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Relative effect of hydraulics, physico-chemistry and other biofilm algae on benthic cyanobacteria assemblages in a regulated river

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Highlights

- We investigated the response of benthic cyanobacteria assemblages to hydraulics, physico-chemical and biotic variables considering three sampling zones differing in terms of velocity variability according to discharge.
- In the studied regulated river, benthic cyanobacteria communities were dominated by potentially toxic genera (>50% of the biovolume).
- The development of cyanobacteria assemblages was limited spatially and temporally by the ecological preferences of genera and high-flow events.
- Warmer water temperatures in mid-summer and higher nutrient concentrations in the end of summer favoured the development of cyanobacteria assemblages.
- The development of Chlorophyta coincides with the decline of cyanobacteria suggesting potential competition between the two phyla.
- Discharges over 100 m$^3$.s$^{-1}$ only had a negative influence on cyanobacteria biovolumes when the flow variability was high for at least two weeks before sampling, and did not influence BC in late summer.
- Biofilm management through flushing flows of 100 m$^3$.s$^{-1}$ is not a viable solution to reduce biofilm development if it lasts less than one day.
Abstract
The development of benthic cyanobacteria currently raises concern worldwide because of their potential to produce toxins. As a result, understanding which measures of biotic and abiotic parameters influence the development of cyanobacterial assemblages is of great importance to guide management actions. In this study, we investigate the relative contributions of abiotic and biotic parameters that may drive the development of cyanobacterial assemblages in river biofilms. First, a 2D hydrodynamic model allowed us to retrace changes in depths and velocities according to discharge at a 4 m$^2$ resolution. From this model, we set up three hydraulic zones in each of the 4 reaches investigated along a 50-km-long river stretch. We further used univariate, multivariate and variance partitioning analyses to assess the contribution of past and present hydraulics, present physical and chemical parameters and algae to the temporal variability of cyanobacterial assemblage composition. The cyanobacteria assemblages were generally dominated by Phormidium sp., Lyngbya sp., Planktolyngbya sp. and Oscillatoria sp., four genera known to contain potentially toxic species. The highest biovolumes of cyanobacteria were present in low velocity zones in early summer and shifted to high velocity zones in late summer, highlighting the major influence of hydraulic parameters on benthic cyanobacteria settlement and development in rivers. Considering the identified genera, biofilms present a potentially high risk of toxin production. Relations between cyanobacterial development, toxin production and environmental parameters need to be further assessed to better estimate this risk.

Keywords:
benthic cyanobacteria; biofilm development; hydraulics; regulated river
1. Introduction

Cyanobacteria blooms have been rising worldwide for the last two decades. Benthic cyanobacteria (BC) are found in rivers and lakes (Mez et al., 1998; Vadeboncoeur et al., 2021). They have raised concern for the last two decades due to the ability of some species to produce cyanotoxins. These toxins can be harmful to aquatic and terrestrial organisms and may cause mammalian neurotoxicosis and death (Quiblier et al., 2013). For example, dog deaths have been reported to be caused by benthic cyanobacteria in New Zealand (Hamill, 2001; Wood et al., 2007), France (Cadel-Six et al., 2007; Gugger et al., 2005), Germany (Bauer et al., 2020), North America (Bouma-Gregson and Higgins, 2015; Puschner et al., 2008), the UK (Edwards et al., 1992) and the Netherlands (Faassen et al., 2012). The rise of intoxications due to BC has translated into an increasing bulk of studies in the last 5 years (Wood et al., 2020), especially focusing on the autecology of BC. While it is widely accepted that BC can have a large ecological niche (Quiblier et al., 2013), the environmental factors allowing their development in rivers are not fully understood and often limited to specific taxa or species. For example, *Phormidium* sp. and *Microcoleus* sp. have been extensively studied, but they may not represent the full diversity of existing development strategies in BC (Wood et al., 2020).

Although studies have proven that temperature and nutrient inputs are major factors acting on planktonic cyanobacteria (Paerl, 1988), the influence of these parameters seems more contrasted for river BC. In rivers, BC also usually develop under warmer water temperatures than the yearly average of the streams, i.e., exceeding 14 °C or 16 °C (in NZ and France, respectively; Echenique-Subiabre et al., 2018; Heath et al., 2015), but the link between their development and nutrient
concentrations is less clear. Studies conducted in eutrophic rivers have shown a preference of certain BC species for nutrient-rich environments (Loza et al., 2013), whereas in oligotrophic rivers, BC species still developed because they were able to use alternative sources of nutrients (McAllister, 2018; McAllister et al., 2018a). For example, Wood et al. (2015) showed that entrapped fine sediments could provide biofilm communities with dissolved reactive phosphorus concentrations on average 320 times higher than the concentration in the water column. In addition, the development of nitrogen-fixing cyanobacteria within biofilms is not inhibited in nutrient-poor environments (Douterelo et al., 2004; Perona et al., 1998). Considering the complex structure and exchanges occurring within biofilms, these nitrogen-fixing cyanobacteria could provide nutrients for other biofilm organisms (cyanobacteria and microalgae) and thus enable the development of complex biofilm communities even in oligotrophic waters (Stolz, 2000).

