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Effect of Temperature, Nutrients and Diuron on Freshwater River Biofilms: A Statistical Approach

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Abstract: The influence of riverine physico-chemical factors on overall physiological status and growth of river biofilms was established in a field data-based model. Two sampling stations were located in the intermediate and downstream watershed areas of the river Morcille (France). Water temperature, suspended matter (SM), dissolved organic carbon (DOC), nutrients (NH_4, NO_2, NO_3, PO_4, Si) and toxicant (herbicide diuron) concentrations in the river were used as independent variables for modeling their effect on biofilm photosynthetic (PS) yield and dry weight (dependent variables). Basis function of 5th degree polynomial to accommodate the non-linear associations between the dependent variable and each of the independent variables followed by multiple linear regression was applied to determine the two endpoints. Data from September 2008 to December 2011 were utilized for model development and 2011 data were used for model validation. Nutrients and DOC, rather than diuron had a significant influence ($p<0.05$) on PS yield and dry weight. This model, therefore, integrated the interaction between co-occurring physico-chemical factors and pollutant to understand the dynamics of biofilm growth.

Keywords: biofilm; diuron; temperature; nutrients; basis function; regression model

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1. Introduction

River biofilms are a layer of microorganisms including bacteria, algae, fungi and protozoans embedded in a complex polymer linked assemblage (extracellular polymeric substances) attached on solid surfaces in a stream. In such ecosystems, biofilms occur at the boundary between substrate and water column. Stream biofilms are sensitive indicators of environmental stress [1] since their development, with regard to structure, taxonomic diversity and function, is extremely reliant on physical and chemical environmental factors [2].

Microbial biofilm community characteristics are determined by (i) physicochemical conditions, such as nutrient availability [3], temperature [4], light regime [5], current flow velocities [6], legacy of pollutant exposure as well as (ii) biotic factors, such biological interactions between microorganisms or predation by grazers [7]. The effects of stressors on stream biofilms have been the objective of many studies that focused on structural endpoints, such as biodiversity and biofilm taxonomic composition and on functional endpoints, such as biomass growth and photosynthetic efficiency [1].

Before its ban in France in December 2008, the phenylurea herbicide diuron was commonly used in urban and agricultural environments causing acute contamination of surface water, predominantly in small streams draining wine-growing areas [8] . While several experimental studies have demonstrated the effects of diuron on structural and functional parameters of biofilm communities [9,10], those are generally difficult to detect *in situ* because of the complex interactions between physical factors and chemical pollutants. The establishment of cause-effect relationships can be facilitated by statistical models, which hold immense potential for quantitatively describing biofilm growth in presence or absence of toxicants as well as variation in temperature and nutrient levels [11].

Previously we developed a statistical model assessing effects of diuron on river biofilm community [12] from empirical data of [10]. Light intensity, temperature and nutrient were kept constant throughout the experimental study, hence changes in biofilm growth due to seasonal variations were not considered in our previous model. To bring new insights, we propose here a statistical model to assess how changes in temperature, nutrient levels and diuron concentrations affect stream biofilm dry weight (total weight of biofilm biomass except the water content) and photosynthetic (PS) yield (the fraction of light energy converted into biomass during photosynthesis) which are indicators of their overall growth and physiological status. This model integrates the interaction between pollutant concentration and the physicochemical parameters of the river environment leading to a better understanding of the dynamics of biofilm physiological status and growth.

2. Materials and Methods

This model has been developed based on published [13,14]and associated unpublished metadata from a field survey conducted from September 2008 to December 2011 in the river Morcille of France. Sampling details are given in the Supplementary Material. Biofilm growth was quantified by PS yield and dry weight of the biofilm (dependent variables) while water temperature, diuron concentration, nutrient concentrations, suspended matter (SM) and dissolved organic carbon (DOC) were considered as independent variables. The correlation coefficients between the predictor or independent variables were less than 0.8. Field survey data demonstrated non-linear relationships between variables. To reduce the effects of non-linearity, polynomial basis functions of 5th degree were introduced before fitting all of them into a linear regression model. The order of polynomial was decided based on forward selection procedure in which the model was successively fitted in increasing polynomial order and the significance of regression coefficients tested at each step of model fitting. The order was increased till the t -test for the highest order term was non-significant.

