

Using Margalef's vision to understand the current aquatic microbial ecology

Ana Rocio Borrero-Santiago¹, Walter Dellisanti^{2,3}, Andrés Sánchez-Quinto⁴,
Javier Moreno-Andrés⁵, Philip Nemoy⁶, Kumari Richa⁷, Patricia Margarita Valdespino-Castillo⁸,
Daniel Diaz-de-Quijano⁹, Vicente J. Ontiveros¹⁰, Simone Fontana^{11,12},
Caterina R. Giner¹³, Isabel Sanz-Sáez¹⁴, Mireia Mestre^{15,16}

¹ Seaweeds Solutions AS, Bynesveien 50 C, 7018 Trondheim, Norway.

(ARB-S) (Corresponding author) E-mail: borrero@seaweedsolutions.com. ORCID iD: <http://orcid.org/0000-0002-8976-4634>

² State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong S.A.R., China.

³ Present address: Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong S. A. R., China.

(WD) (Corresponding author) E-mail: walter.dellisanti@gmail.com. ORCID iD: <https://orcid.org/0000-0003-1336-4578>

⁴ Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México.

(AS-Q) (Corresponding author) E-mail: guitox@msn.com. ORCID iD: <https://orcid.org/0000-0002-9896-1015>

⁵ Department of Environmental Technologies, Faculty of Marine and Environmental Sciences, INMAR—Marine Research Institute, CEIMAR—International Campus of Excellence of the Sea, University of Cádiz, Campus Universitario de Puerto Real, 11510 Cádiz, Spain.

(JM-A) (Corresponding author) E-mail: javier.moreno@uca.es. ORCID iD: <https://orcid.org/0000-0001-9139-4372>

⁶ Department of Maritime Civilizations, Leon Charney School of Marine Sciences, The Recanati Institute of Maritime Studies University of Haifa, Israel.

(PN) E-mail: philip.nemoy@gmail.com. ORCID iD: <https://orcid.org/0000-0003-4599-497X>

⁷ Division of Biological Sciences, School of Science, Technology, Engineering and Mathematics, University of Washington, Bothell WA 98011

(KR) E-mail: kumariricha.szn@gmail.com. ORCID iD: <https://orcid.org/0000-0002-6300-8451>

⁸ Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, USA.

(PMV-C) E-mail: pmvaldespino@lbl.gov. ORCID iD: <https://orcid.org/0000-0002-2998-4627>

⁹ Siberian Federal University, Department of Aquatic and Terrestrial Ecosystems, Krasnoyarsk, Russian Federation.

(DD-d-Q) E-mail: daniquijano@gmail.com. ORCID iD: <https://orcid.org/0000-0002-2231-5957>

¹⁰ Theoretical and Computational Ecology, Centre for Advanced Studies, Blanes-Spain. Integrative Freshwater Ecology Group, Centre for Advanced Studies, Blanes-Spain.

(VLJ-O) E-mail: vicente.jimenez.ontiveros@gmail.com. ORCID iD: <https://orcid.org/0000-0001-8477-2574>

¹¹ Department of Aquatic Ecology, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland.

¹² Present address: Nature Conservation and Landscape Ecology, University of Freiburg, Freiburg, Germany

(SF) E-mail: simonefontana.j@gmail.com. ORCID iD: <https://orcid.org/0000-0003-1194-1909>

¹³ Institute for the Oceans and Fisheries, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. Hakai Institute, P.O. Box 309, Heriot Bay, BC, V0P 1H0, Canada.

(CRG) E-mail: kate.giner@gmail.com. ORCID iD: <https://orcid.org/0000-0002-7267-0260>

¹⁴ Institut de Ciències del Mar, ICM-CSIC, Barcelona, Catalunya, Spain.

(IS-S) E-mail: isanz@icm.csic.es. ORCID iD: <https://orcid.org/0000-0003-0233-7224>

¹⁵ Centro de Investigación Oceanográfica COPAS Sur-Austral, Departamento de Oceanografía, Universidad de Concepción, Concepción, Chile.

