REVIEWS

Microbial oceanography: paradigms, processes and promise

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Abstract | Life on Earth most likely originated as microorganisms in the sea. Over the past ~3.5 billion years, microorganisms have shaped and defined Earth's biosphere and have created conditions that have allowed the evolution of macroorganisms and complex biological communities, including human societies. Recent advances in technology have highlighted the vast and previously unknown genetic information that is contained in extant marine microorganisms, from new protein families to novel metabolic processes. Now there is a unique opportunity, using recent advances in molecular ecology, metagenomics, remote sensing of microorganisms and ecological modelling, to achieve a comprehensive understanding of marine microorganisms and their susceptibility to environmental variability and climate change. Contemporary microbial oceanography is truly a sea of opportunity and excitement.

Phototrophic
Able to fix inorganic carbon using energy from light.

Heterotrophic
Able to acquire metabolic energy by the consumption of particulate or dissolved organic matter.

Microbial oceanography is a new discipline that integrates the principles of marine microbiology, microbial ecology and oceanography to study the role of microorganisms in the biogeochemical dynamics of natural marine ecosystems. Research conducted mostly during the past half-century has built a coherent, conceptual understanding of the role that microorganisms play in the general economy of the sea[1]. In contrast to most terrestrial habitats, life in the sea is dominated, both in terms of biomass and metabolism, by microorganisms from all three domains of life (Bacteria, Archaea and Eukarya; BOX 1). In open-ocean ecosystems, phototrophic microorganisms, mostly Bacteria and unicellular algae (Eukarya), harvest solar energy and produce the organic matter that fuels nearly all heterotrophic processes in the sea. Complex microbial-based food webs that contain a multitude of organisms, from simple bacteria that feed on dissolved organic matter (DOM) to predatory protozoans and micrometazoans, help to transfer and eventually dissipate most of the solar energy that is initially captured during photosynthesis.

A general goal of microbial oceanography is to observe and understand microbial life in the sea well enough to make accurate ecological predictions, for example, of the impact of climate variability on microbial processes in the global ocean. By analogy to a living cell, the ocean has a collective metabolism that is based largely on its dynamic genetic blueprint, with expressed phenotypes that control fluxes of energy and matter. The microbial processes that underlie this collective metabolism are influenced by environmental forcing and are governed by the laws of physics and chemistry.

Ultimately we will need to make observations and conduct experiments at multiple levels of system organization, from genomes to biomes, before a comprehensive understanding of the biology of the oceans can emerge. Therefore, research in microbial oceanography includes laboratory-based studies of model microorganisms and systems, field observations and sample collections from representative marine biomes on relevant time and space scales, and the implementation of *in situ* ecosystem-level experiments that are designed to test explicit hypotheses. Despite recent progress in all of these areas, the scope and effectiveness of the experimental phase of this field has lagged behind the other components[2]. This is largely because marine biomes are not understood well enough to design meaningful ecosystem-level experiments, especially with regard to the temporal and spatial scales that influence microbial processes. This lack of field-based hypothesis testing precludes an intellectual synthesis on the scale required to build accurate predictive global scale ecosystem models[3,4]. This is the grand challenge for the future.

The main purpose of this Review is to assess our current understanding of the structure and dynamics of microbial assemblages in marine ecosystems and to examine the role of these microbial assemblages in our changing world. Where appropriate, a research prospectus for the future is presented.

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Microheterotrophs
Small (2–20 µm) prokaryotic or eukaryotic organisms that are dependent on organic matter.

Bacterioplankton
Bacteria that inhabit the water column of lakes and oceans, either freely suspended or attached to particles.

Voyage of discovery

The field of microbial oceanography began with the discovery of marine microorganisms by Antonie van Leeuwenhoek in 1677 (REF. 5). However, modern investigations of microorganisms and their roles in marine ecosystems, using appropriate sampling techniques and credible methodologies, are only a few decades old[1].

The most basic and relevant information about microbial assemblages in the sea is community structure and organization, distributions, abundances and *in situ* metabolic activities as well as the ecological controls thereof. Research on these topics requires access to the sea, usually aboard large, well-equipped research vessels (>40 m in length and 1,000 tons in displacement), especially if the target habitat is the open ocean. Adequate ships are few in number, expensive (US$20,000–30,000 per day) and often difficult to schedule. As a result, we have much greater knowledge of some regions of the global ocean than others, and we have relatively few observations, if any, from some of the most remote oceanic habitats on Earth (for example, the South Pacific Gyre). Furthermore, marine ecosystems and their microbial assemblages vary in both space and time, so any given expedition will return only a snapshot of the dynamic motion picture that is the ocean.

Box 1 | **Terminology: what's in a name? Everything!**

Historically, the terms microbiology and bacteriology were sometimes used synonymously, and the study of 'marine microbiology' had a focus that was more or less restricted to the study of heterotrophic bacteria that could be isolated from samples of seawater[74,75]. It is now well recognized that marine microorganisms are taxonomically diverse and metabolically complex, and that many of the important groups have not yet been brought into laboratory culture. The term 'microorganism' is now used to describe any small, living organism, typically with a largest dimension of 100–150 µm. This catch-all term includes representatives from all three domains of life (Bacteria, Archaea and Eukarya), as well as acellular viruses. However, this common practice of assigning organisms to the 'micro' class, based strictly on size[76], ignores differences in the evolutionary histories and metabolic capabilities of these organisms.

Furthermore, the term bacteria (and bacterioplankton) has historically been reserved for heterotrophic prokaryotes, although this is no longer acceptable on the basis of our current understanding of life in the sea. Bacteria are not all heterotrophic, and not all heterotrophic prokaryotes are bacteria. The independent discoveries of two major groups of phototrophic marine bacteria, *Synechococcus*[77] and *Prochlorococcus*[78], and the unexpected discovery of pelagic marine archaea[33,34] necessitated a careful re-evaluation of the most basic terminology. Pace[79] has called for a moratorium on the use of the term 'prokaryote' because it is based on the false assumption that Bacteria and Archaea have a common ancestor in evolutionary history, but old habits are likely to die hard. The classification scheme used in this Review is based on the energy- and carbon-acquisition pathways shown in the table below.