Hydrological conditions are also known to influence BC settlement and growth in rivers (Hart et al., 2013; Stevenson, 2009). On one side, high-flow events may remove BC (Heath et al., 2011). Wood et al. (2017) additionally stated that our ability to predict the magnitude of peak flows necessary to scour off Phormidium cover could vary among sampling sites. On the other side flow velocities represent one niche axis for BC species (McAllister et al., 2019, 2018b). However, varying responses to hydraulic constraints occur in the literature. Recent studies indicated that BC could develop at flow velocities ranging from 0.3 to 1.1 m.s\(^{-1}\) (Echenique-Subiabre et al., 2018; Heath et al., 2015). In contrast, other studies from low discharge rivers have shown that BC could develop with velocities <0.1 m.s\(^{-1}\) (Sabater et al., 2003). Finally, Thomson-Laing et al. (2021) found no relationship
between near-bed velocity and accrual rates of *Microcoleus autumnalis* mats. Substrate is also an important component, with large substrate sized (diameter between 5-26 cm) and stable substrate being more favourable to BC development (Echenique-Subiabre et al., 2018; Heath et al., 2015). Other physical parameters, such as light intensity, may also influence BC development. Using water depth as a proxy for received light intensity, Echenique-Subiabre et al. (2018) demonstrated that biofilms expanded widely under low light (when water depth increased), whereas BC thickness increased under high light to protect cells against ultraviolet radiation.

Finally, Wood et al. (2020) suggested potential competition between cyanobacteria and other benthic algae for the colonisation of the substrate. Indeed, cyanobacteria and filamentous green algae (Chlorophyta) can develop during biofilm maturation (see also, Barranguet et al., 2005; Battin et al., 2003; Besemer et al., 2007; Brasell et al., 2015; Majdi et al., 2012; Roeselers et al., 2007; Villanueva et al., 2011), making them potential competitors for space and nutrients necessary for their growth.

As a result, the above-listed BC ecological preferences make them more likely to grow during spring and summer. European climate scenarios predict an increase in water temperature and more variable rainfall patterns that will involve more frequent and intense low-water periods (IPCC, 2018), which might favour BC development (Codd et al., 2017). Such facilitation in development can be exacerbated in regulated rivers. This is the case for the Ain River, a 190 km-long tributary to the Rhône regulated by five dams (built on its main course between 1928 and 1968). During the summer season, this regulation involves long periods of low flow and occasional high-flow events to flush out algal biofilm. This regulated river
represents an appropriate experimental situation for conducting experiments assessing the relative contribution of biotic and abiotic factors to the development of cyanobacterial communities in river biofilms.

In this study, based on our literature review, we hypothesise that the relative contribution of hydraulics, physico-chemistry and the presence of other algae at explaining the composition of benthic cyanobacteria assemblages should vary according to the river regulation regime across summer. In particular, the development of cyanobacteria assemblages should be (i) limited spatially and temporally by hydraulic constraints both in terms of ecological preferences (high velocity, small and unstable substrate), and in terms of high-flow events; (ii) favoured by warmer water temperatures; (iii) weakly impacted by nutrient concentrations; and (iv) limited by intrabiofilm competition with other phyla, such as Chlorophyta.

2. Materials and methods

2.1. Sampling design

Four locations along a 50-km stretch of river, each comprising 270- to 500-m-long reaches, were selected using preliminary surveys of past occurrences of benthic cyanobacteria (SR3A Ain River Authorities; Table 1). Five sampling campaigns were performed during summer 2020 in four locations: 2-3 June (named early June), 29-30 June (late June), 15-16 July (mid-July), 27-28 July (late July) and 24-25 August (late August). At each location, three hydraulic zones (HZ) were delimited from maps resulting from a 2-D hydrodynamic model (INRAE/EDF). This 2-D hydrodynamic model was first used by Judes et al. (2022) for relating the
abundance of fish and invertebrates species to variations of water depth and velocity according to discharge variation. The model predicts mean water depth and velocity in each grid cell (resolution of 4m²) at 40% of the water depth measured from the bottom in the cell for various discharges. This enables the assessment of past local hydraulic variations of water depth and velocity. To extract values from the 2D-hydrodynamic model, we considered discharges ranging from 15 to 40 m³.s⁻¹, the most frequent discharge values during spring and summer on the Lower Ain River (i.e., this range had a frequency ≥ 50% calculated from May to October in the last 5 years according to two gauging stations). In addition, the threshold of 0.8 m.s⁻¹ was used in agreement with literature records to determine zones of velocities exceeding BC preferences (Wood et al., 2020). Finally, we delimited (i) HZ#1 experiencing velocities ≤0.8 m.s⁻¹ for flow between 20 m³.s⁻¹ and 40 m³.s⁻¹ and a low magnitude of variation of velocities with increasing discharges (<0.1 m.s⁻¹ for flow <20 m³.s⁻¹ remaining <0.2 m.s⁻¹ for flow between 20 m³.s⁻¹ and 40 m³.s⁻¹); (ii) HZ#2 experiencing velocity ≤0.8 m.s⁻¹ for a low flow of 15 m³.s⁻¹ and a high magnitude of variation of velocities with increasing discharges (<0.1 m.s⁻¹ for flow <20 m³.s⁻¹ going >0.2 m.s⁻¹ for flow between 20 m³.s⁻¹ and 40 m³.s⁻¹); and (iii) HZ#3 with velocities >0.8 m.s⁻¹ even for flows of 15 m³.s⁻¹ (Fig. 1). It should be noted that depth variations were similar for the three hydraulic zones for flow changes between 15 m³.s⁻¹ and 40 m³.s⁻¹. From the literature review (see introduction), HZ#1 was considered favourable to BC development for all discharge values, HZ#2 was considered favourable at low discharge values (<20 m³.s⁻¹), and HZ#3 was considered unfavourable at all discharge values. Stratified random sampling was further performed according to the surface of each hydraulic zone in the four locations. HZ#1 represented 38% of the surface of the sampled reaches, HZ#2 represented
50% and HZ#3 represented 12%. In coherence with the 2D-model design, units of 4 m² were used as sampling units. In total, 172 units were sampled over summer 2020 (65, 81, and 26 in HZ#1, HZ#2 and HZ#3, respectively, according to the surface of each hydraulic zone).

