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# Flow intermittency affects the nutritional quality of phototrophic biofilms and their capacity to support secondary production

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## Abstract

1. In streams, phototrophic biofilms are considered to be a good-quality resource for consumers and are essential to support secondary production. However, with the increasing occurrence of flow intermittency as a consequence of global climate change, limited information exists regarding the impact of drying and rewetting events on biofilm nutritional quality indicators and their consequences for consumers. This study aims at understanding how river intermittency affects the nutritional quality of phototrophic biofilms. Specifically, we examine the effects of drying and rewetting events on their capacity to support secondary production.
2. Our hypothesis was that the capacity of biofilms to support secondary production relies on their nutritional quality: biofilms characterised by higher contents of long-chain fatty acids, nitrogen and phosphorus are expected to provide a better-quality resource for consumers. We also hypothesised that the nutritional quality of biofilms undergoes changes over time during drying events, and that these changes are influenced by their initial algal composition. This is because the algal composition within biofilms may shift in response to drying events, subsequently impacting the nutritional quality of the biofilms.
3. We grew four phototrophic biofilms in flowing water, each with a different nutritional quality, and then exposed them to a short (3 days) or a long dry period (14 days). Biofilms were sampled 3 days and 18 days after rewetting (post-disturbance and post-recovery) to assess alterations in nutritional indicators relative to their pre-disturbance state through pigment, fatty acid and stoichiometric analyses. We fed these biofilms to Gammarids (*Gammarus fossarum*) for 29 days and measured individual growth, feeding rate and locomotor activity. We also calculated a secondary production index to assess the biofilms' capacity to support higher trophic levels.
4. Our findings revealed that the nutritional quality of biofilms was significantly reduced during the post-disturbance phase. The duration of the dry period had

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minimal effect on this decline. Subsequently, during the recovery phase, nutritional quality indicators improved for biofilms initially dominated by cyanobacteria, while they either remained unchanged or decreased for biofilms initially dominated by diatoms, in comparison to the pre-disturbance state. As a result, biofilms that initially exhibited a high nutritional quality were disrupted by the dry period, depending on the duration. However, the overall effects of dry period on gammarid's response and on secondary production were less pronounced, which is likely to have resulted from changes in the quantity of available resources.

5. Our study demonstrates that a disturbance can modify the expected and effective qualities of biofilms. It highlights that biochemical parameters cannot reliably predict biofilm capacity to support secondary production. Biofilm history of disturbance, among other parameters, must be taken into account.

#### KEYWORDS

dry period, freshwater periphyton, intermittent rivers, nutritional quality, primary consumers

## 1 | INTRODUCTION

Flow intermittency is a global phenomenon observed across all climates, with over half of the global river network experiencing at least 1 day of non-flow per year (Messenger et al., 2021). As a result of global climate change, the duration of dry periods in intermittent rivers and ephemeral streams (IRES) is increasing (Datry et al., 2017), and even some perennial streams and rivers are beginning to experience dry phases. Water intermittency affects the entire biota and alters the trophic structure of the ecosystem by changing interactions between trophic levels (Ledger et al., 2013). Consumers are directly affected by habitat fragmentation and loss of species richness (Acuña et al., 2005; Bogan et al., 2015; Boulton & Lake, 2008), and indirectly affected through changes in food resource composition and availability caused by drought (Lake, 2003). The composition of resources plays a crucial role in determining their palatability or toxicity, making resource composition an essential aspect of food quality (Masclaux et al., 2009). Aquatic consumers generally select high-quality resources (Marcarelli et al., 2011). Food quality depends on the elemental content of resources, such as the carbon (C):nitrogen (N) and C:phosphorus (P) ratios, and the biochemical composition, including the fatty acid (FA) profiles (Burian et al., 2018; Danger et al., 2022; Ruiz et al., 2021). In situations where only low-quality resources are available, consumers can compensate by increasing their feeding rate to maintain their fitness (e.g., Cruz-Rivera & Hay, 2000). Considering the qualitative and quantitative modifications of resources is crucial to fully predict the effects of river intermittency on food-web structure and secondary production.

Phototrophic biofilms—composed of diatoms, green algae and cyanobacteria embedded in an extracellular polymeric matrix, alongside heterotrophic microorganisms such as bacteria, heterotrophic protists or fungi—play a significant role as primary producers in various flowing ecosystems and occupy the base of many food webs (Battin et al., 2016). The importance of this algal-based autochthonous resource in food-web integrity has been demonstrated in

several studies (Allan & Castillo, 2007; Crenier et al., 2017; Kühmayer et al., 2020), particularly in intermittent streams, where consumers assimilate more algal biomass and less allochthonous matter than in perennial systems (Siebers et al., 2019). Low quantities of algal food sources are essential to ensure secondary production (Crenier et al., 2017; Labed-Veydert et al., 2022; Twining et al., 2017). Stream biofilms have a lower C:N ratio than leaf litter (Lau et al., 2009), which enhances their digestibility and quality (Allan & Castillo, 2007). Algae contains higher amounts of essential polyunsaturated FAs (PUFAs) than allochthonous matter (Descroix et al., 2010; Labed-Veydert et al., 2021; Torres-Ruiz et al., 2007). PUFAs are nearly exclusively synthesised *de novo* by phototrophic primary producers, requiring consumers to obtain them from their diet (Brett & Müller-Navarra, 1997; Ebm et al., 2023). However, the lipid composition of primary producers largely differs among taxa. Cyanobacteria, like other prokaryotic organisms, are devoid of sterols (Martin-Creuzburg et al., 2006; Martin-Creuzburg & Bec, 2005) and often lack n-3 series PUFAs. As a result, biofilms dominated by cyanobacteria are generally considered a lower-quality resource than those dominated by eukaryotic algae such as diatoms and green algae (Gergs et al., 2014).

The response of river phototrophic biofilms to desiccation has been extensively investigated. It has been found that, in terms of biomass, the autotrophic compartment is generally more negatively impacted by desiccation than the heterotrophic compartment (Timoner et al., 2012). Additionally, the longer the disturbance lasts, the more autotrophic processes are impacted (Acuña et al., 2015). Response to desiccation varies among algal groups, with cyanobacteria and green algae being more resistant than diatoms, but less resilient (Acuña et al., 2015; Sabater et al., 2017; Timoner et al., 2014). Changes in the composition of biofilms are likely to have consequences for consumers. For instance, it has been shown that the total FA content of biofilms in a Mediterranean stream decreased after a drought (Sanpera-Calbet et al., 2017), although the cascading effects on consumers were not assessed. Another study found

a decrease in the consumption rate of *Physella acuta* when fed with resources exposed to water intermittency (Mas-Martí et al., 2017). These findings suggest that the nutritional quality of phototrophic biofilms in intermittent rivers largely relies on the specific and biochemical compositions of algal communities. However, there is limited information regarding how the nutritional quality of phototrophic biofilms changes during drying and rewetting events and how these changes affect consumers and secondary production.

In this study, we aim at understanding how river intermittency affects the nutritional quality of phototrophic biofilms (pigment content, FA content, stoichiometry) and their capacity to support secondary production. Biofilms characterised by high N and P contents, and high long-chain FA contents (such as diatom-dominated biofilms) are expected to provide a better-quality resource for consumers. We investigated the extent to which the response of biofilms to drying and their recovery from drought is influenced by the intensity of the disturbance and their initial composition. To achieve this, we exposed four biofilms that differed in their algal composition and/or stoichiometry to two dry-period durations followed by rewetting. We measured various indicators that reflect the expected nutritional quality of biofilms before drying, and 3 and 18 days after rewetting. These indicators include pigment analyses, stoichiometric measurements and biochemical composition. Furthermore, we evaluated the effective nutritional quality by feeding metazoan consumers with each produced biofilm and measuring their ecological traits. Firstly, we hypothesised that drying modulates the nutritional quality of biofilms over time. We expect an initial decrease in quality as a result of the lower resistance of diatoms to drying compared to cyanobacteria. Diatom decline should result in a strong decrease of diatom-associated pigments and the long-chain FA content. Following the period of recovery, an increase in nutritional quality is expected. This can be attributed to the higher resilience of diatoms during rewetting, which should result in an increase in the proportion of diatom-associated pigments and long chain FAs. Secondly, we expected that the differences in nutritional quality between biofilms will be amplified as the duration of the dry period increases. Finally, by comparing the expected and effective qualities of biofilms, we tested the hypothesis that the capacity of biofilms to support secondary production is driven by their nutritional quality.

