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The ecology and biogeochemistry of stream biofilms

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Streams and rivers form dense networks, shape the Earth's surface and, in their sediments, provide an immensely large surface area for microbial growth. Biofilms dominate microbial life in streams and rivers, drive crucial ecosystem processes and contribute substantially to global biogeochemical fluxes. In turn, water flow and related deliveries of nutrients and organic matter to biofilms constitute major constraints on microbial life. In this Review, we describe the ecology and biogeochemistry of stream biofilms and highlight the influence of physical and ecological processes on their structure and function. Recent advances in the study of biofilm ecology may pave the way towards a mechanistic understanding of the effects of climate and environmental change on stream biofilms and the biogeochemistry of stream ecosystems.

The perception that most microorganisms live as complex communities that are attached to surfaces has profoundly changed microbiology over the past decades. Most, if not all, bacteria can form biofilms, which are communities of cells embedded in a porous extracellular matrix. Dental plaque, the microorganisms on catheters and implants that cause persistent infections and the fouling of ship hulls and pipework are all examples of biofilms with important implications for public health and industrial processes. Most contemporary biofilm research rests on the discovery made more than 35 years ago by Maurice Lock, Gill Geesey and Bill Costerton: bacteria attached to surfaces dominate microbial life in streams¹⁻³. These microbiologists pioneered research into stream biofilms, also termed periphyton or epilithon, and described them as complex aggregates of bacteria, algae, protozoa, fungi and meiobenthos. The early study of stream biofilms also highlighted the relevance of interactions between microbial phototrophs and heterotrophs for energy fluxes and the role of the biofilm matrix as the site of extracellular enzyme activity and adsorption of dissolved organic matter (DOM)^{3,4}.

Since these early days, the study of the ecology and biogeochemistry of stream biofilms has slowly developed in the wake of thriving research on bacterial biofilms — often comprising

only a single strain, of interest to medical microbiology, rather than the polymicrobial communities found in stream biofilms — and on the microbial ecology of marine and lake planktonic communities⁵. Unlike bacterial biofilms grown in the laboratory, biofilms in streams are continuously exposed to a diverse inoculum that includes bacteria, archaea, algae, fungi, protozoa and even metazoa. These diverse biological ‘building blocks’, when combined with the dynamic flow of streamwater, generate biofilms with inherently complex and varying physical structures that have implications for microbial functioning and ecosystem processes⁶. In streams, biofilms are key sites of enzymatic activity⁷, including organic matter cycling, ecosystem respiration and primary production and, as such, form the basis of the food web.

Why should we study the ecology and biogeochemistry of stream biofilms? Streams sculpt the continental surface, forming dense and conspicuous channel networks that can be thought of as ecological arteries that perfuse the landscape. Streams are connected to their catchments through various surface and subsurface flow paths and notably through the hyporheic zone in the streambed at the interface between groundwater and streamwater⁸. Microbial cells, solutes and particles enter streams through these flow paths and, en route to downstream ecosystems and ultimately to the oceans, they may interact with the biofilms that colonize the large surface area provided by the streambed as a ‘microbial skin’ (BOX 1). As a result, the streambed and its biofilm microbiome contribute to biogeochemical fluxes⁸. Indeed, stream biofilms are now recognized as substantial contributors to global carbon fluxes by degrading organic matter and ultimately emitting an unexpectedly large amount of carbon dioxide into the atmosphere^{9,10}. Microorganisms in streams are also major components of the nitrogen cycle as they denitrify nitrate that they receive from catchment and emit the resulting nitrous oxide or nitrogen gas into the atmosphere^{11,12}. Furthermore, stream biofilms can be viewed as a crucial component of the catchment microbiome that also includes the microbial communities of the phyllosphere¹³ and soil¹⁴. The phyllosphere and the soil crust intercept water, microorganisms and solutes upon their entry into the catchment, whereas stream

biofilms regulate the export of microorganisms and solutes from the catchment. Stream biofilms thus connect the land surface, groundwater, oceans and the atmosphere, and as such they are prominently positioned at the nexus of global biogeochemistry, biodiversity and climate change. In this Review, we describe the ecology and biogeochemistry of stream biofilms at different scales and consider how the biodiversity and functions of these biofilms can influence, and in turn be influenced by, environmental processes.

Biodiversity across spatial scales

Stream biofilms are jungles of biodiversity, and the organisms that are typically found within them span across the entire tree of life. The development of next-generation sequencing methods has enabled a high-throughput profiling of these biofilms that has impressively demonstrated the full breadth and complexity of their microbial diversity (FIG. 1). Depending on light availability, eukaryotic algae (such as diatoms, green algae, chrysophytes, red algae and cryptophytes) and cyanobacteria, together with bacteria and to some extent also archaea, can form biofilms in the benthic zone, whereas bacteria and archaea dominate in the deeper sediments in which phototrophic life is limited. Fungi are probably also an important component of stream biofilms but remain poorly studied^{15,16}. Ciliates, flagellates, nematodes and even young-instar insects (such as midges) are among the top consumers in stream biofilms^{17,18}, and their grazing activity can change the physical structure^{19,20}, community composition²¹ and carbon cycling of biofilms²². Furthermore, viruses have an important role in marine ecosystems²³; for example, in bacterial biofilms²⁴, bacterial viruses (or phages) can infect cells and regulate the dynamics and diversity of bacterial communities. However, little is known of the abundance and relevance of viruses in stream biofilms.

Next-generation sequencing data of 16S rRNA genes (that is, bacterial taxonomic marker genes) of samples from benthic and hyporheic biofilms are becoming increasingly available^{25–27}, which has enabled a more detailed understanding of the composition and diversity patterns of bacterial communities within stream biofilms. These data suggest that the Proteobacteria and Bacteroidetes phyla generally dominate bacterial communities in stream biofilms (FIG.

2). Betaproteobacteria and Alphaproteobacteria are commonly the numerically dominant classes of Proteobacteria in benthic and hyporheic biofilms. The ability of members of the Alphaproteobacteria to degrade humic substances — a major component of DOM in streamwater — and their tendency to form filamentous, and possibly grazing-resistant, morphologies²⁸ may favour the growth of this class of bacteria in biofilms. Within the Bacteroidetes phylum, the Flavobacteriia and Sphingobacteriia classes seem to be of special importance to stream biofilms. Some members of these classes possess the ability to degrade biopolymers, such as cellulose and chitin^{28,29}, that contribute to the high molecular weight fraction of DOM in streams. The gliding motility of many Flavobacteriia is expected to facilitate the colonization of surfaces and subsequent formation of biofilms (and suspended particles) in aquatic ecosystems^{28,29}. This notion is supported by the observation that, under elevated flow velocity, Flavobacteriia adhere better to surfaces than *Pseudomonas aeruginosa*, which is a notable biofilm former³⁰. Furthermore, analyses of co-occurrence networks identified Sphingobacteriia as a key taxonomic group in stream biofilms³¹, which agrees with the involvement of these bacteria in the formation and colonization of suspended aggregates in the ocean and lakes²⁹. Other bacteria that are commonly found in stream biofilms, but at lower relative abundance, include Gammaproteobacteria, Deltaproteobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, Verrucomicrobia, Planctomycetes and Deinococcus–Thermus. Finally, next-generation sequencing data suggest that archaea constitute only a minor component of stream biofilms^{26,32,33}. This finding contrasts with earlier studies using fluorescence in situ hybridization that reported archaea as notable contributors to biofilm communities in oligotrophic glacier-fed streams³⁴ and nutrient-rich streams³⁵. Not unexpectedly, archaea seem to be restricted to specialized niches where, for instance, ammonium-oxidizing archaea outcompete ammonium-oxidizing bacteria³⁶ or, in the hyporheic zone, methanogenic archaea thrive in anoxic pockets³⁷.

Stream biofilm communities are highly diverse with hundreds if not thousands of operational taxonomic units (OTUs) that span over the major taxa outlined above and colonize the

various habitats in streams²⁵ (FIGS 1,2). A major question that arises from these observations is how do these complex communities assemble? Metacommunity theory proposes that the composition and diversity of ecological communities are shaped by the interplay between regional dispersal dynamics, the local environment and biotic interactions³⁸ (BOX 2). Local environmental conditions and dispersal dynamics may differentially affect biodiversity dynamics in biofilms depending on scale. For example, at the sub-millimetre scale, biofilm physical structure and niche differentiation can induce spatial shifts in community composition. During periods of low streamwater flow, when hydraulic stress is reduced, benthic biofilms can develop into thick communities stratified into a canopy that contains algae and cyanobacteria exposed to light; in turn, these phototrophs attenuate the penetration of light into the deeper biofilm layers, thereby shifting the community toward heterotrophs³⁹. Furthermore, depending on the turbulence of the water overlying the biofilms, the biofilm canopy can differentiate into streamers (see below), which can entail microscale shifts in bacterial community composition. This is supported by findings suggesting that niche differentiation and competition are processes responsible for shifts in community composition in streamers⁴⁰.