Source of energy	Source of electrons	Source of carbon
Sunlight (photo-)	Inorganic (-litho-)	CO_2 (-autotroph)
	Organic (-organo-)	Organic (-heterotroph)
Chemical (chemo-)	Inorganic (-litho-)	CO_2 (-autotroph)
	Organic (-organo-)	Organic (-heterotroph)
Radioactive decay (radio-)	Inorganic (-litho-)	CO_2 (-autotroph)
	Organic (-organo-)	Organic (-heterotroph)

Equally as important as access to the sea is a carefully designed and implemented sampling programme that can obtain not only biological samples, but also complementary physical and biogeochemical data. These data are required for the effective interpretation of microbial community structure and dynamics[6] and must include highly resolved and well calibrated data on temperature, salinity, dissolved inorganic and organic nutrients, and dissolved gases. The physical and chemical characterization of the habitat is as important for our understanding of microbial processes as the microbial assemblage itself. Despite several decades of progress in the development of methods, we still lack reliable and routine field methods for determining the distributions, abundances and metabolic activities of marine microorganisms. By comparison, most physical and chemical measurements are precise and often automated. The more complex the structure of the microbial assemblage under investigation, the less accurate the estimates of any bulk processes, such as rates of photosynthesis, microheterotrophic production or microbial growth rates. It is remarkable to think that we can now obtain the exact nucleotide base sequence of the microbial community DNA in a given seawater sample[7], but that we cannot determine the content of living (biomass) carbon, or its turnover rate, with anywhere near the same degree of certainty. This Review does not present, defend or criticize the methods that currently exist, other than to say that better methods will be needed to test more elaborate hypotheses in the future.

Ocean habitats and their microbial assemblages

Microorganisms inhabit all marine ecosystems, from the tropics to the sea ice and from the well-lit surface waters to the deep abyss; they truly are the "unseen majority"[8] (BOX 2). They harvest and transduce solar energy, catalyse key biogeochemical transformations of the nutrients and trace elements that sustain the organic productivity of the oceans, produce and consume most greenhouse gases (for example, carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4)), and are a crucial link in the ocean's carbon cycle. They also represent an enormous and dynamic reservoir of genetic variability that is the basis for evolution by natural selection. A recent report from the American Academy of Microbiology has summarized the state of knowledge and some future challenges in *Marine Microbial Diversity: The Key to Earth's Habitability*[9].

A major breakthrough in the assessment of marine microbial diversity came with the application of molecular phylogeny using nucleotide-sequence analysis of the small-subunit ribosomal (r)RNA gene[10]. This survey method was culture independent, so the rRNA genes report the true phylogenetic diversity of a given habitat, assuming that there is no selection for, or against, specific genes during sample processing. Application of these, and other, nucleic-acid-based methods of natural microbial assemblages has consistently revealed the presence of novel microorganisms, some of which might have novel traits, unique metabolic capabilities or lineages that are isolated branches of the tree of life. Most of

Box 2 | **Diversity of marine habitats**

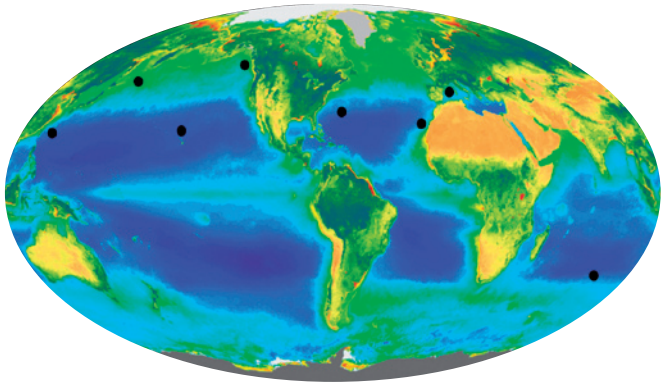
The marine environment is the largest contiguous habitat on Earth. However, even without rigid boundaries, the global ocean is actually a mosaic of semi-isolated habitats that are established and maintained largely by global scale interactions between the atmosphere and the ocean[80]. Ocean circulation, maintained primarily by radiative forcing, basin geometry and the rotational velocity of the Earth around the sun, determines the broad distribution of dissolved nutrients that, along with solar energy, form the basis for life in the sea. The presence and absence of polar ice, the concentrations of atmospheric oxygen and carbon dioxide, the break-up of Pangaea and the subsequent redistribution of continental landmasses, and the emergence of *Homo sapiens*, to name a few planetary benchmarks, have all had major impacts on the ocean as an ecosystem.

The oceans cover approximately 71% of the Earth's surface and are on average 4 km deep. Near the continental boundaries, marine habitats are generally shallower owing to the presence of expansive (10–200 km) continental shelves that are, at most, a few hundred metres deep. Even closer to land, marine habitats are influenced by the input of terrigenous materials (such as sediments, freshwater, organic carbon and nutrients), which increase habitat variability and affect microbial productivity. By comparison, life in the abyss (deeper than 4 km) is characterized by a relatively constant physical and chemical environment.

Microbial assemblages in the sea are highly stratified in the vertical (depth) dimension[81], and vary both in time and space, in large part owing to changes in the physical and chemical characteristics of the habitat. Compared to surface habitats, which have relatively high kinetic energy, deep-ocean circulation is very sluggish. This implies that some regions of the global ocean, for example the deep (deeper than 3,000 m) North Pacific Ocean, might be limited with respect to metabolic energy sources because these ocean regions are very old (up to 2,000 years old, on the basis of radiocarbon age estimates) and far removed from regions of primary solar energy capture. As a result, a decrease in total microbial biomass and metabolic activity with increasing distance from the shore and increasing water depth is generally observed, although many exceptions exist. Among the most important environmental variables are the availability of energy (light and reduced inorganic and/or organic compounds), temperature and hydrostatic pressure. For example, the deep sea, which is characterized by low energy, low temperature and high hydrostatic pressure, typically supports a total microbial assemblage that is <1%, and a total metabolism that approaches 0.1% or less, of that in the overlying surface waters.

A few selected study sites that are thought to be representative of larger biomes (see the black circles on the map) have been identified for the longer-term time-series observations that are required to identify robust relationships between sea microorganisms and climate. These cover a fairly broad range of habitats on the basis of temperature–salinity relationships shown below (see the graph).

The distribution of selected ocean time-series sites where microbial and/or biogeochemical observations have been routinely made are shown along with the characteristic potential temperature–salinity water-mass signatures for each site. From left to right on SeaWiFS (sea-viewing wide field-of-view sensor) ocean colour satellite map: SEATS (18°N, 116°E), KNOT (44°N, 155°E), HOT (22.75°N, 158°W), OSP/PAPA (50°N, 145°W), BATS (32°N, 64°W), ESTOC (29°N, 15.5°W), DYFAMED (43.5°N, 8°E) and KERFIX (50.7°S, 68°E).



these microorganisms have yet to be cultured and, therefore, lack formal descriptions, including physiological characterization.

Sogin *et al.*[11] recently used a massive parallel tag sequencing strategy that targeted hypervariable regions of rRNA to explore the microbial diversity present in the deep sea. Their results identified a large number of mostly low abundance microorganisms, the so-called rare biosphere, that comprise an almost inexhaustible source of genetic potential[11]. A more advanced application of nucleotide-sequence analysis of natural microbial communities is metagenomics[12]. At a single location in the oligotrophic North Atlantic Ocean[13], whole-genome shotgun sequencing identified more than 1.2 million new genes, and a subsequent analysis of surface-water samples along an ocean transect from the North Atlantic to the Eastern Tropical Pacific Ocean has uncovered 1,700 new protein families[14]. Integration of these new perspectives into current paradigms presents an exciting challenge for the discipline of microbial oceanography.