## 2 | MATERIALS AND METHODS

### 2.1 | Biofilm production

#### 2.1.1 | Biofilm inoculation

In order to cultivate biofilms with distinct biochemical and stoichiometric characteristics, various biofilm inocula were used in combination with different culture conditions, including the depletion or non-depletion of P. The phototrophic biofilms were collected from two locations along the Garonne River in south-west France, where different hydrological and nutrient conditions

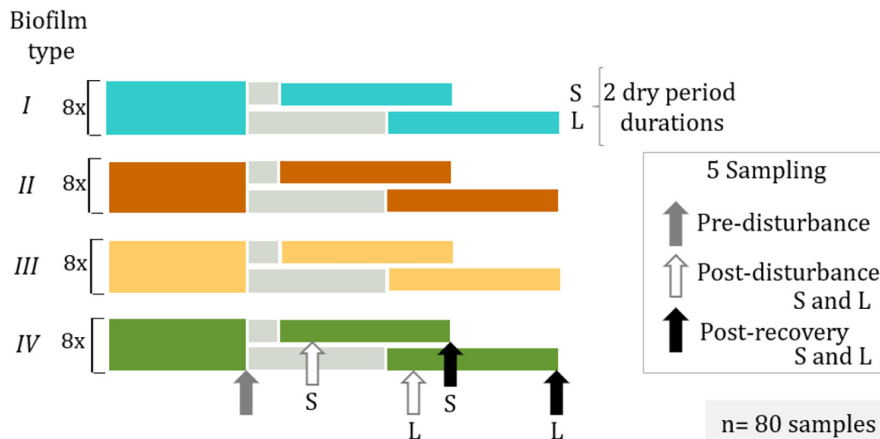
favour different communities. The Aouach site (43.396635, 1.300394, south-west France), is dominated by diatoms, whereas the Toulouse site (43.561740, 1.437750, south-west France), has more green algae. Biofilms were collected by scraping immersed pebbles on the riverbank with a toothbrush directly in river water. Back at the laboratory, the suspensions were homogenised using an ultra-turrax (T25; Janke-Kunkel) and filtered through a 500- $\mu\text{m}$  sieve to remove large mineral particles and macrofauna. To modify the stoichiometry of the biofilm, the Aouach inoculum was cultivated under both standard and P-depleted conditions. To ensure the presence of cyanobacteria in at least one biofilm, a portion of the initial inoculum from Aouach was additionally filtered through a 40- $\mu\text{m}$  sieve to eliminate large diatoms and then enriched with laboratory cultures of several cyanobacteria (*Komvophoron* sp., *Phormidium autumnale* and *Leptolyngbya* sp.), grown in BG11 culture medium (Stanier et al., 1971). This resulted in four different biofilms: Aouach inoculum enriched with cyanobacteria (biofilm I), P-depleted Aouach inoculum (biofilm II), Aouach inoculum (biofilm III), and Toulouse inoculum (biofilm IV).

#### 2.1.2 | Biofilm culture and sampling

The biofilm culture device consisted of 32 channels (eight per biofilm type, 50 cm wide, 8 cm long, 10 cm high) placed in a controlled environment (22°C, neon light intensity: 88  $\mu\text{mol m}^{-2} \text{s}^{-1}$  [ $\pm 7$ ] for 16 h/day). Ceramic tiles (5.76  $\text{cm}^2$ , 54 per channel) were placed at the bottom as growth substrates. During the 14-day biofilm growth period, the channels were connected in pairs (short and long dry periods, four replicates) by sharing the water tank (see Figure 1). Water circulation was maintained by aquarium pumps (Tetra WP-300, 300 L/h). Volvic® water was used as the culture medium, and evaporation was compensated three times per week with demineralised water at the same time as nutrient addition. The same quantity of N (1.4 mg/L N-NO<sub>4</sub>) and P (200  $\mu\text{g/L}$  P-PO<sub>4</sub>) was added to each channel, except for the P-depleted condition (biofilm II), where the quantity of phosphorus was 10-fold lower (20  $\mu\text{g/L}$ ). In the channels hosting biofilms II and III, silica (21.3 mg/L SiO<sub>2</sub>) also was added to favour diatom growth.

In order to characterise each biofilm before drying (pre-disturbance; Figure 1), a first sampling was conducted after the growth period. For this, 11 tiles were collected from each channel, totalling 22 tiles per replicate. The biofilm was gently removed from the tiles with a scraper and resuspended in 4 ml of Volvic® water per tile. After this sampling, the channel pairs were disconnected and dried-out using a vacuum pump. One channel in each pair was subjected to a short dry period of 3 days (S), while the other underwent a longer dry period of 14 days (L). After each dry period, flow resumed in the same condition as during the growth phase, independently in each channel. Biofilms were sampled during rewetting 3 days after flow resumption (post-disturbance) and 18 days after flow resumption (post-recovery). For each sampling, 22 ceramic tiles were retrieved from each channel, and the biofilm was treated as in the

**FIGURE 1** Experimental design: four types of biofilms were grown in a total of 32 channels, with four replicates per biofilm type and dry period duration. After 2 weeks in standard flow condition, we sampled the biofilms (grey arrow) and disconnected the channels in pairs, which were then dried out for 3 days (short dry period: S) or 14 days (long dry period: L). After rewetting, we sampled the biofilms twice, after 3 days (white arrows) and 18 days (black arrows).



pre-disturbance sampling. The biofilms collected are hereafter referred to as pre-disturbance biofilms, post-disturbance biofilms and post-recovery biofilms in the following text.

### 2.1.3 | Biofilm sample analysis

In order to evaluate the nutritional quality of the biofilms, we measured various parameters including biomass, algal pigment composition, stoichiometry and FA composition for each sample. To accurately assess the nutritional quality, each variable was normalised by the weight of biofilm C. This normalisation allows for a comparative analysis of the nutritional content across different biofilms.

In addition, we conducted a second analysis in which the variables were expressed per unit of surface area. This approach considers the quantity of biofilm available for consumption by the consumers. By expressing the variables per surface unit, we could better estimate the nutritional quality of the biofilms in relation to the amount of biofilm that is accessible to the consumers.

#### *Pigment composition*

In order to extract the pigments, 1.5 ml of each biofilm suspension was centrifuged at 10,000g for 5 min at 4°C. The supernatant was removed, and the biofilm was stored at -80°C until being freeze-dried right before pigment extraction. Pigments were extracted in 2 ml of 2% ammonium acetate 1M buffered methanol using the method described by Capdeville et al. (2019). The tubes were sonicated for 3 min and incubated at -20°C for 15 min. After another 3 min of sonication, the tubes were centrifuged at 10,000g for 5 min at 4°C. The supernatant was gently pipetted out and stored at 4°C. Another extraction step was performed on the samples, and the supernatants were pooled, except when the supernatant was not coloured. Pigment analyses were performed using HPLC (LC1200 series; Agilent Technologies), as described in Majdi et al. (2011), from 1 ml of each extract pre-filtered in a glass vial with a 0.2-µm PTFE membrane syringe filter (VWR International). Pigment concentration was quantified by comparing the peak area of the chromatogram with those of standards with the ChemStation software

(Agilent Technologies). To obtain a proportion of each main primary producer, specific pigments of green algae, diatoms or cyanobacteria were summed as described in Allen et al. (2021) and expressed in proportion of the total pigment content (%). The quantity of Chlorophyll (Chl)-a in samples was used as an indicator of the live algal biomass.

#### *Stoichiometric composition*

In order to determine the stoichiometric composition, 1 ml of biofilm suspension was oven dried at 60°C for 24 h in pre-weighed microtubes. Nitrogen and C contents were quantified using a CHN analyser (Flash 2000; Thermo Scientific). Phosphorus quantification was performed using a spectrophotometer at 880 nm (Uvi Light XT5; SECOCAM), after a pre-treatment as described in Courcoul et al. (2022).

#### *Fatty acid composition*

In order to assess the FA composition, 1 ml of biofilm suspension was freeze-dried and pooled from the four replicates of each treatment to ensure an adequate quantity of carbon and for logistic reasons. FA were extracted twice using the method developed by Folch et al. (1957). Biofilms were suspended in clean glassware and in a chloroform: methanol (2:1) mix with two standards (20 µl of 13:0 and 23:0 at 2 mg/ml) to break the bonds between proteins and lipids. To further facilitate the extraction process, samples were exposed to 15 min sonication before incubation at 56°C for 30 min. Next, FA methyl-esters (FAME) were obtained through esterification in acid medium (4% H<sub>2</sub>SO<sub>4</sub> in methanol at 75°C for 2 h). FAME were then diluted in 200 µl of hexane in dark glass vials before quantification. FAME were separated and analysed using an Agilent 6850 gas chromatograph equipped with a J&WDB-WAX capillary column (30 m × 0.25 mm inner diameter) as described in Gerphagnon et al. (2019). Peak areas were expressed as FA quantity according to internal standards and identified using the FA retention time of an external standard Supelco 37 Component FAME Mix (Merck).

In addition to the total FA content, the quantity of poly-unsaturated FAs (PUFAs) was calculated by summing unsaturated FA with more than 18C. Likewise, the quantity of highly-unsaturated

FAs (HUFAs) was calculated by summing 20:4(n-3), 20:5(n-3) and 22:6(n-3) (Bec et al., 2010).

## 2.2 | Feeding experiment

The remaining volume of biofilm suspension was freeze-dried, weighed and stored at  $-20^{\circ}\text{C}$  until the preparation of agarose pellets to feed crustacean amphipods *Gammarus fossarum*, which are generalist consumers.

### 2.2.1 | Selection of individuals

*Gammarus fossarum* individuals were collected from a second-order stream in Vosges (eastern France). The feeding experiment was conducted in river water collected during gammarid capture. In the laboratory, organisms were acclimated in the dark with oxygen supply at  $5^{\circ}\text{C}$  for 5 days. During acclimation, gammarids were fed with alder leaves (*Alnus glutinosa*) collected from the riparian zone of the same stream. Gammarid individuals were sorted to keep only juveniles with similar lengths ( $4.8\text{ mm} \pm 0.3$ ).

### 2.2.2 | Agarose pellet preparation

The agarose pellets were prepared as described by Crenier et al. (2017) at a C concentration of 2%. The biofilm-agarose mixture contained 50% agarose and 50% biofilm based on the biofilm C content quantified previously. The biofilm-agarose mixture was homogenised at  $38^{\circ}\text{C}$  to avoid jellification and then poured into Plexiglas moulds ( $39.27\text{ mm}^3$ ) until solidification. Pellets were stored at  $-20^{\circ}\text{C}$  in Petri dishes until use.

### 2.2.3 | Gammarid feeding

Three gammarids were fed with each biofilm sample ( $n=240$ ). Gammarids were individually disposed in Petri dishes containing 30 ml of river water and kept at  $12^{\circ}\text{C}$  in the dark during the 29 days of the feeding experiment. Once a week, water and biofilm pellets were renewed. Each time, the remaining pellets were stored in microtubes and kept at  $-20^{\circ}\text{C}$  until they were weighed to calculate the biofilm consumed.

### 2.2.4 | Ecological trait analysis

Apart from daily counting surviving gammarids, we also measured several other traits on individuals that survived throughout the feeding experiment. These included growth rate, condition index, feeding rate and median locomotion speed.

#### Growth traits and condition index

The weight growth rate (/day) was calculated as follows:

$$\text{Growth rate} = \frac{(\text{final body dry weight (mg)} - \text{initial body dry weight (mg)})}{\text{initial body dry weight (mg)} * \text{experiment duration (day)}}$$

We measured the final dry weight (in mg) of each individual at the end of the experiment using a precision balance (RADWAG, UYA 2.4.Y). The initial dry weight was estimated from the initial body size (mm) using the following allometric relationship:

$$\text{Initial dry weight} = 0.0609 * \text{initial size}^2 - 0.2897 * \text{initial size} + 0.4291$$

The length was measured between the antennule basis and the telson using VHX-6000\_950F software, on pictures made with a digital microscope (VHX 6000; Kayence). The curved body shape was accounted for using the formula described in Danger et al. (2013):

$$\text{linear state} = \text{curved state} * 0.88$$

We used the Fulton condition factor ( $\text{mg}/\text{mm}^3$ ) to assess the physiological state of gammarids, which was calculated from final weight and length (Ricker, 1975):

$$\text{condition index} = \frac{(\text{body dry weight (mg)})}{\text{body length (mm)}^3}$$

The factor ranged between 0 and 1, with 0 representing poor physiological condition.

#### Feeding rate

The feeding rate ( $\text{mg biofilm mg gammarid}^{-1} \text{ day}^{-1}$ ) was calculated as follows:

$$\text{Feeding rate} = \frac{\text{consumed biofilm weight (mg)}}{\text{final body dry weight (mg)} * \text{experiment duration (day)}}$$

#### Median speed

In order to assess gammarid activity, we measured the median locomotion speed ( $\text{cm}/\text{s}$ ) by tracking each individual's movement for 8 min. To facilitate tracking, each gammarid was placed in Petri dishes wrapped in black tape in a closed and opaque small chamber ( $116 * 50 * 50\text{ cm}$ ) in which light can be easily controlled (LED,  $250\text{ }\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and equipped with a camera (Canon EOS M50).

### 2.2.5 | Secondary production

The secondary production ( $\text{mg cm}^{-2} \text{ day}^{-1}$ ) was calculated from previous indicators as follows:

$$\text{Secondary production} = \frac{(\text{final body dry weight} - \text{initial body dry weight})(\text{mg}) * \text{biofilm total C (mgC/cm}^2\text{)}}{\text{consumed biofilm carbon (mgC)} * \text{experiment duration (day)}}$$

This indicator represents the weight of gammarids that biofilm biomass is able to daily support.



## 2.3 | Data analyses

Statistical analyses were performed using R software (version 3.6.1; R development Core Team). Firstly, we checked the normality of variables and applied transformations when necessary. We then calculated global Pearson correlations between biofilm indicators and gammarid response variables using the *Hmisc* package. To explore potential co-structure between biofilms and gammarid responses, we conducted a general co-inertia analysis on principal component analyses (PCA) carried out on both nutritional quality indicators and gammarid traits responses. The significance of the co-structure was assessed using the RV, which represents the correlation coefficient for the datasets, and we conducted a permutation test to determine the strength of the relationship between the two datasets.

In order to characterise the biofilms based on their pigment composition and evaluate how the composition changes over time, we conducted ANOVA considering the treatment they were exposed to (dry period duration and sampling time). We performed separate PCA analyses on biofilm nutritional quality indicators and gammarid response variables to identify differences between biofilms and sampling times. The coordinates of individuals on the two first axes of the PCAs were extracted as a summary variable. For comparing the dynamics of nutritional quality in the biofilms and the response of gammarids over time, we conducted repeated measures ANOVA (rmANOVA) within dry period duration and sampling time (i.e., pre-disturbance, post-disturbance and post-recovery). If significant, we conducted appropriate *post hoc* analyses (pairwise paired *t*-tests). We also tested differences between biofilm types at each sampling date using ANOVA and performed Tukey's honestly significant difference (HSD) tests when significant, considering that the samples were unpaired. Additionally, we conducted co-inertia analyses for each sampling date to investigate possible co-structure between biofilm nutritional quality indicators and gammarid response variables over time.

Finally, we examined correlations between secondary production and the quantity of biofilm available, as well as gammarid weight gain, before conducting ANOVA analysis and Tukey's HSD tests.

## 3 | RESULTS

The results of Pearson correlation analysis conducted on all biofilm samples indicate that biofilm autotrophy (Chl-*a*/C quantity) was positively correlated with total FA, PUFA, HUFA contents and diatom proportion, but negatively correlated with cyanobacteria proportion (Table 1). Moreover, total FA and PUFA contents showed positive correlations with C:P ratio and diatom proportion, whereas HUFA content exhibited a negative correlation with C:N ratio and cyanobacteria proportion, but a positive correlation with other algal groups, particularly diatoms. The correlation matrix also revealed a negative relationship between cyanobacteria proportion and other main algal groups and the live biomass (i.e., Chl-*a* and total FA content).

Co-inertia analysis conducted between the quality and response of gammarids demonstrated a significant co-structure between biofilm quality indicator and gammarid datasets (permutation test [ $n=999$ ],  $RV=0.27$ ,  $p=0.001$ ). Specifically, gammarid growth rate was positively correlated with Chl-*a*, diatom and green algae proportions, total FA, PUFA and HUFA contents, and C:N ratio (Table 2). The same significant correlations were observed for the condition index of gammarids, except with C:P. As expected, both gammarid growth rate and condition index were negatively correlated with the cyanobacteria proportion. The feeding rate was positively correlated with Chl-*a*, but negatively with green alga proportion. Gammarid median speed increased when C:N ratio and total FA and PUFA contents in biofilms increased.

### 3.1 | Pre-disturbance biofilms

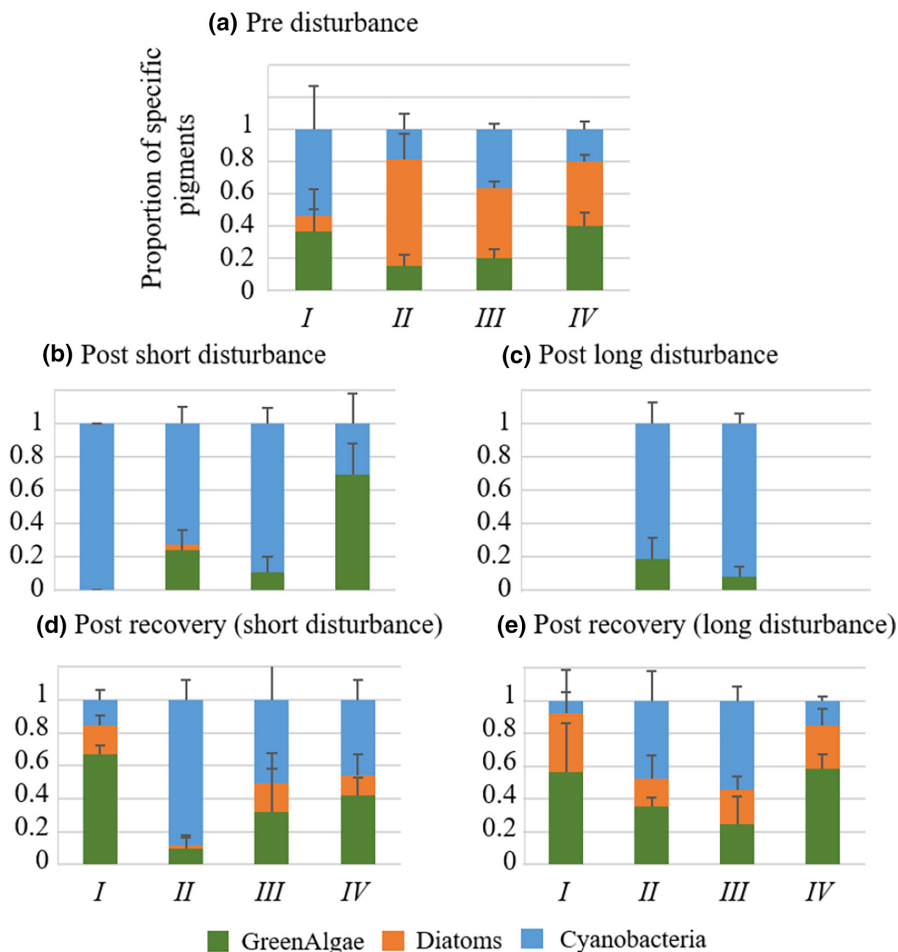
The use of different inocula and culture conditions resulted in the production of contrasted biofilms, as demonstrated by the proportions of the three main primary producer groups (cyanobacteria, green algae and diatoms) (Figure 2a). Biofilm I was dominated by cyanobacteria-specific pigments at more than 54% and green algae-specific pigments at 37%. In biofilms II, 66% of pigments were diatom-specific. Biofilm III was dominated by diatoms (45%) and

TABLE 1 Pearson correlation coefficients (*r*) between the biofilm nutritional quality indicators. Only significant correlations are presented. The asterisks represent the levels of significance:  $0.05 > * > 0.01 > ** > 0.001 > ***$ .

	Chl- <i>a</i>	Total FA	PUFA	HUFA	Green algae	Diatoms	Cyano bacteria	C:N	C:P
Chl- <i>a</i> (mg/mgC)	1								
Total FA (mg/mgC)	0.38***	1							
PUFA (mg/mgC)	0.41***	0.83***	1						
HUFA (mg/mgC)	0.68***	0.46***	0.45***	1					
Green Algae (proportion)				0.32**	1				
Diatoms (proportion)	0.59***	0.56***	0.43***	0.79***		1			
Cyanobacteria (proportion)	-0.28**	-0.28**		-0.60***	-0.6***	-0.42***	1		
C:N	-0.34***			-0.34***				1	
C:P		0.42***	0.41***						1

**TABLE 2** Pearson correlation coefficients ( $r$ ) between the biofilm nutritional quality indicators (rows) and gammarid response variables (columns). Only significant correlations are presented. The asterisks represent the levels of significance:  $0.05 > * > 0.01 > ** > 0.001 > ***$ .

	Gammarids			
	Growth rate (/day)	Condition index (mg/cm)	Feeding rate (mg mg <sup>-1</sup> day <sup>-1</sup> )	Median speed (cm/s)
<i>Biofilms</i>				
Chl- <i>a</i> (mg/mgC)	0.33**	0.33**	0.44***	
Total FA (mg/mgC)	0.54***	0.54***		0.44***
PUFA (mg/mgC)	0.4***	0.42***		0.27*
HUFA (mg/mgC)	0.28*	0.33		
Green algae (proportion)	0.22*	0.31**	-0.24*	
Diatoms (pProportion)	0.40***	0.42***		
Cyanobacteria (proportion)	-0.26*	-0.38**		
C:N	0.27*		-0.29*	0.32**
C:P			-0.36**	



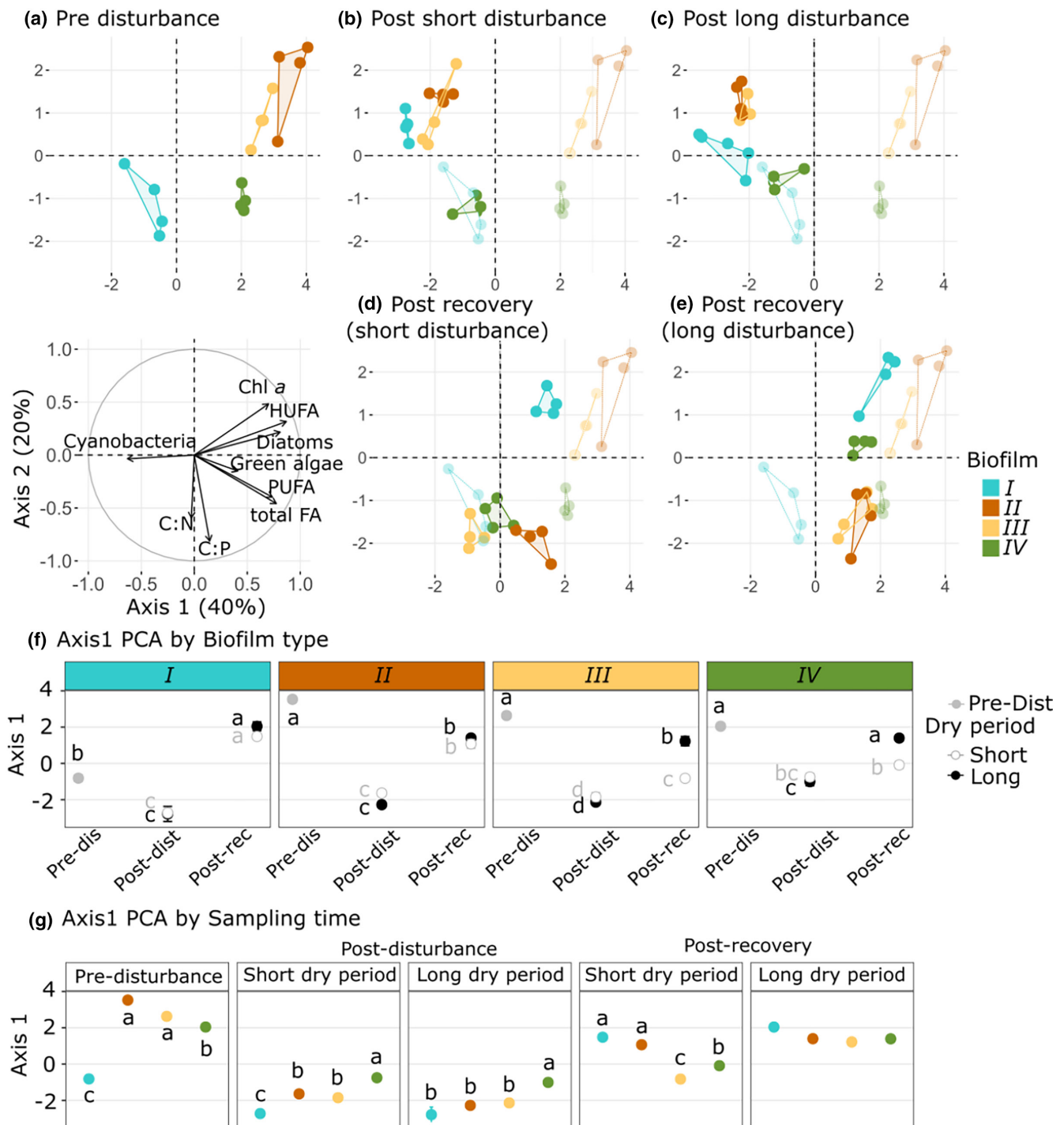
**FIGURE 2** Proportions of the main algal group within biofilms (mean  $\pm$  SD,  $n=4$ ) estimated through pigment analysis, for the pre-disturbance sampling (a), and after a short (left) and long (right) dry period at post-disturbance (b and c) and post-recovery (d and e) samplings. No values are available for biofilms I and IV in (c) because no specific pigment was detected in the samples.

cyanobacteria (36%), whereas biofilm IV showed a dominance of both green algae (40%) and diatoms (40%). The pigment composition was more variable in biofilm I than in other biofilms. The quantity of pigment found in biofilms is reported in Table S1.

The PCA analysis clustered biofilms into three groups on the first two principal components (Figure 3a): biofilm I, biofilms II–III and

biofilm IV. Biofilm I was separated from the others along the first axis because of its higher proportion of cyanobacteria (Figure 3, circle). Biofilms II and III differed from the two others along the second axis, which was mainly negatively correlated with C:P and C:N ratios (Figure 3, circle). The PCA analysis performed on gammarid data distinguished biofilm I from the others on the first axis,

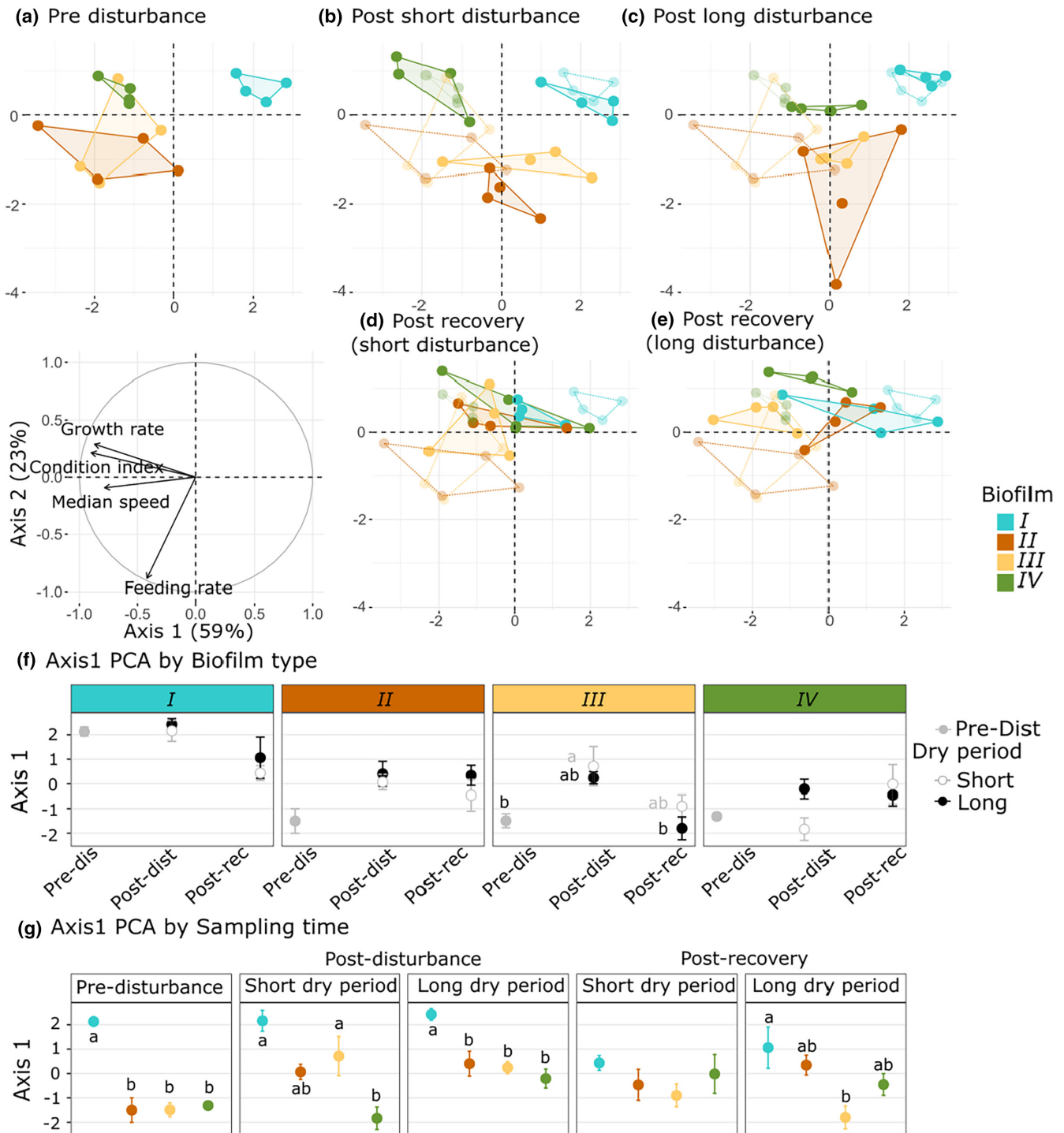




**FIGURE 3** Principal component analysis (PCA) performed on biofilm quality indicators. The resulting points are grouped by biofilm type ( $n=4$ ). Each panel (a–e) represents the points corresponding to one sampling time and dry period duration on the same PCA plan. The pre-disturbance composition has been reported in transparency on each panel to facilitate comparisons. The coordinates of individuals on the PCA first axis (mean  $\pm$  SE,  $n=4$ ) at each sampling time are represented in (f) and (g). The statistical results of the pairwise Student's  $t$ -test performed between treatments for each biofilm type after rmANOVA are shown in (f), while multiple comparison (Tukey's honestly significant difference [HSD]) performed between biofilms type at each sampling time after ANOVA are shown in (g).

and indicated that gammarids fed with biofilm I had lower growth rate and condition index and swam slower than gammarids fed with other biofilm types (Figure 4a). The correlation circle suggested that “good nutritional quality” biofilms were on the left of the ordination, with growth and condition index, physiological and behavioural traits

representative of “healthy” individuals clustering on the top left of the ordination (Figure 4 circle). The first axis of this PCA, which separates biofilms according to their effective nutritional quality for gammarids, was negatively correlated to the first axis of the PCA analysis performed on biofilm data ( $r^2 = -0.49$ ,  $p < 0.001$ ). However, there was



**FIGURE 4** Principal component analysis (PCA) performed on gammarid response variables. The resulting points are grouped according to the biofilm type the individuals were fed with ( $n=4$ ). Each panel (a, b, c, d, e) represents the points corresponding to one sampling time and dry period duration on the same PCA plan. The response of gammarids fed with pre-disturbance biofilms has been reported in transparency on each panel. The coordinates of individuals on the PCA first axis (mean  $\pm$  SE,  $n=4$ ) at each sampling time are represented in (f) and (g). The statistical results of the multiple comparisons performed between treatments for each biofilm type are shown in (f), while those performed between biofilm types at each sampling time are shown in (g).

no correlation between the second axis. This places “good nutritional quality” biofilms on the right half of the PCA ordination, which corresponds to biofilms with a large proportion of diatoms and a large quantity of total FAs, HUFAs and PUFAs (Figure 3, circle).

### 3.2 | Biofilm responses to disturbance

The dry period caused a dramatic change in the proportion of algal groups in the biofilm compared to the pre-disturbance state

(Figure 2a–c). Diatom-specific pigments collapsed in post-disturbance biofilms, regardless of the dry period duration, while the proportion of cyanobacteria-specific pigments drastically increased in all biofilms except in biofilm IV exposed to a short dry period, where green algae became dominant. In post-recovery biofilms, diatom-specific pigments were once again well-represented but did not fully return to the pre-disturbance proportion in biofilms II, III and IV (Figure 2d,e). In post-recovery biofilms, those exposed to a short dry period showed a significantly lower mean diatom proportion ( $13 \pm 13\%$ ) compared to biofilms exposed to a long dry period ( $27 \pm 17\%$ ) (ANOVA,  $F=21.81$ ,  $p=0.025$ ). Additionally, the proportion of cyanobacteria differed between the two groups and showed a trend towards being higher in post-recovery biofilms exposed to a short dry period ( $47 \pm 29\%$ ) than in those exposed to a long dry period ( $29 \pm 20\%$ ) (ANOVA,  $F=2.12$ ,  $p=0.069$ ).

The drying process caused significant changes in the taxonomic, elemental and biochemical composition of the biofilms, resulting in a reduction in their overall nutritional quality. This is evident from the post-disturbance biofilms shifting towards the left side of the graph in Figure 3b,c, regardless of the duration of the dry period. The analysis of the first axis coordinates (Figure 3f) further confirms this trend. At post-disturbance, biofilm IV exhibited the highest nutritional quality compared to other biofilms (Figure 3g). The post-recovery state depended on the biofilm type and, in some cases, on the duration of the dry period (Figure 3d,e). Biofilm I moved towards the upper right panel between the pre-disturbance and post-recovery samplings, suggesting an increase in nutritional quality. Biofilms II and III mainly moved along axis 2, indicating an increase in C:P and C:N ratios. Biofilm IV, however, either moved along the first PCA axis after a short dry period or returned to the same position on the PCA plot as the pre-disturbance state after a long dry period (Figure 3f). After the post-recovery samplings, biofilms I and II proved to be better resources than biofilms III and IV following a short dry period, whereas the post-recovery biofilms exhibited similar nutritional quality across all biofilms after a long dry period (Figure 3g).

### 3.3 | Gammarid response to disturbance

Some mortality was observed for gammarids fed with each biofilm sample (Figure S1). Notably, biofilm I showed the highest mortality rate, with 21 of 60 individuals dying over time, especially when gammarids were fed with biofilms sampled at post-disturbance following the long dry period, where only one third of individuals survived (ANOVA,  $F=2.38$ ,  $p=0.006$ ). There were no other significant differences in gammarid survival.

The drying process resulted in a decrease in biofilm effective nutritional quality for gammarids in biofilms II and III, as indicated by the trend of post-disturbance samples to move towards the right part of the PCA plot (Figure 4b,c). This trend is further confirmed by the significant increase in axis 1 coordinates for biofilm III (Figure 4f). For gammarids fed with biofilms II and

III, the displacement of points along the second axis between post-disturbance and post-recovery states indicated a reduction in gammarid feeding rate after rewetting. The quality of biofilm I tended to increase during rewetting, especially when previously exposed to a short dry period (Figure 4d,f). When comparing gammarid responses between biofilm types they were fed on, it was observed that at post-short disturbance, biofilm IV was the best resource for gammarids. However, after the long dry period its quality did not differ from those of biofilms II and III (Figure 4b,g). At post-recovery following the short dry period, there were no significant differences in gammarid response between all biofilm types. However, after the long dry period, biofilm III was a significantly better resource compared to biofilm I for gammarids, and to a lesser extent biofilms II and IV (Figure 4e,g).

Before the disturbance, there was a strong co-structure between nutritional quality indicators of biofilms and gammarid data sets ( $RV=0.71$ ,  $p=0.001$ ). However, the strength of their relationship decreased with disturbance. At post-disturbance following the short dry period, the RV slightly decreased to 0.64 ( $p=0.001$ ), and there was no co-structure anymore at post-recovery ( $RV=0.22$ ,  $p=0.27$ ). After the long dry period, a significant co-structure between nutritional quality indicators and the response of gammarids persisted, although it decreased over time. At post-disturbance, the RV was 0.43 ( $p=0.006$ ) and at post-recovery it was 0.33 ( $p=0.032$ ).

### 3.4 | Secondary production

Based on measurements of biofilm and gammarid, we estimated the ability of a given surface of biofilm to support secondary production. As expected, we found strong correlation between various indicators of biofilm quality. Secondary production was strongly correlated with the total C quantity in the biofilm ( $r=0.81$ ,  $p<0.001$ ) and with the weight gain of gammarids ( $r=0.64$ ,  $p<0.001$ ). We also found positive correlations between secondary production and measures of living biomass, such as the quantity of Chl-*a* and total FA per surface unit ( $r=0.55$  and  $0.71$ , respectively,  $p<0.001$ ), as well as with indicators of "good" nutritional quality, including PUFA ( $r=0.72$ ,  $p<0.001$ ) and HUFA ( $r=0.38$ ,  $p=0.001$ ). Additionally, the C:N and C:P ratios were positively correlated with secondary production ( $r=0.34$  and  $0.30$ , respectively;  $p<0.006$ ). However, we did not observe any correlation between secondary production and the proportions of different algal groups.

We found that exposure to a dry period did not significantly affect secondary production when measured at post-disturbance for any of the biofilm types tested (rmANOVA; Table 3). Furthermore, although there was a general increase in secondary production after rewetting for all biofilms, this increase was only significant for biofilm III due to the high variability in gammarid weight gain among replicates in the other biofilms (rmANOVA,  $F_{5,15}=12.0$ ,  $p<0.001$ ; Table 3). Carbon quantity increased in all biofilms after recovery with no effect of dry-period duration (rmANOVA,  $F_{5,15}>46.2$ ,  $p<0.02$ ; Table 3). For most biofilm types, gammarid weight gain did not

**TABLE 3** Changes in carbon quantity expressed per surface unit, gammarid weight gain during the feeding experiment, and secondary production (mean  $\pm$  SD,  $n=4$ ). Multiple comparisons (ANOVA) were conducted between biofilm types before disturbance, and pairwise Student's *t*-test (rmANOVA) or Tukey's honestly significant difference multiple comparisons (ANOVA) were conducted between sampling times for each biofilm type. Different letters represent significant differences.

		Carbon quantity in biofilms		Gammarid weight gain		Secondary production	
		rmANOVA	mg/cm <sup>2</sup>	ANOVA	mg	rmANOVA	mg cm <sup>-2</sup> day <sup>-1</sup>
Pre-disturbance	I	$F=7.89$	0.45 ( $\pm 0.06$ ) <sup>b</sup>	$F=11.26$	0.01 ( $\pm 0.04$ ) <sup>b</sup>	$F=11.2$	0.03 ( $\pm 0.02$ ) <sup>b</sup>
	II	$p=0.004$	0.41 ( $\pm 0.19$ ) <sup>b</sup>	$p<0.001$	0.17 ( $\pm 0.08$ ) <sup>a</sup>	$p<0.001$	0.03 ( $\pm 0.01$ ) <sup>b</sup>
	III		0.53 ( $\pm 0.11$ ) <sup>b</sup>		0.18 ( $\pm 0.02$ ) <sup>a</sup>		0.05 ( $\pm 0.02$ ) <sup>b</sup>
	IV		0.78 ( $\pm 0.05$ ) <sup>a</sup>		0.18 ( $\pm 0.03$ ) <sup>a</sup>		0.09 ( $\pm 0.02$ ) <sup>a</sup>
I	Pre-dist	$F=28.6$	0.45 ( $\pm 0.06$ ) <sup>b</sup>	n.s.	0.01 ( $\pm 0.04$ )	$F=3.23$	0.03 ( $\pm 0.02$ ) <sup>ab</sup>
	Post-dist S	$p<0.001$	0.31 ( $\pm 0.14$ ) <sup>b</sup>		-0.01 ( $\pm 0.08$ )	$p=0.036$	0.01 ( $\pm 0.01$ ) <sup>b</sup>
	Post-dist L		0.31 ( $\pm 0.06$ ) <sup>b</sup>		0.02 ( $\pm 0.02$ )		0.02 ( $\pm 0.02$ ) <sup>b</sup>
	Post-recov S		1.06 ( $\pm 0.08$ ) <sup>a</sup>		0.08 ( $\pm 0.07$ )		0.08 ( $\pm 0.04$ ) <sup>a</sup>
	Post-recov L		0.94 ( $\pm 0.21$ ) <sup>a</sup>		0.02 ( $\pm 0.09$ )		0.04 ( $\pm 0.04$ ) <sup>ab</sup>
II	Pre-dist	$F=30.2$	0.41 ( $\pm 0.19$ ) <sup>b</sup>	n.s.	0.17 ( $\pm 0.08$ )	$F=4.97$	0.03 ( $\pm 0.01$ ) <sup>b</sup>
	Post-dist S	$p<0.001$	0.52 ( $\pm 0.08$ ) <sup>b</sup>		0.07 ( $\pm 0.06$ )	$p=0.009$	0.02 ( $\pm 0.01$ ) <sup>b</sup>
	Post-dist L		0.28 ( $\pm 0.08$ ) <sup>b</sup>		0.01 ( $\pm 0.06$ )		0.01 ( $\pm 0.01$ ) <sup>b</sup>
	Post-recov S		1.61 ( $\pm 0.16$ ) <sup>a</sup>		0.13 ( $\pm 0.11$ )		0.13 ( $\pm 0.10$ ) <sup>a</sup>
	Post-recov L		1.17 ( $\pm 0.33$ ) <sup>a</sup>		0.07 ( $\pm 0.04$ )		0.08 ( $\pm 0.03$ ) <sup>ab</sup>
III	Pre-dist	$F=47.1$	0.53 ( $\pm 0.11$ ) <sup>b</sup>	$F=7.56$	0.18 ( $\pm 0.02$ ) <sup>ab</sup>	$F=12.0$	0.05 ( $\pm 0.02$ ) <sup>b</sup>
	Post-dist S	$p<0.001$	0.64 ( $\pm 0.27$ ) <sup>b</sup>	$p=0.004$	0.04 ( $\pm 0.09$ ) <sup>c</sup>	$p<0.001$	0.02 ( $\pm 0.01$ ) <sup>b</sup>
	Post-dist L		0.53 ( $\pm 0.04$ ) <sup>b</sup>		0.07 ( $\pm 0.04$ ) <sup>bc</sup>		0.02 ( $\pm 0.01$ ) <sup>b</sup>
	Post-recov S		1.86 ( $\pm 0.20$ ) <sup>a</sup>		0.14 ( $\pm 0.05$ ) <sup>abc</sup>		0.21 ( $\pm 0.13$ ) <sup>a</sup>
	Post-recov L		1.93 ( $\pm 0.30$ ) <sup>a</sup>		0.25 ( $\pm 0.05$ ) <sup>a</sup>		0.23 ( $\pm 0.05$ ) <sup>a</sup>
IV	Pre-dist	$F=35.0$	0.78 ( $\pm 0.05$ ) <sup>b</sup>	$F=5.03$	0.18 ( $\pm 0.03$ )	$F=5.04$	0.09 ( $\pm 0.02$ ) <sup>ab</sup>
	Post-dist S	$p<0.001$	0.73 ( $\pm 0.08$ ) <sup>b</sup>	$p=0.014$	0.21 ( $\pm 0.06$ )	$p=0.009$	0.11 ( $\pm 0.04$ ) <sup>ab</sup>
	Post-dist L		0.53 ( $\pm 0.05$ ) <sup>b</sup>		0.10 ( $\pm 0.05$ )		0.04 ( $\pm 0.01$ ) <sup>b</sup>
	Post-recov S		1.48 ( $\pm 0.31$ ) <sup>a</sup>		0.08 ( $\pm 0.09$ )		0.14 ( $\pm 0.14$ ) <sup>ab</sup>
	Post-recov L		1.73 ( $\pm 0.28$ ) <sup>a</sup>		0.15 ( $\pm 0.06$ )		0.25 ( $\pm 0.10$ ) <sup>a</sup>

significantly vary between pre-disturbance, post-disturbance and post-recovery, although there was a trend of decreasing weight gain when gammarids were fed with post-disturbance biofilms (Table 3).

When comparing between biofilm types, we found significant differences in the capacity to support secondary production. At pre-disturbance, biofilm IV exhibited a significantly higher capacity to support secondary production compared to the other biofilms (ANOVA,  $F_{3,12}=11.2$ ,  $p<0.001$ ; Figure S2a). This difference persisted after the short dry period (ANOVA,  $F_{3,12}=16.83$ ,  $p<0.001$ ; Figure S2a), but disappeared after the long dry period, with biofilm II exhibiting the lowest capacity to support secondary production (ANOVA,  $F_{3,12}=3.50$ ,  $p=0.05$ ; Figure S2a).

There was no significant difference in secondary production between post-recovery biofilms that had experienced a short dry period (ANOVA,  $p=0.36$ , mean =  $0.11 \pm 0.10$  mg cm<sup>-2</sup> day<sup>-1</sup>). However, after a long dry period, the secondary production of biofilms III and IV ( $0.23 \pm 0.05$  and  $0.25 \pm 0.10$  mg cm<sup>-2</sup> day<sup>-1</sup>, respectively) were higher than those of biofilms I and II ( $0.04 \pm 0.1$  and  $0.08 \pm 0.03$  mg cm<sup>-2</sup> day<sup>-1</sup>, respectively) (ANOVA,  $F_{3,12}$

= 12.68,  $p<0.001$ ; Figure S2a). These differences were consistent with those observed in C quantity in biofilms (Figure S2b) and gammarid weight gain (Figure S2c), although the differences were slight.

## 4 | DISCUSSION

In this study, we investigated how drying and rewetting affect the nutritional quality indicators of phototrophic biofilms and their capacity to support secondary production in river ecosystems, by comparing biofilms with contrasted algal compositions.

### 4.1 | Biofilm response to disturbance

As expected, the overall nutritional quality indicators of biofilms significantly declined during the dry period, with diatoms disappearing for the benefit of cyanobacteria and green algae. This is in

line with several studies arguing that organisms belonging to those groups have the ability to set up desiccation resistance mechanisms (Potts, 1999; Romani et al., 2013). Consistently, PUFAs and HUFAs were nearly undetectable in biofilms at post-disturbance, which aligns with findings from Mediterranean stream biofilms following drought events (Sanpera-Calbet et al., 2017). While drying reduced the different biofilm quality indicators investigated, it did not immediately alter the ranking of nutritional quality between biofilms. However, the nutritional quality of biofilms was significantly modified after the recovery. More specifically, biofilm I exhibited the highest nutritional quality after a short dry period and rewetting, partially confirming our second hypothesis. Such an increase in nutritional quality was attributed to a decline in cyanobacteria and their replacement by recovering diatoms, accompanied by an increase in the contents of HUFAs and PUFAs. A similar trend was observed in a laboratory experiment by Barthès et al. (2015), where cyanobacteria that dominated biofilms before disturbance were replaced by diatoms when the dry period lasted at least 4 weeks. However, the nutritional quality of biofilms II, III and IV either remained constant or decreased compared to pre-disturbance state, which may be explained by only partial recovery of diatom proportion. Therefore, the disturbance, through differential responses among taxonomic groups and communities, modified the relative nutritional quality of contrasted biofilms. However, our experimental design does not preclude the possibility that these effects may be transitory, as biofilms are highly dynamic systems. It would have been interesting to assess the extent to which differences in biofilm qualities persist over time, as the dry-period disturbance may have pushed biofilms onto new dynamic trajectories.

Desiccation is a disturbance that severely affects ecosystems (Sarremejane et al., 2022), including benthic communities and algae (Sabater et al., 2017). In our study, even a short 3-day dry period was sufficient to strongly decrease biofilm nutritional quality indicators. Previous research has highlighted the negative effects of rapid drying on cell viability, even during brief dry periods (Stanley et al., 2004). This might have strong implication over the longer term, as riverbank gravels are frequently exposed to flow fluctuations and desiccation (Ledger et al., 2008). Contrary to our third hypothesis, the PCA on biofilm indicators showed that both short and long dry periods affected the nutritional quality of biofilms likewise after drying. However, for biofilms III and IV, we observed a better recovery of nutritional quality indicators following the longer dry period compared to the shorter one. This recovery was partially linked to a better recovery of diatoms after the longer dry period, consistent with the results of Barthès et al. (2015). These authors suggested that the poor recovery of diatoms after short dry periods was the result of direct or indirect negative interactions with cyanobacteria, which were more resistant. For longer dry periods, the increased susceptibility of cyanobacteria to desiccation may have reduced their negative impact on diatoms, allowing for a better recovery of the latter. A similar mechanism may explain the strong effect of dry-period duration on the algal composition of biofilms II and IV. Although the

dry-period duration did not have a significant effect on the secondary production supported by each biofilm, it did have a significant impact on nutritional quality of the biofilms. The longest dry period tested in our experiment was 2 weeks, which falls within the range of dry periods observed in temperate intermittent rivers (Datry, 2012) and in some Mediterranean streams (Tornés et al., 2021).

## 4.2 | Gammarid response to disturbance

The growth rates of gammarids observed in our experiment were consistent with those found in Crenier et al. (2017), who used different resources but the same method to prepare pellets. This indicates that the quantity of resources was not limiting in our experiment. Gammarid growth and physiological state were found to be dependent on the living biomass and nutritional quality of the biofilm. The more Chl-*a* and FA were available in pellets, the more effectively gammarid individuals grew. Similar results were observed in the growth of *Chironomus* along a concentration of resources with different qualities (Goedkoop et al., 2007). FAs are crucial for the growth of consumers, as shown in several macroinvertebrate consumers (Brett et al., 2017; Torres-Ruiz et al., 2007). By contrast, our experiment suggested a lesser role of C:P and C:N ratios in the nutritional quality of biofilms for gammarids. The second axis of the PCA analysis performed on biofilm indicators, mainly associated with these ratios, was not correlated to gammarid response. However, the feeding rate appeared to be decoupled from resource nutritional quality, despite the fact that a relationship between somatic growth of metazoan and food stoichiometry exists (Elser et al., 2000). Stoichiometric ratios may be more predictive when considering resources with more contrasting C, N and P contents, such as allochthonous versus autochthonous resources. Generally, microalgae exhibit C:N ratios that are, on average, three-fold lower than those of terrestrial organic matter (Brett et al., 2017; Elser et al., 2000).

The feeding rate of gammarids was found to be positively correlated with algal biomass, as consumption increased with higher Chl-*a*, P and N contents in the pellets. However, there was no correlation between the feeding rate and the growth rate or condition index of the gammarids, indicating that it was not influenced by the nutritional quality of the biofilm. It is worth noting that some studies have reported compensatory feeding responses, where consumers increase their feeding rate in response to decreasing food quality (e.g., Flores et al., 2014). The present result may be explained by physiological mechanisms. For instance, Graça et al. (1993) demonstrated that *Gammarus pulex* was able to maintain its growth even when fed a low-quality resource by adjusting its respiration rates.

## 4.3 | Secondary production

Despite significant correlations between the main indicators of biofilm quality and gammarid traits, there are some discrepancies between consumer response and secondary production. These



differences were not entirely consistent with the nutritional quality of the biofilms assessed by several indicators describing their composition. Specifically, the strength of the relationship between the expected and effective qualities of biofilms decreased post-disturbance and post-recovery compared to pre-disturbance. For the long dry period at post-recovery, biofilms II and III exhibited contrasting gammarid responses and secondary production despite having the same nutritional quality. This discrepancy may be explained by the significantly higher C:P ratio in biofilm II grown in P-depleted conditions, compared to biofilm III during the post-recovery sampling. Studies have shown that when fed with P-limited resource, the growth of *Daphnia*—a pelagic filter-feeder—is negatively impacted despite sufficient HUFA availability (Ruiz et al., 2021). Negative effects of P limitation on *Daphnia* growth and reproduction also have been observed in other pelagic ecosystems (Boersma, 2000; Ravet & Brett, 2006). Likewise, a negative effect of P-limitation has been shown on gammarid growth (Danger et al., 2013). However, in our study, stoichiometric compositions alone did not explain all of the discrepancies observed between the nutritional quality of biofilms after recovery and the gammarid response, suggesting that the indicators used to describe biofilm nutritional quality do not fully capture the effective quality of these biofilms. As a consequence of growth under different nutrient conditions, biofilms II and III are likely to have had different specific composition, even though the same algal groups were present. Although we did not monitor the species composition of the biofilms in our experiment, it could have had an effect on the gammarid responses, as the nutritional quality of microalgae is species-specific, even within the same algal group (Nagarkar et al., 2004). The contrasting responses of gammarids also may be linked to a differential production of secondary metabolites. Some of these compounds, produced by phototrophic microorganisms, are able to induce adverse effects on aquatic invertebrates (e.g., Bownik, 2016; Pohnert et al., 2007). Phototrophic biofilms are known to produce a wide range of secondary metabolites, including chemical defences against grazers (Pavia et al., 2012). Nutrient limitation can increase the production of toxic secondary metabolites by microalgae (e.g. Granéli & Johansson, 2003). To fully understand the response of invertebrates to their food, it is necessary to consider the chemical defences in algal resources (Cruz-Rivera & Hay, 2003). Finally, the observed decoupling between nutritional quality and estimated secondary production was explained by differences in C availability between biofilms as the nutritional quality significantly increased in some biofilms after recovery, but the quantity remained too low to increase secondary production. This is consistent with previous studies that have highlighted the reduced importance of food quality when resource quantity decreases because resources are used to maintain metabolism rather than growth (Hartwich et al., 2012; Sterner, 1997). This also suggests the importance of resource quantity for secondary production, as already shown in *Gammarus pulex*, where secondary production was enhanced by the available biomass of leaf-associated biofilms (Franken et al., 2005).

Our findings highlight the substantial impact of flow intermittency on phototrophic biofilms and their role as a primary resource

for consumers. This impact exacerbates the mismatch between biofilm nutritional quality and gammarid response. As a result, relying solely on traditional measures of nutritional quality may not be sufficient. To accurately predict the biofilm's ability to support secondary production in intermittent river ecosystems, it is crucial to consider factors such as the hydric legacy conditions that select certain species, the species composition of the algae, the presence of chemical defence mechanisms and the overall resource availability. Taking these factors into account will provide a more comprehensive understanding of the biofilm dynamics and their influence on secondary production in intermittent rivers.

#### AUTHOR CONTRIBUTIONS

Conceptualisation: CC, SB, MD, VF, JL. Developing methods: CC, SB, AB, MD, VF, CP, MRB, JL. Data analysis: CC. Preparation of figures and tables: CC. Conducting the research, data interpretation, writing: CC, SB, AB, MD, VF, CP, MRB, JL.

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#### CONFLICT OF INTEREST STATEMENT

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

#### DATA AVAILABILITY STATEMENT

The data are available from Figshare (doi: [10.6084/m9.figshare.23807736](https://doi.org/10.6084/m9.figshare.23807736)).

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## SUPPORTING INFORMATION

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