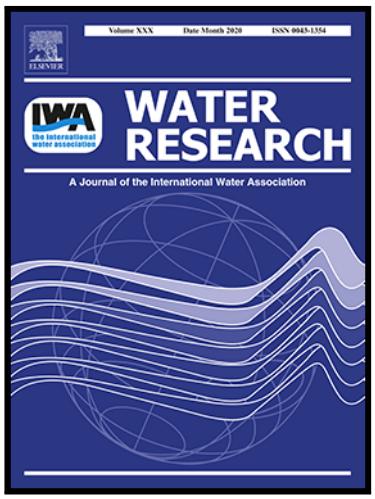

Exploring flow-biofilm-sediment interactions: Assessment of current status and future challenges

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Highlights

Flow-biofilm-sediment interactions in relation to biostabilization are reviewed.

This is supported by a joint workshop, testing feasibility of an integrated approach.

Development in optical tools and molecular approaches increased biofilm understanding

Mechanical understanding of biostabilization have not been well understood.

Challenges include realism, scalability and methodological limitations.

Exploring flow-biofilm-sediment interactions: Assessment of current status and future challenges

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Abstract

Biofilm activities and their interactions with physical, chemical and biological processes are of great importance for a variety of ecosystem functions, impacting hydrogeomorphology, water quality and aquatic ecosystem health. Effective management of water bodies requires advancing our understanding of how flow influences biofilm-bound sediment and ecosystem processes and vice-versa. However, research on this triangle of flow-biofilm-sediment is still at its infancy. In this Review, we summarize the current state of the art and methodological approaches in the flow-biofilm-sediment research with an emphasis on biostabilization and fine sediment dynamics mainly in the benthic zone of lotic and lentic environments. Example studies of this three-way interaction across a range of spatial scales from cell (nm – µm) to patch scale (mm – dm) are highlighted in view of the urgent need for interdisciplinary approaches. As a contribution to the review, we combine a literature survey with results of a pilot experiment that was conducted in the framework of a joint workshop to explore the feasibility of asking interdisciplinary questions. Further, within this workshop various observation and measuring approaches were tested and the quality of the achieved results was evaluated individually and in combination. Accordingly, the paper concludes by highlighting the following research challenges to be considered within the forthcoming years in the triangle of flow-biofilm-sediment:

- Establish a collaborative work among hydraulic and sedimentation engineers as well as ecologists to study mutual goals with appropriate methods. Perform realistic experimental studies to test hypotheses on flow-biofilm-sediment interactions as well as structural and mechanical characteristics of the bed.

- Consider spatially varying characteristics of flow at the sediment-water interface. Utilize combinations of microsensors and non-intrusive optical methods, such as particle image velocimetry and laser scanner to elucidate the mechanism behind biofilm growth as well as mass and momentum flux exchanges between biofilm and water. Use molecular approaches (DNA, pigments, staining, microscopy) for sophisticated community analyses. Link varying flow regimes to microbial communities (and processes) and fine sediment properties to explore the role of key microbial players and functions in enhancing sediment stability (biostabilization).
- Link laboratory-scale observations to larger scales relevant for management of water bodies. Conduct field experiments to better understand the complex effects of variable flow and sediment regimes on biostabilization. Employ scalable and informative observation techniques (e.g., hyperspectral imaging, particle tracking) that can support predictions on the functional aspects, such as metabolic activity, bed stability, nutrient fluxes under variable regimes of flow-biofilm-sediment.

Keywords

1. Biostabilization
2. Erosion threshold
3. Extracellular polymeric substances
4. Bacteria
5. Microphytobenthos
6. Hydrodynamics

Contents

1. Introduction

Microbial life in most water bodies grows in “biofilm”, which are genetically diverse surface-attached aggregates of microorganisms (Archaea, Bacteria, Eukarya) (Flemming and Wuertz, 2019) that are wrapped in a self-produced matrix of extracellular polymeric substances (EPS). Aquatic biofilms are capable of colonizing various soft (e.g., sediment or soil surface) and hard (e.g., stone, plant, pipe or vessel surfaces) surfaces that exist across diverse environments, including streams and rivers (Battin et al., 2016), lakes (Zhang et al., 2020), estuarine (Vijsel et al., 2020) and marine (Yallop et al., 1994) waters, as well as drinking water distribution systems (Chan et al., 2019; Douterelo et al., 2019). Whether growing on mud (epipelic), sand (epipsammic), stone (epilithic) or plant (epiphythic), whether addressed as microphytobenthos (in shallow coastal waters, intertidal flats), microbial mat (among others, in habitats of hot springs, hypersaline ponds, groundwater) or periphyton (on any submerged surface in the aquatic habitat), all communities possess emergent features, such as production of EPS, tolerance towards external stresses, cell-cell communication and collective behaviour as well as synergetic use of nutrients that distinguish them as biofilm (Flemming et al., 2016; Flemming and Wingender, 2010; Flemming and Wuertz, 2019; Gerbersdorf and Wieprecht, 2015; West et al., 2007).

Biofilm lifestyle is distinctly different and more common than planktonic lifestyle, with an estimated 40-80% of cells contributing to the global biomass residing in biofilms (Flemming and Wuertz, 2019). The transition of one microbe from the planktonic to the biofilm lifestyle, and vice-versa, depends on a range of environmental conditions among which the local hydrodynamics are of paramount importance (Berke et al., 2008; McDougald et al., 2012; Wheeler et al., 2019). Hydrodynamics largely dictate initial “touch-down” and the residence time of the microbes on surfaces (Rusconi et al., 2014). When exceeding a certain hydrodynamic force or experiencing a hydraulic

retention time shorter than the doubling-time of the cells, the microbes will disperse again and leave the habitat. Those cells that still stick are selected towards stronger adherence, and the further biofilm development strongly shapes their adjacent physical and chemical surrounding in a reciprocal way (Gerbersdorf and Wieprecht, 2015). Thereby, biofilm growth and its influence on the surrounding strongly depend on the microbial metabolic activity which leads to redox-relevant small-scale stratification and impacts large-scale biogeochemical budgets (Packman, 2013). However, these metabolic processes are determined by mass transfer in the water column and towards the biofilm, which is again controlled by hydrodynamics regulating nutrient supply to the microbes (Gerbersdorf and Wieprecht, 2015). For a comprehensive review on the processes related to surface attachment and subsequent colonization, we refer readers to reviews by Berne et al. (2018), Gerbersdorf and Wieprecht (2015) and Tolker-Nielsen (2015).

When growing on sediment in the bed finer than about 2 mm (clay, silt, sand) (Statzner et al., 1999), biofilms also glue the sediment grains to each other through their EPS matrix (Jones, 2017; Paterson et al., 2018). This, in turn, alters the sediment-bed properties, e.g., density, morphology, size gradation (Fang et al., 2012; Gibbs, 1983; Huiming et al., 2011; Shang et al., 2014), and dynamics, e.g., erosion and transport (Banasiak et al., 2005; Chen et al., 2017; Droppo et al., 2015; Fang et al., 2017; Gerbersdorf et al., 2008; Malarkey et al., 2015; Righetti and Lucarelli, 2010; Vignaga et al., 2013) and, finally, the accumulation and transport of contaminants (Burns and Ryder, 2001; Förstner et al., 2004). The ability of biofilms to increase erosion thresholds by biological actions is an ecologically essential ecosystem function named “biostabilization” (de Brouwer et al., 2005; Gerbersdorf and Wieprecht, 2015; Passarelli et al., 2014; Roncoroni et al., 2019) and has been

reported to mediate sediment erosion, transport, deposition and consolidation (ETDC) cycle in aquatic ecosystems (Paterson et al., 2018). It should be noted that biostabilization can also occur through smoothing of the bed surface and therefore reduction of the hydraulic roughness, as observed over gravel-like hemispheres (Graba et al., 2010). These interactions of sediment and biofilm are critical to the biogeochemical processes at the entire ecosystem level (Packman, 2013). Along with their impact on nutrient fluxes (Battin et al., 2016; Falkowski et al., 2008; Madsen, 2011), biofilms possess further fundamental ecosystem services such as water self-purification (Gerbersdorf and Wieprecht, 2015; Shannon et al., 2008) and they also regulate and mediate primary production and food web processes (Battin et al., 2008; Demars, 2019; Graba et al., 2013).

Such fundamental ecosystem processes and functions of the biofilms are determined by their biodiversity and community composition through the metabolic performance of involved microbial communities (Allan and Castillo, 2007; Besemer, 2015; Loreau et al., 2001). The physical structure, composition and diversity of the biofilms in aquatic ecosystems vary widely depending on the physical (e.g., grain size, porosity), chemical (e.g., sediment nutrient content), biological (e.g., growth rate, cell-cell communication) and environmental (e.g., light, temperature and flow regime) factors (Allan and Castillo, 2007; Stevenson et al., 1996) and processes (Leibold et al., 2004), including interactions with nutrient and organic matter cycling (Battin et al., 2016; Schiller et al., 2007), growth habitat (Salta et al., 2013; Wilhelm et al., 2014) as well as flow and bed topography (Battin, 2000; Risse-Buhl et al., 2017; Woodcock et al., 2013). Indeed, many biofilms have complex morphologies and can develop long, oscillating filamentous structures called streamers (Larned et al., 2011; Nikora,

2010), which not only alter flow dynamism and bed topography, but also mass transport near the bed.

The preceding higher-order effects induced by biofilm formation are of ever-changing nature (Battin et al., 2016) mainly due to complex reciprocal interactions between flow, biofilm and sediment (Gerbersdorf and Wieprecht, 2015) and are also expected to change in a nontrivial way as a result of climate change (Piggott et al., 2015; Zeglin, 2015) and human alteration. However, our understanding of dynamic flow-biofilm-sediment processes nexus in natural water bodies which drive changes in ecosystem processes and functions remain still incomplete (Nikora, 2010; Packman, 2013; Paterson et al., 2018). In order to better manage our water bodies for the benefits of human society and ecosystem functioning as well as to support UN's Sustainable Development Goals, a better understanding of flow-biofilm-sediment interactions – that we call flow-biofilm-sediment triangle – is needed. Challenges include i) creating realistic experimental settings and utilizing a combination of tools and approaches to describe the reciprocal relationships between flow-biofilm-sediment and associated mass transfer, which alters microbial processes and vice-versa and ii) understanding the role of key microbial players (and processes) for biogeochemical and morphological processes (biostabilization) at the entire ecosystem level, and how organismal level functions can be linked to ecosystem functions. These challenges require acquisition of large bodies of information across various spatial scales by studying hydraulics, geomorphology and ecology as an integrated concept using advanced tools and approaches rather than viewing them as subordinately serving the other. The increasing needs for interdisciplinary approaches have been underlined by many recent studies (Battin et al., 2016;

Palmer and Ruhi, 2019; Paterson et al., 2018; Rice et al., 2010; Roncoroni et al., 2019).

