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Microbial mats and the search for minimal ecosystems

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Abstract This article reviews some ecological concepts common to all kinds of ecosystems, describes the characteristics of microbial mats, and focuses on the description of the Ebro Delta microbial mats, to assess whether they fit the concept of a minimal ecosystem. First, microorganisms as components of ecosystems are considered, and some features of microbial life, including ubiquity, size and metabolism, genetic versatility, and strategies to overcome unfavorable conditions, are discussed. Models for ecosystems, regardless of their size, have the same basic components; tropical forests, multilayered planktonic microbial communities, and benthic microbial mats are analogous ecosystems at different scales. The structure – in terms of populations and communities – and ecophysiology of microbial mats are also discussed. The linear distribution of microbial populations along steep gradients of light and hydrogen sulfide allows for the simultaneous presence of different microbial populations. Defining the minimal ecosystem requirements necessary for the survival and proliferation of organisms is crucial in the search for extraterrestrial life and for establishing ecosystems beyond the Earth.

Keywords Minimal ecosystems · Microbial mats · Biodiversity · Biofilms · Ecopoiesis

Introduction

“Ecosystem” is the term coined by the English botanist Arthur Tansley (1871–1955), in 1935, to name an organized unit that comprises the total array of living beings present in a defined area, together with the accompa-

nying physical-chemical environmental factors [40]. An ecosystem is an open system subjected to continuous inputs and outputs, even though it may look static, and where both biotic and abiotic components interact and can modify each other.

Microorganisms are components of all ecological systems integrated into the biosphere. In fact, at one time, when they were the only inhabitants of the planet, prokaryotes were the “founders” of ecosystems. The origin of life (biopoiesis) on Earth can be dated back to some time between 4,000 and 3,500 million years ago, during the early Archaean eon (3,850–2,500 million years ago). Biopoiesis is a physicochemical phenomenon that might have occurred many times and at different places in the universe, including our neighbor planets Venus and Mars, provided that certain circumstances coincided. Nevertheless, if life did originate on any of those planets, it no longer persists [12]. Life on Earth could also have originated independently several times during the early Archaean. If so, those different kinds of life must have become extinct very quickly and soon only one kind survived. This survivor gave rise to the three main forms of life that we know: Bacteria, Archaea and Eukarya.

The origin of ecosystems, or ecopoiesis – a term coined by Canadian geneticist Robert H. Haynes (1931–1998) in 1990 [19] – was crucial for the persistence of life on Earth. Ecopoiesis involved the establishment of trophic chains, in which metabolic products of some organisms served as nutrients – in some cases also as energy sources – for others. The great metabolic diversity exhibited by prokaryotes has contributed to the continued persistence of life on Earth [12, 39]. The result of almost 2,000 million years of evolution, prokaryotes invented most extant metabolic pathways. Nowadays they can be found in a wide range of habitats, including conditions that most other organisms fail to survive. Microbial mats have been considered to be modern analogs of early ecosystems [22], perhaps the *earliest* ecosystems. We discuss here whether they fit the present concept of a minimal ecosystem.

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Microorganisms as components of ecosystems

The axenic-culture paradigm

Our perception of bacteria as unicellular life forms is deeply rooted in the axenic (“pure”)–culture paradigm. Since bacteria can, in a strict sense, be diluted to a single cell and studied in liquid culture, this approach has been exploited to study many bacterial activities. Although growing bacteria in liquid or solid media has been the traditional means to study microbial pathogenesis and to uncover some amazing facets of microbial physiology, in nature, bacteria rarely grow as axenic planktonic cultures. Microbial ecology researchers have long recognized that complex bacterial communities drive the biogeochemical cycling that maintains the biosphere. Until recently, the lack of methods for exploring these communities in situ has hampered detailed analyses. Recent advances in microscopy and molecular techniques, however, have made it possible to examine such communities in situ in great detail and without the bias of laboratory cultures. The observation of a wide range of natural habitats has established that bacteria never function as single individuals. Instead, populations of bacteria arise from individual cells, and metabolically similar populations (i.e. teams of several kinds of bacteria that have a similar metabolism), such as sulfate- and sulfur-reducing bacteria (SRB), constitute groupings referred to as guilds. Sets of guilds, including fermentative, sulfate- and sulfur-reducing, and methanogenic bacteria, which carry out interdependent physiological processes, form microbial communities. In the last decade, molecular techniques have facilitated the detection and identification of many phylogenetic groups that could not be observed before. Even more recently, bioinformatics has made it possible to handle enormous amounts of sequences and data, and by comparing them reveal relationships among genes, proteins, and metabolic pathways of different groups. Table 1 shows the changes in the paradigm for the study of microorganisms, from the dawn of modern microbiology (Pasteur and Koch) to the latest approaches based on the most recent techniques.

Tenacity of life: the power unseen

Life, as we know it, is always based on compounds made of recombining chemical elements (carbon, hydrogen, oxygen, sulfur, phosphorous and others) in aqueous solution. According to Margalef [26], “biology is advanced chemistry and has transmitted to ecology its knowledge about the elements necessarily present in the bodies of organisms”. In 1967, Morowitz published a seminal paper [31], in which he defined the minimum requirements for a self-replicating biological system. From the knowledge at the time, he suggested a minimum cell with 45 functions and a diameter around

Table 1 Conceptual changes in the study of microorganisms

Focus	Main approach (paradigm)	Since (yr)
Axenic cultures (planktonic life)	Disease agents (Pasteur, Koch)	1876
	Special metabolisms (Beijerinck, Winogradsky)	1898
	Industrial agents	1915
	Antibiotic producers (Fleming, Waksman, Dubos)	1928
	Biochemical diversity	1945
	Genetic exchange (Lederberg, Jacob)	1952
	Differences prokaryotes-eukaryotes (Stanier, van Niel)	1954
	Genetic engineering	1973
	Genomics	1995
	Proteomics	1999
	Communities (benthic life)	Bioremediation
Biofilms		1990
Deep subsurface life		1993
Life on Mars		1996

0.1 μm . More recently, he analyzed life from the perspective of physics and defined living beings as complex, organized structures far from equilibrium, which are maintained by the flow-through of energy from sources to sinks [32]. He also advanced the hypothesis that, regarding structure/metabolism, the latter (namely, the reductive citric acid cycle) is the engine of the processes that generated biological structures (namely, proteins and nucleic acids) [33]. Neelson [36] has calculated accurately that even the smallest cells must have a certain size to allow for the minimal number of molecules needed for the simplest kinds of metabolism. He proposed that prokaryotic diversity is expressed in terms of physiology and metabolism whereas that of eukaryotes is expressed in terms of structures and behaviors [37].

