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From the cell to the ecosystem: The physiological evolution of symbiosis

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Abstract Living organisms constantly interact with their habitats, selectively taking up compounds from their surroundings to meet their particular needs but also excreting metabolic products and thus modifying their environment. The small size, ubiquity, metabolic versatility, flexibility, and genetic plasticity (horizontal transfer) of microbes allow them to tolerate and quickly adapt to unfavorable and/or changing environmental conditions. The consumption of resources and the formation of metabolic products by spatially separated microbial populations constitute the driving forces that lead to chemical gradient formation. Communication and cooperation, both within and among bacterial species, have produced the properties that give these organisms a selective advantage. Observations of a wide range of natural habitats have established that bacteria do not function as individuals; rather, the vast majority of bacteria in natural and pathogenic ecosystems live in biofilms, defined as surface-associated, complex aggregates of bacterial communities that are attached to solid substrates and embedded in a polymer matrix of their own production. The spatial configurations of biofilms reach levels of complexity nearing those of multicellular eukaryotes. Microbial consortia have played important roles throughout the history of life on Earth, from the

microbial mats (a type of biofilm) that were probably the first ecosystems in the early Archean, to the complex microbiota of the intestinal tract of different animals.

Keywords Functional bacterial consortia · Energetics · Coordinated metabolism · Microbial mats

Our star, the Sun, began its existence some 5,000 million years ago, in a side of the Via Lactea, the Milky Way. Together with our galaxy, it moves in an almost circular orbit that takes about 226 million years to complete. The sun, like other stars in its class, is accompanied in space by smaller non-luminous bodies that revolve around it, the planets ("wanderers," in Greek). Earth clearly distinguishes itself from Venus and Mars by its non-equilibrium atmosphere and by producing its own light, from volcanoes, large forest fires, and luminous cities. The Earth rotates around its star in one year, and just as a year is not much in the life of a person, neither are 226 million years in the life of Earth.

The origin of life, biopoiesis (*poiesis*, meaning creation and production and the same Greek word from which the word "poetry" derives), may have taken place in our planet several times before and after the Earth had abundant liquid water, some 3850 million years ago. The first autopoietic (self-sustaining) life forms were prokaryotic cells with essentially the same structure as present-day bacteria and archaea. Prokaryotes were the basis from which all other forms of life arose. Subsequent symbiotic associations among prokaryotes gave rise to the ancestors of all the complex and varied biological forms that followed and to the first eukaryotic cells (eukaryopoiesis, *eukaryon* meaning "true nucleus"). The eukaryotic cell is the basis of the structure of protists (protozoans, algae, etc.), the first eukaryotic-celled organisms, as well as fungi, plants, and animals. Indeed, all of these organisms emerged within a prokaryotic world and they have retained their intimate connections with, and dependency upon, prokaryotes. For example, mitochondria, the organelles responsible for aerobic respiration in all eukaryotic cells, are derived from Alpha-proteobacteria. Similarly, chloroplasts, the photosynthetic organelles found in plants and algae, are descendant from a group of aerobic photosynthetic bacteria, the cyanobacteria.

The autopoietic unit, whether a bacterial biont (minimal autopoietic unit) or a holobiont (integrated bionts, i.e., animals or plants, with all of their associated microbiota), is capable of self-maintenance by sensing the environment and adapting to

new circumstances. Complex autopoietic units acquire novel properties when the assembly of their components results in a higher functional-structural complexity. However, autopoiesis alone, while necessary, is not a sufficient condition for life; rather, the continuity of life on Earth requires ecopoiesis, that is, the development of ecosystems, and their maintenance.

Living organisms constantly interact with their habitats, selectively taking up compounds from their surroundings to meet their particular needs but also excreting metabolic products and thus modifying their environment. Communication and cooperation, both within and among bacterial species, is thought to have produced the properties that give these groups a selective advantage. Bacterial cells inevitably produce resources that benefit others. Over time, the recipients of these metablic by-products will forfeit their own, now-redundant synthetic pathways for a dependency that ultimately favors the spread of obligate coevolved partnerships (Sachs and Hollowed 2012). This paradigm suggests that bacteria will engage in interdependent cooperative functional-metabolic interactions in the form of communities and that bacterial cooperation will leave a clear genomic signature (Martínez-Cano et al. 2015) (Fig. 1).
>>> **Approx. here Fig. 1**>>>>

Microorganisms as components of natural ecosystems

How many different forms of life exist and how are they related evolutionarily are long-standing questions in biology. In 1962, Roger Y. Stanier and Cornelis B. van Niel defined "the concept of a bacterium" and thus allowed (micro)biologists to divide living organisms into two primary groups: prokaryotes and eukaryotes. (Guerrero et al. 2002). Although invisible to the naked eye (except when they make up large masses), prokaryotes are essential components of the Earth's biota. Their growth and survival drive the geochemical cycling of the elements, enable the detoxification of many organic and inorganic contaminants, allow essential nutrients present in the biomass of one generation to become available to the next, and result in the maintenance of the conditions required by other inhabitants 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 ($0.25–2.5 \times 10^{30}$ cells). Due to their large population sizes and rapid growth, prokaryotes have an enormous capacity

for genetic diversity and for rapid adaptation to subtle changes in environmental conditions (Whitman et al. 1998; Guerrero and Berlanga 2007; Prosser et al. 2007).

Although microbes are everywhere, they were discovered very late in the history of humankind. Anton van Leeuwenhoek (1632–1723), a Dutch trader from Delft lacking in scientific training, observed the first protists in water in 1674. He called them *beesjes* (beasties), or *cleijne Schepsels* (little creatures), as he wrote in Dutch, or *animalculi* (little animals) in Latin, the term used in the published translation of Leeuwenhoek's letters to the Royal Society of London. Later, in 1683, he observed bacteria for the first time, on the surface of his teeth (Dobell 1958). Our current understanding of microorganisms is the product of three phases of discovery: the microscopic stage, the pathogenic stage, and the ecological stage. In the first, the existence of microorganisms was recognized but they were considered as mere "curiosities," too small to support any important function. In the second stage, work by the fathers of microbiology, Louis Pasteur (1822–1895) and Robert Koch (1843–1910), demonstrated with certainty that microorganisms were the cause of infectious disease and the contaminating agents of food and water. In the third, the pioneering investigations of Martinus Beijerinck (1851–1931) and Sergei Winogradsky (1856–1952) revealed the important role of microorganisms in the recycling of matter in the ecosystem and thus in controlling the evolution of the biosphere.