In this review coupled with an illustrative experiment, we summarize the current understanding of the flow-biofilm-sediment triangle and demonstrate how this three-way interaction and important ecosystem functions such as biostabilization can benefit from a co-application of measurement techniques from various disciplines. The intersection between scientific fields of hydrodynamics, biogeomorphology and microbiology is the theme of this paper. While we focus on flow-biofilm-sediment interactions with implications on fine sediment dynamics mainly in the benthic zone (first cm of the bed) of lotic and lentic environments, some examples from other environments (e.g., medicine) are also presented to provide a more comprehensive picture of the field.

2. Methodology

2.1 General approach

To address the flow-biofilm-sediment triangle and its effects on ecosystem processes with an emphasis on biostabilization (Fig. 1), a joint workshop (three-phase) was held in Stuttgart, Germany between June 2018 and February 2019, bringing together experts from Germany in the relevant areas of hydromechanics, microbial ecology, biochemistry and sedimentation engineering. The main goals of the workshop were i) to consolidate knowledge and identify knowledge gaps in understanding flow-biofilm-sediment interactions through expert discussions and pivotal papers and ii) to perform a pilot experiment to test and discuss how the identified knowledge gaps can be addressed by co-application of modern methods in the fields of hydraulics, sedimentation engineering, microbial ecology and biochemistry. The current knowledge and gaps to elucidate flow-biofilm-sediment interactions were discussed

in the first phase together with the design of the pilot experiment, and the second and third phases were focused on performance of experiments and review-discussion of the results, respectively.

2.2 Pilot experiment

During the pilot experiment, the capabilities of selected promising instruments and methods from different disciplines (shown in black font in Fig. 1) across various spatial scales were exemplarily demonstrated by their co-application on riverine biofilm samples that were quasi-naturally grown on fine sediment at contrasting (high bed shear stress ~0.04 Pa and low bed shear stress ~0.01 Pa) flow conditions in six recirculating flumes, each with dimensions of 3 m long and 0.15 m wide (Schmidt et al., 2015). The specific aims of this pilot experiment were (a) to test the applicability and limitations of the techniques applied and (b) to identify the spatial and temporal scales relevant to better understand the reciprocal interactions in the flow-biofilm-sediment triangle. For the latter, we summarize the various scales applied in different disciplines first (Fig. 1). Selected preliminary data from this pilot work focusing on young (21 days) and mature biofilms (90 days) were utilized in the context of current knowledge and methodological advances that exist in each of the relevant fields for this flow-biofilm-sediment triangle (Fig. 1).

2.3 Structure of the review

This review article provides an update on current scientific knowledge, practices and methodological approaches related to flow-biofilm-sediment interactions, including fine sediment dynamics and outlines how future studies can benefit from an interdisciplinary approach in order to better understand the flow-biofilm-sediment processes nexus. The review is organized into three parts. The first starts from the initial colonization to mature microbial landscapes, thereby focusing on heterotrophic

bacteria and microalgae (Section 3). The second describes the internal architecture, polymeric matrix, community biomass and composition of the biofilm as well as mass transfer (Section 4). The third discusses the mechanical properties of biofilm and biofilm-embedded sediments with specific regard to biostabilization (Section 5). In each of these sections, selected data from the pilot experiment was used to support the challenges and benefits of a comprehensive interdisciplinary approach. Finally, in Section 6, we present research gaps and research challenges in the relevant disciplines based on the condensed knowledge and experience gained during the workshop. Section 7 concludes the review paper.

3. A view from above: Mutual flow – sediment/biofilm interactions

3.1 *Flow - Attachment - Colonization*

In aquatic ecosystems, the mostly turbulent flow is generated by an external supply of energy (e.g., gravity, wind, waves) at the macroscale (bulk) (Kolmogorov, 1941) but gradually passed on to the microscale experienced by the aquatic microorganisms. The hydrodynamic forces affect many aspects of microbial movement, attachment and subsequent biofilm development for which adherence/remobilisation, nutrient supply and metabolic waste removal are of utmost importance. Therefore, understanding the reciprocal interaction between microbial assemblages and near-bed hydrodynamics has direct theoretical and practical implications. Living and moving at the microscale (herein referred as single-cell scale), microorganisms are directly exposed to a local viscous flow characterized by low Reynolds numbers (Re <<1), which in turn interacts with the larger turbulent scales (Tennekes, 1989). Overall, like non-motile macroorganisms, microbes are at the mercy of boundary conditions controlled by the turbulence. While macroorganisms experience flow as an intermittent and chaotic motion, rapid fluid fluctuations appear slower and smoother to microbes that are smaller than the size of

the smallest eddies and thus, embedded within a single whirl of the flow (Wheeler et al., 2019). Nevertheless, most microorganisms are motile and thus, even at the microscale, self-propelling bacteria induce flow perturbations and create spatiotemporal chaos of the otherwise laminar flow. This swimming of a self-propelling bacterium can occur as a random walk, one of the mechanisms being “run-and-tumble” motion (e.g. for *Escherichia coli*). Apart from this individual locomotion, collective motion (e.g. chemotactic waves, swarming) of bacteria might take place (Lauga, 2016; Lauga and Powers, 2009). This behaviour might lead to long-range motions to impact velocity speed and direction (Bratanov et al., 2015), meso-scale turbulence characterized by vortex length scale (Doostmohammadi et al., 2017) as well as collective oscillation as centimetre-scale travelling waves (Chen et al., 2017). Thus, whether via passive (drift, downsweeps) or active (self-propulsion, buoyancy regulation) movements, macro- and microscale interactions between flow and microbes orchestrate together to influence the likelihood of surface contacts as well as detachment/attachment ratios (Characklis and Cooksey, 1983; Tuson and Weibel, 2013; Wey et al., 2009). For instance, it seems difficult for a microorganism to overcome the physical forcing when exposed to higher flow conditions (~0.08 Pa) resulting in delayed attachment as well as growth compared to low (~0.01 Pa) and medium (~0.04 Pa) flow (Schmidt et al., 2018). For further microbial colonization, flow again seems to be the most decisive factor since forcing may increase particle resuspension and light attenuation, limiting metabolic activity and establishment of photoautotrophs within the biofilm (Schmidt et al., 2018).

On the other hand, flow can be highly beneficial to biofilm development in order to maintain nutrient supply and the removal of waste-products. Decisive for these features is the so-called “diffusive benthic boundary layer” (DBBL), usually sub-

millimeter in thickness. Along with surface roughness, the usually turbulent flow above dictates the thickness of this DBBL where viscous flow prevails (Gerbersdorf and Wieprecht, 2015). Accordingly, the DBBL represents the zone between zero velocity at the surface (no slip condition) and turbulent conditions within the water column above. Within this viscous DBBL, molecules are transported by molecular diffusion, driven by a concentration gradient between the bulk fluid and the surface. The diffusion coefficients (*D*aq) are specific for the molecules of interest, and along with the thickness (*L*) of the DBBL determine the transfer velocity (*kL*). Along with the vertical concentration gradient, the external mass transfer towards the surface of the substratum or the developing biofilm (*kL* (m/s) = *D*aq (m^2 s^{-1}) / *L* (m)) is quantified. This external mass transfer is decisive for the replenishment of nutrients or other molecules essential for further biofilm colonization, and the term is used to distinguish it from internal diffusion limitations that might occur within the biofilm (Stewart, 2012). As seen above, the external mass transfer depends on the thickness of the DBBL, which again is controlled by near-bed turbulence and the surface roughness of the biofilm-bound sediment (Nikora, 2010), but difficult to determine experimentally due to its thinness and inherent proximity to the bed surface.

While a growing biofilm under fast local flow conditions, which leads to a relatively thin DBBL with a strong concentration gradient, might be in a favourable situation regarding nutrient replenishment, the risk of immediate detachment or sudden sloughing-off is also enhanced (Zhang et al., 2011). Consequently, the impact of the turbulence has been described as a trade-off between shear forces and nutrient supply to influence the overall lifecycle of microbial assemblages ranging from attachment, colonization, and ongoing growth to dispersal (McDougald et al., 2012).

3.2 *Growth - Topography - Flow*

When the biofilm grows horizontally and in height, it changes the topography of the colonized substratum, rendering the previous surface properties redundant. At first, the biofilm disseminates across the surface to be colonized and the resulting spatio-temporal pattern depends again largely on the flow above. While a hydraulically smooth and more constant flow seems to favour isotropic microcolonies, multidirectional, fluctuating and varying flow velocities allow higher degrees of freedom for colonization (Rossy et al., 2019; Stoodley et al., 1999; Thomas et al., 2013). Hence, growing clusters at a hydraulically rough environment (i.e. turbulent at the roughness-scale) result in anisotropic, star-like structures that may optimize the exploitation of space (Hodl et al., 2014). This is consistent with the data from the pilot experiment, where it was observed that young biofilm featured isotropic growth while more elevated matured biofilm exhibited a preferred growth orientation in alignment with the flow direction (Fig. 2a). While gaining height, the biofilm can either smoothen a formerly rough surface by accruing the “valleys” or enhance the roughness by growing on “hills” to accentuate small differences in surface structure (Picioreanu et al., 1998; Stewart, 2012). In the first scenario, growth in valleys might be favoured since the troughs act as a hideaway to protect from hydrodynamic forces (Barton et al., 2010). The second scenario, the “fingered” biofilm growth, has been proposed to be due to a competitive advantage at flow conditions that impede nutrient replenishment otherwise (Nikora et al., 2002). Generally, hydraulically smooth flow conditions seem to promote the formation of filamentous or stalk-like structures that protrude out of the biofilm and experience a compressed DBBL with a higher supply of nutrients. This is in line with our workshop results on freshwater biofilm where low bed shear stresses (~0.01 Pa) allowed the development of thicker and more heterogeneous biofilm, with elongated filaments (so-called streamers) moving with

the flow. In contrast, medium stresses (~0.04 Pa) resulted in biofilm accumulating close to the surface and forming bungalow-type structures (Gerbersdorf and Wieprecht, 2015).

