Marine biofilms: diversity, interactions and biofouling

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Abstract | Marine biofilms are ubiquitous in the marine environment. These complex microbial communities rapidly respond to environmental changes and encompass hugely diverse microbial structures, functions and metabolisms. Nevertheless, knowledge is limited on the microbial community structures and functions of natural marine biofilms and their influence on global geochemical cycles. Microbial cues, including secondary metabolites and microbial structures, regulate interactions between microorganisms, with their environment and with other benthic organisms, which affects their community succession and metamorphosis. Furthermore, marine biofilms are key mediators of marine biofouling, which greatly affect marine industries. In this Review, we discuss marine biofilm dynamics, including their diversity, abundance and functions. We also highlight knowledge gaps, areas for future research and potential biotechnological applications of marine biofilms.

Biofouling
Biofouling is the colonization of submerged surfaces by microorganisms, plants, algae or small animals; it has destructive effects on the substrate.

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One of the first descriptions of biofilms, decades before the term 'biofilm' was coined, involved marine bacteria[1,2]. The number of marine microorganisms with a biofilm lifestyle is considerably higher than previously thought[3]. Technical advances, including sequencing and imaging, have accelerated biofilm research. However, our knowledge of marine biofilms lags far behind that of biofilms associated with human diseases. Furthermore, information on marine biofilms is primarily drawn from biofilms on artificial surfaces, such as ship hulls, as causal agents of biofouling or biocorrosion[4]. Once immersed in seawater, new surfaces, whether biotic or abiotic, can quickly adsorb organic matter that forms a nutrient-rich layer in a few hours, followed by microbial colonization in less than a day[5]. Therefore, microbial biofilms cover all artificial objects in marine environments, ranging from bottles to oil platforms. Such biofilms cause trillions of US dollars in economic loss annually to maritime industries, such as aquaculture, marine transport, oil and gas, and desalination industries[6,7]. Microplastics and their colonization by microorganisms have become important research topics[8–11].

In addition to man-made surfaces, marine biofilms develop on natural surfaces, including animals, plants, zooplankton, phytoplankton, micro-aggregates and macro-aggregates, and transparent exopolymer particles[3]. Biofilms on living organisms provide a 'second skin' that can be either detrimental to beneficial for the host[12], and can reduce the availability of light, gases, nutrients, chemicals and toxins to plant or animal hosts. For example, biofilms reduce the light available to macroalga by half, which affects their primary productivity and depth distribution[13]. Biofilms on particles and aggregates are hotspots of microbial abundance and activity in the ocean[14–17]. Microbial degradation of marine snow decreases the sinking of particulate organic matter from the surface ocean and subsequent carbon sequestration in the deep ocean and sediments[18,19]. However, the spatial and temporal dynamics of these processes are unclear due to the difficulty of particle sampling and quantification of microbial activity on particles.

Biofilms develop not only on surfaces but also on solid–liquid, liquid–liquid, liquid–gas and solid–gas interfaces[20]. As the largest ecosystem on Earth[21], the ocean provides two such interfaces: the seafloor and sea surface (FIG. 1). Microorganisms in these interfaces are threefold to fivefold more abundant than that in seawater[22], and fluxes of gases and nutrients across these interfaces have key ecological and biogeochemical roles. Materials or cells attaching to these interfaces change the biological, physical and chemical properties of the surface[23], and the abundance, activity and diversity of microbial communities in these interfaces strongly influence the fate of inorganic and organic matter.

Given the major effects of marine biofilms on marine industries, biogeochemical cycles and marine life, for example, metamorphosis, we must understand the diversity, dynamics, assemblage and functions of marine biofilms. In this Review, we discuss microbial diversity and functions, spatial and temporal changes in microbial community structures, microbial interactions of marine biofilms on natural or manufactured surfaces and interfaces, interactions between biofilms and marine benthic organisms, and the effects on maritime industries.

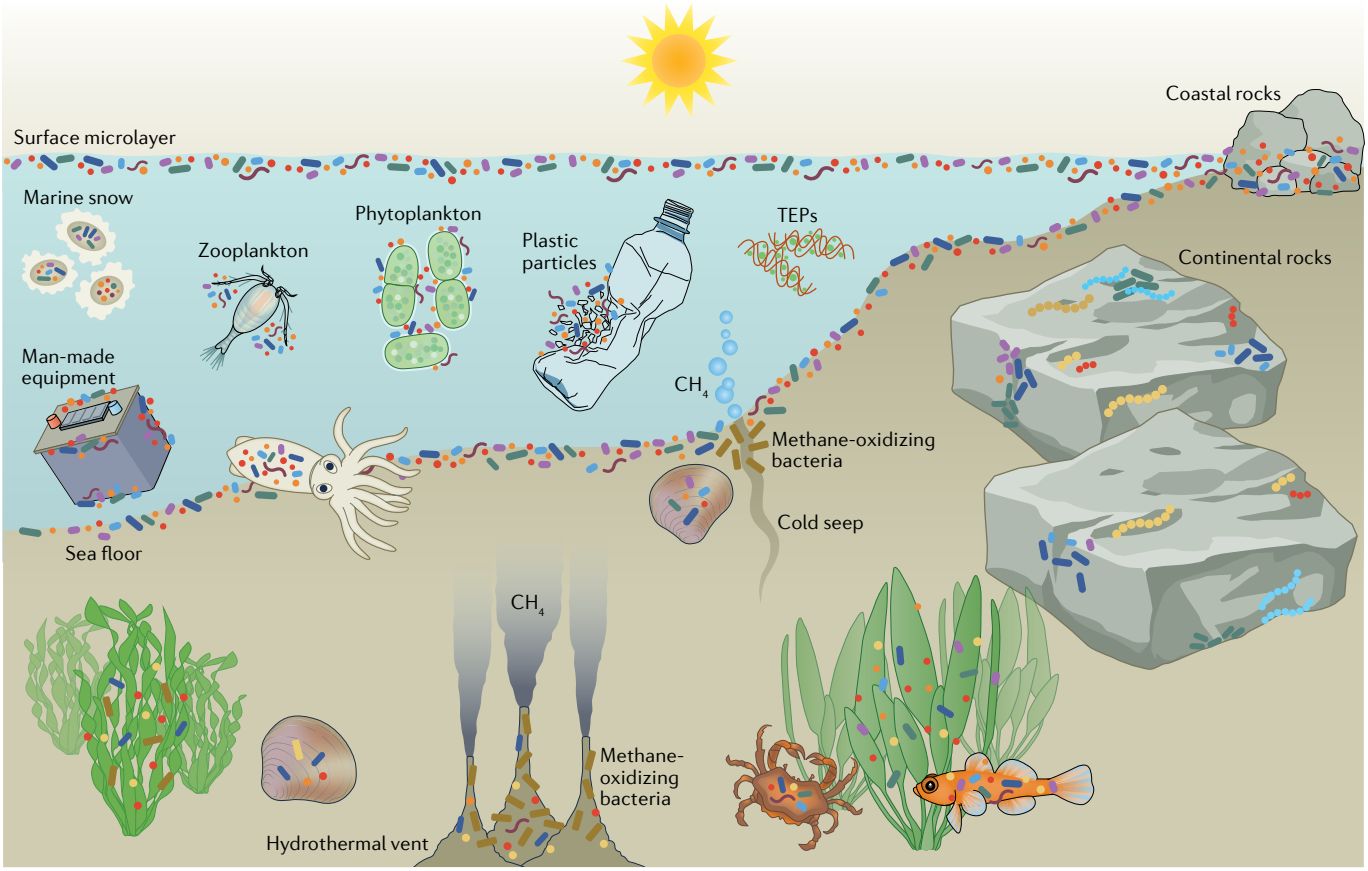

Fig. 1 | **Marine biofilms on different substrates in the ocean.** Microbial cells settle on the surface of microlayers, zooplankton, phytoplankton, transparent exopolymer particles (TEPs), marine snow, macrophytes, squids, crabs, mussels, snails, fishes, cold seeps, hydrothermal vents, rocks, seafloor, plastic particles and other man-made items (for example, submarine equipment) to form biofilms in the global ocean.