Central to biology is cell theory: all life is cellular. Viruses are not living, but *lived* entities. All living systems are self-organized and self-maintained – autopoietic, as they were called by Maturana and Varela [27] – and tend to reproduce themselves with high fidelity. The smallest known minimal free-living systems on Earth, capable of continuous self-maintenance (including macromolecular repair and water retention) and reproduction, are the mollicutes, prokaryotes with almost spherical cells and diameters of around 0.3 μm . *Mycoplasma genitalium*, whose genome was completely sequenced in 1995, has a 580-kb genome, which is thought to be among the smallest possible for a self-replicating organism. Only 470 predicted coding regions were identified, which included genes required for DNA replication, transcription and translation, DNA repair, cellular transport and energy metabolism [11].

Classification and diversity of living organisms

Based on molecular phylogenies, which are established by the analysis of 16S and 18S rRNA sequence data,

Woese et al. [59] proposed distributing all living organisms in three main groups that they called domains. The tree depicting the relationship among these domains was believed to represent the true, natural relationship among extant organisms [59]. The basis for discovering any possible form of life elsewhere in the Universe assumes that evolution followed the same trends that have marked life on Earth. Over the first 3,000 million years, life on Earth was only microscopic, rarely exceeding sizes larger than 1 mm. The size and shape of organisms did not expand markedly until the early Phanerozoic eon (starting 541 million years ago), during which evolution and diversification of animals, plants, fungi, and other “macroorganisms” transformed the biosphere. A major feature underlying evolutionary increases in animal and plant sizes has been their modular construction from reiterated parts [4]. The diversity of life is greater now than it was in microbial Precambrian times. However, microorganisms have proven that they can sustain the biosphere without larger organisms, whereas this is not so for a system without microorganisms. Zoologists have often claimed that “biodiversity is a prerequisite for sustainability”, an equivocal thesis with many limitations [60]. The bulk of genetic diversity in the biota resides in prokaryotes, whereas it is quite limited in eukaryotes [36]. In addition, microbial diversity is not based on the same characteristics that “higher” organisms are defined by, at least regarding the sustainability of the biosphere. The role microorganisms play in an ecosystem depends on their capability to complete the trophic system, which is achieved by means of physiological diversity.

Numbers and production

Although invisible to the naked eye (except when they make up large masses), prokaryotes are essential components of the Earth’s biota. They catalyze unique and indispensable transformations in the biogeochemical cycles of the biosphere. Most of the Earth’s prokaryotes reside in the oceans (1.2×10^{29} cells), in soil (2.6×10^{29} cells), in oceanic subsurfaces (3.5×10^{30} cells) and in terrestrial subsurfaces ($0.25\text{--}2.5 \times 10^{30}$ cells). Out of about 15 million species currently living on Earth, 12 million are probably prokaryotes – although the classical concept of “species” can not be easily applied to prokaryotes (see below). The cellular production rate for all prokaryotes is estimated to be 1.7×10^{30} cells/year and is highest in the open ocean. Due to their large population

sizes and rapid growth, prokaryotes have an enormous capacity for genetic diversity and rapid adaptation to subtle changes in environmental conditions [57]. The rates of primary production in sclerophyllous forests, coniferous forests, and grasslands are close to those observed in microbial mats (prokaryotic communities essentially), whereas tropical jungles have the highest (Table 2). However, if the ratios of carbon assimilation to biomass are taken into account, microbial mats and grasslands are then the highest [53].

Size and metabolism

Many prokaryotes share a common thread with eukaryotes in the mechanism of energy conservation, namely, the generation of a chemiosmotic gradient called the proton motive force (pmf). The pmf implies an electrochemical potential and a pH gradient, and the generation of biologically useful energy in the form of adenosine triphosphate (ATP). Prokaryotes are definitely different from eukaryotes in that they can use a wide range of fuels (e.g. reduced organic and inorganic compounds) and oxidants [nitrate, sulfate, Fe(III), As(IV), Se(VI), etc.) to generate pmf. Eukaryotes utilize only one oxidant (oxygen) and a short number of reductants (glucose, pyruvate, etc.).

The surface to volume ratios (S/V) of the reactants strongly affect chemical reactions. Diffusion limitation generally restricts the maximal size of prokaryotic cells and provides a selective advantage for μm -sized cells at the normally low substrate concentrations in the environment. Living cells often try to optimize this variable [45]. Small bacterial cells, with diameters ranging from 0.5 to a few micrometers, have S/V values 100–1000 times higher than typical eukaryotic cells, whose diameters may range from 20 μm to a few millimeters. During the 1990s, several uncultured bacteria were consecutively announced to be the largest known prokaryotes, the most remarkable being *Epulopiscium fishelsoni* (80 μm in diameter and 600 μm in length) [2], and *Thiomargarita namibiensis* (which may reach 750 μm in diameter!) [46]. Over the years, large bacteria have been described as “megabacteria”, “gigantobacteria” or given names such as *Titanospirillum* [18]. On the other hand, under the collective designation of nanobacteria or ultramicrobacteria, a range of cell forms with diameters in the range of 0.2–0.3 μm have been found [54]. Altogether, the biovolumes of prokaryotic cells may cover a range of more than eight orders of magnitude.

