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Influence of Water Chemistry on the Formation and Stability of Microbial Biofilms in Natural Streams

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Abstract: Streams are dominated by microbial life that is present as microbial biofilms, which drive nutrient cycling and food-web support, but the influence of stream water chemistry on these microbial biofilms and on their physical stability at the catchment scale is not well resolved. This study was conducted for 24 reaches of the Bhavani and Noyyal river basin in southern India covering forest, agricultural and urban land use, to investigate the development of biofilm. The unglazed ceramic tiles were incubated for 28 days, and water samples were collected each week for pH, electrical conductivity, calcium, magnesium, dissolved organic carbon, nitrate, soluble reactive phosphorus, dissolved oxygen and temperature were measured. Biofilm ash-free dry mass, chlorophyll a, extracellular polymeric substances and bacterial density were quantified, while the stability was determined by measuring the percentage of biomass that remained after a standard shear was applied in a laboratory flume. Within the land-use groups, there was significant differences in water chemistry and a single principal component representing a gradient of enrichment and mineralization accounted for 88.4% of the chemical variance. Biofilm biomass showed a positive linear correlation with soluble reactive phosphorus ($R^2 = 0.90$) and the content of extracellular polymeric substance (EPS) was positively correlated with dissolved organic carbon ($R^2 = 0.90$). Biofilm stability increased from forested streams (49.2%) to urban streams (63.7%) and was saturating and correlated with dissolved calcium ($R^2 = 0.74$). Nutrient levels were thus the determining factor for amount of biofilm formed, while divalent cations and polymer production determined the strength of the attached biofilm. It is concluded that regular water-chemistry monitoring can be utilized to predict changes in the benthic microbial community structure, and to guide the protection and restoration of stream ecosystems.

Keywords: Periphyton; Extracellular Polymeric Substances; Divalent Cations; Nutrient Enrichment; Land Use; Ecosystem Health.

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I. Introduction

Streams and rivers are among the most biogeochemically active ecosystems on Earth, and much of the biogeochemical activity is accomplished by microorganisms attached to submerged surfaces rather than by free-floating cells. Stream biofilms, also termed periphyton or epilithon, are complex assemblages of bacteria, archaea, algae, fungi and protists embedded in a self-produced matrix of extracellular polymeric substances (EPS) (Battin et al., 2016). They are deposited on stones, sediments, wood and leaves, and they provide the first encounter between the dissolved nutrients and organic carbon and contaminants and the living parts of the ecosystem. Biofilms have become recognized as an indicator and driver of stream ecosystem health due to their important role in controlling the uptake and transformation of nitrogen, phosphorus and carbon and because they form the basis of food webs that support invertebrates and fish (Battin et al., 2016). The matrix of the biofilm plays a key role in this ecological function. The polysaccharide-protein-lipid-extracellular DNA (EPS) is the major component of the organic matter in biofilms and is believed to contribute mechanical cohesion, adhesion to the substratum, nutrient sorption and desiccation, grazing resistance and protection against toxicants (Flemming & Wingender, 2010). The emergent properties generated in the matrix, such as spatial organization, resource capture and increased tolerance, have been reported to make biofilms an emergent form of microbial life as opposed to a passive conglomeration of cells (Flemming et al., 2016). The integrity of the matrix is the key to whether a community will stand or fail in running waters, where cells are constantly being stressed by hydraulic drag and must withstand it. The sequence of steps in the formation of a biofilm is broadly conserved, with a series of conditioning-film formation, reversible and then irreversible attachment, microcolony growth,