At an intermediate scale (that is, centimetres to metres), the interplay between streambed geomorphology and flow fields creates patchy landscapes. Environmental gradients emanating from hydraulic and microbial processes create potential niches in these landscapes and shape dispersal routes for microbial cells from the streamwater. As a result, environmental heterogeneity increases and can even induce turnover of microbial taxa across streambed landscapes. It was shown, for instance, that the spatial variation of hydraulics over bedforms in experimental streams explained almost half of the variation in biofilm community composition and thereby increased microbial α -diversity over bedforms⁴¹. Neutral models based on flow-induced dispersal of microbial cells indicate that stochastic immigration also contributes to the assembly of local biofilm communities in streambed landscapes with low environmental heterogeneity⁴². That such neutral approaches failed to

explain community turnover in more heterogeneous landscapes suggests sorting by the local environment as an underlying community assembly^{41,42} (BOX 2). These findings agree with the idea that dispersal limitation governs the assembly of microbial communities when sorting by the local habitat is low⁴³, as can be expected for rather homogenous streambed landscapes. Furthermore, these findings concur with the notion that the physical and biological patterns and processes of biofilms co evolve with the streambed environment they inhabit, as the assumptions of neutral models do not apply if there are strong co variances between processes, such as biofilm development and the establishment of environmental gradients in the streambed.

At the multi-kilometre scale of catchments, dispersal dynamics and environmental conditions can also shape bacterial diversity and community composition in stream biofilms. In a study of glacier-fed streams in the European Alps, environmental factors related to catchment geology and altitude (such as pH, temperature and electrical conductivity) sorted microbial taxa from the streamwater that assembled into the biofilm communities³². For instance, the presence of major phyla such as Actinobacteria, Nitrospirae and Verrucomicrobia in biofilms varied with the electrical conductivity of streamwater, whereas the presence of Acidobacteria, Gemmatimonadetes and Proteobacteria in biofilms was related to shifts in pH³². This study also showed that microbial \pm -diversity increased with decreasing altitude, as glacial coverage recedes, which may support the notion that \pm -diversity depends on the size of the catchment from which the metacommunity recruits microbial cells. This is because the recruitment range, and thus the metacommunity size, would increase as relative glacier coverage decreases, thereby diversifying microbial sources (for example, soils, rocks and groundwater) from which stream biofilms communities may ultimately assemble. By contrast, owing to the variety of extreme cold habitats associated with glaciers, α -diversity across glacier-fed streams is elevated when glacier coverage is high³². As glaciers shrink, these habitats and their microbial communities vanish, which translates to a loss of microbial α -diversity in stream biofilms at the multi-kilometre scale.

Patterns of biodiversity dynamics in benthic biofilms also become apparent at the scale of stream networks draining several catchments. In a survey of over 114 streams in a stream network, the average \pm -diversity in benthic biofilms decreased from a greater diversity upstream to a lower diversity in downstream reaches⁴⁴. Strikingly, the survey found that stream confluences induce stepwise reductions in \pm -diversity along this upstream-to-downstream continuum, which the authors of the study tentatively attributed to competitive exclusion. Furthermore, patterns of 2 -diversity identified in the survey indicated that there was a significantly higher degree of variability in community composition among headwaters than among larger downstream reaches that could not be explained by geographical distance⁴⁴. These combined patterns of \pm -and 2 -diversity highlight the crucial role of headwaters as reservoirs of microbial biodiversity at the scale of stream networks. Headwater streams collect diverse microorganisms from soils, and various other sources, in the catchment^{45,46}, which they channel through the stream network to downstream reaches. En route, these microorganisms can colonize bare sediments and invade resident biofilms, thereby experiencing environmental sorting and competitive interactions. As a result, the local diversity of biofilms decreases, and their community composition becomes more uniform in larger streams compared with biofilms in headwaters.

There is also evidence that dispersal dynamics and hydrology, both changing predictably across stream networks, affect the community network structure of microbial biofilms. Analyses of co-occurrence networks generated from 16S rRNA sequencing data showed that community networks tend to fragment into more abundant, but smaller, clusters that may be sensitive to the hydrological regime and dispersal dynamics³¹. The same study also uncovered the role of typical biofilm formers, such as Sphingobacteriia, in the organization of biofilm community networks. This finding is potentially important as it provided the first evidence that biofilm community stability is associated with dispersal dynamics and hydrology, which are processes that are increasingly subject to changes caused by shifts of the hydrological regime induced by climate change and human intervention (for example, damming or inter-

basin diversion of water).

Finally, the roles of geographical distance and large-scale environmental variation on a continental scale were assessed in a survey encompassing 244 streams in New Zealand⁴⁷. This survey showed that environmental conditions were more important than dispersal limitation in biofilm community assembly and diversity. Specifically, the study found that change in land use, such as that entailing the destruction of native vegetation, rather than spatial factors, such as latitude or elevation, most strongly affected community composition and diversity.

Similarly, a survey in New Hampshire, USA, found that environmental factors (notably streamwater pH), rather than geographical distance, best predicted the variation observed between bacterial communities associated with benthic organic matter in streams⁴⁸.

Collectively, these patterns suggest that community composition and biodiversity dynamics of stream biofilms are not random but driven by environmental factors. It is crucial that we understand this relationship, as environmental change (both at global and local scales) may have repercussions on the functioning of biofilms and even on ecosystem processes.

Formation and physical structure

Much of our understanding of the physical structure of stream biofilms has been inspired and guided by the early concepts developed by Lock and colleagues³ and by work on single-species bacterial biofilms grown in flow chambers^{49,50}. Growth and structural differentiation of stream biofilms begins when nascent biofilms are formed from bacterial or algal microcolonies (FIG. 3). Whether algae are important building blocks of the biofilm depends on the availability of light, which gradually declines from the benthic zone to the hyporheic zone of the streambed. In a first step, single diatom cells may attach to the sediment surface, where they form chain-like microcolonies that become entangled and confer physical structure to nascent benthic biofilms⁵¹. Adjacent microcolonies can coalesce, owing to growth through reproduction and short-distance dispersal guided by a combination of colony development on the surface and the direction of streamwater flow⁵². Under reduced flow velocity and laminar flow conditions, coalescing microcolonies can differentiate into star-like

or quasi-polygonal structures that often cover large surfaces of the sediment and develop substantial biomass^{39,52}. The high demands of these biofilms for oxygen, nutrients and dissolved substrates can impede mass transfer of these solutes, causing microorganisms within the interior of the biofilm to become nutritionally deprived^{30,53}. The structural differentiation of the canopy into finger-like structures increases biofilm surface area to replenish crucial solutes⁶; this observation supports early modelling results showing increased canopy differentiation of biofilms exposed to low nutrient concentrations⁵⁴.

As shear stress increases in environments with elevated flow velocity, biofilms can form directed ridges^{39,55} and even centimetre-long streamers that oscillate in the streamwater⁵⁶. Streamers can form from a base attached to the sediment surface and a flexible tail elongated in the flow direction; alternatively, they can develop directly from the canopy of the biofilm. Streamers are complex structures that are composed of diverse building blocks. Some examples have been shown to be constructed from a bacterial backbone embedded in extracellular polymeric substances (EPS), which confers a viscoelastic property, and spiked with abundant diatom cells⁵⁶. Described as ‘garlands’, such streamers may offer microniches to phototrophs by providing favourable access to light, nutrients and carbon dioxide in the streamwater in addition to protection from erosion by tethering to underlying biofilms⁵⁶. Modelling of fluid dynamics around streamers has shown that oscillation enhances solute mass transfer because the streamer both responds to turbulent flow fluctuations and imposes an obstructive drag force that leads to further turbulent dissipation⁵⁷. In flexible canopies of submerged vegetation, this waving motion of the canopy is termed ‘monami’ and results in the formation of a shear layer at the top of the canopy that regulates mass transfer of solutes into the canopy⁵⁸. It could be argued that the oscillation of streamers pumps water and solutes into the biofilms and thereby enhances mass transfer, a notion that is supported by the proposal that biofilms can oscillate as membranes⁵⁹.

Much like bacterial biofilms grown under laboratory conditions⁴⁹, stream biofilms contain voids and channel systems^{6,39,55}. Channel systems in biofilms growing under slow flowing

water can be extensive, whereas biofilms growing in fast flowing water are generally more compact with less spacious voids³⁹. These structural features have important implications for growth and metabolism of cells in the biofilm by regulating both overall mass transfer into the biofilm and spatial distributions of solutes within the biofilm. For example, it has been argued that microorganisms embedded in thin and compact biofilms are readily provided with solutes from the streamwater, whereas microorganisms in thicker and less compact biofilms may rely more on internal sources than on the streamwater for the provision of nutrients and organic matter³⁹. This coupling between physical structure and mass transfer induces chemical and biological gradients in stream biofilms⁶⁰⁻⁶² that are similar to those known to occur in pure bacterial biofilms⁵³.