¹⁶ FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Universidad Austral de Chile, Valdivia, Chile.

(MM) E-mail: mireiamalte@gmail.com. ORCID iD: <https://orcid.org/0000-0003-0986-633X>

Summary: Ramon Margalef was a pioneering scientist who introduced an interdisciplinary approach to ecological studies. His studies were among the first to incorporate various concepts in the literature of aquatic ecology, covering topics such as organisms, ecosystem interactions and evolution. To bring Margalef's approach into current scientific studies, in this review we explore his vision of aquatic ecology within four interrelated fields of study: ecological theory, microbial diversity, biogeochemical cycles and global environmental changes. Taking inspiration from his studies, we analyse current scientific challenges and propose an integrated approach, considering the unifying concept of Margalef's Mandala with the aim of improving future studies on aquatic microbial ecology.

Keywords: aquatic ecology; integrated approach; succession; biogeochemical cycles; global change.

Usando la visión de Margalef para entender la ecología microbiana actual

Resumen: Ramon Margalef fue un científico pionero que introdujo un enfoque interdisciplinario a los estudios ecológicos. Sus estudios fueron de los primeros en incorporar diferentes conceptos en la literatura de la ecología acuática, desde los organismos y las interacciones de los ecosistemas hasta la evolución. Para llevar el enfoque de Margalef a los estudios científicos actuales, en este artículo exploramos su visión de la ecología acuática dentro de cuatro campos de estudio interrelacionados: teoría ecológica, diversidad microbiana, ciclos biogeoquímicos y cambios ambientales globales. Inspirándonos en sus estudios pasados, analizamos en este texto los desafíos científicos actuales y proponemos un enfoque integrado considerando el concepto unificador del Mandala de Margalef con el objetivo de mejorar los estudios futuros sobre la ecología microbiana acuática.

Palabras clave: ecología acuática; enfoque integrado; sucesión; ciclos biogeoquímicos; cambio global.

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INTRODUCTION

Ramon Margalef (1919-2004) was a pioneering scientist who introduced new concepts of ecology and evolution. For example, the Margalef Mandala (Margalef 1978) is a conceptual scheme that explains how variations in external energy can regulate the organismal succession within aquatic ecosystems (Terradas 2015). Similar in appearance to the spiritual symbol, the Margalef Mandala is a model of natural equilibrium describing the influences of physical (turbulence) and chemical (nutrients) forces on the development of ecological succession in aquatic ecosystems (Margalef 1978, Margalef et al. 1979). This idea laid the foundation for the trait-based approach in ecology and was recently corroborated with field data (Villamaña et al. 2019).

Margalef spent the early years of his career meticulously cataloguing plankton in streams and lakes around Spain. Later, he adopted methods from information theory to organize and understand these immense datasets (Margalef 1958, 1968). He used Shannon entropy (Shannon 1949) to express the information contained in the structure of a community (Margalef 1957, 1958). Consequently, he used information theory to relate the basic descriptors such as species abundance, distribution and diversity to energy and information flows in order to define population-in-environment as an irreducible unit of ecological study (Margalef 1968).

Margalef realized that ecology should be studied by considering all variables and forces that are acting on organisms to gain a comprehensive knowledge of the ecosystem. This holistic approach initially contradicted those who focused on one specific parameter to understand ecological, physiological and evolutionary responses of organisms, an approach that was “easily reproducible and faster” in terms of data collection and scientific discussion than Margalef’s approach (Terradas 2015). However, it is now clear that all factors affecting

the planet are acting together, and global processes must be considered as multi-stressor scenarios (Boyd et al. 2018). Margalef’s vision of humanity as an integral part of ecosystems should be considered in future models and theories to promote responsibility for the changes that are increasingly inflicted on nature. He integrated a multitude of concepts from other disciplines to explain ecology. He was a truly multidisciplinary scientist, borrowing from fields such as evolution, information theory and chemistry. In his last works, Margalef applied his ecological thinking to socio-ecological questions (Margalef 1997, 2000a). For example, the inequalities in the availability and consumption of resources, the increase in energy consumption without a parallel increase in efficiency, and the accumulation of CO₂ in the oceans. His ecological thinking led him to study cultural evolution, social change and financial powers (Margalef 2000b).

