# Microbial structuring of marine ecosystems

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Abstract | Despite the impressive advances that have been made in assessing the diversity of marine microorganisms, the mechanisms that underlie the participation of microorganisms in marine food webs and biogeochemical cycles are poorly understood. Here, we stress the need to examine the biochemical interactions of microorganisms with ocean systems at the nanometre to millimetre scale — a scale that is relevant to microbial activities. The local impact of microorganisms on biogeochemical cycles must then be scaled up to make useful predictions of how marine ecosystems in the whole ocean might respond to global change. This approach to microbial oceanography is not only helpful, but is in fact indispensable.

Primary production
The original source of organic material in an ecosystem that is due to carbon dioxide fixation by photosynthetic bacteria, plants or algae, or chemosynthetic microorganisms.

Heterotrophic
The acquisition of carbon and metabolic energy by the consumption of living or dead organic matter.

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One-half of global primary production occurs in the oceans[1,2], and therefore a fundamental problem for oceanographers is to understand how organisms use this carbon to create spatio-temporal patterns of carbon and energy flux. For example: how much is passed into fish[3]; how much is respired and returned to the atmosphere; and how much descends to support the deep-sea biota or to be sequestered on the seafloor[4]. Interest in the carbon cycle has increased recently owing to global problems, such as climate change, coastal eutrophication and over-fishing[5,6]. Historically, oceanographers believed that most primary production moved through a chain of small and large animals[7–9] and microorganisms were largely ignored[10]. However, several remarkable discoveries[11–13] (reviewed in REFS 14–16) that have been made during the past 30 years have shown that bacteria dominate the abundance, diversity and metabolic activity of the ocean (FIG. 1). A large fraction of primary production becomes dissolved (dissolved organic matter; DOM)[17] by various mechanisms in the food web, and this part of the primary production is almost exclusively accessible to heterotrophic bacteria and archaea (together referred to in this Review as bacteria)[9,18,19]. As a result, the uptake of organic matter by bacteria is a major carbon-flow pathway, and its variability can change the overall patterns of carbon flux[8,20,21]. Further, as bacteria use behavioural and biochemical strategies to acquire organic matter — for example, by the expression of enzymes to solubilize particulate organic matter (POM)[22] — they interact with sources of organic matter and modify the ecosystem and carbon cycle in different ways[23,24]. Our aim throughout this Review is to propose a mechanistic and microspatial framework that promotes a better understanding of how bacteria regulate the biogeochemical state of the oceans.

## Ecosystem-level coupling

The effects of bacteria on the carbon flux in the sea have been measured over the past 30 years[25,26]. Although it is impractical to measure the flux of each component of DOM into bacteria, the cumulative carbon flux (bacterial carbon demand; BCD) can be estimated as the sum of the carbon that is assimilated (growth) and respired, which can then be compared with primary production as a measure of the coupling strength (BCD ÷ primary production[27]). This parameter is useful as a global measure of bacterial performance, for example, in concepts that involve the ocean carbon flux or ecosystem-based fisheries[21]. Measurements of coupling strength have been made on many ocean expeditions, in most oceanic locations and in regions that differ in primary production, and this has revealed important geographical and seasonal patterns. The following examples show that the coupling of bacteria with primary production is highly variable and that this variability affects ecosystem functioning.

First, in the eastern Mediterranean bacteria take up most of the primary production, which is consistent with the poor fisheries that are present in this area[28] (although a high BCD might be an effect rather than a cause of poor fisheries). Second, studies carried out on a north–south oceanic transect (53°N in the Atlantic to 65°S in the Southern Ocean)[29] showed latitudinal variation in coupling strength, and, importantly, there were large net-heterotrophic regions. Bacteria took up more DOM than was present as local primary production, which indicates that the spatial or temporal import of organic matter must have occurred. Consequently, these regions have the potential for the net out-gassing of carbon dioxide. More extensive spatial and temporal

Autotrophic
An organism that synthesizes organic carbon from the fixation of inorganic carbon, for example, by photo- or chemosynthesis.

coverage to account for patchy autotrophic processes might, however, reveal a metabolic imbalance[29–31]. Finally, during the summer, bacteria–DOM coupling in the Antarctic Ocean was found to be weak. Perhaps, the bacterial hydrolysis of polymeric substrates and monomer uptake was slowed owing to low temperature and low substrate concentrations. This could result in the storage and temporal export of slow-to-degrade DOM in productive summers (when primary production is high) to support the energy needs of the Antarctic food web during the winter. During winter, a particle-based food web might incorporate bacterial biomass that is produced through the use of DOM[32]. This example illustrates that even when bacteria–DOM coupling is weak, bacteria can still be important for ecosystem functioning. It has been proposed that there is a low-temperature–low-substrate restriction in the Arctic Ocean[33,34]. Conversely, the excessive external input of organic carbon might have deleterious effects on system functioning. In experimental systems, bacteria outgrew coral-reef communities after the addition of DOM[35] or being placed in contact with decaying macroalgae[36].

These examples illustrate that the ability or inability of a bacterial assemblage to grow on specific types of organic matter, either owing to the constraints of community composition or environmental gene expression, can be important for the functioning of globally significant ecosystems. This underscores the need to understand how bacteria function in their natural environment to influence the flux pathways of fixed carbon. From advances in marine genomics and metagenomics[37–42] it can be inferred that enormous bacterial gene diversity is available for assemblage-level bacterial interactions with the ocean. Environmental genomics and proteomics are also yielding insights into bacterial adaptive strategies to ocean life. The challenge is to determine how these strategies are used by bacteria in natural ecosystems, which will require the exploration of the ocean at the micrometre scale — the scale at which the adaptive strategies of bacteria structure marine ecosystems. Bacteria do more than simply cycle carbon; they interact with the whole ocean ecosystem intimately in a multitude of ways[43].