2.2. Field measurements

2.2.1. Benthic algae

Biofilm samples were collected from three cobbles randomly selected within each 4 m²-unit yielding a total of 516 biofilm samples. Both thin periphyton biofilms and thick macroscopic biofilms were sampled (Fig.2). Each sample was scrubbed, homogenised in river water, fixed with Lugol's iodine solution in the field and stored in the dark until microscopic identification in the laboratory. We delimited the area scrubbed on a given pebble using a piece of translucent paper. We further digitised each piece of translucent paper with a scanner and determined the scrubbed area with ImageJ software (Abràmoff et al., 2004).

2.2.2. Environmental variables

At each pebble, we measured the water depth (D in cm) with a meter stick and the average flow velocity of the water column (U in m.s⁻¹, measured at 40% of water depth from the pebble) with an electromagnetic current metre (FLO-MATE 2000, Cometec). At each 4 m²-unit, water temperature (WT), pH and conductivity (EC) were measured with a multiparameter probe (AQUAREAD AP-5000). Water
samples were collected to determine ammonium (NH4), nitrate (NO3) and orthophosphate (PO4) concentrations, following standard colorimetric methods (automatic analyser Smartchem 200, AMS Alliance, Roma, Italy). We estimated the percentage cover of substrate types (sand, gravel, cobble and boulder) and derived the mean grain size (GR). To take into account the recent hydrological changes in the river associated with hydropoaking events, we considered the past 15-day maximal discharge (Q15 in m³.s⁻¹) measured from two gauging stations maintained by the RMC Water Agency: Pont d’Ain (Station V2712010) for site PA and Chazey-sur-Ain (Station V2942010) for sites GI, BU and SJ (see Table 1 for acronyms).

2.2.3. Taxa identification and counting

Biofilm samples were homogenised before identification and enumeration. Subsampling allowed the identification and counting of all algal cells using a light microscope and a Malassez chamber. Photosynthetic communities were identified at the genus level following several guidelines (Bourrelly, 1985, 1970, 1968, 1966; Huber-Pestalozzi, 1968, 1961, 1955, 1941; John et al., 2002; Komárek and Anagnostidis, 2005). Counts were further grouped into phyla for diatoms (Bacillariophyta), Cyanobacteria and Chlorophyta, and less abundant taxa not included in these phyla were grouped as others. The biovolume (µm³.cm⁻²) of each phylum was calculated using PHYTOBS (2021) and ImageJ software (Rasband, 1997), which allowed scaling biovolume values to the pebble surface.

2.3. Data analysis
To compare each hydraulic variable between HZ and each physical and chemical variable between campaigns, we used a Kruskal–Wallis test followed by a Dunn’s test. The combined effect of campaigns and HZ on cyanobacteria biovolumes was investigated with two-way ANOVA and post-hoc Tukey test. Furthermore, to select drivers best explaining the composition of biofilm and cyanobacteria assemblages, we performed a redundancy analysis (RDA; van den Wollenberg, 1977) at each sampling campaign. RDA is a common multivariate technique in ecology that allows to explain variation in a set of response variables (e.g. cyanobacteria taxa) as a function of combinations of explanatory variables (e.g. physical and chemical variables) (ter Braak and Prentice, 1988). Prior to RDA, the variance inflation factor was used to detect multicollinearity between explanatory variables (Dodge, 2008). Only variables with a VIF<4 were included in the RDA models. Variables in RDA models were selected by permutation tests using a forward selection on the adjusted $R^2$. In addition, the hydraulic zone (HZ) was used as a conditioning variable in the model to assess its independent effect on cyanobacteria assemblages. The significance of global RDA, of the first-two RDA axes, and of each physical and chemical variable was further assessed by a permutation test. In addition, variance partitioning (VP) allowed us to quantify the amount of variance (by means of adjusted $R^2$ in RDA; Borcard et al., 1992) in the cyanobacteria biovolumes explained by each group of explanatory variables, i.e. the other biofilm algae (chlorophyta, diatoms, and other algae), hydraulic variables (Q15, U, D, GR) and physical and chemical variables (WT, pH, EC, NH4, NO3, PO4) without removing collinear variables as recommended by Oksanen et al. (2020). Biovolumes were log-transformed prior to statistical analysis to reduce numerical
discrepancies. All analyses were performed with R version 4.1.2 (R Core Team, 2021) using the package vegan (Oksanen et al., 2020).