Oligotrophic
Having low levels of nutrient and algal photosynthetic production (for example, the open ocean).

Radiative forcing
The difference between the incoming radiation energy and the outgoing radiation energy in a given climate system.

Terrigenous
Derived from land or terrestrial ecosystems.

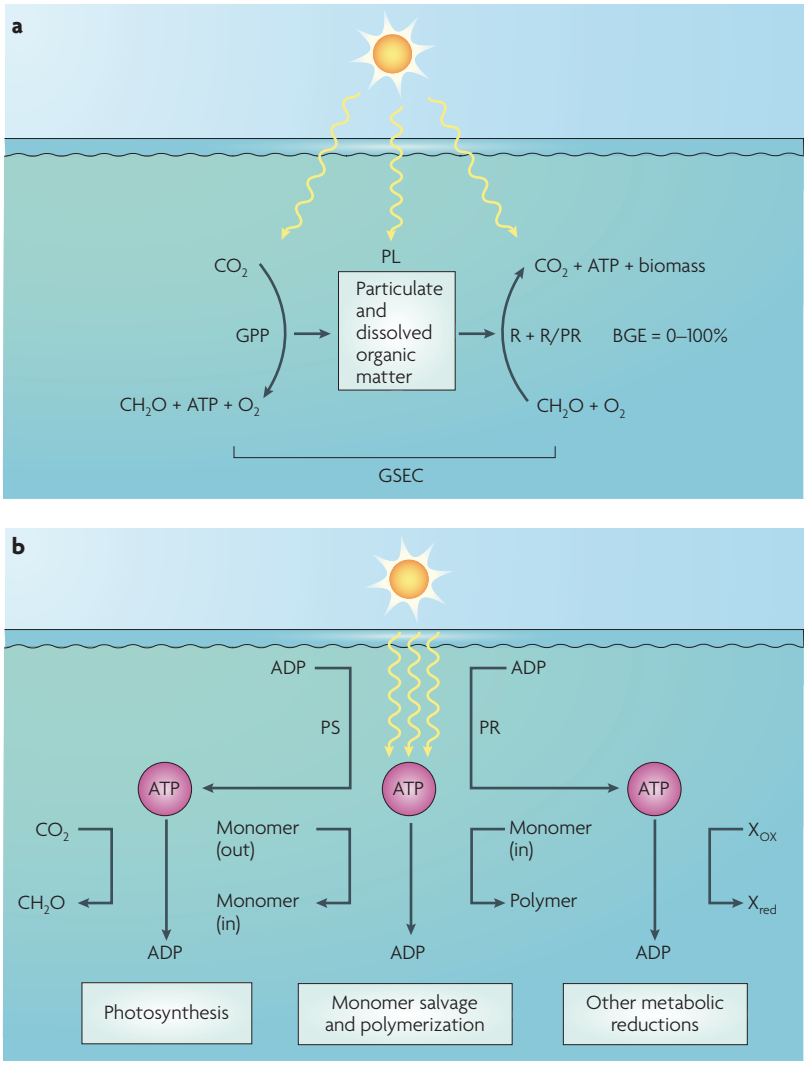


Figure 1 | **Solar energy flux and capture in the sea. a** | Conventional view of gross primary production (GPP) and matter decomposition and/or respiration (R). It is now known, however, that sunlight can also act on dissolved and particulate organic matter through photolysis (PL) and related processes, and sunlight can be used to support a mixotrophic proteorhodopsin (PR)-based metabolism among heterotrophic bacteria (R/PR), possibly by enhancing the overall efficiency of organic matter usage (bacterial growth efficiency, BGE; mol of C used per mol of C assimilated). Consequently, the gross solar energy capture (GSEC) in marine ecosystems is likely to exceed that used strictly for photolithoautotrophic production. **b** | Schematic view of the diverse photophosphorylation reactions, which include photosynthesis (PS) and PR-based ATP production, consumption of ATP for carbon dioxide (CO_2) reduction, monomer transport and polymerization reactions, and other biological redox reactions (for example, N_2 fixation).

A sea of microorganisms: selected case studies

After almost a century of research on the dynamics of marine ecosystems, and in particular nutrient cycles and energy fluxes, it would seem remarkable if new, fundamental metabolic processes were still being discovered. However, they are, and we should expect more to follow. From the many recent advances in the field of microbial oceanography, five 'case studies' ranging from genomes (systems biology) to biomes (systems ecology) are presented with the interconnected themes of energy capture and nutrient cycling on several different scales. Collectively, these five case studies show how observations and measurements can result in hypothesis generation and *in situ* experimentation for the refinement of current global ocean models of microbial biogeochemical dynamics.

Case study 1: proteorhodopsin-based phototrophy. Life on Earth, and especially in marine pelagic ecosystems, is solar powered; energy to support life in the sea is ultimately derived from phototrophy in the euphotic zone (FIG. 1). Until recently, phototrophic energy capture was equated with 'green-plant photosynthesis', namely photolithoautotrophy (BOX 1) or gross primary production (GPP). Theoretical calculations have set an upper limit of approximately 20–30-g dry weight of organic matter per m^2 per day for the GPP of organic matter in the sea[15]. However, physical influences (especially turbulence) on marine ecosystems typically lead to photosynthesis being limited by nutrient availability[16], so only a fraction (generally <10%) of the theoretical production is ever realized[17]. Consequently, life in the sea, especially in surface waters, operates under conditions of excess available energy[16].

Béjà *et al.*[18] detected a novel retinal-binding membrane pigment of the microbial rhodopsin superfamily that was present on a large environmental genome fragment derived from an uncultured marine gammaproteobacterium. Furthermore, they demonstrated experimentally that this new proteorhodopsin (PR) functioned as a light-driven proton pump and suggested that this metabolic process might represent a previously unsuspected mode of solar energy capture for non-photosynthetic microorganisms in marine environments worldwide (FIG. 1). Recently, Martinez *et al.*[19] confirmed a physiological role for PR by observing light-activated proton translocation and coupled photophosphorylation (light-dependent ATP formation) in *Escherichia coli* cells that had been transformed with the PR photosystem gene. This additional light-driven ATP production could be used to effect a more efficient use of organic substrate (that is, higher apparent bacterial growth efficiency; FIG. 1) or for a related metabolic function (for example, chemotaxis), leading to a selective ecological advantage. The cosmopolitan nature, diversity and abundance of PR genes in heterotrophic marine microorganisms have stirred up great interest and excitement owing to the potential for a previously unrecognized pathway of solar energy capture on Earth[20]. But what is the true ecophysiological function (or functions) of PR in the sea?

