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RESEARCH ARTICLE

Disentangling the effects of microalgal diversity and thermal history on freshwater phototrophic biofilms facing heat stress: A thermal dose approach

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Abstract

1. The increase in heatwave intensity and duration is challenging microalgal photosynthesis in shallow waters. In particular, the extent of photosynthesis impairment or limitation due to short extreme heat stress events occurring during a heatwave remains unknown.
2. In this study, we investigated the importance of microalgal diversity and thermal history in response of phototrophic biofilms to severe heat stress. We exposed isolated microalgal strains, synthetic communities previously acclimated at three temperatures, and natural phototrophic biofilms sampled at different seasons to several temperature/duration pairs (from 31 to 44°C for 30, 60, 120 or 180 min). Microalgal photosynthetic activity (F_v/F_M) was measured after each heat stress and was shown to be a function of thermal dose, defined as the product of time and an exponential function of temperature.
3. It appeared that the thermal history plays an important role in tolerance to heat stress, with synthetic communities acclimated at highest temperature being more tolerant. However, the responsiveness (i.e. the strength of the response) to heat stress was driven by the microalgal diversity of the community.
4. *Synthesis*. Our analysis showed that although photosynthetic activity was negatively impacted by heat stress, gross primary productivity was even more strongly and rapidly impacted, highlighting the fact that in situ, even moderate heat stress can significantly impair primary productivity in shallow waters.

KEYWORDS

freshwater microalgae, heatwaves, photosynthetic activity, productivity, thermal dose, thermal tolerance

1 | INTRODUCTION

Many ecosystems are usually exposed to seasonal, as well as daily fluctuations in temperature. In addition to these natural variations, global

warming threatens aquatic ecosystems worldwide through the increase in intensity and frequency of heatwaves (Seneviratne et al., 2021). As photosynthesis, in particular, photosystem II activity, is one of the most heat-sensitive cellular processes (Glatz et al., 1999), it is necessary to

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better understand the effects of heat stress on photosynthetic activity and on the resultant primary productivity. To date, most of previous works on the effects of heat stress on phototrophic organisms have been conducted in marine systems. In pelagic systems, heatwaves generally induce shifts in primary producer community composition (Soulie et al., 2022) together with a decline in species richness (Polazzo et al., 2022) which can ultimately severely impair ecosystem functioning (Frölicher & Laufkötter, 2018). In the intertidal zone, which can already experience extreme heat stress during low tide, both macroalgal and microphytobenthic communities' productivity can be limited by high temperatures (Brito et al., 2013; Laviale et al., 2015; Román et al., 2020; Weitzman et al., 2021). In comparison, the effect of heat stress is poorly documented in freshwater ecosystems, especially for benthic microalgae despite their ecological importance.

Phototrophic biofilms represent the major part of primary production in many shallow water bodies and running aquatic ecosystems (Biggs, 1990; Sánchez et al., 2017). Because of climate change, the prevalence of nonperennial rivers and streams is enhanced (Messenger et al., 2021), and the probability for benthic communities to face heat stress increases (Datry et al., 2014). During low-flow periods, the water level sharply decreases, reducing the thermal inertia of water. Flow cessation enhances the correlation between water and air temperatures, leading to larger daily variations in temperature with notably higher water temperatures in the daytime (Morrill et al., 2005). During heatwaves, some unusually high temperatures can be reached (Gómez et al., 2017; Vidal-Abarca et al., 2002), triggering acute heat stress in phototrophic benthic microorganisms. Given the important functional role of phototrophic biofilms and the predicted climate change scenario, the effects of heat stress on these communities have been understudied.

The individual response to a given temperature is usually defined by the organism's thermal niche, which is species dependent and even strain dependent (Samuels et al., 2021). The thermal niche is defined by a thermal performance curve characterised by an optimum temperature at which performance is maximal (Romaní et al., 2014). Within the curve's optimal range, if the temperature remains below the thermal optimum, some positive effects (e.g. higher growth rate) may be observed with increasing temperature (Angilletta Jr, 2009). When ambient temperature increases over the thermal optimum, several physiological responses are induced that may lead to cell death, depending on the duration and intensity of the stress (Souffreau et al., 2010). Thermal tolerance is associated with cellular stress responses, including the modification of gene expression and the synthesis of heat shock proteins (Horváth et al., 2012). The latter can interact with other peptides and proteins to prevent their denaturation by temperature and the consequent inactivation of cellular processes (Vinocur & Altman, 2005).

The response of communities to heat stress strongly depends on their thermal history, that is, the temperature the community experienced for a given time before the application of the stress. Thermal history can alter thermal niches (Luhring & DeLong, 2017; Padfield et al., 2016) and significantly modify individual tolerance to heat stress. Microalgae previously exposed to temperatures lower than their thermal optimum can enhance their individual tolerance to a new

heat stress, as shown for marine taxa (e.g. Magozzi & Calosi, 2015; Sasaki & Dam, 2019). In contrast, when grown above their thermal optimum, marine diatoms have higher mortality rates during heatwaves (Samuels et al., 2021). For various aquatic taxa, growth at high but sub-lethal temperatures can impair their ability to recover from heat stress (Marbà & Duarte, 2010; Samuels et al., 2021; Siegle et al., 2018). Nevertheless, the tolerance of individuals is not sufficient to infer the response to temperature at the community level. The thermal history induces changes in community composition due to competition and mortality that, in turn, bring changes in tolerance to temperature at the community scale. For instance, in freshwater, cyanobacteria tend to dominate the microalgal community as temperature increases (Watermann et al., 1999). In communities dominated by diatoms, increasing temperatures reduced species diversity (Delgado et al., 2020). Moreover, community diversity may also affect its response to heat stress, in agreement with the insurance hypothesis (Yachi & Loreau, 1999). The association of various species with slightly different thermal niches could increase the general thermal niche of the community, thus allowing better resistance to a changing environment.

Since the effect of heat stress depends on both the duration of the stress and the temperature applied, understanding this effect requires a parameter that summarises both pieces of information. For this purpose, the 'thermal dose' was defined as the product of time and an exponential function of temperature. It has been used for decades in clinical studies, including the application of hyperthermia in cancer therapy (Gerner, 1987). More recently, it was successfully adapted to model the impact of heat stress on microalgal viability and activity to minimise the risk of culture collapses in large-scale outdoor cultivation systems (Béchet et al., 2017). Microalgal viability and photosynthetic activity were both shown to be functions of the thermal dose. The calculation of a 'dose of heat' allowed us to synthesise heat stress and to predict its effect on organisms.

The objective of our study was to understand how phototrophic biofilms respond to short and intense heat stress, as occurs during low-flow periods. We investigated the effects of thermal history and microalgal diversity on the response of photosynthesis to heat using the thermal dose approach. To this end, we performed several temperature experiments using (1) cultivated benthic microalgal strains, (2) simplified microalgal communities obtained by mixing microalgal strains previously acclimated at contrasting temperatures and (3) natural phototrophic communities sampled during different seasons. Each experiment consisted of exposing microalgal samples to a wide range of temperatures (from 31 to 44°C) and durations (from 30 to 180 min). We measured the photosynthetic activity and modelled its response to heat as a function of the thermal dose. We hypothesised that (1) the response of phototrophic biofilms is controlled by the thermal history of the community as the tolerance to heat stress increases with acclimation temperature and (2) the community response to heat stress is also driven by its diversity, which increases its ability to mitigate the heat stress effect. Last, we applied the thermal dose models to temperature fluctuations observed in the field to test and discuss the significance of the thermal dose approach for predicting the effects of heat stress events on river primary productivity.

