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Minireview

Phototrophic marine benthic microbiomes: the ecophysiology of these biological entities

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Summary

Phototrophic biofilms are multispecies, self-sustaining and largely closed microbial ecosystems. They form macroscopic structures such as microbial mats and stromatolites. These sunlight-driven consortia consist of a number of functional groups of microorganisms that recycle the elements internally. Particularly, the sulfur cycle is discussed in more detail as this is fundamental to marine benthic microbial communities and because recently exciting new insights have been obtained. The cycling of elements demands a tight tuning of the various metabolic processes and require cooperation between the different groups of microorganisms. This is likely achieved through cell-to-cell communication and a biological clock. Biofilms may be considered as a macroscopic biological entity with its own physiology. We review the various components of some marine phototrophic biofilms and discuss their roles in the system. The importance of extracellular polymeric substances (EPS) as the matrix for biofilm metabolism and as substrate for biofilm microorganisms is discussed. We particularly assess the importance of extracellular DNA, horizontal gene transfer and viruses for the generation of genetic diversity and innovation, and for rendering resilience to external forcing to these biological entities.

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Introduction

In their natural habitat, the majority of microorganisms are associated with surfaces (Żur *et al*., 2016), either attached to a substratum, as part of an aggregate and on and in sediments (Whitman *et al*., 1998). These surfaces include the exterior of plants and animals, where they form part of the microbiome of these organisms, together with microorganisms living inside these (macro)organisms. Even many pelagic microorganisms (plankton) may form aggregates or bind to surfaces at some time during their life cycle and, although, in numbers free-living microorganisms are predominant, the particle-associated ones are more diverse and show higher activities (Ghiglione *et al*., 2009; Vojvoda *et al*., 2014; Rieck *et al*., 2015). Some benthic microorganisms may thrive part of their life as plankton and are termed pseudoplankton or tychoplankton (Forster *et al*., 2006). This change of lifestyle may, for instance, promote dispersion of the organism. Hence, the question that could be asked is why a benthic lifestyle seems to be preferred.

In contrast to planktonic species that are often limited in readily available nutrients and rarely benefit from direct interactions with other species, the benthic microbiome is characterized by a tight physical coupling between the different members. The nearest neighbour of a planktonic free-living cell is far away in terms of the distance compared to the cell size and any direct cooperation or interaction will be difficult if not impossible (Zehr *et al*., 2017).

Starving microorganisms may attach to surfaces to take advantage of the substrates and nutrients that are associated with them (Kjelleberg *et al*., 1983). It has been reported that attached bacteria were more active than free-living microorganisms (Grossart *et al*., 2006) but explanations for this observation are lacking and other reports rather indicate indirect effects (Iriberri *et al*., 1987). Also, contact with a solid surface stabilizes the

periplasm, even in the absence of a physical connection and may keep exoenzymes close to the cells aiding them to obtain growth substrates and nutrients (ZoBell, 1943). Solid surfaces may bind substrates and lower their molecular activity in solution and consequently decreasing the metabolic rate of the microorganisms, while binding of toxic substances may decrease or alleviate toxicity.

Benthic communities such as biofilms and microbial mats experience diffusion boundary layers. These limit the exchange with the water column and promote the efficient recycling of nutrients and elements within the biofilm (Jørgensen and Revsbech, 1985). Sediment particles and copious amounts of exuded extracellular polymeric substances (EPS) protect the cells against grazing or viral attack (van Loosdrecht *et al*., 1990). This and the physical stability of such communities may be their cue of success.

Benthic microorganisms often develop into a biofilm and may form macroscopic structures such as microbial mats and stromatolites (Stal, 2012) (Fig. 1). Phototrophic microbial mats and biofilms are interesting examples of benthic communities because they are mostly closed and self-sustaining microbial ecosystems (Guerrero and Berlanga, 2016). They occur globally in a variety of habitats, often characterized by more or less extreme environmental conditions that prevent grazing by higher organisms. Examples include hypersaline, hot spring, coastal and intertidal microbial mats (Fig. 1). Cyanobacteria, oxygenic phototrophic microorganisms, are the colonizing organisms because they start enriching the system with biomass, which is synthesized from the fixation of inorganic carbon using solar energy and water as energy and electron donor respectively (Stal, 2012). Diatoms fulfil this role in intertidal mudflats (Underwood *et al*., 2005). Some of the organic carbon is exuded as low molecular weight compounds, while complex, high-molecular carbon compounds are degraded by specialized chemoheterotrophic bacteria (Miyatake *et al*., 2014). Their activity as well as the dark respiration by the phototrophic organisms will deplete the system from oxygen and anaerobic bacteria will further decompose the low-molecular compounds. In the sulfate-rich seawater, notably sulfate- and sulfur-reducing bacteria will end-oxidize the organic matter while producing sulfide (van Gemerden, 1993). Sulfide is oxidized back to sulfur or sulfate by sulfur-oxidizing bacteria, which include anoxygenic phototrophic bacteria such as the purple sulfur bacteria, but also a plethora of autotrophic and heterotrophic chemotrophic bacteria. High levels of sulfide inhibit oxygenic photosynthesis but pockets of ferric iron or anoxygenic photosynthetic bacteria may deplete the sulfide allowing activity of cyanobacteria (de Beer *et al*., 2017). Altogether, these organisms form an intimate community and a small (mm)-scale ecosystem that exhibit macroscopic morphological features (Fig. 1). These systems are also characterized by a spatial structure and physicochemical gradients, which are required to maintain and ascertain cooperation within the biofilm. Microbial mats and biofilms are formed and maintain their architecture by the grace of the altruistic organisms that dominate these systems, even though these living entities may be propagated through single cells that are liberated from them (Kreft, 2004).

Metabolism in biofilms and microbial mats is a joint venture of the participating organisms (van Gemerden, 1993). Syntrophic interactions between two or more different groups or organisms enable metabolic pathways that otherwise would be impossible to perform by any single species (Morris *et al*., 2013). Microorganisms may also benefit from metabolic specialization or division of work as a result of biochemical conflicts within an organism (Johnson *et al*., 2012). This leads to communities with metabolically interdepend microbial groups that exchange a variety of metabolites that drive the cooperation between them (Zelezniak *et al*., 2015).

Cell-to-cell communication is not only possible within a biofilm but is also imperative for the community to coordinate

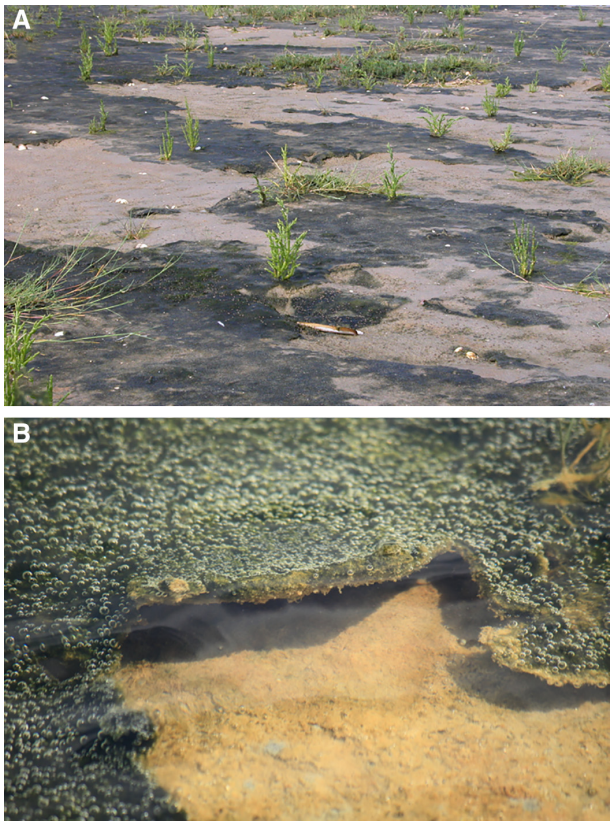

Fig. 1. A. Cyanobacterial mat (the dark patches) on an intertidal sand beach on a North Sea barrier island.
B. Close-up of the cyanobacterial mat showing the coherent structure and the oxygen bubbles trapped in the EPS matrix. [Color figure can be viewed at wileyonlinelibrary.com]

activities and respond to physical and chemical signals and to achieve a division of labor between the different components (Remis *et al*., 2010). The microorganisms of a biofilm or microbial mat are embedded in a matrix of extracellular polymeric substances (EPS) that serve a plethora of different functions such as providing a structural organization of the microorganisms, attachment to a surface, protecting from desiccation and scavenging nutrients, to name a few (Decho, 1990). The medium through which cell-to-cell signalling takes place and nucleic acids and proteins are exchanged plays an important role because it allows molecules to reach the critical concentrations required for these processes. Moreover, since the EPS matrix wraps the cells it may facilitate the dispersion of the biofilm or microbial mat community when sloughed from the surface. Ereshefsky and Pedroso (2015) argue that because of these and other properties, multispecies biofilms should be considered as evolutionary individuals, although, this view has also been strongly criticized (Clarke, 2016). Biofilms and microbial mats exhibit properties that are the result of their function as a physiological entity and which are not seen by any component on its own or together in a planktonic setting and they, therefore, approach the complexity of a multicellular (eukaryotic) organism (Guerrero and Berlanga, 2016). The question of evolutionary individuality is not in the scope of this review. This review will focus mostly on multispecies phototrophic biofilms and microbial mats, emphasizing that these complex systems behave as biological entities with their own characteristic morphology and physiology.

Biofilm formation

Biofilm associated bacteria differ in their morphology and physiology from free-living bacteria. They have been reported to exhibit higher metabolic activity and incorporate more dissolved organic carbon per cell compared to their own free-living state (Kirchman and Mitchell, 1982; Iriberri *et al*., 1987; Grossart *et al*., 2006). In general, immobilized microorganisms often dramatically change their metabolism and physiology and have been considered as a quasi-differentiated state of the organism (Sampathkumar *et al*., 2006) or they could even be mistaken for a completely different organism (Żur *et al*., 2016). Microorganisms adhering to a substrate change their gene expression compared to free-living individuals (Lazazzera, 2005). This was also evident from the differences in the proteome of *Pseudomonas aeruginosa* (Guilbaud *et al*., 2016). Even the type of surface this bacterium adhered to resulted in differences in the proteome. A different set of genes is transcribed in order to achieve these changes and this should be evident from comparing the transcriptomes and proteomes of immobilized and free-living cells (Vilain *et al*., 2004; Sampathkumar *et al*., 2006), although, the identification of a typical immobilized cell as a phenotype has been questioned (Vilain *et al*., 2004).