Microbial ecology only became an independent discipline during the second half of the twentieth century. The first textbook to include the name "microbial ecology" in its title (*Principles of Microbial Ecology*) was published in 1966, by Thomas D. Brock (born in Cleveland, Ohio in 1926). This science has confirmed that the general principles of ecology are applicable to microorganisms and that our knowledge of the microbial world can be integrated into current ecological paradigms (Pedrós-Alió and Guerrero 1994). The study of biodiversity in a particular ecosystem (a forest, a lake, a sea, an animal, or a plant) would be incomplete without the inclusion of microorganisms, since they are essential contributors to the global functioning of the planet and thus to the sustainable development of the biosphere (Maloy and Schaechter 2006).

The field of microbial ecology has experienced revolutionary changes over the past few years due to the impact of new technologies in cellular and molecular biology. Appropriate tools, both technological and intellectual, are needed to quantify biodiversity, the main component of which is microorganisms. An evaluation of

microbial diversity (the microorganisms present), distribution (the spatial and temporal heterogeneity of communities in their environment), and activity (the functions of microorganisms) was limited for many years to the study of the microbiota that could be cultured in the laboratory (axenic cultures). Enrichment and isolation techniques led to the establishment of a series of artificial environmental conditions that allowed the culture of a few organisms, i.e., those adaptable to this fabricated environment. But these in vitro conditions also reflect the ability, persistence, and luck of the researcher; thus, it is not at all surprising that the vast majority of the microbial world remains unculturable (Stahl and Tiedje 2002; Schaechter et al. 2004).

Competition for nutrients and other limiting resources is the major selective force that promotes bacterial adaptations, such as motility to search for nutrients, antibiotic production to inhibit competitors, and adhesion to persist in favorable environments. However, environmental success is defined not only by growth and reproduction but also by the ability of organisms to avoid, tolerate, or defend themselves against natural predators. The basic principles of a biocenosis (biological community) are: (i) the growth of one cell leads to N cells, (ii) N cells are a population, (iii) a population depletes nutrients and accumulates waste, (iv) several populations associate as a guild or community, and (v) the community is the minimal unit of sustainable life. Thus, in our own, previous work (Guerrero et al. 2002; Guerrero and Berlanga 2006), we stated that the growth of each individual population can be expressed by 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 favorable conditions (*K*), such as sufficient nutrients, water, light, pH, and temperature, which promote growth, and on the deleterious conditions (ω), such as outflow, predation, lysis and sedimentation, which reduce the numbers of cells in the population. 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 larger than *K*, then $\mu << 0$, leading to the death of the population.

An ecological community can be considered as the integration of the individual populations composing the community; hence the permanence of a given community

depends on the integration of the individual growth of each population, which can be defined as:

μ community = μP1 + μP2 + μP3 +...+ μPz

Therefore, the size of a population of organisms in the environment is determined by the balance between their specific cell growth and mortality rates (Guerrero et al. 2002; Madsen 2005).

The continuance of a population in an ecosystem depends on both its nutritional needs and its tolerance of abiotic factors . The four basic elements of the Greek cosmology dictate the four basic requirements for life: water (metabolism), air (respiration), fire (heat), and earth (food). The first three establish limits for the existence of microorganisms in different environments, according to Shelford's law of tolerance, which states that that the survival and development of an organism depends on the presence of specific abiotic or biotic factors (e.g. the climatic, topographic, and biological requirements of plants and animals) within a defined range. The fourth element, earth, and therefore food, regulates the biomass of a microorganism according to Liebig's law of the minimum (first developed for agriculture), which states that the yield will be proportional to the amount of the most limiting nutrient (Atlas and Bartha 1998).

Observations of a wide range of bacteria in different habitats, whether environmental, clinical, or industrial, have confirmed that surface attachment is the first step in biofilm development and that the latter is a normal feature of bacterial growth (Kolter 2010; Sachs and Hollowed 2012). Biofilms (the term was coined by Bill Costerton in 1978) are heterogeneous structures composed of different populations of microbial cells. Microorganisms in biofilms are metabolically and functionally integrated consortia that can adopt specific spatial configurations; however, biofilms are also observed in communities with less diversity (Ackermann 2015).

The energetic basis of life

Until the late 19th century, biologists thought that life had only two mechanisms by which it could fulfill its energetic and nutritional needs: (i) the light-fueled intake of simple mineral nutrients (photosynthesis) and (ii) the intake and oxygen-dependent

metabolism (aerobiosis) of complex organic nutrients. However, neither the conversion of light (or other radiant energy used by the organism) into chemical energy by plants nor the more or less oxygen-consuming respiration of animals could explain the metabolism of all living organisms (Guerrero 1998). Instead, an assortment of chemical conversions were eventually shown to satisfy the energetic needs of many cells. The metabolic products formed by microorganisms vary greatly and depend on the initial substrate. Moreover, a single substrate may be decomposed to numerous by-products depending on the nature of the microbial species involved. The manner in which many microorganisms obtain energy differs greatly from the mechanisms of energy production in animals and plants; nevertheless, an understanding of energy production in microbes provided strong evidence for the unity of biochemistry; that is, that in all living cells chemical energy is generated by the transfer of electrons and protons to a variety of acceptors, as described by the general equation of metabolism:

$$AH_2 + B \rightarrow A + BH_2$$

where AH_2 represents any substrate amenable to dehydrogenation, i.e., the "electron donor," and the hydrogen (or electron) acceptor B, at least in the standard reaction, is either an organic compound, as in fermentation, or free oxygen, as in aerobic respiration, or an inorganic compound (anaerobic respiration) containing N, S, or C (such as NO_3^-, SO_4^{2-}, or CO_2) (Guerrero and Berlanga 2006).