Currently, we do not understand how flow through interstitial space may affect the physical structure of hyporheic biofilms, which are expected to differentiate into similar flow-induced structures to those observed in their benthic counterparts. Although bacterial streamers have traditionally only been reported in turbulent flow, studies using microfluidic devices suggest that these structures may even develop in laminar flow^{63,64}, which often predominates in the porous space in the streambed. However, biofilm growth can restrict the flow of water through the porous space and its hydrodynamic exchange with the overlying streamwater, thereby reducing the propagation of solutes and microorganisms into and through the streambed^{65,66}. This represents a strong constraint on the streambed microbiome because delivery of electron acceptors such as oxygen and nitrate from the streamwater generally supports hyporheic metabolism, leading to the formation of vertical gradients of aerobic and anaerobic metabolism and redox structure within streambeds⁸.

Collectively, these observations suggest that coupling of structure and function in biofilms is analogous to that in the streambed. Despite their disparate scales, both biofilms and the streambed are porous systems with advective and diffusive mass transfer, strong internal chemical and biological gradients, and complex structural properties that shelter resident organisms from stream flow. Biofilms and the sedimentary environment of the streambed

may even be considered to have undergone a 'co evolutionary' relationship, as biofilms adapt and evolve in response to the physical and chemical structure of the streambed environment, and simultaneously modify this environment by changing its hydrodynamics^{6,64,65} and establishing chemical gradients⁸. These interactions between the biofilm and the sediment regulate not only physical patterns of the porous space but also the ecology of the biofilms therein. The iteration of this coupling of structure and function across cellular and sedimentary scales may contribute to the performance of stream biofilms as a microbial skin to retain, amplify, transform and reflect matter and microorganisms, and thereby control export fluxes from catchments.

Diversity and function

Elucidating the ecological mechanisms underlying the relationship between biodiversity and function of communities and ecosystems has been a major component of research in animal and plant ecology over the past decades, and more recently in microbial ecology^{67,68}.

Resource partitioning, or positive interactions, among species, often understood as complementarity, are generally recognized as the mechanisms underlying the relationship between biodiversity and resource use^{67,68}, and complementarity requires spatial proximity so that species can readily interact. The close proximity of diverse microorganisms in stream biofilms therefore makes complementarity a powerful ecological mechanism to maximize resource use.

An obvious example of such complementarity is the one of phototrophs and heterotrophs driving carbon cycling in biofilms. On the one hand, diatoms, green algae and cyanobacteria exude organic compounds such as carbohydrates and amino acids, which are highly available to the heterotrophic metabolism^{4,39,69}. On the other hand, respiratory carbon dioxide from these heterotrophs can be assimilated by the phototrophs. This interaction produces benthic biofilms with 'self-sufficient' carbon cycling that is less dependent on external carbon sources if inorganic nutrients and light (as the energy source) are provided^{39,70}.

Owing to the priming effect, complementary interactions between phototrophs and

heterotrophs may also promote the metabolism of the recalcitrant DOM that is often delivered from terrestrial sources into streams. The priming effect enables exudates from phototrophs to provide energy and nutrients (such as nitrate and phosphate) to the biofilm that may otherwise be limiting to microbial growth⁷¹. However, evidence for the priming effect in stream biofilms remains equivocal at present. There is suggestive evidence that the presence of algae stimulates bacterial and fungal growth that is associated with leaf litter^{72,73}, but no evidence for the priming effect could be found in hyporheic biofilms⁷⁴.

The extensive diversity of bacteria in stream biofilms makes it extremely difficult to establish relationships between bacterial diversity and biofilm function. For example, complementarity among algal species has been shown to increase the uptake of nitrate by stream biofilms⁷⁵ but, although it is plausible that this may also occur among bacteria in biofilms, we are not aware of any such study. Studies on the activities of extracellular enzymes increasingly suggest that functional plasticity and functional redundancy may blur the relationship between microbial diversity and function in complex biofilm communities⁷⁶⁻⁷⁸. For instance, one study described biofilm communities with functional plasticity in groundwater-fed streams that may resist environmental fluctuations by adapting their enzymatic machinery, whereas biofilms in glacier-fed streams lacked this plasticity and were instead characterized by specialists able to express specific extracellular enzymes under given conditions⁷⁶. Most of these studies have focused on the function of a single extracellular enzyme at a time. However, it is becoming increasingly clear from the study of multicellular organisms that different species often influence different functions and that studying individual functions, or processes, in isolation may underestimate the level of biodiversity required to maintain multifunctionality (that is, the ability to carry out several enzymatic functions simultaneously)⁷⁹. Existing studies on multifunctionality in biofilms are not conclusive. One study inferred multifunctionality from measurements of the activities of α -glucosidase, α -xylosidase, cellobiohydrolase and leucine-aminopeptidase, which are involved in the final steps of the hydrolysis of polymeric compounds (such as cellulose, hemicellulose and polypeptides)⁸⁰. The study, which analysed

biofilms grown in laboratory-scale bioreactors mimicking the hyporheic zone, showed that the likelihood of sustaining such enzymatic multifunctionality decreases with decreasing microbial diversity, indicative of a low level of redundancy. Another study reconstructed artificial metagenomes to derive 140 orthologues related to carbohydrate and amino acid metabolism in benthic biofilms from alpine streams⁸¹. Analysing the association between inferred metabolic multifunctionality and bacterial diversity revealed a high degree of metabolic redundancy, which means that the biofilm microbiome is able to metabolize a wide range of DOM compounds in the streamwater, regardless of community composition. This is advantageous in an ecosystem in which DOM composition varies continuously, such as the variation that occurs with changing hydrology and season.

A similar pattern of functional gene redundancy was reported in a study of biofilms in a set of streams in New Zealand that had been affected in different ways by land use⁸². This study used functional gene arrays to assess the expression of genes involved in the cycling of nutrients (including nitrate, phosphate and sulphate) and energy metabolism. The expression of these genes was associated with land use in the catchment of the stream. For instance, the expression of sulphur and nitrogen cycling genes in biofilms was significantly different in streams with an urban or native forest catchment compared with streams with an exotic forest or rural catchment. These differences were probably attributable to the varied importance of nitrogen fixation, denitrification and sulphate reduction between these ecosystems⁸².

Furthermore, biofilm communities in streams affected similarly to one another by land use exhibited notable functional redundancy, despite differences in community composition. For instance, bacteria in biofilms in streams draining urban catchments or catchments with native forests had similar functional gene composition to one another, but exhibited marked differences to the functional gene composition of biofilms from streams draining catchments with exotic forests or agricultural land. This functional redundancy may be an advantageous strategy for biofilms to maintain crucial functions in stream ecosystems that are characterized by frequent and pronounced environmental fluctuations. The varied levels of functional

redundancy and metabolic plasticity observed in stream biofilms call for further studies on the functional traits and functional diversity of biofilm communities⁸³, rather than their phylogenetic diversity, to draw conclusions on the functional role of biofilms in stream ecosystems.

Complexity and biogeochemistry

Stream biofilms encapsulate several levels of structural and functional complexity, which are continuously adapted to the prevailing environmental conditions in streams. As such, biofilms in streams could be viewed as a dynamic microbial skin (BOX 1) that is influenced by several physical and chemical processes; however, conversely, biofilms can also themselves influence these processes. The major physical and chemical processes that influence, and are influenced by, stream biofilms include photosynthesis, hydrodynamics and nutrient and organic matter cycling, and are thus dependent on the availability of light, oxygen, nutrients and organic carbon, as well as on the interactions between water flow and streambed topography, and associated hydrodynamic forces and replenishment rates (FIG. 4).

Light. Light is a continuous variable in streams, varying from saturation in open channels to limiting conditions in forested streams, and even to full absence deeper in the streambed. The gel-like properties of the biofilm matrix may also affect light availability in the interior of the biofilm by influencing reflectance across a broad spectrum of incident light⁸⁴. Light availability in biofilms has profound implications on the gross primary production of ecosystems³. For example, light can influence the cycling of carbon, nitrogen and phosphorus by the extracellular enzyme machinery of biofilms; furthermore, the expression of these extracellular enzymes can be adapted according to photosynthesis and the exudation of low-molecular compounds by algae^{26,69,85}. For example, microbial heterotrophs expressed more hydrolytic enzymes (such as α -glucosidase, α -xylosidase and leucine aminopeptidase) and oxidative enzymes (such as phenoloxidase) in ambient light conditions than in shaded conditions⁸⁵. It is generally thought that increased heterotrophic biomass associated with algae or photosynthesis-induced shifts in pH affect the activity of enzymes in the biofilm matrix,

and the products of algal lysis can directly stimulate extracellular enzymes, as has been shown for α -glucosidase in stream biofilms⁷⁰.