The present review resulted from the discussions held during the workshop “2016 Ramon Margalef Summer Colloquia” under the title “Microbes in a changing world: diversity and biogeochemistry”. Inspired by Margalef’s holistic approach, we revisited four interrelated fields of aquatic microbial ecology: ecological theory, microbial diversity, biogeochemical cycles and global environmental changes (Fig. 1). We analysed and showed how several of Margalef’s ideas contributed to all of them and proposed an integrated approach for future research in aquatic microbial ecology. The present article is a subjective review that reflects the bias and perspectives of the authors.

ECOLOGICAL THEORY

It has been argued that truly general laws and rules, i.e. ones that are not contingent on organisms or environments, are lacking in ecology (Lawton 1999). Among the few exceptions are the species-area relationship as the positive power-law relationship between species richness and ecosystem area (Rosenzweig

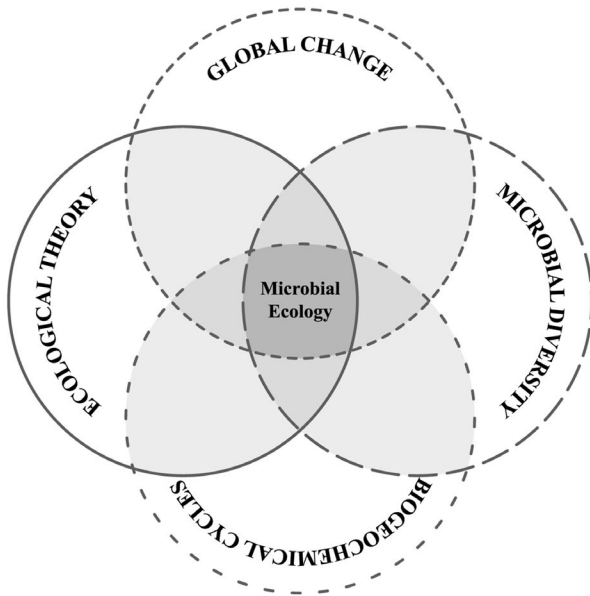


Fig. 1. – The integrated approach to aquatic microbial ecology inspired by the Margalef Mandala.

and Parry 1994) and the metabolic theory of ecology (Brown et al. 2004) as the relationship between metabolic rate, body size and temperature. Margalef always tried to frame his work in a broad context, such as the laws of thermodynamics (Margalef 1975). His work not only included papers distinctly devoted to the microbial world (Margalef 1978), but he also crossed borders and refused to focus on theories that only apply to model systems (Margalef 1963). Therefore, one of his main contributions to ecological theory in microbial ecology was his constant attempt to unify ecological understanding, which he considered a prerequisite for determining speculative explanations of observed patterns in nature. Although Margalef's contributions to ecological theory went far beyond microbial ecology, some are especially important for the study of microorganisms.

Margalef adopted methods from information theory to organize and understand the immense datasets from his studies (Margalef 1958) and extracted insights applicable to many pressing themes in ecology, as was extensively reviewed recently (Sherwin and Prat 2019). Margalef used Shannon entropy (Shannon 1949) to describe the uncertainty that a randomly sampled individual belongs to a previously observed species, and thus the diversity within a community (Margalef 1957). He used information theory to create quantitative descriptors and derive expectations for the species abundance distributions (Margalef 1978). After Margalef's introduction, the use of information theory in ecology became more common (Pielou 1966). It provided the quantitative framework for modern biodiversity studies (Tuomisto 2010) and has been crucial for the development of microbial ecology as we understand it today. Indices based on information theory are essential to analyse the vast amount of sequence datasets in microbial ecology. However, in general, diversity indices should

be used to test hypotheses and make predictions on the drivers of diversity rather than as results in themselves (Shade 2017), as Margalef already realized (Margalef 1991). Just as Margalef moved from using information theory to describe communities to using it to provide expectations, the field of microbial ecology seems to be moving from an observational to a predictive discipline. Conjointly, the observed patterns provide expectations and general relationships that may apply to all organisms (Locey and Lennon 2016).