Giovannoni *et al.*[21] were the first to experimentally evaluate the role of PR in an isolated marine bacterium, *Candidatus* Pelagibacter ubique (SAR11). At the time of their study, P. ubique was the only PR-containing bacterium in pure culture. Their results indicated no difference in growth rates or cell yields of P. ubique cultures, regardless of treatment[21]. The authors proposed that the metabolic benefit of PR expression "may be most evident when organic carbon limitation decreases the ability of cells to generate a proton motive force by respiration."[21]

A few months later, Gómez-Consarnau *et al.*[22] reported that light exposure (180-µmol quanta per m^2 per second) resulted in an increased cell yield in

Pelagic
Water-column portion of marine and fresh-water habitats.

Euphotic zone
Upper realms of the oceans (or lakes) that are penetrated by sufficient amounts of light for photosynthetic organisms to grow.

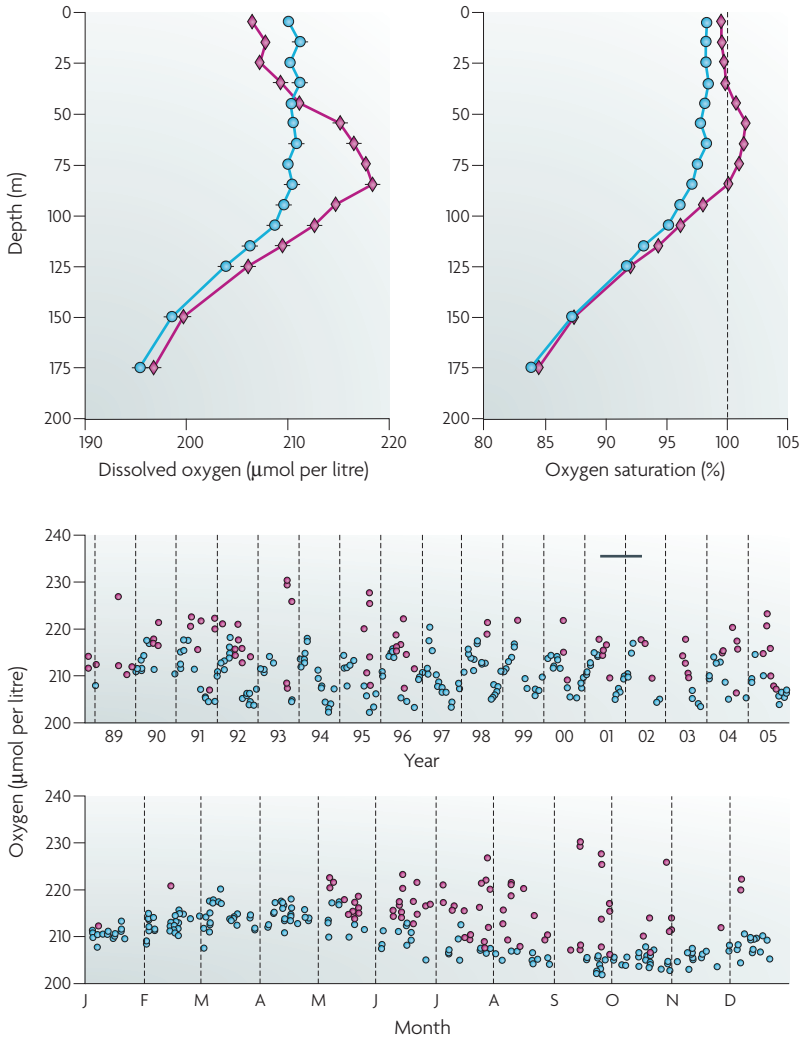


Figure 2 | **Oxygen concentrations and dynamics at the HOT Station ALOHA (22.75°N, 158°W). a** | Dissolved oxygen versus depth profiles of concentration (left) and saturation state relative to air (right). These data are based on a 17-year climatology study with mean winter (blue circles) and summer (red diamonds) values shown (the standard error bars are smaller than the symbols used). **b** | Dissolved-oxygen concentrations versus sampling date from 1989 to 2005 (top) for samples collected at 45±10 m are shown with annual climatology data (bottom). Blue circles are samples that are ≤100% air saturation, and red circles are >100% air saturation. Note interannual and subdecadal scale variability in dissolved oxygen. Specifically, there is >100% air saturation data during 1990–1996 compared to 1997–2001, and there is increased frequency of >100% air saturated concentrations between May–November in the annual climatology data. The solid bar indicates the period of intensive sampling for metabolic balance[26] (see main text). All HOT data are available online from the Laboratory for Microbial Oceanography.

Primary production
Process during which carbon dioxide is incorporated into organic matter by bacteria and algae, using any of a variety of energy sources.

Mixotrophs
Organisms that are part autotrophic and part heterotrophic, such as carnivorous plants.

cultures of a recently isolated PR-containing marine Flavobacterium, compared with growth in darkness. Exposure to light conferred greater metabolic and growth advantages at low to intermediate concentrations of dissolved organic carbon (140–1,100 µmol of C per litre), so phototrophy (and, hence, mixotrophic growth; BOX 1) in *Dokdonia* sp. MED 134 seemed to be facultative. In natural open-ocean marine ecosystems, where dissolved organic carbon is always present at <200 µmol of C per litre, mixotrophy should confer a metabolic advantage and might be the normal mode of existence, perhaps by increasing the organic substrate utilization efficiency as a direct consequence of supplemental phototrophic ATP production (FIG. 1). Ultimately, field experiments will need to be conducted to establish the ecological roles of PR in the energetics of microbial assemblages in the sea.

Case study 2: the metabolic balance of the open sea. In well-lit, near-surface waters, phototrophic microbial oxygen production usually exceeds microbial respiration (that is, GPP>R), so oxygen accumulates; oxygen supersaturations, relative to air equilibrium, are not uncommon in the open sea, especially during the summer (FIG. 2). At greater depths, R exceeds local oxygen-production rates (GPP<R), and the molecular oxygen (O_2) concentration as well as the percentage of air saturation decrease with increasing depth (FIG. 2). Direct measurement of the instantaneous water-column-depth integrated GPP versus R is therefore an explicit contemporaneous assessment of the metabolic balance of that ecosystem. If GPP>R, the system under investigation is in a state of net autotrophy and can sustain the export, or sequestration, of dissolved oxygen, reduced carbon and the potential energy that this represents. However, if GPP<R, the system under investigation is in a state of net heterotrophy, and it must import reduced carbon and oxygen to sustain life.

