization filters; the colonies or phage containing plaques are then lysed by alkaline or enzymatic treatment, after which hybridization is conducted.

Cometabolism the gratuitous metabolic transformation of a substance by a microorganism growing on another substrate; the cometabolized substance is not incorporated into an organism's biomass, and the organism does not derive energy from the transformation of that substance.

Commensalism an interactive association between two populations of different species living together in which one population benefits from the association, and the other is not affected.

Community highest biological unit in an ecological hierarchy composed of interacting populations.

Competition an interactive association between two species, both of which need some limited environmental factor for growth and thus grow at suboptimal rates because they must share the growth-limiting resource.

Competitive exclusion principle the statement that competitive interactions tend to bring about the ecological separation of closely related populations and preclude two populations from occupying the same ecological niche.

Competitive inhibition the inhibition of enzyme activity caused by the competition of an inhibitor with a substrate for the active (catalytic) site on the enzyme; impairment of the function of an enzyme due to its reaction with a substance chemically related to its normal substrate.

Composting the decomposition of organic matter in a heap by microorganisms; a method of solid waste disposal.

Consortium an interactive association between microorganisms that generally results in combined metabolic activities.

Copiotrophic populations organisms adapted to live in habitats with plentiful supply of nutrients (syn: **eutrophic populations**, antonym: **oligotrophic populations**).

Coprophagous capable of growth on fecal matter; feeding on dung or excrement.

Crenarchaeota kingdom of archaea consisting of extreme thermophiles.

Cross-feeding the phenomenon that occurs when two organisms mutually complement each other in terms of nutritional factors or catabolic enzymes related to substrate utilizations; also termed syntrophism.

Culture to encourage the growth of particular microorganisms under controlled conditions; the growth of particular types of microorganisms on or within a medium as a result of inoculation and incubation.

Cyanobacteria procaryotic, photosynthetic organisms containing chlorophyll a, capable of producing oxygen by splitting water; formerly known as blue-green algae.

Cyst a dormant form assumed by some microorganisms during specific stages in their life cycles, or assumed as a response to particular environmental conditions in which the organism becomes enclosed in a thin- or thick-walled membranous structure, the function of which is either protective or reproductive.

Decomposers organisms, often bacteria or fungi, in a community that convert dead organic matter into inorganic nutrients.

Desiccation removal of water; drying.

Detrital food chain a food chain based on the biomass of decomposers rather than on that of primary producers.

Detritivore an organism that feeds on detritus; an organism that feeds on organic wastes and dead organisms.

Detritus waste matter and biomass produced from decom-positional processes.

Direct counting procedures methods for the enumeration of bacteria and other microbes that do not require the growth of cells in culture but rather rely upon direct observation or other detection methods by which the undivided microbial cells can be counted.

Direct viability count a direct microscopic assay that determines whether or not microorganisms are metabolically active, i.e., viable.

Dispersion zone an oligotrophic (nutrient-poor) soil or subsoil space which receives C-substrates mainly as diffusive flux of volatiles (hydrocarbons, alcohols, aldehydes, VFA, etc)

Diversity the heterogeneity of a system; the variety of different types of organisms occurring together in a biological community.

Dormant an organism or a spore that exhibits minimal physical and chemical change over an extended period of time but remains alive.

Ecological niche the functional role of an organism within an ecosystem; the combined description of the physical habitat, functional role, and interactions of the microorganisms occurring at a given location.

Ecological succession a sequence in which one ecosystem is replaced by another within a habitat until an ecosystem that is best adapted is established.

Ecology the study of the interrelationships between organisms and their environments.

Ecosystem a functional self-supporting system that includes the organisms in a natural community and their environment.

Ectomycorrhiza a stable, mutually beneficial (symbiotic) association between a fungus and the root of a plant where the fungal hyphae occur outside the root and between the cortical cells of the root.

Endomycorrhiza mycorrhizal association in which there is fungal penetration of plant root cells.

Endophytic a photosynthetic organism living within another organism.