The formation of a biofilm is often described as a cycle that starts with the reversible attachment of a single microbial cell to a surface. This may be by active migration (swimming) of randomly by physical forces (such as water currents, wind and dry- and wet deposition). The initial reversible attachment may become irreversible though adhesion by appendages of the microorganism or by the exudation of sticky extracellular polymeric substances (EPS) (Kjelleberg and Molin, 2002). For initial adhesion, the surface chemistry is critical and usually characterized by a low surface energy (Ista *et al*., 2004; Anderson, 2016). Surfaces may also be conditioned for adhesion by adherence or adsorption of organic matter from the environment. The biofilm-forming *Pseudomonas aeruginosa* produces extracellular enzymes that alter the EPS by changing physicochemical properties such as viscosity and hydrophobicity and thereby its stickiness as well as the organism's motility (Tielen *et al*., 2010). Cell growth and physical forces (such as water flow) result in a structured biofilm in which the cells are embedded in a matrix of EPS (Bakker *et al*., 2004).

Although, natural biofilms composed of a single species are known, the vast majority are multispecies biofilms. Following the establishment of the initial colonizer, other types or species of microorganisms may join, attracted by a suitable substrate or environment, by signalling compounds emitted by the biofilm organism(s), or just by chance. Continuous growth of the biofilm eventually leads to a structure that becomes unfavourable and the biofilm may start to deteriorate, sloughing small or large aggregates or liberating single cells into the environment, which subsequently move away and result in the dispersion of the biofilm and enables it to colonize other surfaces elsewhere. Whether a multispecies biofilm reproduces as a complete community by dispersion of aggregates or that single cells experience a planktonic phase and that biofilms are always formed *de novo* as described above, can be disputed. In fact, both models may be true. For instance, coastal intertidal microbial mats or diatom biofilms in temperate areas are usually annual and develop *de novo* every year (Stal *et al*., 1985). It is not precisely known whether the new development is from the settlement of colonizing microorganisms introduced by the seawater or that surviving organisms in the sediment are the inoculum. Both possibilities are likely and may depend on the local situation. The structure of established coastal microbial mats often remain throughout the year and seasonal surveys of DNA content have shown that the mat microbiome survives (Bolhuis and Stal, 2011). However, mats developing close to the low water line usually completely disappear

due to physical disturbances leading to erosion and sediment transport (Stal *et al*., 1985). This is also the case for diatom biofilms, which may be heavily grazed during certain periods (Blanchard *et al*., 2001).

Motility of biofilm organisms is an important property. It enables the organisms to continuously migrate and position themselves along the physicochemical gradient to reach an optimum position. In order to do so, the organisms must sense the environment and migrate accordingly. The direction and speed of migration may be controlled by a variety of factors, the most important being light (phototaxis), organic and inorganic chemical species (chemotaxis), water (hydrotaxis) and magnetic field (magnetotaxis) (Stal, 2012). Growth and migration of the microorganisms result in a laminated structure of microbial mats in which different functional groups of microorganisms are positioned vertically stratified along the physicochemical gradient, which is the product of the microbial mat's metabolism.

Marine intertidal areas can be distinguished by their main sediment composition. Roughly, area's exposed by lower energy are usually characterized by muddy sediments composed of fine-grained silt and clays and a low content of sand, while high energy areas (currents, waves) are dominated by more coarse grained sandy sediments. The former is often colonized by diatoms, which form biofilm-like communities, while the latter inhabit mostly filamentous cyanobacteria, that form by their entangled trichomes, dense, robust and coherent communities often designated microbial mats (Stal *et al*., 1985). The reason for this sharp distinction in microbial community by sediment type has not been studied into great depth but may be caused by the differences in transparency, nutrient availability and physical movement of the sand grains (Watermann *et al*., 1999). Both communities develop in intertidal areas, meaning that they are periodically exposed and inundated. The periods of exposure to the air may be lengthy. During inundation, the phototrophic communities may be grazed to the extent that a biofilm or mat is eventually prevented from being formed (Fenchel, 1998; Weerman *et al*., 2011). Hence, inundation must be short but is nevertheless important since the seawater brings nutrients and physical forces may aid in removing any waste.

Muddy sediments are characterized by a low transparency while the seawater may have a high turbidity because of the high content of silt and clays. Hence, only at the surface and during low tide, the diatoms receive enough light for photosynthesis. When inundated, the diatoms migrate into the sediment where they are devoid of light but protected from grazing and find abundant nutrients loaded to the charged mud particles (Paterson, 1989; Epstein, 1997; Van Colen *et al*., 2014). Benthic diatoms have, therefore, only a short window allowing for photosynthesis. These organisms are also known for their potential of heterotrophic growth (Admiraal and Peletier, 1979). Their high speed of migration facilitated by an efficient motility combined with chemotactic behaviour allows them to respond quickly to the changes in this highly dynamic environment (Admiraal and Peletier, 1979; Paterson, 1989; Bondoc *et al*., 2016).

Cyanobacteria, although, many are motile, migrate at much lower speed than diatoms and most of them are incapable of heterotrophic growth on external carbon sources (Stanier and Cohen-Bazire, 1977; Stal, 1995). Cyanobacteria rely mostly on photosynthesis and require a wider time window for that than is possible on mudflats. Quartz sandy sediments are highly transparent and the submerging seawater has a low turbidity. The high energy impacting on intertidal sand flats limit grazing activity. The large, mostly filamentous, cyanobacteria that build microbial mats are robust and resist the physical impact of the large moving sand grains (Stal, 1995; Watermann *et al*., 1999). The uncharged quartz sand grains are low in nutrients but benthic cyanobacteria are nearly self-sustainable and require little nutrients due to their ability of photosynthesis combined with fixing atmospheric nitrogen (N_2) and storing phosphate (Gomez-Garcia *et al*., 2013).

Diversity of microbial mats and diatom biofilms

Microbial mats are bacteria-dominated systems (Bolhuis *et al*., 2014). Even in extreme environments such as hypersaline ponds where Archaea dominate the water (Benlloch *et al*., 2002), the benthic mats are almost exclusively bacterial. The contribution of Eukarya may be considerable in coastal microbial mats but is low in hypersaline mats or hot springs, although, few studies have dealt with them. In the hypersaline mats of Guerrero Negro, Mexico the ratio of bacterial, archaeal and eukaryal ribosomal RNA (rRNA) genes was 90%/9%/1%, although, archaeal metabolism appeared to be more important than expected from their abundance (Robertson *et al*., 2009). Benthic algal mats in Antarctic hypersaline (marine-derived) lakes form and persist above the chemocline in the thin layer where oxygen is low and nutrient concentrations are high (Laybourn-Parry, 2002). In continental Antarctic cyanobacterial mats, the bacterial component of the metagenome was ~98% while Archaea and Eukarya each made only ~1% (Varin *et al*., 2010). The viral component of microbial mats will be discussed separately (see below). A comparison between coastal-, hypersaline- and hot spring microbial mats revealed a decreasing diversity, even though in all systems the diversity was surprisingly high (Bolhuis *et al*., 2014).

Clone libraries of a mixed community of cyanobacteria and diatoms on a coastal intertidal sediment revealed Deltaproteobacteria, Gammaproteobacteria, Planctomycetes, Bacteroidetes, Cyanobacteria and diatoms (chloroplasts) as the main groups (Miyatake *et al*., 2013). A similar community composition was reported from diatom biofilms on another intertidal mudflat with Bacteroidetes and Proteobacteria (Alpha-,~25%, Gamma-,~15% and Delta-, 4%) as the predominant bacterial phyla (Hicks *et al*., 2018). These authors showed in a mesocosm experiment that changes in temperature caused shifts in community composition at lower taxonomic levels but not at the phylum level, which remained more or less constant. They also concluded that there was little functional redundancy within the bacterial component of this diatom biofilm. Miyatake and colleagues (2013) distinguished a 2 cm top layer from the lower 2–5 cm layer of the sediment as well as the 'resident' community (DNA) from the 'active' (RNA) microorganisms. In both fractions, the Deltaproteobacteria were mostly represented by the sulfate-reducing family Desulfobacteraceae and were most abundant in the deeper sediment layer. Gammaproteobacteria were the second-most abundant group and abundantly present in both sediment layers and may comprise anoxygenic phototrophic bacteria or sulfur-oxidizing microorganisms. Remarkably, cyanobacteria and diatoms were also abundantly present in both sediment layers and all resident taxa of these oxygenic phototrophs also appeared in the active community. The activity of these two groups of oxygenic phototrophic organisms was demonstrated by the uptake of organic substrates by these organisms. The deeper sediment layer was dark and anoxic. Hence, these organisms were metabolically active under these conditions. Bacteroidetes and rare taxa ('other') were far more abundant in the 'resident' fraction and most of these taxa were not found in the RNA fraction. They may represent components of the community that were inactive or resting at the time of sampling or consisted of dead cells or even extracellular DNA (see below).