Clearly, one way or the other, if given time, biofilm development changes the bed topography. In the pilot experiment, we measured bed topography by (a) scanning the area with microscope (Axio-Zoom 1.6) and (b) with laser triangulation system (e.g., Noss et al., 2018) where biofilm valleys and peaks are determined by measuring the reflected laser light that falls incidentally onto a receiving object and at a certain angle which depends on the distance of the object (here the biofilm). At the low flow conditions given above, increasing mean and RMS (root mean square) heights over the weeks of growth indicated a rougher topography, while increasing autocorrelation lengths (distance to a different structure) of the surface roughness reflected a more regular surface structure of biofilm (Fig. 2b). Hence, the mature biofilm in week 4 has a remarkably mountainous appearance (Fig. 2c). As compared to the initial conditions, the average biofilm thickness (1.92 to 3.74 mm) as well as surface roughness (0.46 to 1.97 mm) increased significantly along with a reduced flow regime (0.04 to 0.01 Pa), as previously modelled by Head (2013). This increase in roughness appears to (a) reduce the DBBL thickness, (b) increase the surface area and/or (c) induce near-bed flow field fluctuations such as micro-eddies by the protruding structures (e.g., Bishop et al., 1997). Thereby, the effective roughness mediates the friction (=resistance) forces in a way, well beyond the expectation arising from the physical appearance of biofilm (Cowle et al., 2017). Measurements of flow and biofilm growth across the 5 cm patch scale in our pilot experiment suggest a spatially heterogeneous distribution of the Reynolds stress (=total stress tensor in a fluid), which globally increases above the matured biofilm bed as

compared to the initial bare sand bed. The enhanced and varying peaks in Reynolds stress might result in recirculating eddies, turbulent wakes or turbulent bursts (packets of energetic fluid) that penetrate deep into the DBBL to impinge transiently on the biofilm (de Beer et al., 1994a). This way, the mass transfer towards (sweeps to enhance food supply) and out of (ejections to boost waste removal) the biofilm is positively enhanced (Bishop et al., 1997; McDougald et al., 2012; Stewart, 2012) up to a point where detachment and abrasion occurs (Zhang et al., 2011). In the extreme, e.g. smooth flow conditions, the biofilm might become depleted in metabolic substrates and enriched in metabolic waste (Stewart, 2012), while the opposite is true for biofilm growth at rough flow conditions (Biggs et al., 1998); known as the eutrophic effect of the flow.

4. Entering the microbial city

4.1 *Architecture and EPS Matrix*

Biologists developed an early interest in the architecture as well as in the chemical and biological composition of biofilms. Consequently, there are numerous papers dealing with flow-biofilm interactions from young to mature stages of biofilm development. In this context, it has been observed that biofilm matrix, architecture and species composition change significantly along with the hydrodynamics (Azeredo et al., 2017; Risse-Buhl et al., 2020; van Loosdrecht et al., 2002). Observations by several research groups congruently detail that biofilm thickness is inversely related to flow velocities (Graba et al., 2013; Paul et al., 2012; Pereira et al., 2002). Interestingly, biofilm mass follows this trend, but to a much lesser extent. For instance, Paul et al. (2012) reported for one biofilm type thickness reduction from 300 to 100 µm and mass reduction from 0.13 to 0.09 mg TOC cm^{-2} when exposed to increasing shear stress from 2 to 9 Pa. Dreszer et al. (2014) showed elastic sponge-like behavior of biofilm being exposed to varying flow conditions (first three days at 20

L m^{-2} h^{-1}, followed by an increase to 60 L m^{-2} h^{-1} and restoring back to the original flow). Their optical coherence tomography (OCT) measurements revealed that 50% decrease in biofilm thickness at higher velocity was largely due to the collapse of mushroom-like void spaces, while the biofilm mass remained the same (Dreszer et al., 2014). This not only proves the largely visco-elastic nature of biofilm, but also the variations in density of the biofilm matrix along with the flow conditions. By applying various levels of shear stress (from 0.09 to 13 Pa) on the surface of biofilm cultivation plates in an annular reactor, Paul et al. (2012) confirmed the significantly enhanced biofilm density (roughly about three times) with increasing shear stress. The investigations of Pereira et al. (2002) on single species *Pseudomonas* biofilm explained the possible mechanisms behind the observed changes in physiognomy: cells at stronger hydrodynamic conditions secreted more exopolymeric substances per unit volume while void spaces were reduced. The resulting thinner and denser biofilm seems to promote nutrient degradation rates and thus efficiency in wastewater treatment, but caution is warranted for the extrapolation to natural multispecies biofilm (Pereira et al., 2002). First studies in fluvial systems on epilithic biofilms confirmed these effects of turbulent flow on biofilm architecture; however, this was most pronounced at nutrient-rich conditions (Risse-Buhl et al., 2017). Furthermore, Fish et al. (2017) and Polst et al. (2018) attested as well significantly higher production of EPS carbohydrates and EPS proteins at a stronger hydrodynamic regime for biofilms in drinking water pipes and autotrophic stream biofilms, respectively. This was different compared to the results of our pilot experiment, which showed significantly higher carbohydrates content at the lower flow condition, but similar for proteins at both lower and higher flow regimes (Fig. 3). The studies are most likely incongruous since they address different biofilm communities (e.g. heterotrophic bacteria versus microalgae) with varying secretion pattern of polymeric

substances (Pierre et al., 2012; Vu et al., 2009). Moreover, there are some uncertainties as to the broad range of extraction and determination methods used (Delattre et al., 2016). Furthermore, the composition of EPS is highly variable and complex, thus challenging to characterize (Flemming et al., 2016; Frølund et al., 1996; Jahn and Nielsen, 1995; Nielsen et al., 1997, 1996). To the best of our knowledge, there are currently no studies on shifts in EPS quality (e.g., monomer composition, functional groups, structural elucidation) according to various flow conditions although it is eminent that components for structural integrity (e.g., amyloids, Zeng et al., 2015) might be more prevalent at higher flow conditions. That again, will be most likely determined by the dominating microbial species that trigger EPS secretion highly differently depending on their adaptation – an uncharted territory.

4.2 *Microbial biomass and multitrophic relations*

The effect of flow on biofilm biomass is environment-dependent and still inconclusive. While most studies in drinking water distribution systems reported increasing bacterial biomass with increasing flow velocity (Fish et al., 2017; Simões et al., 2007; Torvinen et al., 2007), the others from stream ecosystems showed the opposite for both bacterial and microalgal biomass (Battin et al., 2003; Besemer et al., 2007). Yet, the effect of flow on biomass and diversity of biofilms in streams appears to be season-dependent (Risse-Buhl et al., 2020), suggesting a modulating effect of varying physicochemical parameters and synergistic multitrophic interactions. In our pilot experiment, hyperspectral imaging and quantification of absorption peaks was used to map photopigments across the surface of sedimentary biofilms (Chennu et al., 2013). This technique can be used to non-invasively monitor the distribution and dynamics of chlorophyll *a* and other pigments at very fine spatial scales (Chennu et

al., 2015b) and flexible temporal resolution, providing a comprehensive view of the spatio-temporal evolution of photopigments in biofilms under interesting ecological interactions (Chennu et al., 2015a). The photopigment distributions in our measured biofilms of varying flow regimes, age and sedimentary grain structure (Fig. 4) indicated diversity in spatial structure, succession of new functional groups in older biofilms and represent a generally robust proxy for photosynthetic potential. While studies on pure-cell biofilms have indicated discernibility for diatom-specific photopigments (Fucoxanthin, Jesus et al., 2014), we could not detect this in our studied biofilms embedded in a scattering sediment matrix. However, recent optical modeling work provides promising developments towards fine-tuned applications (Launeau et al., 2018). Besemer et al. (2007), by addressing the community successions of stream biofilms in flumes, gave evidence of higher bacterial species abundance and microalgal biomass within laminar to transitional flow as compared to fully turbulent conditions. Schmidt et al. (2018) verified reduced bacterial cell numbers as well as microalgal biomass at stronger flow conditions, where both flow scenarios (weak vs. strong) were turbulent. Nevertheless, the effects of flow on microbial cell numbers and biomass might be as well of indirect nature. For instance, negative effects on microbial grazer densities (e.g., flagellates, ciliates) by flow potentially generates positive effects in biofilm bacteria as the latter are released from grazing pressure (Risse-Buhl et al., 2020; Wey et al., 2008). Environmental biofilms are in fact multi-trophic consortia including also protistan and micro-metazoan grazers besides prokaryotes and algae (Weitere et al., 2018) and it is this complex microbial cosmos that finally determines biofilm functionality (Arndt et al., 2003; Besemer, 2015). Therefore, we could get revolutionary insights into the microbial world by including the whole microbial web. In this regard, Risse-Buhl et al. (2017) has done pioneering work to address the interaction between near bed turbulence,

flow (ū) and biofilm composition, architecture as well as trophic structure in mountainous stream ecosystems. In this study, the abundance of filamentous autotrophs increased with near-bed turbulent kinetic energy (TKE) which most likely offered shelter to bacteria that remained unaffected in numbers by the increasing flow velocities. Bacteria further benefited from a reduced grazing pressure at faster flowing, more turbulent sites, since the abundance of heterotrophic protists decreased with flow. Results by Risse-Buhl et al. (2017) suggested that near-bed flow might impact the magnitude and direction of matter fluxes through shifts in the microbial food web - thereby possibly affecting ecosystem functioning.

4.3 *Microbial taxonomy (by microscope and molecular techniques)*

Insights into the microbial community composition have been traditionally gained my microscopic evaluation of morphological, taxonomically unique features (Clark et al., 2018). This classical approach is for instance common for the determination of diatom species that – by their appearance and certain requirements - are excellent indicators of different water qualities or various hydrodynamics scenarios. Graba et al. (2013) reported that epilithic biofilms at smooth flow grew much thicker, developed thicker filaments and accommodated multicellular growth forms of diatoms while biofilm at rough conditions were more compact hosting smaller, mobile and unicellular diatoms. This seems to confirm the progression of climax populations at low flow velocities that are subject to minor changes but undergo C-Selection (competition in terms of resources such as nutrients). In contrast, pioneer species dominate at high flow velocities where resources are available, but environmental forcing is strong to experience R-Selection (ruderal strategy to be adapted to disturbed habitats) (Biggs et al., 1998).