Table 2 Primary production and biomass for several terrestrial ecosystem (from [53])

Ecosystem	Primary production (g C assimilated m^{-2} year $^{-1}$)	Biomass (g C m^{-2})	Productivity ratio
Tropical jungle	1000–1500	3,200–40,000	0.03–0.47
Sclerophyllous forest	220–280	5,000–11,000	0.02–0.06
Coniferous forest	150–300	1,000–8,000	0.02–0.30
Grassland	220–740	150–700	0.31–4.93
Microbial mat	234	222–320	0.73–1.05

Genetic plasticity

A species is considered to be a population of organisms that share a common gene pool and can interbreed. New species evolve when the genomes of some individuals within the population change significantly or when some members become geographically isolated such that breeding with the original group is no longer possible. Three characteristics support the idea that the species concept, as it is used in eukaryotes, cannot be applied to prokaryotes: (1) the absence of sexual reproduction linked to meiosis, (2) the existence of various mechanisms (transformation, transduction, conjugation, genome fusion, etc.) that allow horizontal transfer of genes, not only among individuals of the same prokaryotic “species” but also between those that are evolutionarily distant, and (3) the high frequency of heterologous recombination, which allows the acquisition of genetic material that was not present in the original species. While meiosis allows the genetic isolation of genetically different organisms, sexuality started as an exchange of genes (it is not necessarily related to replication nor to increasing the number of individuals). By exchanging their genes, microbes could participate in the evolutionary game. Heterologous recombination makes the bacterial world much more flexible in terms of adaptation than the world of nucleated cells.

Responses to unfavorable conditions

Bacterial populations are frequently exposed to stress due to limitations and changes in their environment (e.g. nutrient availability, temperature, salinity, oxygen). The ability of bacteria to endure stress determines their persistence in an environment. Bacteria often face a “feast or famine” mode of existence, in which long periods of starvation, possibly on the order of months or years, are punctuated by short periods of nutrient availability and rapid growth, often at submaximal rates. Starvation responses, which vary from species to species, divide bacteria into two main classes: differentiating and non-differentiating bacteria. Differentiating bacteria show marked alterations in their cellular ultrastructure, such as the production of endospores or cysts. In non-differentiating bacteria, which comprise most bacteria, starvation-induced changes are confined to a decrease in cell size, protoplast shrinkage, and essentially a profound alteration in their gene expression patterns [28, 38]. These bacteria usually alternate between two phases, one of planktonic cells and another of sessile cells, that build structures known as biofilms (see Table 1).

Some bacteria have evolved a “pack rat” strategy for facing starvation: they store reserve materials including glycogen-like polysaccharides, polyhydroxybutyrate (PHB) and related compounds (polyhydroxyalkanoates), polyphosphates and even sulfur, which can be

found as intracellular or extracellular deposits. Nutrient limitation occurring in the presence of an excess of carbon, phosphorous, nitrogen or sulfur can lead to the deposition of one or more of these reserve materials without any significant effect on the intracellular osmotic pressure [9].

Biological strategies: biofilms and microbial mats

Biofilms (a word coined by John William Costerton in 1978) develop when microorganisms attach to surfaces in aquatic environments and produce exopolysaccharides, which help cells to adhere to submerged surfaces. Biofilms can consist of either a single species or a community consisting of many microbial species, most of which are prokaryotes. Microbial mats can be considered complex biofilms. A major aspect of their species composition is the presence or absence of representatives from the three basic functional groups: primary producers (i.e. autotrophs), consumers, and decomposers. Biofilms without primary producers depend on exogenous sources of organic matter. Single-species biofilms are particularly dependent on their environment not only to provide a source of oxygen and nutrients but also to transport wastes. Microbial mats, with their rich diversity of organisms, are sites of complex elemental transformations [50].

At a higher level of organization, microorganisms in microbial mats or in biofilms make up coordinated functional communities much more efficient than mixed populations of floating planktonic organisms. In fact, biofilms or mats resemble the tissue formed by eukaryotic cells in their physiological cooperativity and in the extent to which they are protected from variations in bulk-phase conditions by a kind of primitive homeostasis provided by the matrix of exopolysaccharides [7]. The analogy with eukaryotic organisms can be extended even to dissemination strategies, in which well-protected communities of cells are the most successful and their genomes are thus the most competitive, whereas planktonic cells are produced to disseminate and to colonize new localizations. The controlled shedding of planktonic cells from biofilms is a major strategy in the bacterial struggle for survival and predominance in aquatic ecosystems. We can consider the survival value of this strategy in the milieu of the early Earth. The advantages that this kind of growth confers to sessile bacteria are: (1) increased availability of nutrients for growth, (2) increased binding of water molecules, which reduces the possibility of desiccation, (3) some protection against UV radiation, (4) the establishment of complex consortia, which allows for the recycling of substances, and (5) easier genetic exchange due to the proximity to progeny and other bacteria [23].

The ability to remain in an optimal or even permissive local environment was one of the most valuable contributions of sessile growth to bacterial survival. The biofilm “phenotype” demonstrates that adhesion and

biofilm development were selected for early in the evolution of bacteria. The persistence of this positive selection for the biofilm phenotype today is evident by the predominance of this sessile mode of growth in most ecosystems [8].