Diversity and dynamics

Unique community structures. Approximately 40–80% of living bacterial and archaeal cells on the planet reside in biofilms[3]. Whereas some host-associated biofilms, such as those attached on human teeth[24], have been extensively studied, microbial diversity in marine biofilms remains largely unknown on a global scale (FIG. 2). Diverse marine microorganisms, including bacteria, archaea, diatoms, fungi, flagellates, ciliates and multicellular eukaryotes, can colonize surfaces[25]. Viruses, extracellular materials and extracellular vesicles are also important components of marine biofilms[26–28]. Molecular techniques have greatly increased our knowledge of community structures of marine biofilms, particularly of bacteria, archaea and diatoms. The number of observed microbial taxa in natural biofilms has drastically increased from less than 50 species identified by culture-dependent methods[29] to about 100 operational taxonomic units (OTUs) identified by different DNA fingerprinting methods[30,31] and >10,000 OTUs by high-throughput sequencing[32]. Confocal laser scanning microscopy[32,33], confocal reflection microscopy[34], contact-angle wettability sensors[33], photoacoustic spectroscopy and scanning electron microscopy have been used to monitor living organisms in marine biofilms[35] as well as their surface properties, structures and photosynthetic ability.

Marine biofilms are distinct from freshwater biofilms[36,37]. Betaproteobacteria (also known as Pseudomonadota) are often the abundant phylum in freshwater biofilms but not in marine biofilms. Furthermore, the microbial community composition in marine biofilms is distinct from that of plankton[15,38–40]. Pennate diatoms (such as *Navicula* and *Nitzschia*) are dominant diatom groups in marine biofilms[25,41]. Sphingomonadaceae of Alphaproteobacteria, Alteromonadaceae of Gammaproteobacteria and Bacteroides are common bacterial taxa in marine biofilms, whereas SAR11, *Prochlorococcus* and *Synechococcus* are abundant in seawater. Similarly, viruses, fungi and other eukaryotes in biofilms on plastic and other substrates[9] are distinct from those in the planktonic environment[27,28,42–47]. Thus, adaptation to a surface-associated lifestyle drives a community structure that differs from planktonic microorganisms[3,15,19].

The microbial community structure, physical conditions, functions and metabolisms of marine biofilms can rapidly change in response to substrate changes (for example, surface wettability[46,48] and substrate type[19,25,32,48–51]) and environmental variables, including salinity, temperature[52], nutrient content[53], ultraviolet radiation[54,55] and flow dynamics[56]. These parameters vary substantially over time and space, leading to dynamic

Biocorrosion
Biocorrosion refers to the deterioration of metal surfaces owing to the presence of biofilms.

Aquaculture
Aquaculture refers to the rearing of aquatic animals or the cultivation of aquatic plants, including breeding, raising and harvesting, for the production of food and commercial products, restoring and creating healthier habitats as well as rebuilding threatened or endangered species populations.

Marine snow
Small organic detritus and inorganic particles drifting towards the seafloor from the upper layers of the water column. Marine snow is formed by dead organisms, faecal matter, sand, soot and other dust.

Metamorphosis
Metamorphosis refers to a biological process of evident and sudden change in animal body structure through cell growth and differentiation after birth or hatching.

Surface wettability
Surface wettability is the tendency of a liquid to spread on or adhere to a solid surface. It is controlled by a balance between adhesive (liquid–surface) and cohesive (liquid–liquid) forces.

patterns of marine biofilms and limited overlap between communities on different substrates and at different locations. Thus, we only summarize the general bacterial community dynamics and highlight biofilms on specific substrates below.

Temporal shifts. Typically, bacteria are the first colonizers of a new surface, followed by unicellular eukaryotes, such as diatoms, dinoflagellates and protozoa, and then multicellular eukaryotes[57] (FIG. 3a). Alphaproteobacteria (such as the Rhodobacteraceae) are pioneer surface colonizers possibly owing to their adaption to relatively oligotrophic environments[58–60]. Gammaproteobacteria (such as *Alteromonas* spp., *Pseudomonas* spp. and *Acinetobacter* spp.) also colonize various substrates within hours, probably because of their adaption to diverse and fast-changing environments[58,61–63]. Flavobacteria are also among initial colonizers but they have hardly been detected by 16S rRNA gene sequencing because of the primer bias[64]. However, detailed information on the microbial community succession over the first hours to days of marine biofilm formation is limited. Biofilm communities on particle surfaces are most diverse in the first 8–20 h, with diversity gradually dropping to a minimum at 36–44 h and rising again, reaching a plateau after 72 h, as shown in laboratory experiments with paramagnetic micro-particles made of chitin as a model system[65]. The growth dynamics and timing of early biofilm communities are dependent on many factors, including substrate characteristics, hydrographic conditions, nutrient availability and free-living microbial communities.

After initial colonization, the number of cells and species generally increases[30,66] and bacterial communities are dynamically dominated by Proteobacteria, Bacteroidetes, Cyanobacteria and Actinobacteria (also known as Actinomycetota)[32,62,67]. For example, the daily shift of microbial communities in marine biofilms developed in a Portuguese Atlantic Port showed a clear switch of the dominant phylum from Proteobacteria to Cyanobacteria and the disappearance of Tenericutes over 30 days of biofilm development[62] (FIG. 3b). The stability of the community increases over time until the biofilm matures[64]. Surface properties can affect the attachment of early microbial colonizers[68], and environmental conditions have a critical role in the development and maturation of marine biofilms[30,50,69–71].

The cell abundance and community composition of mature marine biofilms change over the year, possibly due to seasonal changes in primary productivity, nutrient levels, phytoplankton blooms, physical or chemical factors such as temperature, and salinity[52,72,73]. Such seasonal changes are observed for biofilms colonizing natural and artificial substrates[74–77]. Furthermore, the succession of bacterial communities varies over different seasons. With the development of Portuguese Atlantic Port biofilms, the