$$\boldsymbol{y(n) = \sum_{i=0}^{5}(a_i)x^i} \qquad \text{Eq. (1)}$$

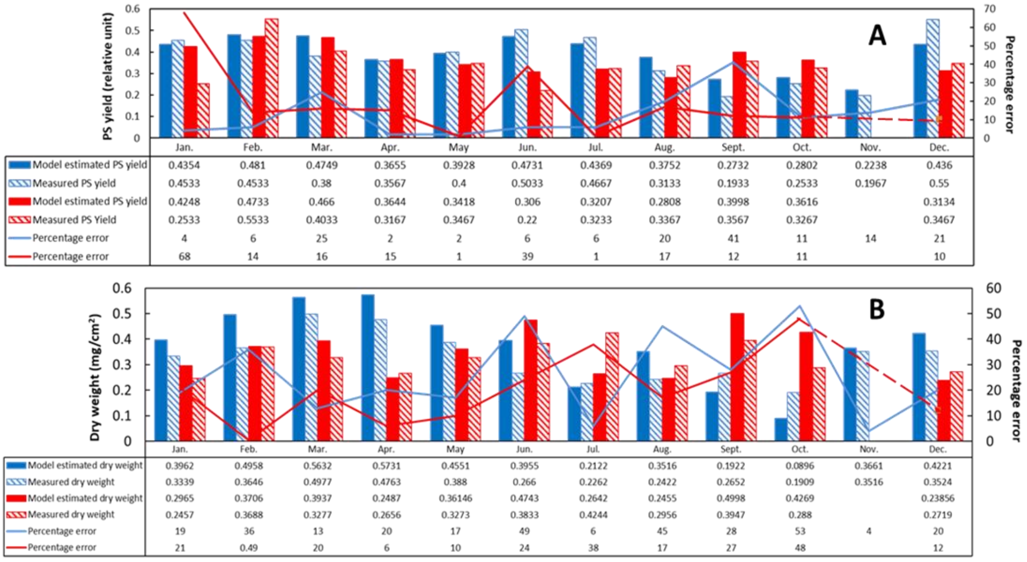
where y(n) represents dry weight or PS yield (dependent variable) and 'n' ranges from 1 to 9 for nine different independent variables, $\boldsymbol{a_i}$ is polynomial coefficients and $\boldsymbol{a_0}$ is the intercept. Basis functions for intermediate and downstream station are shown in Figures S1 to S4. Coefficients of these basis functions for dry weight and PS yield for the stations are listed in Table S1 and S2. Dry weight or PS yield as a function of nitrate [NO_3] concentration [y(1)], phosphate [PO_4] concentration [y(2)], silicon [Si] [y(3)], DOC [y(4)], SM concentrations [y(5)], ammonium [NH_4] concentration [y(6)], nitrite [NO_2] concentrations [y(7)], diuron concentration [y(8)] and temperature [y(9)] measured in intermediate and downstream stations were fitted into a multiple linear regression model (Equation 2) using Python 3.7.6 to obtain the net dry weight or PS yield (y).

$$\boldsymbol{y = A + B\,y(1) + C\,y(2) + D\,y(3) + E\,y(4) + Fy(5) + Gy(6) + Hy(7) + Iy(8) + Jy(9)} \qquad \text{Eq. (2)}$$

When dry weight of biofilm was considered as the dependent variable, coefficients of determination (R^2) of the multiple linear regression model for intermediate and downstream station data were 0.601 and 0.531 respectively. Considering PS yield of biofilm as the dependent variable, coefficients of determination (R^2) of the multiple linear regression model for intermediate and downstream station data were 0.714 and 0.629 respectively (Tables S3 to S6).