maturation and eventual detachment (Besemer, 2015; Hall-Stoodley et al., 2004). Physical and chemical environment is sensitive in each stage. The velocity of flow and the turbulence influence the delivery of cells and solutes to surfaces, the light and nutrients regulate growth of the phototrophic portion, and the nature of the water environment influences surface charge, polymer conformation, and the energy balance of attachment/detachment (Hall-Stoodley et al., 2004). Changes in biofilm formation have cascaded effects on whole ecosystem processes and have been observed in experimental stream mesocosms to have a strong influence on such processes as nutrient retention and respiration (Battin et al., 2003). Thus, water chemistry is a potential master variable for the structure of biofilms, but it has multiple effects. Inorganic nutrients, especially of phosphorus particularly in many tropical and subtropical streams, promote algal and bacterial growth and biomass accretion. The energy and carbon source for the heterotrophic bacteria that are the main producers of EPS is dissolved organic carbon (DOC). Studies on community assembly of biofilm along stream networks have indicated that local environmental conditions are also important in filtering the community as well as the pool of colonists from upstream (Besemer et al., 2012). Meanwhile, biofilms are exposed to chemical stressors, and are extensively applied as early-warning system since biofilms are sensitive to water quality changes (Sabater et al., 2007). Chemistry also plays a role in the functional maturity of the community as the enzymatic activity that remains in the polymeric matrix also evolves with the age of the biofilm and environmental conditions (Romaní et al., 2008). The composition of ions in the water may determine the strength of the biofilm, while the nutrients may determine the amount of biofilm produced. Ionic bridging between adjacent polysaccharide chains can strengthen the gel via a divalent cation, such as calcium (Ca^{2+}) or magnesium (Mg^{2+}), and bind to the negatively charged carboxyl groups. Model biofilms showed that the addition of calcium strongly improves the mechanical stability of the matrix (Körstgens et al., 2001). EC DNA also has roles in structural integrity and it has been shown to interact with cations and other matrix polymers (Das et al., 2013). Most of the mechanistic evidence is from laboratory systems and single-species biofilms, however, many of these are of clinical or engineering interest (Donlan, 2002). The potential effect of natural variation in concentrations of cations across a catchment on the stability of mixed-species stream biofilms is yet little studied in the field. This gap is important for ecosystem management. Rapidly developing catchments will increase nutrient load, organic carbon, conductivity, hardness and reduce dissolved oxygen levels through the simultaneous conversion of forest to agriculture and urban settlement. The increase in biofilm biomass with enrichment may lead to increased cation-driven stabilisation, which also makes the biofilm more difficult to remove with flooding, and thus leave behind thicker biofilms with altered metabolism, decreased habitat quality for grazers and increased retention of contaminants. On the other hand, a thin, nutrient-poor biofilm that can easily be detached can release more carbon downstream in the flushes of the soft forest stream. Therefore, it is important to differentiate between controls on formation and controls on stability to interpret biofilm responses to indicate ecosystem condition. The question was taken up by the present study in the tropical streams originating from the Western Ghats in the southern Indian peninsula where there are short horizontal distances with steep vertical gradients in land use.

The goals were: (i) to describe water chemistry in different reaches of streams that are forested, agricultural, and urban; (ii) to quantify the accumulation of biofilm (biomass, chlorophyll a, EPS, and bacterial density) on a standardized substratum in different reaches; (iii) to measure the mechanical stability of biofilm under controlled shear in different reaches; and (iv) to identify which water chemistry variables best explain the formation or stability of biofilm. We hypothesized that nutrient concentrations would be the major factors controlling biofilm accumulation while divalent cations and EPS concentrations would be the primary factors controlling the resistance to biofilm detachment.

II. Materials and Methods

2.1. Study Area and Site Selection

The study was conducted in Bhavani River basin and Noyyal river basin of the western part of Tamil Nadu, India. Both rivers rise in the monsoon forests of Nilgiri and Western Ghats ranges and run eastwards