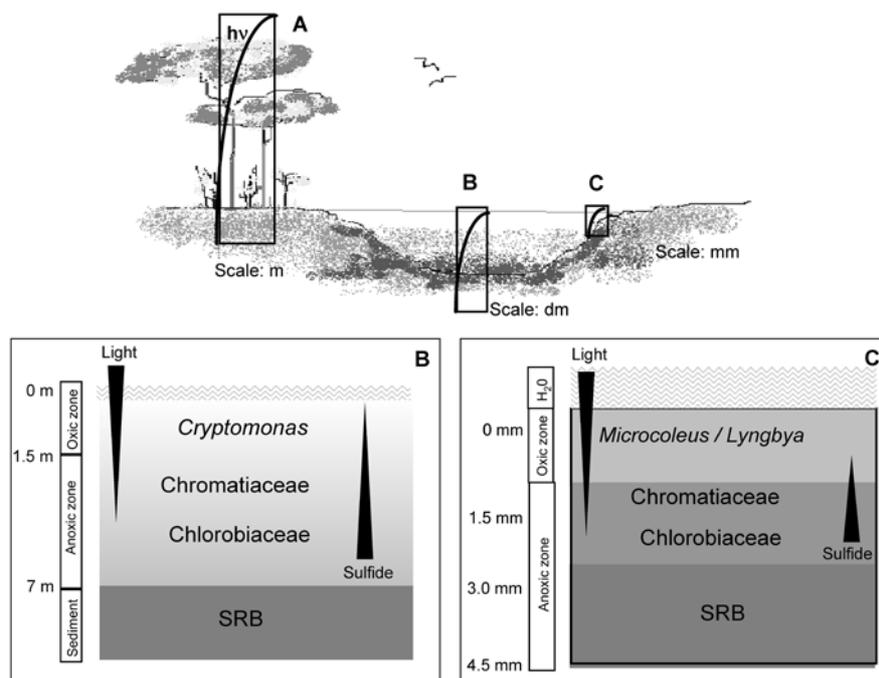
Minimal ecosystems

Ecosystems expand over time and along space. Whereas time is an intrinsic factor with the ecosystem itself, space is an extrinsic factor that contributes to change and also limits the number of effective links between components of the ecosystem. The organization of ecosystems along vertical axes differs qualitatively from that along horizontal ones. Flow can divert the vertical axis. For example, the vertical axis in a lake corresponds with the oblique axis in a river, which may be almost horizontal at some sections. The metabolic activities in the living portion of an ecosystem result in an environment that has an oxidized upper part and a reduced bottom part. There is a net flow of electrons from top to bottom, which are returned to the upper part through the physical environment. Therefore, all ecosystems have a gradient of redox potential. Such a gradient is associated with the vertical distribution of various environmental and biological variables. The physical environment acts as a battery and a repository of potential energy, due to the vertical gradient of redox potential [25]. In ecosystems in which light is the primary energy source, biological communities are usually stratified horizontally, as a consequence of light extinction with depth. Tropical forests, microbial mats, and planktonic communities in stratified lakes are considered to be analogous forms at different scales. The photosynthetic layer expands for many meters in tropical forests; from a few centimeters

to some meters, in multilayered planktonic microbial communities; and for a few millimeters in microbial mats (Fig. 1) [14].

Autotrophic and heterotrophic organisms are major components of ecosystems. Autotrophs fix energy either from light (phototrophs) or from light-independent chemical reactions (chemotrophs), and obtain nutrients from simple inorganic substances such as water, carbon dioxide and nitrates. They are called producers. Heterotrophic organisms, known also as consumers, use, rearrange and decompose the compounds synthesized by autotrophs; they are unable to produce their own nutrients and must obtain them by consuming other organisms. Ecological classifications of the biotic components of an ecosystem, especially when they are microorganisms, are based on the main source of energy utilized. Morphology may be of importance in the ecological relationships of the so-called "higher" organisms. In microorganisms, however, aside from the influence of some properties associated with morphological features (e. g. surface structures involved in the adhesion of cells either to other cells or to inanimate surfaces), there is a stronger connection of physiological characteristics with ecology [42]. Ecological classifications deal with functions, not species. Some species can have more than one energy source, whereas others can even shift their mode

Fig. 1A–C Vertical structuring in several ecosystems in which light is the primary energy source corresponds to the same ecological theater but one in which different actors play their roles at different scales. **A** Forest ecosystem, in which photosynthesizers can span vertically for meters. **B** Multilayered planktonic microbial community in a lake, in which the layering of different populations spans vertically for decimeters. **C** Microbial mat, in which layering of different populations spans vertically for millimeters. (SRB: sulfate-reducing bacteria) (Modified from [14])



of nutrition and function either as autotrophs or heterotrophs, depending on the availability of energy source and organic matter [40].

In theoretical ecology, a working model of a minimal ecosystem usually has five basic components: (1) properties, which are stated variables; (2) forces, which are outside energy sources or casual forces that drive the system; (3) flow pathways, which are energy or material transfers that connect properties with one another and with forces; (4) interactions, which are functions by which forces and properties modify, amplify or control flows; and (5) feedback loops, which are circuits through which matter or energy flow and influence an “upstream” component or flow. The boundary for the system can be either natural, such as the shore of a lake, the lake itself being the system, or arbitrary, delimiting an area such as a section of a beach or of a block of forest [40].

Energy is a necessary input in any ecosystem. Traditionally, the sun was considered to be the ultimate energy source for the biosphere; and life was thought to be directly or indirectly dependent on solar radiation. In fact, the overwhelming source of modern biological energy is the flux of solar radiation, which is photosynthetically converted into biologically useful forms [32]. In 1977, deep-sea vents were discovered at the sea bottom, several kilometers from the ocean surface. Although organisms thrive there, they depend on oxygen in the water that was produced in the photic zone to oxidize the sulfide from the vents. Other organisms rely on matter, again produced by photosynthesis, falling from the upper water layers. In the 1980s, however, an array of independent reports described the findings of microbial life in the deep subsurface. These communities comprised both chemolithotrophic and heterotrophic bacteria, whose metabolisms are independent of photosynthesis and therefore of solar energy [13]. Several kinds of metabolism explain how different microorganisms are able not only to live miserly, but also thrive at several kilometers depth. All bacteria found so far in the deep subsurface are very specialized. Although these microbes differ in some respects, the specific kinds of metabolism found at the deepest habitats have already been described in bacteria from specialized habitats at the surface.