Another of Margalef's contributions was the focus on species traits as important integrative variables for advancing ecological theory. Traits represent a "common value" for studying the responses of disparate organisms to environmental gradients and ecosystem processes. Margalef's Mandala represents an ecosystem as a bidimensional space described by two major factors affecting phytoplankton growth dynamics: nutrients and turbulence (Margalef 1978, Margalef et al. 1979). These external factors describe the preferred environmental conditions for functional groups of phytoplankton and how the pulses of energy can determine a new start of species succession. Although the Mandala was proposed in the context of phytoplankton succession and functional morphology, the rationale behind it involved a greater concept because both nutrients and turbulence are related to external energy supply (Margalef 1978). Later developments of this framework helped unify the four basic concepts of community ecology that bridge ecological and evolutionary scales in microbial communities: mutation, selection, dispersal and drift (Vellend 2016). The modern interpretations and extensions of the Margalef Mandala prove that his approach to ecological theory and community assembly processes remains valid (Wyatt 2014). Moreover, following the classical Margalef Mandala (based on phytoplankton succession), the prokaryote and protist successions could also be drawn using similar schemes with slight variations in the axes, moving from inorganic nutrients to organic matter (Pinhassi and Hagström 2000).

Succession, as ecological maturity, was a life-long concern for Margalef. He defined it as "the transformation of an excess of available energy into a future increase in biomass" (Margalef 1963). More mature ecosystems are the most stable ones, allowing an increase in information via structures or behaviour and a decrease in the energy needed to maintain them. The definition of succession based on thermodynamics (less mature systems are characterized by a higher ratio of primary production to biomass) also generates solid expectations on directional flows of energy and information across systems of unequal maturity: a net transfer of energy in the form of biomass occurs from the less mature to the more mature systems. Furthermore, based on these general principles, Margalef predicted the consequences of human exploitation and rising temperatures on ecosystems in terms of reduced diversity and stability (Margalef 1963), which is of a shocking topicality. These insights can be applied to microbial ecology and coincide with current studies of succession in microbial communities (Ortiz-Álvarez et al. 2018).

MICROBIAL DIVERSITY

Microbial communities are critical components of important global processes. Species composition is important, among other things, for the stability and proper functioning of ecosystems and their services (Goswami et al. 2017), and one of the main challenges in microbial ecology has been the estimation of species diversity (Vitorino and Bessa 2018). Today, we strive to understand the patterns of community structure and its dynamics in terms of ecosystem health and function (Shade 2017). Microbial ecologists have worked persistently to increase the sampling effort, making advancements in molecular techniques and developing mathematical measurements of diversity known as “diversity indices” (Fuhrman and Hagström 2008). Diversity indices use either incidence (presence/absence) or relative abundance of species when comparing diversity in two or more communities (Schroeder and Jenkins 2018). Quantitative approaches to functional and taxonomic diversity such as the Rao coefficient and the Simpson index have been applied (Botta-Dukát 2005). Other strategies measure functional diversity by functional richness and evenness (Goswami et al. 2017). Margalef actively contributed to the development of novel diversity indices: the Margalef index (d) includes abundance in the determination of diversity (Margalef 1951), while the Shannon index (H') accounts for evenness plus richness (Margalef 1957, Moseman et al. 2009; Table 1).

Each method that analyses diversity has limitations and biases. Several analysis pipelines are available for a set of high throughput sequencing data (used to easily calculate diversity; Pylro et al. 2014). However, the limitations and biases start from the very beginning, including sampling (agreement on sampling protocols between laboratories must be reached; Prosser 2010) DNA/RNA extraction, PCR/primer bias, sequencing