2 | MATERIALS AND METHODS

Three similar heat stress experiments were conducted on isolated microalgal strains cultivated at 20°C (Experiment 1), on synthetic communities consisting of a mix of three microalgal strains previously acclimated at 15, 20 and 25°C before heat stress experiment (Experiment 2) and on natural biofilms sampled in autumn, winter, midsummer and at the end of summer (Experiment 3).

2.1 | Biological material

2.1.1 | Algal strains

The experiments were conducted on six nonaxenic microalgal strains: two green algae, *Uronema conferviculum* and *Scenedesmus* sp., both isolated from rivers in southwestern France; two diatoms, *Gomphonema parvulum* (SAG-1032-1) and *Nitzschia palea* (CPCC-160); and two cyanobacteria, *Komvophoron* sp. (southwestern France) and *Nostoc* sp. (PCC 7120). All strains were cultivated under controlled conditions (20°C, light/dark cycle 16 h/8 h, 30 µmol. m⁻².s⁻¹). Diatoms and green algae were grown in COMBO medium (Kilham et al., 1998), and cyanobacteria were grown in BG-11 medium (Stanier et al., 1971). All these strains are common benthic species, except *Scenedesmus* sp., a planktonic microalgae that is often found in river biofilms due to sedimentation (Lowe & Gale, 1980). *Scenedesmus* sp., *Gomphonema parvulum* and *Komvophoron* sp. were also acclimated at 15°C or 25°C for 5 months in the same light and medium conditions as for strains cultivated at 20°C. To ensure the good physiological state of the culture, the medium was renewed 3 days before each experiment.

For Experiment 1, the cell density of each microalgal strain suspension was chosen so that the chlorophyll fluorescence signal was high enough to be easily detected by the PHYTO-PAM instrument (Phytoplankton Analyser, Heinz Walz GMBH). The chosen concentrations depended on cell biovolume and pigment concentration and covered a range from 5.10⁶ to 8.10⁷ cell.ml⁻¹. For Experiment 2, synthetic microalgal communities were produced by mixing one strain of each main algal group, that is, *Scenedesmus* sp., *Gomphonema parvulum* and *Komvophoron* sp. in equivalent biomass proportions. Three synthetic communities were produced with the same microalgae acclimated at 15°C, 20°C or 25°C. The cell concentrations of strains in the mix were chosen to reach a total biovolume of approximately 1 10⁶ µm³.cell⁻¹ (Table S1).

2.1.2 | Natural communities

Experiment 3 was performed on natural phototrophic biofilms. Biofilms were sampled four times throughout the year, in November 2019 (autumn), January 2020 (winter), July 2020 (midsummer) and September 2020 (endsummer), on a gravel bank in the river Garonne (43°23'47.5"N 1°17'58.9"E, southwestern France). Biofilms were

scrapped from 15 hand-sized pebbles with a toothbrush directly on site and suspended in river water. Returning to the laboratory, the 1-l suspension was homogenised in river water with an ultraturax (T25, Janke-Kunkel) at 1000g before filtration to 500 µm to remove coarse sediments and macrofauna. For each sampling date, the biofilm was diluted so that the initial F₀, a proxy of algal biomass measured with PHYTO-PAM after dark adaptation, was the same. The diluted suspension was kept overnight at 20°C in the dark with bubbling before heat stress exposure.

To assess the thermal history of natural communities in the river, water temperature was measured every 5 min from November 2019 to September 2020 with one data recorder (TG-4100, TinyTag) positioned at the bottom of the water line. Additional biofilm samples were preserved in formaldehyde (4%) for microscopic analysis to characterise the microalgal composition. After a first observation under a microscope that was completed to identify soft bodied algae, sulphuric acid (98%) was added to eliminate organic matter and to allow for only diatom frustules to remain for diatom species identification. For each dominant species, the average length and width were retrieved from the literature (Table S2), and the theoretical biovolume was estimated using the appropriate geometric formula (Hillebrand et al., 1999). The relative abundance of each dominant species could then be calculated based on biovolume: only species that represented more than 1% in terms of biovolume were considered to calculate diversity indices. The taxonomic diversity dominating each sample was assessed using richness, Shannon diversity and Pielou equitability indices.

2.2 | Heat stress exposure

Microalgal and biofilm suspensions were exposed to temperatures from 31 to 44°C, with one-degree increments, for 30, 60, 120 or 180 min. The tested temperature ranges were adjusted for each strain based on preliminary range-finding experiments. The first set of preliminary experiments (data not shown) was performed to design an alternative approach to performing each experimental treatment (heat stress) at a similar point in the day/night cycle of our cultures. Thus, the following approach was used: heat stresses were applied between 8 am (2 h after the start of the usual light period) and 6 pm, and the temperature conditions were randomly distributed along the day of experiment (e.g. stresses at 35, 38 and 41 degrees in the morning and stresses at 36, 37, 39 degrees in the afternoon). Moreover, prior to and during heat stress exposure, each biological material was kept under low light condition for 12 h to ensure circadian rhythm does not influence the response of microalgae to temperature. Stress exposures were conducted in a water bath in 1.5-ml tubes with 200 µl of biofilm or microalgal suspension in each, in six replicates for natural biofilms (Experiment 3) and four replicates for laboratory cultures (Experiments 1 and 2) since variability between replicates was lesser with single strains and synthetic communities than natural biofilms. For each experiment, unstressed 1.5-ml tubes were used as controls ($n = 4$ for Experiments 1 and

2, $n = 6$ for Experiment 3). At the end of each exposure, the tubes were placed in a chamber at 20°C or at temperature acclimation for Experiment 2 under low light intensity (neon tubes, $5 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 24 h to recover. The recovery duration was based on Béchet et al. (2017); it allowed optimisation of the numerous measurements.

2.3 | Photosynthetic activity

The photosynthetic activity was estimated 24 h after heat stress using the pulse amplitude modulated (PAM) fluorescence technique with a PHYTO-PAM instrument (Phytoplankton Analyser, Heinz Walz GMBH) following the protocol described by Barthès et al. (2015). For the measurements, microalgal or biofilm suspensions were first transferred from the tubes into black 96-well plates and then dark-acclimated for 20 min. The maximum photochemical quantum yield of photosystem II (F_v/F_m , Kitajima & Butler, 1975) of each sample was estimated by placing the optic fibre above each well. The obtained values were expressed as the percent of the mean value of the corresponding controls.

2.4 | Thermal dose

To analyse the response of photosynthetic activity for each combination of exposure duration and temperature, we adapted the concept of thermal dose that Béchet et al. (2017) developed to model the viability and photosynthetic activity of *Dunaliella salina* cultures in response to high temperatures (Figure 1).