through the interspersion of cropland and plantations and peri-urban settlements in Coimbatore city. This region is of tropical climate with rainfalls in the south-west monsoon season (June – September) and north-east monsoon season (October – December) with a relatively stable dry season. Twenty-four third- and fourth-order wadeable stream reaches were chosen from catchment land-use maps and field reconnaissance and divided into three groups of eight reaches: forested (more than 80% natural vegetation in the upstream catchment), agricultural (more than 50% cropland and plantations) and urban (more than 30% built-up area or receiving treated or untreated domestic effluent). The physical habitat of the reaches to be sampled was selected to be as similar as possible to eliminate physical habitat effects. At the time of sampling, all reaches were dominated by cobble and gravel substrates, had riffle–run sequence, mean wetted width from 4 to 11 m and mean depth from 0.2 to 0.5 m. A 50 m study reach was placed at each reach and the flow velocity in each reach was maintained at approximately 0.25 to 0.45 m s^{-1} by the positioning of the substrates in similar riffle locations. Field work was conducted during the post-monsoon dry season (January – March 2024) when discharge was relatively stable and the chances of flooding scouring during incubation were low.

2.2. Water Sampling and Chemical Analysis

Water samples were taken once a week for 28 days (five times per reach) at each reach. In situ measurements of temperature, pH, electrical conductivity (EC) and dissolved oxygen (DO) were made using an in situ multiparameter calibrated probe. Grab samples were obtained in acid-washed polyethylene bottles, stored on ice and filtered by pre-combusted 0.7 µm glass-fibre filters within six hours. The DOC was filtered, acidified, and measured using a total organic carbon analyser by high temperature catalytic oxidation. Nitrate-nitrogen (NO_3^--N) and soluble reactive phosphorus (SRP) were measured by cadmium reduction method and ascorbic acid molybdenum-blue method respectively, with a UV–visible spectrophotometer. Calcium and magnesium were measured using flame atomic absorption spectrophotometry after adding nitric acid. Reagent blanks, duplicate analysis of each 10th sample and certified reference standards were used for analytical quality check, and recoveries ranged from 94% to 106%. The five weekly values were averaged for each reach to get the mean exposure concentration at each reach for the incubation period.

2.3. Biofilm Colonisation and Sampling

Biofilms were grown on unglazed ceramic tiles (4.7 × 4.7 cm, area 22.1 cm^2) that offer a uniform rough surface which is inert and commonly used in stream periphyton studies. City blocks were placed on the streambed surface in riffles and the surface on which the tiles were attached was pre-cleaned, combusted and oriented upward with a parallel surface in the direction of the stream flow. Twenty-four tiles were deployed per reach and left to colonise for 28 days, which was long enough to allow mixed algal–bacterial communities to get to a mature stage in warm tropical streams. Six tiles per reach were removed after 7, 14 and 21 days to describe accrual and all other tiles were removed after 28 days for the main analyses. Tiles retrieved were put in individual sterile containers with filtered stream water, kept in a cool and dark place and processed within 8 hours.

2.4. Biofilm Structural Variables

The upper surface of each tile was removed of biofilm using a sterile nylon brush and scalpel, and the biofilm slurry was transferred to a known volume of filtered stream water, homogenised and divided into sub-samples. The gravimetric method was used to determine ash-free dry mass (AFDM) after drying at 60 °C and combustion at 500 °C for 4 h. Chlorophyll a extraction and estimation was done by using 90% buffered acetone for 24 hours in dark at 4 °C and using correction factors for phaeopigments. Bacteria were fixed with formaldehyde, sonicated, stained with 4′,6-diamidino-2-phenylindole (DAPI) and filtered onto black polycarbonate membranes, with 20 or more random fields counted per filter. Under gentle agitation (to reduce cell lysis), extracellular polymeric substances were extracted with a cation-exchange resin and the carbohydrate fraction was determined by the phenol–sulphuric acid method and expressed as glucose

equivalents. The EPS extract was analysed for protein content using a modified Lowry assay and the protein-to-carbohydrate ratio calculated. Each of the structural variables is reported per unit area of tile.