Microbial mats as models for minimal ecosystems

Microbial mats are layered microbial communities (LMC) made up of accretionary cohesive microbial populations that grow at sediment-water (occasionally sediment-air) interfaces. Most mats stabilize unconsolidated sediments and grow actively; they can be several millimeters to a few centimeters thick, and develop along a variety of microgradients established between water and sediments. The formation of microbial mats is an extremely ancient biological phenomenon, as communities of different types of microbes covered the early

Earth. Generation after generation of bacteria in the uppermost layers died from radiation exposure, but their remains shielded those organisms in the lower layers, which accumulated sand and sediment to form a cohesive mass. Microbial mats dominated Archean landscapes. Today they can still be found in a variety of habitats including the hypersaline lagoons and sabkhas of Baja California, Mediterranean Spain, the Persian Gulf, western Australia, as well as alkaline lakes (e.g. Kenya), hot springs, sulfur springs and deep-sea hydrothermal vents. They best document their presence in the fossil record by producing laminated sedimentary rock structures called stromatolites. Stromatolites are organo-sedimentary structures produced by trapping, binding, and/or precipitation as a result of the growth and metabolic activity of microorganisms [44, 51]. The persistence and abundance of stromatolites throughout most of geological time attest to the evolutionary success of microbial mat ecosystems. Stromatolites are found in rocks as old as 3,500 million years from the Warrawoona Group of Western Australia [24].

Ecological diversity of microbial populations and communities

Microbial populations rarely occur alone in nature but rather interact with each other forming complex communities. Communities can be regarded as assemblages of microbial heterogeneous populations living together at a given place or habitat. The community is the highest biological unit in an ecological hierarchy [3]. Community structures can be more or less stable depending on their degree of development. This usually involves the orderly and sequential succession of populations. The integrating mechanisms of the members of the community and the permanence of the community modify habitat conditions that allow the evolution of new communities. In different parts of the Earth where physical environments are similar, the communities comprise either ecologically similar species or ecological counterparts. Natural assemblages can be regarded as biological self-regulating communities, and can become stable and persistent after many years of Darwinian evolution [15]. LMC are a special kind of natural assemblage in which the gradients change sharply along the vertical axis.

Ecological diversity is considered to be a function of both the number of different types (richness or variety) and the relative importance of individual elements among these types (evenness or equitability). Species richness can be expressed by simple ratios between total species and total numbers. It measures the number of species in the community, but not how many individuals of a species are present. Equitability, which measures the proportion of individuals among the species, indicates whether there are dominant populations. Various indices have been proposed as measures of diversity that incorporate both aspects, richness and evenness.

The Shannon-Weaver index is the most common diversity index used by ecologists [3]. It is based on information theory and expressed in the average number of bits that each symbol (here each individual) can carry as a message bearer. It states that $H = -\sum p_i \log_2 p_i$, where p_i stands for the representation of each species ($\sum p_i = 1$) in the whole unit. This formula is equivalent to that of Brillouin, which expresses statistical entropy, as proposed by Boltzman, divided by the total number of elements (individuals) N , as $H = (1/N) \log_2 [N! / N_a! N_b! \dots N_m!]$, where N_a stands for the individual number of species a , ..., and N_m for the individual number of species m [26].

Modifications in environmental conditions of an ecosystem frequently lead to changed proportions of members of one specie, so diversity will be $H' = H + 1/N \log_2(N_a/N_b + 1)$. If $N_a < N_b$, diversity decreases, because one rare species was replaced by another more numerous. By contrast, if $N_a > N_b$, diversity increases [3].

Population identification is the first step to establish relationships between the whole (community) and its parts (populations). The study of microbial communities has raised questions about their composition, structure, and stability and about the activity and function of the individual inhabitants. In the past, knowledge of microorganisms in the environment depended mainly on studies of axenic cultures in the laboratory. In fact, microbial physiology and genetics can be investigated in great detail in cultivated isolates. Most microorganisms, however, have so far resisted cultivation efforts [41]. From most habitats studied, less than 1% of the microorganisms observed by microscopy have been brought into culture [1].

Different techniques, such as light microscopy and scanning or transmission electron microscopy, have been used to characterize microorganisms from LMC [10, 49]. Confocal laser scanning microscopy has offered significant advantages in microbial ecology studies [48]. Recently, new microscopic techniques to study in situ the morphological, structural and geophysicochemical characteristics of benthic communities have been described. These methods combine SEM using a back-scattered electron (BSE) detector in conjunction with an auxiliary X-ray energy-dispersive spectroscopy (EDS) microanalytical system [58].

Molecular biological techniques to detect and identify microorganisms by molecular markers such as 16S rRNA or functional genes is now more frequently used than microscopy to explore microbial diversity and to analyze the structure of microbial communities [34]. The chemical analysis of sediments can indicate function or metabolic activity in prokaryotes, which morphology cannot. Lipids analysis, especially fatty acids, is a quantitative means of measuring viable microbial microorganisms, microbial community composition [56], and community nutritional/physiological status [35]. Nonetheless, the combination of different methodologies allows a more representative picture of the distribution and abundance of microorganisms in complex communities (Fig. 2).

Population growth

The basic principles of a biocenosis are: (1) the growth of one cell leads to N cells, (2) N cells are a population, (3) a population depletes nutrients and accumulates wastes, (4) several populations associate in a guild or in a community, and (5) the community is the minimal unit of sustainable life.

In general, the growth of each individual population can be expressed by the adaptation of the Monod's equation:

$$dP/dt = \mu P$$

where P is the population density in a given time (t) and μ is the specific growth rate of the population. The value of μ depends on both favorable conditions (K) such as nutrients, water, light, pH and temperature, and deleterious conditions (ω) such as outflow, predation, lysis and sedimentation, which reduce the numbers of cells in the population [14]. If $K > \omega$, then $\mu > 0$, and the population increases, whereas if $K < \omega$, then $\mu < 0$, and the population decreases. In the second case, if ω is much higher than K , then $\mu \ll 0$, leading to the death of the population.

The growth of a community depends on the growth of each population, which can be defined as:

$$\mu_{community} = \mu P_1 + \mu P_2 + \mu P_3 + \dots + \mu P_z$$

If there are only energy and matter fluxes, matter will become exhausted and lead to death. If there is energy flux and matter recycling, however, the ecosystem will persist. In fact, since the establishment of the earliest ecosystems, life has persisted on Earth, and should last until the cosmic end of the planet.