On the basis of conceptual models of the structure and function of open-ocean ecosystems, together with direct observations of near-surface oxygen-saturation state and organic matter export from the euphotic zone, a net autotrophic state (GPP>R) is predicted for the open ocean. So it was a surprise when del Giorgio *et al.*[23] and Duarte and Agusti[24] systematically analysed the extant global ocean database of GPP and R values and concluded that 80% of the ocean's surface is expected to be heterotrophic. If open-ocean ecosystems are indeed net heterotrophic, how do they obtain the energy (or organic matter) that is required to sustain them? A concerted intellectual and research effort ensued to address this important issue.

As with any ecological study, meaningful results are only obtained if the experimental sampling design is rigorous, taking into account time and space variability, and the analytical methods are appropriate and accurate. Williams *et al.*[25] reasoned that periodic analysis (for example, monthly measurements) of an open-ocean habitat for a full year might provide a more accurate estimate of the true metabolic state if, for example, there were seasonal variations or short-term metabolic decoupling. However, their year-long study in the North Pacific Subtropical Gyre (NPSG), which was based on bottle incubations, revealed that the 0–150-m euphotic zone was consistently in metabolic deficit (GPP<R) with a shortfall of approximately 9 mol of O_2 per m^2 per year, a value equivalent to nearly 40% of the annual GPP[25].

Karl *et al.*[26] proposed that apparent net heterotrophy in open-ocean habitats was a paradoxical manifestation of a highly variable ecosystem with respect to the metabolic balance. They provided time-series data from

Autotrophy
The acquisition of metabolic energy from the fixation of inorganic carbon, for example, by photo- or chemosynthesis.

a deep-ocean mooring in the NPSG that revealed a complex pattern of net ecosystem metabolism, based on changes in the concentrations of O_2, that were characterized by short-lived, aperiodic bursts of O_2 accumulation[26]. Consequently, open-ocean ecosystems should be viewed as time- and space-variable mosaics with regards to microbial metabolism, an important point made several decades earlier during a previous controversy over microbial metabolisms in the sea[27]. The physical mechanisms responsible for sustaining this intermittent O_2 accumulation are not well understood, even in broad terms. It is plausible that resource control through the aperiodic delivery of inorganic nutrients from below the euphotic zone[28–30] (the nutrient-loading hypothesis) might create and sustain the metabolic heterogeneity of the open sea.

McAndrew *et al.*[31] tested the nutrient-loading hypothesis[26] by tracking the short-term (over days) metabolic response of nutrient-depleted surface microbial assemblages following the addition of deep-water nutrients. Within a few days, photoautotrophic biomass and GPP increased and community metabolism shifted from a near-balanced (GPP=R) or slightly net heterotrophic (GPP<R) state to one that was demonstrably net autotrophic (GPP>>R). They also observed a significant shift in the size spectrum of chlorophyll-containing cells present, from small (<2 µm in diameter) to large (>10 µm in diameter) silicon-containing cells, which were presumably diatoms (FIG. 3). These 'blooms' of relatively large, net autotrophic plankton assemblages, if subsequently removed from the system by gravitational settling or zooplankton grazing, would leave behind an excess of oxygen that was roughly proportional to the organic matter exported. This oxygen could then support net heterotrophy until the next nutrient-injection event. In this way, intermittent net autotrophy fuels a steadier rate of R, which often exceeds GPP. The high rates of metabolism that are required to sustain 'net heterotrophic' systems (GPP<R) depend on a continuous supply of usable DOM. Typically, DOM is concentrated in near-surface marine ecosystems (60–100 mmol of C per m^3) relative to sub-euphotic zone waters, implying a sustained local production from the combined processes of excretion, exudation, grazing and cell death. It has been suggested that atmospheric deposition of usable organic matter might supplement the DOM that is produced *in situ*[32], at least in selected marine habitats.

Future field studies on the net metabolic balance of the sea will need to consider time as one of the key variables for the control of microbial processes in the sea. Future studies should also include a careful analysis of DOM production, inventories and chemical characterization, including microbial bioavailability. Furthermore, new metabolic models could be constructed to accommodate non-traditional modes of solar energy capture (see case study 1) and, perhaps, energy dissipation. In this regard, direct measurements of energy flux (for example, ATP-pool turnover; FIG. 1) might be desirable, as ATP is the common energy currency of life.

Case study 3: ecology of mesopelagic crenarchaea. One of the most important recent contributions to microbial oceanography was the independent discovery of non-thermophilic, planktonic archaea[33,34]. Until discovered, planktonic members of the domain Archaea had been mistakenly identified as Bacteria and had been enumerated, characterized and modelled as such. Once a systematic methodology for identifying individual members of the two domains was established, it became evident that marine group I crenarchaea were dominant components of coastal and open-ocean marine systems[35–37]. Indeed, a comprehensive set of observations over a 2-year period at Station ALOHA (a long-term oligotrophic habitat assessment) revealed that the abundance of archaea was equal to, or slightly greater than, the total abundance of heterotrophic bacteria at water depths greater than approximately 500 m[37]. A recent review by DeLong[38] provides both a historical account of this discovery and a new ecological framework for the role of crenarchaea in open-ocean marine systems.

The discovery of deep-sea archaea is starting to provide some answers to a long-standing puzzle in marine microbiology — the process of nitrification. Most of the fixed nitrogen that enters the deep sea (deeper than 200 m), usually in the form of sinking particles, is in the most reduced form (ammonia; NH_3/NH_4^+, valence state –3), whereas essentially all the fixed nitrogen that accumulates over time is in the most oxidized form (nitrate; NO_3^-, valence state +5). The two-step process of nitrification (ammonia → nitrite → nitrate) that yields energy for the growth of specialized chemolithoautotrophic nitrifying bacteria was thought to be responsible for the accumulation of nitrate in the deep sea[39].