2.5. Biofilm Stability Assay

Mechanical stability was evaluated in the laboratory using a recirculating flume, with a rectangular test section (1.2 m long and 0.10 m wide), filled with water from the reach selected for the test. To simulate moderate spate conditions found in the study streams, three tiles were installed face-down in the flume bed at day-28, and subjected to a ramped flow rate until a bed shear stress of 0.5 Pa was achieved, with each tile corresponding to one reach in the flume. The velocity profiles were measured using an acoustic Doppler velocimeter and shear stress estimated. A shear stress holding time of 15 minutes was used for each tile. The biomass not removed by the filter from the flume water was collected after the filter and the biomass adhering to the tile was removed and determined as above. Stability was represented as the percentage of the initial AFDM remaining after shear exposure. Reach water was used in the flume to ensure that the ionic environment in the flume was the same as during growth.

2.6. Statistical Analysis

The number of replications (n) was 24 reach. Bacterial density and SRP were log-transformed if necessary for parametric tests and tests for normality of variables were performed using Shapiro–Wilk tests, as were tests for homogeneity of variance using Levene's test; untransformed means are reported. One-way analysis of variance (ANOVA) and Tukey's honestly significant difference test were used to determine differences between land-use groups. The nine chemical variables were standardised in order to summarise the main chemical gradient using principal component analysis (PCA) on the correlation matrix of the nine standardised chemical variables. Spearman rank correlations and least-squares regression were used to investigate the relationship between individual chemical variables and responses to biofilm. Many chemical variables co-varied significantly, so collinearity was checked using variance inflation factors, and the most parsimonious predictor of biofilm stability was determined using forward stepwise regression. A second-order polynomial was used to compare with a linear model of the relationship between stability and calcium, using the adjusted coefficient of determination and also an F test for the quadratic term. All calculations were done in R version 4.3 at significance level $\alpha = 0.05$.

III. Results

3.1. Water Chemistry Across the Land-Use Gradient

The water chemistry in streams varied significantly among the three land-use categories and all nine measured streams had significant group effects (one-way ANOVA, $p < 0.001$ for all; Table 1). The electrical conductivity increased by almost 8 folds from forested reaches (98.7 ± 14.3 µS cm^{-1}) to urban (773.3 ± 99.2 µS cm^{-1}) reaches, and calcium and magnesium also increased similarly from 9.9 to 52.2 mg L^{-1} and 3.5 to 18.9 mg L^{-1} respectively. Nutrient concentrations were similar. The concentrations of soluble reactive phosphorus in the forested reaches were 6.5 µg L^{-1} but increased to 46.3 µg L^{-1} in agricultural reaches and 101.9 µg L^{-1} in urban reaches, while nitrogen in the form of nitrate increased from 0.23 to 3.64 mg L^{-1}. The ratio of DOC for urban streams to forested streams was about 4:1. In contrast, dissolved oxygen decreased in forested reaches and increased in urban reaches (4.8 mg L-1 in urban to 8.3 mg L-1 in forested), and water temperature was 2.4 to 3.6 °C higher in agricultural and urban reaches, due to less riparian shading and effluent inputs.

The PCA showed the chemical variables were clustered together on one major gradient. The first principal component (PC1) accounted for 88.4 % of the chemical variance, where the loadings of pH, EC, calcium, magnesium, DOC, nitrate, SRP and temperature were almost equal and positive (0.30-0.34), and the dissolved oxygen was negative (−0.34). Only 3.7% of the variance was accounted for by the second component. The PC1 gradient was distinctively occupied by forested, agricultural and urban sites

respectively, showing that the gradient was a mixture of enrichment and mineralisation caused by land use, not separate gradients in each of the solutes.