3. Results

3.1. Environmental context

The first two campaigns occurred after a long period of high discharges >100 m³.s⁻¹ (maximum discharge = 224 and 117 m³.s⁻¹ in early and late June, respectively; Fig. 3). The early June campaign occurred 15 days after the last dam release event, while the late June campaign occurred only 5 days after the last dam release event. The two July campaigns were performed during low and stable flows (12 to 17 m³.s⁻¹). From late July to August, dam releases led to three flushing flow events (maximum discharge = 101 m³.s⁻¹, 99.3 m³.s⁻¹ and 97.9 m³.s⁻¹, respectively) in order to scour off algae and cyanobacteria. The latter flushing event occurred 3 days before the last sampling campaign (late August). As we could not measure flow velocities in the field for these high discharges, they were predicted using the 2D-hydrodynamic model (Fig.1). At 100 m³.s⁻¹, velocities ranged from 1.3 to 1.8 m.s⁻¹ in most of the river section, while at 200 m³.s⁻¹ velocity could reach 2.5 m.s⁻¹ in the middle of the river section.

The water velocity increased from HZ#1 to HZ#2 and HZ#3 as expected (Fig. 4; Kruskal–Wallis test, P<0.0001, n = 172). The depth was significantly lower in HZ#1 (Kruskal–Wallis test, P<0.0001), while the mean grain size was coarser in HZ#2 and HZ#3 than in HZ#1 (Kruskal–Wallis test, P<0.0001). NH4 concentrations were significantly lower in early June than in the other sampling campaigns and
significantly lower in late August than in mid-July (Table 2; Kruskal–Wallis test, \( P<0.0001 \)). The NO3 concentrations were the highest in early June and the lowest in mid-July (4.0 mg. L\(^{-1}\) and 1.4 mg. L\(^{-1}\), respectively; Kruskal–Wallis test, \( P<0.0001 \)), while concentrations in late June, July and August covered a similar range (i.e., between 2.3 and 2.8 mg.L\(^{-1}\)). PO4 concentrations decreased significantly between early and late June and reached the lowest values in mid-July (Kruskal–Wallis test, \( P<0.0001 \)). Finally, the water temperature was significantly higher in late July and lower in mid-July (Kruskal–Wallis test, \( P<0.0001 \)).

3.2. Biofilm composition

The mean cyanobacteria biovolume significantly differed over time and hydraulic zones (\( F_{8,157}= 5.36, p<0.0001 \)). It tended to decrease during the summer period, ranging from 4.33E+07 to 1.18E+07 \( \mu \text{m}^3.\text{cm}^{-2} \). Overall, cyanobacteria biovolume was the highest in June (maximum in late June with 55. E+07 \( \mu \text{m}^3.\text{cm}^{-2} \)) and the lowest in July (Tukey test, \( P<0.05 \)). All campaigns combined, biovolumes were significantly lower in HZ#1 than in HZ#2 and HZ#3 (Fig. 5; Tukey test, \( P<0.05 \)). Investigation of the combined effects of HZ and campaign revealed a high temporal variability of cyanobacteria biovolumes in HZ#1, with mean biovolumes being higher in the June and August campaigns than in the July campaigns (Tukey test, \( P<0.01 \)), while no significant differences appeared for HZ#2 and HZ#3. These low biovolumes in HZ#1 occurred during campaigns characterised by stable low flow (mid-July and late July).

A total of 23 genera of cyanobacteria were identified in the biofilm samples, 10 of which are known to comprise potentially toxic species such as *Anabaena* sp.,

(supplementary information; Table S1).

The 4 most abundant genera of cyanobacteria (mean relative contribution to total cyanobacteria biovolume >10%) included Lyngbya sp., Phormidium sp., Planktolyngbya sp., Oscillatoria sp. (17.2%, 29.4%, 20.2% and 17.2%, respectively).

These genera were also the most frequently found in the samples (occurrence >10%). They were present at each campaign, with the exception of Planktolyngbya sp. being absent in mid-July only. Indeed, Phormidium sp. was the major component of cyanobacteria biovolume (Fig. 6A). Its biovolume reached a maximum in mid-July and late July during the low flow period and was significantly at its lowest in June and August during periods of high flow variability. Phormidium sp. biovolumes were significantly lower in HZ#1 than in HZ#2 (Tukey test, \( P < 0.05 \)). Planktolyngbya sp. was the second major component of cyanobacteria biovolume (Fig. 6B); Planktolyngbya sp. contribution significantly decreased from June to July (from 3-5% to <0.4%) and was highest in late August (22.1%) after hydropeaking events.

Planktolyngbya sp. biovolumes were significantly lower in HZ#1 than in HZ#2 and HZ#3 (Tukey test, \( P < 0.01 \)). Lyngbya sp. was more prominent in early June than in July (Fig. 6C). During the low-flow period in mid-July, it was more abundant in HZ#3 than in HZ#1 and HZ#2 (Tukey test, \( P < 0.05 \)). Finally, Oscillatoria sp. did not exhibit any spatiotemporal trends in its biovolumes across the campaigns or HZ (Fig. 6D).

Diatoms dominated the total biofilm composition in June, while Chlorophyta increased greatly from mid-July (supplementary information, Table S2 and Fig. S1).