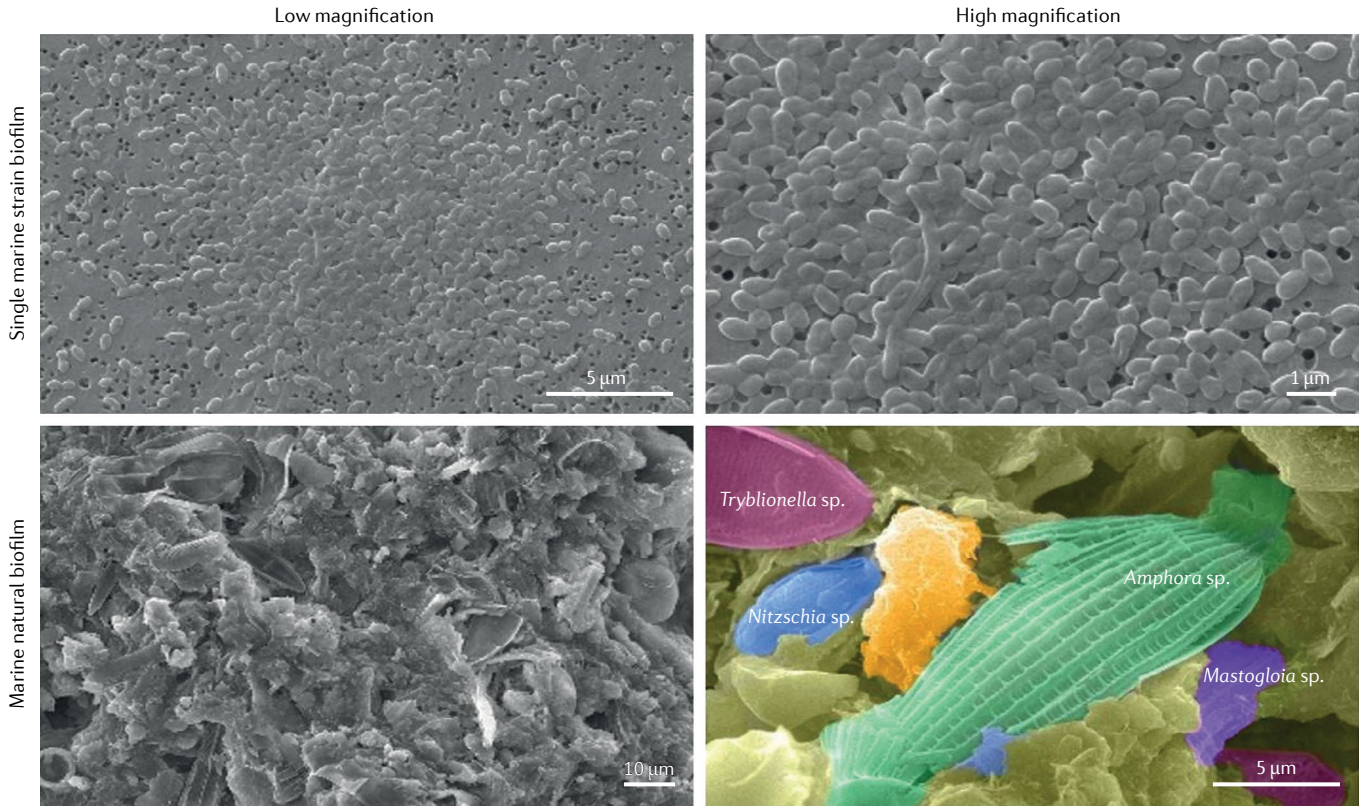

Fig. 2 | **Composition of natural marine biofilms.** Representative scanning electron microscopy images of a single marine strain biofilm and a natural marine biofilm at different magnifications. Top: Marine bacterial strain *Erythrobacter* sp. HKB8-formed biofilm at 48 h on polycarbonate membrane. Bottom: A 21-day natural marine biofilm formed on polycarbonate membrane at HKUST pier, Clear Water Bay, Hong Kong (22.34° N, 114.27° E). Typical microbial species shown in the high-magnification (5,000×) image (bottom right): *Amphora* sp. (turquoise), *Nitzschia* sp. (royal blue), *Tryblionella* sp. (hot pink), *Mastogloia* sp. (blue violet) and matrix secreted from cells (orange). Scale bars are as indicated.

dominant Proteobacteria was replaced by Cyanobacteria in spring but remained as dominant taxa in winter[62] (FIG. 3b,c). In the deep Mediterranean Sea, a biofilm was dominated by Gammaproteobacteria, Epsilonproteobacteria and Sphingobacteria in winter and by Flavobacteria, Betaproteobacteria and Deltaproteobacteria in summer[71].

Spatial changes. There are major metabolic differences between the sunlit upper waters and dark deep waters. In coastal or euphotic waters, photoautotrophic diatoms and cyanobacteria are major primary producers of biofilms on different types of artificial surfaces[40], whereas in deep-sea hydrothermal vent fields, chemolithoautotrophs belonging to Epsilonproteobacteria (for example, *Sulfurovum* spp. and *Arcobacter* spp.) are dominant primary producers[78–82], and biofilms have key ecological roles in this ecosystem[80]. Sulfate-reducing and methanotrophic bacteria and archaea dominate cold seep biofilms[50,51]. Gammaproteobacteria are more abundant than Alphaproteobacteria in biofilms on artificial substrates in the deep Mediterranean Sea, with the opposite pattern observed in coastal biofilms[67,71]. Given that temperature and salinity are homogeneous in the deep sea, the origin and biogeochemical characteristics of the surrounding water masses may determine distinct deep-sea biofilm communities[71]. Diverse archaea are also detected in deep-sea biofilms[81,82].

Low temperatures and extreme seasonal variations in irradiation and light hours may lead to specific microbial biofilm communities in polar oceans[70,83,84], with psychrophiles dominating[85] such as *Polaribacter* spp., *Psychrosphaera* spp. and *Psychrobacter* spp.[86]. Sea ice is a widespread surface for biofilm development in polar regions[87,88], where low temperatures drive the diversification of microbial communities[89–91]. Some sea ice-dwelling biofilm bacteria have been found both in the Antarctic and Arctic[92].

Most studies on spatial dynamics of marine biofilms are conducted in mid-to-low latitude coastal oceans[23,31,40]. An investigation of 101 biofilms grown with the same protocol in 8 locations from coastal Pacific and Atlantic Oceans revealed the existence of ubiquitous microbial