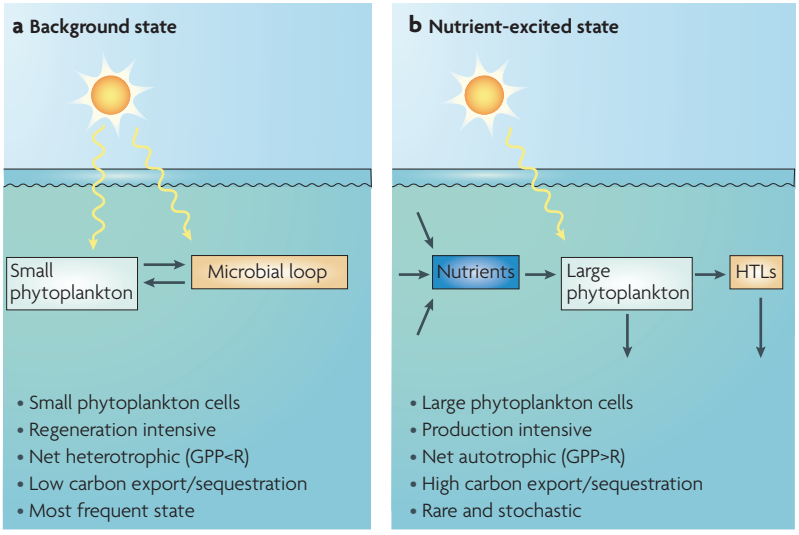


Figure 3 | **Effects of nutrient perturbations on open-ocean ecosystems.** The schematic shows a representation of the effects of nutrient perturbations on open-ocean ecosystems, including changes in microbial community structure, dynamics and function. Nutrients include carbon dioxide, nitrate, phosphate and trace elements. Phytoplankton include pico, small (mostly Bacteria) and large (mostly Eukarya) organisms. Grazers and predators, including zooplankton and fish, are the main occupants of higher trophic levels (HTLs). Whereas panel **a** shows the background state, panel **b** shows the nutrient-excited state, which is established by the rapid introduction of new nutrients into the system either from the atmosphere or from below the euphotic zone. GPP, gross primary production; R, respiration.

Recently, Pearson *et al.*[40] and Wuchter *et al.*[41] provided evidence that might indicate that mesopelagic zone archaea have an autotrophic metabolism. More recently, the first example of nitrification in the archaeal domain was discovered with the isolation of a chemolithoautotrophic NH_4^+-oxidizing marine archaeon, named strain SM1 (REF. 42). SM1, which has been assigned the candidate status *Nitrosopumilus maritimus*, can grow in the laboratory with ammonia as a sole energy source by converting it to nitrite, and can use CO_2 as the sole carbon source. It is tempting to hypothesize that archaea might have a role in, or even control, sub-euphotic zone nitrification, or at the very least the oxidation of NH_4^+ (see REFS 43–45).

Metagenomic surveys have also shown that some archaea harbour unique ammonia monooxygenase (*amoA*) genes[13,46], and polymerase chain reaction (PCR) primers designed to specifically target archaeal *amoA* sequences have detected *amoA* in various coastal and open-ocean habitats[47]. Mincer *et al.*[48] recently investigated the quantitative distribution of putative nitrifying genes and phylotypes in a picoplanktonic genome library from Station ALOHA. They uncovered a deeply branching crenarchaeal group that is related to a hot spring clade, indicating that the *amoA*-containing archaea in the mesopelagic zone might be more diverse than previously suspected. Furthermore, as nitrite (the presumed end-product of archaeal ammonium oxidation) does not accumulate in the deep sea, the activities of these specialized archaea must be tightly coupled to nitrite-oxidizing microorganisms, presumably nitrifying bacteria[49]. In this regard, Mincer *et al.*[48] found a positive correlation between *Nitrospina*, a nitrite-oxidizing bacterium, and crenarchaea, which suggests a possible syntrophic metabolic relationship for mesopelagic zone nitrification.

The pathways and diversity of carbon and energy flow through mesopelagic archaea are not well characterized. Ingalls *et al.*[43] used the natural distribution of radiocarbon in archaeal membrane lipids to quantify the bulk carbon metabolism of archaeal assemblages at two depths in the subtropical North Pacific Ocean near Hawaii, USA. Their compound-specific radiocarbon analyses and isotopic mass balance model for carbon assimilation implied a predominantly (83%) autotrophic (CO_2 assimilation) metabolism for the whole archaeal population. This analysis was based on the source of carbon and does not preclude the use of alternative energy sources, including organic matter (BOX 1).

Other reduced inorganic or organic substrates could serve as energy sources for mesopelagic and deep-sea archaea. Although the only isolate that is available seems to grow as an obligate chemolithoautotroph[42], mesopelagic zone archaea assimilate amino acids under simulated *in situ* conditions[50,51], which suggests either a chemoorganoheterotrophic or a mixotrophic mode of metabolism for selected groups. It has also been suggested that mesopelagic archaea might function as a sink for D-amino acids in the interior of the ocean[52]. Furthermore, the uncultivated marine crenarchaeote *Cenarchaeum symbiosum* — which seems to fix CO_2 by a modified 3-hydroxypropionate cycle rather than by the Calvin–Benson cycle that is used by most marine microbial autotrophs — also has genes for a near complete tricarboxylic acid cycle, which would enable the oxidative metabolism of various organic compounds[53]. Well designed *in situ* rate experiments that can distinguish between bacterial and archaeal metabolic processes, including ammonia and nitrite oxidations, perhaps using specific inhibitors, will be necessary to provide us with a better understanding of coupled carbon- and nitrogen-cycle processes in the ocean's interior.

Case study 4: a microbial observatory in the sea — Station ALOHA. Long-term studies in microbial oceanography are predicated on the straightforward assertion that certain processes, such as climate-driven changes in microbial community structure and productivity, as well as natural or anthropogenic changes in nutrient loading and habitat changes, are time-dependent processes and must be studied as such. Time-series investigations in microbial oceanography must be conducted with the explicit recognition of the interdisciplinary connection between physics, chemistry, biology and geology and should target strategic locations that are deemed to be representative of larger biogeographical provinces or biomes.

In October 1988, a deep-water station, dubbed Station ALOHA, was established in the NPSG (BOX 3). Open-ocean ecosystems such as Station ALOHA are characterized by low concentrations of fixed, biologically available nitrogen, which would seem to make them suitable niches for the selection and proliferation of N_2-fixing microorganisms. However, until recently this flux pathway had been ignored both in conceptual paradigms and in models of oceanic ecosystems[54]. We currently estimate that as much as 50% of the resupply of bioavailable nitrogen at Station ALOHA is derived from local microbial N_2 fixation. The independent lines of evidence for this include: summer-time drawdown of salinity-normalized total dissolved inorganic carbon in the absence of nitrate or other forms of fixed nitrogen; direct measurements of N_2 fixation and attribution of metabolic activity to different size classes and phylogenetic groups of N_2-fixing microorganisms; assessment of the molar N:P stoichiometries of surface-ocean dissolved and particulate matter pools and the development of a one-dimensional model to calculate nitrogen and phosphorus mass balances; and seasonal variations in the natural ^{15}N isotopic abundances of particulate matter that is exported to the deep sea and collected in bottom-moored sediment traps[55–58].

If the biomass of N_2-fixing microorganisms and the rates of N_2 fixation in the NPSG are increasing over time owing to climate-coupled changes in the environment[54], then the phosphorus-stressed biome would become even more phosphorus-limited. Ecological consequences might include changes in the standing stocks and turnover rates of dissolved and particulate phosphorus and alterations in the C:N:P composition of newly produced biomass, which might select for microorganisms that do not require as much phosphorus for growth[59,60] or for slower growing microorganisms with lower phosphorus requirements owing to reduced rRNA[61]. At Station ALOHA there still seems to be a surplus of phosphate (in the form of inorganic phosphorus or P_i), even though the inventory has

Picoplankton
Organisms that are suspended in the water column that are less than 2 mm in size.