An extensive study of the biodiversity of a coastal microbial mat using a massive tag sequencing approach was conducted by Bolhuis and Stal (2011). They concluded that the biodiversity of these mats was among the highest reported for any benthic microbial ecosystem (average Chao and Shannon, 2853 and 5.9 respectively). Also, it became clear that these coastal microbial mats are Bacteria dominated and that Archaea were low in abundance and also much less diverse (average Chao and Shannon, 182 and 2.9 respectively). The seasonal differences were minor, indicating that the composition of the resident organisms was more or less constant. More pronounced differences were found between mats that developed at different locations along the tidal gradient. Most likely, the salinity gradient that varied from nearly freshwater in mats developing close to the dunes, alternating fresh- (rain and upwelling groundwater) and marine water (brackish) in the intermediate mats and marine water between the high and low water mark is the driving force behind the extant community diversity. Cyanobacteria were in low abundance in the marine mat and made up 18% and 16% of the community in the freshwater and brackish mats respectively. Proteobacteria were abundant in all mats and represented on average about 38% of the community. However, there were remarkable differences when considering the subdivisions of the Proteobacteria. Alphaproteobacteria and Betaproteobacteria were particularly important in the freshwater mat. The latter was virtually absent in the brackish and marine mats, in which the Deltaproteobacteria and Gammaproteobacteria dominated. This distribution of Proteobacteria and Cyanobacteria hints strongly to salinity as a driving factor. Bacteroidetes and Actinobacteria were the other two abundant groups and showed less notable differences along the tidal gradient. These two groups are important as chemoheterotrophic organisms, specialized in the degradation of complex organic matter and play, therefore, a crucial role in the carbon cycle in the mats (Coskun *et al*., 2018). Sulfate-reducing bacteria and (phototrophic) sulfur-oxidizing bacteria belong roughly to the Deltaproteobacteria and Gammaproteobacteria, respectively, and this explains their higher abundance in the marine and brackish mats. Non-sulfur photosynthetic bacteria are often Alphaproteobacteria and were more abundant in the freshwater mats. Nevertheless, all of these groups were abundantly present in all three mat types because they fulfil crucial functions within this ecosystem.

There are two problems with the analysis of diversity in these microbial mats. First, the heterogeneity of microbial mats and the small sample size for nucleic acid extraction may give highly variable results, and secondly, DNA may reveal a far higher diversity because of the presence of inactive, dormant or dead organisms or even extracellular DNA. In order to deal with these problems, Cardoso and colleagues (2017) co-extracted DNA and RNA from replicate samples taken randomly from a mat and at regular times during a 24 h day. The idea was that the community composition would not change during 1 day and that differences, therefore, must be attributed to heterogeneity. The DNA-recovered 16S rRNA gene diversity was assumed to represent the resident community, while the RNA-recovered 16S rRNA diversity would represent the active community. Cardoso and colleagues (2017) did not find major differences during the 24 h day, indicating that the cells did not vary their ribosome content during a day. These authors did however find considerable

differences between the 'resident' and 'active' fractions of the mat community. Remarkably, they found that 95% of the active fraction consisted of cyanobacteria, but represented 60% of the resident community. The latter number was much higher than previously reported by Bolhuis and Stal (2011), which may be explained by the progressing techniques of nucleic acid extraction and amplicon sequencing but also by the continuous development and changing structure and community composition of these coastal microbial mats and their sedimentary environment. The results of Cardoso and colleagues (2017) emphasize the fact that these systems are true 'cyanobacterial mats'. The other members of the community were Proteobacteria (~40% and ~5% in the resident and active fractions respectively) and Bacteroidetes. This is in line with the previous analyses of these mats. The resident cyanobacteria were mostly represented by filamentous non-heterocystous cyanobacteria of the genera *Microcoleus* (*Coleofasciculus*) and *Lyngbya.* The active cyanobacteria were about equal filamentous non-heterocystous and heterocystous (N_2-fixing) cyanobacteria in addition to lower numbers of unicellular cyanobacteria, confirming previous analyses of coastal microbial mats.

The millimetre scale vertically stratified communities and steep physicochemical gradients give ample opportunities for niche differentiation and the co-existence of competing microorganisms, but few studies actually considered aspects of phylogenetic clustering and trait diversity along these physicochemical gradients. Armitage and colleagues (2012) observed remarkable differences in microbial diversity when analysing different layers of a salt marsh microbial mat. They concluded that habitat filtering was an important factor for the assembly of these mat layers, except for the top layer of cyanobacteria where the phylogenetic diversity was high and trait richness relatively low.

Biodiversity of ecosystems has often been mentioned as an essential factor for performing ecosystem function. In intertidal diatom biofilms it was shown that photosynthesis was either positively correlated with diatom biodiversity or had a unimodal relationship (highest photosynthesis at a certain 'optimum' biodiversity and a lower photosynthetic rate both at higher and lower biodiversity) and that these relationships were site specific (Forster *et al*., 2006). These authors also noticed that biodiversity was not related to the standing stock of diatom biomass.

Viruses in microbial mats and biofilms

Although, not actual living organisms, viruses outnumber any other biological entity (Danovaro *et al*., 2008a). Viruses multiply by infecting their (microbial) hosts, causing their death and recycle their organic matter and nutrients in the environment (Fig. 2). Therefore, viruses play

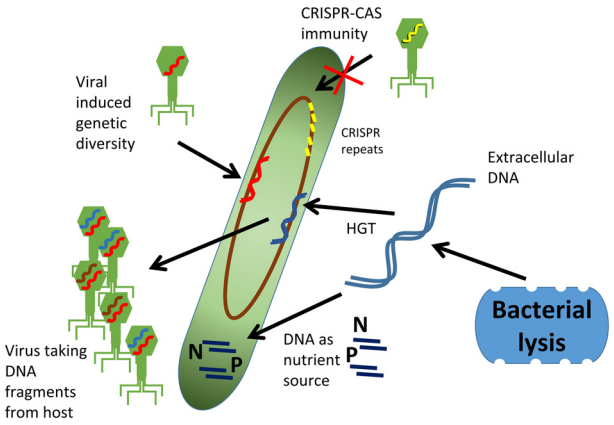

Fig. 2. Schematic representation of the generation of genetic diversity through horizontal gene transfer (HGT) in bacteria.
The source of genetic material for HGT is present as extracellular DNA released by lysing cells in the biofilm's EPS matrix and as part of the viral genome that can be integrated in the host chromosome. Upon viral lysis, novel combinations of genomic DNA can be integrated in the viral genome and increase genetic diversity upon subsequent infections. The CRISPR-CAS system indicated at the top induces a bacterial immune response that prevent reinfection by previously identified viruses. [Color figure can be viewed at wileyonlinelibrary.com]

undoubtedly an important environmental role including marine sediments and more specifically microbial mats and biofilms (Montanié *et al*., 2015). There is only a limited amount of data available with respect to the occurrence and activity of viruses in phototrophic biofilms and microbial mats. Examples taken from the better studied deep-sea sediments may help to understand their role.

Danovaro and colleagues (2015) estimated that two-third of the total number of microbes and viruses in the top 0.5 m of the global deep-sea sediments (1.5×10^{29}) is represented by viruses, although, their biomass is only < 1%. It has been estimated that viral-induced mortality of benthic heterotrophic microorganisms in deep-sea sediments may be as high as 80% (Danovaro *et al*., 2008b). This mortality increases with depth and in the seafloor below 1000 m virtually all heterotrophic production is recycled through the viral shunt which seems to affect Archaea more than Bacteria (Danovaro *et al*., 2016). Viral mortality seems to be low in (oxygenated) freshwater sediments (Fischer *et al*., 2003; Filippini *et al*., 2006). It is not clear why this is the case but it might be attributed to a low virus-to-bacterium ratio, despite the high viral abundance in these sediments. Also in anoxic coastal sediments virus-induced lysis of bacteria was moderate despite the high abundance of viruses in these sediments compared to the water column and the viral shunt was considered unimportant (Glud and Middelboe, 2004; Middelboe and Glud, 2006).

In deep-sea sediments viral production and decay rates have been shown to be in equilibrium and were correlated to bacterial production (Corinaldesi *et al*., 2010).

These authors also reported that in deeper sediment layers viruses seemed to be preserved to a certain extend. The viral shunt contributed significantly to the release of carbon and hence is a crucial process for the sediment biogeochemistry and is probably an important factor that determines the composition of the microbial community (Corinaldesi *et al*., 2012; 2014) although, others concluded that nutrient availability rather than viral infections controls diversity and community composition in marine benthic environments (Hewson *et al*., 2003). Naturally, viral lysis also recycles nutrients and it is, therefore, difficult to distinguish what is actually the controlling factor. Moreover, viral lysis may be favourable to those organisms not affected and taking advantage of the recycled nutrients. Danovaro and Serresi (2000) showed that marine sediments contained up to three orders of magnitude more viruses compared to the water column but that the virus-to-bacterium ratio was much lower, although, Mediterranean deep-sea sediments seemed to be an exception to this rule. This was attributed to the low doubling times of the bacteria in these sediments (Danovaro *et al*., 2002; Mei and Danovaro, 2004). This could hint to a higher retention and lower decay of viruses at higher depth in subsurface (marine) sediments (Danovaro *et al*., 2005) and that also the potential of these sediment viruses to infect bacteria is low. It is also possible that viruses from the water column deposit in the sediment and do not find their appropriate hosts. The benthic microbial community differs from its planktonic counterpart. In contrast, viruses decay rapidly at the deep-sea sediment surface through the activity of extracellular enzymes and the input of the released carbon, nitrogen and phosphorus represent an important resource for the microbial community (Dell'Anno *et al*., 2015).

Carreira and colleagues (2015) reported that photosynthetic microbial mats in intertidal sediments harbour high densities of viruses (2.8×10^{10} g^{-1}) up to three orders of magnitude higher than in other benthic habitats, but in the same order of magnitude of some groups of bacteria (Visscher *et al*., 1992; Carreira *et al*., 2013). Also deep-sea chemosynthetic microbial mats contain such high numbers of viruses and very high virus-to-bacteria ratios (Kellogg, 2010). These high ratios were attributed to the high microbial activity in microbial mats. The heterogenous distribution of substrates in photosynthetic microbial mats probably caused a lack of correlation of the distribution of bacteria and viruses. It has been proposed that the microbial loop dominates during times of high resources in a phototrophic biofilm (i.e. when photosynthetic production is high in spring) and the viral shunt during summer when resources have been depleted (Montanié *et al*., 2014). The metagenome of Arctic cyanobacterial mats revealed only 0.1% of the reads attributed to viruses, mainly cyanophages and phages of Alfaproteobacteria, Betaproteobacteria and Gammaproteobacteria (Varin *et al*., 2010). Also other authors have observed the lack of correlation between bacteria and viruses in marine sediments. Bettarel and colleagues (2006) suggested that sediments preserve viruses very well and that that was the reason for their high abundance. Middelboe and colleagues (2011) suggested that the decay of viruses in subsurface marine sediments is low and that they may persist for many (even millions of) years. Low numbers of infected bacteria may hint that viruses do not proliferate in some marine benthic sediments (Bettarel *et al*., 2006). It is possible that the viruses may have been deposited from the water column and that the sediment bacteria are not their host (Säwström *et al*., 2009).