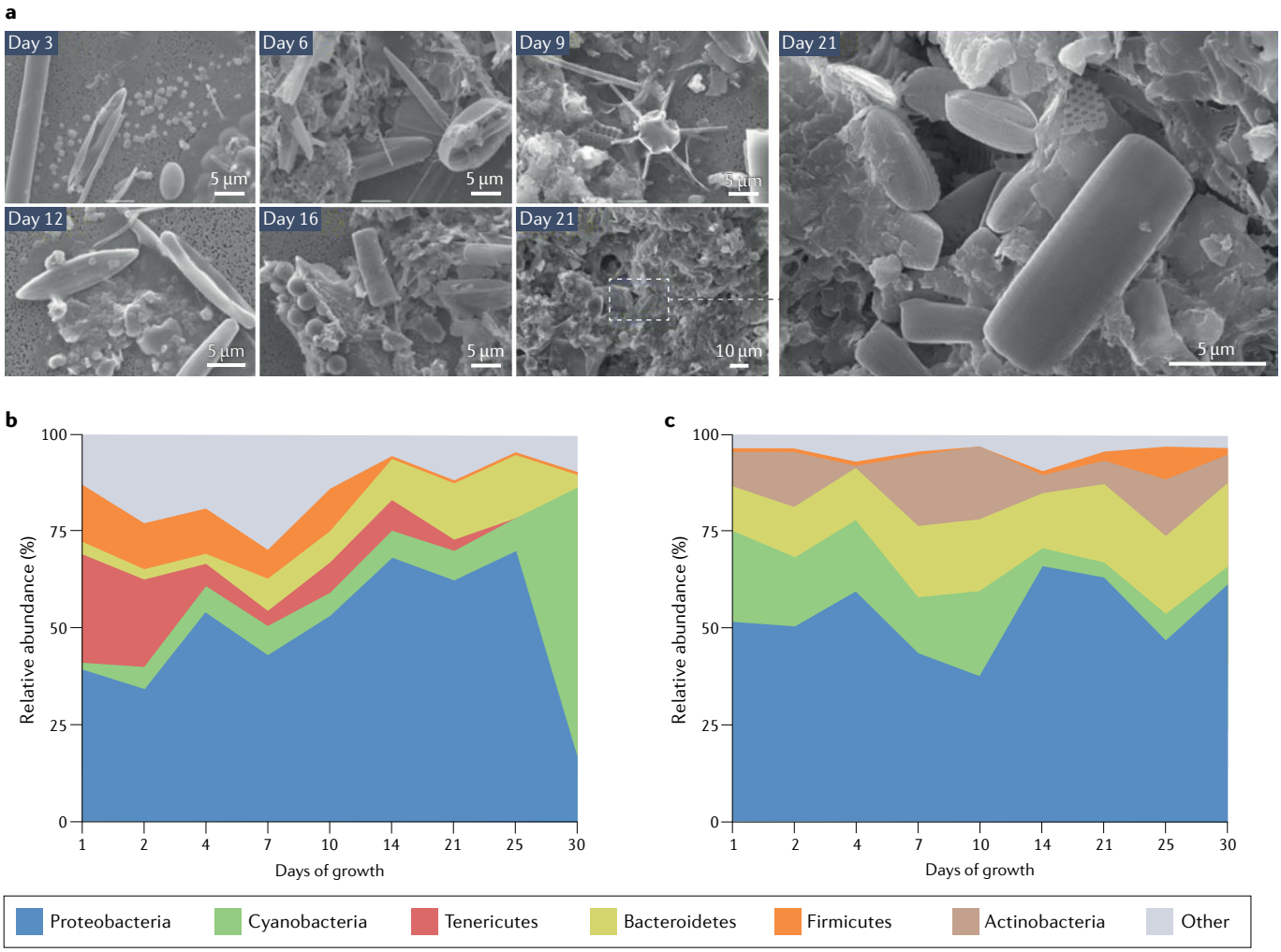

Fig. 3 | **The temporal succession of marine biofilms.** Representative scanning electron microscopy images of natural marine biofilms collected at different time points: at 3, 6, 9, 12, 16 and 21 days of growth on a polycarbonate membrane at HKUST Pier, Hong Kong (22.34° N, 114.27° E). Scale bars are as indicated (part **a**). Dynamic composition of the top five most abundant phyla of marine biofilms collected from a Portuguese Atlantic Port (41.10°N, 8.43°W) in spring (part **b**) and winter (part **c**). The marine biofilms were developed on stainless steel foil plates and triplicates were collected. The relative abundance was generated based on the Supplementary Table 2 of REF.[62]. For clarity of presentation, the figure shows the top five most abundant phyla only in each season, whereas the other phyla were combined as 'Others'. Parts **b** and **c** adapted from REF.[62], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

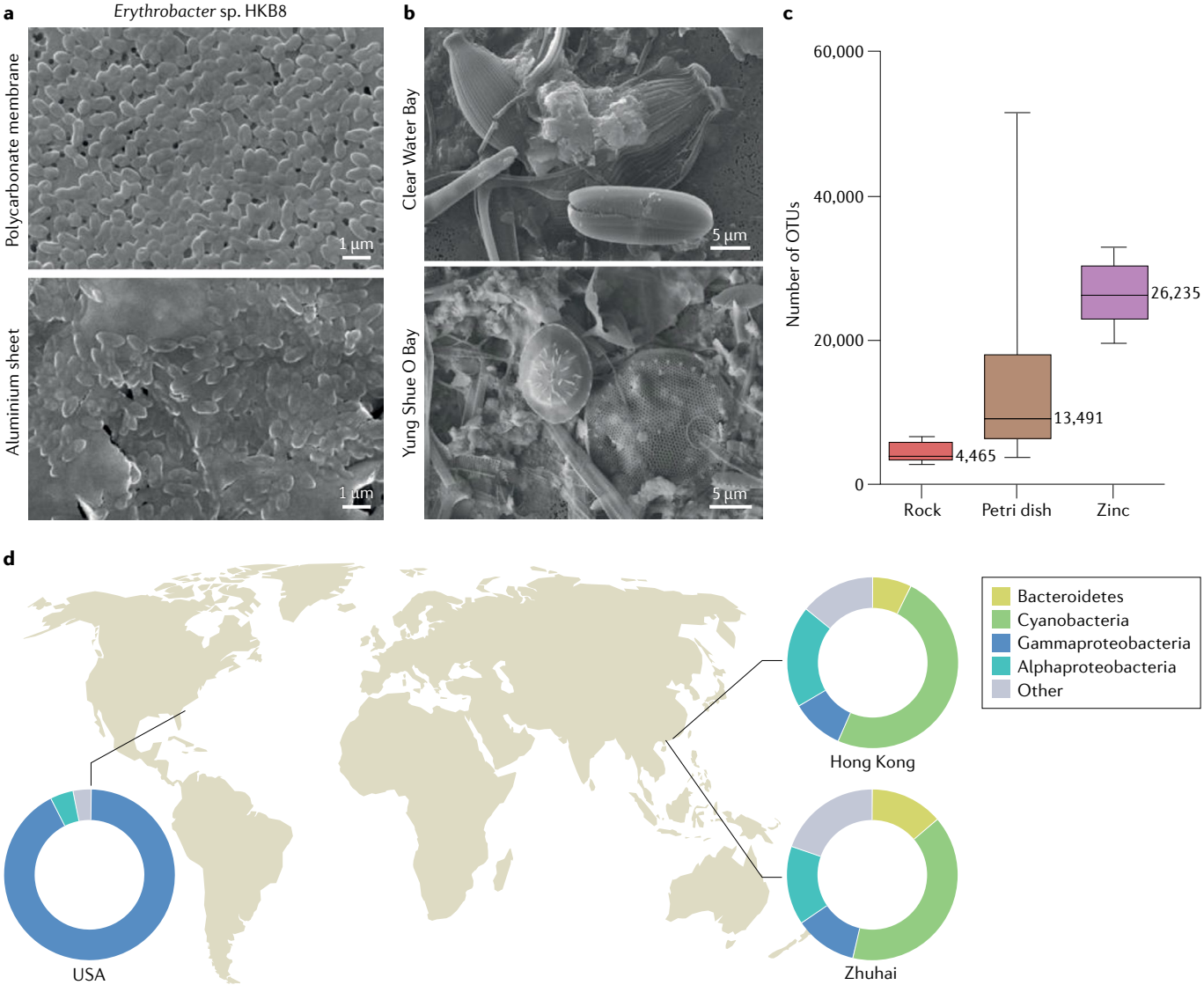

Fig. 4 | **Influence of substrate and location on microbial biofilm diversity. a** | Scanning electron microscopy images of mono-strain biofilms formed by the marine bacterium *Erythrobacter* sp. HKB8 on a polycarbonate membrane (top) and aluminium sheet (bottom) at 48 h. **b** | Scanning electron microscopy images of 9-day-old natural marine biofilms collected from two locations: (top) HKUST Pier, Hong Kong (22.34° N, 114.27° E); (bottom) Fish Farm, Yung Shue O Bay, Hong Kong (22.43° N, 114.28° E). **c** | Operational taxonomic unit (out) distribution of marine biofilms developed on different types of substrates. The marine biofilm samples developed on different substrates (natural rock ($n = 7$), Petri dish ($n = 73$) and zinc panel ($n = 12$)) were selected from a previous study[32] (deposited in the NCBI database under BioProject accession no. PRJNA438384) and the OTU distribution was replotted. The average number of OTUs is shown, and the quartiles and extremums are indicated in the figure. **d** | Microbial composition of marine biofilms at different locations (phylum level). Biofilm samples developed on Petri dishes in Hong Kong (22.34° N, 114.27° E), Zhuhai (21.70° N, 114.35° E) and the USA (31.42° N, −81.30° W) were selected from a previous study[32] (deposited in the NCBI database under BioProject accession no. PRJNA438384), and the microbial composition at the phylum level (except for those at the class level of Proteobacteria) was replotted.

groups and functions[42]. Cyanobacteria are the dominant biofilm component in the western Pacific (Hong Kong and Zhuhai) but are the minority in biofilms from the western Atlantic, where Verrucomicrobia dominate (FIG. 4). Biofilm community structure and biodiversity varied substantially by location and the driving forces for this diversity vary in different local environments[23,31,40].