3.3. Drivers of benthic cyanobacteria biovolume
Abiotic and biotic variables used to investigate their influence for explaining cyanobacteria assemblages at each campaign are available in the supplementary information (Table S3). The overall variance of cyanobacteria biovolume explained by the RDA models ranged between 17.3 and 41.7%, (i.e. constrained models using the selection of significant variables), with the first canonical axis explaining between 16.0 and 25.7% of the total variance (Table 3). Past 15-day maximum discharge (Q15) and local hydraulic variables (D, depth; U, flow velocity; and GR, mean grain size) had a prominent influence throughout summer ($R^2$ ranging from 0.06 to 0.165). In addition, chlorophyta biovolume explained 11.5% of the cyanobacteria biovolume variance in mid-July. Finally, physical and chemical variables were mostly influential in late August, with NH4 and NO3 being responsible for 11.2 and 10.3% of cyanobacteria biovolume variance, respectively.

The amount of variance explained by each group of explanatory variables ranged between 20.9 and 32.9% (Figure 7). Hydraulic variables were the main source of variation in each campaign (variance explained ranged from 14.5% to 19.4%). Physico-chemistry and the biovolume of non-cyanobacteria algae represented a secondary source of variation. The biovolume of other algae was influential in late June and mid-July (14.0% and 10.5%). Physico-chemistry was a significant source of variation only in early June (13.4%).

4. Discussion

Our study showed that past flow and local hydraulic patterns mainly influenced the development of BC, as expected from our first hypothesis. However,
contrary to our first hypothesis, BC development was not limited by high water velocities, which facilitated the development of some genera. Only periods with high flow magnitudes and variations altered their development. However, large-sized and stable substrates facilitated cyanobacterial development, as expected from our first hypothesis. In agreement with our second hypothesis, warmer temperatures favoured BC development when water temperature was the highest. In contrast, nutrient concentrations had only a minor effect on BC, validating our third hypothesis. Finally, in agreement with our fourth hypothesis, a negative correlation between BC and other biofilm algae developments suggested intrabiofilm competition in at least two campaigns (mid-July and late July).

4.1. Hydraulic conditions

The total cyanobacterial biovolume was overall smaller in HZ#1, and most genera were positively related to high current velocity variation across discharge (HZ#2 and HZ#3). Among the four most abundant cyanobacteria genera, some occurred during stable flow periods (*Phormidium* sp. and *Oscillatoria* sp.), while some others occurred during periods of high flow variations (*Lyngbya* sp. and *Planktolyngbya* sp.). However, our study does not allow us to discuss in detail the ecological preferences of each genus despite scrutinising this pattern, which needs further investigation in the future. Our result showing that the most abundant cyanobacteria genera were associated with high velocities (HZ#2 and HZ#3) is somewhat in contradiction with the results of Sabater et al. (2003), who worked on a low discharge river but in agreement with studies conducted in active dynamic rivers (Bouma-Gregson et al., 2019; Echenique-Subiabre et al., 2018; Heath et al., 2015;
McAllister et al., 2019). This suggests that BC might be adapted to specific river habitats, with different communities from one river to another depending on their flowing regimes. In addition, as suggested by Wood et al. (2020), macroinvertebrate colonisation might be reduced in hydraulic zones with high velocities, which may limit grazers (see Hart et al., 2013 and McAllister et al., 2019 in Wood et al., 2020). This could enable biofilms and BC to develop at higher biovolumes at high velocities.

RDA and variance partitioning showed that hydraulic variables were the major drivers of BC biovolumes, and past flow and local hydraulic variables were influential at every campaign. High discharge events (>100 m$^3$.s$^{-1}$) decreased BC biovolume as expected (e.g. Heath et al., 2011; Wood et al., 2017) in early summer (early June and late June), whereas in late August, they did not induce any decrease in BC biovolume. According to Heath et al. (2011), BC development increases when the river discharge is below half the yearly average and water temperature increases. In the Ain River, all the sampling campaigns occurred for discharge below half the yearly average of 51.5 m$^3$.s$^{-1}$, but hydraulic conditions preceding sampling campaigns differed greatly between campaigns. Even though Q15 in late August was of similar magnitude to that in the June campaigns, it is possible that its short duration (less than a few hours) was not sufficient to induce a decrease in BC biovolumes. The high flow events of June presented a higher flow variation and duration, which might be a key to reduce BC development, along with a high discharge magnitude. Moreover, this suggests that during periods of favourable environmental conditions on already developed BC, high discharge events may not be as effective in decreasing BC biovolumes compared to early colonisation stages. This may be seen as counterintuitive since as benthic algal biomass increased, drag...
also increases, and high-flows can more easily remove biomass (e.g. Biggs et al., 1998). For example, in New Zealand benthic cyanobacteria decreased at 2-3x median/mean flows (Heath et al., 2011; Wood et al., 2017). Translated to the Ain River that has a yearly average discharge of 103 m$^3$.s$^{-1}$, this would mean that flushing flows with discharges from 206 to 309 m$^3$.s$^{-1}$ would be necessary to reduce BC development. In our study, the highest discharge event in late August was of ~ 100 m$^3$.s$^{-1}$ (close to the yearly average discharge). At this discharge value, flow velocities range from 1.3 to 1.8 m.s$^{-1}$ in most of the river section, which is slightly superior to previously recorded velocities for BC development (e.g. 1.1 m.s$^{-1}$; Heath et al., 2015) but might still be fitting their ecological niche. This discharge value may not be high enough to scour off benthic cyanobacteria, and higher discharged should be considered (for example, 200 m$^3$.s$^{-1}$).