Table 1: Water Chemistry and Biofilm Characteristics (Day 28) in Forested, Agricultural and Urban Stream Reaches (Mean ± sd, n = 8 Reaches Per Group). All Variables Differed Significantly Among Groups (One-Way Anova, $p < 0.001$)

Variable	Unit	Forested	Agricultural	Urban
Water chemistry				
pH	–	7.12 ± 0.11	7.59 ± 0.19	7.99 ± 0.30
Electrical conductivity	µS cm^{-1}	98.7 ± 14.3	332.8 ± 56.4	773.3 ± 99.2
Calcium (Ca^{2+})	mg L^{-1}	9.9 ± 1.2	31.8 ± 4.1	52.2 ± 8.4
Magnesium (Mg^{2+})	mg L^{-1}	3.5 ± 0.6	11.2 ± 2.1	18.9 ± 2.9
Dissolved organic carbon	mg L^{-1}	2.07 ± 0.43	4.41 ± 0.94	9.27 ± 1.22
Nitrate-nitrogen	mg L^{-1}	0.23 ± 0.05	1.61 ± 0.32	3.64 ± 0.65
Soluble reactive phosphorus	µg L^{-1}	6.5 ± 2.1	46.3 ± 10.6	101.9 ± 21.5
Dissolved oxygen	mg L^{-1}	8.27 ± 0.30	7.06 ± 0.46	4.79 ± 0.51
Water temperature	°C	22.7 ± 0.7	25.1 ± 0.7	26.3 ± 1.0
Biofilm characteristics				
Ash-free dry mass	mg cm^{-2}	1.42 ± 0.28	2.78 ± 0.36	4.65 ± 0.59
Chlorophyll a	µg cm^{-2}	1.36 ± 0.91	3.91 ± 0.98	6.53 ± 0.96
EPS carbohydrate	µg glucose eq. cm^{-2}	10.2 ± 1.4	19.8 ± 2.0	31.5 ± 3.0
Bacterial density	× 10^8 cells cm^{-2}	2.43 ± 0.31	3.98 ± 0.67	9.02 ± 1.89
Biomass retained after shear	%	49.2 ± 3.8	60.7 ± 4.9	63.7 ± 4.4

EPS, extracellular polymeric substances. Stability was measured as the percentage of ash-free dry mass retained after 15 min at 0.5 Pa bed shear stress.

3.2. Biofilm Formation

Biofilm accrual continued in all reaches during the 28-day incubation, and differed with respect to both rate and amount of accretion depending upon land use. At day 28, AFDM was 1.42 ± 0.28 mg cm^{-2} in forested reaches, 2.78 ± 0.36 mg cm^{-2} in agricultural reaches and 4.65 ± 0.59 mg cm^{-2} in urban reaches ($F = 111.8$, $p < 0.001$), and all three groups differed significantly from one another. Chlorophyll a showed a nearly fivefold increase along the same gradient ($F = 58.7$, $p < 0.001$), and bacterial density increased from 2.4 × 10^8 to 9.0 × 10^8 cells cm^{-2} ($F = 69.6$, $p < 0.001$). The most sensitive measured structural variable was the EPS carbohydrate content, which rose by threefold (10.2 to 31.5 µg glucose equivalents cm^{-2}) ($F = 180.9$, $p < 0.001$; Table 1). Chemical predictors of biofilm biomass were quite weak as single predictors with the strongest being SRP (silicorated phosphorus). Day-28 AFDM increased linearly with SRP (AFDM = 1.31 + 0.032 × SRP; $R^2 = 0.90$, $p < 0.001$; Figure 1a), and chlorophyll a showed a similarly strong linear association with SRP ($R^2 = 0.90$, $p < 0.001$). The best correlation was obtained between EPS and DOC (EPS = 6.26 + 2.72 × DOC; R2 = 0.90; $p < 0.001$), which fits with heterotrophic bacteria acting as the source of ECP for polymer production. There was a positive relationship between the density of bacteria and both SRP and DOC. Therefore, the nutrients and organic carbon supply variables were most strongly correlated with the variables used to describe the amount of biofilm formed.