The nature of an electron donor is determined by the electron acceptor, in accordance with thermodynamic principles, which state that an energy-yielding reaction must always involve electron flow from a more negative to a more positive redox potential. Organisms that use electron acceptors of relatively low negative potential are restricted to electron donors that are even more negative, e.g., hydrogen and certain organic compounds in the case of methanogens, acetogens, and sulfur/sulfate reducers, or iron reducers. By contrast, organisms that use more positive electron acceptors can oxidize a greater variety of substances, as demonstrated by aerobic lithotrophs; thus, certain nitrate reducers (anaerobic respiration) can oxidize sulfide, ferrous iron, methane, nitrite, and ammonia (Vallino et al. 2003; Guerrero and Berlanga 2006).

Anaerobic microorganisms can use protons as terminal electron acceptors for the oxidation of organic compounds forming hydrogen. The low redox potential of the redox pair H^+/H_2 ($E^{o\prime} = -414$ mV) has an energetic deficiency compared to, for

example, the redox pair O_2/H_2O, which is used in aerobic respiration and has an $E^{o\prime}$ of +818 mV. It is energetically difficult to reduce protons using the redox mediators NADH and $FADH_2$: the $E^{o\prime}$ of the redox pair NAD^+/NADH is –320 and that of $FADH/FADH_2$ –220 mV. Other common redox mediators are ferredoxins (Fd); the Eo′ of the redox pair Fd(ox)/Fd(red) is –398 mV. Many bacteria grow in obligate syntrophy with methanogens or other hydrogen-utilizing bacteria (e.g., sulfate-reducing bacteria). In these cases, hydrogen-consuming bacteria are essential to maintain the low concentrations of hydrogen that make the reaction sufficiently exergonic to support energy conservation (Stams and Plugge 2009; Sieber et al. 2012) (Fig. 2).

>>>> **Approx. here Fig.2**>>>>>

Other forms of cooperation among microorganisms allow thermodynamically unfavorable metabolic reactions. For a long time, organisms capable of anaerobic growth on methane and ammonium compounds could not be detected. This led to the belief that ammonium and methane were inert under anoxic conditions. However, recent studies using molecular techniques have shown that anaerobic methane oxidation can be carried out by a syntrophic consortium made up of an archaeon and a sulfate-reducing bacterium, although it has been suggested that some archaea can oxidize CH_4 without the need for a syntrophic bacterial partner (Boetius et al. 2000; Marlow et al. 2014). Presently, the microbial oxidation of methane is thus believed to proceed only with oxygen or sulfate. The details of the association are still unclear, but it appears that the archaeal partners oxidize methane and transfer its electrons to bacteria, which reduce sulfate to sulfide, generating energy to power cellular functions. But, the anaerobic oxidation of methane coupled to nitrate reduction (denitrification) is also possible (Raghoebarset al. 2006) and is carried out by a consortium consisting of two microorganisms, a bacterium representing a new division (or phylum) of *Bacteria* and an archaeon distantly related to marine methanotrophic *Archaea*. Anaerobic ammonium oxidation (referred to as "anammox") is mediated by a monophyletic, deeply branching group of bacteria, the Planctomycetes. These organisms produce sterols, contain ether lipids (in the anammox case), and have a proteinaceous cell wall comparable to that of *Archaea*, most likely without peptidoglycan. In addition, they have a differentiated cytoplasm with membrane-bound intracytoplasmic compartments that are unique for each species (Fuerst 2005; Fuerst and Sagulenko 2011). *Candidatus* "Brocadia anammoxidans" (the first anammox bacteria to be discovered) and *Candidatus* "Kuenenia stuttgartiensis," *Candidatus* "Scalindua sorokinii," and *Candidatus*

"Anammoxoglobus propionicus" share the same metabolism and have a similar ultrastructure, characterized by the presence of an "anammoxosome" (Fuerst and Sagulenko 2011).

All living systems produce two forms of usable energy: (i) the energy-rich chemical bonds of ATP and (ii) transmembrane ion gradients. In metabolic evolution, carbon dioxide respiration with hydrogen (methanogenesis and acetogenesis) was probably the first type of catabolism (Downs 2006; Falkowski et al. 2008), together with several forms of respiration. However, the latter would have been limited by the availability of substrates and/or electron acceptors. The wide variety of biochemical modes of existence reflects billions of years of evolution, adaptation, and niche differentiation. Hence, although there is enormous genetic diversity in nature, there remains a relatively stable set of core genes coding for the major redox reactions essential for life and biogeochemical cycles (Falkowski et al. 2008). Six major elements (H, C, N, O, S, and P) constitute the major building blocks for all biological macromolecules (Guerrero 1998; Falkowski et al. 2008). The biological fluxes of the first five of these elements are driven largely by microbially catalyzed, thermodynamically controlled redox reactions.

Microbial mats: A case study

Microbial mats exemplify functionally integrated, self- sustaining, laminated microbial consortia that develop in the physical-chemical microgradients established at the interfaces of water and solid substrates. They are an extremely ancient biological phenomenon, as the early Earth was probably covered by communities of different types of prokaryotes. Microbial mats dominated Archean landscapes. Their presence is best documented in the fossil record in laminated sedimentary rock structures called stromatolites, which are found in rocks as old as 3,500 million years from the Warrawoona Group of Western Australia (Lowe 1980) and the Buck Reef Chert, South Africa (Tice et al. 2004).. The persistence and abundance of stromatolites throughout most of geological time corroborate the evolutionary success of microbial mat ecosystems.