Nutrients. Biogeochemical cycling in stream biofilms is not solely related to light but also to nutrient availability^{86,87,88}, which is often increased by agricultural land use. The degradation of complex macromolecules by extracellular enzymes can constitute up to 80% of the microbial activity in biofilms⁷, and these enzymes are therefore fundamental to biogeochemical fluxes of carbon, nitrogen and phosphorous in streams^{86,89}. The stoichiometry of biosynthetic enzymes that metabolize each of these nutrients relates to the stoichiometry of the respective nutrient in the microbial biomass, as well as to the stoichiometry of organic matter cycling. This stoichiometry ensures that a high availability of energy and carbon will shift the repertoire of metabolic enzymes from those specializing in nutrient biosynthesis to those specializing in respiration and metabolic consumption⁹⁰. In addition to the abundance of extracellular enzymes, bioavailability of the nutrients metabolized by these enzymes is also an important determinant of community function. For example, one study found that the availability of inorganic nitrogen and phosphorous to stream biofilms affects the net primary production of these biofilms and consequently the exudation, from algae, of the labile and low-molecular-weight organic matter that supplies the metabolism of microbial heterotrophs^{87,88}. The study found that a greater proportion of this newly fixed organic carbon was retained in the heterotrophic bacterial biomass in streams depleted in inorganic nitrogen and phosphorous than in streams enriched in these inorganic nutrients. This work is important because it draws a mechanistic link between light, inorganic nutrients and carbon cycling and the potential availability of fixed carbon to downstream ecosystems.

Hydrodynamics. Channel geomorphology and hydrodynamics jointly affect the mass transfer of solutes from the streamwater to biofilms and the metabolism of these solutes within biofilms. For example, one study of benthic biofilms showed that glucose, which has high bioavailability, and arabinose, which has low bioavailability, are differentially taken up in turbulent flow compared with laminar flow⁶⁰. Combining microautoradiography and

microscopy, the study showed that arabinose is taken up by bacterial cells in the interior of the biofilm in the event of glucose depletion arising from the thickness of the biofilm. These fine-scale observations support earlier work suggesting that, when transport of solutes from the streamwater into the biofilm becomes limiting, the metabolism of organic molecules by heterotrophs is limited by the ability of the molecule to diffuse into the biofilm, rather than its intrinsic bioavailability⁶.

In addition to the type of flow, heterogeneity of flow is also an important factor in mass transfer in stream biofilms. The heterogeneous distribution of light, DOM and nutrients in streams, together with the heterogeneous geomorphology and flow conditions, has implications for the interactions between biofilms and ecosystem processes. Work with experimental streams has shown that the spatial heterogeneity of streambed topography and related flow conditions not only increases the mass uptake of DOM from the streamwater but also augments the diversity of the organic compounds that biofilms remove from the DOM pool⁹¹. These results provided the first support for the widely held assumption that physical heterogeneity controls resource use, and even the diversity of resources used, in stream biofilms. The findings also corroborated an older study that examined stream reaches in which near-bed flow velocity and turbulence intensity fluctuated, which demonstrated that habitat heterogeneity influences the primary productivity and respiration of benthic biofilms⁹². An experiment using phototrophic biofilms with eight algal species further highlighted the relationship between physical habitat heterogeneity, diversity and nitrate uptake as an important ecosystem process in streams⁷⁵. These studies suggested that the complementary use of resources by microbial communities, such as through facilitation or niche partitioning (see above), is the mechanism underlying enhanced ecosystem processes in physically heterogeneous streams. The interplay between the spatially heterogeneous environment of the streambed and its microbial skin may offer various opportunities for the processing of organic matter and nutrients and ensuing biogeochemical fluxes. Viewing stream biofilms as a microbial skin may therefore explain the high performance of stream ecosystems and the

contributions of these ecosystems to large-scale biogeochemical fluxes of oxygen, inorganic nutrients and carbon⁹⁻¹².

Conclusions and perspectives

What have we learned in the 35 years since Lock, Geesey and Costerton's exciting discovery that biofilms dominate microbial life in streams? Today, stream biofilms are recognized as complex communities that harbour an immense degree of biodiversity across all three domains of life and that differentiate into highly structured architectures. We also understand stream biofilms as hotspots of enzymatic and metabolic activities that drive fundamental ecosystem processes and biogeochemical cycles. Biofilm structure and function are intimately linked to the physical forces exerted by the flow of water and at the same time biofilms can also change the dynamics of water flow. This has a major effect on mass transfer into biofilms that can even result in changes to ecosystem function and biogeochemical fluxes.

Several crucial questions relating to the biology of stream biofilms remain to be solved. The stream biofilm microbial community is inherently diverse but the various interactions within and between the domains of life in the biofilm remain poorly explored. An integrated 'omic' analysis that blends metagenomics, metatranscriptomics and metaproteomics will be required to unravel key functional capabilities of major taxa and to map these onto interaction networks. This should also offer a new framework to relate microbial phylogeny to functional traits in complex communities⁸³. Furthermore, we would need to relate key functions and traits to the measurement of fluxes, as this would allow us to assess the potential relevance of microbial processes to ecosystem processes and related biogeochemistry. Finally, the differentiation of stream biofilms into complex physical structures and the role of each of the various biological building blocks that contribute to this process also warrant more attention, if we are to be able to mechanistically link biodiversity to biofilm function. Combining mass spectrometry techniques with chemical tagging and time-lapsed imaging⁹³ may open new and exciting avenues to disentangle these complex relationships. The development of novel microfluidics devices may further advance this field by constraining biofilm architecture to

facilitate observational studies relevant to biofilm ecology⁹⁴. However, fine-scale observations on biofilm structure and function must be scaled to stream ecosystems. No doubt, this is the least trivial part of the story, as the nature of stream biofilms and ecosystems is inherently complex and heterogeneous, and new physical and mathematical models are thus required to address this challenge. It is clear that future progress in stream biofilm research will require interdisciplinary studies that bridge life sciences, environmental sciences and engineering and that are rooted in ecology and biogeochemistry. This is imperative to better integrate stream biofilm research, and more generally stream microbial ecology, into the larger context of aquatic sciences. For instance, it is only now that the sensitivity of stream biofilms to climate warming and global environmental change is beginning to be recognized^{25,95}. These environmental changes include shifts in precipitation patterns, damming and water diversion that profoundly alter the hydrological regime. Furthermore, the conversion of land into agricultural use increasingly delivers nutrients into streams, resulting in eutrophication, and concomitant deforestation of the vegetation of stream banks shifts the light regime and channel geomorphology. The larger ecological and biogeochemical consequences of such shifts for the microbial skin are yet to be explored.

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References

1. Geesey, G. G., Mutch, R., Costerton, J. W. & Green, R. B. Sessile bacteria: an important component of the microbial population in small mountain stream. *Limnol. Oceanogr.* 23, 1214–1223 (1978).
This study represented the first evaluation of the abundance and biomass of bacteria in the streamwater in benthic biofilms. It also presents the first microphotographs to depict the spatial organization of benthic biofilms.
2. Costerton, J. W., Geesey, G. G. & Cheng, K. J. How bacteria stick. *Sci. Am.* 238, 86–95 (1978).
3. Lock, M. A., Wallace, R. R., Costerton, J. W., Ventullo, R. M. & Charlton, S. E. River epilithon: toward a structural–functional model. *Oikos* 42, 10 (1984).
This is the first study to propose a conceptual model of stream biofilm structure and function, including the interactions of biofilms with DOM.
4. Haack, T. K. & McFeters, G. A. Nutritional relationships among microorganisms in an epilithic biofilm community. *Microb. Ecol.* 8, 115–126 (1982).
This study reported the first quantification of carbon fluxes between algae and microbial heterotrophs in stream biofilms.
5. Findlay, S. Stream microbial ecology. *J. N. Am. Benth. Soc.* 29, 170–181 (2010).
6. Battin, T. J., Kaplan, L. A., Denis Newbold, J. & Hansen, C. M. E. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* 426, 439–442 (2003).
7. Romani, A. M. et al. Relevance of polymeric matrix enzymes during biofilm formation. *Microb. Ecol.* 56, 427–436 (2008).
8. Boano, F. et al. Hyporheic flow and transport processes: mechanisms, models, and biogeochemical implications. *Rev. Geophys.* 52, 603–679 (2014).
9. Battin, T. J. et al. Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* 1, 95–100 (2008).