## Microscale interactions

***Spatial distribution of microorganisms.*** Most oceanographic studies assume that bacteria take up homogeneously distributed DOM. This premise relies on the assumption that the DOM that is released from any source diffuses homogenously into the bulk phase before it is taken up by an organism. This view is changing, however, with the recognition of bacterial *in situ* behavioural and physiological responses to DOM-production loci and gradients. The detection of high abundances of decomposer bacteria ($10^6$ per $ml^{11}$) has led to the suggestion that the numbers and activity of primary producers (such as cyanobacteria and algae),

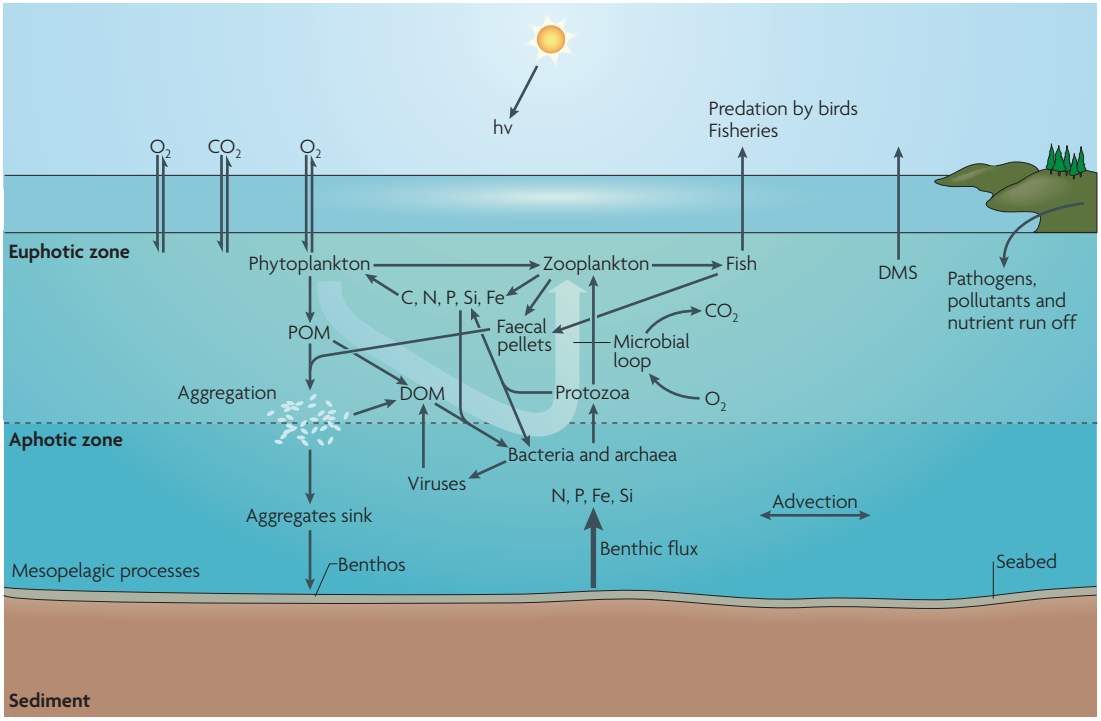


Figure 1 | **Microbial structuring of a marine ecosystem.** A large fraction of the organic matter that is synthesized by primary producers becomes dissolved organic matter (DOM) and is taken up almost exclusively by bacteria. Most of the DOM is respired to carbon dioxide and a fraction is assimilated and re-introduced into the classical food chain (phytoplankton to zooplankton to fish). The action of bacteria on organic matter plays a major part in carbon cycling through DOM. It therefore influences the air–sea exchange of carbon dioxide, carbon storage through sinking and carbon flux to fisheries. DMS, dimethylsulphide; hv, light; POM, particulate organic matter.

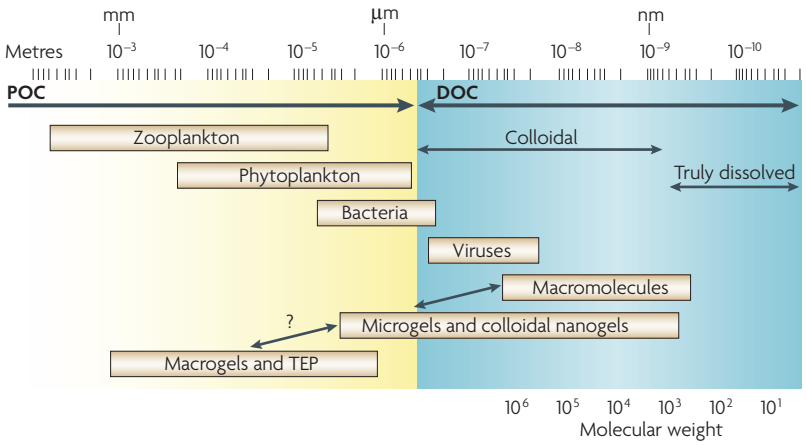


Figure 2 | **The size range of organic matter and microbial interactions in the ocean.** Organic matter has traditionally been divided into dissolved organic matter and particulate organic matter, based on filtration. Here, the full size range of organic matter is shown, from monomers, polymers, colloids and gel particles to traditional particles (divided into particulate organic carbon (POC) and dissolved organic carbon (DOC)). These organic nutrient pools form an intricate three-dimensional architecture of polymers, colloids, gel particles and large aggregates. Living plankton and dead organisms are thought to be embedded in a cobweb-like structure of organic matter that is dynamic over time and space (not shown). The organic matter architecture provides the spatial context within which microorganisms — bacteria, archaea, microalgae, protists and viruses — interact with each other and with the environment. TEP, transparent exopolymer particle. Modified, with permission, from (REF. 72) © (2004) Elsevier Science.

decomposers (such as bacteria) and predators (such as viruses and protists) are similar (Fig. 1). Typically, 1 $mm^3$ (or µl) of surface seawater — considered in this Review to be the bacterium's microenvironment — contains 10,000 viruses; 1,000 bacteria; 100 *Prochlorococcus* cells; 10 *Synechococcus* cells; 10 eukaryotic algae; and 10 protists, although the numbers are highly variable[16,18,44]. The close proximity (0–1 mm) of individual cells suggests that there is potential for many cell–cell interactions.