The remarkably high biodiversity found within a microbial mat is compressed into a few millimeters and includes photosynthetic bacteria, aerobic heterotrophs, fermenters, anaerobic heterotrophs (notably, sulfate-reducing species), and

chemolithotrophs (especially, sulfur-oxidizing species) (Guerrero et al. 2002). This rich diversity accounts for the complex elemental transformations that characterize microbial mats (Guerrero and Berlanga 2013; Ufarté et al. 2015). Resource consumption and the generation of metabolic products by microbial populations are the driving forces in the formation of chemical gradients (Brune et al. 2000). The chemical properties of the mats fluctuate daily and seasonally. During the day, oxygenic photosynthesis operates in the uppermost layers. At night, however, the mats become anoxic and high in hydrogen sulfide concentrations, as a result of ongoing sulfate reduction in the absence of photosynthesis. The high O_2 consumption in the mat leads to a low O_2 concentration, which together with low night-time O_2 penetration confines the oxic zone to the upper 0.2 mm of the mat. At noon, high rates of oxygenic photosynthesis result in a strong increase in O_2 concentrations and thus into greater oxygen diffusion, to a mat depth of up to 2 mm. In the mats of temperate environments, such as those in the Western Mediterranean (Ebro Delta, Spain, and Camargue, France) and Guerrero Negro (Baja California, Mexico), three main chemical zones have been described: the oxic/photic (~0–2 mm depth) zone, the low sulfide (~2–4 mm depth) zone, and the high sulfide (~5 mm and deeper) zone (Bolhuis et al 2014; Nielsen et al 2015).

Two complementary, culture-independent approaches that can be used to assess microbial biodiversity, including within microbial mats, are metagenomic shotgun and targeted-gene amplicon sequencing. In a continued study in Guerrero Negro, Baja California, Mexico, the detected sequences within a mat represented > 20 Bacteria phyla (Fig. 3) (Ley et al. 2006; Harris et al. 2013). Molecular surveys of the 16S:18S rRNA contained in microbial mats have determined the large predominance of bacteria (90 %), followed by archaea (9%), and eukaryotes (1 %) (Ley et al. 2006; Robertson et al. 2009; Harris et al. 2013; Carreira et al. 2015). Archaeal diversity within the oxic zone but also in the deep anoxic layers consists mainly of members of the Euryarchaeota. By contrast, although few crenarchaeal sequences are found above the first 2 mm of a mat, their numbers increase with depth (Harris et al. 2013). The eukaryotic diversity of the Guerrero Negro mats is surprisingly low, consisting mainly of bacterivorous nematodes (Feazel et al. 2008), whereas the bacterial diversity in the same mats is enormous. However, while bacteria collectively exhibit broad metabolic capabilities and can occupy many chemical niches, the metabolic versatility of eukaryotes is limited, even though these organisms are capable of survival under high sulfide, fermentative, anoxic conditions.

>>>>> **Approx. here Fig. 3** >>>>>

Microbial mat diversity is apparently stable over a period of hours during the daily cycle, with the exception of those microorganisms that migrate vertically or undergo changes in abundance, especially after periods of intense photosynthetic activity (Villanueva et al. 2007). Diversity is generally thought to be desirable for ecosystem stability; that is, more complex systems are more robust than simpler ones and thus less vulnerable to environmental changes. In the case of mats, their robustness is based in part on redundancy, in which multiple units perform the same or very similar functions inside the system. If one such unit fails to survive a challenge, e.g. in the form of stress, it can be replaced by many other, almost functionally identical units, thus repairing and maintaining the system. The stability and function of a microbial community also depends on nutritional interactions among community members, such as the cross-feeding of essential small molecules synthesized by a subset of the population (Berlanga et al. 2009; Seth and Taga 2014; Knelman and Nemergut 2014).

Metabolic connectivity as a driver of symbiont cooperation: Microbial mats

Oxygenic photosynthesis on Earth profoundly affected both geochemical and biological evolution. Much of this new productivity probably occurred in microbial mats, which, as discussed above, incorporate a broad range of photosynthetic and aerobic/anaerobic microorganisms in extremely close physical proximity. The potential contribution of these systems to global biogeochemical change would have depended on the nature of the interactions among the resident microorganisms. Linking the biogeochemical processes observed in natural microbial communities with their associated metabolic pathways and assigning these pathways to specific groups remains a daunting task.

In microbial mats light is the primary energy source that drives carbon fixation during daylight hours. Photosynthate is accumulated, often as glycogen, through both oxygenic and anoxygenic photosynthesis. This organic matter serves as substrates for the growth of a broad array of microorganisms. Bacterial production of low-molecular-weight nitrogen and sulfur compounds provides substrates for the energy and electron flow of anaerobic ecosystems. It thus forms the basis of a subset of the microbial interactions that take place within the mat, such as those between phototrophic green sulfur bacteria and chemolithotrophic, sulfur-reducing bacteria, in which sulfur

compounds are exchanged between the partners, and syntrophic associations between fermentative bacteria and methanogenic archaea or sulfate-reducing bacteria (Guerrero et al. 2002; Sieber et al. 2012) (Fig. 4).

>>> **Approx. here Fig. 4** >>>>

In the dark, cyanobacteria degrade their carbon reserves under anoxic conditions by fermentation, resulting in the production of low-molecular-weight organic acids and hydrogen (Hoffmann et al. 2015). Those organic acids are further oxidized by methanogenic bacteria and by sulfate-reducing bacteria, often syntrophically with other microorganisms. In microbial mats, sulfate-reducing bacteria outcompete methanogens because of the high concentration of sulfate in the seawater.