For each tested temperature, we first used the Weibull equation to model the response of the photosynthetic activity $Y(t)$ (% of the control) as a function of the exposure duration t (min) using Equation 1, where λ is the abscissa of the inflexion point (min) and n is the shape factor (Figure 1a):

$$Y(t) = Y(0) \exp\left(-\left(\frac{t}{\lambda}\right)^n\right). \quad (1)$$

We assumed a normal distribution of model errors, that is, discrepancies between the data and the theoretical model. Only temperatures that induce at least a 20% loss in photosynthetic activity were considered to ensure a reliable estimation of parameters; otherwise, the model was unable to estimate λ . Second, the inflexion points λ produced by Equation (1) were expressed as an exponential function of temperature T (°C) using Equation (2), where $T_{0,Y}$ is the temperature (°C) at which photosynthetic yield reaches zero, and a_Y is the exponential coefficient (°C⁻¹) (Figure 1b):

$$\log(\lambda) = a_Y(T - T_{0,Y}). \quad (2)$$

For each experiment and for each biological material, the obtained linear regression was tested through ANCOVA to test whether a_Y or $T_{0,Y}$

could be fixed among communities or strains depending on the experiment for parsimony. For Experiments 2 and 3, the a_Y was thus fixed, as coefficients were not significantly different among communities.

Based on both Equations (1) and (2), the thermal dose d_Y (min) was defined for each combination of temperature and time as follows:

$$d_Y = t \exp(-a_Y(T - T_{0,Y})). \quad (3)$$

Finally, the photosynthetic activity $Y(t)$ (% of the control) was represented as a function of the thermal dose d_Y (min) by fitting a Weibull model:

$$Y(t) = Y(0) \exp\left(-\left(d_Y\right)^n\right). \quad (4)$$

For each experiment, as there was no relationship between n and temperature (Equation 1, Table S3), we used for Equation (4), the mean value of the estimations obtained using Equation (1). The obtained parameter λ' (min) is the inflexion point, and b (% min⁻¹) is the slope. In our study, the inflexion point λ' was used as a tolerance parameter, as it represents the thermal dose the algal sample was able to tolerate: the higher the lambda, the higher the thermal dose required to disrupt photosynthetic activity (Figure 1c). The slope of curve b was used as a responsiveness parameter, as it represents the ability of algae to cope with stress: the higher the slope is, the quicker the response to heat stress is initiated (Figure 1d).

Data analysis was conducted in R software (v 3.6.1) using the R package `DRC`. For each parameter, 95% confidence intervals were calculated using the function '`confint.drc`' and were used to test differences in responses to heat stress between biological materials. If confidence intervals did not overlap, differences were considered significant.

2.5 | Application to field conditions

To test the significance of the thermal dose approach for predicting the effects of heat stress events on in situ primary productivity, it was necessary to establish the link between photosynthetic activity and river phototrophic biofilm primary productivity. We performed an additional experiment on a natural phototrophic biofilm to establish the relationship between the loss of photosynthetic activity and the loss of gross primary productivity (GPP) due to heat.

The biofilm was collected in September 2020, and the suspension was prepared as described before. In this experiment, we assumed that the relationship between productivity and photosynthetic activity does not depend on exposure duration. One 30-min heat stress was applied to phototrophic biofilm suspensions in triplicate for 12 temperatures ranging from 32°C to 44°C. For each of the three replicates, one 15-ml tube was filled with 7.2 ml of biofilm suspension and placed in a water bath at an adequate temperature. After 30 min, the tube was removed and placed in a water bath at 20°C. Unstressed controls were prepared the same way and maintained at 20°C in triplicate. After homogenisation, 200 µl of each

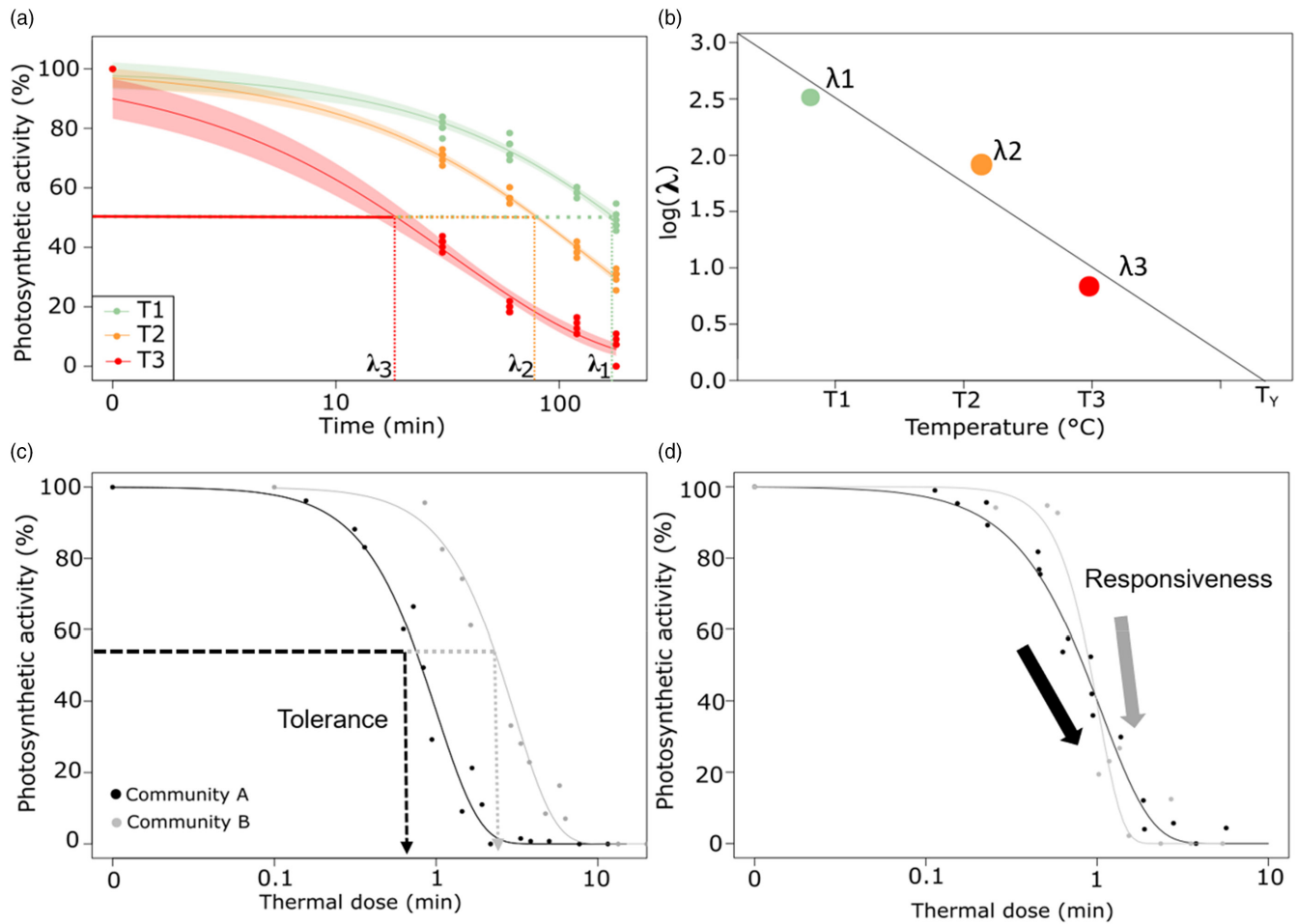


FIGURE 1 Thermal dose approach adapted from B chet et al. (2017)). (a) The inflexion point λ was estimated for each tested temperature using the Weibull relationship between the photosynthetic activity and the exposure duration according to Equation (1). (b) The linear regression between $\log(\lambda)$ and temperature allowed us to estimate the exponential coefficient a_Y and the estimated temperature at which the photosynthetic activity reaches zero $T_{0,Y}$ using Equation (2). Both parameters were required for the thermal dose calculation using Equation (3). In plots (c) and (d), the photosynthetic activity was represented as a function of the thermal dose using Equation (4). Curve parameters represent tolerance (inflexion point, λ') and responsiveness (slope, b) to thermal dose. In (c), communities have the same responsiveness to heat stress, but community A is more tolerant than community B, while in (d), both community tolerances are similar, but community A is less responsive than community B.