3.3. Biofilm Stability

There were also significant differences in the stability of the biofilms between land-use groups ($F = 23.9$, $p < 0.001$), where the percentage of biomass remaining after a shear of 0.5 Pa was calculated. The mean biomass retention of biofilms in forested reaches was 49.2 ± 3.8%, whereas the mean biomass retention in agricultural and urban reaches was 60.7 ± 4.9% and 63.7 ± 4.4%, respectively. The results of Tukey post hoc comparisons indicated that there were significant differences between forested biofilms and agricultural biofilms ($p < 0.001$) and between forested biofilms and urban biofilms ($p < 0.001$), but there

were no significant differences between agricultural and urban biofilms. The forested and agricultural reaches then became very stable, with little change in biomass, nutrients and EPS beyond that point, even though biomass, nutrients and EPS increased throughout the urban group. Stability correlated positively with calcium ($\rho = 0.78$, $p < 0.001$), pH ($\rho = 0.74$), magnesium ($\rho = 0.73$), EC ($\rho = 0.67$), SRP ($\rho = 0.66$) and DOC ($\rho = 0.65$), and negatively with dissolved oxygen ($\rho = -0.70$). Because of the high correlation among the chemical variables, forward stepwise regression only found calcium to be a significant predictor of stability in a linear model, accounting for 69% of the variance ($p < 0.001$). A second order polynomial provided the best fit ($R^2 = 0.74$, $p < 0.001$; Figure 1b), and showed a saturating response with stability grasping rapidly at low calcium concentrations and leveled off at approximately 66% retention at near 65 mg L^{-1} of calcium. Stability was also positively correlated with the EPS carbohydrate content ($r = 0.74$, $R^2 = 0.55$, $p < 0.001$), but this correlation was less than that with calcium, and the correlation between conductivity and stability ($R^2 = 0.48$) was smaller, indicating that the effect of ionic strength was primarily on divalent cations rather than total dissolved solids.

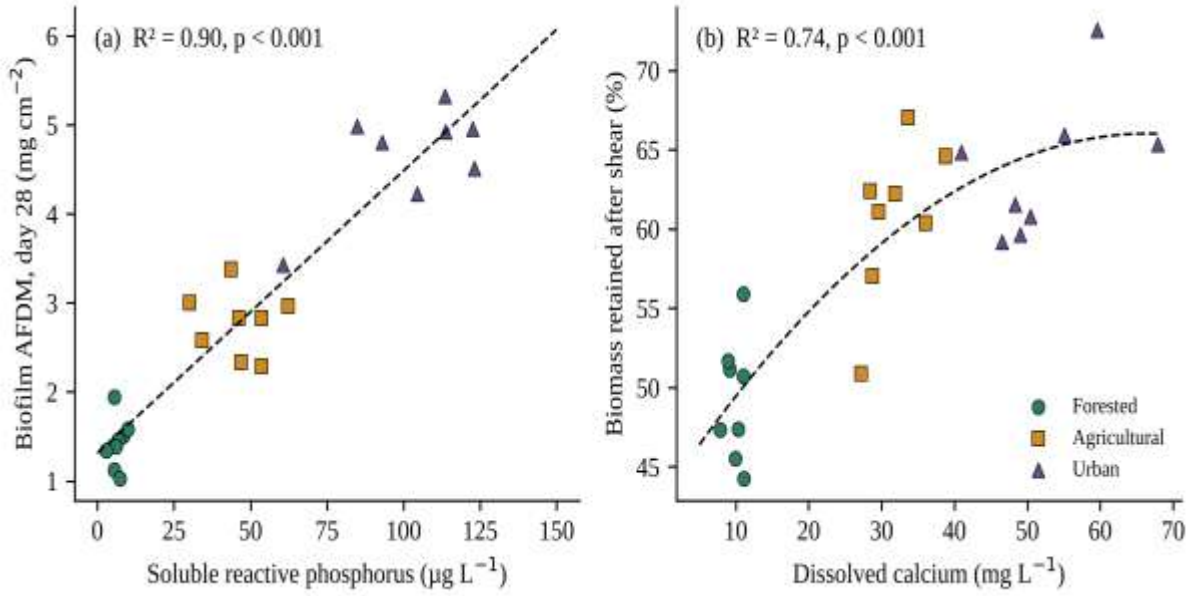


Figure 1: Relationships Between Stream Water Chemistry and Biofilm Properties Across 24 Reaches. (a) Day-28 Biofilm Ash-Free Dry Mass (AFDM) Versus Soluble Reactive Phosphorus, with Linear Fit. (b) Biofilm Stability (Percentage of Biomass Retained After 0.5 PA Shear) Versus Dissolved Calcium, with Second-Order Polynomial Fit Showing Saturation Near 65 mg l^{-1}