Box 3 | **Hawaii ocean time-series program — it's HOT**

Station ALOHA (22.75°N, 158°W), which is located in deep water (4,800 m) well beyond the physical and biogeochemical influence of the Hawaiian Ridge, is thought to be representative of the eastern portion of the North Pacific Subtropical Gyre (NPSG), one of the largest contiguous biomes on Earth[54,82]. The initial goals of the Hawaii ocean time-series (HOT) program were: to observe and interpret the seasonal and interannual variability in water-mass structure and its relationship to gyre fluctuations and climate; to develop a decade-scale climatology of microbial and biogeochemical processes, including community structure, primary and export production and nutrient inventories and fluxes; and to estimate the air-to-sea exchange of biogenic gases, including carbon dioxide (CO_2)[82]. A sampling frequency of approximately monthly research cruises was selected as a compromise between logistics (including funding) and best estimates of the characteristic time-scales of variability for the ecosystem processes that we initially set out to observe and interpret — namely low frequency (>1 year) changes[83,84]. A set of core measurements was selected that would provide the data needed to calibrate, validate and improve biogeochemical models, mostly by obtaining a more comprehensive understanding of ecosystem controls of microbial community structure and dynamics. Over the intervening 2 decades, additional core measurements and continuous data collection capabilities from unattended moorings and remotely operated vehicles have improved our understanding of the time–space domains of variability, but much more still needs to be done.

Numerous unexpected discoveries, some serendipitous, have already been made, including the discovery of microorganisms, metabolic processes and paradigms, as well as several emerging climate–microorganism connections. Selected examples are listed below.

- Numerical dominance of picophytoplankton from the genera *Prochlorococcus* and *Synechococcus* and the discovery of a new unicellular N_2-fixing cyanobacterium.
- Probable role for photomixotrophy in solar energy capture.
- Numerical importance of crenarchaea in the distribution of picophytoplankton, especially at depths below the euphotic zone.
- Photoautotrophic rates of CO_2 fixation that are 2–3-fold higher than was previously thought (17 year mean = 500 mg of C per m^2 per day) and a temporal increase of nearly 50% of the rate of CO_2 fixation during the period of observation (1988–present).
- The importance of N_2 fixation as an approximately equal source, together with nitrate assimilation, of 'new nitrogen production'.
- The importance of bacterial and archaeal nitrification, and the possible role of bacterial phosphonate degradation, as modes of metabolism that could potentially generate nitrous oxide and methane, two potent greenhouse gases that might be released into the atmosphere.
- Importance of spatio–temporal variability, including mesoscale eddies and aperiodic photoautotrophic blooms, which led to the demise of the 'climax community' concept.
- Evidence for within-decade to decade-to-decade scale variability in microbial structure and function in response to changes in climate forcing.

decreased by nearly 80% over the past 2 decades[62], most likely as a result of N_2 fixation. Further reduction of P_i, often to subnanomolar concentrations, can be expected along with a selection for alternative 'phosphorus capture' mechanisms. The mechanisms could include novel enzymes that can mobilize older, semi-labile organic phosphorus residues including carbon–phosphorus bonded phosphonates (FIG. 4) as well as a further shift in cell size and activity spectra towards smaller, slower growing microorganisms. This has numerous potential effects on the trophic structure and might select for smaller predators, thereby altering top-down grazing control of microbial populations and, therefore, nutrient-cycling rates (also see case study 5). Without an adequate re-supply of phosphorus and other nutrients, these phosphorus-stressed open-ocean ecosystems could lose biomass, biodiversity and possibly their ability to respond to habitat variability and climate change.

A key negative feedback to enhanced N_2 fixation, decoupling of nitrogen and phosphorus cycles and the export of high N:P organic matter is the eventual build-up of a sub-euphotic zone nutrient reservoir that has an elevated N:P ratio relative to cellular needs[63]. As these regenerated nutrients slowly feed back into the euphotic zone, they will select against N_2 fixers because there will be other sources of nitrogen available for the growth of competing microorganisms that do not need to invest cellular energy to convert N_2 into biomass. This could lead to another shift in community structure, ecological stoichiometry, grazing control and organic matter export under the newly established nutrient regime. This alternation between nitrogen limitation and phosphorus (or phosphorus/iron) limitation in the NPSG is predicted to occur on an approximately 20–50-year cycle on the basis of the estimated residence time of nutrients in the upper mesopelagic zone reservoir[54,63]. The extent to which greenhouse-gas-induced warming and other changes to the surface ocean will affect the dynamics of these proposed alternative ecosystem states, or create new ones, is currently unknown. However, it seems almost certain that the global dimensions of subtropical gyres will expand, and if this happens then the open ocean will become more stratified and nutrient depleted, setting the stage for changes in microbial population structure and function.

Case study 5: ecosystem-level nutrient-perturbation experiments. Field measurements to assess the ecosystem response to a controlled nutrient perturbation have

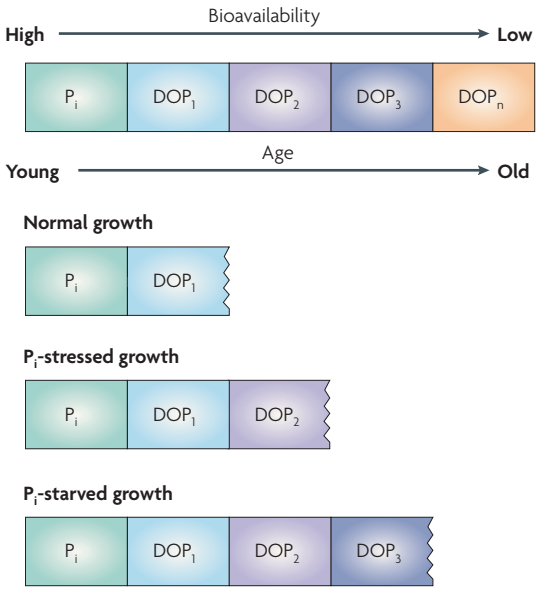


Figure 4 | **The relationships between inorganic phosphorus and dissolved organic phosphorus.** The schematic shows the hypothetical relationships between inorganic phosphorus (P_i) and dissolved organic phosphorus (DOP) bioavailability and microbial growth. Shown at the top is the spectrum of different DOP compound classes ($DOP_1 \rightarrow DOP_n$) as well as their relative bioavailability and, hence, probable age. Under 'normal' phosphorus-sufficient microbial growth, P_i and perhaps a small fraction of the total DOP support microbial growth. As communities become 'P_i stressed' and, eventually, 'P_i starved' there is a greater reliance on the older, more refractory, DOP compound classes. The selection for these alternative phosphorus-capture pathways is under genetic control and can, therefore, lead to changes in community structure and function with significant effects on the coupled cycles of carbon, nitrogen and phosphorus in the marine environment.

usually been conducted in closed containers that contained millilitres to litres of seawater, with incubation periods of a few days at most. The underlying assumption is that the microbial dynamics in these small closed containers accurately reflect the *in situ* rates of metabolism and biosynthesis in the sea, but this assumption is generally impossible to verify because there are no 'uncontained' control samples for comparison with the static incubation treatments. An alternative approach is to conduct a whole-ecosystem experiment to eliminate the shortcomings of containment experiments[64].