artefacts of cutoff for defining operational taxonomic units, development of consistent reference databases, and choice of including or excluding singletons/doubletons (operational taxonomic units that occur only once or twice, respectively, in each dataset). (Goodrich et al. 2014). Furthermore, many studies have focused on a single sampling point, leaving out spatio-temporal variability. Transects, temporal series and diversity patterns at different scales should become the norm (Jones et al. 2012). As the technique applied to analyse samples depends strongly on the question posed by the microbial ecologist, there is a need for good working protocols regarding the wide ranges of techniques to determine microbial diversity (PCR-based 16S/8S studies, metagenomics, etc.), accompanied by data analysis and publication of the raw data. A common bioinformatics pipeline is also needed to compare results from multiple studies because pipelines have an inherent bias, and using distinct pipelines delivers different results (Logares et al. 2012). As computers provide a completely controlled environment, implementing a common pipeline with low biases should be easier than implementing a universal molecular laboratory protocol. Although biases and limitations cannot be eliminated, a strong effort to take them into account and thus analyse datasets carefully is essential. The correct choice of diversity index is an important issue. Many indices have been proposed to estimate biodiversity (Goswami et al. 2017), but there is no consensus on which is most appropriate and informative in each context (Morris et al. 2014). Margalef’s attitude and legacy can help solve this conundrum: by constantly investigating links between different scientific disciplines, he suggested that only a careful mathematical interpretation of a given formula assures a correct ecological interpretation. From this perspective, an index should be chosen based on what it truly measures (e.g. for testing specific hypotheses), acknowledging the dif-

Table 1. – List of some quantitative indices to measure functional and taxonomic diversity.

Index	Symbol	Description	Formula	Reference
Rao coefficient	$H_D(p)$	Quadratic entropy	$H_D(\mathbf{p}) = \sum_{i=1}^n \sum_{j=1}^n p_i p_j d_{ij}$	Rao (1982)
Simpson index	D	Species diversity	$1 - \sum_{i=1}^k \frac{n_i(n_i - 1)}{n(n - 1)}$	Simpson (1949)
Evenness index	J'	Species evenness	$J' = \frac{H'}{H'_{\max}}$	Pielou (1966)
Shannon index	H'	Species entropy	$H'_{\max} = - \sum_{i=1}^S \frac{1}{S} \ln \frac{1}{S} = \ln S$	Shannon and Weaver (1949)
Margalef index	d	Species richness	$D_{Mg} = \frac{S-1}{\ln N}$	Margalef (1951)

ferent facets of biodiversity (and that most indices are not simply interchangeable).

The microbial community composition plays a crucial role in the health (functionality) of an ecosystem (Astudillo-García et al. 2019). A decrease in species richness (the number of species inhabiting a particular niche) and evenness (how evenly the species are distributed) might have a negative impact on the ecosystem's productivity and stability, resulting in overall decreased functionality (Goswami et al. 2017). Therefore, assessing the phylogenetic and functional diversity of microbial communities allows a more precise and efficient way of describing an ecosystem. The recognition of functional groups of species helps to understand functional diversity. Also, community diversity can be explained hierarchically, by describing the diversity of functional groups using methods such as cluster analysis for functional traits (Lepš 2005). The significant effects of functional diversity on the productivity and functioning of an ecosystem can be quantitatively explained by the sampling effect model and the niche differentiation model (Goswami et al. 2017). A sampling effect suggests that the increase in diversity of an ecosystem increases the probability that it harbours competitive species, thus making it more productive "by change". By contrast, the niche differentiation model assumes that habitats are spatiotemporally heterogeneous and occupied by species with different traits that allow them to compete for resources, be productive and thrive in the prevailing conditions. Likewise, niche complementarity and species redundancy can be explained using functional traits, which underlie the mechanisms by which diversity affects ecosystem functioning (Goswami et al. 2017). The rivets and idiosyncratic models relate functional diversity and species richness to ecosystem functioning. The rivet model suggests that the ecological function of different species sharing the same niche space overlaps (Ehrlich and Ehrlich, 1981), while the idiosyncratic model suggests that the ecological function of a particular niche varies proportionately with an increase in species richness (Lawton 1994).