Functional and chemical diversity. A large-scale metagenomic investigation showed that 37.7% of open reading frames and >11,000,000 non-redundant genes derived from marine biofilms were undetected in seawater samples, and over 57% of open reading frames in the biofilm samples were unique[32]. These results indicate considerable unexplored diversity, novel metabolic pathways and unknown functions. Signal transduction genes are abundant in marine biofilms[93], and genes related to transposase and heavy metal transport are enriched in biofilms that develop on metals or hydrothermal chimneys[94–96]. Antibiotic and metal-resistance genes are frequently observed in plastic-associated biofilm communities[60,97,98]. Compared with phylogenetic diversity, the functional diversity is more specific for certain substrates and environments.

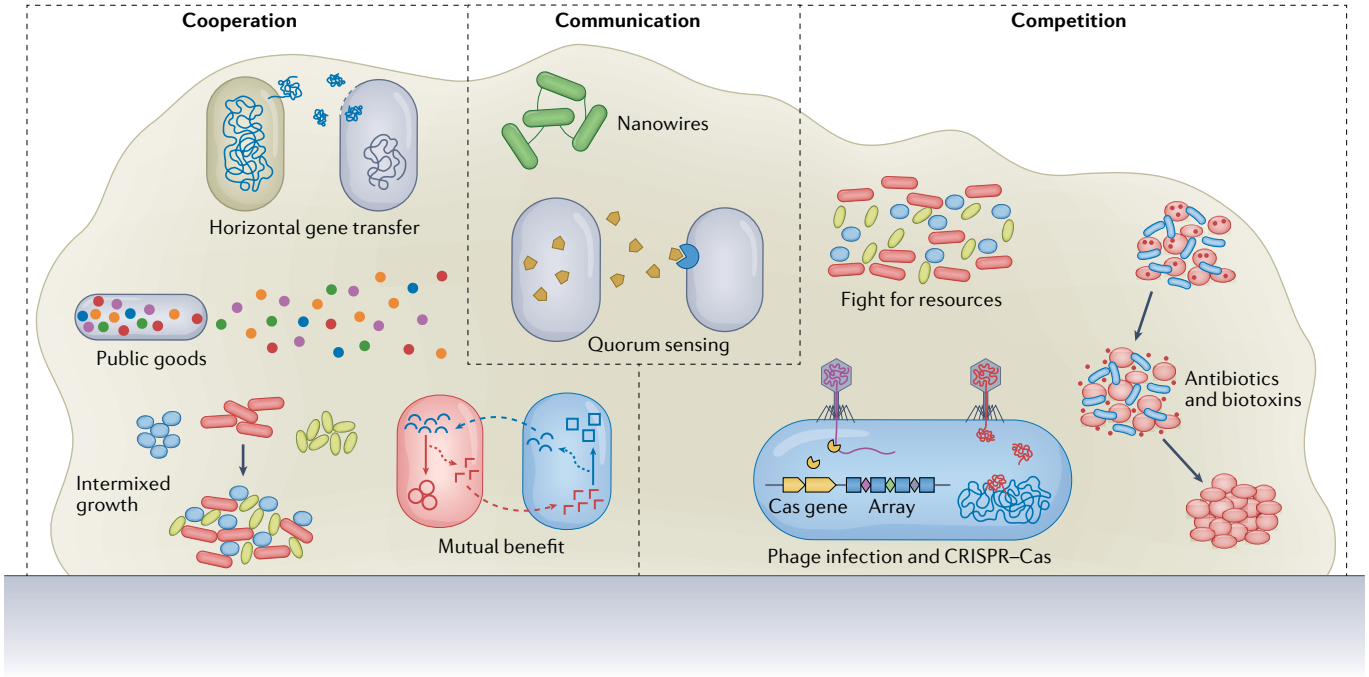

Fig. 5 | **Microbial interactions in marine biofilms.** Cooperation can help microorganisms gain advantages, for example, through compounds that promote collaboration, the uptake of nutrients and horizontal gene transfer. Competition is pervasive in multispecies biofilms owing to limited space and resources; it drives evolution and has an essential role in shaping the biofilm structure and physiological activities. Chemical communication (such as quorum sensing) and electrical communication (such as nanowires) regulate social behaviours in microbial communities.

The chemical composition, including extracellular polymeric substances (EPS) and metabolites, of marine biofilms changes through community succession[30]. Chemical profiles of marine biofilm extracts showed qualitative and quantitative changes at different ages[30]. Metagenomic analyses of natural marine biofilms revealed 1,148 biofilm-specific biosynthetic gene clusters distributed in 20 microbial phyla[32], indicating that biofilm microorganisms can produce different bioactive compounds to adapt to changing environments[32]. Various chemical cues, such as quorum sensing (QS) and signalling molecules, can modulate biofilm formation, assembly and succession[40,99]. Intriguingly, the presence of QS inhibitors shifts the compositions of microbial communities in biofilms from being dominated by Gram-negative bacteria to domination by Gram-positive bacteria[100].

Chemical cues can initiate phenotypic changes, for example, promoting access to nutrients or stress resistance. However, studies investigating in situ chemical diversity and cues of natural marine biofilms are rare. One study looked at the production of acyl-homoserine lactone (AHL) during biofilm formation using AHL reporter strains and gas chromatography–mass spectrometry (GC-MS)[101], and another investigated the chemical profiles of marine biofilms at different ages and on different substrates using GC-MS[30]. These studies showed that marine biofilms release different chemical compounds dynamically under changing environmental conditions, and the additive effects of these chemical compounds eventually determine whether biofilms are inducing or inhibiting the attachment of marine larvae[30].

Microbial interactions

Bacterial, archaeal or eukaryotic microorganisms attach to surfaces through diverse mechanisms, creating complex interactions between cells in natural biofilms[19]. Although the social behaviours of several model microorganisms have been well investigated, our knowledge of interactions in natural marine biofilms is limited and several theories have been proposed. The species-sorting theory argues that the selection pressure of local abiotic and biotic environmental conditions allows particular species from microbial pools to form a community[51,102]. Incubation conditions and particular environmental parameters such as seasons, temperature and light can be an effective selective force[103,104]. Furthermore, microbial interactions mainly occur between neighbouring cells[105,106], which in turn shape the spatial structure within biofilms[75]. Thus, the spatial distribution of microbial cells has a crucial influence on the cooperative and competitive interactions in marine biofilms[107,108] (FIG. 5).

Cooperation and competition. Cooperative behaviours provide biofilm-dwelling microorganisms with an advantage over their free-living counterparts[109], for example, through enhanced stress resistance and nutrient uptake. One collaborative behaviour is the release of compounds or the performance of functions that benefit nearby cells[110]. Biofilm-dwelling *Vibrio cholerae* cells secrete chitinase and the nutrients released by chitin digestion can be used by non-producers without metabolic cost[111]. Horizontal gene transfer is the main approach by which antibiotic resistance genes spread and accumulate in marine biofilms[112,113]. The production

Marine benthos
Organisms that are living in or on the surface of the continental shelf and seafloor (sediments and rocks).

of cooperative compounds, such as EPS, makes bacteria in biofilms 10–1,000-fold more resistant to antimicrobial agents and harsh conditions[114] and promotes the aggregation and structuring of microbial cells in marine biofilms[75]. Symbiosis is an important form of cooperation and the formation of biofilm-like aggregates on the mucus surface of the Hawaiian bobtail squid *Euprymna scolopes* may be the best symbiotic example in the marine ecosystem[115,116].

Given space and resource limitations, competition is pervasive in marine biofilms; it serves as the crucial driving force of evolution and has an essential role in shaping the community structure and physiological activities of biofilms[117]. The secretion of toxins and antibiotics is a common strategy in microbial interspecies and intraspecies competition[118,119]. The marine bacterium *Bacillus licheniformis* strain EI-34-6 and *Bacillus subtilis* strain II-111-5 produce antimicrobial compounds to kill competitors when grown in biofilms[120]. Finally, phages and CRISPR systems have been identified in marine biofilms[32,47].