Whole-ecosystem nutrient-perturbation experiments have recently been carried out in selected marine habitats by adding dissolved iron along with an inert tracer (usually a gas such as sulphur hexafluoride (SF_6)) to map the effected volume and to control for diffusive and advective processes[65,66]. Major advantages of these open ecosystem experiments are the ability: to investigate the impact of larger organisms such as mesozooplankton or even fish on microbial communities; to examine longer time-scale effects over days to months; and to study supply and loss processes subsequent to the experimental perturbation. In addition to these iron-fertilization experiments, a P_i-enrichment study was recently conducted in the Eastern Mediterranean Sea[67].

The Eastern Mediterranean Sea is one of the most oligotrophic regions on Earth, at least with respect to the distributions of suspended and dissolved particulate matter[68]. Comprehensive field investigations conducted over the past few decades have suggested that phosphorus limits biological production in this region[69]. In May 2002, a multidisciplinary team of scientists conducted the cycling of phosphorus in the Eastern Mediterranean (CYCLOPS) project, a large-scale P_i-fertilization experiment on a ~16-km^2 patch of open sea at 33.3°N, 32.3°E (REF. 67).

Following the addition of a diluted mixture of phosphoric acid to increase the P_i concentration by two orders of magnitude to ~110 nM (SF_6 was also added) in this phosphorus-limited habitat, there was a 40% decrease (rather than the predicted 40-fold increase) of chlorophyll in the microbial assemblage compared to control regions outside the P_i-enriched patch. The chlorophyll 'hole' developed over approximately 5 days, before returning to background levels after a period of about a week. Rates of primary production and phytoplankton growth after P_i addition were also lower[67]. In contrast to the response observed for the photoautotrophic assemblage, the addition of P_i stimulated 'bacterial' production (as measured by ^{14}C–Leu incorporation) and resulted in the accumulation of particulate phosphorus, presumably a result of the net growth of the chemoorganoheterotrophic assemblage. Rates of N_2 fixation also increased following P_i addition[70], but the microorganisms responsible were not identified.

Another unexpected, and still unexplained, result of P_i addition to the phosphorus-stressed Eastern Mediterranean Sea habitat was the observed order of magnitude increase in copepod egg production, starting just 2 days after P_i enrichment. Two possible trophic pathways were proposed to explain this rapid transfer of phosphorus to higher trophic levels. The first, termed trophic by-pass, proposed that the heterotrophic microbial loop (heterotrophic bacteria → protozoans → copepods) was favoured over the phytoplankton → copepod food chain[67]. Excess energy flowing to copepods stimulated reproduction and, hence, egg production. Alternatively, the addition of P_i to a phosphorus-limited habitat might have resulted in a 'luxury uptake' of phosphorus by heterotrophic bacteria and, possibly, phytoplankton, resulting in a change in the C:N:P stoichiometry, but not in the abundance of the organisms, a process termed trophic tunnelling[67]. The phosphorus-enriched prey could transfer the extra phosphorus to predator biomass, including ciliates and copepods, and the enhanced phosphorus content could stimulate growth and reproduction, as indicated by the enhanced rate of copepod egg production. Food quality, rather than food quantity, determines the reproductive response at the higher trophic levels in this model.

Additional details of this elegant P_i-addition field experiment — the first of its kind ever carried out in the open sea — have recently been reported in a special volume of *Deep-Sea Research*[71]. The unprecedented

results obtained during the CYCLOPS project required a new conceptual framework with much more complex and possibly non-linear trophic interactions. Because this was a 'one off' experiment, it is not clear whether the results obtained are reproducible, whether the concentration of exogenous phosphorus is a crucial variable (for example, it is unclear whether the results scale on phosphorus loading), or whether the results obtained are unique to hyperoligotrophic ecosystems. Perhaps more importantly, this field experiment shows that manipulation of ocean ecosystems can yield entirely unexpected results, and this message is central to the current debate over the intentional fertilization of the ocean to offset carbon emissions[72].

Future prospects, challenges and opportunities

These case studies reveal the importance of, and the need for, comprehensive analyses — ranging from genomes to biomes, coupled to interdisciplinary physical and chemical observations of broad temporal–spatial scales — before a comprehensive understanding of the role of microorganisms in oceanic ecosystems can be achieved. Since the beginning of the anthropocene, human-induced changes have affected even the most remote oceanic biomes. We now recognize that climate change, including but not limited to greenhouse-gas-induced surface-ocean warming and acidification[73], will probably affect microbial community structure and dynamics. More cryptic, but no less important, is the issue of endangered microbial species and extinction. Unless we know which species are around today, we will never be able to recognize biodiversity changes when they occur. Because each macrofaunal species can harbour numerous microbial symbionts that are found nowhere else[9], marine microbial extinctions must already be happening at an alarming rate.

Significant progress towards a comprehensive and meaningful integration of novel genomic and environmental metagenomic data sets with oceanographic and biogeochemical observations and field experiments will require focused teams, with an explicit commitment to collaborate, because the scope of the challenges and opportunities is too large for any individual laboratory or research group.

Selected challenges for the future include: understanding how the information encoded in marine microbial genomes manifests itself at the ecosystem level; understanding the interlinked roles of microorganisms in energy capture and dissipation, in carbon, nutrient and trace element cycling and in carbon sequestration; developing new ecological theories, if necessary, to deal with the unique characteristics of microorganisms, including but not limited to the 'species' concept and lateral gene transfer; using comprehensive ecological models that are based on and compared with multifaceted observations of the ocean on a broad range of scales to understand and predict changes in marine microbial community structure and function as anthropogenic influences alter ocean climate; establishing collaborations among teams of scientists from various disciplines who do not routinely communicate in order to devise creative solutions to long-term ecosystem-scale problems; and training the next generation of scientists to tackle the future challenges of science and society, including environmental policy, social responsibility and public education at all levels.

The recent establishment of the Center for Microbial Oceanography: Research and Education (C-MORE) should provide a physical setting and incentive for these collaborative efforts to occur within an initial focus on microbial processes in the NPSG.

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Competing interests statement
The author declares no competing financial interests.

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