10. Raymond, P. A. et al. Global carbon dioxide emissions from inland waters. *Nature* 503, 355–359 (2014).
11. Mulholland, P. J. et al. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* 452, 202–205 (2008).
12. Beaulieu, J. J., Tank, J. L. & Hamilton, S. K. Nitrous oxide emission from denitrification in stream and river networks. *Proc. Natl Acad. Sci. USA* 108, 214–219 (2011).
13. Vorholt, J. A. Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* 10, 828–840 (2012).
14. Daniel, R. The metagenomics of soil. *Nat. Rev. Microbiol.* 3, 470–478 (2005).
15. Baschien, C., Manz, W., Neu, T. R., Marvanova, L. & Szewzyk, U. In situ detection of freshwater fungi in an Alpine stream by new taxon-specific fluorescence in situ hybridization probes. *Appl. Environ. Microbiol.* 74, 6427–6436 (2008).
16. Bärlocher, F. & Murdoch, J. H. Hyporheic biofilms — a potential food source for interstitial animals. *Hydrobiologia* 184, 61–67 (1989).
17. Hakenkamp, C. C. & Morin, A. The importance of meiofauna to lotic ecosystem functioning. *Freshw. Biol.* 44, 165–175 (2000).
18. Dopheide, A., Lear, G., Stott, R. & Lewis, G. Molecular characterization of ciliate diversity in stream biofilms. *Appl. Environ. Microbiol.* 74, 1740–1747 (2008).
19. Lawrence, J. R., Scharf, B., Packroff, G. & Neu, T. R. Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. *Microb. Ecol.* 44, 199–207 (2002).
20. Böhme, A., Risse-Buhl, U. & Küsel, K. Protists with different feeding modes change biofilm morphology. *FEMS Microbiol. Ecol.* 69, 158–169 (2009).
21. Wey, J. K., Jürgens, K. & Weitere, M. Seasonal and successional influences on bacterial community composition exceed that of protozoan grazing in river biofilms. *Appl. Environ. Microbiol.* 78, 2013–2024 (2012).
22. Risse-Buhl, U. et al. Tracking the autochthonous carbon transfer in stream biofilm

- food webs. *FEMS Microbiol. Ecol.* 79, 118–131 (2012).
23. Jacquet, S., Miki, T., Noble, R., Peduzzi, P. & Wilhelm, S. Viruses in aquatic ecosystems: important advancements of the last 20 years and prospects for the future in the field of microbial oceanography and limnology. *Adv. Oceanogr. Limnol.* 1, 97–141 (2010).
 24. Sutherland, I. W., Hughes, K. A., Skillman, L. C. & Tait, K. The interaction of phage and biofilms. *FEMS Microbiol. Lett.* 232, 1–6 (2004).
 25. Zeglin, L. Stream microbial diversity responds to environmental changes: review and synthesis of existing research. *Front. Microbiol.* 6, 454 (2015).
 26. Romani, A. M. et al. Shifts in microbial community structure and function in light- and dark-grown biofilms driven by warming. *Environ. Microbiol.* 16, 2550–2567 (2014).
 27. Timoner, X., Borrego, C. M., Acuña, V. & Sabater, S. The dynamics of biofilm bacterial communities is driven by flow wax and wane in a temporary stream. *Limnol. Oceanogr.* 59, 2057–2067 (2014).
 28. Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D. & Bertilsson, S. A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol. Biol. Rev.* 75, 14–49 (2011).
 29. Kirchman, D. L. The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* 39, 91–100 (2002).
 30. Zhang, W., Sileika, T. & Packman, A. I. Effects of fluid flow conditions on interactions between species in biofilms. *FEMS Microbiol. Ecol.* 84, 344–354 (2013).
 31. Widder, S. et al. Fluvial network organization imprints on microbial co occurrence networks. *Proc. Natl Acad. Sci. USA* 111, 12799–12804 (2014).
 32. Wilhelm, L., Singer, G. A., Fasching, C., Battin, T. J. & Besemer, K. Microbial biodiversity in glacier-fed streams. *ISME J.* 7, 1651–1660 (2013).
 33. Besemer, K. et al. Unraveling assembly of stream biofilm communities. *ISME J.* 6, 1459–1468 (2012).

34. Battin, T. J., Wille, A., Sattler, B. & Psenner, R. Phylogenetic and functional heterogeneity of sediment biofilms along environmental gradients in a glacial stream. *Appl. Environ. Microbiol.* 67, 799–807 (2001).
35. Olapade, O. A. & Leff, L. G. Seasonal response of stream biofilm communities to dissolved organic matter and nutrient enrichments. *Appl. Environ. Microbiol.* 71, 2278–2287 (2005).
36. Merbt, S. N., Auguet, J. C., Casamayor, E. O. & Marti, E. Biofilm recovery in a wastewater treatment plant-influenced stream and spatial segregation of ammonia-oxidizing microbial populations. *Limnol. Oceanogr.* 56, 1054–1064 (2011).
37. Buriánková, I. et al. Identification of methanogenic archaea in the hyporheic sediment of Sitka stream. *PLoS ONE* 8, e80804 (2013).
38. Leibold, M. A. et al. The metacommunity concept: a framework for multi-scale community ecology. *Ecol. Lett.* 7, 601–613 (2004).
39. Battin, T. J., Kaplan, L. A., Newbold, J. D., Cheng, X. & Hansen, C. Effects of current velocity on the nascent architecture of stream microbial biofilms. *Appl. Environ. Microbiol.* 69, 5443–5452 (2003).
40. Besemer, K., Hödl, I., Singer, G. & Battin, T. J. Architectural differentiation reflects bacterial community structure in stream biofilms. *ISME J.* 3, 1318–1324 (2009).
41. Besemer, K., Singer, G., Hödl, I. & Battin, T. J. Bacterial community composition of stream biofilms in spatially variable-flow environments. *Appl. Environ. Microbiol.* 75, 7189–7195 (2009).
42. Woodcock, S., Besemer, K., Battin, T. J., Curtis, T. P. & Sloan, W. T. Modelling the effects of dispersal mechanisms and hydrodynamic regimes upon the structure of microbial communities within fluvial biofilms. *Environ. Microbiol.* 15, 1216–1225 (2013).
43. Wang, J. et al. Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. *ISME J.* 7, 1310–1321 (2013).
44. Besemer, K. et al. Headwaters are critical reservoirs of microbial diversity for fluvial

networks. *Proc. Biol. Sci.* 280, 20131760 (2013).

This is the first study that shows how \pm - and 2 -diversity of biofilm bacteria change across a stream network; the study discusses metacommunity dynamics and hydrology as potential drivers of biodiversity dynamics.

45. Crump, B. C., Amaral-Zettler, L. A. & Kling, G. W. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME J.* 6, 1629–1639 (2012).
46. Ruiz-González, C., Niño-García, J. P. & del Giorgio, P. A. Terrestrial origin of bacterial communities in complex boreal freshwater networks. *Ecol. Lett.* 18, 1198–1206 (2015).
47. Lear, G. et al. The biogeography of stream bacteria. *Glob. Ecol. Biogeogr.* 22, 544–554 (2013).
48. Fierer, N., Morse, J. L., Berthrong, S. T., Bernhardt, E. S. & Jackson, R. B. Environmental controls on the landscape-scale biogeography of stream bacterial communities. *Ecology* 88, 2162–2173 (2007).
49. Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R. & Lappin-Scott, H. M. Microbial biofilms. *Annu. Rev. Microbiol.* 49, 711–745 (1995).
50. Parsek, M. R. & Tolker-Nielsen, T. Pattern formation in *Pseudomonas aeruginosa* biofilms. *Curr. Opin. Microbiol.* 11, 560–566 (2008).
51. Celler, K., Hodl, I., Simone, A., Battin, T. J. & Picioreanu, C. A mass-spring model unveils the morphogenesis of phototrophic *Diatoma* biofilms. *Sci. Rep.* 4, 3649 (2014).
52. Hödl, I. et al. Biophysical controls on cluster dynamics and architectural differentiation of microbial biofilms in contrasting flow environments. *Environ. Microbiol.* 16, 802–812 (2014).
53. Stewart, P. S. & Franklin, M. J. Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* 6, 199–210 (2008).
54. Wimpenny, J. & Colasanti, R. A unifying hypothesis for the structure of microbial

- biofilms based on cellular automaton models. *FEMS Microbiol. Ecol.* 22, 1–16 (1997).
55. Neu, T. Development and structure of microbial biofilms in river water studied by confocal laser scanning microscopy. *FEMS Microbiol. Ecol.* 24, 11–25 (1997).
- This is one of the first studies to use confocal laser scanning microscopy to comprehensively describe the physical structure of river biofilms.**
56. Besemer, K. et al. Biophysical controls on community succession in stream biofilms. *Appl. Environ. Microbiol.* 73, 4966–4974 (2007).
57. Taherzadeh, D., Picioreanu, C. & Horn, H. Mass transfer enhancement in moving biofilm structures. *Biophys. J.* 102, 1483–1492 (2012).
58. Nepf, H. M. Flow and transport in regions with aquatic vegetation. *Annu. Rev. Fluid Mech.* 44, 123–142 (2012).
59. Vignaga, E. et al. Erosion of biofilm-bound fluvial sediments. *Nat. Geosci.* 6, 1–5 (2013).
60. Singer, G., Besemer, K., Hochedlinger, G., Chlup, A. K. & Battin, T. J. Monomeric carbohydrate uptake and structure–function coupling in stream biofilms. *Aquat. Microb. Ecol.* 62, 71–83 (2011).
61. Lawrence, J. R., Swerhone, G. D. W., Kuhlicke, U. & Neu, T. R. In situ evidence for microdomains in the polymer matrix of bacterial microcolonies. *Can. J. Microbiol.* 53, 450–458 (2007).
62. Arnon, S., Gray, K. A. & Packman, A. I. Biophysicochemical process coupling controls nitrogen use by benthic biofilms. *Limnol. Oceanogr.* 52, 1665–1671 (2007).
63. Rusconi, R., Lecuyer, S., Guglielmini, L. & Stone, H. A. Laminar flow around corners triggers the formation of biofilm streamers. *J. R. Soc. Interface* 7, 1293–1299 (2010).
64. Drescher, K., Shen, Y. & Bassler, B. L. Biofilm streamers cause catastrophic disruption of flow with consequences for environmental and medical systems. *Proc. Natl Acad. Sci. USA* 110, 4345–4350 (2013).