Endospores thick-walled spores formed within a parent cell; in bacteria, heat-resistant spores.

Endosymbiotic a symbiotic (mutually dependent) association in which one organism penetrates and lives within the cells or tissues of another organism.

Endosymbiotic evolution theory that bacteria living as endosymbionts within eucaryotic cells gradually evolved into organelle structures.

Enrichment culture any form of culture in a liquid medium that results in an increase in a given type of organism while minimizing the growth of any other organism present.

Epilimnion the warm layer of an aquatic environment above the thermocline.

Epiphytes organisms growing on the surface of a photo-synthetic organism, e.g., bacteria growing on the surface of an algal cell.

Epizootic an epidemic outbreak of infectious disease among animals other than humans.

Estuary a water passage where the ocean tide meets a river current; an arm of the sea at the lower end of a river.

Eubacteria procaryotes other than archaebacteria.

Eucaryotes cellular organisms having a membrane-bound nucleus within which the genome of the cell is stored as chromosomes composed of DNA; eucaryotic organisms include algae, fungi, protozoa, plants, and animals.

Euphotic the top layer of water, through which sufficient light penetrates to support the growth of photosynthetic organisms.

Eurythermal microorganisms that grow over a wide range of temperatures.

Eutrophic containing high nutrient concentrations, such as a eutrophic lake with a high phosphate concentration that will support excessive algal blooms.

Eutrophication the enrichment of natural waters with inorganic materials, especially nitrogen and phosphorus compounds, that support the excessive growth of photosynthetic organisms.

Evolution the directional process of change of organisms by which descendants become distinct in form and/or function from their ancestors.

Extreme environments environments characterized by extremes in growth conditions, including temperature, salinity, pH, and water availability, among others.

Extreme thermophiles organisms having an optimum growth temperature above 80˚ C.

Fastidious an organism with stringent physiological requirements for growth and survival; an organism difficult to isolate or culture on ordinary media because of its need for special nutritional factors.

Floc a mass of microorganisms cemented together in a slime produced by certain bacteria, usually found in waste treatment plants.

Flocculate to aggregate or clump together individual, tiny particles into small clumps or clusters.

Food web an interrelationship among organisms in which energy is transferred from one organism to another; each organism consumes the preceding one and in turn is eaten by the next higher member in the sequence.

Fungi a group of diverse, unicellular and multicellular eucaryotic organisms, lacking chlorophyll, often filamentous and spore-producing.

Fungicides agents that kill fungi.

Fungistasis the active prevention or hindrance of fungal growth by a chemical or physical agent.

Grazers organisms that prey upon primary producers; protozoan predators that consume bacteria indiscriminately; filter-feeding zooplankton.

Greenhouse effect rise in the concentration of atmospheric CO_2 and a resulting warming of global temperatures.

Gross primary production total amount of organic mat ter produced in an ecosystem.

Growth rate increase in the number of microorganisms per unit of time.

Guild populations within a community which use the same resources.

Habitat a location where living organisms occur.

Halophiles organisms requiring NaCl for growth; extreme halophiles grow in concentrated brines.

Heterotrophs organisms requiring organic compounds for growth and reproduction; the organic compounds serve as sources of carbon and energy.

Hot springs thermal springs with a temperature greater than 37°C.

Humic acids high-molecular-weight irregular organic polymers with acidic character; the portion of soil organic matter soluble in alkali but not in acid.

Humus the organic portion of the soil remaining after microbial decomposition.

Hyperthermophiles organisms having an optimum growth temperature above 80°C; some grow best at 110°C.

Hypolimnion the deeper, colder layer of an aquatic environment; the water layer below the thermocline.

In situ in the natural location or environment.

Ex situ outside the natural environment, under artificial laboratory conditions (∼ in vitro).

In vitro in glass; a process or reaction carried out in a culture dish or test tube.

In vivo within a living organism.

Indigenous native to a particular habitat.

Lichens a large group of composite organisms consisting of a fungus in symbiotic association with an alga or a cyanobacterium.