We note that all microbial trophic groups are in close proximity, so that the adaptive biology of bacteria occurs in a microspatial context that integrates the interactions between all of the trophic groups. For instance, bacteria might respond behaviourally and metabolically to the DOM that is produced by a range of mechanisms, such as algal exudation and cell lysis[18], predation by viruses, which releases prey DOM[45], and the action of protists, which egest food vacuoles that contain DOM and regenerated N, P and $Fe^{2+}$ (REFS 46,47). Such DOM hot spots might occur in the microenvironment of a bacterium over short timescales, perhaps even minutes, owing to the high abundance of microorganisms.

***Motility.*** Motility and sensing enable bacteria to adapt in environments that contain DOM gradients. Dark-field microscopy has revealed that motility is common in natural assemblages of marine bacteria[48–50], although the fraction of bacteria that are able to swim ranges from 5 to 70%. Motility might enable bacteria to achieve spatial coupling with a DOM source, such as a living or dying alga, or a protist[51,52].

***Hydrolytic enzymes and hydrolysis-uptake coupling.*** Marine bacteria hydrolyse polymers and particles using cell-surface-bound hydrolytic enzymes or ecto-hydrolases (protease, glucosidase, lipase, phosphatase, nuclease and chitinase)[22,53–59]. These enzymes, together with membrane-bound transporters, make the bacterial surface reactive for organic matter transformation and uptake. This is the final step that couples bacteria to primary production in the ocean. The bacterial surface is the dominant biotic surface, and it is proposed that 0.1–1 $m^2$ of the bacterial surface per $m^3$ of seawater interacts intimately with DOM[60]. A pelagic bacterium that swims through organic matter leaves a stream of monomers and oligomers behind it. This led J. Stern to refer to bacteria as the "perfect swimming stomachs" (REF. 21).

Bacteria express multiple (multiphasic) transporters that have $K_m$ values that range from nanomolar to millimolar, consistent with adaptation to environments that contain DOM gradients[61–64] (FIG. 2). This raises the question of whether the distinction that is made between oligotrophic and eutrophic bacteria, which is usually based on culturing studies, actually reflects the microspatial adaptations of different strains to nutrient hot spots. Perhaps, bacteria simply move to an appropriate distance from a DOM gradient, so that they can effectively use the carbon resources that are available. The *in situ* behaviour of strains — such as the widely distributed oligotrophic *Candidatus* Pelagibacter ubique (SAR11)[65,66] bacterium or the high-nutrient-loving Roseobacter-clade members — when viewed in such a microspatial context could lead to new predictions about their ecology and distribution[67,68].

A biochemical mechanism that tightly couples the transport and hydrolysis of organic matter would be a useful adaptation in ocean environments, where diffusion rates are high[69] (FIG. 2). The existence of such a coupling mechanism has been difficult to demonstrate experimentally, but genomic, metagenomic and proteomic data might provide hints as to how bacteria obtain sufficient nutrients in an environment such as the ocean.

## A bacterium's-eye view of organic matter

In a sense, microbial oceanography has a long history of studying bacteria at the microscale, including studies on the physiology and growth performance of bacteria that are present in seawater, attached to a particle or clustered around algae and detritus. Traditional distinctions between DOM and POM have been based on filtration methods that have used filters with a pore size of 0.45 µm. However, recent research shows that organic matter in seawater is replete with transparent gels that form tangled webs of components in the form of colloids that are approximately 10 nm long ($10^8$ colloids per ml[70,71]) and mucus sheets and bundles that are up to 100 µm long (1,000 sheets or bundles per ml[72,73]). Colloids, sheets and bundles interact to form macromolecular networks that are 100 µm or more long. The polymeric components of transparent gels are probably derived from microorganisms. Phytoplankton and bacteria produce

Pelagic
Relating to or occurring in the oceanic water column.

Oligotrophic
An aquatic environment that has low levels of nutrients and primary production (for example, high mountain lakes or the open ocean).

Eutrophic
A marine or lake environment with a high nutrient concentration and high levels of primary production.

Phytoplankton
Composed of microscopic plants and photosynthetic cyanobacteria. These are the main primary producers in marine food webs, ranging in size from 1 µm to approximately 100 µm.

cell-surface mucus, which can either be released or, alternatively, solubilized by cell-surface-acting enzymes. Phytoplankton release condensed polysaccharide particles by exocytosis, which form gels in seawater[74]. Most (90–95%) of the DOM is refractory to degradation by bacteria and it has been proposed that some DOM components serve as stable scaffolds of a gel architecture that is inaccessible to bacterial ectohydrolases[75]. Potentially, gels are a nutrient sink and represent a mosaic of nutrient hot spots. This nutrient pool is huge, as approximately 10% of all the DOM ($70 \times 10^{15}$ grams of carbon) that is found in the ocean is present in the form of gels. This represents a carbon pool that is larger than all of the carbon that is present as biomass in the ocean[72]. Depending on their surface properties, gels might adsorb DOM components from seawater, and this could be significant given the large surface area of these gels. Bacteria can attach to gels[76,77] and it is possible that they hydrolyse the nutrients that are present on the gel surface using cell-surface hydrolases[78]. This, in turn, might alter the local architecture of the gel and the nutrient dynamics at the microscale.