The viral diversity of marine benthic systems is largely unknown, which is partly due to lacking bioinformatic tools to analyse metagenomic datasets (Tangherlini *et al*., 2016). In deep-sea sediments the viromes were dominated by dsDNA viruses (Corinaldesi *et al*., 2017). RNA viruses were also detected but their actual presence and role is uncertain and is probably due to erroneous assignment by the available sequence databases. Although, some viruses may infect multiple hosts and even taxonomically distant organisms (and as such contribute to interspecies and even interdomain gene transfer), the majority seem to be specialized to infect single species. The viral diversity is, therefore, to be expected to greatly exceed that of the microbial world, but in fact very little is known about the virome in marine sediments (Danovaro *et al*., 2008a).

The role and fate of EPS in benthic phototrophic communities

Microphytobenthos exudes extracellular polymeric substances (EPS), which may be as much as 70%–80% of the fixed carbon (Goto *et al*., 1999; Smith and Underwood, 2000; Underwood and Paterson, 2003). There may be two reasons for these organisms to exude EPS. One is overflow metabolism. The photoautotrophic organisms fix CO_2 into organic matter and synthesize structural cell material. However, in order to be able to do this the cell need nutrients such as among others nitrogen, phosphorus, sulfur and iron. When these nutrients are not in sufficient supply, carbohydrates are produced which do not contain these elements. In this way, the cell can also divert the energy that it necessarily harvests from the sunlight through its pigments (Fields *et al*., 2014; Levitan *et al*., 2015). Under nutrient replete conditions, 40% of the carbon fixation is used for the synthesis of amino acids (Levitan *et al*., 2015). Excess electrons could otherwise produce reactive oxygen species that could damage

DNA, membranes, proteins and other macromolecules (Hu *et al*., 2008). Neutral lipids are particular efficient storing excess electrons (Rawat *et al*., 2013). Carbohydrates are stored intracellularly and serve as carbon- and energy storage. These intracellular storage compounds are used for the synthesis of structural cell material as soon as nutrients become available (Kroth *et al*., 2008; Bellinger *et al*., 2009). Hence, photosynthetic carbon fixation and growth (synthesis of structural cell material) may be temporally separated (Mitbavkar and Anil, 2004; Underwood *et al*., 2005). However, intracellular storage capacity is limited and excess fixed carbon may be exuded as EPS, which is usually more complex than the intracellular stored carbohydrate (mostly glycogen in cyanobacteria and chrysolaminaran in diatoms) and may contain glycolipids, proteins, nucleic acids, uronic acids and sulfated-sugars. EPS contain enzymes such as β-glucosidase that degrade polysaccharides (Romaní *et al*., 2008). Cyanobacteria as well as diatoms may utilize the released glucose from EPS under dark anoxic conditions (Miyatake *et al*., 2014).

Exudation of EPS may also be the result of motility of the organism. Both diatoms and cyanobacteria migrate while exuding extracellular polymeric substances. In addition, EPS is instrumental in the attachment of microorganisms to a surface through hydrophobic interactions and co-flocculation mediated by certain charged groups in these molecules (Bar-Or and Shilo, 1988). Moreover, variations in these properties may enable organisms to detach and disperse. Anionic EPS may be released into the medium and bind organisms and clay particles in the water column via divalent cation bridges and transporting them to the microbial mat, making the water column above it more transparent. The divalent nature of these EPS also enabled binding metals, which may be beneficial both for scavenging toxic and micronutritional elements (Bender *et al*., 1994).

EPS is distinguished in material that is closely associated with the cell and may be part of a structured sheath or capsule or unstructured cell-bound material. EPS may also be unattached to the organism. It is exuded into the environment and forms the matrix of the biofilm or the mat in which the organisms are embedded. This is usually unstructured mucilage (Flemming and Wingender, 2010).

Extraction of EPS from benthic phototrophic communities is problematic and prone to biases. Depending on the protocol used, operational fractions of EPS are obtained, that is, the extracted material is the result of the applied method (Stal, 2010). Most methods distinguish two major fractions: a glucose-rich largely neutral polymer and a more complex charged carbohydrate that is composed of a variety of monomeric sugars, and other components. The former is obtained by simple (warm) water extraction. The latter fraction is often tightly bound to the sediment because the charged groups bind to cations and form bridges with other polymers and with the sediment. This fraction can be extracted by EDTA. Stal (2010) conceived that the glucose from the water-extractable EPS is consumed transforming it to the EDTA-extractable EPS. Hence, the diatoms would produce only one type of EPS, which is rich in glucose and amenable to microbial degradation. This is supported by the work of Moerdijk and colleagues (2018b), who found that carbon label appeared later in EDTA-EPS and that this label was more evenly distributed among the carbohydrate monomers. The more refractory EDTA-EPS remains and renders stability to the sediment, may bind nutrients or immobilize (toxic) metals, and is only slowly degraded. For instance, proline and glycine in the amino acid fraction of EDTA-EPS may be involved in the adhesion between microorganisms and determine the rheological properties of the EPS matrix (Redmile-Gordon *et al*., 2015). Diatoms such as the benthic *Phaeodactylum tricornutum* have been shown to produce EPS containing modular adhesive proteins as part of a supramolecular adhesive structure (Dugdale *et al*., 2006).

There is a tight coupling between microalgae and bacteria in biofilms where the latter group consumes the organic matter produced by the former and is supported by the presence of extracellular enzymes (Ylla *et al*., 2009). Addition of glucose to such biofilm causes shifts in the community composition and breaks down the coupling between algae and bacteria. Miyatake and colleagues (2014) showed that part of the carbon exuded by the diatoms was consumed by Gammaproteobacteria, Bacteroidetes and Deltaproteobacteria, which represented 21%, 8% and 7% of the 16S rRNA clone libraries in this diatom biofilm. These groups equally consumed organic matter produced by the diatoms. Hence, there appeared no specialization among these heterotrophic bacteria. The authors distinguished a fast initial consumption, which was attributed to low-molecular compounds leaking from the diatoms, followed by a slower process, which was attributed to the decomposition of the more complex and high-molecular EPS, which first had to be degraded by extracellular enzymes to low-molecular compounds. A similar carbon transfer was found in a freshwater chemoautotrophic benthic community, although, in this case the consumers were more diverse and seemed to have a higher specialization (Coskun *et al*., 2018). These differences may be attributed to the method, which was fundamentally different from the one used by Miyatake and colleagues (2014). The latter authors also showed that only the water-extractable EPS was consumed. Interestingly, not only the heterotrophic bacteria consumed the EPS but also the diatoms used it. Staats and colleagues (2000) showed in axenic cultures of the benthic diatom *Cylindrotheca closterium*, when grown under

an alternating light–dark cycle that this organism exuded EPS during the light and consumed it during the dark. Hence, it seems that diatoms may use EPS as a storage compound in addition to the intracellular chrysolaminaran. While they possess the enzymes that produce these polymers, it is anticipated that they might also possess enzymes or mechanisms of uptake and hydrolysis.

Moerdijk and colleagues (2018a,b) conducted a comprehensive seasonal study of an intertidal diatom biofilm using a carbon stable isotope labelling – and – chasing method. They found that the highest carbon fixation occurred during early spring and that the fixed carbon was mainly (80%) stored as glucose. In summer, a much lower percentage of the fixed carbon was exuded as extracellular polymeric substances compared to the other seasons (9% and 21% respectively). Although, this may not seem surprising, it has important ecological implications. Moerdijk and colleagues (2018a) hypothesized that the physiology of the microphytobenthos was fundamentally different in summer compared to the rest of the year. In summer, more of the fixed carbon was stored as neutral lipids (Hu *et al*., 2008; Hockin *et al*., 2012) compared to the rest of the year (27% and 12% respectively) and the partition of carbon among the different components changed. Also, the exuded EPS had a different composition and it was supposed that in summer the exudation of EPS was predominantly associated with migration of the diatoms. During summer, the physiology of the diatoms may have changed as the result of higher irradiation and temperature, depletion of inorganic nitrogen and the concomitant shift to urea as nitrogen source. This could have decreased the production of carbohydrate at the expense of neutral lipids, and triggered higher motility (Consalvey *et al*., 2004; Guschina and Harwood, 2006; Fields *et al*., 2014; Levitan *et al*., 2015). This may also have been influenced by the high grazing pressure and bioturbation of fauna in the intertidal sediment during summer.

The carbon isotope label applied by Moerdijk and colleagues (2018a) on a natural intertidal diatom biofilm disappeared quickly by (dark) respiration of chrysolaminaran, exudation and subsequent loss of EPS in the overlying water during inundation, and by bacterial consumption (Goto *et al*., 2001; Miyatake *et al*., 2014; Moerdijk *et al*., 2018b). Apart from being resuspended in the overlying water or being consumed by bacteria, EPS may also be reabsorbed by the diatoms themselves (Staats *et al*., 2000; Miyatake *et al*., 2013; 2014).

Moerdijk and colleagues (2018b) demonstrated that there was a close coupling of the biomass and production of diatoms and bacteria during spring, when productivity was highest and which became weaker during the rest of the year. Based on the carbon stable isotope labelling experiment, these authors concluded that the low-molecular SCOA (short-chain organic acids) were the most important substrates for the bacteria and that the sulfate-reducing bacteria were the group benefiting most of these substrates released by the diatoms. During summer and autumn, the production of SCOA was low and other bacterial groups consumed the diatom exudates. Hence, the changing physiology of the diatoms controlled the composition of the bacterial community and generated the bacterial diversity. The study showed that the water-extractable fraction of EPS was the primary substrate for heterotrophic bacteria. Clearly, the EDTA-fraction was recalcitrant to bacterial degradation (Giroldo *et al*., 2003).

It was discovered that during spring when productivity and EPS exudation were highest, the EDTA fraction contained a high amount of threonine. This amino acid has been attributed a role in algal defence (Buhmann *et al*., 2016) and it might, therefore, decreased the bacterial consumption of EPS, which would allow the diatoms to use a greater portion for re-assimilation. Alternatively, threonine or threonine-containing amino acids or polymers could serve as signalling compound or adherence and defence processes (Buhmann *et al*., 2016).