65. Pintelon, T. R. R., Picioreanu, C., van Loosdrecht, M. C. M. & Johns, M. L. The effect of biofilm permeability on bio-clogging of porous media. *Biotechnol. Bioeng.* 109, 1031–1042 (2012).
66. Chen, C., Packman, A. I. & Gaillard, J. F. Pore-scale analysis of permeability reduction resulting from colloid deposition. *Geophys. Res. Lett.* 35, L07404 (2008).
67. Naeem, S., Duffy, J. E. & Zavaleta, E. The functions of biological diversity in an age of extinction. *Science* 336, 1401–1406 (2012).
68. Loreau, M. et al. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294, 804–808 (2001).
69. Ylla, I., Borrego, C., Romaní, A. M. & Sabater, S. Availability of glucose and light modulates the structure and function of a microbial biofilm. *FEMS Microbiol. Ecol.* 69, 27–42 (2009).
70. Romaní, A. M. et al. Biofilm structure and function and possible implications for riverine DOC dynamics. *Microb. Ecol.* 47, 316–328 (2004).
71. Guenet, B., Danger, M., Abbadie, L. & Lacroix, G. Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology* 91, 2850–2861 (2010).
72. Danger, M. et al. Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect? *Ecology* 94, 1604–1613 (2013).
73. Kuehn, K. A., Francoeur, S. N., Findlay, R. H. & Neely, R. K. Priming in the microbial landscape: periphytic algal stimulation of litter-associated microbial decomposers. *Ecology* 95, 749–762 (2014).
74. Bengtsson, M. M. et al. No evidence of aquatic priming effects in hyporheic zone microcosms. *Sci. Rep.* 4, 5187 (2014).
75. Cardinale, B. J. Biodiversity improves water quality through niche partitioning. *Nature* 472, 86–89 (2011).
76. Freimann, R., Bürgmann, H., Findlay, S. E. G. & Robinson, C. T. Bacterial structures and ecosystem functions in glaciated floodplains: contemporary states and potential future shifts. *ISME J.* 7, 2361–2373 (2013).

77. Frossard, A., Gerull, L., Mutz, M. & Gessner, M. O. Disconnect of microbial structure and function: enzyme activities and bacterial communities in nascent stream corridors. *ISME J.* 6, 680–691 (2012).
78. Wagner, K. et al. Functional and structural responses of hyporheic biofilms to varying sources of dissolved organic matter. *Appl. Environ. Microbiol.* 80, 6004–6012 (2014).
79. Hector, A. & Bagchi, R. Biodiversity and ecosystem multifunctionality. *Nature* 448, 188–190 (2007).
80. Peter, H. et al. Multifunctionality and diversity in bacterial biofilms. *PLoS ONE* 6, e23225 (2011).
81. Wilhelm, L. & Besemer, K. Altitudinal patterns of diversity and functional traits of metabolically active microorganisms in stream biofilms. *ISME J.* 9, 2454–2464 (2015).
82. Dopheide, A., Lear, G., He, Z., Zhou, J. & Lewis, G. D. Functional gene composition, diversity and redundancy in microbial stream biofilm communities. *PLoS ONE* 10, e0123179 (2015).
83. Martiny, J. B. H., Jones, S. E., Lennon, J. T. & Martiny, A. C. Microbiomes in light of traits: a phylogenetic perspective. *Science* 350, aac9323 (2015).
84. Decho, A. W. et al. Sediment properties influencing upwelling spectral reflectance signatures: the ‘biofilm gel effect’. *Limnol. Oceanogr.* 48, 431–443 (2003).
85. Rier, S. T., Shirvinski, J. M. & Kinek, K. C. In situ light and phosphorus manipulations reveal potential role of biofilm algae in enhancing enzyme-mediated decomposition of organic matter in streams. *Freshw. Biol.* 59, 1039–1051 (2014).
86. Van Horn, D. J., Sinsabaugh, R. L., Takacs-Vesbach, C. D., Mitchell, K. R. & Dahm, C. N. Response of heterotrophic stream biofilm communities to a gradient of resources. *Aquat. Microb. Ecol.* 64, 149–161 (2011).
87. Lyon, D. R. & Ziegler, S. E. Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnol. Oceanogr.* 54, 439–449

- (2009).
88. Ziegler, S. E., Lyon, D. R. & Townsend, S. L. Carbon release and cycling within epilithic biofilms in two contrasting headwater streams. *Aquat. Microb. Ecol.* 55, 285–300 (2009).
 89. Findlay, S. E. G., Sinsabaugh, R. L., Sobczak, W. V. & Hoostal, M. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnol. Oceanogr.* 48, 1608–1617 (2003).
 90. Sinsabaugh, R. L. & Follstad Shah, J. J. Ecoenzymatic stoichiometry and ecological theory. *Annu. Rev. Ecol. Evol. Syst.* 43, 313–343 (2012).
 91. Singer, G., Besemer, K., Schmitt-Kopplin, P., Hödl, I. & Battin, T. J. Physical heterogeneity increases biofilm resource use and its molecular diversity in stream mesocosms. *PLoS ONE* 5, e9988–e9911 (2010).
 92. Cardinale, B. J., Palmer, M. A., Swan, C. M., Brooks, S. & Poff, N. L. The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. *Ecology* 83, 412 (2002).
 93. Watrous, J. D. & Dorrestein, P. C. Imaging mass spectrometry in microbiology. *Nat. Rev. Microbiol.* 9, 683–694 (2011).
 94. Liu, J. et al. Metabolic co dependence gives rise to collective oscillations within biofilms. *Nature* 523, 550–554 (2015).
 95. Piggott, J. J., Salis, R. K., Lear, G., Townsend, C. R. & Matthaei, C. D. Climate warming and agricultural stressors interact to determine stream periphyton community composition. *Glob. Chang. Biol.* 21, 206–222 (2015).
- This is the first experimental study to unravel the effects of global warming and eutrophication on algal and bacterial diversity in stream biofilms.**
96. Downing, J. Global abundance and size distribution of streams and rivers. *Inland Wat.* 2, 229–236 (2012).
 97. Drummond, J. D. et al. Retention and remobilization dynamics of fine particles and microorganisms in pastoral streams. *Wat. Res.* 66, 459–472 (2014).

98. Cadenasso, M. L. et al. An interdisciplinary and synthetic approach to ecological boundaries. *BioScience* 53, 717–722 (2003).
99. Strayer, D. L., Power, M. E., Fagan, W. F., Pickett, S. & Belnap, J. A classification of ecological boundaries. *BioScience* 53, 723–729 (2003).
100. Flemming, H. C. & Wingender, J. The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633 (2010).
101. Freeman, C. & Lock, M. A. The biofilm polysaccharide matrix -a buffer against changing organic substrate supply. *Limnol. Oceanogr.* 40, 273–278 (1995).
- This study shows that the biofilm matrix serves as a notable carbon reservoir for microbial heterotrophs during deprivation of carbon from external sources.**
102. Battin, T. J. et al. Microbial landscapes: new paths to biofilm research. *Nat. Rev. Microbiol.* 5, 76–81 (2007).
103. Garcia-Pichel, F. & Wojciechowski, M. F. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. *PLoS ONE* 4, e7801 (2009).
104. Logue, J. B. & Lindström, E. S. Biogeography of bacterioplankton in inland waters. *Freshw. Rev.* 1, 99–114 (2008).
105. Martiny, J. B. H. et al. Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* 4, 102–112 (2006).
106. Araya, R., Tani, K., Takagi, T., Yamaguchi, N. & Nasu, M. Bacterial activity and community composition in stream water and biofilm from an urban river determined by fluorescent in situ hybridization and DGGE analysis. *FEMS Microbiol. Ecol.* 43, 111–119 (2003).
107. Rendueles, O. & Ghigo, J. M. Mechanisms of competition in biofilm communities. *Microbiol. Spectr.* 3, MB-0009-2014 (2015).