Lignin a class of complex polymers in the woody material of higher plants, second in abundance only to cellulose.

Limnetic zone in lakes, the portion of the water column excluding the littoral zone where primary productivity exceeds respiration.

Lithosphere the solid part of Earth.

Lithotrophs microorganisms that live in and obtain energy from the oxidation of inorganic matter; chemo-autotrophs.

Littoral situated or growing on or near the shore; the region between the high and low tide marks.

Mesophiles organisms whose optimum growth is in the temperature range of 20–45°C.

Methanogens methane-producing procaryotes; a group of archaea capable of reducing carbon dioxide or low-molecular-weight fatty acids to produce methane.

Methylation the process of substituting a methyl group for a hydrogen atom.

Mineralization the microbial breakdown of organic materials into inorganic materials brought about mainly by microorganisms.

Mixotrophs organisms capable of utilizing both autotrophic and heterotrophic metabolic processes, e.g., the concomitant use of organic compounds as sources of carbon and light as a source of energy.

Most probable number (MPN) a method for determination of viable organisms using statistical analyses and successive dilution of the sample to reach a point of extinction.

Mutualism a stable condition in which two organisms of different species live in close physical association, each organism deriving some benefit from the association; symbiosis.

Mycelia the interwoven mass of discrete fungal hyphae.

Mycobiont the fungal partner in a lichen.

Mycorrhiza a stable, symbiotic association between a fungus and the root of a plant; the term also refers to the root-fungus structure itself.

Net primary production amount of organic carbon in the form of biomass and soluble metabolites available for heterotrophic consumers in terrestrial and aquatic habitats.

Neuston the layer of organisms growing at the interface between air and water.

Neutralism the relationship between two different microbial populations characterized by the lack of any recognizable interaction.

Niche the functional role of an organism within an ecosystem; the combined description of the physical habitat, functional role, and interactions of the microorganisms occurring at a given location.

Nitrogen fixation the reduction of gaseous nitrogen to ammonia, carried out by certain procaryotes.

Nitrogenase the enzyme that catalyzes biological nitrogen fixation.

Numerical taxonomy a system that uses overall degrees of similarity and large numbers of characteristics to determine the taxonomic position of an organism; allows organisms of unknown affiliation to be identified as members of established taxa.

Obligate aerobes organisms that grow only under aerobic conditions, i.e., in the presence of air or oxygen.

Obligate anaerobes organisms that cannot use molecular oxygen; organisms that grow only under anaerobic conditions, i.e., in the absence of air or oxygen; organisms that cannot carry out respiratory metabolism.

Obligate intracellular parasites organisms that can live and reproduce only within the cells of other organisms, such as viruses, all of which must find suitable host cells for their replication.

Obligate thermophiles organisms restricted to growth at high temperatures.

Oligotrophic lakes and other bodies of water that are poor in those nutrients that support the growth of aerobic, photo-synthetic organisms; microorganisms that grow at very low nutrient concentrations.

Osmophiles organisms that grow best or only in or on media of relatively high osmotic pressure.

Osmotic pressure the force resulting from differences in solute concentrations on opposite sides of a semipermeable membrane.

Osmotolerant organisms that can withstand high osmotic pressures and grow in solutions of high solute concentrations.

Parasites organisms that live on or in the tissues of another living organism, the host, from which they derive their nutrients.

Parasitism an interactive relationship between two organisms or populations in which one is harmed and the other benefits; generally, the population that benefits, the parasite, is smaller than the population that is harmed.

Pathogens organisms capable of causing disease in animals, plants, or microorganisms.

Pelagic zone the portion of the marine environment beyond the edge of the continental shelf, comprising the entire water column but excluding the sea floor.

Pest a population that is an annoyance for economic, health, or aesthetic reasons.

Pesticides substances destructive to pests, especially insects.

Photoautotrophs organisms whose source of energy is light and whose source of carbon is carbon dioxide; characteristic of plants, algae, and some procaryotes.

Photoheterotrophs organisms that obtain energy from light but require exogenous organic compounds for growth.