Finally, a larger grain size facilitated the settlement of cyanobacteria, as expected. This result is in agreement with previous results that showed a larger BC coverage on stable substrates (cobbles and boulders), and BC coverage increased with grain size (Echenique-Subiabre et al., 2018; Heath et al., 2015).

4.2. Warming temperatures and nutrient concentrations

BC development occurred despite the oligo-mesotrophic water quality of the Ain River (according to nutrients thresholds provided by Loza et al., 2013), suggesting non-limiting nutrient concentrations. Our results showed evidence for nutrient concentrations influencing BC biovolume only in late August. According to the reviews of Quiblier et al. (2013) and Wood et al. (2020) on drivers of BC development, nutrients and other physical and chemical variables have been
reported to have an effect on BC in only a few studies. Moreover, their effect is reported to be more important during the implantation phase of BC, which seems consistent with our findings. Indeed, in our study, physical and chemical variables had an influence in late summer after flushing flows that could have greatly modified the ecosystem and led to a new colonization phase.

Nutrient concentrations only influenced BC biovolumes in late August: cyanobacteria biovolumes were influenced positively by NH4 and negatively by NO3. NH4 availability probably favoured BC, as NH4 is the most easily assimilated nitrogen source (Bryant, 1994). The fact that nutrient concentrations did not influence cyanobacteria biovolumes at other campaigns suggests that their development was not nutrient-limited in the Ain river. Indeed, important BC proliferations have been observed in very clear and oligotrophic rivers with lower NH4, NO3 and PO4 concentrations than those measured in the Ain river (McAllister, 2018; McAllister et al., 2018a). As nutrient uptake is faster in taxa with small cell sizes, such as cyanobacteria, it can explain their dominance in low-nutrient conditions (Reynolds, 1989). Moreover, the increase in BC development and biofilm thickness during summer might have allowed the uptake of phosphorus in sediments (Wood et al., 2015), independently from dissolved phosphate concentrations.

Water temperature positively influenced BC development in late July. At this campaign, the water temperature was the highest (20.9 ± 0.1 °C). This result is coherent with findings showing that BC development is associated with temperatures warmer than the seasonal average (>14 °C in New Zealand rivers, Heath et al., 2015; >16 °C in the Tarn River, France, Echenique-Subiabre et al., 2018).
4.3. Intrabiofilm competition

In mid and late July, the cyanobacterial assemblages were negatively correlated with those of other biofilm algae, suggesting a trend of intrabiofilm competition between BC and other benthic algae (diatoms, chlorophyta and other less abundant algae). This pattern could result from real spatial competition or from different environmental preferences. Indeed, low and stable flow associated to flow regulation might have allowed the fast development of other biofilm algae (mean discharge = 14.9 m$^3$.s$^{-1}$ and Q15 = 66.2 m$^3$.s$^{-1}$ in mid-July), along with an increase in NH4 concentrations, temperature and light availability. Under these conditions, we hypothesise that the development of Chlorophyta was faster than that of cyanobacteria and limited the growth of cyanobacteria. Indeed, under stable conditions, the Chlorophyta growth rate is higher than that of cyanobacteria under the same temperature conditions (Lürling et al., 2013). The growth of macroalgae might have caused a light limitation for cyanobacterial cells in the biofilm, with the filaments leading to shading (Dodds et al., 1999). However, cyanobacteria development continued in high velocity zones and deep water zones, suggesting that the competition with other algae for space and resources was more intense in zones with low and stable velocity (e.g. HZ#1). Indeed, Chlorophyta were the most prominent in HZ#1. Their development might have been limited by hydraulic constraints in June and late August, leaving space for cyanobacteria to grow. This would be coherent with findings showing that filamentous green algae have a low tolerance to shear stress (Biggs and Thomsen, 1995). This pattern could also result from the successional stages of biofilm development in river ecosystems. Biofilm
development generally starts with bacteria and diatoms (Azim and Asaeda, 2005; Stal, 2012, 1995), followed by filamentous algae, mostly Chlorophyta, as a late successional stage (Burns and Walker, 2000; Vadeboncoeur et al., 2021), which is consistent with our findings showing the growth of Chlorophyta and other algae overgrowing cyanobacteria in biofilms.

5. Outcome

This study allowed us to show the different environmental preferences of cyanobacteria genera. Even if our RDA models had a low explanatory power when constrained with the selection of significant variables, constrained models explained between 35.7 to 59.2% of the variance of BC biovolumes. This low explanatory power might be the result of the temporal variability in explanatory variables. Different variables influenced BC biovolumes over time, and the same variable could influence BC biovolumes differently at different campaigns, depending on the current combination of environmental conditions. This unexplained variance might also come from the number of genera implied in the model (23 genera), whose responses to environmental variables could differ, inducing more variability. Overall, a dominant pattern of environmental preferences was observed. Further analysis considering each genus could help to determine their specific preferences.