Diatoms may have other ways in limiting the amount of EPS going to other organisms. For instance, benthic diatoms accumulate dimethylsulfoniopropionate (DMSP) as osmoprotectant. When this is liberated into the environment, for example, after an osmotic down-shock, DMSP is decomposed into the gas DMS and acrylate. The latter may have a bacteriostatic effect limiting the consumption of EPS by bacteria (Slezak *et al*., 1994).

Extracellular nucleic acids and their transfer in benthic microbial communities

High molecular free DNA is present in considerable amounts in the environment and is mostly originating from microorganisms (Fig. 2). More than 90% of the total DNA in deep-sea sediments may be extracellular and is bound to sediment particles where it seems to be protected from nucleases (Dell'Anno and Danovaro, 2005). Nevertheless, a substantial part of the extracellular DNA can be a source of carbon and nutrients for the community. Corinaldesi and colleagues (2014) showed that viral lysis of bacteria was the main source of extracellular DNA in deep hypersaline anoxic basins and that it contained the signature of past and present infections. In microbial mats and biofilms, EPS may contain considerable amounts of extracellular DNA (Flemming and Wingender, 2010). Cell lysis is certainly one of the mechanisms by which DNA is released in the environment but there may be other processes as well. For example, DNA could be released without affecting the cell integrity and may be related with the development of competence, sporulation in spore-forming microorganisms or the formation of

membrane-derived vesicles containing DNA (Lorenz and Wackernagel, 1994). Distinguishing between intracellular and extracellular DNA in sediments is not easy (Pietramellara *et al*., 2009). DNA will bind to sediment particles and humic acids, may be caught into EPS, or attach to the exterior of microorganisms. Methods to extract this extracellular DNA may also affect the integrity of cells and, hence, release intracellular DNA. In order to circumvent these problems, one could focus on RNA, which is said to be unstable outside a living cell. However, to our knowledge no reports have been published about the half-life of (r)RNA in (anoxic) marine sediments.

In order to be able to take up extracellular (high-molecular) DNA, the cells have to be in a so-called 'competent' state. In most microorganisms, competence is transient and is developed under conditions that prevent normal growth and are often hostile to the cell or during the stationary phase. Also, bivalent cations such as Ca^{2+} and Mg^{2+} and monovalent Na^{+} ions play a role in some species. Development of competence is a complex process that involves a variety of genes and their products and, therefore, requires that the organism is metabolically active. *Bacillus subtilis* releases DNA while in the process of becoming competent (Sinha and Iyer, 1971). Also, *Pseudomonas aeruginosa* actively exudes DNA in the environment through vesicles. This extracellular DNA seemed to be critical for the formation of a biofilm by this bacterium (Whitchurch *et al*., 2002). The exudation of DNA by biofilm-forming bacteria is strongly dependent on the species and whether or not a multispecies biofilm is considered (Steinberger and Holden, 2005). These reports were all from laboratory experiments and it is questionable whether the mechanisms derived from these experiments can be extrapolated to highly complex and diverse natural biofilms and microbial mats.

Microbial mats are highly diverse ecosystems with a large variety of functional groups and thousands of species. Many species that have been identified belong to the rare biosphere, that is, occur in very low abundances. Since most of the rare biosphere has been identified based on the sequences of extracted DNA it has been proposed that it is possible that these sequences do not actually belong to living organisms but rather originate from extracellular DNA (Reid and Buckley, 2011). The sequence identity of extracellular DNA differs from the resident community in marine sediments (Corinaldesi *et al*., 2005; 2014). Although, extracellular DNA most likely originates from the resident microbial community, the possibility cannot be excluded that it may have been imported from elsewhere, which would explain the difference with the signature of the resident community. Nevertheless, this DNA can be taken up, and it will thereby have an important function not only as a source of nutrients but also may contribute to the adaptive and evolutionary capacity of the community.

DNA binds to quartz sand and clays in the presence of NaCl or divalent ions such as $MgCl_2$, the latter being up to 100 times more efficient (Lorenz and Wackernagel, 1994). Moreover, divalent ions such as Mg^{2+} or Ca^{2+} are required for genetic transformation. DNA that is immobilized in these sediments appears to be resistant to DNase and keeps it transforming activity (Lorenz *et al*., 1988; Pietramellara *et al*., 2009). Also, DNase does not prevent genetic transformation. Extracellular DNA in sediments is mostly double stranded and high molecular, both prerequisites for transformation. In fact, microorganisms seemed to be more attracted to particles with DNA attached and adhered more frequently to them. The close vicinity of microorganisms and extracellular DNA in microbial mats and biofilms should offer excellent conditions under which natural transformation can take place (Orsi, 2018). Because nucleic acids can also be used as a substrate by microorganisms, freshwater sediments and soils may, therefore, not show extensive natural transformation.

Horizontal gene transfer

Gene transfer occurs among Bacteria, Archaea and Eukarya (Heinemann, 1991; Garcia-Vallvé *et al*., 2000; Rest and Mindell, 2003). In nature, gene transfer takes place by three fundamentally different mechanisms (Roberts *et al*., 2001). (i) Transformation is the process of the uptake of foreign extracellular genomic DNA or plasmids by a (naturally) competent host and its incorporation into the genome of the host by recombination. (ii) Conjugation is the process during which two 'mating' cells physically connect and exchange DNA in the form of plasmids or transposons through this connection. (iii) Transduction is the transfer of DNA through the infection by bacteriophages (viruses). The phage may carry DNA of a donor and have it integrated into the genome of the host after it became infected (Fig. 2). The phage may also acquire DNA from the target cell after it has infected that cell and replicate it in its viral genome and this phage is subsequently released into the environment after lysis of the host (Sullivan *et al*., 2006). Inside the phage, the DNA is stable and resistant to physical factors and chemicals. This persistence in the environment can be considerably enhanced when these viral particles are attached to minerals and other particles. Transfer of genes between different bacteria depends on the host range of the phage, which may be small or large.

Due to their high standing stock biomass and cell density and a physiology that is characterized by nutrient deficiency, biofilms and microbial mats are ideal environments

in which genetic material of various sources can be exchanged by any of the known mechanisms (Ghigo, 2001) (Fig. 2). Microorganisms in biofilms and microbial mats share the same habitat, which aids to the success of horizontal gene transfer as it is unlikely that genetic information is transferred that mismatches with the environment (Cohan and Koeppel, 2008; Philippot *et al*., 2010). Especially the high cell density may allow recombination that in itself would be highly unlikely in diluted plankton communities (Levin and Bergstrom, 2000). Nevertheless, horizontal gene transfer is obviously more frequent and more successful among closely related organisms (Popa *et al*., 2011). Among other factors, sequence identity facilitates recombination. Also, DNA from closely related organisms is recognized and preferentially taken up (Hamilton and Dillard, 2006). Gene transfer is not only facilitated in and by a microbial mat but the consequent greater genetic adaptability of the community also allows the development of this microbial community under dynamic environmental conditions (Molin and Tolker-Nielsen, 2003). Bacterial evolution and the acquisition of new properties is accelerated by the fact that these organisms can readily acquire genes from other microorganisms using a variety of processes. Horizontal gene transfer allows microorganisms to adapt to new niches and is, therefore, critical for the formation of biofilms and microbial mats and consequently also lies at the basis of the diversity in these microbial ecosystems (Wiedenbeck and Cohan, 2011). Hence, horizontal gene transfer may be an inherent property of biofilms and evolved for the benefit of these communities. Also proteins can be transported in biofilms from an organism that has the genomic capability of synthesizing them to organisms without this capability and hence extend their metabolic capacities without transfer of genetic code (Remis *et al*., 2010).

Horizontal transfer of genes from mat-forming Cyanobacteria may have introduced the formation of trichomes, chemotaxis and the exudation of EPS into the chemotrophic bacteria *Beggiatoa.* This may have provided them with homologue mat formation traits, which, in the rock record, might then be erroneously attributed to Cyanobacteria (Flood *et al*., 2014).

Horizontal gene transfer of the gene cluster coding for phycobiliproteins in the planktonic picocyanobacterium *Synechococcus* resulted in the formation of a population of different pigmented ecotypes that were sometimes indistinguishable, or at least highly similar, from their 16S rRNA gene or ribosomal internally transcribed spacer (ITS) sequences (Haverkamp *et al*., 2009). These red- and blue–green pigmented ecotypes coexisted in their habitat while sharing different spectral parts of the available light (Stomp *et al*., 2004; 2007). Apart from their remarkable different colours, the phenotypes of these ecotypes differed in size and growth rate (red-pigmented ecotypes were larger and their maximum specific growth rate was faster, probably reflecting the higher nutrient availability at the greater depth where they were more abundant) (Stal, unpublished). Hence, based on their 16S rRNA sequence these organisms would be considered the same species, but based on their phenotype (and genotype of the phycobiliprotein gene clusters; Six *et al*., 2007) these ecotypes may fulfil the definition of an ecological species that share an environmental niche (Ward *et al*., 2006). Similarly, the evolution of differently pigmented *Pseudanabaena*, a filamentous cyanobacterium with red, blue–green and flexible (complementary chromatic adaptation) phenotypes, may have been achieved through lateral gene transfer of phycobiliprotein gene clusters (Acinas *et al*., 2009). Although, these examples were not from a benthic habitat, both *Synechococcus* and *Pseudanabaena* occur in benthic habitats such as microbial mats where they experience the same environmental forces, be it on a micrometre scale rather than (10th's of) meters. For instance, the hot spring microbial mat unicellular cyanobacterium *Synechococcus* has adapted to different temperature, nutrient and light conditions (Bhaya *et al*., 2007). *Synechococcus* and *Roseiflexus*, a phototrophic filamentous green non-sulfur bacterium from mats of Octopus Spring in Yellow Stone Park (United States), were ecologically distinct according to their location along the temperature gradient of the spring and the depth at which they occurred in the mat. These ecotypes may have arisen through adaptive radiation by mutation or horizontal gene transfer and as a result of the actual ecological niche in which they occur or have invaded and thereby being selected as competitively superior (Ward *et al*., 2006). Coexistence of ecotypes with different adaptations to ecological conditions would increase the resilience of an ecosystem towards changes in these conditions, as there is a chance that one ecotype is optimally adapted to the new situation. Hence, community composition is intimately connected to ecosystem function. *Synechococcus* may have acquired genes for iron-, phosphate- and nitrogen utilization pathways through horizontal gene transfer according to differences in nutrient fluxes in the mat and thus contributed to the diversification and ecotype evolution along the temperature gradient of the spring (Bhaya *et al*., 2007). Ecotypes occurring downstream at the lower temperature areas were adapted to lower nutrient concentrations. In salterns, cyanobacterial variants were detected in hypersaline microbial mats that seemed to be adapted to certain levels of salinity and were accordingly distributed along the salt gradient (Nübel *et al*., 2000).