Ebro Delta microbial mats and ecophysiological processes

The Ebro Delta, the third largest delta in the Mediterranean, with 320 km², is located at the northeastern coastline of the Iberian Peninsula (0°35'E–0°56'E; 40°33'N–40°47'N). Light may be the key environmental factor determining the structure of the community. The LMC of the Ebro Delta is composed of communities of oxygenic photoautotrophs (diatoms, cyanobacteria – *Microcoleus* and *Lyngbya*), anoxygenic photoautotrophs [purple sulfur bacteria (PSB) – *Chromatium* and *Thiocapsa* – , green sulfur bacteria (GSB) – *Chlorobium*], chemoautotrophs (colorless sulfur bacteria such as *Thiobacillus* and *Beggiatoa*), heterotrophs (several metabolic kinds of bacteria, among them the characteristic morphophysiological group of the spirochetes) and sulfate-reducing bacteria (SRB) [16, 30]. The largest known free-living spirochete was first identified and described from Ebro Delta mats. It was named *Spirosymplokos deltaeiberi*, and can be up to 100 μm × 0.4–0.3 μm [17]. Recently, a huge, highly motile,

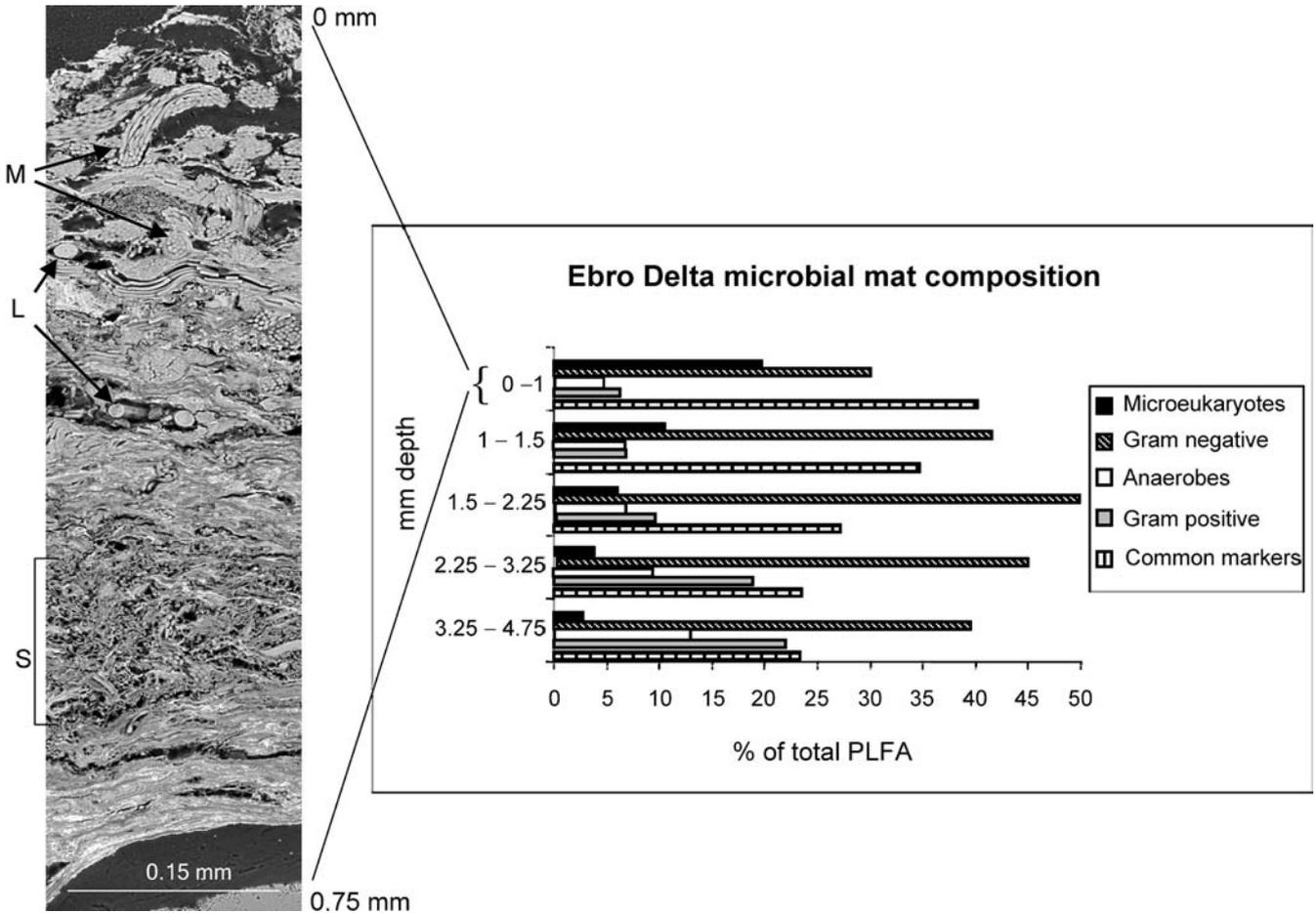


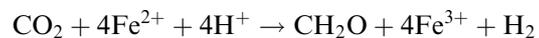
Fig. 2 Integrated polyphasic study of the microbial mats from the Ebro Delta (Tarragona, Spain). *Left*: Micrograph obtained by scanning electron microscope-backscattered electron (SEM-BSE) microscopy of the top 0.75 mm vertical section of the mat [Wierzchos et al, unpublished results], which offers an in situ vision of the structure and distribution of the microorganisms in the mat. The most abundant microorganism is the filamentous-sheathed cyanobacterium *Microcoleus chthonoplastes*, showing different states of lithification, from the thriving trichomes on the top, to the reduction of filaments inside each sheath, in the middle, until the flat, empty, hardened, compacted sheaths at the bottom. *Lyngbya* sp. is also seen in a few places. M, *Microcoleus*; L, *Lyngbya*; S, mostly empty sheaths. *Right*: An estimation of the relative taxonomic composition from the surface to a vertical section 4.75 mm in depth of the mat as determined by a lipid biomarkers assay; *PLFA* phospholipid fatty acids [35]

sulfur-storing spirillum with a special kind of motility, *Titanospirillum velox*, has been described [18] (Fig. 3).