3. Results

The model was developed using data from September 2008 to December 2011 from the intermediate and downstream station. In the upstream station concentrations of diuron were mostly below detection limit of 0.001 µg/L and thus could not be used for model development. Model validations were done by comparing measured dry weight and PS yield from the field study and model estimated dry weight and PS yield for the year 2011 (Figure 1). The mean absolute percentage error (MAPE) between field measured and model estimated PS yield was 13.2% for intermediate and 18.5% for downstream station. MAPE between field measured and estimated dry weight was 25.8% for intermediate and 20.3% for downstream station. The t-test results indicated that nutrients (NO_3, NO_2, PO_4 and DOC) had a significant influence ($p<0.05$) on PS yield and dry weight (Table S3-S6). The coefficients of determination of the multiple linear regression of PS yield ranged from $R^2 = 0.63$-0.71 ($p<0.01$) and those of the dry weight ranged from $R^2 \approx 0.53$-0.60 ($p<0.01$) for the two sampled locations.



	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
🟦 Model estimated PS yield	0.4354	0.481	0.4749	0.3655	0.3928	0.4731	0.4369	0.3752	0.2732	0.2802	0.2238	0.436
▨ Measured PS yield	0.4533	0.4533	0.38	0.3567	0.4	0.5033	0.4667	0.3133	0.1933	0.2533	0.1967	0.55
🟥 Model estimated PS yield	0.4248	0.4733	0.466	0.3644	0.3418	0.306	0.3207	0.2808	0.3998	0.3616		0.3134
▨ Measured PS Yield	0.2533	0.5533	0.4033	0.3167	0.3467	0.22	0.3233	0.3367	0.3567	0.3267		0.3467
— Percentage error	4	6	25	2	2	6	6	20	41	11	14	21
— Percentage error	68	14	16	15	1	39	1	17	12	11		10

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
🟦 Model estimated dry weight	0.3962	0.4958	0.5632	0.5731	0.4551	0.3955	0.2122	0.3516	0.1922	0.0896	0.3661	0.4221
▨ Measured dry weight	0.3339	0.3646	0.4977	0.4763	0.388	0.266	0.2262	0.2422	0.2652	0.1909	0.3516	0.3524
🟥 Model estimated dry weight	0.2965	0.3706	0.3937	0.2487	0.36146	0.4743	0.2642	0.2455	0.4998	0.4269		0.23856
▨ Measured dry weight	0.2457	0.3688	0.3277	0.2656	0.3273	0.3833	0.4244	0.2956	0.3947	0.288		0.2719
— Percentage error	19	36	13	20	17	49	6	45	28	53	4	20
— Percentage error	21	0.49	20	6	10	24	38	17	27	48		12

Figure 1. Comparison of measured and model estimated (A) photosynthetic (PS) yield and (B) dry weight at intermediate (blue) and downstream (red) stations for the survey year 2011. Biofilms could not be collected in November 2011 in the downstream station due to major high flow event. The lines indicate error (as percentage) between the measured and model estimated PS yield and dry weight.

4. Discussion

4.1. Effect of temperature

The bacterial production and algal biomass of biofilm communities in temperate lotic ecosystems are mainly regulated by temperature and light intensity [15,16]. Experimental studies demonstrated that diuron bioaccumulation and resultant toxic impact on biofilms is controlled by temperature [17]. The elevated temperature during the summer can enhance bacterial development [18], whereas the lower temperature during winter can stimulate algal growth indicated by increase in chlorophyll *a* concentrations [6]. Although the daylight is reduced in winter, the biofilm communities of the Morcille river (which is bordered by many riparian trees) experience greater light intensity during this season compared to summer when the canopy cover is dense. Hence increase in light intensity due to loss of canopy cover during winter stimulated algal growth [6]. However, as per our model results, temperature did not have a significant influence on the biofilm growth in the two stations (Table S3-S6).