Mats are dominated by Proteobacteria, especially those related to sulfur cycling. For instance such as purple sulfur bacteria belonging to the Alpha clade comprising the purple non-sulfur bacteria (e.g., *Rhodobacterales*), the Gamma clade (e.g., *Chromatiales* and the sulfate-reducing bacteria belonging to the Delta clade (e.g., *Desulfobacterales* and *Desulfovibrionales*). Bacteroidetes are mainly heterotrophic organisms that are active in multiple layers in the mat, where they are important for carbon cycling. In marine ecosystems, members of this phylum decompose high-molecular-mass dissolved organic matter (Harris et al. 2013). Chloroflexi, Chlorobi, Acidobacteria, and Actinobacteria are minor phyla in hypersaline mats, but they contribute significantly to carbon and sulfur cycling (Bolhuis et al. 2014). The presence in microbial mats of very diverse and stable populations of spirochetes suggests their involvement in a well-integrated metabolic symbiosis (i.e., permanent physiological cooperation) with other specialized populations in the mats, where they maintain a coordinated functional and stable community (Berlanga et al. 2008). The main compounds produced by spirochetes are acetate, H_2, and CO_2, all of which are normally consumed by sulfate-reducing bacteria and by methanogens, two groups highly represented in microbial mats. The metabolic capabilities of spirochetes, such as nitrogen fixation, went unrecognized for many years (Lilburn et al. 2001; Desai and Brune 2012) but it is now clear that these species supply carbon sources and electron donors to other members of the mat community and thus form a dynamic population essential for the continued functioning of this ecosystem. They are a ubiquitous component of the oxic-anoxic gradient of microbial mats, where they compete effectively with other heterotrophic organisms for soluble sugars (Berlanga et al. 2008).

Although molecular hydrogen is not abundant in the biosphere, the metabolic diversity of microbial mats is reflected in a diverse potential for H_2 metabolism (Lee et al. 2014). Cyanobacterial H_2 production plays an important role in fueling anaerobic processes in the mats, such as sulfate reduction and anoxygenic photosynthesis. Hydrogen is consumed by anaerobic mineralization processes, including as an electron donor in sulfate reduction and in methanogenesis. Anoxygenic photosynthetic bacteria can also utilize hydrogen as an electron donor (Nielsen et al. 2015). The sulfate-reducing bacteria in cyanobacterial mats are predominantly hydrogenotrophs. The night-time fermentation of stored light energy can explain the close association between filamentous Chloroflexi and of the sulfate-reducing bacteria with cyanobacterial filaments (Lee et al. 2014). The close links between cyanobacteria and Chloroflexi observed in other microbial mat environments suggest that this association is a general characteristic of mats and that it plays an important role in anoxic carbon cycling (Burow et al. 2012).

The ubiquity of syntrophic metabolism in many anoxic environments (e.g., microbial mats and the intestinal tract) emphasizes that metabolic cooperation among microbial species is often the rule rather than the exception and that the consortium is the catalytic unit of anaerobic metabolism.

Coda

All living beings on Earth depend on prokaryotes, which fuel a complex web of interconnected metabolic pathways. Prokaryotes are present in all places where life is possible and they are able to thrive in a wide variety of environmental conditions, ranging from ideal (from the "macroorganism" point of view) to extreme (unthinkable for inhabitance by more evolved or recent forms). Without knowledge of microorganisms, our understanding of biology would be very limited: We would not know that life is possible under conditions of extreme temperature, salinity, or pH; photosynthesis would be understood as aerobic and oxygenic (when, actually, it developed in prokaryotes as anaerobic and anoxygenic), and the longest-living beings (such as redwoods) would not be older than a 1,000 years old (whereas the spores of *Bacillus* are "as old as the hills").

As Louis Pasteur wrote (Guerrero and Berlanga 2009): "I have taken my drop of water from the immensity of creation, and I have taken it full of the elements

appropriate to the development of inferior beings. And I wait, I watch, I question it, begging it to recommence for me the beautiful spectacle of the first creation. But it is dumb, dumb since these experiments were begun several years ago; it is dumb because I have kept it from the only thing man cannot produce, from the germs which float in the air, from Life, for Life is a germ and a germ is Life."

Conflict of interest The authors declare that they have no conflict of interest.

References

Ackermann, M. (2015). A functional perspective on phenotypic heterogeneity in microorganisms. *Nature Review of Microbiology*, doi: 10.1038/nrmicro3491.

Atlas, R. M., & Bartha, R. (1998). *Microbial ecology. Fundamentals and applications* (4th ed). Benjamin Cummings, Menlo Park, CA.

Berlanga, M., Aas, J. A., Paster, B. J., Boumenna, T., Dewhirst, F. E., & Guerrero, R. (2008). Phylogenetic diversity and temporal variation in the *Spirochaeta* populations from two Mediterranean microbial mats. *International Microbiology*, 11, 267–274.

Berlanga, M., Paster, B. J., & Guerrero, R. (2009). The taxophysiological paradox: changes in the intestinal microbiota of the xylophagous cockroach *Cryptocercus punctulatus* depending on the physiological state of the host. *International Microbiology*, 12, 227–236.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel F., et al. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, 407, 623–626.

Bolhuis, H., Cretoiu, M. S., & Stal, L. J. (2014). Molecular ecology of microbial mats. *FEMS Microbiology Ecology*, doi: 10.1111/1574-6941.12408.

Brune, A., Frenzel, P., & Cypionka, H. (2000). Life at the oxic-anoxic interface: microbial activities and adaptations. *FEMS Microbiology Reviews*, 24, 691–710.

Burow, L. C., Woebken, D., Marshall, I. P. G., Lindquist, E. A., Bebout, B. M., Prufert-Bebout, L., Hoehler, T. M., Tringe, S. G., Pett-Ridge, J., Weber, P. W., Spormann, A. M., & Singer, S. W. (2012). Anoxic carbon flux in photosynthetic microbial mats as revealed by metatranscriptomics. ISME Journal, 7, 817–829.

Carreira, C., Piel, T., Staal, M., Stuut, J-B. W., Middelboe, M., & Brussaard, C. P. D. (2015). Microscale spatial distributions of microbes and viruses in intertidal photosyntheic Microbial mats. *Springer plus*, doi: 10.1186/s40064-015-0977-8.

Desai, M. D., & Brune, A. (2012). *Bacteroidales* ectosymbionts of gut flagellates shape the nitrogen-fixing community in dry-wood termites. *ISME Journal*, 6, 1302–1313.