The architecture of gels has been detected using stains that target proteoglycan, protein and DNA (FIG. 3). These methods might not be sensitive enough to reveal all of the details of gel architecture, and some, or even most, of the gel might be too diffuse to be detected by imaging technology. Techniques that are useful for macromolecular-level imaging, such as atomic force microscopy[79–81], could also be useful for studying marine gels. In addition, the architecture of organic matter is based on information obtained using methods that can only detect structures in two dimensions, after sample collection on filters. One important, but challenging, goal is to develop methods that can visualize and biochemically characterize the microscale architecture of the organic matter gel matrix in relation to the distribution of bacterial taxa in various physiological states. Confocal laser microscopy might be useful, as gels can be viewed in three dimensions, and this method might be able to more closely pinpoint the physical relationship between bacteria and the organic matter of these gels. Fluorescently labelled lectins, and other probes for biochemical composition, might be useful for determining the composition of gels[82,83]. Microspatial viscosity is probably variable[84] (for example, on the algal surface or near a lysing dinoflagellate) and this might influence bacterial adaptive behaviours[85,86], such as motility, microspatial distribution and *in situ* physiological states[87]. Individual bacteria might experience different microspatial nutrient concentrations at different positions within the organic matter matrix. Also, bacteria could export inhibitory molecules that become bound to the gel matrix, so enabling them to compete with other bacteria by niche modification. Clearly, much remains to be learnt about the structural and chemical dynamics of the microscale architecture of organic matter and its relationship with bacteria.

Research on gel architecture has altered how microbial oceanographers think about the function of bacteria in marine ecosystems. Microspatial architecture provides huge surfaces for bacterial attachment and interactions. Indeed, the enormous genetic diversity of marine bacteria[37–39,88,89] might be explained by the ability of gels to provide niche diversity in seemingly homogeneous ocean waters. For example, many, or all, bacteria that are currently considered to be free-living, for example, SAR11, might in fact be attached to the gel matrix. This would mean that oligotrophs, such as SAR11, do not compete directly for dissolved solutes. Acinas and colleagues[90] suggested that the clusters of microdiversity that they detected in pelagic bacteria might be due to the presence of pelagic microniches. As primary productivity and DOC generally decrease offshore[91,92], the hot spots of DOM production and bacterial activity might be less abundant. A bacterium's-eye view of organic matter offers a more elaborate and dynamic spectrum of choices, rather than the traditional dichotomy that is derived from regarding bacteria as either attached or free living.

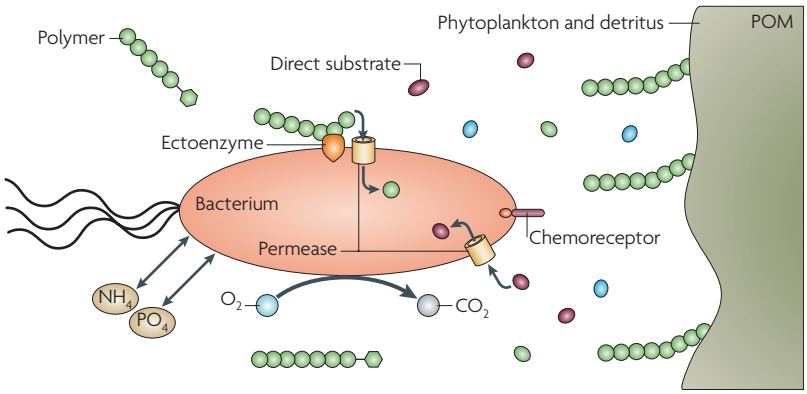


Figure 3 | **Adaptive strategies of bacteria in the ocean.** The adaptions that are shown are relevant to the structuring of marine ecosystems by bacteria at the nanometre–millimetre scale, but they also affect ocean-basin-scale processes, including the cycling of carbon, nitrogen and phosphorus and the biogeochemical behaviour of organic matter (for example, sinking). The strategies that are depicted here include motility, environmental sensing, permeases and cell-surface hydrolases. They enable coupling between bacteria and organic matter. For example, phytoplankton or cell debris, depicted as particulate organic matter (POM) and bacteria. Polymers also require hydrolysis to direct substrates before trans-membrane transport. Note the ability of bacteria to take up, as well as release, $NH_4$ and $PO_4$.

**In situ *growth rate variability.*** A major physiological variable among oceanic bacterial assemblages is the ability to grow over a broad range of growth rates, from nearly zero to more than one doubling per day[12,19,25,93,94]. These growth rates — in combination with population size and biomass — are reflected by the variation in BCD and bacteria–phytoplankton coupling. Marine isolates typically grow fast in enriched culture media (although notably SAR11 cannot grow on rich media[95]). We propose that high growth rates occur periodically in nutrient-rich microzones[96–98], for example, near to, or on, nutrient-rich particles[99,100], plankton surfaces or in the guts of animals. Thus, the slow average-growth rates that are typically observed for pelagic assemblages do not preclude the possibility that a small fraction of the assemblage might be growing rapidly[101–103].

***Bacterial growth efficiency (BGE).*** This is a crucial variable for ecosystem function. The BGE compares the fraction of assimilated carbon that is respired with the fraction that is used to increase bacterial biomass. The BGE for natural assemblages is usually 10–30%, but this can vary widely from 1 to 40%; this means that 60–99% of all assimilated carbon is respired[27,104–106]. The variation in BGE can affect the role of bacteria in carbon cycling, owing to alterations in the partitioning of carbon between carbon dioxide and biomass, which is accessible to animals. BGE varies between oligotrophic and eutrophic ecosystems[107]. A mixed population has a range of BGE's that are dependent on the physiological state of individual bacteria and their spatial and temporal interactions with organic matter[107]. It is important to place BGE in a microspatial context. A goal should be to determine how the environment affects the respiration, growth and phylogenetic identity of individual cells and thereby influences BGE on the microscale.

## Bacterial coupling to primary production

Bacterial performance (as measured by growth, respiration and other metabolic activities) is constrained by the fact that bacteria are obligate osmotrophs, whereas primary production mainly produces particulate and polymeric organic matter. As a result, the *in situ* physiological attributes and adaptations of bacteria must be responsive to the production of DOM in the microenvironment of the bacterium. Another adaptive challenge for the bacterium is to position itself optimally in relation to DOM production. For example, is it adapted to growth in DOM-production hot spots or does it exploit the environmental volume-fraction at the tail-end of DOM gradients? Methods that have been developed to determine the growth rate and phylogenetic identity of individual cells within natural assemblages constitute powerful tools to relate *in situ* physiology and taxonomy in the microenvironment[93,94,108–111].