IV. Discussion

4.1. Nutrients and Organic Carbon Govern Biofilm Formation

Phosphorus was the main resource limiting biofilm buildup in the study streams, as shown by the strong linear relationships between SRP and biomass and with chlorophyll a. This is in line with the consensus that the nutrient response of the phototrophic and heterotrophic parts of stream biofilms is rapid, and that the biomass of stream biofilms reflects nutrient conditions over a span of days to weeks (Battin et al., 2016; Besemer, 2015). Forested streams naturally had low levels of phosphorus and even moderate agricultural inputs were enough to double biomass. The trend of increasing urban reaches (SRP>100ugL-1) indicates that biofilm building was not complete for the range of phosphorus concentrations sampled and this has implications for the management of eutrophication in downstream reservoirs that draw water from such urban basin. The intimate association of EPS and DOC provides a heterotrophic aspect to this image. Bacteria are the main producers of matrix polysaccharides and their EPS production is frequently stimulated

when there is an excess of carbon compared to other nutrients (Flemming & Wingender, 2010). The matrix polymers in the urban sections were presumably abundant because of the large source of labile organic carbon in the form of domestic effluent, and because of this, the bacterial density there was also concentrated. The EPS content of urban biofilms is higher than in older ones and as the resource environment changes as biofilms mature, this may be attributed to an additional larger community, but also to a more heterotrophic community. Such an interpretation is supported by both the evidence that the composition and function of biofilms in streams is shaped by local environmental conditions Besemer et al., (2012) and by the evidence of the mesocosms that changes in biofilm structure lead to changes in ecosystem processes (Battin et al., 2003).

4.2. Divalent Cations Govern Biofilm Stability

The key discovery of this study was that the chemical factors that influence the stability of the biofilm were in part different from those that influence the formation of the biofilm. The shear resistance was most closely predicted by calcium and the relationship was saturating; that is, most of the increase in shear resistance took place in the soft forested waters and the moderately hard agricultural waters. This is similar to the cation-bridging mechanism where Ca^{2+} ions bind to adjacent matrix polymers that have negative groups, which leads to greater cohesion and elastic strength. In model biofilms, calcium has been found to greatly enhance the mechanical stability of the matrix Körstgens et al., (2001) and in natural, mixed-species stream biofilms it has been found to be significantly effective over a realistic range of water hardness (field data). These curves indicate that saturation occurs at around 50-65 mg L^{-1} calcium, indicating that at levels beyond this range, further increases in calcium have minimal effect on stability. In addition, it describes the reasons for the lack of significant differences in the stability of the agricultural and urban biofilms despite very different biomass and EPS. Beyond this point, other factors may be limiting. Substratum attachment might be compromised in thicker biofilms with lower internal oxygen levels, counteracting cohesive benefits of a cation-rich matrix, to form anoxic basal layers. In addition, the extracellular DNA and proteins play a role in matrix integrity and interact with divalent cations Das et al., (2013) and Donlan, (2002) that can influence the efficiency of divalent cation bridging at high enrichments. Detachment is a natural occurrence in the life cycle of a biofilm Hall-Stoodley et al., (2004) and the strength of the cohesive forces and the intensity of growth, sloughing and grazing events dictate the amount of biomass remaining. The less close the approach to calcium with respect to stability, the more important it is that the ions present in solution are the ones and not the total ionic strength. Sodium, a monovalent ion, is added to the effluent and can bind to matrix sites but may result in a weakening of the matrix. Difficulty in separating individual ion effects in the field is a consequence of co-variation of individual ions along land-use gradients; the single principal component indicates this. Experiments using manipulative techniques that would adjust only the calcium and not the nutrients or conductivity in stream-side channels would be a valuable next step.