Box 1. Biofilms as a “microbial skin”

The global surface area of streams and rivers is estimated at 662,041 km² (REF. 96). One cubic metre of sedimentary bed material of these streams and rivers provides, on average, a potential surface area of 100 m² for gravel of 5 cm diameter and up to 1000 m² for grains that are ten times smaller. Considering an average streambed depth of 1 metre, the global surface area potentially available for microbial colonization of streambeds would thus range up to 662,041,000 km². In principle, this would provide up to five square metres of sediment surface area available for microbial colonization for each square metre of catchment land surface area (excluding the polar caps and major deserts).

Assuming that there are on average 10⁷ to 10⁹ microbial cells per square centimetre of sediment surface in streams and rivers^{3,6}, we expect that 5 × 10¹¹ to 5 × 10¹³ cells in the streambed receive and process matter from each square metre of catchment land surface area. We view this large microbial surface in streams as a crucial component of a ‘microbial skin’ that covers the catchment and that also includes the microbiomes of the phyllosphere¹³ and soils¹⁴ (see the figure, parts a and b). On its journey through a catchment, via surface runoff or subsurface flow paths, water entrains solutes, particles and microorganisms from rocks, soils and vegetation into the streams. Hydrodynamic coupling of groundwater and streamwater ensures continuous mixing of water, replenishment of key substrates and nutrients, and opportunities for suspended microorganisms from terrestrial and aquatic sources to interact with established biofilms⁹⁷. The streambed microbiome is thus continuously exposed to, and challenged by, invading microorganisms.

The notion of the microbial skin refers to the dynamic nature of stream biofilms as an ecological boundary^{98,99} and should not be understood to mean the presence of a resilient interface between the water and the substrate that biofilms coat. In fact, stream biofilms, as interfaces for solute exchange and biological interactions, share functional and structural properties with interfaces of boundaries that structure ecological communities, ecosystems and landscapes, and that control fluxes of organisms, materials, energy and genetic

information therein^{98,99}.

In streams, biofilms adsorb, retain, amplify and transform organic substances and nutrients in the matrix^{100,101} (see the figure, part c), thereby accumulating substances that are otherwise highly diluted in the streamwater, such as dissolved organic carbon or contaminants. These solutes become concentrated in biofilms via adsorption to the matrix or incorporation into microbial biomass and subsequent release to the extracellular space^{100,101}. Biofilms have reflective characteristics similar to those provided by an ecological boundary; the resident microbiome may sort and reflect invading microorganisms¹⁰² with consequences for local community assembly and downstream release of microorganisms. Biofilms also have protective features; the matrix protects embedded cells from erosion¹⁰⁰, which seems to be particularly important in ecosystems characterized by continuous loss of water and contained solutes and particles, including microbial cells. Furthermore, polymeric components of the biofilm matrix can stabilize sediments from erosion, as can filamentous cyanobacteria that produce supracellular ropes^{59,103}, thereby protecting the local environment from physical disturbance.

Box 2. Metacommunity ecology

Metacommunity ecology is derived from the field of community ecology and is concerned with explaining biodiversity dynamics and spatial patterns of species distribution and abundance. The theory of metacommunity ecology has become an important way to think about how different spatial scales relate to one another in the ecology of both multicellular organisms¹⁰⁴ and microorganisms¹⁰⁵.

A metacommunity is defined as a set of local communities that are linked by the dispersal of several potentially interacting species¹⁰⁴. Metacommunity ecology encompasses several theoretical perspectives, among which ‘species sorting’, ‘mass effects’ and ‘neutrality’ may prove most relevant for microbial biofilms¹⁰⁴.

The species sorting perspective emphasizes the role of the local environment and biotic interactions as drivers of local community composition¹⁰⁴, in which differences between local communities are caused by environmental heterogeneity. Dispersal is global but the strength of this factor is not high enough to maintain species in less favourable habitats, and thus communities are expected to have a distance–decay of similarity relationship along environmental distances (that is, the degree of environmental variation) rather than geographical distances.

The mass effect perspective focuses on the effect of immigration and emigration dynamics on local community composition¹⁰⁴. This perspective acknowledges the importance of environmental heterogeneity but assumes that local populations can be rescued from competitive exclusion by migration from habitat patches where they are good competitors. Communities are expected to have a distance–decay of similarity relationship along both environmental and geographical distances.

The neutral perspective assumes that all species are, on average, ecologically similar¹⁰⁴. Community dynamics derives from immigration and emigration, extinction and genetic drift. Communities are expected to have a distance–decay of similarity relationship along geographical, but not environmental, distances.

Metacommunity theory posits that high dispersal rates and short generation times of microorganisms render geographical distances less important than the local environment for community assembly and ensuing biodiversity patterns¹⁰⁶. For ecosystems with low residence times, such as streams, mass effects through high dispersal are assumed to maintain microbial populations even in less favourable habitats, thereby shifting community composition away from strict dependence on local environmental conditions¹⁰⁵. This seems particularly true for the streamwater. The few studies that have compared microbial communities in the streamwater with those in stream biofilms have found contrasting community structures between the two, with a higher diversity of taxa in streamwater^{32,33}. The inoculation of soil bacteria in stream water is likely to be a major driver of this difference^{57,58}. Streamwater bacteria have also been reported to be less active and their communities more temporally fluctuating than their biofilm counterparts¹⁰⁶.

We note that residence times in biofilms, and more generally in the porous space of the streambed, are higher than in the streamwater itself^{6,8}, and microorganisms therein are therefore expected to be more responsive to the local environment than those in the streamwater. As such, biofilm assembly can be assumed to result from a complex interplay of immigration from the community in the streamwater, environmental sorting and biotic interactions. Biotic interactions between immigrants and the established biofilm, including the ability of immigrants to compete successfully with resident community members, may be relevant for biofilm community assembly, owing to the close proximity between microbial cells¹⁰⁷. The notion of species sorting as a major mechanism underlying local biofilm community assembly is supported by the observation that random immigration of microorganisms from the streamwater into benthic biofilms was not sufficient to explain the assembly of biofilm communities across a wide range of streams^{32,33}.

Figure legends

Figure 1: Biodiversity of a benthic biofilm across all three domains of life. The biodiversity of the benthic biofilm represents a microbial ‘jungle’ that spans all three domains of life: from insect larvae to diatoms and their parasitic fungi to ciliate and flagellate grazers, in addition to a diverse ensemble of bacteria and, in specialized niches, small populations of archaea. Displayed are the taxonomic affiliations and relative abundances of small subunit rRNAs from biofilms in an Alpine stream (M.M.B. unpublished observations).

Figure 2. Major bacterial classes in stream biofilms. Next-generation sequencing of the 16S rRNA gene reveals consistent patterns of bacterial community composition in hyporheic and benthic biofilms from various streams, ranging from glacier-fed streams to streams in the boreal zone. Betaproteobacteria, and often also Alphaproteobacteria, typically dominate these communities. The values shown are derived from 454 pyrosequencing studies of samples from boreal streams⁴², which are rich in dissolved organic carbon (DOC) (that is, more than 30 mg C l⁻¹), from various alpine streams^{32,33,44,81} (which have DOC concentrations ranging from 1 to 3 mg C l⁻¹) and from glacier-fed streams⁴¹ (which have DOC concentrations below 0.5 mg C l⁻¹). The taxonomic affiliation was estimated using a confidence threshold of 70%. Arrows show environmental conditions potentially relevant for biofilm communities, which vary along altitudinal and lateral gradients. While temperature, nutrients and dissolved organic carbon concentrations can explain taxonomic variation along altitudinal gradients, disturbance regime and seasonal fluctuations separate glacier-fed from groundwater-fed alpine streams.

Figure 3: Physical structure and “building blocks” of stream biofilms. a | Optical coherence tomography (OCT) image of a benthic biofilm with coalescent microcolonies and ridges; the inset shows a cross-section with internal voids (black areas) and a high-roughness canopy. Scale bar represents 500 µm. b | Stream biofilms can form elongated streamers that float in the water and that can grow up to several centimetres long, as shown in this OCT image. Scale bar represents 500 µm. c,d | Cryosections combined with epifluorescence

microscopy reveal the structural and compositional complexity of stream biofilms. Diatoms are shown by autofluorescence (red) and bacteria by DAPI staining (blue). e | Depending on light availability, biofilms can include abundant algae, which coexist with bacteria. Stream biofilms can form conspicuous patterns, resembling honeycombs, that include elongated diatoms, extracellular polymeric substances (EPS) and bacteria. Scale bar represents 100 μm . f | A cross-section of a benthic biofilm imaged with a confocal laser scanning microscope. Various diatoms confer structure to stream biofilms and provide a scaffold to bacterial growth and EPS production. g | In phototrophic benthic biofilms, *Diatoma* spp. can form filamentous microcolonies that become entangled and form a structural scaffold for further bacterial growth; the inset shows a scanning electron microscopy picture of *Diatoma* spp. cells. The second inset shows the interface between neighbouring cells. Images in parts a and b are courtesy of Kevin Roche, Northwestern University, Evanston, Illinois, USA. Images in parts c and d are courtesy of Robert Niederdorfer, University of Vienna, Austria. Part e is reprinted with permission from REF. 39, American Society for Microbiology. Part g is from REF. 51, Nature Publishing Group.