Bacteria, such as SAR11 (REFS 65,66), members of the Roseobacter clade, including the alphaproteobacteria Roseobacter-clade-affiliated cluster[67,68,112], and members of the Bacteroides clades[113,114], might be good models for defining the adaptive strategies that are used by bacteria in relation to DOM-production regimes in microenvironments. Available substrates are maintained at picomolar to nanomolar concentrations in the bulk phase — Hedges and colleagues estimated that "$10^{12}$ diverse organic molecules...[are]...dissolved in every millilitre of seawater…", which are "dynamically shaped and buffered by microbiological action" — and, therefore, DOM production forms DOM gradients against the background of extremely low bulk-phase concentrations.

In proposing how bacteria adapt to use low or high DOM concentrations it is important to recognize that the conversion of POM to DOM involves many different phytoplankton organisms that use varied mechanisms of DOM production. Therefore, numerous adaptive strategies might be used by diverse bacterial taxa.

Phytoplankton are organic matter production loci. The main processes that convert up to half of all the primary production into DOM must occur before substantial amounts are transferred through multiple trophic levels and respired. The most likely mechanisms of DOM formation are direct processes, such as phytoplankton exudation and lysis (by virus attack or nutrient stress), bacterial interactions with live phytoplankton (for example, commensalism or predation) and the bacterial enzymatic degradation of recently dead phytoplankton and protists. The aggregation of phytoplankton and phytoplankton detritus could facilitate bacterial access to DOM. Finally, protists that exhibit boom-and-bust growth cycles could enable bacteria to use protist biomass.

***Bacteria–phytoplankton interactions.*** One way for bacteria to metabolically couple to primary production would be to swim up to phytoplankton and use cell-surface hydrolases to kill them[115]. Bacteria have various adaptations that allow them to attach to dead phytoplankton and cause hydrolysis[116]. If bacteria are randomly distributed, each bacterium would be a few hundred micrometres away from the nearest phytoplankton cell[9] — a distance that most motile marine bacteria could traverse rather quickly (although many bacteria are non-motile and would not use this strategy). Bacteria can cluster around phytoplankton[117] to create considerably higher concentrations than the average for seawater, which is $10^6$ bacteria per ml[85]. Tight spatial coupling between marine bacteria (*Pseudoalteromonas haloplanktis* and *Shewanella putrefaciens*) and a motile alga (*Pavlova lutheri*) has been filmed and referred to as the 'pestering' of algae, because the bacteria closely tracked motile phytoplankton[52]. It seems that some phytoplankton–bacteria associations might even be species specific[118–120].

Phytoplankton produce surface mucus, polysaccharides and proteoglycans, which might serve as a protection from bacteria; this is analogous to corals, which convert substantial photosynthate into mucus for defence against microbial invasions[121]. Some phytoplankton also produce inhibitory compounds[122]. Mucus creates a region around the phytoplankton cell that is rich in organic matter, and is known as the phycosphere[117]. The nitrogen- and phosphorus-depleted mucus could adsorb nitrogen- and phosphorous-rich materials, such as polymers and colloids, from seawater, resulting in the development of a rich gel medium. Although much research has been done on bacteria that are attached to phytoplankton it has been technically difficult to study the *in situ* physiology of bacteria (growth, respiration and antibiotic synthesis) in the phycosphere. It is also likely that the community composition of bacteria in the phycosphere will differ from that in the local ocean environment. Significant diurnal alterations in the bacteria–phytoplankton relationship are to be expected, but these need to be addressed in a microspatial context. As the phycosphere is organically rich it has been proposed that it could support the proliferation of human pathogens or other bacteria that are adapted to high-nutrient environments[97].

Although bacteria can cluster near, or attach to, phytoplankton[52,69,123], the biochemistry of the bacteria–phytoplankton interaction is poorly characterized.

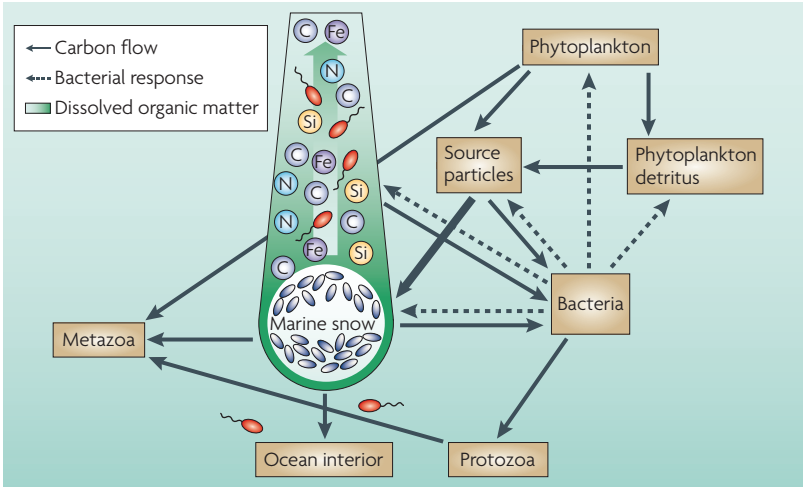


Figure 4 | **Microbial cycling of carbon in marine snow.** The aggregation of organisms and organic matter to form sinking marine snow plays a major part in ocean ecosystems. Marine snow is highly colonized by bacteria, presumably because it is formed from pre-colonized source particles that transport carbon, nitrogen, phosphorus, iron and silicon into the ocean's interior. The high hydrolytic enzyme activities of bacteria convert the aggregate organic matter into non-sinking dissolved organic matter (DOM), forming plumes in the ocean. Free-living bacteria that are attracted to such DOM-rich hot spots respire carbon to carbon dioxide and their biomass production feeds into the pelagic foodweb. Colonizing bacteria might adapt by releasing their progeny into the plume. The intensely colonized marine-snow aggregates create hot spots of life, and the death of bacteria, viruses (decay), protozoa and metazoa — thus having substantial roles in the structuring of the marine ecosystem.