Photosynthesis the process in which radiant (light) energy is absorbed by specialized pigments of a cell and is subsequently converted to chemical energy; the ATP formed in the light reactions is used to drive the fixation of carbon dioxide, with the production of organic matter.

Phototaxis the ability of bacteria to detect and respond to differences in light intensity, moving toward or away from light.

Phototrophs organisms whose sole or principal primary source of energy is light; organisms capable of photophosphorylation.

Phycobiont the algal partner of a lichen.

Phytoplankton passively floating or weakly motile photosynthetic aquatic organisms, primarily cyanobacteria and algae.

Phytoplankton food chain a food chain in aquatic habitats based on the consumption of primary producers.

Plankton collectively, all microorganisms and invertebrates that passively drift in lakes and oceans.

Plasmid an independent self-replicating DNA molecule, which compared to a bacterial chromosome carries relatively few genes which are not essential for survival under nonselective growth conditions.

Plate counting method of estimating numbers of microorganisms by diluting samples, culturing on solid media, and counting the colonies that develop to estimate the number of viable microorganisms in the sample.

Predation a mode of life in which food is primarily obtained by killing and consuming animals; an interaction between organisms in which one benefits and one is harmed, based on the ingestion of the smaller organism, the prey, by the larger organism, the predator.

Predators organisms that practice predation.

Prey an animal taken by a predator for food.

Primary producers organisms capable of converting carbon dioxide to organic carbon, including photoautotrophs and chemoautotrophs.

Profundal zone in lakes, the portion of the water column where respiration exceeds primary productivity.

Proto-cooperation synergism; a nonobligatory rela-tion-ship between two microbial populations in which both populations benefit.

Protonmotive force potential chemical energy in a gradient of protons and electrical energy across the membrane.

Protozoa diverse eucaryotic, typically unicellular, non-photosynthetic microorganisms generally lacking a rigid cell wall.

Psychrophile an organism that has an optimum growth temperature below 20°C.

Psychrotroph (or psychroactive microbe) a mesophile that can grow at low temperatures.

Pure culture a culture that contains cells of one kind; the progeny of a single cell.

Recalcitrant a chemical that is totally resistant to microbial attack.

Rhizosphere an ecological niche that comprises the surfaces of plant roots and the region of the surrounding soil in which the microbial populations are affected by the presence of the roots.

Rhizosphere effect evidence of the direct influence of plant roots on bacteria, demonstrated by the fact that microbial populations usually are higher within the rhizosphere (the region directly influenced by plant roots) than in root-free soil.

Self-purification inherent capability of natural waters to cleanse themselves of pollutants based on biogeochemical cycling activities and interpopulation relationships of indigenous microbial populations.

Seston all material, both organic and inorganic, suspended in a waterway; all the fine particulate matter which drifts passively in lakes, seas and other bodies of water, including living organisms.

Soil horizon a layer of soil distinguished from layers above and below by characteristic physical and chemical properties.

Solfatara hot, sulfur-rich environment; a volcanic area or vent which yields sulfur vapors, steam, and the like.

Solid waste refuse; waste material composed of both inert materials – glass, plastic, and metal – and decomposable organic wastes, including paper and kitchen scraps.

Stenothermophiles microorganisms that grow only at temperatures near their optimal growth temperature.

Stenotolerant highly specialized and therefore having a narrow tolerance for a specific growth factor.

Succession the replacement of populations by other populations better adapted to fill the ecological niche.

Symbiosis an obligatory interactive association between members of two populations, producing a stable condition in which the two organisms live together in close physical proximity to their mutual advantage.

Synecology the study of the ecological interrelationships among communities of organisms.

Synergism in antibiotic action, when two or more antibiotics are acting together, the production of inhibitory effects on a given organism that are greater than the additive effects of those antibiotics acting independently; an interactive but nonobligatory association between two populations in which each population benefits.

Syntrophism the phenomenon that occurs when two organisms mutually complement each other in terms of nutritional factors or catabolic enzymes related to substrate utilization; also termed cross-feeding.