Signal transduction. QS is one of the most important mechanisms regulating bacterial interactions in biofilms[121–123] (FIG. 5). Several QS signals have been discovered in model bacteria, including AHL, autoinducer 2 (AI-2) and oligopeptide-two-component-type QS[124–127]. In addition, the second messenger also regulates formation. Cyclic adenosine monophosphate influences irreversible bacterial attachment during biofilm formation of *Pseudomonas aeruginosa* PAO1 (REF.[128]) and *V. cholerae*[129], and bis-(3′-5′)-cyclic dimeric guanosine monophosphate influences the switch between a planktonic motile lifestyle and biofilm adhesion[122,130,131]; however, little is known about signalling in marine biofilms. In one example, the cellulose synthesis gene *bcsQ* of *Persephonella marina* controls the secretion of exopolysaccharides and biofilm formation, indirectly influencing larval settlement of mussels[132]. AHL production regulates the physiology and activity of subtidal biofilms[133]. Signalling molecules can change the composition of marine biofilms by regulating the relative abundance of specific microbial taxa as supported by a recent study on the roles of signal transduction in natural biofilm development[134]. The further analysis of signal transduction systems in 101 marine biofilms that developed on different surfaces and at different locations in various oceans revealed that the expression of signalling-related genes in biofilm samples was substantially higher than that in planktonic cells[93]. Furthermore, the taxonomic affiliation of signal transduction genes in marine biofilms differed from that in seawater samples, with the potential for numerous inter-phyla interactions between bacteria in marine biofilms and the water column[93].

Given that almost all knowledge on microbial cell–cell interactions is derived from either laboratory experiments with model strains or computational modelling, studies on species interactions and social behaviours in marine biofilms are rare. We lack knowledge on how microorganisms interact and communicate with each other in marine biofilms and how they respond to environmental changes. These knowledge gaps prevent unveiling microbial interactions in natural microbial communities and more research on microbial interactions in natural marine biofilms is needed.

Benthos interactions and biofouling

Substrate–biofilm interactions. Biofilms developing on biotic surfaces differ from those developing on abiotic surfaces because of differences in selective forces (FIG. 4). As mentioned, biofilms on the surface of marine benthos are called a second skin[12]. Chemical cues produced by hosts and modification of their surfaces modulate microbial attachment and select which microorganisms settle[51], leading to lower microbial diversity in epibiotic biofilms than in biofilms on the abiotic surface. Overall, microbial density per square centimetre in epibiotic biofilms varies from 2×10^2 to 8×10^8 cells on macroalgal blades[13,75] or from $0–1.5 \times 10^2$ (REF.[135]) to 8×10^8 cells[136] on marine invertebrates. Different species of marine macroalgae[137] and sponges[138] in the same habitat may support different epibiotic bacterial communities, whereas the same algal or animal species from different environments or locations may have relatively consistent epibiotic communities[138,139]. Proteobacteria and Bacteroidetes often dominate on macroalgae[140,141], whereas Actinobacteria, Bacteroidetes and Proteobacteria dominate on fish[142] and Gammaproteobacteria on the carapace of the deep-sea squat lobster *Munidopsis alvisca*[143]. The kelp blades of *Saccharina latissimi* harboured 239 OTUs from 16 phyla[141], the carapace of *M. alvisca* harboured 2,037 bacterial and 364 archaeal amplicon sequencing variants[143], and the skin of the fish *Perca fluviatilis* harboured 5,778 amplicon sequencing variants belonging to 52 phyla[142], highlighting the diversity of epibiotic biofilms. Epibiotic biofilms can change the surface chemical and physical properties and impede trans-epidermal gas and fluid exchange, thus modulating host–environmental interactions. Furthermore, released chemical cues can influence the behaviour of other microorganisms, pathogens and hosts[16].

Surface tension and molecular topography affect the initial microbial colonization. Corroded metallic alloys can provide energy for sulfate-reducing bacteria (SRB), leading to SRB dominance in mature biofilms[144–146]. By contrast, non-corroded materials support microorganisms not relying on this energy source, for example, Saprospiraceae, which dominate biofilms on polyvinyl chloride[147,148]. However, several studies showed that substrate type has a relatively minor role in shaping bacterial communities compared with water depth or environmental variables[25,67,71]. Furthermore, substrates influence early biofilms more strongly, whereas the microbial community structure and dynamics of matured biofilms on different surfaces are similar due to the 'converging phenomenon of old biofilms'[30]. Biofilms from different substrates showed conservation of function (for example, biofilm formation, stress response and environmental adaptation) in the genes of widespread phyla such as Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes[32]. Another study concluded that changes in biofilm diversity or function in response to different substrate characteristics and environmental factors remain elusive because of limited in-depth analysis[40].

Macrofouling
The formation of complex benthic community on man-made marine surfaces after biofilm formation, leading to the substantial build-up of biological and abiotic materials that affects the performance and function of marine surfaces.

Microfouling
Biofilm development on man-made marine surfaces, leading to changes in the physical and chemical properties of the surfaces.

In addition to biofilm–substrate interactions, marine biofilms directly interact with organisms mediating macrofouling[25]. Marine biofilms can modify and/or mask surface topographies and properties, which influences macrofouling colonization[25]. In particular, bacterial secondary metabolites are cues for larval settlement and EPS produced by diatoms induce larval colonization[25].

Microfouling and marine corrosion. Biofilm formation on man-made surfaces causes marine corrosion (microfouling)[149]. Mature biofilms show environmental gradients, for example, in oxygen and pH. Bacteria growing in the anaerobic zone in contact with the surface can use metal compounds from the material as resources, for example, through extracellular electron transfer[150]. Extracellular reduction of metal ions, used as terminal electron acceptors, is required by certain metal-respiring bacteria[151,152]. Thus, free electrons can be released and subsequently used by the bacteria for respiration, which can lead to microbially influenced corrosion. For example, the iron-corroding strain *Desulfovibrio ferrophilus* IS5 required effective biofilm formation for electron uptake on a cathodic surface, although the mechanism of electron uptake from the cathode may be distinct from that in iron corrosion[148]. Hydrogenase-positive microorganisms commonly use H_2 as an electron carrier, whereas anaerobic microbial iron corrosion bypasses H_2, that is, anaerobic microorganisms obtain electrons from metallic iron in a more direct manner than via hydrogen consumption[153,154]. Similarly, direct iron-to-microorganism electron transfer is feasible in stainless steel corrosion[155]. In addition, biofilms harbour a high concentration of organic acids, which are corrosive to metals and concrete[108,152]. Biofilm EPS can function as an electron transporter between biofilms and metals by selectively chelating cations[156,157]. Therefore, the development and maintenance of biofilm structures can have important roles in marine biocorrosion.

Common bacteria in metal corrosive biofilms include SRB belonging to the Deltaproteobacteria (including *Desulfovibrio*, *Desulfobacter*, *Desulfotignum* and *Desulfuromonas* species), iron-oxidizing bacteria belonging to the Zetaproteobacteria, which are non-cultivable, and sulfide-oxidizing bacteria. Although several of these bacteria have been demonstrated to be directly related to corrosion, corrosion is likely multifactorial in natural environments. A common corrosion process may involve sulfate reduction, nitrate reduction, metal reduction, sulfur oxidation, metal oxidation and fermentation processes. Different species in natural biofilms develop a cascade of biochemical processes and contribute to more severe corrosion than single species[158,159]. The co-existence of iron-oxidizing bacteria (which have a direct role in corrosion) and iron-reducing bacteria results in the continuity of the corrosion process[160]. The synergistic growth of SRB and sulfide-oxidizing bacteria produces sulfuric acid and accelerates the corrosion of concrete and carbon steel[161,162]. Conversely, biofilm formation on metal surfaces can also inhibit corrosion[160,163] through several mechanisms, including neutralization of the corrosive agents by bacterial aerobic respiration[160,164], generation of protective films[158,160] and inhibition of the growth of corrosion-inducing bacteria through secretion of antimicrobials[158]. Much is still to be learned about biofilm formation and surface corrosion in the marine environment.