Do bacteria produce either endohydrolases or exohydrolases, or both, and can bacteria couple hydrolysis and the uptake of DOM in the phycosphere? Endohydrolase action could release polymers into the microenvironment that might then be accessible to exohydrolases, which could, in turn, release monomers that bacteria can take up. The released polymers might be detectable as transparent gel particles in seawater[124,125].

*Microscale processes in the phycosphere.* Because the cell-associated phycosphere has fundamental implications for the adaptive biology of bacteria and phytoplankton, we must address microscale processes within the phycosphere at the mechanistic level. The relationship between clustering bacteria and phytoplankton is probably complex and variable over short timeframes. Clustered bacteria could benefit the phytoplankton cell by enhancing nutrient regeneration in the phycosphere at the expense of dissolved organic nitrogen and phosphorus. The inorganic nutrient hot spot that surrounds the phytoplankton cell could make its microenvironment eutrophic in what might otherwise be oligotrophic water according to bulk seawater analysis. This hot spot of nutrients could also receive protist contributions owing to the intense grazing of bacteria by bacterivorous protozoa, which would release regenerated nitrogen, phosphorus and iron into the microenvironment. However, if seawater becomes depleted in dissolved organic nitrogen and dissolved organic phosphorus then the presence of clustering bacteria might also reduce the release of dissolved inorganic nitrogen and dissolved inorganic phosphorus, thus further limiting primary productivity. Stressed phytoplankton might be unable to defend themselves by the release of mucus or antibiotics[122] and clustering or colonizing bacteria could then kill the phytoplankton for use as a growth substrate. The nutrient-hot-spot premise[9], if supported, would change our concepts and models of the nutrient regulation of primary production. Current models assume there is homogeneity in nutrient distribution. This is another example where further study of a microspatial process promises to yield insights into the regulation of primary production and carbon cycling.

The phycosphere might also be important for the use of dimethylsulphoniopropionate (DMSP) by bacteria. Many marine algae produce this osmolyte and release some of it into the microenvironment. Some marine bacteria, including SAR11 and Roseobacter clades, use DMSP as a source of energy[109,126,127]. Intracellular pools of DMSP are present in algae at approximately 0.2–0.5 M, although bulk seawater concentrations are approximately 10 nM. DMSP and extracts of *Pfiesteria piscicida* function as chemoattractants for *Silicibacter* spp. strain TM1040, an alphaproteobacterium that can grow in close association with the dinoflagellate *P. piscicida*. Chemotaxis might enable *Silicibacter* spp. strain TM1040 to remain close to *P. piscicida*[128]. It would be of interest to know whether community composition and the growth environment in the phycosphere affects the metabolic fate of DMSP[129,130]. This could affect the rate of bacterial hydrolysis of DMSP to dimethyl sulphide (DMS)[131,132]. As DMS can affect climatic processes, understanding the regulation of the conversion of DMSP to DMS at the microscale could lead to more informed global climate models.

The action of cell-surface-associated bacterial protease and glucosidase on diatom surfaces might reduce diatom 'stickiness' and the aggregation potential. This enzymatic 'pruning' of the diatom mucus would require microscale interactions between diatoms and bacteria; a high concentration of bacteria in the phycosphere would probably increase the rate of this process[133]. Diatom aggregation can form large, rapidly sinking aggregates known as marine snow (FIG. 4), which are important for the export of organic matter to the ocean's depths[134] as well as the demise of algal blooms. Understanding the microscale action of bacterial hydrolases on diatom surfaces could help us to predict the timing of bloom termination and the regulation of carbon export in an ecosystem context — this would represent a genome–biogeochemistry connection.

***Interaction of bacteria with protists.*** Protists consume more than half the primary production in many ecosystems[135]. How can we reconcile the large grazing pressure on phytoplankton by protists with an equally high bacterial carbon demand? Perhaps when the BCD and protist grazing are both high, a large fraction of the organic matter that is ingested by protists is released through the lysis of these protists by viruses or other factors[136]. Bacteria can rapidly colonize dead protists and degrade fresh protist detritus. However, it remains to be determined whether heterotrophic bacteria cause significant protist

Chemotaxis
The sensing by bacteria of chemical gradients, and movement up or down a gradient towards or away from a chemical source.

Dimethyl sulphide
(DMS). A sulphur-containing organic chemical compound that is a breakdown product of dimethylsulphoniopropionate (DMSP). It is also produced by the metabolism of methanethiol by marine bacteria that are associated with phytoplankton.

Marine snow
Composed of organic aggregates more than 0.5 mm in diameter. These macroscopic particles are enriched in organic matter and are inhabited by a rich and diverse community of phytoplankton, protozoans and bacteria.

mortality in an 'eat them before they eat you' scenario. In this hypothetical scenario, protists are the processors of primary production, their enzymes convert the POM of prey to DOM and their newly synthesized biomass is consumed by bacteria as predators. Protists are important grazers of small, highly abundant phytoplankton, such as *Synechococcus, Prochlococcus* and picoeukaryotes[135]. Therefore, protist processing of primary production might be one mechanism that increases the coupling of bacteria to primary production.

***Interactions of bacteria with detritus.*** Dead phytoplankton cells are a source of organic matter for bacteria, which readily colonize such material to form a detritosphere[133,137] (FIG. 4). Bacterial colonization and enzymatic hydrolysis could convert detritus to DOM. However, not all bacteria attach to dead phytoplankton. In an experimental system, dense clusters of bacteria were recorded swimming around a killed dinoflagellate, and these were modelled to predict bacterial interactions with DOM hot spots[51,123,138]. Depending on the particle sinking rate, the effects of bacteria on dead phytoplankton cells might result in DOM plumes (as discussed later).