Margalef suggested that microbial diversity is expansive and dynamic. He proposed that "the real structure of an ecosystem is a property that remains out of reach." (Margalef 1963). In other words, diversity is not a question that can be addressed by a single observation. According to Margalef, continuous monitoring is needed to understand the diversity of dynamic and complex microbial ecosystems, which is indeed the way microbial ecologists are operating nowadays (Mestre et al. 2020). For example, remote sensing has been proposed as a promising technology for the continuous and high-frequency monitoring of biodiversity and ecosystem functions at large spatial scales (Pettorelli et al. 2018). Also, automated and continuous monitoring of phytoplankton communities through in situ scanning-flow cytometry increases our ability to predict future changes in biodiversity and the functions it sustains (Thomas et al. 2018).

Until the last decade, methodological constraints such as the inadequacies of conventional microscopy

and cultivation-dependent techniques limited progress in the field (Jing et al. 2013). However, the advent of molecular techniques, particularly 'next-generation sequencing', has increased our understanding of microbial communities (Heidelberg et al. 2010; Sánchez-Quinto and Falcon 2019), with current estimates of microbial diversity being at least 100 times greater than previously thought (Vitorino and Bessa 2018). Nevertheless, in line with Margalef's intuition, microbial ecologists report that, even with modern tools and techniques, it is not easy to identify true microbial diversity and mapping of variations in community structure in space and time in the complex and highly dynamic marine environment (Biller et al. 2018). Some investigations indicate that the extent—and therefore the functional potential—of marine microbial biodiversity are limitless and seem to grow greater as new techniques emerge to measure them (Heidelberg et al. 2010).

BIOGEOCHEMICAL CYCLES

Margalef's efforts to integrate micro- and macroscopic visions of ecosystems inspire how biogeochemistry should be studied in the era of discoveries in the microbial world. Margalef strived to integrate different fields of ecology (matter–energy–biodiversity) with a common quantitative currency, which is why he applied information theory and thermodynamics to ecology (Margalef 1957, 2000a). Although he focused on the study of primary production—limiting nutrients rather than on biogeochemistry itself, Margalef honoured the holistic nature of the Vernadskian biosphere as an innovative planetary vision of life in which biogeochemistry plays a key connective role (Margalef 1992). Therefore, it is highly likely that the possibility of linking microbial community structure with quantification of its contribution to biogeochemical cycling rates would have been of concern to Margalef, should he have had access to current microbial ecology discoveries and toolboxes.

Today, modern molecular techniques, namely omics, have clarified the role of microbes in some biogeochemical processes. The findings range from gene discovery to stoichio-genomics, including gene evolution over geological time and the mapping of genes, transcripts and proteins in the environment (Young et al. 2012). Nevertheless, the inference of biogeochemical rates from omics/molecular datasets remains a challenge. Simultaneous measurements and correlations between functional gene expression and biogeochemical rates have offered unconnected results suggesting that different regulation mechanisms operate at protein and cell levels (Hultman et al. 2015). Consequently, it is crucial to pay attention to single-cell processes. These are one of the missing links between the three steps of the central dogma of molecular biology, namely replication, transcription and translation (from DNA to RNA to protein), assessed by omics, and the actual effect of microbes on biogeochemical rates.

In this context, the phosphorus (P) cycle is one of the most important and probably deserves as much attention in the ocean as it has been paid in freshwaters