Microfouling and biofilm–benthos interactions. Marine benthos have critical roles in structuring and stabilizing marine ecosystems and maritime industries such as mariculture and biofouling control. Most benthic organisms have a pelagic form (larva or spore) in their life cycle, which controls the distribution of the population across a sizeable geographic scale and locally through the selection of the optimal surface to attach to for transition to the sessile juvenile and adult form. Given that biofilms cover all marine interfaces, the interaction between settling larvae and spores and chemical and physical signals produced by biofilms determines the attachment, subsequent population dynamics, community structure, and function of benthic ecosystems (FIG. 6). The dynamic interaction between marine biofilms and settling larvae and spores is widespread among animal taxa[104] and has been studied for almost 90 years[2,165,166]. Most studies have been conducted under laboratory conditions[134,167–169] and extrapolation to the natural environment is challenging. Nevertheless, several general observations have emerged.

Bacterial isolates from natural biofilms show varied effects on the settling larvae and spores of marine benthos, ranging from stimulation and inhibition to no effects[29,170]. Bacteria in the same genus also display differences in mediating larvae and spore attachment[29,171], indicating that the phylogenetic relationship of microorganisms is a poor predictor of bioactivity. The bioactivity of natural marine biofilms depends on the combined effects of all microorganisms present and the relative abundance of individual bacterial species. Whether the bacterial cell density of marine biofilms is a good indicator of bioactivity remains controversial. Some studies have suggested that biofilm bioactivity increases with increased bacterial cell density[172,173]; however, other studies have seen no correlation between larval attachment and biomass and cell density of bacteria and diatoms in biofilms[31,174,175].

Larvae–biofilm interactions are mediated by chemical cues, which can be soluble or bound to the biofilm surface. A monospecies biofilm experiment showed that surface-bound rather than soluble cues from biofilm bacteria trigger larval settlement of the tube-building polychaete *Hydroides elegans*[2], whereas other experiments revealed that bacterial metabolic activity in biofilms is essential for settlement of the same worm species[55,56]. These contradictory results can be explained by observations in other studies revealing that the same larval species can respond to different cues from various bacteria in natural biofilms[174], whereas different larval species respond to the same cues in various ways[162]. Biofilms with different microbial communities, cell densities and physiological conditions produce various chemical cues such as QS molecules, including N-dodecanoyl-L-homoserine lactone (C12-HSL), which is involved in larval settlement of *H. elegans*[55]. The inhibition of QS systems in natural biofilms by

Phage tail-like structures
Protein structures produced by *Pseudoalteromonas luteoviolacea* that can stimulate larval metamorphosis of the tube-building polychaete *Hydroides elegans*.

QS blockers inhibited larval settlement of the bryozoan *Bugula neritina*[176]. Chemical cues for larval settlement of marine benthos inevitably include soluble and surface-bound molecules and interactions are complex.

Biofilms can attract zoospores of the green alga *Ulva* by the release of signalling molecules[177]. The structure of the acyl side chain of AHLs affects the settlement of *Ulva* zoospores[178]. When AHL production of polymicrobial biofilms is disrupted by *Shewanella* sp., *Ulva* zoospore settlement is inhibited[179]. Similarly, biofilms lose their attractiveness to *Ulva* zoospores when the homoserine lactone ring produced by biofilms is destroyed[99]. These results highlight the importance of signalling molecules from biofilms in regulating spore settlement of macroalgae.

Temporal and spatial changes in community structure and densities of bacteria in natural biofilms affect larval settlement of different marine species and marine larvae have discriminative behaviour towards biofilms originating from various environments, leading to spatial variation in larval recruitment[30,31,175,180]. Sufficient evidence shows that a shift in the dominant microbial species composition of biofilms drives the initial larval attachment.

Arrays of phage tail-like structures (specifically, metamorphosis-associated contractile structures (MACs)) produced by a marine bacterium trigger the metamorphosis of the tubeworm *H. elegans*[181]. MACs are the only known bacterial structure that can activate larval settlement under laboratory conditions. Based on this finding, a model has been proposed to describe the effect of bacterial factors (solubility, surface binding and injection into host cells) on animal metamorphosis[162]. Given that gene homologs with similar structures as MACs are highly diverse and widely distributed in natural marine biofilms[182], how MACs affect larval settlement of *H. elegans* in natural biofilms remains to be determined.

Overall, marine biofilms release different chemical compounds under various environmental conditions, and the combined effects of these chemical compounds eventually determine the bioactivity of marine biofilms for the initial colonization of marine benthos[2,160,183]; however, understanding of the fundamental mechanisms of larvae–biofilm interactions is still at its infancy.

Economic impacts of marine biofilms. Biofilm development (microfouling) affects the structure and function of the benthic community on man-made marine surfaces (and thus macrofouling). However, the formation of the benthic community will destroy the initial biofilm on the surface and create new surfaces for new biofilm

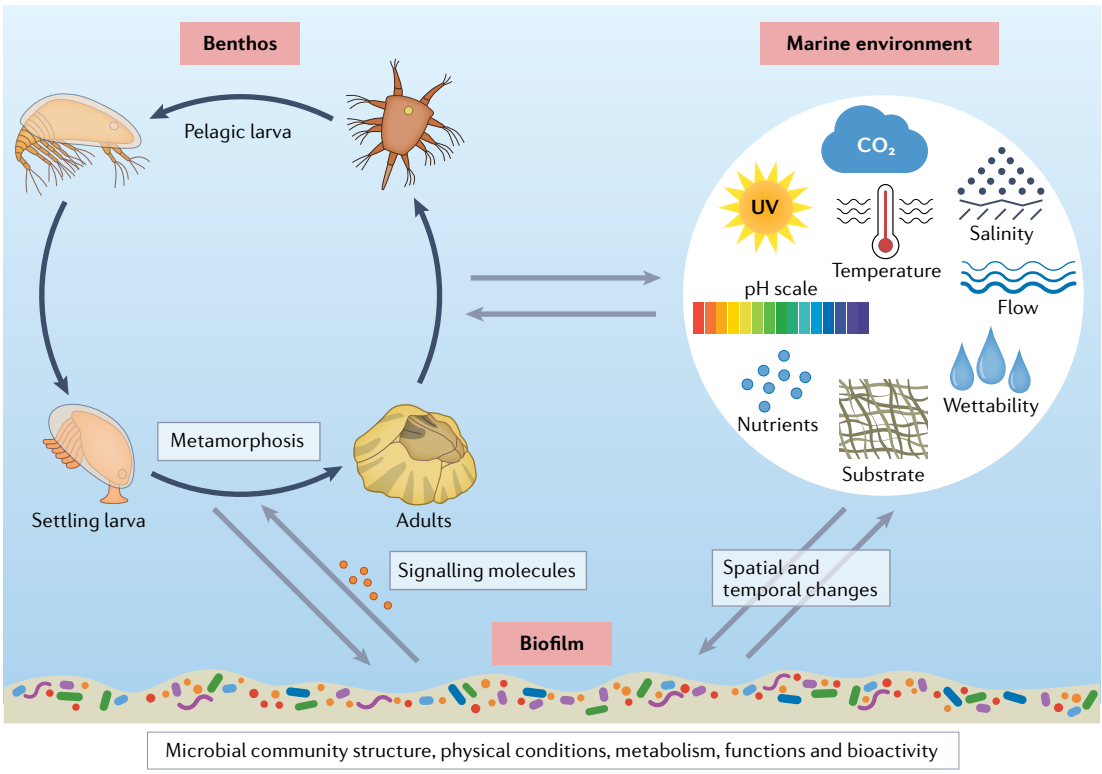

Fig. 6 | **Interactions of marine biofilm with the benthic ecosystem and marine environment.** Environmental conditions, such as ultraviolet (UV) radiation, gases, temperature, pH, salinity, flow dynamics, surface wettability, nutrient content and substrate type, influence the life cycles of the marine benthos (larva and adults). These environmental factors also shape microbial community structures, physical conditions, microbial metabolism and bioactivities in biofilms. Biofilms, in turn, can attract or drive away settling larvae and spores, influencing the initial colonization, recruitment patterns and population dynamics of marine benthos. Dynamic changes in signalling molecules are responsible for the complicated interactions of microorganisms in biofilms, between biofilms and the environment, and between biofilms and benthic ecosystems.