Diatoms attract bacteria, and freshly lysed diatoms comprise organic matter hot spots that support the robust growth of bacteria. Because diatoms require silicon for growth, the mechanisms of silicon recycling are important. Unexpectedly, it was found that bacteria that do not require silicon regulate the regeneration of silicon from dead diatoms[116]. Although bacteria use their ectohydrolases to solubilize organic matter, the action of these enzymes also removes the protective proteoglycan silaffins from the silicon frustules[139] and it is the silaffin proteins that normally prevent the dissolution of silicon[116]. This process requires colonization by bacteria, and those bacteria with high cell-specific protease activity cause more rapid dissolution. In view of the importance of diatom production, and the regeneration of silicon, the regulation of the silicon cycle by bacteria could affect the carbon-export flux. Consequently, an understanding of the biochemical basis of this process should be incorporated into biogeochemical models.

This is an example of a bacteria-mediated process at the microscale that results in the regulation of the silicon cycle and coupled silicon–carbon cycles in the global ocean. Bacterial enzymatic action involves a trivial amount of carbon that is contained in the proteoglycan covering and is readily accessible to bacteria. However, the action has a major effect on the biogeochemical behaviour of silicon. This might suggest that bacteria have a more general ability that allows them to de-mineralize living or dead silicoflagellates, radiolarians or calcified phytoplankton, most notably *Emiliania huxleyi*. However, whether bacteria really do de-mineralize these organisms will depend on the relationship between the mineralized structure and organic matter[140].

## Marine snow and nutrient hot spots

***Aggregation state of the ocean.*** The aggregation of organic matter is a fundamentally important process in the functioning of marine ecosystems. Aggregation generally increases the sedimentation rate of organic matter[141]; the degree of aggregation influences the residence time of component particles in the upper ocean, where they are acted on by bacteria that respire carbon and regenerate nutrients. Sinking aggregates are a dominant conduit for the export flux of organic matter, the variation of which influences carbon storage. The components of the organic matter continuum tend to aggregate to varying degrees forming a dynamic size spectrum. Bacteria can inhibit, as well as enhance, the aggregation state of the system (by reducing the stickiness with hydrolases or increasing it by mucus production)[78,133]. Aggregates can attract microorganisms, such as bacteria, protists and viruses, as well as gels, colloids and cell debris. Although most aggregates are microscopic, some are large, such as marine snow, which is visible underwater, and has been extensively studied for its ecosystem significance. Next, we discuss a model that illustrates the significance of microscale activities of bacteria in marine snow, and which has implications for ocean-basin and global-scale processes.

***Marine snow.*** Bacteria colonize marine snow in population densities that reach $10^8$–$10^9$ per ml[142]. The expression of several ectohydrolases, such as protease, lipase, chitinase and phosphatase, is high, but glucosidases are not well expressed. This enzyme complement digests some of the DOM in marine snow[78]. However, the colonizing bacteria use only a fraction of the hydrolysate (weak coupling)[78,143] and, therefore, the sinking marine snow leaves an extended plume of DOM behind them that other bacteria can use. One model predicts that approximately half of all the BCD in the ocean is satisfied by bacterial interaction with these plumes[144] (FIG. 4).

Marine-snow plumes help to retain nitrogen, phosphorus and iron within the upper mixed layer of the ocean and this supports primary productivity. In addition, as the glucosidase activity of bacteria in marine snow is low, a disproportionate amount of carbon could sink below the upper mixed layer, thus increasing the carbon:nitrogen and carbon:phosphorus ratios in aggregates and causing carbon storage. Because marine snow is organically enriched, bacterial growth might occur with high growth efficiency[145]. One strategy that might enable such loose organic matter–bacteria coupling to be adaptive is for the attached bacteria to release their progeny into the plume[145,146]. Thus, the microscale interactions of bacteria could influence fundamental biogeochemical processes, such as carbon storage and the regulation of carbon flux, as well as provide a microspatial framework for understanding the behavioural and biochemical strategies of free-living bacteria that might be adapted to using the DOM at high concentrations in the plumes. Such strategies might also be used by bacteria at depth[143] because marine snow can sink to hundreds or even thousands of metres[147]. Further, elucidating the *in situ* expression of ectohydrolytic enzymes should help us to understand the biochemical bases of the interactions of bacteria with organic matter, and link carbon storage and carbon biogeochemistry with gene expression. Whether bacterial action will increase or decrease the

net efficiency of the biological carbon pump cannot currently be predicted, but we think that better models will be possible through such studies on the biochemical bases of bacteria-organic-matter interactions.

How bacteria influence the aggregation and biogeochemical fate of carbon is also relevant to concerns about ocean fertilization and carbon sequestration, in which the goal is to maximize aggregation and export carbon flux. Our discussion in this Review suggests that models that predict the outcome of ocean fertilization should include the roles of bacteria and, specifically, the *in situ* expression of selected ectohydrolases[148].

Many bacteria are non-motile, notably pelagic Bacteroidetes and SAR11 (REF. 65). Bacteroidetes are specialized for colonizing aggregates such as marine snow[113,114,149]. How do non-motile bacteria accumulate to form such large populations on marine snow, which has such a short residence time in the upper ocean? Perhaps, non-motile bacteria, being particle specialists, attach first to the highly abundant small gel particles[113,114], which are in the 10-µm length range and are several orders-of-magnitude more abundant than marine snow (typically 1–10 aggregates per litre in the upper ocean)[72]. Aggregation with larger particles and agglomeration with other materials, such as phytoplankton or detritus, forms marine snow. Therefore, the source particles[150] would contribute diverse bacteria, including non-motile bacteria, that have been selected for by attaching to the particles that eventually aggregate to form marine snow. Future metagenomic surveys should consider these seascapes of organic matter, which potentially contain an immense diversity of bacteria that are attuned to the nature and dynamics of their miniscule worlds.