Thermal stratification division of temperate lakes into an epilimnion, thermocline, and hypolimnion, subject to seasonal change; zonation of lakes based on temperature where warm and cold water masses do not mix.

Thermal vents hot areas located at depths of 800–1,000 m on the sea floor, where spreading allows seawater to percolate deeply into the crust and react with hot core materials; life around the vents is supported energetically by the chemoautotrophic oxidation of reduced sulfur.

Thermocline zone of water characterized by a rapid decrease in temperature, with little mixing of water across it.

Thermophiles organisms having an optimum growth temperature above 40°C.

Tolerance range the range of a parameter, such as temperature, over which microorganisms survive.

Transposons translocatable genetic elements; genetic elements that move from one locus to another by non-homologous recombination, allowing them to move around a genome.

Trickling filter system a simple, film-flow aerobic sewage treatment system; the sewage is distributed over a porous bed coated with bacterial growth that mineralizes the dissolved organic nutrients.

Trophic level the position of an organism or population within a food web: primary producer, grazer, predator, etc.

Trophic structure the collection of steps in the transfer of energy stored in organic compounds from one to another.

Trophozoite a vegetative or feeding stage in the life cycle of certain protozoa.

Turbidostat a system in which an optical sensing device measures the turbidity of the culture in a growth vessel and generates an electrical signal that regulates the flow of fresh medium into the vessel and the release of spent medium and cells.

Ultraviolet light (UV) short wavelength electromagnetic radiation in the range 100–400 nm.

Vectors organisms that act as carriers of pathogens and are involved in the spread of disease from one individual to another.

Vesicular-arbuscular mycorrhiza a common type of mycorrhiza characterized by the formation of vesicles and arbuscules.

Viable nonculturable microorganism microorganisms that do not grow in viable culture methods, but which are still metabolically active and capable of causing infections in animals and plants.

Viable plate count method for the enumeration of bacteria whereby serial dilutions of a suspension of bacteria are plated onto a suitable solid growth medium, the plates are incubated, and the number of colony-forming units is counted.

Virus a noncellular entity that consists minimally of protein and nucleic acid and that can replicate only after entry into specific types of living cells; it has no intrinsic metabolism, and its replication is dependent on the direction of cellular metabolism by the viral genome; within the host cell, viral components are synthesized separately and are assembled intracellularly to form mature, infectious viruses.

Volatile organic compounds (VOC) vaporizes into the atmosphere.

Water activity (a_w) a measure of the amount of reactive water available, equivalent to the relative humidity; the percentage of water saturation of the atmosphere.

Xenobiotic a synthetic product not formed by natural biosynthetic processes; a foreign substance or poison.

Xerotolerant able to withstand dry ness; an organism capable of growth at low water activity.

Yeasts a category of fungi defined in terms of morphological and physiological criteria; typically, unicellular, saprophytic organisms that characteristically ferment a range of carbohydrates and in which asexual reproduction occurs by budding.

Zymogenous term used to describe opportunistic soil microorganisms that grow rapidly on exogenous substrates.

REFERENCES

1. Camill P (2002/2004) Encyclopedia of life science. Nature/Wiley
2. Kronstad JW, Kaiser D (2000) Growth and development signals and their transduction. Editorial overview. Curr Opin Microbiol 3:549–552