4.2. Effect of nutrients

Anthropogenic sources can increase nutrient loading, which may additionally affect the response of lotic ecosystem towards several stressors [19]. At the river Morcille an enhanced algal density from upstream to downstream was reported in winter, despite slight increase in diuron concentrations[2]. The increase in algal density could be explained partially due to the increase in nutrient concentrations. NH_4, NO_2, NO_3, PO_4 and DOC are the principal sources of heterotrophic nutrition. Nitrogen and phosphorous are nutrient supplements to autotrophic nutrition. Si is the main component for the formation of the diatom frustules. Our model shows significant ($p<0.05$) positive influence of NO_2, NO_3, PO_4 and DOC on the biofilm endpoints in the two stations (Table S3-S6). This is in accordance with previous observations in River Jauron (central France) where increased algal activity from August to November of 2003 were observed with concomitant increase in phosphate levels despite increased herbicide concentrations during September and October [20].

4.3. Effect of the diuron

Diuron can impact microalgal populations by lowering chlorophyll *a* levels and primary productivity and by altering community structure and species diversity [9,21]. Consequently, chronic diuron exposure can potentially inhibit growth of the lotic biofilm community [22]. During the study period, the average diuron concentrations in the two stations varied from 0.0047 μg/L to 3.2 μg/L. Within this concentration range neither of the measured functional endpoints of the biofilms were significantly affected by diuron (Table S3-S6).

4.4. Functional endpoints

Previous studies found that biofilm photosynthetic efficiency was the most sensitive indicator of stress compared to other endpoints such as biomass and chlorophyll *a* [23,24]. Our result matches these previous studies, where the coefficients of determination of the multiple linear regression of PS yield ($R^2 \approx 0.63$-0.71) is greater than the coefficients of determination of dry weight ($R^2 \approx 0.53$-0.60) for the two sampled location. Additionally, MAPE for PS yield determined for the two stations (13-18.5%) were less compared to the MAPE for dry weight (20-26%).

The primary factor determining light penetration, nutrient uptake as well as effect of toxicants [25] on such algal mats is the depth and thickness of these lotic biofilms. The effectiveness of photosynthesis is significantly influenced by the incident light. Dense biomass of these biofilms prevent uniform light transmission and dispersion at different depths, which linearly reduces the rate of photosynthetic activity as depth increases [26], thus affecting PS yield or dry weight. Additionally, nutrient distribution through the algal mat is uneven. Nutrient absorption by individual cells within the mat depends on its penetration depth. Even when sensing the same average nutrient concentration, individual cells in a biofilm may consume the nutrient at different rates [27]. In areas with strong positive curvature as opposed to flat areas, nutrients reach the deeper regions of the biofilm. Incorporation of biofilm thickness and light intensity into our model as independent variables, would enhance the precision of model-estimated dependent variables (PS yield or dry weight) .Unfortunately neither light intensity nor biofilm depth/thickness were measured during this field survey, obviating inclusion of these variables in the model. Nevertheless, flexibility of our developed model provides an opportunity for annexation of additional variables in future studies.

4.5. Model Significance

This type of modelling approach provides insight into the sensitivities of different biological endpoints towards nutrients and toxicants. Since growth and response of lotic biofilms depends on several factors, such multiple linear regression models are able to assess large number of variables and interrelations between them, and are therefore efficacious in defining biological processes. Our model shows that within the given diuron concentration limits used in the model the herbicide did not influence PS yield or the dry weight of the biofilms. Instead, the various nutrients and DOC played a significant role in enhancing biofilm growth (positive correlation) in the Morcille river. Such models can be used for any other toxicants in combination with other physical factors affecting biofilm growth and function.

5. Conclusions

The linear regression model explained 63-71% of the variance in the PS yield and 53-60% of the variance in the biomass dry weight due to the influence of the different dependent variables. Nutrients (NO_3, NO_2, PO_4) significantly contributed towards biofilm growth and PS yield. DOC, the nutrient for heterotrophic metabolism positively influenced dry weight in the intermediate station. Diuron in the modelled concentration range (maximum concentration 3.2 μg/L) did not affect the two functional end points. Therefore, our results all converge to reveal that this model can efficiently assess the effect of co-occurring factors on river biofilm community

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