(Margalef 1998, Letscher and Moore 2015). The crucial steps of this cycle include interactions with chemical species (e.g. aluminium) and with living beings, in which phosphatases play a key role in the utilization of dissolved organic phosphorus and polyphosphate. Margalef's foundational experiments and assumptions about the role of microorganisms (i.e. plankters) in the regeneration of phosphate by phosphatases (Margalef 1950) have been confirmed, expanded and detailed over time (see Boavida 2012). In the framework of microbial enzymatic activities (i.e. phosphatases) contributing to phosphorus cycling, recent advances include (i) the discovery of diverse pH-dependent metallo-phosphatase isoenzymes in prokaryotic and micro-eukaryotic plankton genomes (Sebastian and Ammerman 2009), (ii) the development and use of single-cell fluorescent substrates in environmental plankton samples (Polaske et al. 2016), (iii) new strategies of analytical determination that address a large diversity of P-substrates in the environment, and (iv) the development of mathematical models for phosphatase activity in microalgae (Ghyoot et al. 2015). This multifaceted approach has helped understand why the results of enzymatic activity (i.e. ecological function) can be poorly correlated with any of the omics approaches (gene presence, gene expression and protein presence). The reason is that fine modulation of the enzyme activity is due, for example, to isoenzyme gene diversity, the dependence of different isoenzymes on temperature, substrate concentration (i.e. kinetic properties) and metallic cofactors, microbe-specific metabolic responses (such as lipid remodelling), and the physiological status of different individuals in a population (Valdespino-Castillo et al. 2014). Furthermore, phosphatase research is paving the path to understanding other microbial enzyme classes that play a role in the phosphorus cycle (phosphonate esterases, phosphodiesterases and phytases, e.g. Dyhrman et al. 2007) and, by extension, to all the important cycles (e.g. carbon, nitrogen and silicon). Experimental designs on microfluidic platforms and analytical tools for assessing the intracellular chemical composition (atomic force microscopy, chemical imaging, etc.) might also help parameterize the mathematical models. Like the Margalef Mandala, therefore, a multifaceted approach is required to investigate the role of microbes in linking the biogeochemical cycles through the study of omics.

GLOBAL CHANGE

Global changes in the natural environments were not new to Margalef. The biosphere as a whole entity was discussed by Margalef in his book *Our Biosphere* (1997), in which he introduced new theoretical concepts of organization of ecosystems, ranging from stratification of marine plankton to global regulation, that were extraordinarily advanced for the ecology of the time. Indeed, the idea contained in the Margalef Mandala of relating the composition of phytoplankton to specific parameters of the water column could help us to predict future changes in marine microbial community composition, especially regarding recent global

changes in ocean biogeochemistry, such as warming, acidification, deoxygenation and bio-invasions.

Currently, the oceans are experiencing rapid and unprecedented shifts in biogeochemical parameters as a result of global change. Anthropogenic activities are having impacts on atmospheric and oceanic carbon dioxide (CO₂), nutrient pollution and the introduction of non-native organisms, which are critical issues for world oceans and ecosystems in general. For instance, atmospheric CO₂ is rising to unprecedented levels (+415 ppm in 2020; Dlugokencky and Tans 2020), leading to direct and indirect effects on marine organisms (Borrero-Santiago et al. 2016a) that may modify succession and evolution. One of the consequences of the rising CO₂ levels is the warming of marine waters, which favours stratification, reduces oxygen solubility and enhances respiration rates of marine organisms (Gruber 2011). Increased levels of CO₂ dissolved in seawater lead to a reduction of carbonate availability and a lowering of pH, causing acidification stress (Gattuso et al. 2011). Together with changing oceanic circulation, these factors might be responsible for the recent and future decreases in the ocean's oxygen content and in the formation of oxygen minimum zones (OMZs), which are defined as water masses with dissolved oxygen concentrations declining by 0.09 to 0.34 μmol kg⁻¹ y⁻¹ (Levin 2018). The microbial degradation of organic matter, and subsequently the oceanic carbon pump, might be altered in OMZs, leading to a fall in N₂ production by the microbial community and modifying chemical and biological gradients in the water column (Bertagnolli and Steward 2018).

Human influence is not only related to changes driven by increasing CO₂ in the atmosphere. The introduction of non-native organisms is currently a challenge in many parts of the oceans. These introductions or invasions may be accelerated by global environmental changes but are also being enhanced by direct anthropogenic drivers such as the transfer of ballast water and aquaculture (González-Ortegón and Moreno-Andrés 2021). For these invasions to occur, microbes need to be transported from a source to a foreign environment, but invasions are also determined by competitive interactions. Specific examples of microbial transfer that have been reported include evidence of bacilli endospores dispersed over long distances by ocean currents and the spreading of toxic cyanobacteria (Fastner et al. 2007) as well as invasive protists (Wyatt and Carlton 2002). Microbial invasions can be divided into four steps: (i) introduction, (ii) establishment, (iii) growth and spread, and (iv) impact (Levine et al. 2004). The ecological rules that drive microbial interactions within the community are decisive (Locey and Lennon 2016). Additionally, anthropogenic perturbations induce instability in the ecosystem and may thus alter the potential for invasion (González-Ortegón and Moreno-Andrés 2021). These aspects were mentioned by Margalef (Margalef 1963, 1975), who predicted the consequences of the use of natural resources by humans and highlighted its impacts.