Dobell, C. (1958). *Anton van Leeuwenhoek and his little animals.* Rusell & Rusell, New York.

Downs, D. M. (2006). Understanding microbial metabolism. *Annual Review Microbiology*, 60, 533–359.

Falkowski, P. G., Fenchel, Y., & Delong, E. F. (2008). The microbial engines that drive Earth's biogeochemical cycles. *Science*, 320, 1034–1039.

Feazel, L. M., Spear, J.R., Berger, A. B., Harris, J.K., Frank, D. N., Ley, R. E., & Pace, N. R. (2008). Eucaryotic diversity in a hypersaline microbial mat. *Applied Environmental Microbiology*, 74, 329–332.

Fuerst, J. A. (2005). Intracellular compartmentation in *Planctomycetes*. *Annual Review Microbiology*, 59, 299–328.

Fuerst, J. A., Sagulenko, E. (2011). Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. Nature Review Microbiology, doi: 10.1038/nrmicro2578.

Guerrero, R. (1998). Crucial crises in biology: life in the deep biosphere. *International Microbiology*, 1, 285–294.

Guerrero, R., Piqueras, M., & Berlanga, M. (2002). Microbial mats and the search for minimal ecosystems. *International Microbiology*, 5, 177–188.

Guerrero, R., & Berlanga, M. (2006). Life's unity and flexibility: the ecological link. *International Microbiology*, 9, 225–235.

Guerrero, R., & Berlanga, M. (2007). The hidden side of the prokaryotic cell: rediscovering the microbial world. *International Microbiology*, 10:157–168.

Guerrero, R., & Berlanga, M. (2009). The evolution of microbial life: paradigm changes in microbiology. *Contributions to Science*, 5, 53–60.

Guerrero, R., & Berlanga, M. (2013). An integrative ecogenetic study of minimal ecosystems: the microbial mats of Ebro Delta and the Camargue (Western Mediterranean). *Contributions to Science*, 9, 117–139.

Harris, J. K., Caporaso, J. G., Walker J. J.,Spear, J. R., Gold, N. J., Robertson, C. E., Hugenholtz, P., Goodrich, J., McDonald, D., Knights, D., Marshall, P., Tufo, H., Knight, R., & Pace, N. R. (2013). Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. *The ISME Journal*, 7, 50–60.

Knelman, J. E., & Nemergut, D. R. (2014). Changes in community assembly may shift the relationship between biodiversity and ecosystem function. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2014.00424.

Kolter, R. (2010). Biofilms in lab and nature: a molecular geneticist's voyage to microbial ecology. *International Microbiology*, 13, 1–7.

Harris, J. K., Caporaso, J. G., Walker, J. J., Spear, J.R., Gold, N. J., Robertson, C.E., Hugenholtz, P., Goodrich, J., McDonald, D., Knights, D., Marshall, P., Tufo, H., Knight, R., & Pace, N. R. (2013). Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. *The ISME Journal*, 7, 50–60.

Hoffmann, D., Maldonado, J., Wojciechowski, M. F., & Garcia-Pichel, F. (2015). Hydrogen export from intertidal cyanobacterial mats: sources, fluxes and the influence of community composition. *Environmental Microbiology*, doi: doi:10.1111/1462-2920.12769.

Lee, J. Z., Burow, L. C., Woebken, D., Everroad, R. C., Kubo, M. D., Spormann, A. M., Weber, P. K., Pett-Ridge, J., Bebout, B. M., & Hoehler, T. M. (2015). Fermentation couples Chloroflexi and sulfate-reducing bacteria to Cyanobacteria in hypersaline microbial mats (2014) *Frontiers in Microbiology*, doi: 10.3389/fmicb.2014.00061.

Ley, R. E., Harris, J.K., Wilcox, J., Spear, J. R., Miller, S. R., Bebout, B. M., Maresca, J. A., Bryant, D. A., Sogin, M. L., & Pace, N. R. (2006). Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. *Applied Environmental Microbiology*, 72, 3685–3695.

Lilburn, T. G., Kim, K. S., Ostrom, N. E., Byzek, K. R., Leadbetter, J.R., & Breznak, J. A. (2001). Nitrogen fixation by symbiotic and free-living spirochetes. *Science*, 292, 2495–2498.

Lowe, D. R. (1980). Stromatolites 3,400-Myr old from the Archean of Western Australia. *Nature*, 284, 441–443.

Madsen, E. L. (2005). Identifying microorganisms responsible for ecologically significant biogeochemical processes. *Nature Review Microbiology*, 3, 439–446.

Martínez-Cano, D. J., Reyes-Prieto, M., Martínez-Romero, E., Partida-Martínez, L. P., Latorre, A., Moya, A., & Delaye, L. (2015). Evolution of small prokaryotes genomes. *Frontiers in Microbiology*, doi:10.3389/fmicb.2014.00742.

Maloy, S., & Schaechter, M. (2006). The era of microbiology: a Golden Phoenix. *International Microbiology*, 9, 1–8.

Marlow, J. L., Steele, J. A., Ziebis, W., Thurber, A.R., Levin, L.A., & Orphan, V. J. (2014). Carbonate-hosted methanotrophy represents an unrecognized methane sink in the deep sea. *Nature Communications*, doi:10.1038/ncomms6094.

Nielsen, M., Revsbech, N.P., & Kühl, M. (2015). Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2015.00726.

Pedrós-Alió, C., & Guerrero, R. (1994). Prokaryotology for the limnologist. In Margalef R (ed) Limnology now. A paradigm of planetary problems. Elsevier, Amsterdam, pp 37–57.

Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green, J.L., Green, L.E., Killham, K., Lennon, J. L., Osborn, M., Solan, M., van del Gast, C. J. , & Young, J. P. W. (2007). The role of ecological theory in microbial ecology. *Nature Review Microbiology*, 5, 384–392.

Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J. P., Ettwig, K. F., Rijpstra, I. C., Schouten, S., Damsté, J. S. S., Op den Camp, H. J. M., Jetten,M. S. M., & Strous, M. (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, 440, 918–921.

Robertson, C.R., Spear, J. R., Harris, J. K., & Pace, N. R. (2009). Diversity and stratification of Archaea in a hypersaline microbial mat. *Applied Environmental Microbiology*, 75, 1801–1810.

Sachs, J. L., & Hollowed, A. C. (2012). The origins of cooperative bacterial communities. *mBio*, doi:10.1128/mBio.000099-12.

Schaechter, M., Kolter, R., & Buckley, M. (2004). Microbiology in the 21st century: Where are we and where are we going? Report for American Academy for Microbiology, ASM Press, Washington DC

Seth, E. C., & Taga, M. E. (2014). Nutrient cross-feeding in the microbial world. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2014.00350.

Sieber, J.R., McInerney, M.J., &Gunsalus, R.P. (2012). Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. *Annual Review Microbiology*, 66, 429–452.

Stahl, D.A., & Tiedje, J. M. (2002). Microbial ecology and genomics: a crossroads of opportunity. Report American Academy for Microbiology, ASM Press, Washington, DC

Stams, A. J. M., & Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nature Review Microbiology*, doi:10.1038/nrmicro2166.

Tice, M. M., & Lowe, D. R. (2004) Photosynthetic microbial mats in the 3,416-Myr-old ocean. *Nature*, 431, 549–552.

Ufarté, L., Potocki-Veronesse, G., & Laville, É. (2015). Discovery f new protein families and functions: new challenges in functional metagenomics for biotechnologies and microbial ecology. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2015.00563.

Vallino, J. J. (2003). Modeling microbial consortiums as distributes metabolic networks. *Biological Bulletin*, 204, 174–179.

Villanueva, L., Navarrete, A., Urmeneta, J., Geyer, R., Guerrero, R., & White, D. C. (2007). Monitoring diel variations of physiological status and bacterial diversity in an estuarine microbial mat: an integrated biomarker analysis. *Microbial Ecology*, 54, 523–531.

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proceeding of the National Academy of Sciences of the United States of America*, 95, 6578–6583.

Figures

Fig. 1 The Earth in the early Archaean Eon (~3,500 Ma ago). Microbial mats were probably the earliest ecosystems on Earth. The Moon was much closer to the Earth than today. The proximity of the Moon determined high tides, which were crucial to the survival of many organisms and thus to the maintenance of life. (Drawing by C. Puche, with permission of ©*Metode*).

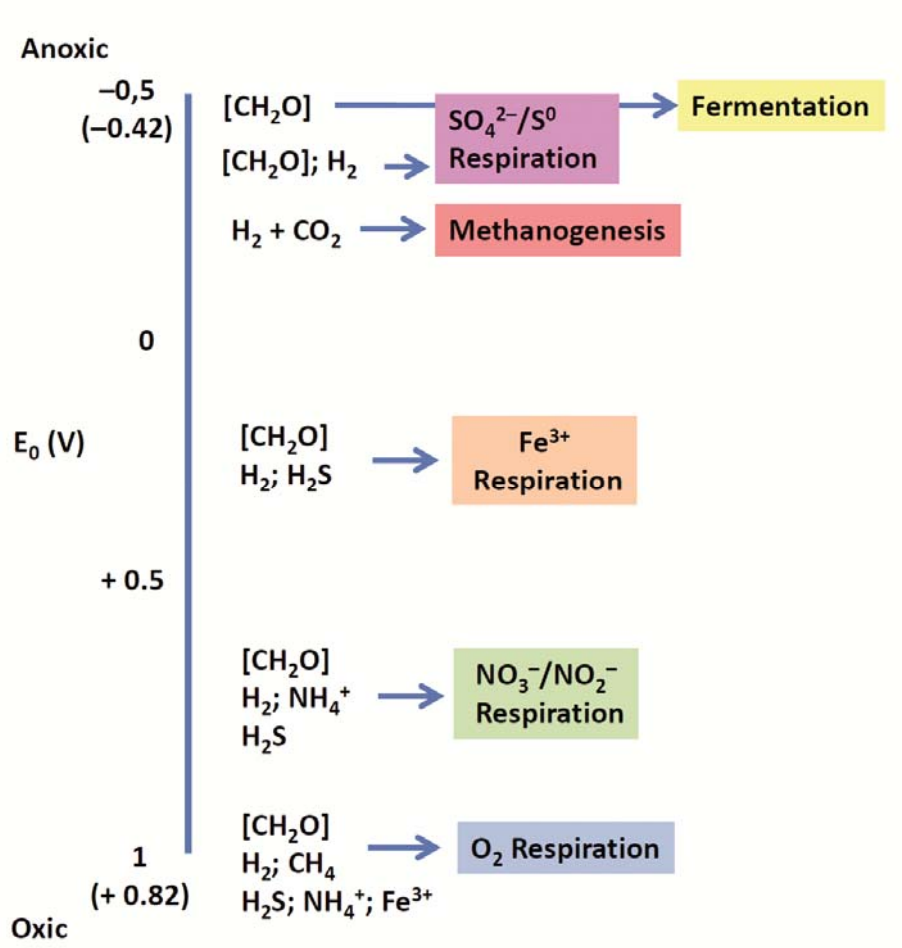

Fig. 2 Microbial metabolism is driven by biochemical processes. The redox reactions are arranged in order from the most electronegative E_0' (top) to the most electropositive E_0' (bottom) (at pH 7).