Microcoleus (Coleofasciculus) chthonoplastes is a cosmopolitan mat-forming cyanobacterium. Some authors noticed the lack of cyanobacterial *nif* (*nifH*) genes in *M. chthonoplastes* mats that were shown to

possess nitrogenase activity (Steppe and Paerl, 2002; 2005). Because *nifH* sequences obtained from these mats belonged to sulfate-reducing bacteria, these authors suspected that sulfate-reducing bacteria rather than cyanobacteria were responsible for the N_2 fixation. However, Bolhuis and colleagues (2010) discovered a complete *nif* cluster in the genome of the type strain. Remarkably, most of this cluster, including the structural genes *nifHDK*, appeared not to be of cyanobacterial origin but was related to genes from Deltaproteobacteria or Chlorobi. Bolhuis and colleagues (2010) concluded that *M. chthonoplastes* must have obtained (part of) this *nif* cluster through horizontal gene transfer, possibly from a sulfate-reducing bacterium. Because this gene cluster was present in all available strains of *M. chthonoplastes*, isolated from distant microbial mat environments around the globe, this event of gene transfer must have occurred early in the evolution of this organism, most likely in a microbial mat setting.

M. chthonoplastes and sulfate-reducing bacteria are co-occurring in marine microbial mats and both groups of organisms are, therefore, well-adapted to this environment. The transfer of a long stretch of DNA encompassing nine genes is high for a horizontal gene transfer event between such divergent organisms. This horizontal gene transfer may have been successful because the gene cluster must have been adapted for that environment (Cohan and Koeppel, 2008).

Complicating factor in this story is that hitherto it has not been possible to grow *M. chthonoplastes* diazotrophically (on N_2 as the only source of nitrogen) nor that this organism expressed its *nif* genes in culture. The tested conditions included anoxic and anoxygenic conditions and the addition of sulfide. None of these were successful in obtaining nitrogenase activity or *nif* gene expression (authors' unpublished results). Using primers, specific for *M. chthonoplastes nifHDK*, it was shown that these genes were expressed in natural mats of this cyanobacterium, which could be taken as evidence that under natural conditions expression of the *nif* genes is possible. Preliminary experiments indicated that growing *M. chthonoplastes* as a mat on sand might result in the expression of the *nif* genes and in the detection of nitrogenase activity. These unpublished results need confirmation but when proven true, it emphasizes the importance to study mat-forming microorganisms immobilized on sediment rather than in liquid culture, which is hitherto standard.

Another example of a horizontal gene transfer event was found in the marine benthic (mat-forming) unicellular chlorophyll-*d* containing cyanobacterium *Acaryochloris*, strain HICR111A, which contained the whole cluster of structural genes for nitrogenase and the genes for all associated enzymes and co-factor biosynthesis (Pfreundt *et al*., 2012). In this case, the cluster seemed to be obtained from obligate marine benthic unicellular *Synechococcus.* This shows that horizontal gene transfer events in benthic microbial communities such as microbial mats may be far more common than hitherto anticipated and also that, when occurring in the same environment, large stretches of DNA encompassing many genes may be transferred. In the case of *Acaryochloris*, strain HICR111A it was shown beyond any doubt that these genes were expressed and led to nitrogenase activity. As is common in these unicellular diazotrophs, nitrogenase activity was confined to the dark and required anoxic conditions and the transcription of *nif* and associated genes peaked shortly before nitrogenase activity was detected (Pfreundt *et al*., 2012).

CRISPR in microbial mats and biofilms

Many Bacteria and Archaea possess CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), which are acquired DNA sequences that serve as an immunity defence against phages (Makarova *et al*., 2011) (Fig. 2). It is conceived that CRISPR may be exchanged by horizontal gene transfer via megaplasmids, which would explain their similarity and commonness among Bacteria (Godde and Bickerton, 2006). Whole genome sequencing of bacteria originating from various habitats revealed that they possess 'adaptive immunity' against phages and that they all contain CRISPR loci (Jansen *et al.,* 2002; Bolotin *et al.,* 2005). CRISPR loci and their CRISPR-associated (Cas) genes function as an adaptive immune system protecting Bacteria and Archaea from invading genetic entities (Bergh *et al.,* 1989) (Fig. 2). Repeat elements of the CRISPR locus are intercalated with short 'spacer' sequences that match phage genomes (or mobile genetic elements such as plasmids) and trigger the immunity response on the basis of sequence identity (Marraffini and Sontheimer, 2010). The sequence and length of the repeat-spacer array are conserved within the specific CRISPR locus but can diverge within the same genome. The mechanism of the active immunity requires transcription of the CRISPR locus, followed by cleavage of the transcript within repeat sequences by Cas endoribonucleases (van der Oost *et al.,* 2014). This liberates small non-coding RNAs named CRISPR-rRNAs (crRNAs), which specify the target for RNA-guided Cas nucleases that defend the cell from infection by degrading invading genomes (Deveau *et al.,* 2008; Hale *et al.,* 2009). CRISPR-Cas systems can behave in an adaptive fashion through acquisition of new spacer sequences from invading elements, thereby conferring sequence-specific immunity. However, very little is still known regarding the complete role and functionality of such type of immunity in wild/natural communities. Overall, there is a lack of investigation into biotic factors (such as viruses and virus-like

entities) that lead to accumulation of certain type of CRISPRs in biofilms and microbial mats. Heidelberg and colleagues (2009) used CRISPR spacer sequences extracted from the genomes of two thermophilic *Synechococcus* isolates, from a phototrophic mat in Octopus Spring. Subsequently, they searched for viral contigs from previously published water metaviromes from the Octopus and Bear Paw Springs in Yellowstone National Park (United States) (Schoenfeld *et al*., 2008). Furthermore, CRISPR spacers and nucleotide motive frequencies linked to viral contigs were applied to known hosts using a metavirome obtained by Multiple Displacement Amplification (MDA) of virus-like particles from a mat in Octopus Spring (Davison *et al.,* 2016), as well as reference genomes from dominant species (*Synechococcus* sp., *Roseiflexus* sp. and *Chloroflexus* sp.) previously described in the same microbial mat. A key finding from these studies was the link between viruses and their hosts, indicating their co-evolution and an effective 'arms race' within hot spring phototrophic mats. We conceive that CRISPR sequences might be crucial for the generation of genetic diversity and hence the resilience of microbial mats and multispecies biofilms in general.

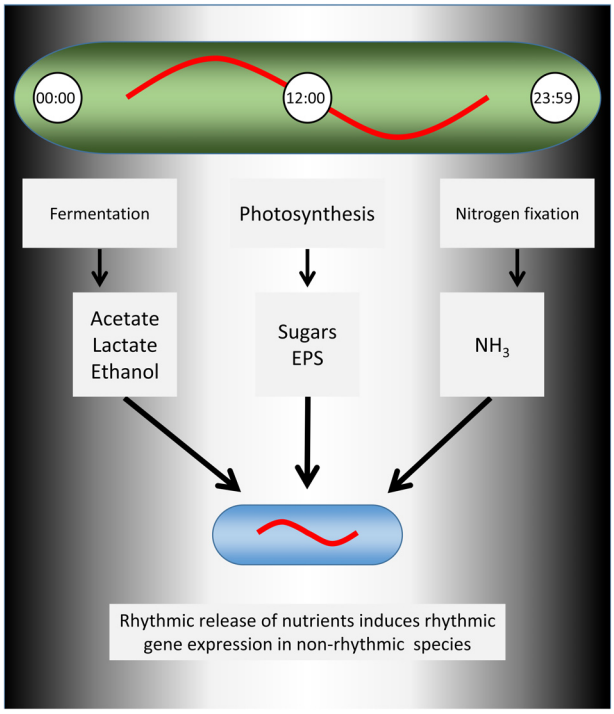

Fig. 3. Circadian control in tightly coupled microbial communities. Schematic representation of the choirmaster – choir theory describing the induction of rhythmic gene expression in non-rhythmic bacteria (choir) through the rhythmic extrusion of substrates by a circadian clock controlled cyanobacterium (Choirmaster) (Hörnlein *et al*., 2018). [Color figure can be viewed at wileyonlinelibrary.com]

Circadian rhythm in microbial mats

While a biological clock is common in eukaryotes, among the other domains Bacteria and Archaea only Cyanobacteria have been shown to possess a clock that produces a circadian rhythm for numerous processes. The circadian clock in Cyanobacteria has been well studied but only in some cultured model strains under laboratory conditions. Hörnlein and colleagues (2018) were the first to study the rhythmic transcription of genes in a natural cyanobacterial mat (Fig. 3). They identified rhythmic genes belonging to the eukaryotic diatoms and Cyanobacteria, but interestingly also to Proteobacteria and Bacteroidetes, both groups of Bacteria that play essential roles in the mat community. About 50% of the rhythmic genes belonged to the Proteobacteria. One of the questions these authors addressed was whether the circadian clock of cyanobacteria (and/or diatoms) would also force rhythmic gene transcription in other, anoxygenic phototrophic and chemotrophic bacteria in the mat. If this would be the case, the microbial mat might function as a biological unit in which the individual parts cooperate synchronously and as a living entity. Interestingly, Hörnlein and colleagues (2018) found homologues of the cyanobacterial clock genes in other mat bacteria, although, none possessed the full set of clock genes present in Cyanobacteria. Nevertheless, the possibility cannot be excluded that a molecular clock is more widespread than previously thought. The majority of the rhythmic genes in the studied cyanobacterial mats was expressed maximally during early morning and included among others genes involved in pigment synthesis, photosynthesis, carbohydrate metabolism and CO_2 and N_2 fixation and of course, the clock genes *kaiABC* and the circadian input kinase *cikA* that resets the clock. Another interesting observation was the cyclic expression of the peroxiredoxin gene *prx.* Many of the transcripts of this gene belong to Bacteroidetes. Peroxiredoxin redox cycles has been thought to function as an alternative circadian rhythmicity in Bacteria and Archaea (Edgar *et al*., 2012). Many of the rhythmic genes of the Cyanobacteria in the microbial mat were involved in CO_2 and N_2 fixation and photosynthesis and expressed in the early morning, confirming what is known from laboratory studies (Ito *et al*., 2009). Nevertheless, axenic laboratory cultures provide a very different environment and the lack of interaction with other organisms may result in a different behaviour compared to a natural environment and microbial community. Therefore, extrapolation from a laboratory experiment to the natural environment may not always be meaningful.