3. Panikov NS (1999) Fluxes of CO_2 and CH_4 in high latitude wetlands: measuring, modelling and predicting response to climate change. Polar Res 18:237–244
4. Azam F, Fenchel T, Field JG (1983) The ecological role of water column microbes in the sea. Mar Ecol Prog Ser 10:257–263
5. Atlas RM, Bartha R. Microbial ecology. Fundamentals and applications. 99. Benjamin/Cummings, Redwood City, CA
6. Gregory PH (1973) The microbiology of the atmosphere. Wiley, New York
7. Panikov NS (1995) Microbial growth kinetics. Chapman & Hall, London, 378
8. Blackman FF (1905) Optima and limiting factors. Ann Bot London 19:281–293
9. Boyd PW, Watson AJ, Law CS, et al. (2000) A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature 407:695–702
10. Panikov NS, Pirt SJ (1978) The effects of cooperativity and growth yield variation on the kinetics of nitrogen or phosphate limited growth of *Chlorella* in a chemostat culture. J Gen Microbiol 108:295–303
11. Odum EP (1983) Basic ecology (Revised). W.B. Saunders, Philadelphia, PA, p 613
12. Panikov NS, Gorbenko AJ (1992) The dynamics of gas exchange between soil and atmosphere in relation to plant-microbe interactions: fluxes measuring and modelling. Ecol Bull 42:53–61
13. Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP (2001) Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 25:365–404
14. Gause GF The struggle for existence, vol 163. Williams and Wilkins, Baltimore, p 34
15. (1992) The American Heritage Dictionary of the English Language. Houghton Mifflin Company, Boston
16. Mac Arthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton, NJ, 203
17. Monod J (1942) Recherches sur la croissance des cultures bacteriennes. Hermann, Paris
18. Panikov NS (2005) Microbial processes: kinetics. In: Hillel D (ed) Encyclopedia of soils in the environment. Elsevier, Oxford, pp 463–479
19. Winogradsky SN (1949) Microbiologie du Sol. Masson et Cie Editeurs, Paris, p 861
20. Ierusalimsky ND (1963) The fundamentals of microbial physiology, vol 146. Publ. House of USSR Akademy of Sciences, Moscow
21. Hungate RE (1962) Ecology of bacteria. In: Gunsalus IC, Stanier RY (eds) The bacteria. Academic, New York, pp 95–119
22. Staley JT, Gosink JJ (1999) Poles apart: biodiversity and biogeography of sea ice bacteria. Annu Rev Microbiol 53:189–215
23. Hugenholz P, Goebel BM, Pace NR (1988) Impact of culture-dependent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:476–574
24. Junge K, Imhoff F, Staley T, Deming JW, (2002) Phylogenetic diversity of numerically important arctic sea-ice bacteria cultured at subzero temperature. Microb Ecol 43:315–328
25. Kasahara Y, Hattori T (1991) Analysis of bacterial populations in a grassland soil according to rates of development on solid media. FEMS Microbiol Ecol 86:95–102
26. Torsvik VL, Daae L, Goksoyr J (1995) Extraction, purification, and analysis of DNA from soil bacteria. In: Van Elsas JD, Trevors JT (eds) Nucleic acids in the environment: methods and applications. Springer, Heidelberg, pp 29–48
27. Ingham ER, Griffiths RP, Cromack K, Entry JA (1991) Comparison of direct vs fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. Soil Biol Biochem 23:465–471