## Conclusion and future prospects

Microbial oceanography is a field that is caught between scales — microbial processes must be understood at the scale of the individual microorganism, but yet we want to understand the cumulative influence of microbial processes on the ocean as a biogeochemical system. We have argued that understanding the biochemical bases of how bacteria interact with the ocean system at the nanometre (molecular) to millimetre scale can provide insights into globally significant biogeochemical processes. Therefore, an understanding of nanoscale biochemistry can be extended hierarchically[151] to the global ocean and the Earth's biogeochemistry. Indeed, some insights are not accessible by large-scale studies alone. We need a robust understanding of microscale biogeochemistry and how it fits with ocean and global biogeochemical studies of all scales. This should result in models of the biochemical bases for the interactions among organisms and the environment.

We need additional methods and instruments that can measure individual bacteria-cell *in situ* growth and respiration rates in natural seawater in three dimensions, without perturbing these assemblages by using filters. It might also be possible to study other basic ecological interactions, such as grazing and phage lysis. Methods and instruments that can be used to study the activities of microorganisms in the context of their ecosystem are on the horizon. They should enable us, for example, to interrogate the chemical and physical characteristics of the environmental architecture in relation to bacterial diversity, distribution and activity. With the current momentum in nanotechnology, nanobiology and advanced imaging we see no reason why microbial ecologists cannot explore the oceans at the nanometre–millimetre scale. Eventually, one goal of microbial oceanographers should be to understand carbon cycling and to visualize microbial interactions that affect the biogeochemical state of the ocean.

Perhaps it is stating the obvious, but we would probably not be concerned for the health of corals or tropical forests if they were invisible. The microscale architecture of the ocean and its relationship with much of the diversity in the sea may well be delicate, and could be sensitive to new patterns of enzyme expression or activity that might arise owing to warming and acidification. It is conceivable that exploration of the ocean at the microscale will yield novel measures of the ocean's biogeochemical state, or ocean health. We stress the need for a concerted research effort in microscale biogeochemistry as a discipline that is integrated with environmental genomic and ecosystem research and climate science.


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### Competing interests statement
The authors declare no competing financial interests.

### DATABASES
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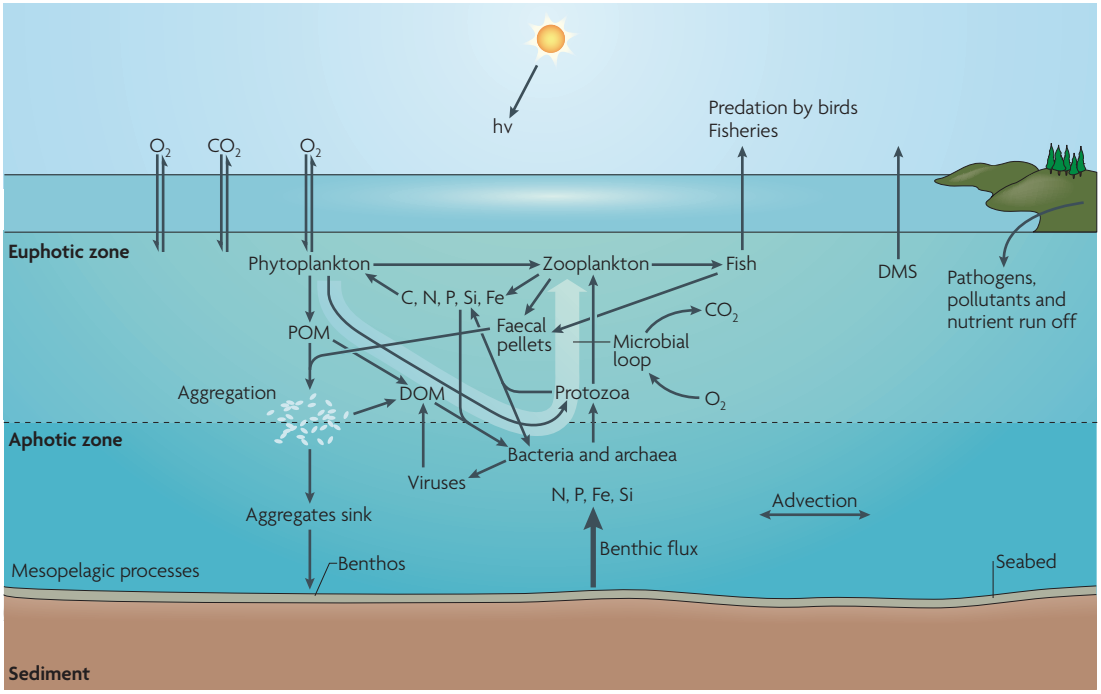
**ERRATUM**

# Microbial structuring of marine ecosystems

*Farooq Azam and Francesca Malfatti*

*Nature Reviews Microbiology* **5**, 782–791 (2007), doi: 10.1038/nrmicro1747

In the above article, an arrow was missing from figure 1. The correct figure is shown below. In the same article, the legend to figure 4 should have indicated that the figure was first published in reference 145. We wish to apologize to the author, and to readers, for any confusion caused.




**CORRIGENDUM**

# Microbial structuring of marine ecosystems

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In the above article, the following passage of text on page 783 might have been misleading: 'The detection of high abundances of decomposer bacteria ($10^6$ per $ml^{[1]}$) has led to the suggestion that the numbers and activity of primary producers (such as cyanobacteria and algae), decomposers (such as bacteria) and predators (such as viruses and protists) are similar (Fig. 1).' It should have read: 'The detection of high abundances of decomposer bacteria ($10^6$ per $ml^{[1]}$) has led to the suggestion that every microlitre of seawater contains all the components of the microbial loop: primary producers (such as cyanobacteria and algae), decomposers (such as bacteria) and predators (such as viruses and protists) (Fig. 1).' The authors apologize to the readers for any confusion caused.