In cyanobacterial mats and other communities in which there is a direct coupling of the photosynthetic produced organic matter and its consumption by chemotrophic organisms a synchronization between these processes

seems to be of eminent importance (Gasol *et al*., 1998). A circadian clock that acts on the whole community may be the most obvious mechanism to achieve this and such periodicity has been found in the metatranscriptome of some oceanic microbial communities (Poretsky *et al*., 2009; Ottesen *et al*., 2014).

Hörnlein and colleagues (2018) proposed a theory that in cyanobacterial mats the Cyanobacteria (and possibly diatoms) act as a choirmaster, directing the rest of the community (the choir; Fig. 3). Only Cyanobacteria (and algae) possess a fully functional circadian clock, which is entrained by the daily light-dark cycle, while the molecular clocks of other organisms respond to the cyclic release of organic matter or other factors such as oxygen or pH and alkalinity by the phototrophs. Besides a partial molecular clock, quorum sensing might be another mechanism that could contribute to the regulation and cyclic behaviour of gene expression in the community (Danino *et al*., 2010; Decho *et al*., 2010).

Quorum sensing in microbial mats and biofilms

Quorum sensing is one form of extracellular cell-to-cell communication and represents one of the two small molecule signalling pathways in Bacteria, the other being intracellular, involving cyclic dinucleotides (Camilli and Bassler, 2006). Both signalling pathways are thought to control processes such as multicellularity, biofilm formation and virulence. Quorum sensing involves the release of small molecules extracellularly that can be detected by the population and triggers gene expression when a certain threshold of the concentration of this signalling compound (autoinducer) is exceeded (quorum). Hence, gene expression is changed in response to changes of the density of that population (Decho *et al*., 2011). The production of acyl homoserine lactones (AHLs) allow bacteria to control certain quorum-sensing processes through the control of gene expression. This is important because the action in question will only be successfully completed when a certain threshold of the population is present. Among these actions are biofilm formation (Davies *et al*., 1998), competence for DNA uptake and the production of antibiotics, to name a few. Some organisms that produce these autoinducers interfere at the same time with the quorum sensing process of other, competing, organisms and may interrupt them (Ji *et al*., 1997).

Not much is known about quorum sensing in microbial mats. Bachofen and Schenk (1998) reported about the presence of AHLs in microbial mats. Also Decho and colleagues (2009) reported the presence of a large variety of *N*-acylhomoserine lactones (AHLs) in stromatolithic microbial mats in the Bahamas. These authors also noted that short-chain AHLs were lower in abundance during daytime. Based on laboratory experiments in which the lability of AHLs at high pH was demonstrated, the authors suggested that the high pH in these microbial mats, resulting from photosynthetic CO_2 fixation, might have been the cause of the daily fluctuations of the short-chain AHLs. Many of the other supposed functioning of quorum sensing in microbial mats was extrapolated from what is known about this process and from the biogeochemistry, physiology and microbial diversity of microbial mats (Decho *et al*., 2010).

The development of a biofilm or a microbial mat involves a series of processes such as adhesion, the production of EPS, motility and chemotaxis and phototaxis that require tight tuning. Several models predict the development of a biofilm structure based solely on the response of individual biofilm microorganisms to substrate concentration (Wimpenny and Colasanti, 1997) or substrate gradient (Van Loosdrecht *et al*., 1997). However, the regulation of expression of the genes coding for these processes is difficult to envision without the involvement of cell-to-cell signalling. Quorum sensing is a possible candidate to achieve this, even when its role in biofilm and microbial mat formation has not yet been elucidated (Kjelleberg and Molin, 2002). Moreover, complex communities composed of many different functional groups of microorganisms such as diatom biofilms and microbial mats most likely depend on intraspecies and interspecies communication but little is known whether this indeed plays a role. Signalling may not always be required and cells in a colony or biofilm may organize themselves without it (Cho *et al*., 2007). The discovery of compounds that inhibited quorum sensing in extracts of cyanobacterial mats may even hint to a detrimental effect of signalling (Dobretsov *et al*., 2011; Abed *et al*., 2013).

In order to make quorum sensing functional, dense aggregates of microorganisms are usually required. However, a confined single cell would be able to initiate the quorum sensing process and be stimulated to form a biofilm (Carnes *et al*., 2010). The EPS matrix could play a role to provide a structure in which signalling compounds accumulate, constrain their diffusion and remain close to the producing cells (cell-to-cell calling distance) (Decho *et al*., 2011). Especially, when the EPS contains a high amount of charged ions, it may decrease the diffusion of solutes such as AHLs and accumulate and react with the biofilm matrix or other biofilm components. Moreover, the EPS matrix itself may alter the signalling compound or affect the signalling process and biofilms produce a variety of compounds that inhibit the quorum sensing process (Lazar, 2011).

Sharif and colleagues (2008) reported the production of the AHL N-octanoyl homoserine lactone in the biofilm-forming unicellular cyanobacterium *Gloeothece* and demonstrated changes in the expression of 43 proteins in this organism. These authors speculated how this could be

involved in the formation of a biofilm. In a biofilm, Cyanobacteria may experience light and nutrient limitations, which might require a concerted action in carbohydrate- and amino acid metabolism that could be modulated by quorum sensing. However, the production of AHL that is reported to be labile under alkaline conditions in a phototrophic biofilm that raises the pH to a high level when photosynthesizing in the light questions its function. Moreover, the autoinducer might also affect other microorganisms in the biofilm (cross talk) or may be degraded and used as carbon and nitrogen source by biofilm organisms, which would lead to a diminishing of the quorum sensing signal. The high levels of sulfide produced during the night in marine microbial mats may also react with low-molecular signalling compounds such as AHLs. To date, neither of these supposed actions have actually been shown in microbial mats and biofilms.

The purple non-sulfur anoxygenic phototrophic bacterium *Rhodopseudomonas palustris* produces the quorum sensing autoinducer p-coumaroyl-homoserine lactone from environmental, plant-derived, p-coumaric acid (Schaefer *et al*., 2008). The group of purple non-sulfur anoxygenic phototrophic bacteria, including the species *R. palustris* is important in marine microbial mats. The fact that this organism, in contrast to other AHL-producing bacteria, which use intracellular fatty acids, uses an extracellular substrate for the production of this autoinducer gives food for thought of the possibility of other environmental metabolites and more complex intraspecies and interspecies and even interkingdom signalling.

Cable bacteria and the sulfur cycle

Direct interspecies and intraspecies electron transfer and extracellular electron transfer between microorganisms and minerals are important processes in sediment microbial communities. The various forms in which such electron transfer occurs have recently been extended and reviewed by Shi and colleagues (2016) and Lovley (2017). Anaerobic oxidation of methane is possible because of direct electron transfer from methanogenic archaea to sulfate-reducing bacteria living in consortia probably through nanowire-like structures that connect these organisms (Wegener *et al*., 2015), although, also other mechanisms may be instrumental in the transport of electrons in these type of consortia (McGlynn *et al*., 2015). Conductive minerals such as magnetite may also allow the transport of electrons between different species such as *Geobacter* and methanogens (Kato *et al*., 2012). Benthic microbial communities are often enriched in minerals, especially iron and manganese and the interactions of microorganisms with these metals may greatly enhance biogeochemical reactions.

In marine sediments there is a great diversity of sulfur-oxidizing bacteria. Members of the *Beggiatoa* family are filamentous, motile bacteria that form thick, often whitish mats on the surface of sulfide rich sediments. *Beggiatoa* oxidizes sulfide to elemental sulfur, which is stored inside their cells and the refractivity of these sulfur globules render the white appearance to these mats. *Beggiatoa* stores nitrate intracellularly in vacuoles, which is used to anaerobically oxidize the intracellular elemental sulfur to sulfate (Sayama, 2001; Sayama *et al*., 2005). When available, *Beggiatoa* can also use oxygen as electron acceptor to oxidize the intracellular elemental sulfur to sulfate. Nitrate is reduced to ammonia rather than denitrified to N_2 and, therefore, this nutrient remains available for the sediment microbial community. Remarkably, even some sulfate-reducing bacteria contribute to the oxidation of sulfide with nitrate by inversing the pathway of dissimilatory sulfate reduction (Thorup *et al*., 2017). Similar as *Beggiatoa*, *Desulfurivibrio alkaliphilus* oxidizes sulfide initially to elemental sulfur, which is subsequently oxidized to sulfate. These sulfur-oxidizing bacteria compete with cable bacteria and the activity of these groups may be separated seasonally (Nielsen and Risgaard-Petersen, 2015). Cable bacteria have recently been discovered and extensively been studied as astonishing examples of interspecies and intraspecies electron transfer (Fig. 4). This discovery has changed our view on sediment biogeochemistry. Cable bacteria have been found globally in a variety of different benthic environments characterized by moderate to high sulfide concentrations (Burdorf *et al*., 2017). Cable bacteria belonging to the family Desulfobulbaceae transport electrons in marine and freshwater sediments over distances as long as 10 mm and more (Pfeffer *et al*., 2012) (Fig. 4). These organisms are the only known in the family to be filamentous. They form chains of thousands of cells that stick with one end in the anoxic layer of the sediment rich in reduced sulfur and with the other end in the oxic zone. The positioning of the cells along the vertical gradient is aided by the motility of cable bacteria (Malkin and Meysman, 2015; Bjerg *et al*., 2016). Electrons obtained from the anaerobic oxidation of the reduced sulfur are transported through the chain of cells to the oxygenated top layer of the sediment where they are used to reduce oxygen to water, which has been demonstrated in isolated living cable bacteria in the laboratory (Bjerg *et al*., 2018). As much as half of the oxygen reduction in these sediments may be attributed to cable bacteria. Oxygen may be replaced by nitrate and/or nitrite as electron acceptor (Marzocchi *et al*., 2014). The consumption of protons leads to an increase in pH that can be measured with microsensors and which is now usually considered as evidence for the presence and activity of cable bacteria. It is obvious that electric transport along such long distances is infinitely faster than diffusion, giving cable bacteria an important advantage over other microorganisms that depend on the latter. However, such