The Mediterranean Sea has experienced several periods of desiccation, the last of them only some 5 million years ago (Miocene) when it dried up completely. A study of the geochemical balance of the oceans indicates that the deposition of very large salt bodies in isolated basins, such as the Miocene Mediterranean, has happened every 100 millions years or so, and that those “saline giants” have served to keep the salt concentration in Earth’s oceans rather constant throughout time [20]. In mats, fluctuations of anaerobic and aerobic conditions are

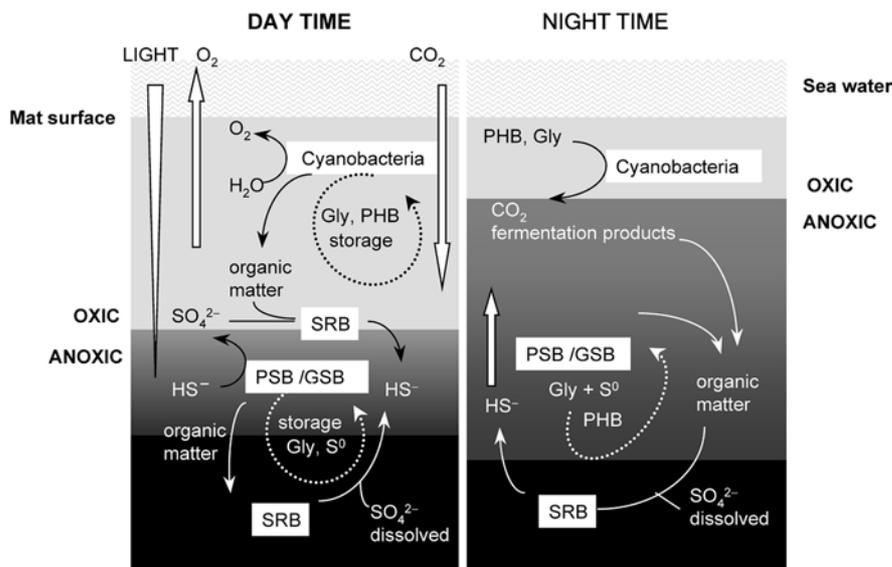
common. These fluctuations may occur seasonally, diurnally, or even more frequently. Oxygenic photosynthesis of cyanobacteria in the vicinity of sulfide results in the establishment of a sharp redoxcline of less than 1 mm. Diffusion over these small distances is very rapid, and the redoxcline migrates through the photosynthetically active layer with diurnal changes of light intensity. Diurnal oscillations in oxygen and sulfide expose cyanobacteria to varying sulfide concentrations, and SRB to periodic oxic conditions [6]. Sulfate reduction was long considered to be an obligate anaerobic process; however, many sulfate reduction measurements in a variety of habitats show maximal rates of sulfate reduction close to sediment surfaces that are exposed to oxygen [47, 52].

The diurnal migration of the redoxcline within the euphotic zone of the cyanobacterial mats causes periodical releases of Fe^{2+} ions from the pool of FeS ; these ions serve as a major sink for the sulfides produced by the SRB. Several mat-forming cyanobacterial isolates could photoassimilate CO_2 efficiently by using Fe^{2+} ions, which serve as yet another alternative electron donor for photosynthesis in these strains:

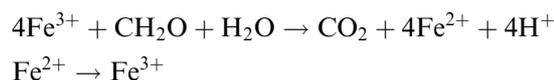


If the pH is ≥ 5 , then the ferric ion produced would precipitate as ferric hydroxide [$\text{Fe}(\text{OH})_3$]. To complete

Fig. 3 Ecophysiology of the day-night carbon and sulfide cycles carried out by different populations in a typical marine microbial mat community (Ebro Delta). (*PHB* : Poly- β -hydroxybutyrate, *Gly*: glycogen)



the iron cycle, bacteria would reduce Fe^{3+} ion to Fe^{2+} using the organic molecules produced:



Ferrous ion is a good potential electron donor for photosynthesis in cyanobacteria and may operate at the spectrum of redox potential values of -50 to $+50$ mV, which is typical for the photic microzone during the daily transition from fully reducing conditions at night to high oxygen concentrations at noon and the opposite transition in the late afternoon. Banded iron formations are thought to be a result of the oxidation of ferrous ion by oxygenic photosynthesis and were taken as geological proof for the accumulation of oxygen during the Precambrian era. Ferrous-ion-dependent photosynthesis may be another mechanism for deposition of the banded iron formation, which does not necessarily involve oxygen. Just beneath the surface of the microbial mat-water interface, the environment can be anoxic and rich in Fe^{2+} . The processes that occur in this area may well represent processes that occurred on a more extensive scale in an ancient anoxic world [43].

Lithification process in Ebro Delta microbial mats: the way to stromatolites

Microorganisms have left significant evidence of their presence and activities in sediments. We are just beginning to appreciate the intimate juxtaposition and interdependence of minerals and microbes. The widespread occurrence of microorganisms in sediments contributes to the immobilization of some metals through a continuum of sorption and precipitation reactions. Depending on the prevailing environmental conditions and activity of indigenous microbial populations, individual cells can facilitate the nucleation and growth of distinct

minerals. Mineral precipitation may be promoted by: (1) changes in the microenvironmental chemical conditions and hence saturation state caused by microbial metabolic processes, (2) nucleation on surfaces of microorganisms (e.g. the cell envelope is very important for calcification) or microbial products. Thus, benthic microbes form sediments, contribute to their development and can modify them significantly.