Currently, we were able to show that hydraulic variables prominently shaped BC assemblages in rivers, with varying responses over time. Discharges over 100 m$^3$.s$^{-1}$ only had a negative influence on cyanobacteria biovolumes when the flow variability was high for at least two weeks before sampling, and did not influence BC in late summer. Therefore, biofilm management through flushing flows of 100 m$^3$.s$^{-1}$
is not a viable solution to reduce biofilm development if it lasts less than one day. Higher discharge variability, or longer flushing flows, should be considered to truly affect BC development. Flushing flows of 200 m$^3$.s$^{-1}$ might be more effective for scouring off benthic cyanobacteria by exceeding their ecological preferences in terms of flow velocity. More importantly, discharge is a parameter that depends on the studied river, therefore more precise hydraulic indicators, such as flow velocity, should be used to make management recommendations. In light of our study, it appears that flushing flows should induce flow velocities reaching 1.3 to 2.5 m.s$^{-1}$ in order to scour off benthic cyanobacteria. Further investigations could enable the depiction of more precise hydraulic limitations to their development. Indeed, the results from the 2-D hydrodynamic model could be used more extensively by deriving fine hydraulic parameters from hourly discharge chronicles on the river during the sampling period. For example, the number of hours above or under a certain velocity value could be taken into account in a predictive model, as Judes et al. (2022) performed to describe past hydraulic variables in their study. This opens new perspectives in terms of biofilm management in regulated rivers in the future.

Similar to the recent study of Bauer et al. (2022) on benthic cyanobacteria, our biofilm samples contained a variety of cyanobacteria genera, with only a few dominant ones containing potentially toxic species. Among all identified cyanobacteria genera, 10 out of 23 included species known to produce cyanotoxins, and the four most abundant genera found in our samples are potentially toxic (Phormidium sp., Planktolyngbya sp., Lyngbya sp., Oscillatoria sp.). Cyanotoxins produced by these genera are mostly neurotoxic (Cadel-Six et al., 2007; Gugger et al., 2005; Lajeunesse et al., 2012; Puschner et al., 2008; Seifert et al., 2007; Wood
Therefore, BC development in the Ain River is associated with a high toxicity potential, even though genetic analyses or toxin extraction would be needed to better assess this toxic risk. Further analysis assessing toxin production by these biofilms would determine the risk associated with their development and, more importantly, determine the environmental parameters favouring cyanotoxin production.

**Declaration of competing interest**

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.

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**References**
Abrâmoff, M.D., Magelhaes, P.J., Ram, S.J., 2004. Image Processing with ImageJ.

Biophotonics International 11, 36–42.


https://doi.org/10.1079/9780851990965.0015


https://doi.org/10.1080/09670260400009882


https://doi.org/10.3390/toxins12110726


IPCC, 2018. Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas
emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty.


Table 1. Sampling site characteristics (PA=Pont d’Ain, BU=Buchin, GI= Giron, SJ=St Jean de Niost)

<table>
<thead>
<tr>
<th>Site</th>
<th>GPS coordinates (Lambert 93)</th>
<th>Distance to Allement dam (km)</th>
<th>Mean T°C (^1)</th>
<th>Median discharge ((m^3.s^{-1}) (^1)</th>
<th>Geomorphology (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>880109.5 ; 6552094.0</td>
<td>13.6</td>
<td>18.2 ± 3.0</td>
<td>14.5</td>
<td>Low lateral mobility, incised, paved</td>
</tr>
<tr>
<td>BU</td>
<td>873932.5 ; 6535675.0</td>
<td>35</td>
<td>18.9 ± 3.0</td>
<td>19.8</td>
<td>Low lateral mobility, stable channel</td>
</tr>
<tr>
<td>GI</td>
<td>872498.5 ; 6533919.5</td>
<td>38.6</td>
<td>18.9 ± 3.0</td>
<td>19.8</td>
<td>Low lateral mobility, stable channel</td>
</tr>
<tr>
<td>SJ</td>
<td>873516.0 ; 6529243.3</td>
<td>43.6</td>
<td>18.9 ± 3.0</td>
<td>19.8</td>
<td>Lateral mobility, dynamic channel</td>
</tr>
</tbody>
</table>

\(^1\) Calculated for the summer periods of the past 5 years (2014-2019, early June to late August)

\(^2\) From Rollet et al. (2014)
Table 2. Average values (n = 29; 30; 43; 40; 30; respectively) of physical and chemical variables measured at the five sampling campaigns in 2020 on the Lower Ain River.