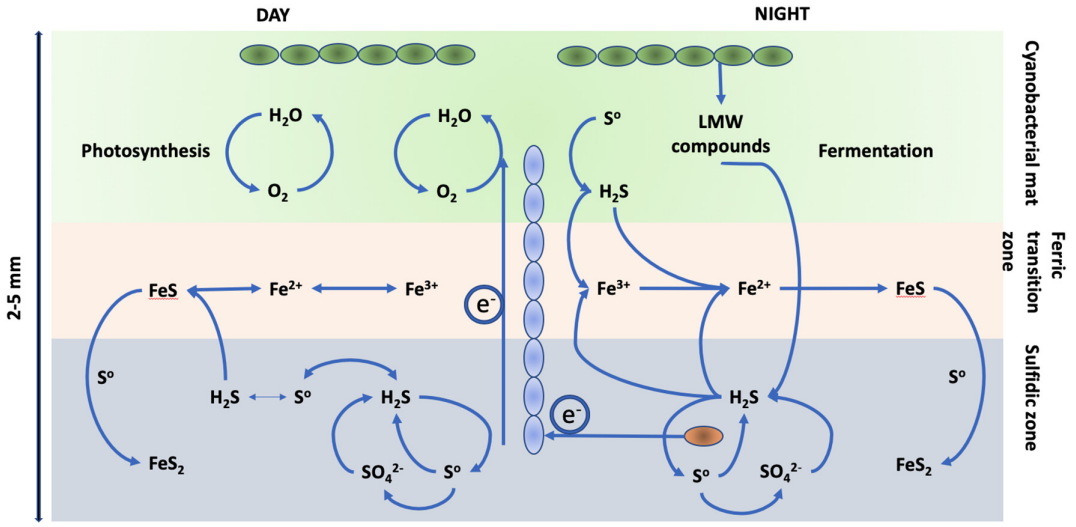

Fig. 4. Simplified scheme of a cyanobacterial mat with an iron 'firewall' between the oxygenic photosynthetic layer of cyanobacteria and the permanent anoxic sulfidic layer.
Ferrous iron reacts with oxygen to ferric iron preventing it from reaching the sulfidic layer, while ferric iron reacts with sulfide, preventing this toxic compound to accumulate in the oxygenic layer. These reactions may be chemically as well as biologically mediated. Cable bacteria overcome the separation of the sulfidic and oxygenic layers by forming a chain of cells through which electrons generated from the oxidation of sulfide are transported to the oxygenated layer where they reduce oxygen. Other sulfide oxidizers may benefit by donating electrons to these cable bacteria and hence also overcome the spatial separation of electron donor and acceptor. It is, however, uncertain whether cable bacteria are important in microbial mats, considering the small-scale character of the system and the short distance of the various layers. In the dark, Cyanobacteria produce low molecular organic compounds by fermentation that fuel sulfate- and sulfur reducing bacteria. A 'mini-sulfur cycle' of the 2-electron reaction between elemental sulfur and sulfide is probably more important than the 8-electron reduction–oxidation between sulfate and sulfide, which requires more steps and perhaps several different microorganisms. Iron sulfide (FeS) may react with elemental sulfur to the more stable mineral pyrite. It should be noted that the sulfur cycle is far more complex than depicted here. [Color figure can be viewed at wileyonlinelibrary.com]

organisms may nevertheless find their niche, using reduced sulfur compounds and transfer the electrons by (direct) interspecies transfer to the cable bacteria (Vasquez-Cardenas *et al*., 2015) (Fig. 4). The fact that the electric transport was through a wire of bacteria was demonstrated through intersecting the wire or by placing filters with pore sizes too small for the bacteria to pass (Pfeffer *et al*., 2012). To date, the process has not been fully elucidated. However, the chain of bacterial cells indicated structures that run along the periplasmatic space that seem to be insulated from the environment and that supposedly could serve as electrical wires and conduct electrons (Pfeffer *et al*., 2012).

Cable bacteria remove sulfide from the deeper sediment layers which results in acid formation and a consequent decrease in pH (Malkin *et al*., 2017). This acidification causes the dissolution of FeS and carbonates and the oxidation of the released ferrous iron results in the deposition of solid ferric iron minerals (Sulu-Gambari *et al*., 2016). The deposited ferric iron minerals have been conceived to serve as a buffer between preventing the toxic free sulfide to be released in the overlying hypoxic water (Canfield, 1989; Seitaj *et al*., 2015). A layer of solid phase iron oxides has been observed in coastal microbial mats (Fig. 4). This layer occurs always between the oxygenic layer of cyanobacteria on top and the anoxygenic purple layer of phototrophic sulfur bacteria (Stal, 2001). The deposition of these iron oxides may be the result of the activity of anoxygenic phototrophic bacteria that use ferrous iron (e.g. FeS) as electron donor or be a pure chemical phenomenon of iron oxidation, facilitated by the evolution of oxygen by the cyanobacteria or by cable bacteria. In microbial mats this layer of ferric iron oxides may act as a firewall between the layers of oxygenic and anoxygenic phototrophs, protecting the cyanobacteria from sulfide and the purple sulfur bacteria from oxygen (Stal, 2001) (Fig. 4). This mechanism might even be instrumental at the level of individual cyanobacteria. The mat-forming *Geitlerinema* (at the time assigned as *Microcoleus*) accumulates iron in the EPS sheath of the organism and in contact with sulfide it turns black from the precipitated FeS. This protects the organism from the toxic form of hydrogen sulfide, while it also keeps the level of oxygen down, facilitating the evolution of oxygen and minimizing losses by photorespiration and the oxygenase activity of the CO_2-fixing enzyme RubisCO (Stal, 2001).

Conclusion

Phototrophic biofilms such as microbial mats and benthic diatom communities are largely self-sustained and closed microbial ecosystems. They develop a macroscopic

habitus and have a common physiology which makes them biological entities that resemble an organism. The physiology of this entity is more than the sum of the physiologies of the individual microorganisms. The dense biomass in these biofilms is diverse and associated with viruses all embedded in a matrix of extracellular polymeric substances (EPS) that contains considerable amounts of extracellular nucleic acids. This allows the fast and efficient exchange of genetic information leading to an optimum adaptation of the biofilm to the prevailing conditions. In order to fine-tune the activities of individual cells and functional groups of organisms, through its phototrophic organisms the biofilm possesses a biological (circadian) clock that is directed through the daily light cycle as 'Zeitgeber' and enforced on the whole community. Cell-to-cell communication (quorum sensing) is another mechanism to synchronize populations of cells and to coordinate their activities with other functional groups of organisms in the biofilm. Last but not least, extracellular electron transport allows biogeochemical processes over large distances, shortcutting geochemical gradients such as of oxygen and sulfide that were previous thought to be separated. Recent insights in all these processes have fully revised our thinking about the functioning of biofilms and strengthened the concept of a biological entity for these microbial ecosystems. However, many questions are still open. An exciting new approach and a way forward would be to synthetize artificial biofilms using co-cultures of isolated biofilm microorganisms of different phyla and functional groups grown on solid substrates (such as quartz sand) and in varying levels of complexity.

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Minireview

Phototrophic marine benthic microbiomes: the ecophysiology of these biological entities

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Summary

Phototrophic biofilms are multispecies, self-sustaining and largely closed microbial ecosystems. They form macroscopic structures such as microbial mats and stromatolites. These sunlight-driven consortia consist of a number of functional groups of microorganisms that recycle the elements internally. Particularly, the sulfur cycle is discussed in more detail as this is fundamental to marine benthic microbial communities and because recently exciting new insights have been obtained. The cycling of elements demands a tight tuning of the various metabolic processes and require cooperation between the different groups of microorganisms. This is likely achieved through cell-to-cell communication and a biological clock. Biofilms may be considered as a macroscopic biological entity with its own physiology. We review the various components of some marine phototrophic biofilms and discuss their roles in the system. The importance of extracellular polymeric substances (EPS) as the matrix for biofilm metabolism and as substrate for biofilm microorganisms is discussed. We particularly assess the importance of extracellular DNA, horizontal gene transfer and viruses for the generation of genetic diversity and innovation, and for rendering resilience to external forcing to these biological entities.

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Introduction

In their natural habitat, the majority of microorganisms are associated with surfaces (Żur *et al*., 2016), either attached to a substratum, as part of an aggregate and on and in sediments (Whitman *et al*., 1998). These surfaces include the exterior of plants and animals, where they form part of the microbiome of these organisms, together with microorganisms living inside these (macro)organisms. Even many pelagic microorganisms (plankton) may form aggregates or bind to surfaces at some time during their life cycle and, although, in numbers free-living microorganisms are predominant, the particle-associated ones are more diverse and show higher activities (Ghiglione *et al*., 2009; Vojvoda *et al*., 2014; Rieck *et al*., 2015). Some benthic microorganisms may thrive part of their life as plankton and are termed pseudoplankton or tychoplankton (Forster *et al*., 2006). This change of lifestyle may, for instance, promote dispersion of the organism. Hence, the question that could be asked is why a benthic lifestyle seems to be preferred.

In contrast to planktonic species that are often limited in readily available nutrients and rarely benefit from direct interactions with other species, the benthic microbiome is characterized by a tight physical coupling between the different members. The nearest neighbour of a planktonic free-living cell is far away in terms of the distance compared to the cell size and any direct cooperation or interaction will be difficult if not impossible (Zehr *et al*., 2017).

Starving microorganisms may attach to surfaces to take advantage of the substrates and nutrients that are associated with them (Kjelleberg *et al*., 1983). It has been reported that attached bacteria were more active than free-living microorganisms (Grossart *et al*., 2006) but explanations for this observation are lacking and other reports rather indicate indirect effects (Iriberri *et al*., 1987). Also, contact with a solid surface stabilizes the