While these insights are very valuable on the microalgae level, addressing the occurrence of certain bacterial species requires metagenomics approaches. Nowadays, it has become possible to decipher the previously unprecedented diversity of biofilms using high-throughput technologies (referring to next-generation sequencing (NGS)). In terms of community composition, results of our pilot experiment of prokaryotic 16S ribosomal ribonucleic acid (rRNA) and eukaryotic 18S rRNA suggest varying responses of the prokaryotic (bacterial) and (micro-) eukaryotic ("higher" cells) species. Species diversity (number of species and number of individuals per species) was significantly higher at high flow condition (0.04 Pa) for the bacterial community while microalgal species flourished at the low flow (0.01 Pa). Additionally, bacterial species that are filamentous or well-known to have EPS-coding genes were more dominant at the mentioned high flow velocities (Fig. 5). This indicates clear shifts of the bacterial community as a response to the hydraulic regime. Few studies took it even further to the level of gene expression in order to reveal microbial responses to mechanical stress by shear flow, but this is so far restricted to the single-cell level (Persat et al., 2015; Thomen et al., 2017)

4.4 Mass transfer (towards, out of and within the biofilm)

The above briefly discussed biofilm architecture, EPS quantity, biomass and community composition is decisive for the mass transfer towards and within the biofilm. Mass transfer has implications for both, the resupply of nutrients and the removal of waste-products as stated earlier and is largely influenced by the hydrodynamic features. To follow the transport of dissolved molecules into and within the biofilm, mainly microelectrodes have been used so far (Beyenal and Babauta, 2013; de Beer et al., 2018; Sønderholm et al., 2017). For instance, vertical profiles of oxygen microelectrodes allowed the calculation of the DBBL thickness for oxygen

(depending on the flow conditions above) as well as metabolic activity (photosynthesis, respiration) and the resulting penetration depth and micro-niches within a biofilm or biofilm-inhabited sediment (de Beer et al., 1994b; Gerbersdorf et al., 2004; Jorgensen and Revsbech, 1985). Using oxygen microelectrodes in our pilot experiment, vertical profiles of oxygen concentration at the water-biofilm-sediment interface were recorded in the transition from light to dark (Fig. 6a). While oxygen peaks and concentrations within the first 5 mm of depth were clearly decreasing over darkness, the oxygen concentration below the photic zone never decreased to zero, indicating some advection. Based on the calculated photosynthetic and respiration rates, it could be stated that the metabolic activity was quite low in our system as compared to e.g. studies from intertidal flats (de Beer et al., 2005), microbial mats (Nübel et al., 2002) and alkaline lakes (Wieland and Kühl, 2000). That proves the unbroken popularity of microsensors to determine physiological responses and essential functions of biofilms at high spatial and temporal resolution. Some investigations took it even one step further to address flow pattern *in situ* within cell clusters or voids of single-cell or multi-species bacterial biofilm by tracking the movement of microscopic fluorescent particles with the help of confocal microscopy (de Beer et al., 1994a; Thomen et al., 2017). Other studies directly determined local mass transfer coefficients applying modified limiting current techniques (LCT) within the biofilm (Yang and Lewandowski, 1995). As a result, the non-uniformity of local mass transfer processes within biofilms became apparent by their large fluctuations that were explained by irregularities in biofilm microstructures comprising channels, voids and cell clusters. While diffusion was prevailing in cell clusters, liquid flow (convection and diffusion) occurred within the biofilm voids (de Beer et al., 1994b, 1994a; Yang and Lewandowski, 1995). Moreover, the flux from the bulk water into the biofilm was enhanced by the elevated biofilm structure being twice as high as

compared to a planar surface (de Beer et al., 1994b). With these valuable insights that can be gained at high spatial resolutions, it is not surprising that microsensor studies have skyrocketed in the last decades. Right now, mass transfer and local conversion rates of various molecules (e.g. N_2O, H_2S, NO_3^-, NO_2^-) can be determined by a large range of sensors to calculate the distribution of a suite of metabolic activities (de Beer, 2011). To give just one example, one of the more recent developments, the hydrogen peroxide (H_2O_2) microsensor, was applied in our pilot experiment. While photosynthesis produces H_2O_2 as damaging by-product, it is usually scavenged by catalase to avoid cell damage. However, bursts of H_2O_2 up to 100 – 200 µM were observed when the sensor tip touched a diatom colony – most likely a defence mechanism of these microalgae against predators that has not been noticed before (Fig. 6b). Again, this allows novel information on prey-predator relation and resulting functions at microscale level. In order to move on from the fragile micrometre-sized glass electrodes towards more robust sensors, macroelectrodes with sensing tips in centimetre range were developed as well as the simultaneous determination of molecules in two-dimensional arrays by optodes was pursued (Glud et al., 2000). These optodes based on luminescence quenching are superior to electrochemical sensors in many ways such as obstruction of the local flow field, hysteresis or cross-sensitivity (Kautsky, 1939; Tengberg et al., 2006). Still, there are new challenges associated like response time, drift, long-term stability in organic-rich environments and data processing (Bittig et al., 2018; Glud et al., 1994; Tengberg et al., 2006). Nowadays, both types, electrochemical and luminescent-based microsensors are pushed manifold. However, to particularly address biofilm-flow interaction is still rare (Glud et al., 1998; Kühl et al., 2007) but has unbowed potential to unravel links between morphology and functionality of biofilms.

5. Mechanical stability of biofilm and its inhabited environment as one essential ecosystem function

5.1 Biofilm functions for its inhabitants and for the ecosystem

As opposed to a single planktonic lifestyle, the biofilm offers high survival, persistence and reproduction potential to the embedded microbes (Flemming and Wuertz, 2019). On one hand, there is co-metabolism and an enhanced availability of essential resources, resulting in significantly higher metabolic activity. This has attracted extensive attention since it links to the important microbial ecosystem functions such as biodegradation, self-purification or drinking water provision (see Introduction and the references therein). On the other hand, protection from environmental stressors is a key factor for biofilm members. The EPS matrix controls material fluxes largely by its internal porosity and permeability that determine fluid flow conduits and their connectivity (Flemming et al., 2016). Since slow diffusion processes prevail and adsorption occurs, toxicants such as antibiotics or disinfectants may be intercepted in the outer layers of a biofilm, which also represents a huge problem in medical treatment (Bjarnsholt et al., 2011). All of that is possible by the cohesive and adhesive forces binding microbes to each other as well as to their substratum and conferring their overall mechanical and structural integrity that is largely impacted by the predominant flow conditions. Thanks to this stability, biofilm eventually colonizes all kinds of interfaces whether unintentionally (e.g., biofouling in pipes and on ship-hulls) or encouraged (e.g., in waste-water treatment, on membranes). Therefore, understanding mechanical properties of aquatic biofilms and biostabilization potential of biofilm-bound sediment have important implications not limited to aquatic ecosystems.

5.2 The challenges with biofilm-induced mechanical stability

Characterizing the mechanical stability of biofilm and biofilm-enclosed environments is thus of broad and significant concern, but remains a challenging task despite a body of work. Part of the problem is that, traditionally, it was attempted to remove biological effects, while nowadays, biology is often brought to the laboratory with little consideration of natural settings (Paterson et al., 2018). In this regard, laboratory-grown biofilm are often based on distinct microbial strains growing at conditions that are difficult to compare and often lack natural relevance (e.g. single-species biofilm and nutrient supply that do not occur in natural rivers (Vignaga et al., 2012)). When testing for the biostabilization effect in the laboratory, a range of engineering devices are applied that act at different size scales while addressing different forces (e.g. vertical jets versus horizontal bed shear stress (Vardy et al., 2007; Widdows et al., 2007)). Moreover, examination of erosion thresholds are more complicated in biofilm-embedded sediments since they behave very differently compared to the traditionally used abiotic particle-size fractions (e.g., they erode in aggregates and chunks rather than in single-grain mode (Thom et al., 2015)). Last but not least, investigating development of biofilm over time requires non-destructive methods, but most approaches require bed failure to occur (Jonsson et al., 2006).

Overall, flow-microbe interactions and implications for mechanical stability of biofilm have received special attention since this process understanding might help to control (eradicating harmful or encouraging beneficial) biofilm by optimizing cleaning procedures (e.g., in drinking water pipes) or improving operational parameters (e.g., in rotating biological contactors). In the following, we will present a brief selection of such studies focusing on the mechanical strength of a biofilm in relation to flow from

single-cell level to bulk biofilm measurements in the range from several millimeters to several centimeters (Wagner et al., 2010b).

5.3 Single-cell approaches to determine adhesive forces

Atomic Force Microscopy (AFM) has been widely used to determine the elasticity and adhesive capacity of single bacterial or microalgal cells linking the results to different cell surface biomolecules with implications to the initial stages of biofilm colonization (Wright et al., 2010). The AFM technique can be applied in a static or dynamic mode, measures in the range from piconewtons to several nanonewtons and allows 3D mapping of surfaces within a limited area (Boudarel et al., 2018). Since the technique has been widely used to study initial attachment, only few studies relate to reciprocal microbe-flow interactions that become most interesting in later stages of biofilm development. Lim et al. (2008) gave proof of the positive relation between morphological parameters such as surface coverage and roughness as well as flow rate in biofilms growing on glass beads within microfluidic cells. To shed light on the internal structure of biofilm, passive particle tracking microrheology (PTM) or active optical tweezer (OT) and magnetic tweezer (MT) techniques have been successfully implemented (see reviews by Ahmad Khalili and Ahmad, 2015; Azeredo et al., 2017). These approaches allow the determination of spatially and temporally varying adhesive strength as well as the quantification of shear stresses required for detachment while operating in the sub-piconewton (pN) to several hundreds of pN (<1 pN) range (Castelain et al., 2012; Picioreanu et al., 2018). Using MT as a more robust approach for actively moving cells, Galy et al. (2014) developed a 3D map of mechanical biofilm properties and demonstrated decreasing elastic compliance in *Escherichia coli* biofilms being exposed to increasing shear stress. This research provided valuable insights of the heterogeneity of biofilm showing

variations in shear compliance in the order of two magnitudes within close proximity (Galy et al., 2014). Again at microscale, microfluidics are an integral part in the study on mechanical properties of growing biofilms, often combined with microscopy to monitor biofilm formation during growth and biofilm deformation due to applied stress such as pressurized air or flow (see review by Karimi et al., 2015). Hohne et al. (2009) established such an approach to examine the Young modulus and relaxation time of two bacterial strains while imaging their deflection due to varying air pressure with confocal laser scanning microscopy (CLSM). Thomen et al. (2017) pursued the growth of *E.coli* to reveal the previously unknown bacterial strategy to settle in low shear stress regions before strategically expanding from these bases towards areas of high shear stress that were impossible to colonize before. Hou et al. (2018) applied attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy as well as CSLM to give evidence that the individual *Staphylococcos aureus* bacterium produced two to five times more EPS polysaccharides at high shear conditions as compared to low shear stress. That also extended to the entire biofilm as was shown by tribometrically measured coefficients of friction (CoF), confirming that EPS quantity is considered relevant for mechanical strength (Hou et al., 2018).