28. Jenkinson DS, Ladd JN (1981) Microbial biomass in soil: measurement and turnover. In: Paul EA, Ladd JN (eds), Soil biochemistry. Marcel Dekker, New York, pp 415–471
29. Jenkinson DS, Powlson DS (1976) The effect of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol Biochem 17:57–63
30. Parkinson D, Coleman DC (1991) Microbial communities, activity and biomass. Agric Ecosyst Environ 34:333
31. Perfil'ev BV, Gabe DR (1969) Capillary methods of investigating micro-organisms. Oliver and Boyd, Edinburgh
32. Staley JT (1971) Growth rates of algae determined in situ using an immersed microscope. J Phycol 7:13–17
33. Hagström, Larsson U, Hörstedt P, Normark S (1979) Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl Environ Microbiol 37:805–812
34. Meynell GG (1959) Use of superinfecting phage for estimating the division rate of lysogenic bacteria in infected animals. J Gen Microbiol 21:421–437
35. Brock TD (1971) Microbial growth rates in nature. Bacterial Rev 35:39–58
36. Romanenko VI (1964) Heterotrophic CO_2 assimilation by bacterial flora of water. Microbiology 33:679–683
37. Overbeck J, Daley RJ (1973) Some precautionary comments on the Romanenko technique for estimating heterotrophic bacterial production. Bull Ecol Res Comm 17:342–344
38. Laws EA (1984) Improved estimates of phytoplankton carbon based on 14C incorporation into chlorophyll a. J Theor Biol 110:425–434
39. Karl DM (1979) Measurement of microbial activity and growth in the ocean by rates of stable ribonucleic acid synthesis. Appl Environ Microbiol 38:850–860
40. Aristovskaya TV (1975) Quantity, biomass and productivity of soil bacteria. In: Rodin LE, Smirnov NN (eds) Resources of the biosphere (Synthesis of the Soviet Studies for the International Biological Programme). Nauka, Leningrad, pp 241–259
41. Catler DW, Crump LM, Sandon HA (1923) A quantitative investigation of the bacterial and protozoan population of the soil with an account of the protozoan fauna. Philos Trans R Soc Lond B Biol Sci 211:317–350
42. Gray TRG, Williams ST (1971) Microbial productivity in soil. In: 21st Symp. Soc. Gen. Microbiol. Cambridge Univeristy Press, Cambridge
43. Svensson BH, Rosswall T (1980) Energy flow through the subarctic mire at Stordalen. In: Sonesson M (ed) Ecology of a subarctic mire. Ecol. Bull. (Stockholm) 30:283–301
44. Chapman SJ, Gray TRG (1986) Importance of cryptic growth, yield factors and maintenance energy in models of microbial growth in soil. Soil Biol Biochem 18:1–4
45. Parkinson D, Domsch KH, Anderson JPE (1978) Die Entwicklung mikrobieller Biomassen im organischen Horizont eines Fichten standortes. Oecol Plant 13:355–366
46. Lynch JM, Panting LM (1980) Cultivation and the soil biomass. Soil Biol Biochem 12:29–33
47. Gilmanov TG, Bazilevitch NI (1983) Conceptual balance model of organic matter turnover in ecosystem as a theoretical basis of monitoring. In: Theoretical basis and implementation of ecological monitoring. Nauka, Moscow, pp 7–57
48. Thrush BA (1977) The chemistry of stratosphere and its pollution. Endeavour 1:3–6
49. Anonymous (2004) "Marine Ecosystems". "Hydrology". "Inland water ecosystem." Encyclopedia Britannica from Encyclopedia Britannica Online

50. Epstein SS (2003) Microbial interactions. In: Encyclopedia of life sciences. Macmillan/Nature,/www.els.net
51. Pikitch EK, Santora C, Babcock EA, Bakun A, Bonfil R, Conover DO, Dayton P, Doukakis P, Fluharty D, Heneman B, Houde ED, Link J, Livingston PA, Mangel M, McAllister MK, Pope J, Sainsbury KJ (2004) ECOLOGY: ecosystem-based fishery management. Science 305:346–347
52. Fredrickson JK (1992) DOE explores subsurface biosphere. ASM News 58:183
53. Nilsson M (2002) Mire ecosystems. Encyclopedia of life sciences. Macmillan/Nature, www.els.net
54. van Breemen N (1995) How Sphagnum bogs down other plants. Trends Ecol Evol 10:270–275
55. Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecol Appl 1:182–193
56. Freeman C, Ostle N, Kang H (2001) An enzymic 'latch' on a global carbon store. A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. Nature 409:149
57. Poindexter JS (1981) Oligotrophy. Fast and famine existence. Adv Microb Ecol 5:63–89
58. Ayres R (1992) Toxic heavy metals: materials cycle optimization. Proc Natl Acad Sci USA 89: 815–820
59. Piasecki B (1992) Industrial ecology: an emerging management science. Proc Natl Acad Sci USA 89:873–875
60. Wang LK and Aulenbach DB (2004) Implementation of Industrial ecology for industrial hazardous waste management. In: Wang LK, Hung Y-T, Lo HH, Yapijakis C (eds), Handbook of industrial and hazardous wastes treatment. Marcel Dekker and CRC Press, New York, 1–13