<table>
<thead>
<tr>
<th></th>
<th>Discharge (m$^3$.s$^{-1}$)</th>
<th>Q15 (m$^3$.s$^{-1}$)</th>
<th>T°C</th>
<th>pH</th>
<th>NH4 (µg.L$^{-1}$)</th>
<th>NO3 (mg.L$^{-1}$)</th>
<th>PO4 (µg.L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early June</td>
<td>17.1</td>
<td>69.8</td>
<td>19.2 ± 1.8</td>
<td>8.1 ± 0.6</td>
<td>10.7 ± 4.3</td>
<td>4.0 ± 0.9</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td>Late June</td>
<td>20.1</td>
<td>124.4</td>
<td>19.5 ± 1.7</td>
<td>8.2 ± 0.1</td>
<td>28.2 ± 14.8</td>
<td>2.5 ± 0.9</td>
<td>3.5 ± 2.6</td>
</tr>
<tr>
<td>Mid-July</td>
<td>14.9</td>
<td>66.2</td>
<td>18.6 ± 0.8</td>
<td>8.1 ± 0.4</td>
<td>32.9 ± 14.6</td>
<td>1.4 ± 0.5</td>
<td>2.1 ± 2.0</td>
</tr>
<tr>
<td>Late July</td>
<td>13.5</td>
<td>45.9</td>
<td>20.9 ± 0.1</td>
<td>8.1 ± 0.3</td>
<td>29.7 ± 8.8</td>
<td>2.3 ± 0.7</td>
<td>4.1 ± 2.9</td>
</tr>
<tr>
<td>Late August</td>
<td>10.9</td>
<td>99.0</td>
<td>19.9 ± 1.2</td>
<td>8.3 ± 0.2</td>
<td>21.8 ± 10.5</td>
<td>2.8 ± 1.0</td>
<td>4.6 ± 3.0</td>
</tr>
</tbody>
</table>
Table 3. Evolution of the influence of biofilm algae (green), local hydraulics (light blue), past hydraulics (dark blue) and physico-chemical variables (pink) on the biovolume of 23 genera of cyanobacteria at 5 sampling campaigns in 2020 on the Ain River (RDA models) (GR, mean grain size; Q15, maximum discharge 15 days before sampling; D, water depth; U, flow velocity; Chloro, biovolume of Chlorophyta; Others, biovolume of other biofilm algae; NH4, ammonium concentration; NO3, nitrate concentration; EC, conductivity). Dot size is proportional to the score of each genus or each variable along the first RDA axis. Closed dots represent positive scores, open dots represent negative scores, and the absence of points shows the absence of a genus in samples in this campaign. For a given genus and a given variable, a similar dot filling indicates a similar positive effect of the variable on the genus (e.g., Q15 and Planktolyngbya in early June or GR and Phormidium in late June), whereas different dot filling (open vs. closed) indicates a negative effect of the variable on the genus (e.g., Q15 and Phormidium in late June). In this table, genera are separated into two groups according to their potential toxin production and are ordered according to their relative contribution to the total cyanobacteria biovolume, from the most to the least contributing genus. The percentage of variance explained by the conditional model, the constrained model, and the constrained model including only the selection of significant variables and the two RDA axes are indicated in the last five lines.
* model using the selection of significant variables

** statistically significant from a permutation test
**Figure 1.** Example of delimitation of the three hydraulic zones from a 2-D hydraulic model in the upstream site (PA). (A) Distribution of water velocity for a discharge of 15 m$^3$.s$^{-1}$ (light blue ≤0.8 m.s$^{-1}$, dark blue >0.8 m.s$^{-1}$), allowing us to identify HZ#3 in dark blue; (B) distribution of velocity variation for discharges between 15 and 20 m$^3$.s$^{-1}$ (light blue <0.1 m.s$^{-1}$, dark blue >0.1 m.s$^{-1}$), allowing us to identify HZ#1 and HZ#2 in light blue; (C) distribution of velocity variation for discharges between 20 and 40 m$^3$.s$^{-1}$ (light blue <0.2 m.s$^{-1}$, dark blue >0.2 m.s$^{-1}$), allowing us to discriminate HZ1 from HZ2; (D) selection of the final hydraulic zones with HZ#1 (light grey), HZ#2 (medium grey), and HZ#3 (dark grey). Water velocity predictions for discharges of 100 m$^3$.s$^{-1}$ (E) and 200 m$^3$.s$^{-1}$ (F) (yellow ≤0.8 m.s$^{-1}$, orange [0.8-1.3] m.s$^{-1}$, light red [1.3-1.8] m.s$^{-1}$, and dark red [1.8-2.5] m.s$^{-1}$).
Figure 2. Examples of sampling plots presenting mostly thin periphyton biofilms (A) and mostly thick macroscopic biofilms (B). Macroscopic biofilms were identified by their dark-green colour (C).
Figure 3. Hourly discharge and water temperature at the gauging station and multiparameter probe of Chazey-sur-Ain (RMC Water Agency, Station V2942010) during summer 2020. Vertical blue bars correspond to the 5 sampling campaigns. The absence of temperature data in late June was due to a probe malfunction.
Figure 4. Variation in mean current velocity (A), depth (B) and mean grain size (C) across the three HZs ( Significant Kruskal–Wallis tests and Dunn’s tests of multiple comparisons are shown with an asterisk).
Figure 5. Average biovolume of cyanobacteria collected at the 5 sampling campaigns separated by hydraulic zone (Significant Tukey tests of multiple comparisons are shown with an asterisk).
Figure 6. Mean relative contribution of the four most abundant genera (mean contribution > 15%) to the total biovolume in cyanobacteria at the 5 sampling campaigns and in each hydraulic zone: HZ#1 in light grey, HZ#2 in grey, HZ#3 in dark grey. (A) Phormidium sp., (B) Planktolyngbya sp., (C) Lyngbya sp., (D) Oscillatoria sp.
* statistically significant from a permutation test (P<0.05)

Figure 7. (A) Percentage of variance of the cyanobacteria biovolumes explained by the three groups of explanatory variables: other biofilm algae in green (chlorophyta, diatoms, and other algae), hydraulic variables in blue (Q15, U, D, GR) and physical and chemical variables in pink (WT, pH, EC, NH4, NO3, PO4). The residual variance at each campaign equals 75.1%, 69.1%, 68.9%, 79.1% and 67.1%, respectively. (B) Tests of significance of the amount of variance explained by the three explanatory tables alone or in combination (X1 = Physico-chemistry, X2 = Other algae, X3 = Hydraulics, n = number of plots).