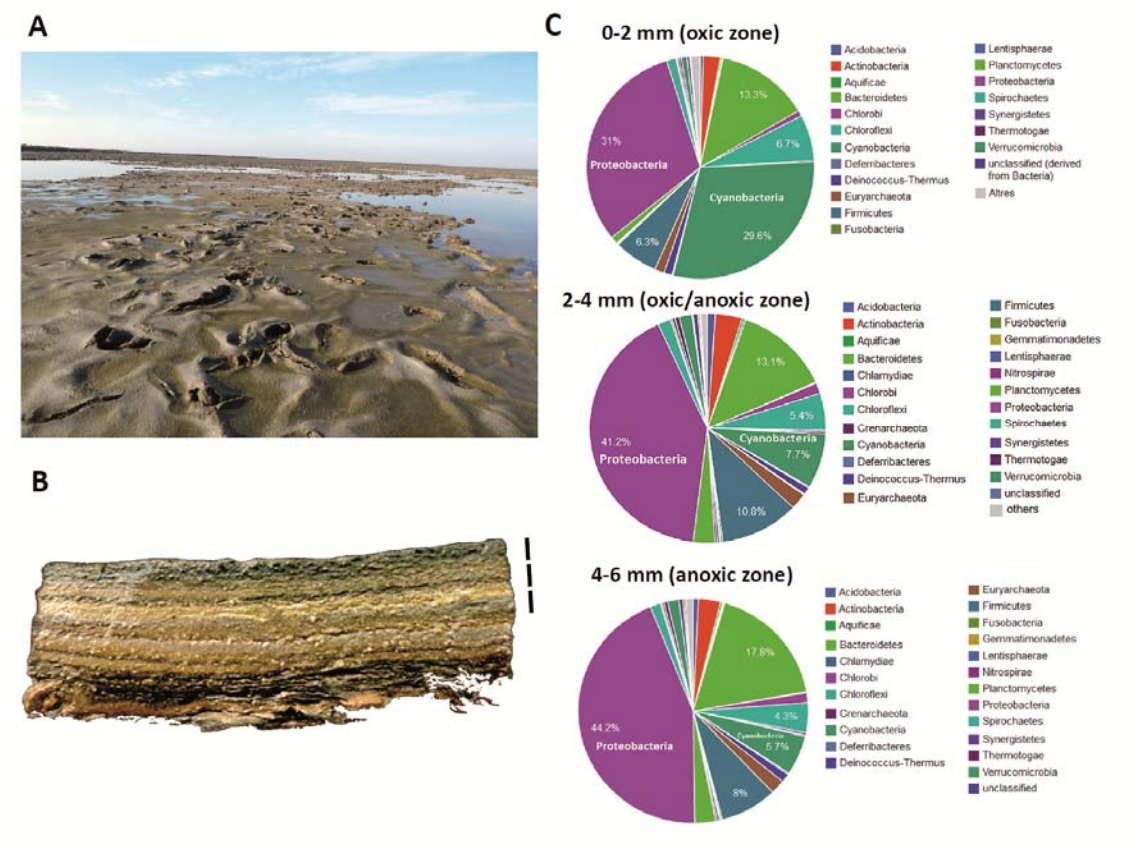

Fig. 3 a Landscape of the Camargue (France), showing extended microbial mats. **b** A cross-section of these microbial mats. **c** Bacterial composition at the phylum level as determined at three depths in a microbial mat: 0–2 mm, 2–4 mm, and 4–6 mm.

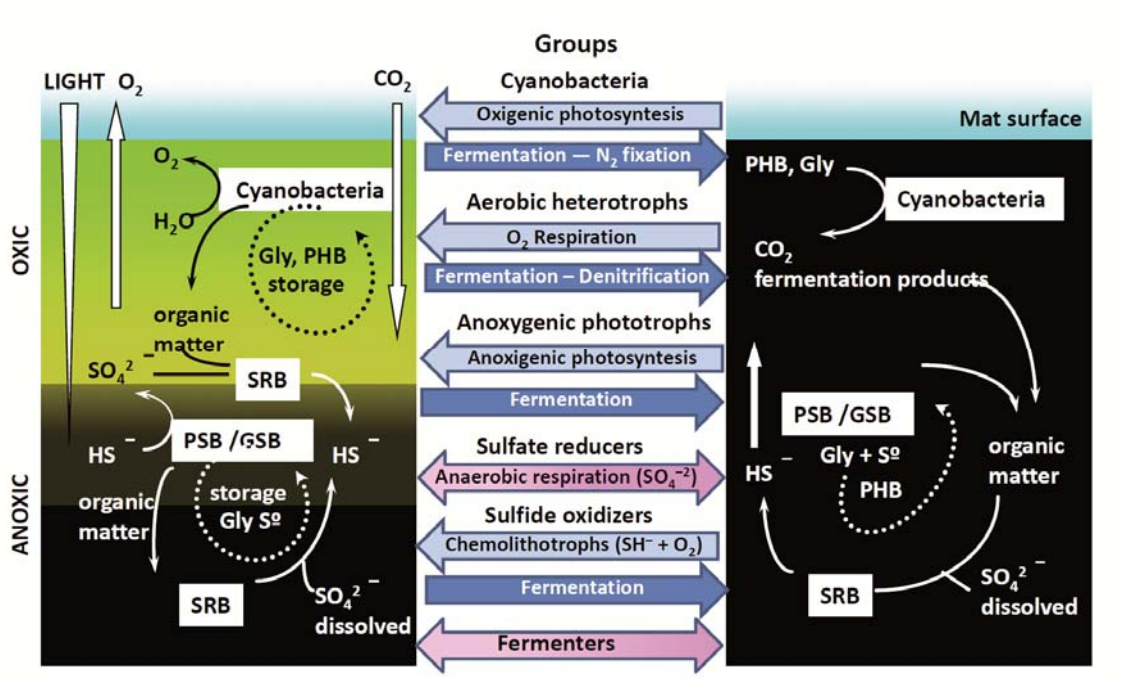

Fig. 4 Ecophysiology of the day–night carbon and sulfide cycles carried out by different populations of a typical marine microbial mat community (Ebro Delta). PHB: poly-β-hydroxybutyrate, Gly: glycogen, SRB: sulfate-reducing bacteria, PSB/GSB, purple/green sulfur bacteria (anoxygenic phototrophs). (Adapted from Guerrero et al. 2002).