5.4 Structural visualization and mechanical strength at the mesoscale

While the microscale is certainly very important to learn about activities and functions of biofilms at high resolution, it remains difficult to extrapolate these insights to the dimensions of an entire biofilm (mm-cm range) which is of greater interest if it comes to flow-biofilm interaction and the resulting mechanical strength (Wagner and Horn, 2017). Knowledge on mechanical strength is needed to re-think anti-biofouling measures, to manage biofilm growth in technical systems such as bioreactors, membranes or drinking water distribution pipes or simply to better understand the

mode of action that is behind the effects recorded in biostabilization of sediments. Therefore, other techniques such as optical coherence tomography (OCT) or optical coherence elastography (OCE) (Larin and Sampson, 2017) which has a more holistic view on biofilm structure could be explored. OCT allows to work on this mesoscale in micrometer resolution and represents a fast, non-invasive, *in situ* imaging technique that gives depth-resolved structural information which does not require staining. Thus, employing near-infrared light allows deeper penetration into the biofilm in comparison to CLSM and does not need fluorophores that might interfere with local properties of the biofilm (Azeredo et al., 2017; Picioreanu et al., 2018). However, our own OCT measurements in the pilot experiment suggest that while surface topography is easy to image, it can be difficult to capture the internal structure as well as to differentiate between biofilm and sand particles. Still, OCT clearly visualized how the microbes filled the sand grain space with biofilm maturation (Fig. 7). In young biofilms, filaments were visible at the mentioned low flow conditions (0.01 Pa) while at high flow (0.04 Pa), the growth seems delayed again (Fig. 7). This influence of various flow scenarios in the initial stages of biofilm growth is expected to manifest later in variations in thickness, morphology as well as hydraulic resistance of the mature biofilm as has been revealed by other OCT measurements (Dreszer et al., 2014).

Exploring the usage of rotating disk electrodes (RDE), Boulêtreau et al. (2011) confirmed varying riverine biofilm thickness and elasticity that both were significantly higher at low flow conditions (0.1 versus 0.45 m s^{-1} over 21 days). In contrast to OCT, RDE examines the biofilm as homogenous bulk material which is similar to the application of rheometers that are commonly used for studying viscoelastic material although being destructive (Boudarel et al., 2018). Biofilm comprises both an elastic

(or solid-like) and a viscous (or liquid-like) part that stores and dissipates energy during strain deformation. In our workshop, we also tested viscoelastic properties of young and mature biofilms using a rotational rheometer. Conducting dynamic tests in the oscillatory mode of the rheometer in our pilot experiment documented clearly the viscoelastic behavior of the biofilms at varying stages of maturity. Beforehand, some biofilm samples (young biofilm) were cultivated *in situ* on rheometer disks with the same substrate tested simultaneously with other devices, while other samples (mature biofilm) were transferred later onto those disks at termination of the experiment. Due to the roughness of the substrate, both sample preparation methods showed similar results even though the *in situ* growth has a clear advantage of ensuring an overall structural integrity. The recorded phase angle highlighted the strong dependence of the viscoelastic properties on the maturity of the biofilm. The results showed a low phase angle of $\tan\delta = 0.16$ for the matured biofilm, indicating less viscous and more elastic behavior, while this value increased for the young biofilm to $\tan\delta = 0.28$ (Fig. 8). However, no clear relation could be detected between the phase angle and the prevailing flow conditions, although the margin of the phase angle has been slightly larger for biofilms exposed to lower flow conditions (Fig. 9). For a more comprehensive interpretation however, it is necessary to link the results on rheological properties to structural features (e.g., streamers or flat biofilm) and chemical composition (polymer type) in future studies. Then it would also be possible to structurally explain stronger adhesion and lower detachment rates at higher shear stresses as it has been previously observed by recording stress-strain tests and creep-compliance curves in *Pseudomonas aeruginosa* biofilm samples (Stoodley et al., 2002).

5.5 Erosion vulnerability and adhesive capacity in sediment research

As we can see from Sections 5.1–5.4, there exists promising work highlighting mechanical properties of biofilm in interaction with the flow at various scales (see also review by Araújo et al., 2019). However, none of the research includes the substratum, except for our presented OCT (Fig. 7) and rheometer measurements (Fig. 8) where biofilm grew on fine sand. This is, of course, different in sedimentation engineering where the erosive response of the substratum towards hydrodynamic forcing is central. However, biofilms were and sometimes still are neglected in this research field (Paterson et al., 2018; Righetti and Lucarelli, 2007). That changed some decades ago when it was increasingly recognized that biofilms are ubiquitously distributed and impact significantly the dynamics of the ETDC (erosion – transport – deposition – consolidation) sediment cycle (Black et al., 2002). With the consensus on the importance of biostabilization, the portfolio of methods to address sediment stability has broadened. The classical approach in hydraulic research and engineering utilizes erosion flumes or chambers in which the flowing water eventually causes bed failure to occur (Aberle et al., 2003; Jonsson et al., 2006; Noack et al., 2015; Widdows et al., 2007). With growing interest in the biology mediating the erosive response, devices with smaller footprints capable for usage in the field were developed to pursue mechanical failure and sloughing-off at higher temporal and spatial resolution (Vardy et al., 2007). However, to follow-up gradual changes in the attachment and increasing cohesion of substratum by young biofilms, non-destructive methods with higher sensitivity were needed. Magnetic Particle Induction (MagPI) system has been developed to determine the adhesive capacity of growing biofilm at the patch scale with a small footprint, but large enough to get meaningful results on biofilm-embedded sediment stability (Gerbersdorf et al., 2018; Larson et al., 2009). Using 50% particle clearance, the MagPI indicated significantly lower adhesiveness

at low flow condition (1241 ± 97 mA) as compared to high flow condition (834 ± 59 mA) in our pilot experiment. This is in line with the results of Graba et al. (2013) who performed a sloughing test on 40-day-old biofilms and showed an inverse relation between the proportion of detached biomass and the average value of friction velocity during growth. The higher stability might be related to enhanced secretion of extracellular polymeric substances at high shear stresses as stated earlier (Brading et al., 1995; Fish et al., 2017). Whether or not this translates into a higher biostabilization capacity of the biofilm within the sediment at high flow regimes, is currently unknown (Gerbersdorf and Wieprecht, 2015). In our pilot experiment, by investigating the erosion failure of the samples within the SETEG-flume (Noack et al., 2015), the critical shear stress necessary to erode the biofilm-sediment complex was 40-fold higher as compared to the bare sand (Fig. 9). However, there was no statistically significant difference between the two contrasting flow scenarios. This might be explained by the growing mode of these samples where the biofilm covered the underlying substratum like a carpet. Erosion often occurred suddenly at the edges of our sample holders, followed by a severe resuspension of the bare, unprotected sediment beneath the biofilm carpet, rather than indicating a true failure of the biofilm-sediment surface (see S1 and S2 for videos). The starting position of the erosion at the edges of the cartridges can be attributed to a sudden change in surface roughness upstream and downstream of the measurement location. Hence, while cultivating biofilms in special cartridges (Schmidt et al., 2015) or coupons (Singer et al., 2010, 2006) facilitates *in situ* and easier measurements using a multitude of instruments, it is critical for erosion tests to exclude unwanted effects of sudden roughness change. It is further deemed advisable to adapt Shield's-like erosion schemes to biofilm-embedded material (Shields, 1936; Thom et al., 2015).

6. The gaps in hydromechanics, biofilm and sediment research and lessons to learn

In biofilm research, the single-cell scale has been naturally favoured to decipher details on biofilm attachment, quorum sensing, morphology and/or detachment (Kim et al., 2016; Mukherjee and Bassler, 2019; Wheeler et al., 2019). As we have reported above, reciprocal flow-biofilm interactions have been experimentally explored within micro-fabricated channels that mostly range from micrometres to millimetres and analysed mainly by microsensors (de Beer et al., 1994a; Yang and Lewandowski, 1995) and/or imaging techniques. For the latter, Thomen et al. (2017) visualized the trail lengths of 1-µm fluorescent particles via microscopically derived z-stack images in millifluidic channels. Magnetic Resonance Microscopy (MRM) is another promising method for the investigation of transport phenomena, which is capable of simultaneously imaging the development of flow field and biofilm structure in a non-invasive, less time-consuming way while covering quantitatively relevant areas (Gjersing et al., 2005; Manz et al., 2005, 2003). For instance, Wagner et al. (2010a) addressed the response of biofilm to various flow gradients and shear rates by scanning the flow field with MRM. Herrling et al. (2017) successfully elucidated water diffusion within five different types of biofilm structures by pulsed field gradient-nuclear magnetic resonance (PFG-NMR). As Morgenroth and Milferstedt (2009) stated, "…a biofilm with a total area of 1 m^2 is not simply the sum of biofilm grown in 1,000 flow channels, even though the total areas roughly correspond." In order to study the effect of laminar, transient and fully turbulent conditions on biofilm, other laboratory studies (Morgenroth and Milferstedt, 2009) went up to the patch scale which is between several millimetres to centimetres. Collectively, these experiments gave essential insights into detailed processes of biofilm development at various hydrodynamic settings. However, their relevance for aquatic environments is still

largely debatable due to difficulties in reproducing natural conditions in the laboratory. Challenges include representing natural temporal and spatial variability of flow (single-cell scale to ecosystem scale) and biofilm (multitaxa and multispecies communities) or generating fully-developed turbulent flow conditions and irregular surfaces (e.g., mixed sediments, spatial heterogeneity of roughness) at the measurement section.