4.3. Implications for Stream Ecosystem Health and Management

The separation between formation and stability has important implications. biofilms are thicker and more difficult to remove in enriched and hard-water streams thus have a greater chance of surviving moderate spates. Streambed surface and interstitial habitat quality can be impacted by persistent thick biofilms, oxygen dynamics can be changed at the streambed, and sorbed metals, pesticides and other contaminants can be held longer. Biofilms are sensitive and adaptable to time-integrated exposure to chemical stressors, making them good candidates for monitoring effects on biological communities (Sabater et al., 2007). Based on the results shown here, indicator programmes should also incorporate aspects of the stability of the biofloc or EPS rather than biomass or chlorophyll a as this is a different aspect of the chemical environment. Nutrient reduction is the most direct means of limiting excessive biofilm accumulation, but may not be sufficient to re-establish the thin and easily renewed biofilms that are characteristic of reference forested streams if water hardness is maintained high by weathering of catchment water or liming or effluent inputs. Therefore, the nutrient and ionic status should be taken into account when deciding restoration targets for biofilm condition. The presence of a strong single chemical gradient here also indicates that routine water-quality measurements, routinely collected by regulatory agencies, can

be valuable tools for predicting likely changes in the structure of benthic microbial communities and identify reaches where the communities are likely to change. This is a step towards linking traditional chemical monitoring with the ecosystem-based assessment, a key objective of integrated aquatic ecosystem management.

4.4. Limitations

There are a few caveats that must be mentioned. The study was performed in a single dry season and changing light, temperature and discharge throughout the seasons could affect the relative influence of chemical drivers. The use of artificial ceramic substrata standardised the surface properties but does not fully match the development of biofilm on natural stones. The flume assay used only one shear stress and the effect of larger shear stresses during floods could be different. Finally, the high collinearity among the chemical variables is typical of land use gradients and makes it difficult to be certain about the effects of individual solutes. Even with these caveats, the pattern is consistent across the 24 reaches and there is consensus with existing mechanisms that the conclusions are valid.

V. Conclusion

In this study it is demonstrated that the water chemistry affects stream biofilms in two ways that are partly independent of each other: formation and stability. Chemistry was found to be correlated to a single gradient of enrichment and mineralization for 24 tropical stream reaches across a gradient of land use (forested, agricultural and urban). Phosphorus supply was the primary factor controlling the amount of biofilm formed (biomass, chlorophyll a and bacterial density) and these increased linearly with soluble reactive phosphorus, with no evidence of saturation. The amount of extracellular polymeric substances (EPS) produced closely tracked the dissolved organic carbon (DOC) with an increasing heterotrophic contribution for enriched streams. The stability of the biofilm, on the other hand, was most closely correlated with dissolved calcium and followed a saturating curve. The resistance to shear rose rapidly from soft forested waters to moderately hard agricultural waters, but then flattened out, with no difference in stability between agricultural and urban biofilms, despite the many orders of magnitude difference in biomass. The findings confirm the hypothesis that nutrients control biofilm amount and divalent cations control the strength of the biofilm, through bridging of the matrix polymers. Results suggest that nutrient reductions are not enough to achieve reference biofilm conditions when water hardness is high, for the management of ecosystems. Stability/matrix measures must therefore be incorporated into the biofilm monitoring, in addition to biomass and chlorophyll a. The chemical drivers identified here are those that are routinely measured by water-quality agencies and therefore provide a practical guide for predicting change in benthic microbial communities and for water-quality restoration targets. These relationships should be examined further in different seasons and flood conditions, and by manipulative experiments, to differentiate the effects of individual ions in future.

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Conflict of Interest

The authors declare that there is no known conflict of interest existing which would affect the study results.

Data Availability

Data to support the findings of this study are available on request from the corresponding author.

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