Microbial cells promote sediment accretion by selectively incorporating sediment particles [21]. Such preferential incorporation of mineral matter with bacteria may frame-build or guide precipitation to the construction of organosedimentary structures with different sizes and shapes. Various participating taxa may reflect external shaping and/or internal textures. We observed evidence of the beginning of the lithification process in Ebro Delta microbial mats at approximately 2 mm depth. Live cells, including *Microcoleus chthonoplastes* and *Lyngbya* sp., can be observed, as well as some dead cells and empty or collapsed sheaths (Fig. 4). Studies of mineral precipitation at early stages with SEM-BSE and EDS show that the mineral is composed by $\text{Ca}_3(\text{PO}_4)_2$ (apatite), and that the sheaths are critical in the accretion process [44, Wierzchos et al., unpublished results]. These accumulations occupy voids or intercellular space. Cell shapes serve as templates to create mineral irregular forms. It has been suggested that lithification in flat laminated microbial mats does not occur at the surface but rather at the bottom after the cyanobacteria have died. Mineralization (e.g. calcification) of dead cyanobacterial material is probably due to carbonate precipitation by heterotrophic bacteria living on and from the organic material of the sheaths [5, 29]. In fact, carbonate production by heterotrophic bacteria is potentially much higher than autotrophic or chemical sedimentation in most environments [21, 44]. However, Vissher et al. [55] have shown that lithification in modern marine stromatolites occurs at the surface and is mediated by a community of SRB.

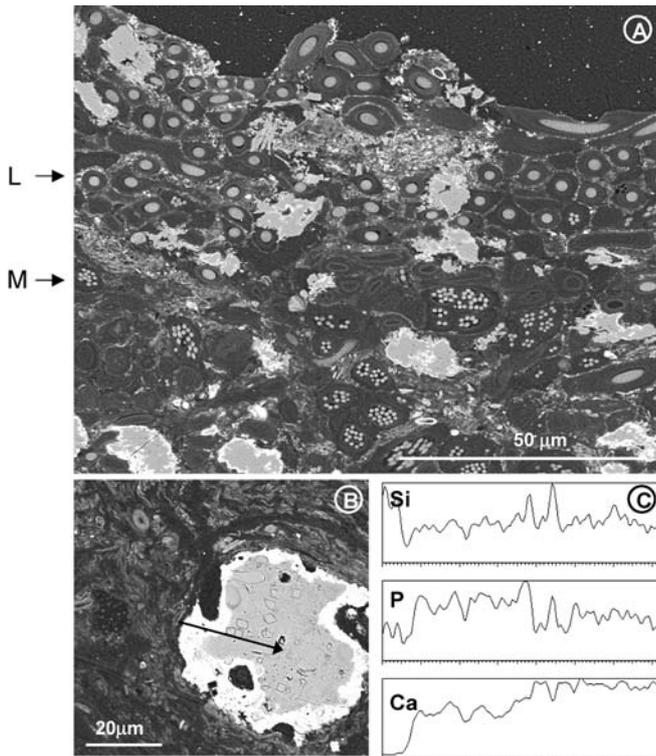


Fig. 4A–C Scanning electron microscope-backscattered electron (SEM-BSE) micrographs from an Ebro Delta microbial mat. **A** Transversal section of the upper layer shows the filamentous cyanobacteria *Microcoleus* and *Lyngbya*. These microorganisms produce extracellular polymeric substances that bioimmobilize or trap sedimentary particles brought by the flowing waters. **B** SEM-BSE image corresponding to a 4-mm-depth section of the mat. A calcium phosphate grain embedded with diatoms, which reflects the lithification process, is shown. **C** Scan-line analysis performed by energy dispersive X-ray spectroscopy (EDS) shows the presence and relative quantity of different elements within an *arrow*, in this case, the *arrow* in **B**. (Courtesy of J. Wierchos and C. Ascaso)

Concluding remarks

Life is a consequence of gradients as well as of limitations of space and number of transitional states; it may not need energy and chemical sources other than those that occur in the natural development of planetary bodies. The observation of extant life in the subsurface of Mars or in another body in our solar system might be the discovery of the 21st century. Perhaps life is present in many places in the Universe, lurking in solid planets surrounding many stars [12]. One may wonder how life beyond Earth would be manifested. The only possibility for life to survive anywhere is by evolving ecosystems, as happened on our planet. Given conditions similar to those of the early Earth, microbial mats would seem to be the simplest of the self-organized structures that may have first appeared. Our primitive atmosphere was reduced and reducing, like the atmospheres of other planets where life could also originate. Although it is not possible to know how life started and how the first living beings organized, the acquisition of an internal mechanism was surely the critical

step. Provided an active structure persisted, the path to success was by switching and controlling the flow of photons from solar radiation, which provided organisms with reducing power and the possibility of oxidizing their surroundings. A reasonable scenario is that those thriving primary producers multiplied rapidly and their activity led to the formation of an oxidized atmosphere whose composition gradually approached that of the present one [26].

Not all kinds of habitats where only microbial communities develop can be considered minimal ecosystems, not even ecosystems. For example, bacterial communities growing in the guts of termites and other wood-eating insects are not ecosystems because they do not contain primary producers. Microbial mats operate as almost closed systems with persistent oxidation-reduction gradients and restricted vertical flows. At the top, photosynthesis supplies energy in an oxic environment. Under the driving force of sunlight, the components interact and feedback flows become established. Thus, microbial mats fulfil the basic requirements for minimal ecosystems [26].

As Gene Odum¹ once stated, “ecology is a discipline that emphasizes a holistic study of both parts and wholes. While the concept of the whole being greater than the sum of the parts is widely recognized, it tends to be overlooked by modern science and technology, which emphasizes the detailed study of smaller and smaller units on the theory that specialization is the way to deal with complex matters. [...] to understand and properly manage a forest, we must not only be knowledgeable about trees; we also need to know about the unique characteristics of the forest as it functions in its entirety.” Let us paraphrase Odum and add that, to understand microbial mats, we must not only be knowledgeable about the microorganisms that make up them; we need to know about the unique characteristics of the mat as it functions in its entirety.

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¹[Note added in proof: Eugene P. Odum (1913–2002) died on August 10, 2002. We offer this article as an homage to his memory and his everlasting contribution to our understanding of Earth's (macro)ecosystems.]

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