Ideally, biofilm should be grown in a most natural-like setting to allow a typical community composition and matrix structure at environmentally relevant flow patterns. Obviously, this might differ severely in simulating technical or natural habitats but in both cases, this requires experimental facilities that are beyond the small scale of the microfluidic channels. Despite increasing number of mesocosm studies, our understanding of near-bed flow dynamics is currently hampered by the lack of velocity measurements at the flow-biofilm-sediment interface (e.g., Nuy et al., 2018; Risse-Buhl et al., 2017). While the interactions between flow and biofilm occur predominantly at mesoscale (100 µm to 10 cm in vertical length), most studies concerning flow-biofilm reciprocity usually represent flow with a single (bulk) value for the entire channel either as a depth-averaged or cross-sectional average velocity (and discharge) (Moulin and Eiff, 2012 and references therein) or temporally-averaged flow and turbulence parameters far above the bed (Risse-Buhl et al., 2017; Risse-Buhl et al., 2020; Singer et al., 2010), ignoring the heterogenous characteristics of the flow at local biofilm scale (µm to cm) and its dispersive contribution to mass flux. This can mainly be attributed to frequent use of acoustic-based (e.g., acoustic doppler velocimeter or ADV) or magnetic field based (e.g., electromagnetic current meter or ECM) instruments in both laboratory and field studies, which have difficulties in measuring near-bed mean flow (<5 mm) and

turbulence (<10 mm) due to acoustic interference of the bed (Koca et al., 2017; Voulgaris and Trowbridge, 1998) and/or sensor size. On the other hand, applying hot-film anemometers, Biggs et al. (1998) demonstrated clearly the influence that the biofilm has on the close-by flow pattern 2 mm above its surface while there was no measurable effect to the far more uniform mean velocity of the mid-water column. Hydrodynamic fluctuations with local shear stress peaks are critical to mass transfer (Stoodley et al., 1999; Voermans et al., 2017), with important consequences for biofilm to modulate ecosystem health and services. This is particularly important for biofilms with streamers which oscillate with the flow and modulate mass transfer (Nikora, 2010; Larned et al., 2011). Ultimately, only local flow conditions are relevant to describe the forcing at microscale and are not easily inferred from mean bulk velocities (Graba et al., 2013). The introduction of modern, optical and non-intrusive techniques such as Particle Image Velocimetry (PIV) allows high resolution measurements of flow patterns close to the biofilm (~1-2 mm) in standard configurations. PIV is based on visualization and computation of the displacement of small tracer particles in a flow, captured by two subsequent images (see reviews by Adrian et al., 2011; Westerweel et al., 2013). Since it allows for both quantitative measurements at larger areas (few dm^2) and visualization of flow structures, PIV provides physical insights into the behaviour of flow and biofilm interactions, thereby offering various advantages over traditional methods (i.e., ADV, ECM). Despite its costly and complicated setup, low-cost PIV systems have recently been developed for use in the laboratory (Cierpka et al., 2016) and in the field (Cameron et al., 2013; Koca et al., 'Unpublished results'). Thus, instead of following traditional single-point or vertical profile measurements, it is now possible and timely to characterize flow pattern near biofilm at high resolution (Koca et al., 2016). Nevertheless, the challenge remains to measure this at sub-millimetre scales in fully-turbulent, fully-rough and

fully-developed environments, which, unavoidably, must be performed in relatively large flow facilities on the several-meter scale for a variety of controlled flow and environmental conditions (Packman, 2013; Vignaga et al., 2013). Indeed, it would be desirable to describe scales small enough to include the viscous sublayer on the biofilm and grains while simultaneously capturing the full turbulent spectrum. Only then can the mechanisms behind biofilm growth and mass and momentum flux exchanges between biofilm and the water be elucidated.

Research on flow-sediment interaction has a long tradition in engineering science which is motivated *inter alia* by the huge economical aspect to maintain waterways and harbours for shipping as well as flood control measures (Voermans et al., 2017). Sediment dynamics from bedload such as rolling gravel to suspended load of fine particles is of uttermost importance for the hydrological, geomorphological and ecological functioning of aquatic systems including rivers, lakes, reservoirs, estuaries and coastal zones (Forstner and Westrich, 2005). Along with sediment properties, the hydrodynamic regime is decisive for the transport, deposition and finally spatial distribution of sediments. Hence, fine-grained particles such as silt and clay usually settle in low energetic habitat while coarser sediment deposit in areas of high energy impact where frequent collisions of sand particles (“rolling”) occur (Van Rijn, 1993). This in turn impacts the settlement of microbes with a higher likelihood to develop in fine sediments since these small particles feature (a) a high surface to volume ratio, (b) offer plenty of binding sites to trap nutrients and (c) offer more protection for sensible shells such as diatom frustules that might be destroyed in rolling (Delgado et al., 1991; Gerbersdorf and Wieprecht, 2015). Mediation of fine sediment characteristics by the developing microbial assemblages then changes their erosive response to hydraulic forcing as described in Sections 1 and 5.5. While the onset of

motion of non-cohesive sediment particles such as sand or gravel is generally predicted by using the Shields diagram (Shields, 1936), there is no valid approach for fine cohesive sediment because of the biological features inhabited (Black et al., 2002). In order to better predict the behaviour of fine sediment and the often-associated pollutants at extreme (e.g., 100-year flood) or management scenarios (e.g., flushing), experiments have been largely performed in laboratory flumes with sediments that were retrieved and transferred from the field (Forstner and Salomons, 2008; Gerbersdorf et al., 2007; Haag et al., 2001). However, there are increasing efforts towards *in-situ* measurements in order to avoid unwanted changes due to transport of sediment from field to flume and sediment aging (Aberle et al., 2003; Noack et al., 2015; Witt and Westrich, 2003).

Overall, although fine sediments and their erosional behaviour have thus received increasing attention over the last decades with or without the consideration of biofilm, their link to the temporally and spatially highly varying pattern of hydrodynamics (e.g. TKE) and bed topography co-evolving with biofilm growth have not been studied yet (Gerbersdorf and Wieprecht, 2015; Hannah et al., 2004; Rice et al., 2010). On a much smaller scale, some studies addressed the sloughing-off phenomena of biofilm at varying shear stresses by measuring biofilm weight losses or the amount of eroded sand/biofilm mixtures (Grün et al., 2016; Pique et al., 2016). Consequently, future studies should further explore the flow-biofilm-sediment processes nexus in order to better understand biostabilization and sediment dynamics which have key implications for morphodynamics, aquatic habitat, water quality and beyond. This requires integrated investigations of hydrodynamics, biogeomorphology and microbiology. An example of such integrated approach was illustrated in this review paper combined with a pilot experiment. Based on the co-application of state-of-the-

art methods from different disciplines (black font in Fig. 1) on quasi-naturally grown biofilm-bound sediment developed at contrasting flow conditions, we have made following observations about advantages and challenges associated with the tested methods:

- Flow affects time of settlement, growth direction and subsequent topography of biofilm-bound sediment (Fig.2). In turn, the biofilm growth increased considerably the magnitude and heterogeneity of Reynolds shear stress (not shown here). While the measurements of spatially and temporally varying flow in combination with motion of streamers are still challenging, PIV may become a key method in studying natural flow-biofilm interactions at high resolution.
- Obtaining topography by scanning microscopy (Fig. 2a-b) was far too time-consuming for larger areas, instead laser triangulation system showed similar results (Fig. 2c) in a fraction of the time previously needed and is thus an excellent choice for characterizing topography at patch scale (cm^2-dm^2). Microscopy is needed for higher resolutions at spatially limited spots in order to e.g., visualize key components of the biofilm (members and EPS moieties) by fluorescence signals.
- With application of microsensors, we have observed bursts of H_2O_2 when touching a diatom colony, suggesting a likely defense mechanism of these microalgae against predators. Therefore, using microsensors, one can also gain insights into how microbes cope with friendly and unfriendly neighbours in their close surrounding (Figs 6 and 7). Microsensor measurements could be used together with PIV measurements and OCT images in analyzing the external and internal mass and momentum transfer by combining substrate distribution, flow dynamics and biofilm structure. Even though it is not tested

here, the application of MRM holds promise for studying internal and external diffusion and flow patterns.

- OCT is a beneficial imaging technique for characterizing internal structure of biofilm without disturbing or damaging the samples. However, since the probe depth is limited, it remains to be tested how suitable this technique is for analyzing biofilm-embedded sediment over depths larger than 2 mm.
- By biochemical and microbiological analyses, it was observed that quantities of polymeric substances play a minor role in explaining the mechanisms of attachment and binding (Fig. 3), suggesting the role of key microbial players and functions in biostabilization.
- By applying molecular techniques, we have observed significant shifts in species composition and diversity at both prokaryotic and eukaryotic level (Fig. 5). The link between functionality and diversity of key players is matter of ongoing debate in ecology (Besemer, 2015; Dang and Lovell, 2016; Leibold et al., 2004), and to bring this idea in biofilm research would be a great research concept for hypothesis building.
- Microalgae seem to play a dominant role in biostabilizing the sediment with impact according to the particular groups or species involved (Fig. 5). Thus, the possibility to monitor by hyperspectral imaging the density, composition and distribution of phototrophic biofilms (Fig. 4) creates a strong predictor for sediment stability and metabolic activity.
- While magnetic particle induction techniques is highly sensitive for measuring adhesiveness of biofilm-bound sediment at high resolution (< 5mm), it is restricted to measurements at the sediment surface. Furthermore, measurements are challenging if streamers are abundant. Until now,

determination of the stability of deeper layers has been limited to erosion flumes.

7. Conclusions

Recent advances in measurement techniques have provided a wealth of knowledge regarding individual domains of biofilm research, yet the understanding of flow-biofilm-sediment processes is still at its infancy. Studying flow-biofilm-sediment interactions are of importance to better understand ecological functions and engineering processes and to help establish healthy aquatic ecosystems. Most of the insights on the sediment-ecology relation derive from investigations on macroorganisms and macrophytes. This is surprising since microbes are the first colonizers to dictate subsequent colonization by higher trophic levels and they e.g. predominately affect the flux of matter on larger scales. Moreover, the link to the substratum in which microorganisms settle is mostly missing. Therefore, integrative and interdisciplinary approaches are needed that simultaneously and equally address the complex and non-linear ways in which sediment properties and biofilm interacts with the hydrodynamics at a scale of µm to cm (single-cell and patch scale) before extrapolating the theoretical knowledge to the larger scales for environmental management purposes. While field studies are essential, process understanding comes from controllable and repeatable conditions addressed in laboratory experiments which should cross disciplinary boundaries to avoid oversimplification and unrealistic settings (e.g., use of isolated species, inappropriate physical conditions, unconsidered wall effects or short test sections).