Environmental microbiology and microbiome science have progressively implemented these concep-

tual developments, and several reports in the last few decades have explored microbial system diversity in relation to community, environmental or climatic stability to understand species invasions. Microbiome studies of species invasions provide a new dimension for understanding the mechanisms underlying these invasions. In addition to competition and synergistic relationships, which are hot topics of microbiome research, at least two other emergent properties of microbial systems seem to be important players with respect to microbial invasions: immediate or delayed responses of horizontal gene transfer (Emamalipour et al. 2020) as a short-term exchange of genetic information; and pulse-coordinated responses, such as *quorum sensing*, as a regulation of gene expression according to microbial population. The latter has been found to be an important challenge in aquaculture (Fuente et al. 2015). Finally, the speed of ecosystem change is unquestionably another relevant element in the instability and adaptive responses of biological systems.

In addition to warming, acidification, deoxygenation and bio-invasions, the accumulation of plastic in the ocean is a major concern today. Plastic is currently the most common form of marine debris, and negative impacts of plastic pollution on the marine environment are documented at every level of biological organization, from molecules to ecosystems (Guo and Wang 2019). The microbial inhabitants of plastic marine debris termed “the plastisphere” (Amaral-Zettler et al. 2020) are an emerging source of genetic and metabolic diversity that may profoundly change our views of global marine matter and energy budgets.

Following Margalef’s approach to humanity as a part of natural ecosystems, we should include the changes brought about by anthropogenic impacts into the natural fluctuation of marine microorganisms. The generated matrix will help to predict changes in microbial distribution and functionality.

FUTURE PERSPECTIVES

Ramon Margalef introduced innovative ideas in the study of ecology, particularly in microbial ecology, as discussed in this review. He considered that unifying ecological understanding was a prerequisite for going beyond speculative explanations of observed patterns in nature, and that quantifying differences in biological and ecosystem structures is fundamental to the understanding of the mechanisms underlying ecological processes.

It may seem surprising that concepts and ideas developed decades ago can improve the way we study microbial ecology today. Margalef did not have access to many of the techniques and data that are now available, but his thinking is instrumental in avoiding the risk of data dredging. There is an ongoing debate about the way science should be defined and conducted (e.g. “Defining the scientific method” 2009), with some researchers more inclined towards data-driven research and others towards hypothesis-driven research. Without pretending to resolve this debate, we argue that Margalef is a prime example of how a combination of both approaches,

namely predicting patterns and inferring mechanisms, can be extremely effective in revealing ecological laws and rules (see also e.g. Fontana et al. 2021).

Taking inspiration from Margalef’s teaching, we propose an integrated conceptual approach for microbial ecology (Fig. 1), which includes ecological theory, microbial diversity, biogeochemical cycles and global change. This new representation visualizes the interdisciplinary nature of microbial ecology as an intersection of related research fields. As an example, ecosystem structures are fundamental to the integration of information theory and diversity-related concepts into ecology (Margalef 1957, 1985). As we have explained at length in this review, we believe improvements should be made particularly in three areas of ecological studies: sampling strategy, data analysis and ecological interpretation.

Considering Margalef’s view, microbial ecologists must have a clear perspective that improving a multidisciplinary approach between biologists, bioinformaticians, statisticians and oceanographers will be key to understanding microbial ecology as a whole. All variables and factors are directly and indirectly intercorrelated. They cannot be fully understood without considering all the others. Today, the scientific community needs to develop new concepts and an integrated vision of all disciplines in microbial ecology. To move forward, it might be necessary to look back: it is back to the future with Ramon Margalef!

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