Figure 4. Controls on the complexity of stream biofilms. A broad suite of coupled physical, chemical and biological processes control the physical structure, community composition and function of biofilms in streams. These processes are hierarchically positioned under the common umbrella of climate and Earth surface processes and, therefore, susceptible to climate change and global environmental change (such as changes to land use). The effects of streambed topography, hydrodynamics, dispersal dynamics and the availability of dissolved organic matter (DOM) and nutrients on biofilm features are modulated by their spatial and temporal heterogeneity in stream ecosystems. Similarly, spatial and temporal heterogeneity, in addition to properties inherent to biofilm communities, such as metabolic plasticity and functional redundancy, modulate the impacts of biofilms on stream ecosystem processes. Micro-scale processes in biofilms may also feedback on larger scale physical and chemical controls. At the same time, physical and chemical controls may directly affect

ecosystem processes. Process coupling across various scales and the adaptive response of biofilms to the dynamic environment makes biofilms complex systems. Dashed arrows indicate feedbacks and modulating interactions.

Glossary

Periphyton: Traditionally considered to be a phototrophic biofilm that coats benthic substrates in stream ecosystems.

Epilithon: Traditionally considered to be a biofilm that grows on stones in stream ecosystems.

Meiobenthos: Invertebrates living in aquatic ecosystems that have a body size typically not exceeding one millimetre.

Ecosystem respiration: The respiration by both heterotrophic and autotrophic organisms within an ecosystem, in which heterotrophic respiration generates carbon dioxide from the breakdown of organic compounds.

Primary production: The generation of organic carbon from carbon dioxide by photosynthesis, which uses light as an energy source.

Catchments: Drainage basin of streams or rivers delineated by the watershed and within which water from rain, snow or ice melt converges at the valley bottom to contribute to streamwater flow.

Hyporheic zone: The zone in the streambed sediment in which streamwater interacts with groundwater, as driven by hydrodynamic exchange. Typically considered to be a habitat with high rates of biodiversity and biogeochemical reaction.

Reflective characteristics: The ability of an interface, or ecological boundary, to partially or entirely return matter, energy or organisms.

Phyllosphere: The microbial communities colonizing the above-ground surfaces that are provided by terrestrial plants.

Benthic zone: The upper zone of the streambed; the benthic zone is notable for its direct interface with streamwater flow and its exposure to light.

Humic substances: A complex and heterogeneous mixture of polydispersed materials formed by biochemical and chemical reactions during the decay of plant tissue. This mixture is a major contributor to dissolved organic matter in aquatic ecosystems.

Co occurrence networks: Graphical visualization of potential relationships, between species or other entities, that have been derived from correlation analyses.

Operational taxonomic units: (OTUs). Classification of microorganisms on the basis of an operational definition for species distinction that applies a percentage similarity threshold to 16S rRNA sequences.

Flow fields: Flow patterns that are generated by a moving liquid over and around solids.

Bedforms: Geomorphological features that develop at the interface of fluid and a movable bed, such as dunes and ripples on the beds of streams and rivers. Bedforms affect near-bottom hydraulics and hydrodynamic exchange with porewater in the streambed.

β -diversity: The compositional similarity of ecological communities and the species turnover therein.

Neutral models: In the context of biodiversity, models that assume that individuals of all ecologically similar species are competitively equal and that the stochasticity of demographic processes, such as immigration, birth and death, drive local community assembly.

α -diversity: Local species diversity in a habitat or ecosystem, often referred to as species richness or Shannon or Simpson diversity.

Competitive exclusion: Ecological process whereby two (or more) species that use the same resources cannot stably coexist.

Headwaters: The smallest streams in a fluvial network and where streamflow is generated.

Laminar flow: The flow of water in parallel layers that are not disrupted. Laminar flow often fosters copious biofilm growth, as turbulence-induced erosion of microbial biomass is low.

Drag force: A force that acts on any solid objects exposed to water flow; the drag comes from forces caused by pressure distributions over the surface of the object.

Hydrodynamic exchange: The exchange of water masses driven by the pressure differences that occur over rough streambeds.

Priming effect: Phenomenon in which labile dissolved organic matter (DOM) compounds facilitate the breakdown of apparently recalcitrant DOM compounds by microbial

heterotrophs. The mechanism is unclear but may involve the provision of energy for the expression of extracellular enzymes that degrade recalcitrant DOM.

Recalcitrant DOM: Dissolved organic matter (DOM) that is resistant to degradation by microbial heterotrophs.

Functional plasticity: The capacity of an ecological community to accommodate environmental changes by adjusting the overall performance of dominant phylotypes.

Functional redundancy: A concept that relates changes in ecosystems to species loss, in which species that perform similar roles in communities can substitute for one another with little effect on the functioning of the community and ecosystem.

Functional gene arrays: DNA array technology for assessing functional gene diversity and distribution in microbial communities.

Microautoradiography: Method that visualises and quantifies the uptake of a radioactively labelled compound at the level of single cells.

Eutrophication: The process in which increased nutrient inputs drive an increase in algal biomass in aquatic systems, which in turn causes anoxia as a result of the breakdown of these algae by microbial heterotrophs.

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Key Points

1. Biofilms dominate microbial life in stream ecosystems. These matrix-enclosed and surface-attached microbial communities are ubiquitous, prolific and highly active at the interfaces of the streambed. The biofilm mode of life is advantageous in streams with a fast flow of water and continuous export of nutrients and organic matter.
2. Biofilms in streams can be considered a 'microbial skin', regulating the processing and export of nutrients and organic matter from catchments and influencing the dispersal of microorganisms and their biodiversity dynamics at the scale of entire stream networks.
3. Interactions between the growth of biofilms, streamwater flow and substratum chemistry produce emergent environmental complexity in the streambed.
4. Proteobacteria and Bacteroidetes often dominate the communities of stream biofilms. Flavobacteriia and Sphingobacteriia seem to be especially important members of these communities. Archaea are found within niche microenvironments established by the metabolic activity of other microorganisms.
5. High biodiversity in stream biofilms is supported by continuous input of microorganisms from upstream catchments, environmental sorting induced by habitat heterogeneity (ranging from the scale of the biofilm to large stream networks) and episodic disturbance from streamwater flow.
6. New interdisciplinary approaches are needed to link structure and function of biofilms to their environment and, ultimately, to ecosystem processes and biogeochemical fluxes in streams. This is crucial to understand and predict implications of global ecosystem change and climate change on the microbial ecology and functioning of stream ecosystems.

Author biographies

Tom J. Battin is a professor of ecohydraulics at the Ecole Polytechnique Fédérale de Lausanne, Switzerland. His research focuses on the ecology of biofilms in streams and the implications of biofilms for ecosystem processes. He is interested in the physical, chemical and biological process coupling that makes these biofilms so diverse and successful in the biogeochemical cycle. His research also includes the study of large-scale carbon fluxes at the scale of entire stream networks. Tom Battin's webpage.

Katharina Besemer pursued postdoctoral research at the University of Glasgow, UK, and recently moved to the University of Vienna, Austria. Her research focuses on the structure and function of microbial communities in streams and rivers, particularly on the distribution of microbial biodiversity in fluvial landscapes, ranging from millimetres to kilometres. Her current work emphasizes the role of stream confluences as conspicuous nodes in fluvial networks on microbial diversity and community structure.

Mia M. Bengtsson is a junior group leader at the University of Greifswald, Germany. In 2011, she obtained her Ph.D. from the University of Bergen, Norway, studying bacteria in kelp forests and has done postdoctoral research at the University of Vienna and the WasserCluster Lunz, Austria, on the ecology of stream biofilms and lake planktonic communities. She is interested in how biotic interactions shape microbial communities and likes to pursue this topic on global, ecosystem, experimental and molecular scales.

Anna M. Romani is an associate professor at the University of Girona, Spain. She received her Ph.D. in Biology in 1997 at the University of Barcelona, Spain, and subsequently held research positions at the University of Wales, Bangor, UK, at the Max-Planck-Institute for Limnology, Schlitz, Germany, and at the Evolutionary Biology Center, Uppsala, Sweden. Her main research expertise covers river ecology, specifically the role of the microbial biofilm on organic matter cycling with emphasis on microbial interactions and functions, biofilm formation, and global change effects.

Aaron I. Packman is a professor of environmental engineering and science at Northwestern

University, Evanston, Illinois, USA. His research focuses on water, sediments and microbiota, with particular emphasis on the basic processes that control interfacial dynamics in aquatic systems and the coupling of physical processes with biological and biogeochemical processes. Important applications include benthic microbial ecology, nutrient and carbon cycling, contaminant dynamics, ecosystem degradation and restoration, waterborne disease transmission, and control of biofilms in human infection and engineered water systems.

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