In this Review, we summarized the current state of the knowledge and methodological approaches in the flow-biofilm-sediment research with an emphasis

on biostabilization and fine sediment dynamics mainly in the benthic zone of lotic and lentic environments. Specifically, we combined a literature review with the results of a pilot experiment that was conducted in the framework of a joint workshop, with the aims of i) consolidating expert knowledge from different scientific fields, but all with the same goal directed towards flow-biofilm-sediment triangle, ii) identifying knowledge gaps and iii) exploring the feasibility of different instruments to address these gaps. In the pilot experiment, co-application of advanced methods with capabilities to visualize at cell-, micro- and mesoscales at high spatial resolution in controllable laboratory conditions has facilitated investigations into flow dynamics (Particle Image Velocimetry), bed topography (laser triangulation system and light microscopy) , biofilm structure (optical coherence tomography), mass transfer (microsensors), microbial community (16s and 18s rRNA gene sequencing) as well as mechanical characteristics (rheometer) and biostabilization potential (magnetic particle induction and erosion flume). Based on the evaluation of feasibility of these techniques, we also provided research insights, methodological limitations, existing research gaps and future research directions that have potential to make important contribution to the field of biostabilization.

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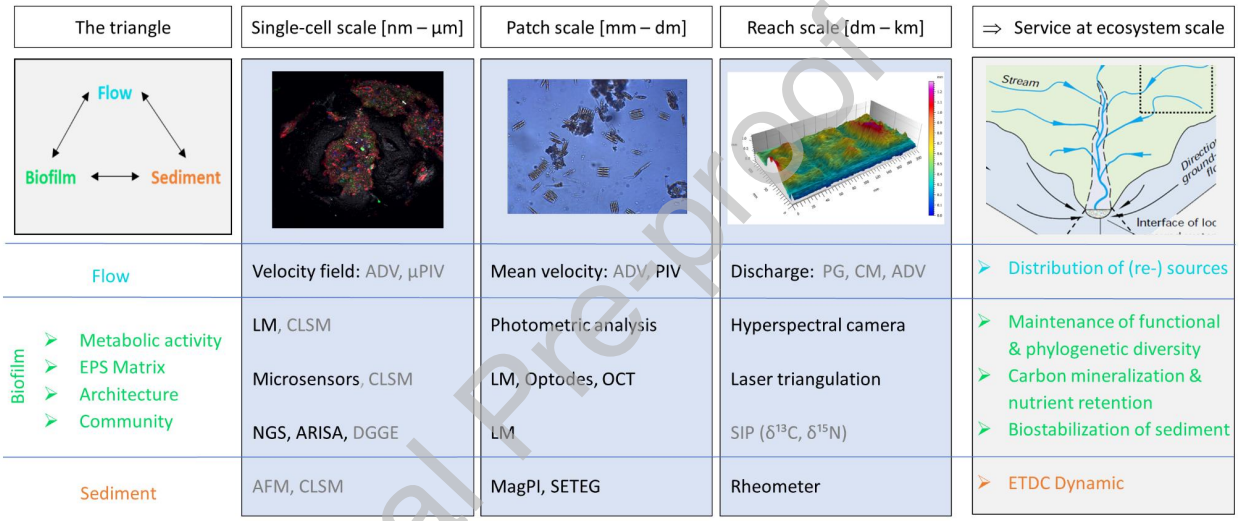
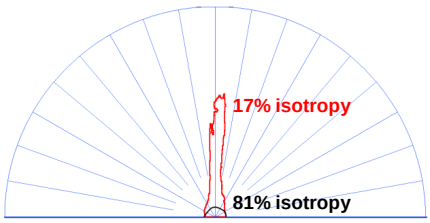


Fig. 1: How to investigate the interactions of flow-biofilm-sediment. Addressing various scales and applying appropriate techniques used in different disciplines. This figure is the result of a knowledge consolidation exercise through expert discussions during the joint workshop that was held between June 2018 and February 2019 in Stuttgart. Accordingly, the instruments reported in black font were employed in our pilot experiment as a contribution to this review paper.

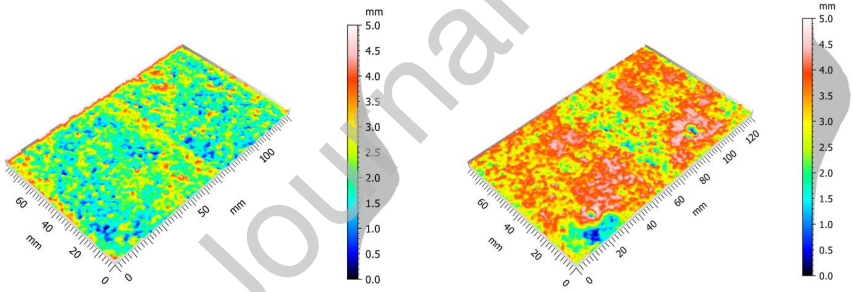
(A)



(B)

21-day biofilm
AR height: 0.53 mm
RMS height: 0.69 mm
Skewness = -0.03
Autocorrelation = 3.33 mm

90-day biofilm
AR height: 0.63 mm
RMS height: 0.81 mm
Skewness = -0.14
Autocorrelation = 7.78 mm



(C)

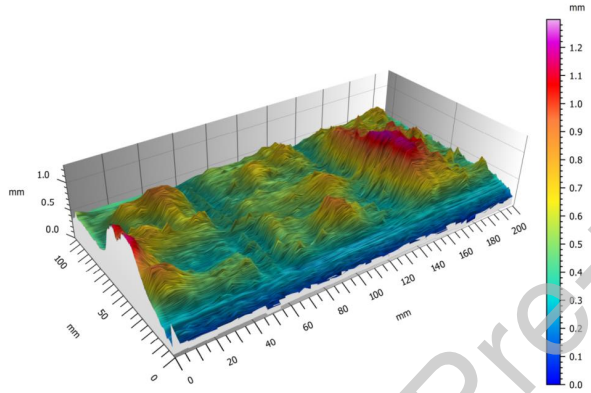


Fig. 2: Comparison of biofilm growth at different stages of development in the pilot experiment. (A) Young biofilm surface (black line) exhibits isotropic properties (81%) without any directional properties while mature biofilm surface (red line) shows anisotropic properties (17%) with a preferred orientation along with the flow direction. (B) Biofilm topography changes over the weeks followed by light microscopy (LM). (C) Mountainous appearance of mature biofilm after 4-week growth scanned by laser triangulation (LS) system.

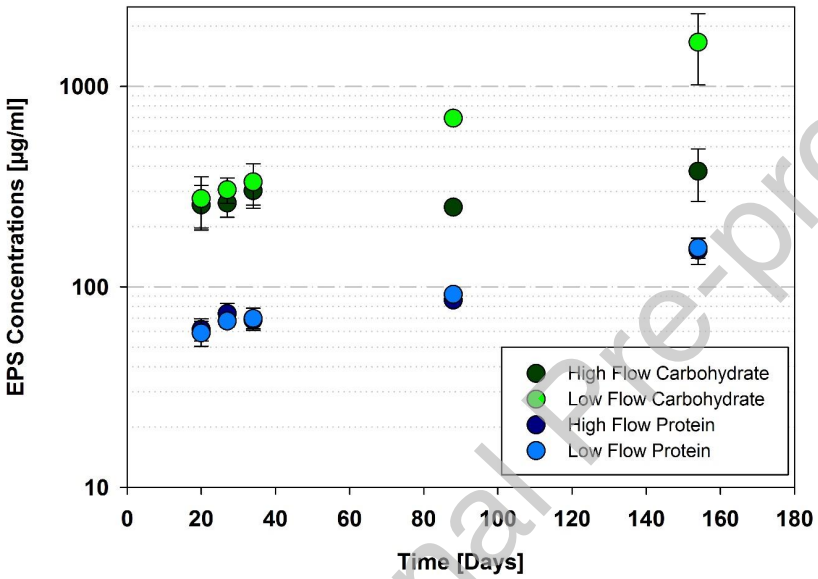


Fig. 3: Water-extractable (colloidal) extracellular polymeric substances EPS concentrations over time. Indicated are carbohydrates (green) and proteins (blue) at high (dark colours) and low (light colours) flow conditions.

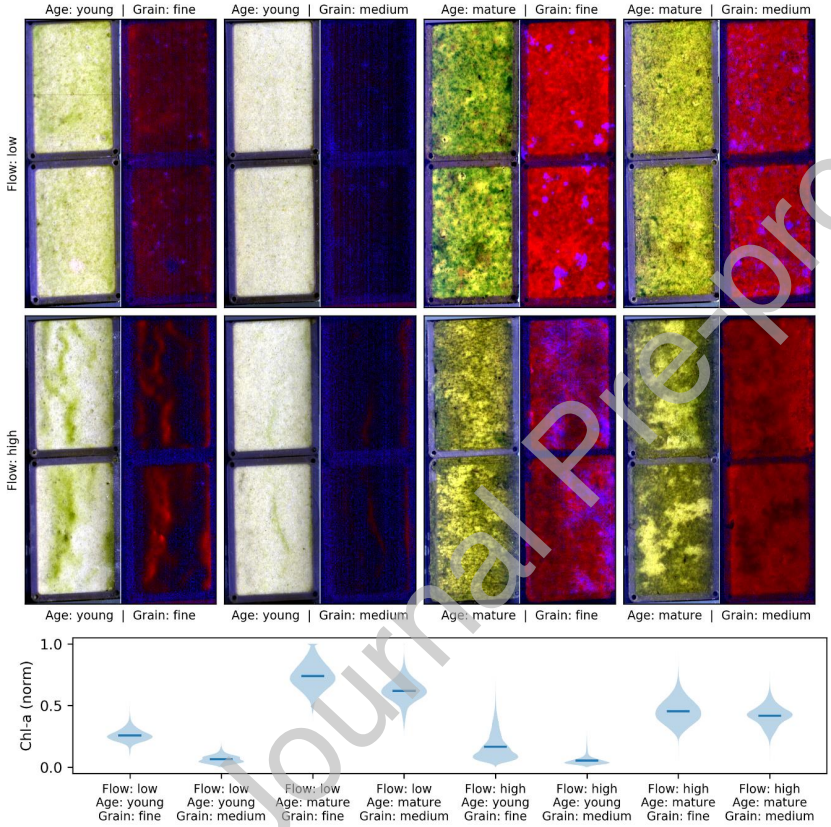


Fig. 4: Photopigment distributions derived from hyperspectral imaging. Monitored is the surface of sedimentary biofilms under various flow conditions, biofilm maturity (age) and sediment grain structure (see panel edge labels). Each condition shows the natural (true color) view of the surface alongside a false-color composite of the abundance of Chl a (in red) and Phycoerythrocyanin (in blue), with a common colormap scale for each pigment across all abundance maps. The Chl a abundance was calculated using the log-corrected MPBI centered at 675 nm (see reference in text: Chennu et al., 2013), and represents a proxy for photosynthetic biomass in the biofilm. Phycocyanin abundance was calculated using second derivative at 625 nm (see reference in text: Chennu et al., 2015a), but was not shown as it correlated completely with Chl-A map. The Phycoerythrocyanin abundance was calculated using second derivative at 575 nm, and was generally patchy across the surface but with higher values in the fine-grained sediment. The spatial patterns of Chl a was heterogeneous at mesoscales, but showed a directionality (perpendicular to flow) in young biofilms in medium-grained sediment under high flow velocities. The statistical distribution of the Chl a values from the ~1.2 million pixels in each abundance map is shown in the lower panel, indicating that age of the biofilm was the primary correlation to Chl a level, with values slightly lower for coarser sediments.