formation. Therefore, interactions between microfouling and macrofouling on surfaces are dynamic and continuous, which makes biofouling control on marine structures difficult and causes substantial economic loss to maritime industries. The global cost of corrosion is about 2.5 trillion USD, as estimated by the Worldwide Corrosion Authority in 2008 (REF.[184]), and 20% of this expenditure can be attributed to microbiologically influenced corrosion[130]. Biofouling on ship hulls substantially increases the resistance and power consumption of naval vessels, reducing the cruising speed by up to 86%[7]. The US Navy estimated that the annual cost related to hull fouling for its surface fleet reaches up to 160 million dollars[185]. The attachment of fouling organisms can block pipelines, valves and cooler nozzles. Biofouling affects the function of acoustic equipment, buoys, nets and other facilities. For the aquaculture industry, given the competition for food, oxygen and other resources, the health of cultured organisms is affected, resulting in their reduced fitness and yield[186]. The economic costs associated with biofouling control in aquaculture are 5–10% of production costs[187]; thus, controlling biofouling would lead to an increase in the annual production of the aquaculture industry[188].

Conclusions and future perspectives

Our knowledge of diversity, community structure, functions and interactions of microorganisms, particularly for eukaryotes and viruses, in natural marine biofilms on a global scale is in its infancy. No single study has identified all the bacteria, archaea and eukaryotes in a marine biofilm as a whole. With the current lack of knowledge on organisms in biofilms and their functions, understanding their interactions and integrated ecological functions is impossible. Thus, our comprehension of marine biofilm succession, especially in the early or very early stages, is incomplete. Still, such knowledge is crucial for an in-depth understanding of biofilm development, microbial biofouling and biocorrosion. With the advancement of omics technologies, our understanding of microbial biofilms, particularly of model strains related to public health, has been substantially improved; however, overall, the application of omics technologies in natural marine biofilms is still scarce.

Integrated genomics, metagenomics, metatranscriptomics and metabolomics will facilitate the in-depth exploration of the microbial diversity and functions in biofilms and the temporal dynamics of microbial populations, cells, proteins and chemicals, revealing the molecular and ecological mechanisms of biofilm development and providing a holistic view of biofilm biology[189]. However, given that most microorganisms in marine biofilms are uncultured, most genomic information has been generated by metagenomics and genome binning[31], which unavoidably lead to errors. New bioinformatics pipelines need to be developed to reduce such errors, whereas the cost for omics analysis should be further reduced. An artificial intelligence-based multi-omics platform that can predict pathways associated with different biofilm phenotypes should also be developed[189].

The cellular heterogeneity of monomicrobial and polymicrobial biofilm communities has been well documented, particularly in human health-related biofilms[190,191]. Several techniques and protocols have been developed to trace variations in cell phenotypes, physiology, metabolic activities, responses to environmental change, biological processes and underlying molecular mechanisms of biofilm formation. Single-cell transcriptomics[190] and multiple luciferase reporter systems[191] have revealed single-cell states and dynamics of gene expression in vivo with high spatial and temporal resolution in complex microbial communities[190]. These techniques can be adapted for marine biofilms in the future, which will provide new opportunities for biofilm research in the coming decade.

The abundance, diversity, community structure and activity of biofilm bacteria affect the flux of matter in the ocean. However, quantitative assessment of this influence is rare as it requires sensitive techniques for in situ measurements. Electrochemical camera chips[192] and surface-enhanced resonance Raman scattering spectroscopy[193] are useful for monitoring the spatial distribution of metabolites[132] and/or signalling molecules[133]. However, these methods need to be refined before they can be applied to natural marine biofilms. Although a spatial metabolomics pipeline (MetaFISH) has been developed to link the genotype and metabolic phenotype of microorganisms in situ at a scale relevant to microbial interactions[194], the broad application of this method in natural marine biofilms needs to be tested. The identification of novel biosynthesis pathways producing compounds with diverse potential activities, such as anti-bacterials, anti-biofilm agents, anti-biofouling agents and novel drugs, shows promise. Meanwhile, the roles of chemical molecules in the formation and development of biofilms in model bacteria have been extensively investigated, but the chemical signals produced by dominant but uncultured microorganisms in natural marine biofilms remain uncharacterized. The characterization of the biosynthesis and natural functions of signalling molecules in natural biofilms is extremely difficult and key questions remain poorly understood. Which chemical signals are produced and at which stage of biofilm development can they be detected? What is the percentage of uncultured (and potentially uncultivable) bacterial species that produce QS molecules? Are there any unknown QS molecules with important roles in biofilm development? Are there specific QS molecules produced by bacteria in biofilms in comparison with planktonic communities? The combination of molecular and biochemical methods will facilitate effective discoveries of the biosynthesis, production and dynamics of signals in marine biofilms. Such progress will provide insights into biofilm control and manipulation in biofouling and biocorrosion of maritime industries.

The influence of biofilms on marine biogeochemical cycles, such as carbon flux, is generally overlooked in present ecological and biogeochemical models. Given the huge number of microbial cells attached to diverse ocean interfaces, this represents a substantial knowledge gap. Therefore, future work on the global carbon cycle should include biofilm-driven flux and processes to evaluate the effect of biofilms on biogeochemical cycling and the global climate.

Microbial fuel cells
In a fuel cell system, the microbes on the anode oxidize reduced compounds (known as fuel or electron donors) and divert electrons to high-energy oxidized compounds (also known as oxidizing agents or electron acceptors) on the cathode to generate an electric current through an external electrical circuit.

Finally, local, regional and global environmental changes shape microbial community structures and functions in biofilms, which in turn affect the settling larvae or spores of marine benthos. Thus, although studies investigating the impact of climate change on marine biofilms and the larvae of invertebrates are very limited[195–197], warming oceans probably pose considerable influence on the colonization, formation and development of marine biofilms and the benthos. A better understanding of how environmental change affects microbial community structures and biofilm functions and of how biofilms affect the initial colonization of marine benthos will provide critical knowledge about the interaction among environment, biofilms and benthic ecosystems. A stepwise approach must be implemented to decipher the complex interactions between microorganisms, underlying molecular mechanisms and chemical signals responsible for mediating interactions within the biofilm, between biofilms and marine benthos, and between natural biofilms and the ambient environment. Of course, marine biofilms also hold great biotechnological potential[4], including as sources of bioactive compounds such as antibiotics[112,114,119], mediators of biofouler settlement, recruiters of aquaculture species[183], controllers of biocorrosion[159], cleaners of organic pollutants[19] and as microbial fuel cells, highlighting the potential for the application of biofilms in maritime industries in the future.

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