tube was immediately transferred into a black 96-well plate and kept for 20 min in the dark before measuring photosynthetic activity with the PHYTO-PAM instrument. The remaining volume of biofilm suspension was transferred into an 8-ml transparent vial closed with a gas tight lid for the assessment of oxygen production with FireSting oxygen sensors (PyroScience). Measurements were conducted under low magnetic agitation. Concentrations of dissolved oxygen were monitored every 5 s for 5 min under light (neon lamp, $90 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to measure the net primary productivity (NPP), followed by 5 more min in the dark to measure oxygen consumption (respiration). The GPP was calculated as the sum of NPP and respiration. Losses of photosynthetic activity and GPP were expressed as the percent of the mean values of the corresponding controls. The relationship between the loss of photosynthetic activity and the loss of GPP was then described through regression of a logarithmic equation.

Then, we used the thermal doses previously calculated on mid-summer and endsummer biofilms for every combination of exposure duration and temperature to evaluate the potential impact of the summer temperatures measured in the field in a disconnected branch of the river from mid-July to August 2020. The effective thermal doses inducing 10%, 25%, 50%, 75% and 90% photosynthetic activity loss were estimated using the function 'ED' of the package DRC. The thermal dose values were then projected on a heatmap using effective thermal doses as thresholds to visualise the potential loss of photosynthetic activity for different combinations of duration and temperature. This heatmap scale was transformed to visualise the potential loss of GPP using the relationship between photosynthetic activity and GPP previously established. The high summer temperature events measured in the field were positioned on the heatmaps considering the heat stress events independently.

3 | RESULTS

3.1 | Microalgal strains

Green algae had the highest photosynthetic activity, with a mean (\pm SD) F_V/F_M in controls equal to 0.75 (\pm 0.01) and 0.70 (\pm 0.01) for *Scenedesmus* sp. and *U. conferviculum*, respectively. For diatom controls, it was equal to 0.68 (\pm 0.05) for *G. parvulum* and to 0.70 (\pm 0.01) for *N. palea*. Cyanobacteria had the lowest photosynthetic activity, with values of 0.33 (\pm 0.02) for *Komvophoron* sp. and 0.32 (\pm 0.01) for *Nostoc* sp. The threshold temperature inducing a negative response of photosynthetic activity was variable between species. Most of the strains were impacted by heat stress at a temperature equal or above 39°C (Figure S1), while for *Scenedesmus* sp. the threshold temperature was 41°C. *Nostoc* sp. was impacted by all tested temperatures, but F_V/F_M never reached 0, while 1 h of exposure at 41°C was enough to fully inhibit photosynthetic activity in *Komvophoron* sp.

The effects of exposure duration and temperature on photosynthetic activity were synthesised using the thermal dose approach (Figure 2a). The tolerance of microalgal strains to heat stress did not significantly differ between algal groups or species (Figure 2b). However, diatom tolerance appeared to be more variable than for other strains, as shown by the relatively high 95% confidence interval in *G. parvulum* and *N. palea* in comparison to other species. In contrast to tolerance, responsiveness to heat stress was strain dependent (Figure 2c). *Nostoc* sp. was less responsive than other species, including the other cyanobacteria species *Komvophoron* sp.,

while diatoms, and notably *Gomphonema* sp. were more responsive than cyanobacteria and green algae.

3.2 | Synthetic communities

The mean F_V/F_M (\pm SD) of the control synthetic communities was 0.63 (\pm 0.01), 0.66 (\pm 0.01) and 0.55 (\pm 0.01) for communities acclimated at 15°C, 20°C and 25°C, respectively. The tested temperatures above 41°C had a significant effect (Figure S2). The thermal dose approach showed that tolerance to heat stress increased with the acclimation temperature (Figure 3a,b): the synthetic community was three times more tolerant to heat stress when composed of microalgal strains acclimated at 25°C than with microalgae acclimated at 20°C. The synthetic community obtained by mixing microalgae acclimated at 15°C was less tolerant than that obtained by mixing microalgae acclimated at 20°C. In contrast to tolerance, the responsiveness of synthetic communities to heat stress did not depend on the acclimation temperatures (Figure 3c).

3.3 | Natural communities

Unstressed samples had a mean (\pm SD) F_V/F_M of 0.55 (\pm 0.02) in autumn, 0.62 (\pm 0.02) in winter, 0.37 (\pm 0.02) in midsummer and 0.52 (\pm 0.03) in the endsummer. All tested temperatures had a negative effect on the photosynthetic activity of phototrophic biofilms. The response of biofilms was gradual (Figure S3). Thermal dose analysis

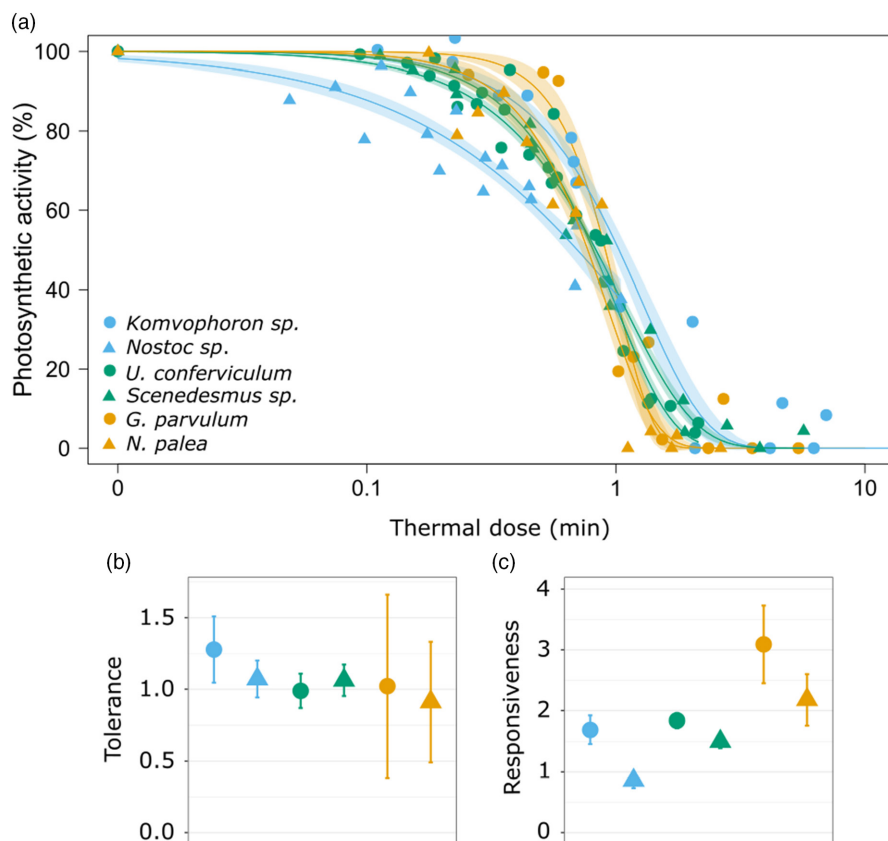


FIGURE 2 (a) Photosynthetic activity response (% control) of microalgal strains (cyanobacteria, green algae and diatoms) to thermal dose (eq. min), (b) tolerance (λ' , inflexion point) to heat stress and (c) responsiveness (b, slope) to heat stress for microalgal strains acclimated at 20°C for cyanobacteria (blue), green algae (green) and diatoms (brown). Model-specific confidence intervals and estimated parameters \pm 95% confidence interval are represented for each microalgal strain ($n = 4$).

highlighted differences in tolerance and responsiveness to heat stress between communities (Figure 4). Winter biofilms were the least tolerant to heat stress, endsummer biofilms were the most tolerant, and

midsummer and autumn biofilms had the same intermediate value of tolerance (Figure 4b). The endsummer community was less responsive to heat stress than the autumn and winter communities (Figure 4c).

FIGURE 3 (a) Photosynthetic activity response (% control) of synthetic communities to thermal dose (eq. min) depending on acclimation temperature, (b) tolerance (λ' , inflexion point) to heat stress and (c) responsiveness (b , slope) to heat stress for synthetic communities acclimated at 15°C (blue), 20°C (pink) and 25°C (red). Model-specific confidence intervals and estimated parameters $\pm 95\%$ confidence intervals are represented for each synthetic community ($n = 4$).

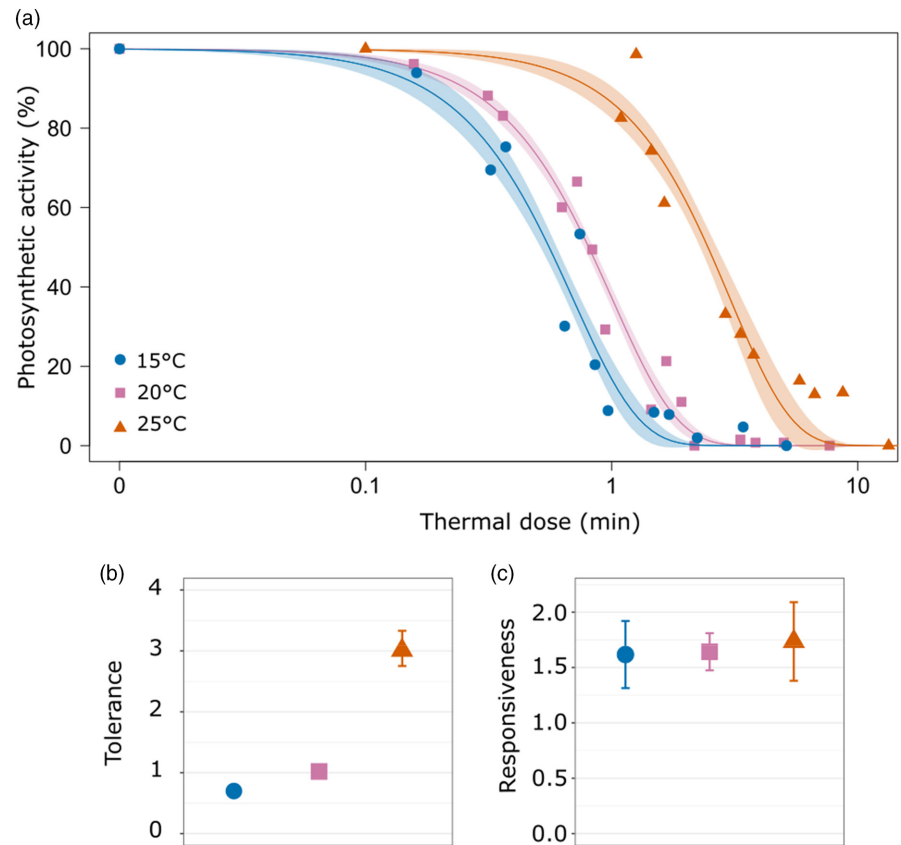
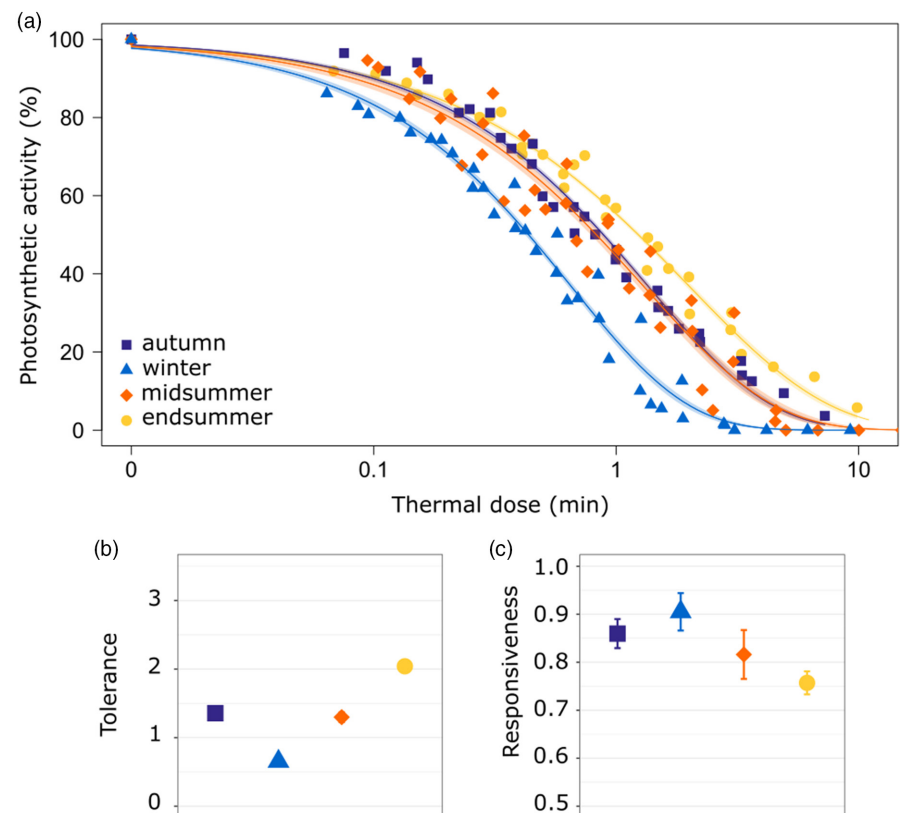


FIGURE 4 (a) Photosynthetic activity response (% control) of natural biofilms to thermal dose (eq. min) depending on the sampling period, (b) tolerance (λ' , inflexion point) to heat stress and (c) responsiveness (b , slope) to heat stress for natural communities in autumn (purple), winter (blue), midsummer (orange) and endsummer (yellow). Model-specific confidence intervals and estimated parameters $\pm 95\%$ confidence intervals are represented for each natural community ($n = 6$).



The temperatures recorded in the river 27 days before sampling are reported in Table 1a and Figure S4. For autumn and winter, the mean temperature and amplitude were relatively low compared to the two other communities, while for midsummer and endsummer, the temperatures experienced were higher but also more variable, as shown by the larger thermic amplitude. The midsummer algal community had the lowest species richness, diversity and equitability indices (Table 1b). For autumn biofilms, large (biovolume $>500\mu\text{m}^3$) and very large ($>1000\mu\text{m}^3$) species accounted for 80% of the total biovolume. For winter and endsummer biofilms, very large species represented more than 60% of the total biovolume. In contrast, in the midsummer biofilm, the species contributing more to the total biovolume were small (biovolume $<200\mu\text{m}^3$) (Figure S5). It was dominated at 80% of the total biovolume by the cyanobacteria *Leptolyngbya* sp. (Figure S6). The other biofilms were mainly composed of diatoms. In winter and endsummer biofilms, the high-sized diatom *Diatoma vulgare* was the dominant species in biovolume (60% and 40% total biovolume, respectively). In the autumn biofilm, *D. vulgare* and *Nitzschia* species dominated (30% of the biovolume contribution each; Figure S6). In addition, autumn and endsummer biofilms were more diversified, and species were more evenly distributed (Table 1b).

3.4 | Comparison between experiments

Communities acclimated at higher temperatures (i.e. synthetic community acclimated at 25°C and endsummer phototrophic biofilm) were more tolerant than other tested biological materials (Figure 5a). On the other hand, the responsiveness was more variable and hardly predictable within strains, as it seems to depend on the observed species. Generally, natural communities were less responsive than microalgal strains and synthetic communities (Figure 5b).

3.5 | Application to field conditions

The last objective of the study was to compare the models of biofilm physiological response to heat stress to in situ conditions to provide insights into the potential impact of a summer heat wave in the natural environment. To do this comparison, we performed a new heat stress experiment to relate photosynthetic activity and GPP (Figure 6). In this experiment, unstressed biofilms had a mean F_V/F_M (\pm SD) of 0.54 (\pm 0.01) and a mean GPP (\pm sd) of 0.015 (\pm 0.001) $\text{mg O}_2\cdot\text{h}^{-1}$. After exposure at 44°C for 30 min, F_V/F_M decreased to

TABLE 1 (a) In situ thermal history in natural biofilms. Mean temperature represents the mean water temperature experienced during the 27 days before biofilm sampling, values in brackets are the mean minimal and the maximal temperatures measured during this period. Mean (min and max) daily temperature amplitudes represent the mean (min and max) of water temperature daily amplitudes over the 27-day period and (b) specific diversity indices of the algal community

	Autumn	Winter	Midsummer	Endsummer
(a)				
Mean temperature (min-max) (°C)	11.5 (11.0–12.2)	7.5 (7.0–8.4)	19.7 (18.2–21.6)	21.6 (19.0–24.8)
Mean daily temperature amplitude (min-max) (°C)	1.2 (0.7–3.4)	1.4 (0.4–4.5)	3.4 (0.8–8.1)	5.8 (2.6–9.7)
(b)				
Species richness	15	14	9	17
Diversity (Shannon)	2.21	1.61	0.78	2.12
Equitability (Pielou)	0.82	0.61	0.36	0.75

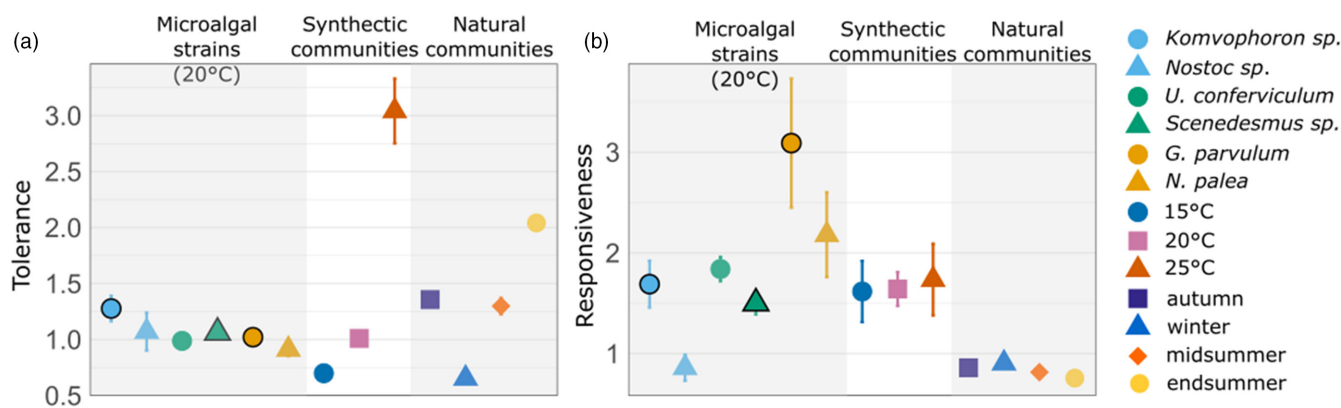


FIGURE 5 (a) Tolerance (λ' , inflexion point) and (b) responsiveness (b , slope) to heat stress of microalgal strains ($n = 4$), synthetic communities ($n = 4$) and natural communities ($n = 6$). Each point represents the mean \pm 95% confidence interval of parameters for different tested samples in each experiment. Strains used in synthetic communities are represented with black outlines.

0.11, and GPP decreased to 0 mg O₂·h⁻¹. The loss of GPP due to heat stress logarithmically increased with the loss of photosynthetic activity (ANOVA, $r^2 = 0.955$, p value < 0.001, Figure 6). From 35 to 39°C, GPP was three times more affected by heat stress (loss from 0% to 60%) than the loss of photosynthetic activity (loss from 0% to 20%). In contrast, from 40 to 44°C, the GPP loss decreased, while photosynthetic activity was strongly reduced (from 30% to 80% loss).

Then, we used the in situ monitoring water temperatures to identify extreme events during which primary productivity can be impacted. These events were then projected on heatmaps constructed for the midsummer and endsummer biofilms (Figure 7a). For that projection, thermal doses were calculated for combinations of exposure duration and temperatures and translated into levels of effects from previous results of GPP loss (Figure 6). The effects of heat

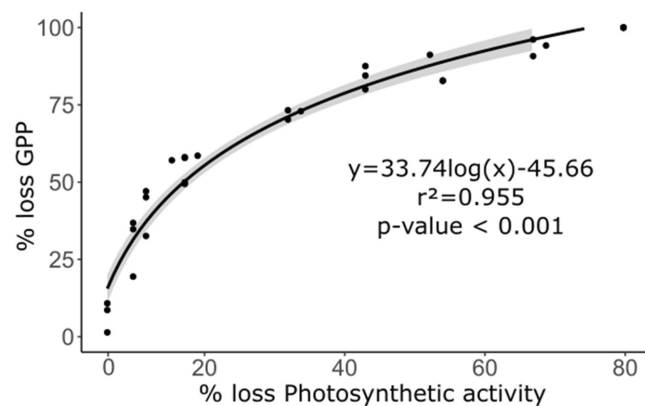


FIGURE 6 Relationship between the loss of photosynthetic activity (% F_v/F_m) and the loss of gross primary productivity (% GPP) of natural biofilms after exposure to one 30-min heat stress at temperatures from 35 to 44°C.

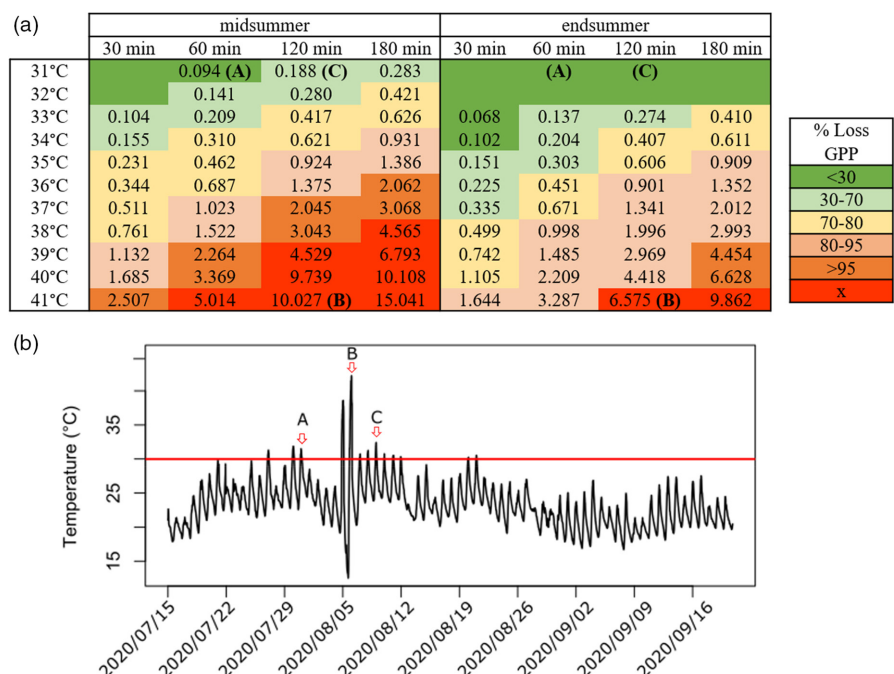
stress depended on the biofilm sampling period and on the level of effects measured (i.e. GPP; Figure 7a): endsummer biofilms could be affected by heat stress from 33°C, while exposures of midsummer biofilms at 31 and 32°C for more than 1 h could induce losses of GPP.

Finally, we used heatmaps to estimate the effect of temperatures measured in situ on natural communities. The water temperature exceeded 30°C in the afternoon several days in summer (Figure 7b), especially between the 8th and 12th of August, with events higher than 31°C for 180 min. Temperature exhibited extreme values on the 6th and 7th of August, reaching 41°C during more than 2 h on the 7th. We positioned three heat stress events (i.e. A, B and C) on heatmaps as examples to estimate their potential effect on natural communities in the field. Event A, which corresponded to exposure at 31°C for 60 min, only impacted the midsummer community by inducing a 30% loss of GPP. Event C corresponding to exposure at the same temperature for 120 min would induce between 30% and 70% loss of GPP. In contrast, the endsummer community would not be impacted by events A and C, as the water temperature would remain below the critical threshold of 33°C. For both midsummer and endsummer communities, event B would have induced a complete inhibition of GPP.

4 | DISCUSSION

We used the thermal dose approach to investigate the relative contribution of thermal history and diversity to the response of benthic freshwater microalgae to extreme heat stress. The thermal dose approach allows us to clearly and easily compare the response of photosynthetic activity between experiments thanks to the two metrics calculated using the Weibull model. We compared Weibull with logistic model, and selected the Weibull model because it fitted

FIGURE 7 (a) Heatmaps for midsummer and endsummer biofilms transformed into levels of effects depending on the threshold calculated from photosynthetic activity and primary production losses. Values inside boxes are thermal doses calculated from models (in min). Empty boxes indicate that heat stress has no effect. (b) Measured temperature in the field from mid-July to August 2020. The red line corresponds to a threshold of 30°C, and A, B and C correspond to the chosen peaks, with: 60 min at 31°C, B: 120 min at 41°C and C: 120 min at 31°C. The data logger was positioned in a branch of the Garonne River, temporally disconnected from the main stream during the low water period.



our data the best (lowest AIC). In addition, it is commonly used for dose–response representations in ecotoxicological studies, as it was found to improve the fit compared to other models (Forfait-Dubuc et al., 2012; Ritz, 2010). It has been widely used in food research for modelling microbial heat inactivation as a nonlinear response (Smelt & Brul, 2014; van Boekel, 2002). The thermal dose we used in our study enables a full and more refined examination of community responses compared to a simple threshold analysis of ED50.

4.1 | Effect of thermal history

The temperature at which microalgal strains grow controlled the tolerance of their photosynthetic activity to heat stress, confirming our first hypothesis. Indeed, all our microalgal strains, which were cultivated in the same laboratory conditions for one decade prior, showed the same tolerance to heat stress, while the synthetic communities acclimated at different temperatures exhibited significant differences in tolerance. The higher the acclimation temperature is, the better the synthetic microalgal communities tolerate high heat doses. Such increased tolerance has been observed in marine copepods: a 4°C increase during development enables better thermal tolerance (Sasaki & Dam, 2019). Similarly, phytoplankton communities acclimated to thermal conditions above their optimum exhibited a better tolerance to heat stress (Baker & Geider, 2021). In our study, the relationship between acclimation temperature and tolerance of photosynthetic activity of synthetic microalgal communities was nonlinear, with an increase in tolerance four times higher when acclimation temperature increased from 20°C to 25°C than from 15°C to 20°C. For two species belonging to the genera *Scenedesmus* and *Gomphonema*, 25°C was the optimal growth temperature (Guedes et al., 2011; Rose, 2008), and it is likely that species grown near their optimal temperature could exhibit a better tolerance to heat stress. Such an increase in tolerance might be due to changes in cell physiology owing to growth temperature. For instance, it has been shown that marine diatoms grown at 35°C accumulate more saturated and monounsaturated fatty acids at the cost of polyunsaturated fatty acids than diatoms grown at lower temperatures (Scholz & Liebezeit, 2013). A similar phenomenon was observed in natural communities, with an increase in the concentration of polyunsaturated fatty acids in cooler autumn (Whorley & Wehr, 2018). The decrease in unsaturated fatty acids has been considered responsible for the high-temperature tolerance of photosynthesis in a mutant of *Chlamydomonas reinhardtii* (Sato et al., 1996). Fatty acid profiles seem to be important to tolerate heat stress since a thermotolerant diatom, *Chaetoceros muelleri*, displayed greater changes in fatty acid composition than *Phaeodactylum tricorutum*, which is thermosensitive (Rousch et al., 2003). Such biochemical changes could explain the observed increased tolerance to heat stress in the experiment where synthetic communities were grown at 25°C.

The comparison between the results observed for natural communities sampled in winter and sampled in summer also tends to highlight the effect of thermal history on biofilm tolerance to heat

stress. In the natural environment, thermal conditions combined with other factors, such as irradiance, can induce the selection of more thermophilic species. For instance, some species from the genera *Achnanidium*, *Cocconeis* and *Nitzschia* are able to survive at very high temperatures (Delgado et al., 2020) and were present in summer and autumn but not in winter in our samples (see Figure S6). Another explanation for the better tolerance of summer biofilms to heat stress could be the high amplitude in temperature experienced by the biofilms before the experiment (see Figure S4). It has been shown that regular temperature fluctuations can have positive effects, such as increased growth rates (Gill et al., 2022). Similarly, in another experiment conducted on the marine diatom species *Thalassiosira pseudonana*, it was shown that growth in a fluctuating environment enhanced the tolerance to heat stress through increased genetic divergence over generations (approximately 1 year; Schaum et al., 2018). In addition, an experiment conducted on two seagrass species pointed out that previous exposure to thermal stress enhanced the ability to cope with heat stress compared to unstressed plants, notably through the reduced expression of genes related to stress (Nguyen et al., 2020).

4.2 | Effect of microalgal diversity

In addition to thermal history, microalgal assemblage diversity is likely an important factor for explaining the heat stress tolerance of freshwater biofilms, as biofilms sampled in midsummer and at the end of summer displayed slight differences in their tolerance while experiencing very similar thermal conditions. Microalgal species richness and diversity might explain such differences in community response to heat stress. The midsummer biofilm was less diversified than the endsummer biofilm, which exhibited the highest microalgal richness and diversity. In addition, endsummer biofilm showed the lowest evenness. When communities are dominated by a single or low number of species, their tolerance/resistance to environmental stress is reduced, as has been shown in denitrifying bacterial communities exposed to salinity stress (Wittebolle et al., 2009), while a higher diversity induces better resistance to environmental stresses, as shown in a meta-analysis conducted on microbial communities and plants (Hong et al., 2022). While tolerance to heat stress did not significantly differ between the studied microalgal strains, the responsiveness of photosynthesis varied between microalgal species. Differences in responsiveness to heat stress were overall significant when comparing responses of laboratory assemblages with natural ones. Whatever the sampling period, complex microalgal communities were less responsive to increasing heat stress than microalgal strains and synthetic communities, validating our second hypothesis. The reduced variability in responsiveness for natural communities supports the insurance hypothesis. In a study where the diversity level of ciliates and bacteria was manipulated, the authors showed that differences in species response to environmental changes helped stabilise the effects of temperature fluctuations (Leary & Petchey, 2009). To

explain the link between diversity and stability, Loreau and de Mazancourt (2013) notably argued that the response of species to stressors is asynchronous and that species do not respond at the same speed to stress. Such mechanisms might explain the observed difference in responsiveness between synthetic and natural communities and the buffering effect of diversity observed in our study (Figure 5b). This is consistent with another study carried out on phytoplankton communities exposed to heat stress (+4°C for 7 h per day), which demonstrated that natural communities were better adapted to the disturbance than artificial communities (Striebel et al., 2016). In an experiment carried out on phytoplankton where the species richness was manipulated and exposed to different heat stress (+4°C), the authors highlighted the importance of complementarity between species to maintain growth and phosphorus content (Schabhüttl et al., 2013). Similar results have been observed in other microbial communities living in soil ecosystems: they showed enhanced stability after exposure to heat stress due to increased diversity (Tardy et al., 2014). These results support the importance of diversity in communities to reduce the responsiveness of photosynthesis to heat stress.

4.3 | Field application of thermal dose

The objective of this study was to apply the thermal dose model to data collected in the field to predict the response of phototrophic biofilms *in situ*. On some days in summer, the temperature measured in the river exceeded 30°C for several hours (Figure 7b). Despite the relatively low number of such critical days, our analysis highlighted the fact that a short extreme event, for instance, 1-h exposure at 35°C, could decrease the photosynthetic activity by 30% 24 h after the stress. The effects of such short extreme events on benthic primary productivity were all more significant, as the relationship between photosynthetic activity and GPP was nonlinear (Figure 6). A 30% loss in photosynthetic activity would correspond to at least a 70% loss of GPP. In addition, the response of biofilm depended on the community composition, with the mid-summer biofilm being less tolerant to heat stress than the end-summer biofilm. Contrary to the photosynthetic activity that can be maintained at a quite high level thanks to a few more resistant species, GPP depends on biofilm biomass, with lower biomass implying lower productivity and therefore a stronger decrease due to heat stress.

The application of the thermal dose approach to natural communities and heatmap construction provided interesting information regarding the potential impact of extreme heat temperature events in the field. Some additional experiments should be conducted to decipher the potential additive properties of thermal doses. In the field, the temperature increase occurs more gradually than what was experimentally tested, generating a succession of several heat stresses, such as those applied in this study (e.g. 1 h at 31°C + 2 h at 32°C + 1 h at 33°C). When facing a succession of such events during summer heat waves, phototrophic biofilms

may acclimate and ultimately maintain a certain level of primary productivity. Several studies have pointed out the enhanced stability of microbial communities after repeated disturbances, such as heat stress (Renes et al., 2020; Samuels et al., 2021) or acidification stress (van Moorsel et al., 2021). However, other studies have underscored opposite results, with a decreasing stability of communities because of stress repetition (e.g. Jurgburg et al., 2017). Prior exposure to high temperatures can increase the sensitivity of organisms to heat stress. For instance, in a study conducted on marine diatoms grown at different temperatures and exposed to different heat wave intensities, it was shown that when grown at suboptimal temperatures, the survival of algae decreased after a heatwave (Samuels et al., 2021). Similarly, the thermal tolerance and survival of copepods was reduced when organisms were previously exposed to prior sublethal temperatures (Siegle et al., 2018). The response of aquatic microbial communities to repeated heat stress could depend on the intensity, frequency and duration between repetitions (Dal Bello et al., 2019) but also on the predictability of temperature fluctuations (Gill et al., 2022).

5 | CONCLUSIONS

Our work provides clear insight into the effects of short heat stress on the tolerance and responsiveness of microalgae at various levels of diversity. We found that the tolerance of communities to stress depended on thermal history or temperature acclimation, while the responsiveness of the photosynthetic activity of the whole community rested on its diversity because natural communities were less responsive than synthetic communities. In addition, we found that a slight decline in photosynthetic activity could have a major impact on oxygen productivity and that during low flow periods in intermittent rivers or in shallow waters, when the thermal inertia is low, primary productivity can be strongly reduced. This may threaten the whole ecosystem as trophic chains would be impacted. The thermal dose approach appears to be a relevant tool to understand and compare the effect of heat stress and could be useful to predict the impact of a summer heat wave in the field, even if it needs further investigation.

AUTHOR CONTRIBUTIONS

Martin Laviale, Joséphine Leflaive and Stéphanie Boulêtreau conceived the ideas. The methodology was designed with the help of Camille Courcoul. Camille Courcoul, Joséphine Leflaive and Stéphanie Boulêtreau proceeded the laboratory work. Camille Courcoul analysed the data with the help of Vincent Baillard, Elise Billoir and Martin Laviale. Camille Courcoul led the writing with the help of Joséphine Leflaive and Stéphanie Boulêtreau. All authors contributed to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.14058>.

DATA AVAILABILITY STATEMENT

The data are available from Figshare <https://doi.org/10.6084/m9.figshare.20485311> (Courcoul et al., 2022).

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