(A)

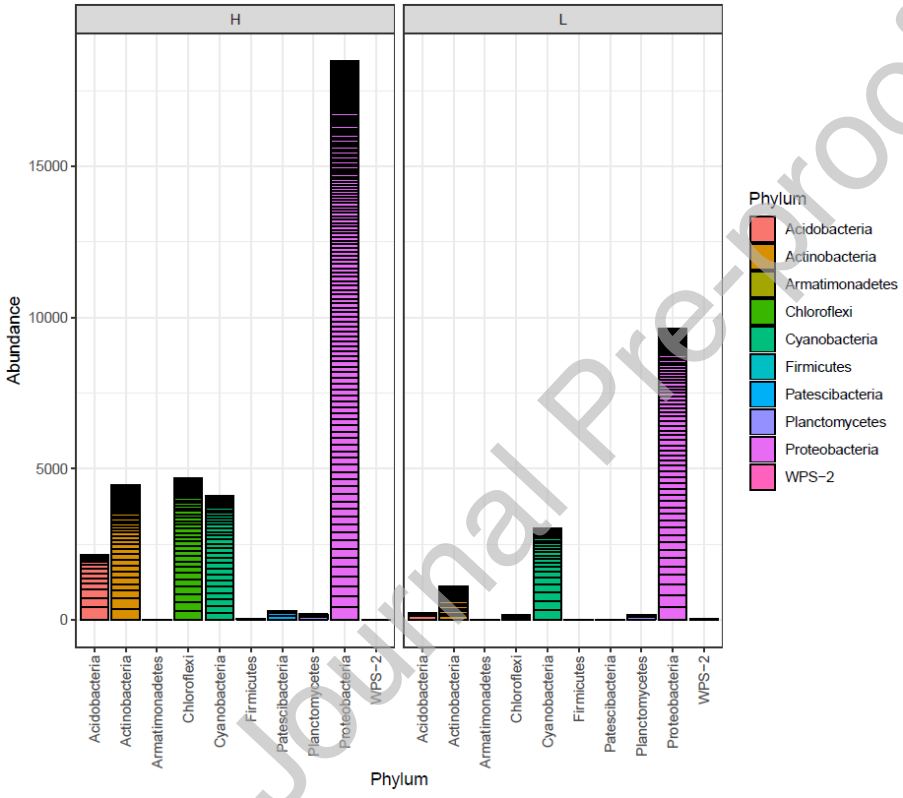
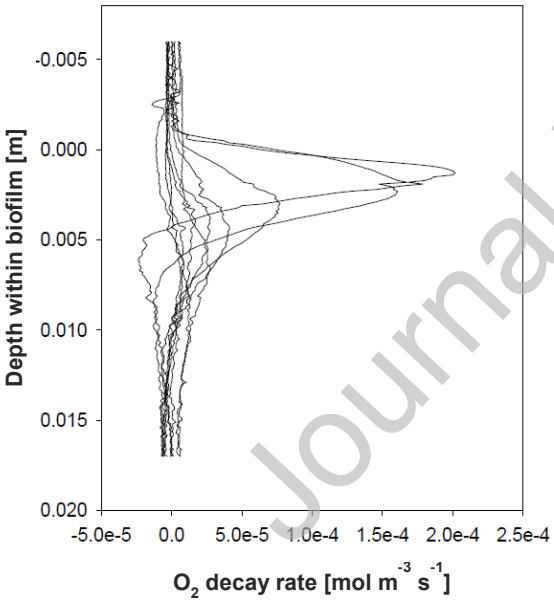


Fig. 5: Molecular Fingerprinting of the community composition. Next Generation Sequencing NGS of 16S rRNA (prokaryotic organisms). Groups of individuals that are genetically closely related are organized in OTUs (operational taxonomic units) for the high (=H) flow conditions (left) and the low (=L) flow conditions (right).

(A)



(B)

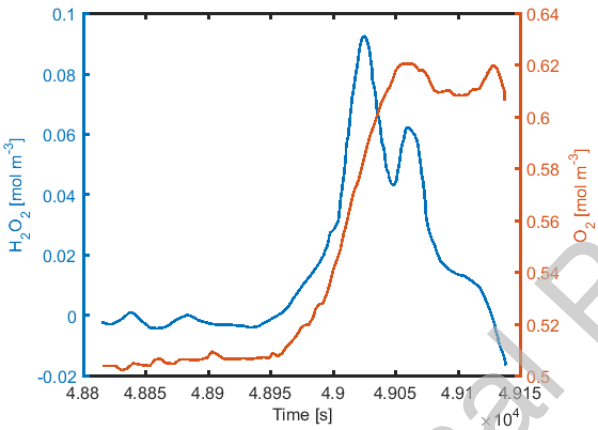
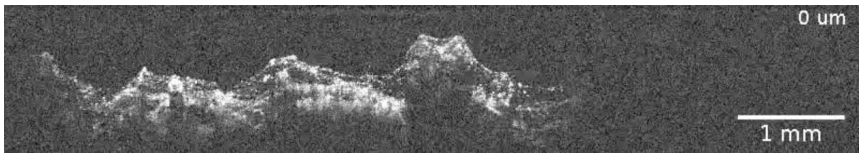
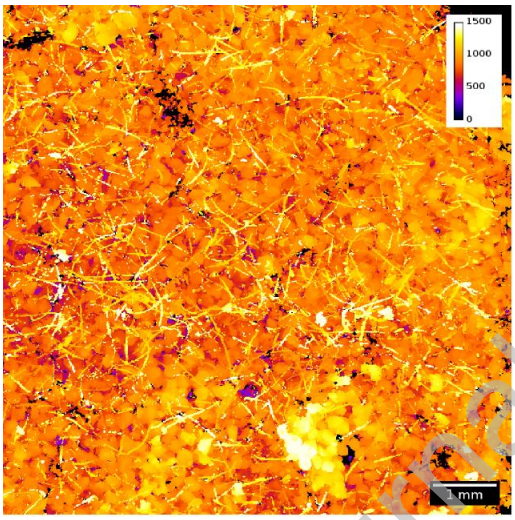


Fig. 6: Microsensor Profiles. (A) Oxygen respiration rates determined by subsequent oxygen profiles in transition from light to dark. (B) Hydrogen peroxide burst after touching a diatom colony with the sensor tip.





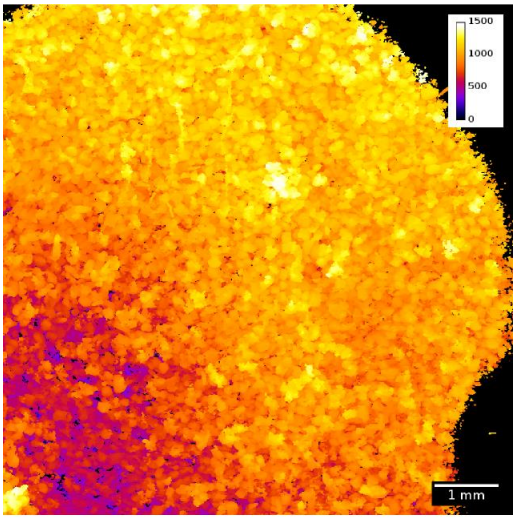


Fig. 7: Optical coherence tomography OCT images. Sand grains embedded by biofilm showing young growing biofilm with filamentous structure at low flow (above) and delayed attachment at high flow (below).

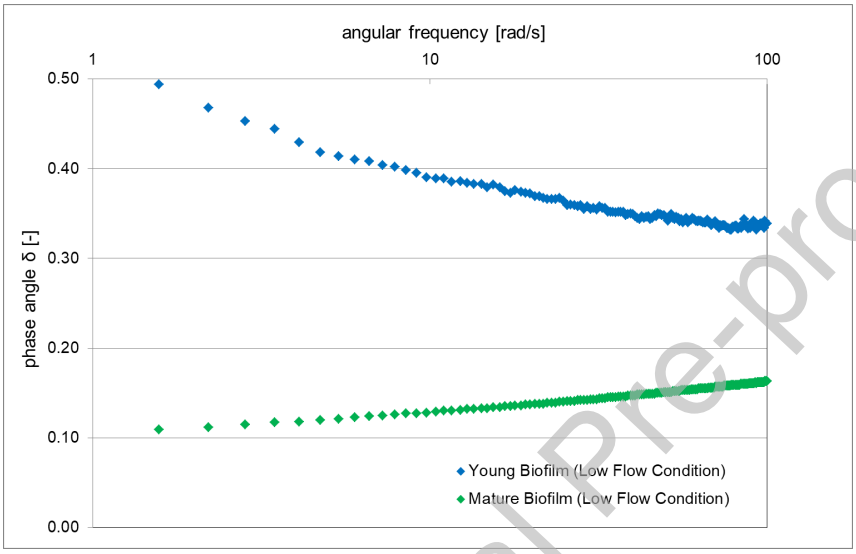


Fig. 8: Rheometer Measurements. Phase angles of the mature and young biofilm samples measured by rheometer under low flow conditions

Fig. 9: Erodibility tests. Biofilm-sediment complex is exposed to increasing shear stress in the erosion flume SETEG (Strömungskanal zur Ermittlung der tiefenabhängigen Erosionsstabilität von Gewässersedimenten). Left is the bare sand that acts as the control (critical shear stress: 0.3 Pa), right is the mature biofilm after 6 weeks of growth (critical shear stress: 12 Pa after failure at the edges). Please see also S1 and S2 videos in the supplementary material.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract