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# **Dissipation of pesticides by stream biofilms is influenced by hydrological histories**

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## 1 Abstract

2 To evaluate the effects of hydrological variability on pesticide dissipation capacity by stream  
3 biofilms, we conducted a microcosm study. We exposed biofilms to short and frequent droughts  
4 (daily frequency), long and less frequent droughts (weekly frequency) and permanently immersed  
5 controls, prior to test their capacities to dissipate a cocktail of pesticides composed of tebuconazole,  
6 terbuthylazine, imidacloprid, glyphosate and its metabolite aminomethylphosphonic acid. A range of  
7 structural and functional descriptors of biofilms (algal and bacterial biomass, extracellular polymeric  
8 matrix (EPS) concentration, microbial respiration, phosphorus uptake and community-level  
9 physiological profiles) were measured to assess drought effects. In addition, various parameters were  
10 measured to characterise the dynamics of pesticide dissipation by biofilms in the different  
11 hydrological treatments (% dissipation, peak asymmetry, bioconcentration factor, among others).  
12 Results showed higher pesticide dissipation rates in biofilms exposed to short and frequent droughts,  
13 despite of their lower biomass and EPS concentration, compared to biofilms in immersed controls or  
14 exposed to long and less frequent droughts. High accumulation of hydrophobic pesticides  
15 (tebuconazole and terbuthylazine) was measured in biofilms despite the short exposure time (few  
16 minutes) in our open-flow microcosm approach. This research demonstrated the stream biofilms  
17 capacity to adsorb hydrophobic pesticides even in stressed drought environments.

## 18 Keywords

19 Microbial communities; hydrological variability; pesticide cocktail; artificial streams; pesticide  
20 dissipation

## 21 1. Introduction

22 Among freshwater microbial communities, benthic consortia, henceforth called biofilms, are  
23 assemblages of heterotrophic and autotrophic microorganisms (algae, fungi, bacteria, and  
24 cyanobacteria, among others) embedded in a self-produced matrix of extracellular polymeric  
25 substances (EPS) composed of polysaccharides, proteins, glycoproteins, and phospholipids, enhancing  
26 interactions among microbial cells (Battin *et al.* 2016). In streams, microbial cells can grow on hard  
27 surfaces such as cobbles and rocks, on soft surfaces (*i. e.*, leaf litter or wood) or in sediments, where  
28 they can obtain nutrients (Romaní *et al.* 2013) and organic compounds (including pesticides)  
29 (Edwards and Kjellerup 2013) from either the water column or the substratum itself. Biofilms  
30 contribute to the self-depuration capacity of stream waters, an important ecosystem service resulting  
31 from the removal of nutrients from water column (Saltarelli *et al.* 2021). Less studied is the  
32 attenuation capacity of pesticides such as diuron (Vercaene-Eairmal *et al.* 2010; Chaumet *et al.*  
33 2019), glyphosate (Klátyik *et al.* 2017; Carles *et al.* 2019) or mixtures of pesticides (*e. g.*, mesotrione,

34 S-metolachlor, and nicosulfuron; Carles *et al.* 2017). Pesticide contamination is considered one of the  
35 greatest threats to freshwater ecosystems (Malaj *et al.* 2014; Bernhardt, Rosi and Gessner 2017).  
36 Agricultural activities (*i. e.*, vineyards or cereal crops (Fernández *et al.* 2015; de Souza *et al.* 2020;  
37 Rydh Stenström, Kreuger and Goedkoop 2021; Bordin *et al.* 2022) modulate the quantity and  
38 mixtures of pesticides reaching surface waters (Cui *et al.* 2020). Aquatic biofilms have the ability to  
39 deal with pesticide contamination through accumulation, transformation and/or degradation processes  
40 (Lawrence *et al.* 2001; Sabater *et al.* 2007; Edwards and Kjellerup 2013). Biofilm's EPS matrix may  
41 contribute to pesticide dissipation thanks to its sorption capacity in sequestering cations, anions,  
42 apolar compounds and particles from the water phase (Schorer and Eisele 1997; Flemming and  
43 Wingender 2001).

44 Pesticide dissipation capacity of biofilms can be affected by global change environmental stressors,  
45 including those related to climate change (*i. e.*, increased severity of flood and drought events),  
46 affecting the overall streams hydrology. Hydrological variations due to climate change and/or  
47 anthropic activities (*i. e.*, irrigation or hydropeaking) can cause different frequencies and durations of  
48 drought events in streams. Hydrological variations caused by hydropeaking, generating short and  
49 frequent droughts (daily frequency, Li and Pasternack 2021), and by agricultural practices, generating  
50 longer and less frequent droughts (weekly frequency, Courcoul *et al.* 2022), are those mainly affecting  
51 rivers and streams in south-western Europe. Hydrological changes (*i. e.*, drought episodes and high  
52 flow events) have been shown to be one of the most relevant stressors to biofilm structure and  
53 function (Romero *et al.* 2019). The study of droughts frequency and duration in aquatic microbial  
54 communities has been addressed in experimental manipulation in the field or using mesocosms (Colls  
55 *et al.* 2021). It has been observed that different duration and frequency of droughts decrease microbial  
56 densities and affect metabolism as occurred on extracellular enzymatic activities (Timoner *et al.* 2012;  
57 Romaní *et al.* 2013), microbial respiration (Gionchetta *et al.* 2020a), phosphorus uptake capacity  
58 (Proia, Romaní and Sabater 2017) or carbon degradation ability by microbial communities (Perujo,  
59 Romaní and Martín-Fernández 2020). It has been described that long-term drought (5 months)  
60 exposure induces metabolic changes in biofilms, increasing the degradation of recalcitrant organic  
61 matter, and enhancing the formation of EPS in sediment microbial communities (Gionchetta *et al.*  
62 2019). Drought-related effects on biofilms may thus enhance biofilms capacity to degrade recalcitrant  
63 organic molecules, including pesticides.

64 Temporal water scarcity and dry conditions in freshwater ecosystems induce structural and functional  
65 adaptations in biofilms (Timoner *et al.* 2012; Feckler, Kahlert and Bundschuh 2015; Romero *et al.*  
66 2019), such as EPS production. EPS contributes to the stability of the biofilm structure, commonly  
67 influenced by environmental changes such as temperature, nutrients availability and hydrological  
68 pressures (Flemming *et al.* 2023) as observed in several studies (Schmitt *et al.* 1995; Zhang *et al.*

69 2014; Gionchetta *et al.* 2019). Pesticide molecules retained in the EPS (Lubarsky *et al.* 2012;  
70 Chaumet *et al.* 2019) can be degraded by extracellular enzymes in the EPS (Flemming, Neu and  
71 Wingender 2016) or by intracellular oxidative processes (*e. g.* Krauss *et al.* 2011). Therefore, EPS is  
72 expected to enhance the capacity of biofilms to retain and degrade toxic molecules, provide structural  
73 stability, and increase their stress resistance to contaminants (Zhang *et al.* 2015). Accordingly, the  
74 importance of exploring pesticide interactions with aquatic biofilm communities and their dissipation  
75 on aquatic ecosystems is essential not only for single pesticide molecules but for complex cocktails of  
76 pesticide molecules present in the aquatic environment (Mayer *et al.* 1999; Stehle and Schulz 2015).

77 The main objective of this study was to investigate how modified hydrological scenarios (short  
78 droughts of high frequency and long droughts of short frequency) may affect the capacity of biofilm  
79 for pesticide dissipation. Specifically, we aim to decipher which structural (*i. e.*, biofilm and algal  
80 biomass, bacterial cell density and viability, EPS content, algal composition and diatoms viability)  
81 and functional (*i. e.*, microbial respiration, phosphorus uptake capacity and carbohydrates  
82 metabolism) changes in the autotrophic and heterotrophic components of drought-exposed biofilms  
83 could be responsible for changes in pesticide dissipation capacities. The study is methodologically  
84 addressed as environmentally realistic as possible by: i) working with a cocktail of pesticides [one  
85 fungicide (tebuconazole), two herbicides (terbuthylazine, glyphosate and its metabolite  
86 aminomethylphosphonic acid), and one insecticide (imidacloprid)] selected among the most common  
87 pesticide groups detected in European watersheds (Mohaupt *et al.* 2020); ii) the combination of  
88 different pesticide molecules can induce different responses in microbial communities due to their  
89 physicochemical properties, concentration in water and biofilm characteristics, thus modifying their  
90 toxicokinetic and toxicodynamic properties, which can affect their dissipation in the aquatic  
91 environment (Hernández, Gil and Lacasaña 2017); and iii) assessing the pesticide dissipation in a  
92 continuous open-flow approach. While most studies examine pesticides' dissipation by aquatic  
93 microbial communities in batch approaches (*e. g.*, Carles *et al.* 2017; Rossi *et al.* 2021) and by  
94 ecotoxicological studies (*e. g.* diuron and triclosan; Proia *et al.* 2011; diuron, imazalil, prochloraz,  
95 simazine and chlorpyrifos; Romero *et al.* 2019), the novelty of this study was to assess the dissipation  
96 of a short pulse of a pesticide cocktail by biofilms in a continuous open-flow approach. Our main  
97 hypothesis is that biofilms affected by different hydrological scenarios would present specific  
98 structural and functional attributes influencing their performance to dissipate cocktails of pesticides in  
99 stream ecosystems. We specifically examined the total biofilm biomass and the specific EPS  
100 accumulation as potentially participating in the dissipation of the pesticide cocktail. We expected that  
101 biofilms with greater EPS content, specifically those exposed to longer droughts of low frequency (*e.*  
102 *g.* Gionchetta *et al.* 2019), would accumulate more pesticide molecules, improving their dissipation  
103 capacity. However, droughts are also expected to reduce total biofilms biomass and thus reduce the

104 sorption capacity for pesticides. Apart from the EPS role, microbial densities and metabolisms highly  
105 affected by droughts could potentially impair the responses of biofilm microorganisms to their  
106 functional capacities for ecosystem services, including pesticide dissipation. Our second hypothesis is  
107 that hydrophobic molecules present in the pesticide cocktail (tebuconazole and terbuthylazine,  $\log K_{ow}$   
108 = 3.70 and 3.40, respectively) are expected to accumulate more than hydrophilic molecules  
109 (glyphosate and AMPA,  $\log K_{ow}$  = -3.20 and -2.17, respectively) in biofilms due to their intrinsic  
110 physicochemical differences (Bonnineau *et al.* 2021; Desiante, Minas and Fenner 2021).

## 111 2. Materials and methods

### 112 2.1. Experimental design

113 Nine artificial streams were setup in the laboratory to test the effect of droughts frequency and  
114 duration on stream biofilms' structure and functions, including their capacity for pesticides  
115 dissipation. These artificial streams, henceforth called microcosms, were composed by PVC channels  
116 with 1% of slope (length x width x depth = 2 x 0.014 x 0.010 m) connected with a 35 L plastic tank  
117 (upstream) and to a 20 L glass aquarium downstream (Figure 1). The system was filled with  
118 dechlorinated tap water (BRITA P1000; active carbon filter) and recirculated using a pump (New-Jet  
119 1200, 1200 L h<sup>-1</sup>). A 0.5 cm plastic mesh was placed at 5 cm from the top of the channel to reduce  
120 water turbulence inside the channel generated by the water tank release. The microcosms were  
121 exposed to 14 h light and 10 h dark cycle (EASY LED, 6800 K full spectrum, Aquatlantis) mimicking  
122 the late of Spring photoperiod. Air temperature was fixed at 18 °C.

123 Aquatic biofilms were grown on unglazed glass tiles fixed on concrete slabs placed during three  
124 weeks in the Veyre stream (France – 45°40'15.7''N, 3°06'57.8''E; Table S1). Biofilms were then  
125 transported to the laboratory and placed at the bottom of each microcosm and acclimated to laboratory  
126 conditions for four weeks. The water recirculating in the microcosms was renewed twice a week  
127 during the experiment to avoid nutrients depletion in the system and provide new microbial inoculum  
128 (1 L per microcosm at each water renewal) from the stream where biofilms were grown.

129 After the acclimatisation phase of biofilms, three different hydrological treatments were simulated in  
130 triplicate in the microcosms: a control condition with biofilms permanently immersed in water (IC), a  
131 condition with biofilms exposed to short droughts of high frequency (HF\_SD = 1 day immersed + 1  
132 day drought, daily frequency; hydropeaking scenario), and a condition with long droughts of low  
133 frequency (LF\_LD: 1 week immersed + 1 week drought, weekly frequency; agriculture scenario).  
134 Sampling of water and biofilms were always performed in wet and light conditions. Water and  
135 biofilm samples were collected from each microcosm at S1 = day 14, S2 = day 28, S3 = day 42, S4 =  
136 day 45 and S5 = day 56. Biofilm samples were obtained by scrapping glass tiles and suspending

137 scrapped materials in 40 mL of previously filtered water (0.2  $\mu\text{m}$  pore size nylon filters, Merck) from  
 138 the corresponding microcosm. Structural (total biofilm biomass, extracellular polymeric substances  
 139 concentration, microalgae and bacteria cell densities and viability, chlorophyll-*a* concentration) and  
 140 functional (community-level physiological profiles, microbial respiration, and phosphorus uptake  
 141 capacity) descriptors of biofilms were measured at each sampling time for each hydrological  
 142 condition in triplicate.

143 After four weeks (day 44) of biofilms exposure to the three hydrological treatments, 10 mL of a  
 144 cocktail of pesticides composed by terbuthylazine (TBT; 4.5  $\text{mg L}^{-1}$ , purity >98%, CAS: 5915-41-3),  
 145 tebuconazole (TBZ; 35  $\text{mg L}^{-1}$ , purity >98%, CAS: 107534-96-3), imidacloprid (IMID; 35  $\text{mg L}^{-1}$ ,  
 146 purity >98%, CAS: 138261-41-3), glyphosate (GLY; 35  $\text{mg L}^{-1}$ , purity >98%, CAS: 1071-83-6) and  
 147 its metabolite aminomethylphosphonic acid (AMPA; 35  $\text{mg L}^{-1}$ , CAS: 171259-81-7), all of them  
 148 obtained from Sigma-Aldrich (Table 1), was spiked at the top of each channel (before stream  
 149 biofilms) in continuous open-flow conditions to reach effective concentrations of 8.12  $\mu\text{g L}^{-1}$  (TBT)  
 150 and 63.18  $\mu\text{g L}^{-1}$  (TBZ, IMID, GLY, and AMPA) in the bottom of each artificial stream. The lower  
 151 concentration of TBT compared to the rest of molecules in the cocktail is explained by its lower  
 152 solubility in water. After the spiking, a total of 12 water samples were collected at the end of each  
 153 channel (after stream biofilms) in order to quantify the mass of pesticides remaining after being in  
 154 contact with stream biofilms (Figure 1). The previous day of pesticides spiking, 10 mL of a  
 155 conservative tracer solution ( $\text{NaCl}$ , 5.54  $\text{g L}^{-1}$ ) was added at the top of each channel and electrical  
 156 conductivity (EC) was recorded at the end of the channel to determine the hydrological characteristics  
 157 (water velocity, discharge) of each microcosm following methods described in the Stream Solute  
 158 Workshop (1990) (Table S2). From these hydrological data, we determined the specific sampling  
 159 times to collect the 12 water samples at the end of the channels and quantify the mass of pesticides  
 160 remaining (*i. e.* not accumulated or transformed by biofilms). Samples were taken at a frequency  
 161 ranging from a few seconds to a few minutes, after spiking (time 0) and up to 22.5 minutes. Water  
 162 samples consisted of 50 mL of non-filtered water to determine concentrations of neutral pesticides  
 163 (TBT, IMID and TBZ) and 50 mL of filtered water (0.45  $\mu\text{m}$  sterilized cellulose filter, Merck) to  
 164 determine concentrations of GLY and AMPA. Samples were stored at -20  $^{\circ}\text{C}$  until analyses. At the  
 165 end of the experiment, three glass tiles were scrapped from each microcosm and filtered in a pre-  
 166 weighted GF/F filter (Whatman) to determine concentrations of pesticides accumulated in the  
 167 biofilms. Biofilms in filters were lyophilized and stored at -20  $^{\circ}\text{C}$  until analyses of pesticides content.

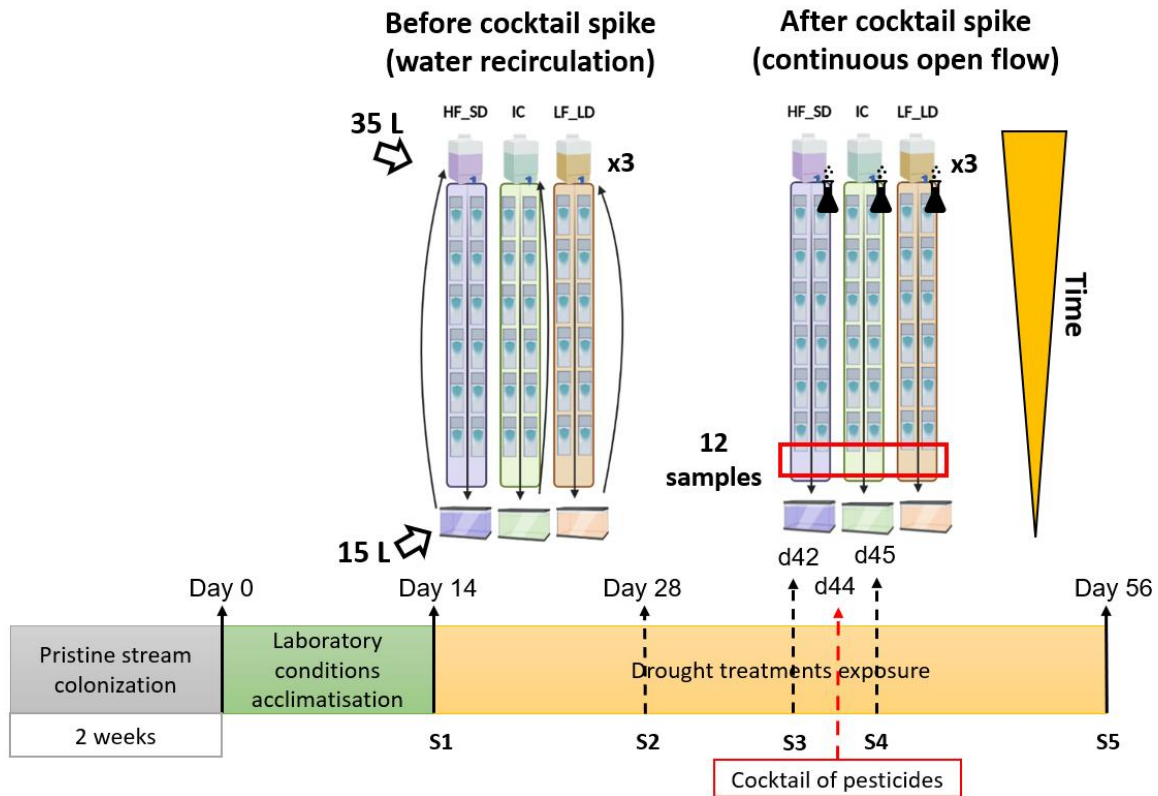
168 *Table 1. Cocktail pesticide molecules characteristics: solubility, octanol-water partition coefficient*  
 169 *(Log  $K_{ow}$ ), and dissipation time in water (DT50).*

Pesticide molecule	Solubility ( $\text{mg L}^{-1}$ )	Log $K_{ow}$ (partition octanol/water)	DT50 water phase (days)
Tebuconazole (TBZ)	36.0	3.70	120-597

Glyphosate (GLY)	10500.0	-3.20	13.8-301
AMPA	146.6	-2.17	9.28-9.64
Terbutylazine (TBT)	6.6	3.40	22.40
Imidacloprid (IMID)	610.0	0.57	184

170

171



172

173 *Figure 1. Experimental design of hydrological treatments before (recirculating conditions) and after*  
 174 *(open flow conditions) the spike of the pesticide cocktail. Hydrological treatments are named as*  
 175 *HF\_SD (high frequency and short duration of drought), LF\_LD (low frequency and long duration of*  
 176 *drought), and IC (immersed control). Samples collection calendar before and after the spike of the*  
 177 *pesticide cocktail (in red) is also represented (S1 = day 14; S2 = day 28; S3 = day 42; S4 = day 45;*  
 178 *S5 = day 56).*

## 179 2.2. Physical and chemical analyses of water

180 Physicochemical parameters such as electrical conductivity, dissolved oxygen concentration and  
 181 saturation, water temperature (Pro DSS 4-port Digital Sampling System, YSI, U.S), light intensity  
 182 (Testo 545 lux meter) and pH (FiveEasy F20, Mettler Toledo) were measured at each sampling date  
 183 and microcosm. Water samples for dissolved nutrients concentration determination were previously  
 184 filtered through 0.2  $\mu\text{m}$  nylon filters (Merck) and analysed through spectrophotometric methods  
 185 following Murphy & Riley (1962) for  $\text{P-PO}_4^{3-}$  and TDP (after basic digestion) and using the  $\text{N-NO}_3^-$   
 186 kit-test (Spectroquant  $\text{\textcircled{R}}$ , Merck). Dissolved organic carbon (DOC), total dissolved carbon (TDC),



187 dissolved inorganic carbon (DIC) and total dissolved nitrogen (TDN) were analysed using a TOC  
188 sampler (TOC<sub>VC</sub>PN, Shimadzu, Japan).

## 189 2.3. Biofilm structural analyses

### 190 2.3.1. Bacterial density and viability

191 Total bacterial density (TBD) in biofilm samples was measured after sonicating twice for 60s to  
192 favour bacteria disaggregation followed by a centrifugation step at 800 g for 60 s. The supernatant  
193 obtained was diluted in a Tris-EDTA buffer solution x100 (1 M Tris, 0.1 M EDTA) and double  
194 stained with 100 µM SYBR Green and 1 mg mL<sup>-1</sup> propidium iodide (PI) in order to distinguish live  
195 from dead bacterial cells (Invitrogen) according to Borrel *et al.* 2012. The results are given in  
196 percentage of live cells and total bacterial density (TBD) per unit of biofilm surface area in cm<sup>2</sup>.

### 197 2.3.2. Chlorophyll-a concentration, microalgal density and total biofilm biomass

198 Chlorophyll-*a* concentration was measured as a proxy for algal biomass. Chlorophyll-*a* concentration  
199 was determined following Jeffrey & Humphrey (1975) using acetone 90 % as extractant. To improve  
200 pigment extraction, biofilm extracts were sonicated for 4 min at 37 KHz (Ultrasonic bath FB 1504,  
201 Fischer Scientific). The extracts were centrifuged at 800 g for 5 min at 6 °C and then absorbances at  
202 430, 665 and 750 nm were measured in the supernatant using BIOMATE 3S UV-Visible  
203 Spectrophotometer (Thermo Fisher Scientific). Chlorophyll-*a* concentrations are expressed as µg chl-  
204 *a* per cm<sup>2</sup>.

205 On S5 (day 56), microalgal density was assessed using an aliquot (1 mL) from biofilm suspensions.  
206 From each homogenized subsample, 125 µL were dropped onto a Nageotte counting chamber  
207 (Marienfeld, Germany), after appropriate dilution (10 to 100-fold). Ten counting grids were randomly  
208 analysed and the number of cells of live chlorophytes, diatoms and cyanobacteria, as well as dead  
209 diatoms, were enumerated. Results were expressed as cells cm<sup>-2</sup> and as percentage of diatoms viability  
210 (Morin *et al.* 2010).

211 Total biofilm biomass was measured from biofilm suspensions that were first centrifuged at 800 g  
212 during 20 min to pellet the biofilm and afterwards, dried in aluminium trays at 60 °C for 48 h to obtain  
213 dry weight (DW) and burned at 450 °C for 5 h to obtain ash free dry weight (AFDW) for organic and  
214 inorganic composition of biofilm. Biofilm DW and AFDW were weighted in a precision balance  
215 (PRECISA, 80A-200M, SWISS QUALITY). Results were expressed as mg AFDW cm<sup>-2</sup>.

216 Biofilm microbial carbon (C) was calculated using conversion factors permitting to transform  
217 chlorophyll-*a* and bacteria to algal and bacterial carbon units. Briefly, algal biomass was calculated  
218 based on the ratio algal carbon : chlorophyll-*a* = 60 (Geider, MacIntyre and Kana 1996) and bacterial

219 biomass considering  $2.2 \times 10^{-13}$  g C  $\mu\text{m}^3$  (Bratbak and Dundas 1984) with a mean bacterial cell  
220 biovolume of  $0.1 \mu\text{m}^3$  (Theil-Nielsen and S ndergaard 1998).

### 221 2.3.3. Extracellular polymeric substance matrix in biofilms

222 The concentration of glucose equivalents in the extracted extracellular polymeric substances (EPS)  
223 matrix in biofilms were measured using a cation exchange resin (CER, Dowex Marathon C sodium  
224 form, Sigma-Aldrich). CER was previously conditioned following (Roman  *et al.* 2008). Afterwards,  
225 the content of polysaccharides in the extracted biofilm EPS was measured by the phenol/H<sub>2</sub>SO<sub>4</sub> –  
226 Assay (Dubois *et al.*, 1956) at 485 nm absorption (UV-1800 spectrophotometer, Shimadzu). EPS was  
227 expressed as  $\mu\text{g}$  glucose-equivalent  $\text{cm}^{-2}$ .

## 228 2.4. Biofilm functional analyses

### 229 2.4.1. Community level physiological profiles (CLPP)

230 CLPP was tested by 96-well Biolog Ecoplates   which produce a purple gradient response in relation  
231 to carbon substrates utilisation. Biofilm suspensions were diluted at equal densities  $1 \times 10^6$  cells  $\text{mL}^{-1}$   
232 and inoculated to Ecoplates (150  $\mu\text{L}$  per well). Plates were incubated at 22.5  C and absorbance at  
233 596 nm was measured every 24 h (up to 192 h) using a plate reader (Multiskan<sup>TM</sup> FC Microplate  
234 Photometer, ThermoFisher Scientific). Most wells achieved sigmoid colour saturation, establishing  
235 their average well colour development (AWCD) close to 96 h. Raw absorbance data were corrected  
236 by the absorbance of the blank wells (Gionchetta *et al.* 2020b).

237 Different CLPP descriptors were determined at 96 h of incubation such as functional richness (S),  
238 average well colour development (AWCD), Shannon-Wiener diversity index ( $H'$ ) and evenness ( $E$ )  
239 calculated as means of evaluating diversity in carbon source consumption. Additionally, kinetic  
240 parameters such as slope of AWCD, half-time to reach 50% of the slope (DT50) and  $\beta_{\text{max}}$  associated to  
241 the plateau level of the sigmoidal function were determined using the R package *sigmoid*. Carbon  
242 sources were divided in different groups according to Fr c *et al.* (2012) and Gryta *et al.* (2014) for  
243 further analyses (more details in Table S3).

### 244 2.4.2. Microbial respiration

245 Microbial respiration of biofilm communities was measured using the resazurin method according to  
246 Gionchetta *et al.* (2020a). An entire colonized glass tile from each microcosm was incubated with 100  
247  $\mu\text{g L}^{-1}$  resazurin (final concentration) in buffered pH 8 water from the corresponding microcosm.  
248 Incubations were run in the dark at 18  C, under soft orbital shaking (40 rpm). Additionally, an abiotic  
249 sample (sterile water without biofilm) was also incubated and subtracted from biofilms respiration.

250 The monitoring of resazurin reduction into resorufin was performed by fluorescence measurements at  
251 two excitation/ emission wavelengths ( $\lambda_{\text{ex}}$  602/  $\lambda_{\text{em}}$  616;  $\lambda_{\text{ex}}$  570/  $\lambda_{\text{em}}$  585) on the

252 spectrofluorometer (SFM 25, Kontron Instruments, Italy) at 2, 4, 6, 24 and 48 h, respectively.  
253 Microbial respiration in biofilms was expressed as  $\mu\text{g}$  resazurin  $\mu\text{g}$  microbial  $\text{C}^{-1}$  hour $^{-1}$ .

### 254 2.4.3. Phosphorus uptake capacity

255 Phosphorus uptake rate of biofilms was determined at S3 (day 42), S4 (day 45) and S5 (day 56),  
256 following the methodology described by Proia et al., (2017). We used  $\text{KH}_2\text{PO}_4$  as stock solution  
257 to sextuplicate the background concentration of inorganic phosphate ( $50 \mu\text{g L}^{-1}$  to  $350 \mu\text{g L}^{-1}$ ,  
258 respectively) in water. An entire colonised glass tile from each microcosm was incubated for 300 min  
259 in a 100 mL flask containing dechlorinated tap water with an stream water inoculum (1:32 v:v) and  
260 the spike of the stock solution. Incubations were performed under controlled temperature ( $18 \text{ }^\circ\text{C}$ ) and  
261 light conditions (14h:10h, light:dark cycle), under orbital agitation (70 rpm). Further inorganic  
262 phosphorus ( $\text{P-PO}_4^{3-}$ ) concentrations were analysed in 5 ml water samples collected at 1, 30, 60, 90,  
263 120, 180, 300 min after the spike of the stock solution using the Spectroquant kit-test for phosphate  
264 (Merk). An abiotic control was run in parallel to ensure a non-significant abiotic decrease in  
265 phosphorus concentration during the experiment. Phosphorus uptake was normalised by microbial  
266 carbon and time units.

## 267 2.5. Pesticide dissipation

### 268 2.5.1. Pesticides analyses

#### 269 2.5.1.1. HPLC-MS/MS analysis for neutral pesticides from water and biofilm samples

270 Water samples for neutral pesticides (IMID, TBZ and TBT) analyses were previously filtered on  
271 Whatman® Puradisc 13 syringe filters (cellulose acetate membrane, pore size  $0.45 \mu\text{m}$ ), from which 1  
272 mL was fortified at  $10 \text{ ng } \mu\text{L}^{-1}$  of an internal standard solution (imidacloprid d4, tebuconazole d6 and atrazine d5).  
273 The samples were further analysed through HPLC-MS/MS. For neutral pesticides in biofilm matrixes, 10 mg  
274 (dry weight) of lyophilized biofilm sample were fortified at  $1 \text{ ng } \mu\text{L}^{-1}$  of a surrogate solution  
275 (monuron d6, prometryn d6, simazine d5), and extracted twice on acetonitrile before evaporation in  
276 nitrogen. The resulting pellet performed a solid phase extraction (SPE) purification step by  
277 Chromabond HR-X SPE cartridges (3 mL, 60 mg, Macherey-Nagel, France) placed on a Visiprep  
278 (Supelco), conditioned initially with methanol:UPW (5mL, v/v). The final elution step was performed  
279 by flushing the cartridge with acetonitrile, previously dried under nitrogen, and fortified at  $10 \text{ ng } \mu\text{L}^{-1}$   
280 of the initial solution of internal standards.

281 Both the neutral pesticides from water samples and biofilms were analysed with Dionex Ultimate  
282 3000 HPLC (Thermo Fisher Scientific, Villebon-sur-Yvette, France). Chromatographic separation was  
283 performed with a Gemini-NX C18  $3\mu\text{m}$ , 110 A,  $100 \times 2 \text{ mm}$  with a Security Guard cartridge Gemini-NX C18  $4 \times$   
284  $2.0 \text{ mm}$  (Phenomenex, Le Pecq, France). Detection was performed with an API 2000 tandem mass spectrometer  
285 (Sciex, Villebon-sur-Yvette, France). Further details about mass parameters and chromatographic conditions can

286 be found in Lissalde *et al.* 2011, Poulier *et al.* 2021. The limits of quantification in these analyses are provided in  
287 Table S4. For further details of the chemical analysis see Supplementary material B.

### 288 2.5.1.2. HPLC-MS/MS analysis for glyphosate and AMPA from water samples

289 GLY and AMPA analyses were performed following the recommendations of the project ISO/DIS 16308  
290 (Water Quality – Determination of GLY and AMPA- Method using high performance liquid  
291 chromatography (HPLC) with tandem mass spectrometry detection). The water samples were  
292 analysed by HPLC-MS/MS with the same instruments for IMID, TBZ and TBT. Reversed phase  
293 separation was performed on a X-Bridge C<sub>18</sub> 3.5 µm, 2.1 x 50 mm protected by a precolumn X-Bridge C<sub>18</sub> 2.1 x  
294 10 mm (Waters, Le Pecq, France). Further details related to mass spectrometry parameter or gradient elution can  
295 be found in Fauvelle *et al.* 2015. Limits of quantification were 0.05 ng mL<sup>-1</sup> for both compounds, as indicated in  
296 Table S4. Further details of the chemical analysis were detailed in Supplementary material B.

### 297 2.5.1.3. Bioconcentration factor (BCF) in biofilms

298 The BCF in stream biofilms at the end of the pesticide cocktail pulse was only calculated for neutral  
299 pesticide molecules (IMID, TBT and TBZ) (Wang 2016) as the ratio between the pesticide  
300 concentration in biofilms and in water (Eq.1). This factor was calculated considering both absorption  
301 and adsorption processes by biofilms. This parameter determines the capacity of biofilms to uptake  
302 the pesticide cocktail from the water column.

$$303 \quad BCF = \frac{[Pesticide]_{Biofilms}}{[Pesticide]_{Water}} \quad (Eq. 1)$$

### 304 2.5.2. Pesticide dissipation determination by biofilms

305 The pesticide dissipation capacity of biofilms was measured using a mass balance approach (*e. g.*  
306 Baynes, Dix and Riviere 2012). The difference between the mass of pesticides spiked at the upper part  
307 (10 mL of the pesticide cocktail described in section 2.1) and the lower part of the channel was  
308 calculated by integrating the trapezoid area under the curve (AUC) resulting from the 12 water  
309 samples collected from each channel (Eq.2) (Baynes, Dix and Riviere 2012) normalised by the water  
310 flow ( $Q$ ) (Eq.3). AUC integration of the different pesticide concentrations over time ( $dt$ ) was obtained  
311 by *DescTools* R package. Pesticide dissipation rates (%) measured for biofilms in the three  
312 hydrological treatments were corrected by the abiotic pesticide dissipation in the microcosm system.

$$AUC = \int_0^{\infty} Concentration * dt \quad (Eq. 2)$$

$$Q = \frac{EC \text{ Stock solution NaCl} * Volume \text{ stock solution NaCl added}}{\int_0^{\infty} EC * dt} \quad (Eq. 3)$$

313 Other parameters were measured in the mass balance approach to calculate the dynamics of pesticide  
314 dissipation in the different experimental treatments including: i) the peak height (in  $\mu\text{g L}^{-1}$ ), ii) the  
315 time to rise the peak height (in seconds), and iii) the asymmetry of the peak (ratio between peak  
316 height and width). These parameters have been used to compare dissipation between hydrological  
317 treatments and pesticide molecules.

## 318 2.6. Statistical analyses

319 Water physicochemical characteristics (temperature, dissolved oxygen, conductivity, nutrients, among  
320 others) and biofilm descriptors (total biomass, EPS content, bacterial density and viability,  
321 chlorophyll-*a* concentration, respiration, phosphorus uptake capacity, and CLPP parameters) were  
322 compared among hydrological treatments and time by two-way repeated measures ANOVA (factor I  
323 = hydrological treatment, factor II = time). For this two-way ANOVA test we considered data from  
324 S2 to S5 and excluded S1 since hydrological treatments were not yet applied in the latter. Natural  
325 logarithm transformation was applied to all these descriptors to achieve normality and  
326 homoscedasticity of the data according to the tested factors. Post-hoc pairwise t-student p-adjusted  
327 Bonferroni's tests were performed to determine differences between hydrological treatments and time  
328 by *rstatix* R package, excepting chlorophyll-*a* concentration by Kruskal-Wallis with pairwise Wilcox  
329 tests. Moreover, microalgal densities and diatom viability measured in the last sampling day (S5)  
330 were tested through one-way ANOVA accompanied by a post-hoc test as described above, except for  
331 cyanobacteria densities which was tested through Kruskal-Wallis.

332 Raw CLPP data was analysed by compositional data (CoDa) following repeated measures of  
333 multivariate analysis of variance (MANOVA) (Perujo, Romaní and Martín-Fernández 2020) and the  
334 Pillai statistical test to distinguish potential functional fingerprinting differences between hydrological  
335 treatments, with graphical representation in canonical variate plots. All measured variables were first  
336 assessed by Pearson's and Spearman's correlations to check for collinearity previous to Principal  
337 Component Analysis (PCA) by *corrplot*, *FactoMineR* and *factoextra* R packages. The PCA permitted  
338 to distinguish structural and functional responses descriptors according to hydrological treatments and  
339 time effects before and after the spike of the cocktail of pesticides (S3-S4-S5).

340 Finally, the capacity of biofilms to dissipate the cocktail of pesticides (% of dissipation, peak height,  
341 time to reach the peak and peak asymmetry for each molecule) were compared between hydrological  
342 treatments using the non-parametric Kruskal-Wallis test with pairwise Wilcox-tests with p-adjusted of  
343 Benjamini-Hochberg (BH) in non-parametric tests (*tidyr* R package), excepted for peak asymmetry  
344 that were compared using the parametric one-way ANOVA test accompanied by Tukey test pairwise  
345 comparisons (*multcomp* and *multcompView* R package). All statistical analyses were set at 5% of  
346 significance level using RStudio version 2022.07.2+576.

## 347 3. Results

### 348 3.1. Water physicochemical characteristics

349 Water physicochemical parameters were monitored in the nine microcosms at each sampling date  
350 (Table S1) and showed non-significant differences between hydrological treatments (Table S5).  
351 However, time differences were observed for electrical conductivity (EC), pH, dissolved oxygen  
352 (concentration and saturation) and dissolved organic carbon (DOC) (Table S5). For instance, the  
353 percentage of oxygen saturation in water was slightly reduced from S2 to S4 and increased again at S5  
354 independently from treatments (Table S5; Bonferroni's tests  $< 0.05$ ). EC and pH showed a significant  
355 reduction after spiking the pesticide cocktail (S3 - S4) in the three treatments and recovered further at  
356 S5 (Table S5; Wilcoxon rank sum test (WRST)  $< 0.05$ ). Otherwise, the average DOC concentration in  
357 all microcosms decreased progressively during the experiment from  $6.9 \pm 0.0 \text{ mg L}^{-1}$  at S1 to  $3.3 \pm 0.7$   
358  $\text{mg L}^{-1}$  at S5 (Table S5; Bonferroni's tests  $< 0.05$ ). N-NO<sub>3</sub>, TDN, P-PO<sub>4</sub>, TDP concentrations and  
359 light did not show neither differences among hydrological treatments nor among sampling times  
360 (Table S5).

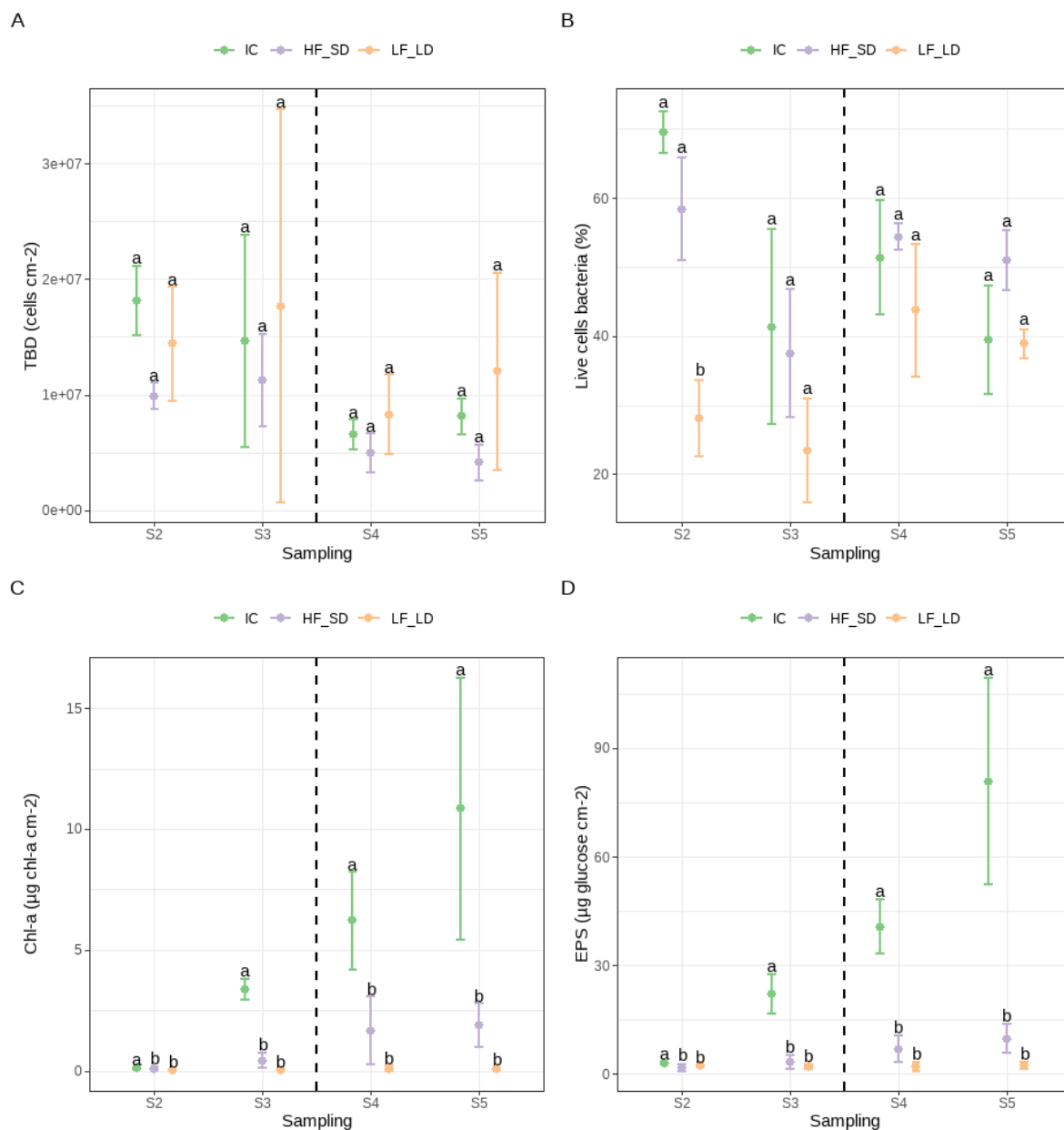
### 361 3.2. Biofilm structure in different hydrological treatments

362 Some biofilm structural parameters responded to hydrological treatments. Total bacterial density  
363 (TBD) in biofilms was unaffected by hydrological treatments (Figure 2.A; Table 2), whereas the  
364 percentage of live bacterial cells were significantly lower in LF\_LD compared to the other treatments  
365 (Figure 2.B; Table 2). Those differences in the percentage of live bacterial cells were more marked at  
366 the beginning than at the end of the experiment (Figure 2.B). Otherwise, algal biomass measured as  
367 chlorophyll-*a* (Chl-*a*), microalgal density and total biofilm biomass consistently decreased in drought  
368 treatments (HF\_SD and LF\_LD) compared to the permanently immersed control IC (Figure 2.C and  
369 Figure S1.A; Table 2 and Table S6). Indeed, total biofilm biomass was positively correlated with Chl-  
370 *a* concentration when considering the three hydrological treatments together (Spearman coefficient =  
371  $0.7816, p < 0.0001$ ).

372 The density of green algae was overall dominant in biofilms and droughts (HF\_SD and LF\_LD  
373 treatments) tended to favour green algae density over that of diatoms and cyanobacteria (Figure  
374 S1.A). The lowest Chl-*a* concentrations and total microalgal densities were measured in LF\_LD  
375 compared to HF\_SD and IC treatments. Surprisingly, diatoms presented low mortality despite the  
376 different hydrological treatments applied (Figure S1.B; Table S6). Extracellular polymeric substances  
377 (EPS) concentration in biofilms was also affected by droughts and strongly correlated with total  
378 biofilm biomass (Spearman coefficient =  $0.75; p < 0.0001$ ) (Figure 2, Table 2). Droughts drastically  
379 reduced EPS concentration in biofilms (Figure 2.D), although EPS normalised by the microbial  
380 carbon did not show differences among hydrological treatments (Figure S2; Table S7).

381 Some of the measured biofilm structural parameters (*i. e.*, chl-*a* concentration, percentage of live  
382 bacterial cells, and EPS concentration) varied also over time (Table 2). While the chl-*a* concentration  
383 increased in all treatments between S2 to S5, the percentage of live bacterial cells showed an  
384 interaction between time and hydrological treatments (Table 2). The percentage of live bacteria were  
385 significantly lower in LF\_LD comparing to HF\_SD and IC treatments at S2, but such differences  
386 disappeared in the other sampling dates (Figure 2.B). Moreover, the percentage of live bacteria  
387 slightly increased after spiking the pesticide cocktail from  $42.59 \pm 7.64\%$  in S3 to  $46.94 \pm 10.55 \%$  in  
388 S4 when considering all the treatments together (time effect: Table 2, Bonferroni's test  $P < 0.05$ ,  
389 Figure 2.B). Finally, EPS concentration in biofilms progressively increased over time in IC but  
390 remained rather low and stable in drought treatments (Figure 2.D).

391



393

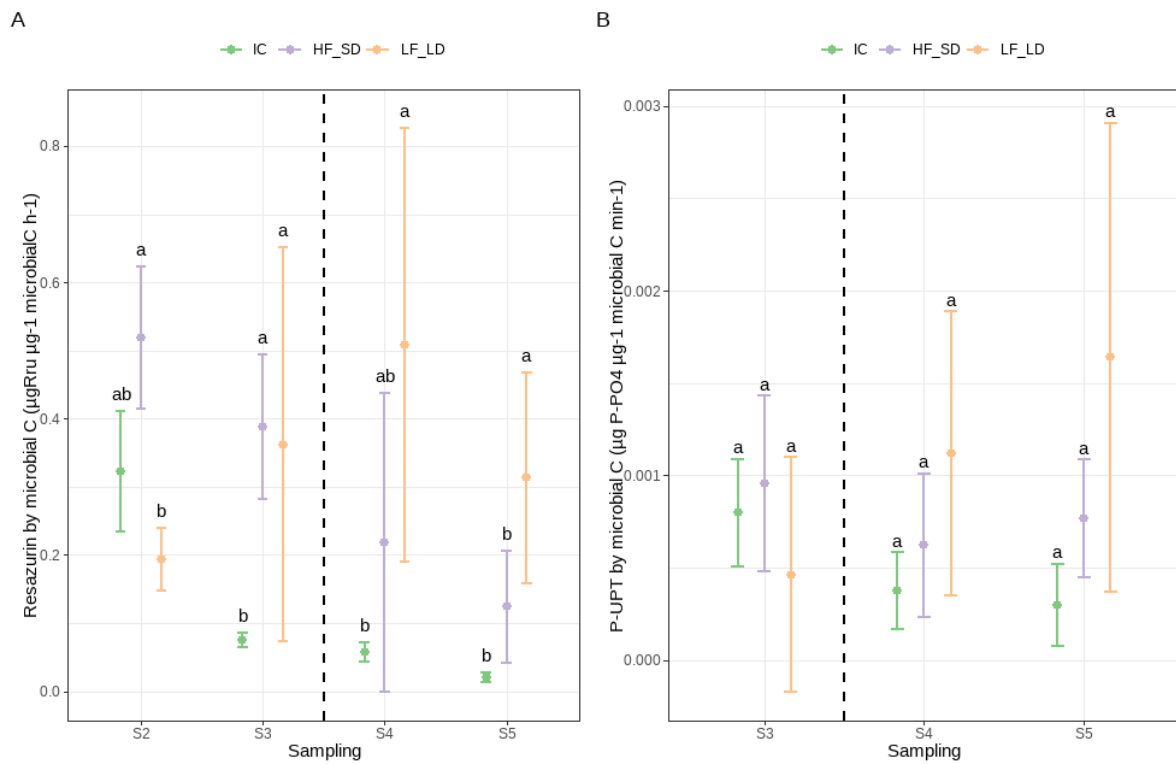
394 *Figure 2. Total bacteria density (A), percentage of live bacteria (B), chlorophyll-a concentration (C)*  
 395 *and EPS normalised by microbial C (D) in the three hydrological treatments (IC: immersed controls;*  
 396 *HF\_SD: high frequency and short duration drought; LF\_LD: low frequency and long duration*  
 397 *drought) in different sampling days. Different letters indicate significant differences among*  
 398 *treatments based on Tukey post-hoc tests (descriptors A, C, D) and Wilcoxon rank-sum test*  
 399 *(descriptor B). The dashed line represents the spike of cocktail of pesticides. Values represented are*  
 400 *means and standard deviation (n = 3).*

### 401 3.3. Biofilm function in different hydrological treatments

402 Biofilm respiration normalised by microbial carbon was increased by droughts. Respiration was  
 403 higher in LF\_LD condition, followed by HF\_SD and finally the control (IC) (Figure 3.A), but at the  
 404 same time, interactive effects between treatment and time were observed for respiration (Table 2).



405 While respiration decreased in the IC and HF\_SD treatments between S2 to S5, it was maintained or  
 406 even increased in the LF\_LD treatment (Table 2; Bonferroni's tests < 0.05). Biofilm respiration was  
 407 correlated with phosphorus (P) uptake rate when considering all the treatments together (Pearson  
 408 coefficient = 0.496, p = 0.010) despite the high variability of P uptake in biofilms subjected to the  
 409 LF\_LD treatment (Figure 3.B). Slightly higher phosphorus uptake rate was observed in LF\_LD and  
 410 HF\_SD compared to IC after the application of pesticides (S4 and S5), though differences between  
 411 hydrological treatments were not statistically significant. P uptake capacity in biofilms slightly  
 412 changed over time (Table 2).



413

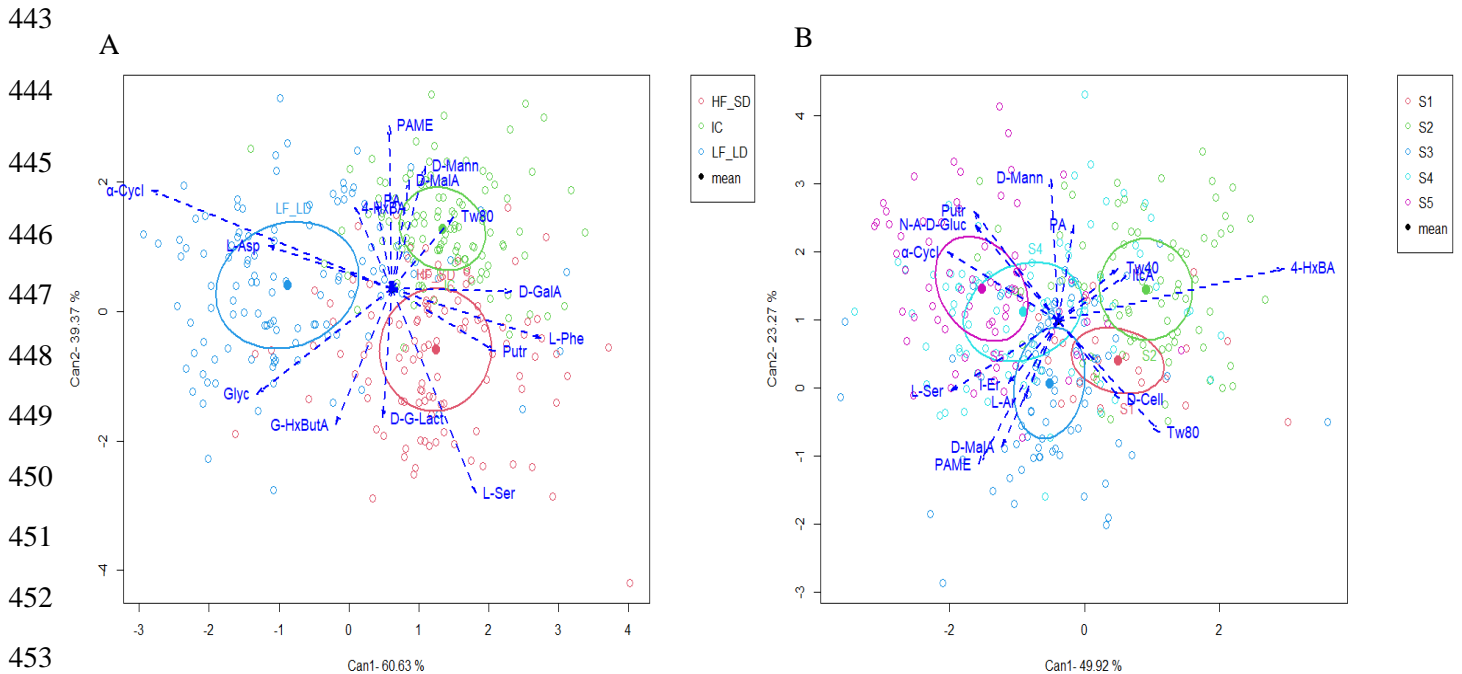
414 *Figure 3. Microbial respiration (A) and phosphorus uptake normalised by microbial carbon (B) in the*  
 415 *three hydrological treatments (IC: immersed controls; HF\_SD: high frequency and short duration*  
 416 *drought; LF\_LD: low frequency and long duration drought) and sampling days. Different letters*  
 417 *indicating significant differences among treatments based on Tukey post-hoc test. The dashed line*  
 418 *represents the spike of the pesticide cocktail. Values represented are means and standard deviations*  
 419 *(n=3).*

420 Global community-level physiological profile (CLPP) parameters revealed that the time to dissipate  
 421 50% of substrates (DT50) showed interactive effects between hydrological treatments and time (Table  
 422 2; Table S8). Differences were observed in HF\_SD ( $44.34 \pm 3.74$  h) and LF\_LD ( $39.72 \pm 4.62$  h)  
 423 compared to IC ( $54.35 \pm 5.15$  h) (Table 2; Tukey tests < 0.05) only at S4. The rest of CLPP  
 424 descriptors (slope and Shannon index) were not affected by hydrological treatments but mostly by  
 425 time effects (

426 Table 2). In particular, higher values of the Shannon index were measured at S5 ( $3.06 \pm 0.06$ )  
 427 compared to S2 ( $2.56 \pm 0.07$ ) (Table 2; Tukey tests  $< 0.05$ ; Table S8).

428 When comparing the specific C substrates utilisation among hydrological treatments, a significantly  
 429 lower carboxylic and ketonic acids and amines or amides catabolism, but not for amino acids  
 430 catabolism, were observed in LF\_LD compared to HF\_SD and IC (Bonferroni tests  $< 0.05$ ). Biofilm  
 431 communities had major tendency to consume polymers such as  $\alpha$ -cyclodextrin ( $\alpha$ -Cycl) and glycogen  
 432 (Glyc), and the amino acid L-asparagine(L-Asp) in LF\_LD treatment (Figure 4.A), whereas D-  
 433 mannitol (D-Mann), pyruvic acid methyl ester (PAME), Tween 80 (Tw80) and D-malic acid (D-  
 434 MalA) is mostly consumed in the IC treatment. Finally, biofilms exposed to HF\_SD mostly consumed  
 435 L-serine (L-Ser), D-galactonic acid  $\gamma$ -lactone (D-G-Lact) and putrescine (Putr) (Figure 4.A; Table 3).

436 Carbon sources utilisation in biofilms did not statistically differ among sampling times compared to  
 437 hydrological treatments (Figure 4.B, Table 3). Additionally, the incubation time of CLPP  
 438 compositional data was embedded between 96 and 168 h (73.44 % total variance of canonical axes),  
 439 excepting the compounds of tween 80 (Tw 80), i-erythritol (i-Er), putrescine (Putr),  $\gamma$ -hydroxybutyric  
 440 acid (G-HxButA), 4-hydroxy benzoic acid (4-HxBA),  $\beta$  -methyl-D-glucoside ( $\beta$ -M-D-Gluc), D-  
 441 galactonic acid  $\gamma$ -lactone (D-G-Lact), pyruvic acid methyl ester (PAME) and L-asparagine (L-Asp),  
 442 for which the AWCD was achieved before 72 hours of incubation (Figure S3; Table S9).



454 *Figure 4. Canonical variates plot by hydrological treatments (A) and sampling dates (B) from CLPP*  
 455 *canonical data.*

456

457

458 Table 2. Results of two-way ANOVA and  $\chi^2$  and p values of Kruskal-Wallis non-parametric tests from  
 459 biofilm descriptors measured in the three hydrological treatments. P-values below 0.05 are  
 460 represented in bold. Chl-a = Chlorophyll-a; TBD = Total bacteria density; LC = Live cells  
 461 percentage; EPS normalised by microbial C = Extracellular polymeric substance normalised by  
 462 microbial carbon; Resazurin normalised by microbial C = microbial respiration by Resazurin method  
 463 normalised by microbial carbon; P-UPT normalised by microbial C: Phosphorus uptake normalised  
 464 by microbial carbon; Slope of AWCD: slope of average well colour development of Biolog method;  
 465 DT50 of AWCD: dissipation time at 50%.

S2-S3-S4-S5

	TWO-WAY ANOVA repeated measures			Kruskal-Wallis		
	Treatment	Time	Treatment x Time	Treatment	Time	Treatment x Time
Total biofilm biomass (mg AFDW cm <sup>-2</sup> ) (Ln)	<b>0.020</b> <b>F=12.137</b>	n.s.	n.s.	<b>&lt;0.0001</b> <b><math>\chi^2=20.655</math></b>	<b>0.037</b> <b><math>\chi^2=8.495</math></b>	<b>&lt;0.0001</b> <b><math>\chi^2=30.920</math></b>
Chl-a ( $\mu\text{g chl-a cm}^{-2}$ )	n.s.	n.s.	n.s.			
TBD (cells cm <sup>-2</sup> ) (Ln)	n.s.	n.s.	n.s.			
LC (%)	<b>0.029</b> <b>F=9.693</b>	<b>0.002</b> <b>F=17.837</b>	<b>0.004</b> <b>F=5.913</b>			
EPS ( $\mu\text{g glucose cm}^{-2}$ )				<b>0.0001</b> <b><math>\chi^2=18.362</math></b>	<b>0.047</b> <b><math>\chi^2=7.949</math></b>	<b>0.0019</b> <b><math>\chi^2=29.426</math></b>
Resazurin by microbial C ( $\mu\text{g Rru } \mu\text{g microbial}$ $\text{C}^{-1} \text{h}^{-1}$ ) (Ln)	<b>0.005</b> <b>F=26.187</b>	<b>0.013</b> <b>F=8.864</b>	<b>0.004</b> <b>F=6.096</b>			
P-UPT* by microbial C ( $\mu\text{g P-PO}_4 \mu\text{g}$ $\text{microbial C}^{-1} \text{min}^{-1}$ ) (Ln)	n.s.	<b>0.029</b> <b>F=32.973</b>	<b>0.006</b> <b>F=36.607</b>			
Slope of AWCD ( $\text{h}^{-1}$ )	n.s.	<b>0.020</b> <b>F=7.274</b>	n.s.			
DT50 of AWCD (h)	n.s.	<b>0.031</b> <b>F=5.942</b>	<b>0.029</b> <b>F=3.547</b>			
Shannon diversity of AWCD	n.s.	<b>0.020</b> <b>F=7.315</b>	n.s.			
Carbohydrates (%)	n.s.	n.s.	n.s.			
Carboxylic and ketonic acids (%)	<b>0.001</b> <b>F=58.585</b>	<b>0.033</b> <b>F=5.832</b>	n.s.			
Amino acids (%)	n.s.	n.s.	n.s.			
Polymers (%) (Ln)	n.s.	n.s.	n.s.			
Amines or amides (%)	<b>0.004</b> <b>F=29.997</b>	n.s.	n.s.			

\* (P-UPT only S3-S4-S5)

466

467 Table 3. Type II Repeated Measures MANOVA Tests of CLPP and Pillai statistic tests.

	Df	Pillai test	approx F	num Df	den Df	Pr(>F)
Time	3	0.0494	0.277	3	16	n.s.
Treatment	2	0.4218	5.836	2	16	0.0125*
Time:Treatment	6	0.4042	1.809	6	16	n.s.

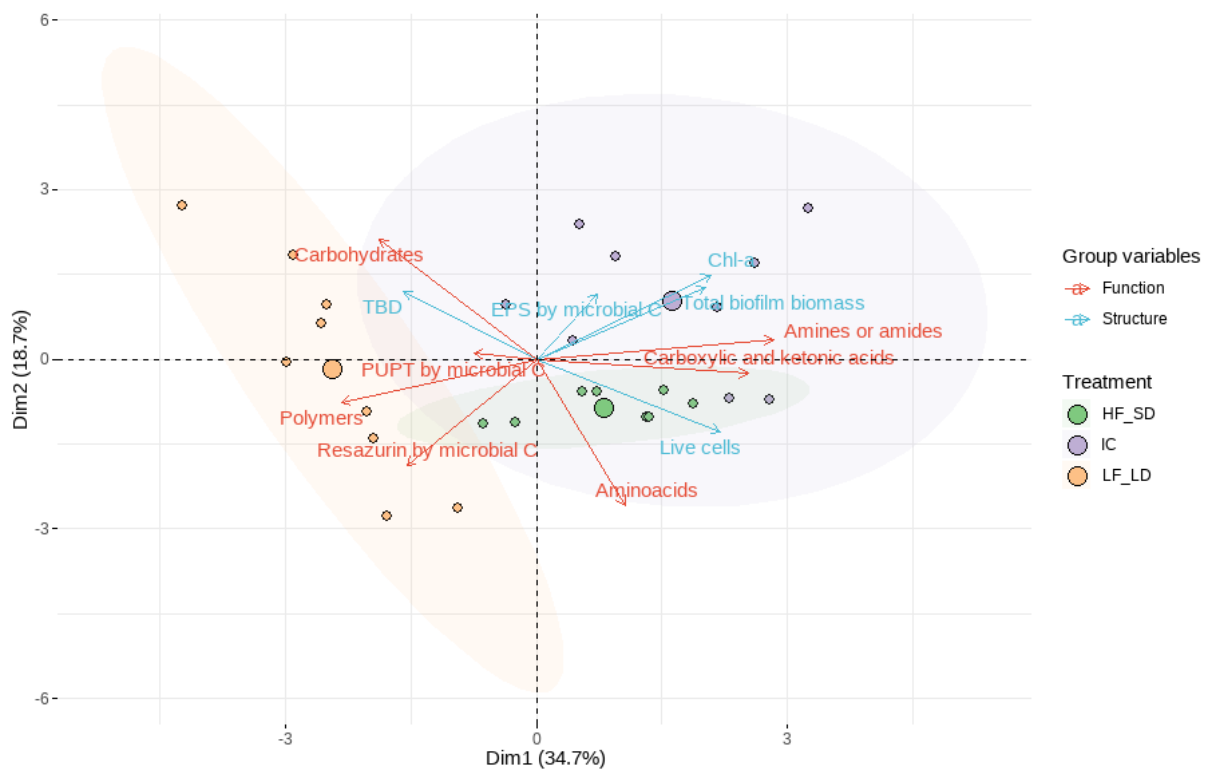
Signif.codes: n.s. = non-significant; (\*) = significant difference (p-value < 0.05)

468

469 3.4. Relationships between biofilm structural and functional responses to hydrological  
470 treatments

471 The hydrological treatments clearly affected the structural and functional characteristics of biofilms  
472 (53.40 % of total explained variance in the first two dimensions of the PCA; Table S10) and separated  
473 biofilms exposed to LF\_LD from those exposed to HF\_SD and IC treatments (Figure 5). For instance,  
474 biofilms in the LF\_LD condition presented lower microbial biomass but higher microbial respiration  
475 (*Resazurin by microbial C* in the PCA) and catabolism of polymers and carbohydrates compared to  
476 biofilms in the HF\_SD and IC treatments. Biofilms in HF\_SD and IC had higher algal biomass and  
477 high catabolism for amines or amides, and carboxylic and ketonic acids. Differences between HF\_SD  
478 and IC biofilms were weaker, excepting for the relative abundance of live bacterial cells and amino  
479 acids catabolism that was higher in HF\_SD compared to IC.

480



481

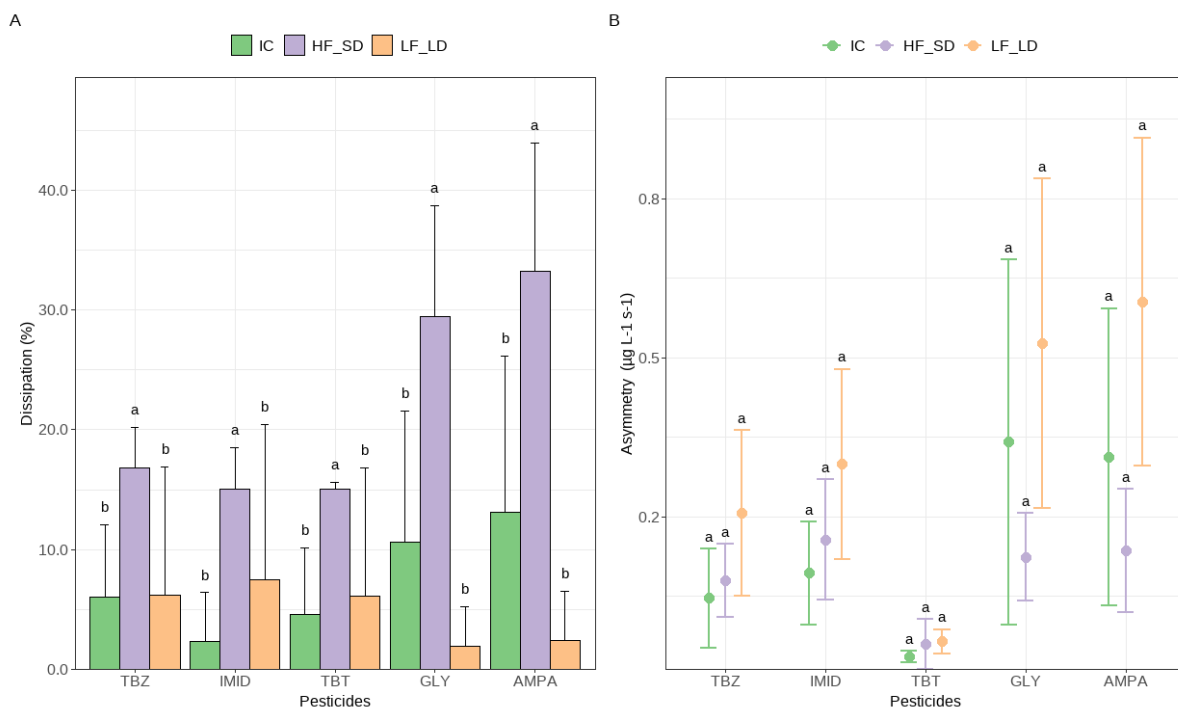
482 *Figure 5. Principal component analysis of the structural and functional biofilm variables in the three*  
483 *hydrological treatments at S3, S4 and S5 of the experiment.*

484 3.5. Pesticide dissipation capacity of biofilms

485 Biofilms showed an overall low capacity to dissipate the pesticide cocktail with averages of  
486 dissipation rates ranging from 2 to 35 % (imidacloprid and AMPA, respectively; Figure 6.A).  
487 However, statistical differences in pesticides dissipation among hydrological treatments were

488 observed ( $\chi^2= 19.681, p < 0.0001$ ) (Figure 6.A). The dissipation percentages of the five molecules of  
 489 the cocktail were generally higher in biofilms exposed to HF\_SD compared to biofilms exposed to  
 490 LF\_LD and IC treatments (Wilcoxon rank sum test (WRST)  $< 0.05$ ). When comparing the dissipation  
 491 rates for each pesticide molecule separately, the lowest average dissipation percentage was measured  
 492 for neutral pesticides such as imidacloprid (IMID) ( $8.28 \pm 8.92$  %), terbuthylazine (TBT) ( $8.57 \pm 7.75$   
 493 %) and tebuconazole (TBZ) ( $9.68 \pm 8.29$  %) and the highest for glyphosate (GLY) ( $14.00 \pm 14.23$  %)  
 494 and its metabolite aminomethylphosphonic acid (AMPA) ( $16.24 \pm 16.09$  %), although those  
 495 differences were not statistically significant (Table 4).

496



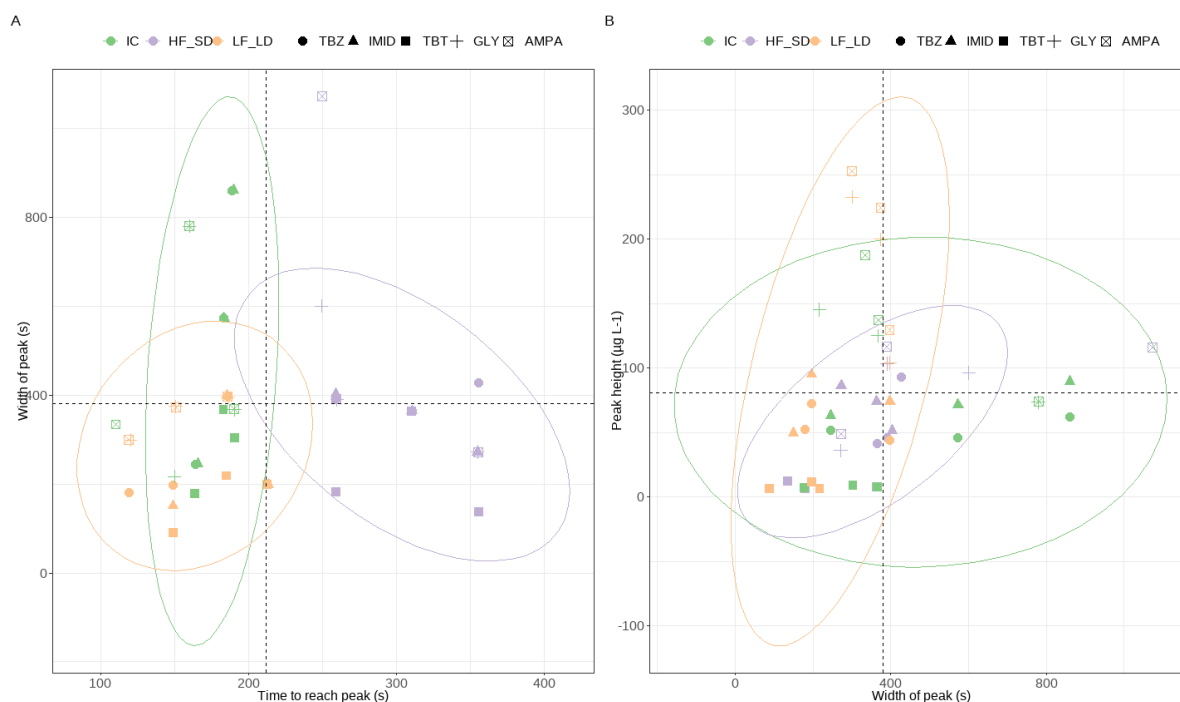
497

498 *Figure 6. Dissipation rates (%) of the pesticides (AMPA = aminomethylphosphonic acid, GLY =*  
 499 *glyphosate, IMID = imidacloprid, TBT = terbuthylazine, TBZ = tebuconazole) by biofilms exposed to*  
 500 *different hydrological treatments (IC = immersed control, HF\_SD = high frequency and short*  
 501 *duration drought, LF\_LD = low frequency and long duration drought) (A), asymmetry of pesticide*  
 502 *peaks after the different hydrological treatments (B). Different letters indicate significant differences*  
 503 *among treatments based on Wilcoxon rank-sum test (A) and Tukey post-hoc tests (B). Values*  
 504 *represented are means and standard deviations (n=3).*

505 Other toxicokinetic parameters measured during the experiment reinforced the differences in  
 506 pesticides dissipation between hydrological treatments. For instance, all pesticide concentration peaks  
 507 in channels were reached earlier in LF\_LD ( $164.53 \pm 31.85$  s) and IC ( $164.53 \pm 31.85$  s) than in  
 508 HF\_SD treatments ( $300.67 \pm 45.49$  s) suggesting a greater interaction between pesticides and biofilms  
 509 in the latter (Table 4; WRST  $< 0.05$ ; Figure 7.A).

510 According to the mass balance approach of the pesticide molecules, no statistical differences in height  
511 and asymmetry of peaks for the five pesticide molecules were observed between hydrological  
512 treatments (Table 4 and Figure 7.B), though the peak asymmetry presented clear differences among  
513 pesticide molecules ( $F= 16.000$ ,  $p < 0.0001$ ). The highest peak asymmetry was measured for  
514 glyphosate and AMPA suggesting lower interaction between those molecules and the biofilms.  
515 Moreover, this peak asymmetry for GLY and AMPA was higher in LF\_LD followed by IC and  
516 HF\_SD treatments (Tukey tests  $< 0.05$ , Figure 6.B). Similar differences on peak asymmetry between  
517 treatments were observed for TBZ, TBT and IMID. TBT presented the lowest peak asymmetry values  
518 ( $0.04 \pm 0.02 \mu\text{g L}^{-1} \text{ s}^{-1}$ ) due to the low concentration spiked compared to the other molecules from the  
519 cocktail (Tukey tests  $p < 0.05$ ). Peak asymmetry was calculated by dividing height and width of  
520 pesticide peaks that were also significantly different among pesticide molecules (WRST  $< 0.05$ ; Table  
521 4). Peak heights of TBZ ( $56.46 \pm 16.62 \mu\text{g L}^{-1}$ ) and IMID ( $72.24 \pm 16.19 \mu\text{g L}^{-1}$ ) were lower  
522 compared to those of GLY ( $123.90 \pm 61.00 \mu\text{g L}^{-1}$ ) and AMPA ( $143.02 \pm 66.64 \mu\text{g L}^{-1}$ ) (Figure 7.A).  
523 Otherwise, peak width of GLY ( $411.00 \pm 174.93 \text{ s}$ ) and AMPA ( $476.47 \pm 268.28 \text{ s}$ ) were higher  
524 compared to those of TBZ ( $404.13 \pm 211.30 \text{ s}$ ) and IMID ( $384.83 \pm 219.33 \text{ s}$ ). The lowest peak height  
525 ( $8.21 \pm 2.42 \mu\text{g L}^{-1}$ ) and width ( $226.17 \pm 97.88 \text{ s}$ ) were measured for TBT (Figure 7.A and Figure  
526 7.B).

527 Differences in pesticides accumulation in biofilms were not observed between hydrological  
528 treatments, despite a trend of higher accumulation in the treatment LF\_LD (Table 5). Differences  
529 were observed among pesticide molecules accumulation in biofilms ( $\chi^2= 21.147$ ,  $p < 0.0001$ ; WRST  
530  $< 0.05$ ), specifically when comparing averages of TBZ ( $12.30 \pm 5.97 \mu\text{g g}^{-1}$  dry weight (DW) biofilm)  
531 to TBT ( $0.65 \pm 1.67 \mu\text{g g}^{-1}$  DW biofilm) and IMID ( $< \text{LOQ}$ ) in the three hydrological treatments.  
532 Despite the lower concentration of TBT in the cocktail respect to the other pesticides, this molecule  
533 tends to accumulate in biofilms together with TBZ. Accumulation of neutral pesticides (TBZ, TBT  
534 and IMID) in biofilms was highly correlated with BCF (Spearman coefficient = 0.97;  $p < 0.0001$ ;  
535 Table 5). The highest BCF values were measured for the TBZ and TBT in LF\_LD (Table 5) despite  
536 the lack of significance among hydrological treatments.



537

538 *Figure 7. Pesticide peak width against time to reach the peak (A) and peak height against peak width*  
 539 *(B) (AMPA = aminomethylphosphonic acid, GLY = glyphosate, IMID = imidacloprid, TBT =*  
 540 *terbutylazine, TBZ = tebuconazole) in different hydrological treatments (IC = immersed control,*  
 541 *HF\_SD = high frequency and short duration, LF\_LD = low frequency and long duration) are*  
 542 *represented.*

543

544 *Table 4. Results of  $\chi^2$  and  $p$  values of Kruskal-Wallis non-parametric test,  $F$  and  $p$  values of one-way*  
 545 *ANOVA.  $p$  values below 0.05 are represented in bold.*

	Treatment	Pesticide
<i>Dissipation (%)</i>	<b>&lt;0.0001 <math>\chi^2=19.681</math></b>	n.s.
<i>Peak height (<math>\mu\text{g L}^{-1}</math>)</i>	n.s.	<b>&lt;0.0001 <math>\chi^2=31.768</math></b>
<i>Peak width (s)</i>	n.s.	<b>0.033 <math>\chi^2=10.495</math></b>
<i>Time to reach peak (s)</i>	<b>&lt;0.0001 <math>\chi^2=29.987</math></b>	n.s.
<i>Peak asymmetry (<math>\mu\text{g L}^{-1} \text{s}^{-1}</math>)</i>	n.s.	<b>&lt;0.0001 <math>F=16.000</math></b>
<i>Bioaccumulation (<math>\mu\text{g pesticide g}^{-1} \text{ DW biofilm}</math>)</i>	n.s.	<b>&lt;0.0001 <math>\chi^2=21.147</math></b>
<i>BCF (<math>\text{L s}^{-1} \text{ kg}^{-1}</math>)</i>	n.s.	<b>&lt;0.0001 <math>\chi^2=18.647</math></b>

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548

549 *Table 5. Concentration of pesticides ( $\mu\text{g pesticide g}^{-1}$  dry weight biofilm) and associated*  
 550 *bioconcentration factors (BCF) ( $\text{L s}^{-1} \text{kg}^{-1}$ ) of pesticides (IMID = imidacloprid, TBT = terbuthylazine,*  
 551 *TBZ = tebuconazole) in the different hydrological treatments (IC = immersed control, HF\_SD = high*  
 552 *frequency and short duration drought, LF\_LD = low frequency and long duration drought). <LOQ:*  
 553 *under the limit of quantification ( $0.1 \mu\text{g IMID g}^{-1}$  biofilm extracts). Values are means  $\pm$  standard*  
 554 *deviations ( $n=3$ ).*

		<b>Treatment</b>		
<b>Pesticide</b>		<b>Immersed control (IC)</b>	<b>High frequency and short duration drought (HF_SD)</b>	<b>Low frequency and long duration drought (LF_LD)</b>
Pesticide concentrations	Imidacloprid (IMID)	<LOQ (n.a.)	<LOQ (n.a.)	<LOQ (n.a.)
	Terbuthylazine (TBT)	$0.12 \pm 0.12$	$0.14 \pm 0.12$	$1.70 \pm 2.94$
	Tebuconazole (TBZ)	$10.61 \pm 4.27$	$9.31 \pm 7.31$	$16.99 \pm 4.49$
BCF	Imidacloprid (IMID)	<LOQ (n.a.)	<LOQ (n.a.)	<LOQ (n.a.)
	Terbuthylazine (TBT)	$0.12 \pm 0.13$	$0.11 \pm 0.10$	$1.19 \pm 2.06$
	Tebuconazole (TBZ)	$1.26 \pm 0.13$	$1.08 \pm 0.84$	$2.97 \pm 1.24$

555 n.a. (not accumulated)

556 

## 4. Discussion

557 Drought treatments simulated in this experiment mimic the effects caused by agricultural practices  
 558 and hydropeaking in rivers and streams from south-western Europe, producing different frequencies  
 559 and durations of droughts that affect the structure and function of aquatic microbial communities  
 560 (Acuña, Hunter and Ruhí 2017). In agricultural and urban watersheds, other stressors may affect  
 561 aquatic microbial communities, such as pesticides (Dai and Dong 2014; Sharma *et al.* 2019; Meftaul  
 562 *et al.* 2020; Jabiol *et al.* 2022). While loads of pesticides reaching streams are expected to decrease,  
 563 pesticide concentrations in stream waters are expected to increase in watersheds subjected to droughts  
 564 (Palma *et al.* 2021; Chow *et al.* 2023). Understanding how biofilm capacities to dissipate a cocktail of  
 565 pesticides in streams will change in the context of climate change are of special importance for the  
 566 future management of pesticide-contaminated watersheds. The present study shows that hydrological  
 567 variations (long and short droughts) can modify the biofilm structure and functions, including their  
 568 capacity to dissipate a cocktail of pesticides. In this section, we first addressed the specific effects of  
 569 droughts on the structure and functions of biofilms (section 4.1) and then the effects of droughts on  
 570 pesticide dissipation capacity by biofilms (section 4.2).



#### 571 4.1. Biofilm responses to droughts

572 Long and short droughts applied in our experiment reduced total biofilm biomass, and especially the  
573 biomass corresponding to the autotrophic component (chlorophyll-*a* concentration, microalgal  
574 density) rather than that of the heterotrophic component (bacterial cell viability and/or total bacterial  
575 density). This result suggests that microbial autotrophs are more sensitive than microbial heterotrophs  
576 to droughts. Indeed, it has been demonstrated that aquatic microbial heterotrophic communities  
577 exposed to long drought showed higher xerotolerance, characterised by higher resistance and  
578 resilience to droughts, than autotrophic communities developed under the same treatments (Acuña *et al.*  
579 *et al.* 2015). Bacterial density and cell viability did not decrease in our experiment as observed in other  
580 experiments (*i. e.*, Timoner *et al.* 2012 and Courcoul *et al.* 2022). As suggested in those studies, the  
581 run-off of dead cell debris (mostly from algal origin) in our biofilms could have been fuelling carbon  
582 and nutrients to bacteria and helping them to resist during droughts. In addition, our experiment show  
583 that green algae seem to be more resistant compared to cyanobacteria and diatoms to droughts as  
584 observed in other studies (*i. e.*, Zlatanović *et al.* 2018; Courcoul *et al.* 2022).

585 Various studies in the literature observed that biofilms exposed to intermittent droughts (weeks to  
586 months) became more active (*i. e.* in terms of bacterial production and extracellular enzymatic  
587 activities) compared to biofilms not exposed to droughts (Marxsen, Zoppini and Wilczek 2010;  
588 Timoner *et al.* 2014; Coulson *et al.* 2022). In our study, long droughts significantly increased biofilm  
589 respiration and modified the diversity of carbon sources utilisation. The functional responses of  
590 biofilms to droughts in our experiment were weaker than those observed in Gionchetta *et al.* 2019,  
591 Coulson *et al.* 2022 and Miao *et al.* 2023. This difference was probably explained by the short  
592 duration droughts in our experiment (from 1 day to 1 week) compared to these studies (from 5 weeks  
593 to 5 months). Regarding the shifts in carbon substrates utilisation, the increase in decomposition of  
594 polymers and carbohydrates, even carboxylic acids, by biofilms exposed to long droughts in our  
595 experiment has been already observed (Barthès *et al.* 2015). Specifically, glycogen polymers have  
596 been considered a potential resource for xerotolerant bacteria's metabolism under drought stress due  
597 to carbon accumulation in the cells (Lebre, De Maayer and Cowan 2017) and consequent  
598 decomposition, as well as carbohydrates, amines and phenolic compounds (Freixa *et al.* 2016).

599 Despite long droughts have been shown to favour EPS development in biofilms (Gionchetta *et al.*  
600 2019; Coulson *et al.* 2022), contrary to our hypothesis, our results showed higher EPS content in  
601 immersed controls than in drought-exposed biofilms. This lack of EPS production in drought-exposed  
602 biofilms (both short and long droughts) could be associated with i) the short duration of droughts  
603 compared to other studies (*e. g.*, Timoner *et al.* 2014; Gionchetta *et al.* 2019) that would have not  
604 permitted a proper EPS synthesis by microbial cells and/ or ii) the sampling strategy employed in our  
605 study, which consisted of systematic sampling under wet conditions irrespective of the drought

606 treatment, thus favouring the EPS removal when the water flow resumed. The rewetting phase, as  
607 observed in Gionchetta *et al.* 2019, showed that biofilm previously exposed to drought stress reduced  
608 the EPS below the permanent wet conditions, as observed in our experiment. Surprisingly, the  
609 concentration of EPS increased in biofilms exposed to permanent wet conditions (IC) during the  
610 length of the experiment. This result can be explained by the strong correlation observed between  
611 total biofilm biomass and EPS concentration, suggesting that algal growth was probably responsible  
612 for EPS production in the biofilm.

#### 613 4.2. Influence of droughts in biofilm ability to dissipate pesticides

614 Biofilms are sites for the accumulation and degradation of pesticides transported by flowing waters  
615 (Rheinheimer dos Santos *et al.* 2020). It is well recognised that thicker biofilms (greater total biofilm  
616 biomass) would have a stronger sorption capacity for pesticides (Guasch, Admiraal and Sabater 2003;  
617 Paule *et al.* 2015). Within the biofilm components, a well-developed EPS matrix offers a physical  
618 interface capable of adsorbing polar and non-polar pesticides (Fernandes *et al.* 2020). The results  
619 from our study did not entirely agree with this statement, nor with our initial hypothesis, since  
620 biofilms exposed to short droughts (thinner and lower EPS concentration) presented higher pesticide  
621 dissipation rates, and a longer interaction time with pesticides compared to biofilms permanently  
622 immersed control (thicker and higher EPS concentration). There are several possible explanations for  
623 these results: i) the specific EPS quality could influence pesticides adsorption (Mahto *et al.* 2022); ii)  
624 the specific functional fingerprint (greater use of carbohydrates, amino acids, amines and/or phenolic  
625 compounds) could enhance developed EPS structures by increasing the cation-anion electron  
626 exchange (Bonnineau *et al.* 2021) and therefore increasing their respective bioconcentration factors  
627 (BCFs); ii) the increased live bacteria (after the pesticide spike, e.g., Courcoul *et al.* 2022); iii) the  
628 increased dead microalgae (generating more sorption sites for pesticides, e.g., Proia *et al.* 2013); or iv)  
629 other non-measured variables such as biofilm architecture (sinuosity, thickness, density and  
630 viscoelasticity of the matrix, and electrostatic interactions, as observed in Battin *et al.* 2003 and  
631 Flemming *et al.* 2023).

632 The accumulation of hydrophobic pesticides associated with the accumulation of microbial biomass,  
633 or EPS specifically (Headley *et al.* 1998), in biofilms could explain the presence of tebuconazole and  
634 terbuthylazine in biofilms from our experiment. Imidacloprid (IMID), glyphosate (GLY) and/or  
635 AMPA accumulation in the biofilm was not detected and this could be explained by the lower  $\log K_{ow}$   
636 of these molecules (IMID = 0.57; GLY = -3.20; AMPA = -2.17) compared to tebuconazole (TBZ =  
637 3.70) and terbuthylazine (TBT = 3.40), as suggested in our initial hypothesis. The highest peak  
638 asymmetry values were measured for AMPA and glyphosate, two hydrophilic molecules, highly  
639 soluble in water that weakly interacted with biofilms and did not accumulate on them compared to  
640 long exposure to these molecules as observed in Carles *et al.* 2019 (27 days of glyphosate exposure).

641 This result shows the difficulty of stream biofilms to accumulate glyphosate and AMPA molecules  
642 due to low contact time between the biofilm surface and water that could imply less capacity for  
643 biofilms to dissipate them, increasing the probability of their release to surface waters and  
644 groundwaters (Poiger *et al.* 2017). We observed tebuconazole and terbuthylazine accumulation even  
645 if the contact between biofilms and the pesticides lasted for only a few minutes in our experiment  
646 ( $16.38 \pm 3.87$  minutes). This result is in agreement with another study focused on the herbicide diuron  
647 (Chaumet *et al.* 2019), whereas contrasts with other works in which longer exposure times were  
648 tested (48 hours to days, e.g., Romero *et al.* 2019; Courcoul *et al.* 2022). Specifically, tebuconazole  
649 was accumulated  $12.30 \pm 5.97 \mu\text{g g}^{-1}$  DW of biofilm in different hydrological treatments in our  
650 experiment compared to the accumulation of below  $0.05 \mu\text{g g}^{-1}$  DW of biofilm in Tlili, Montuelle,  
651 Bérard, & Bouchez, 2011. The different results between this experiment and our study could be  
652 explained by the exposure conditions (chronic versus acute) and concentrations spiked to biofilms  
653 ( $0.5 \mu\text{g L}^{-1}$  compared to  $63.18 \mu\text{g L}^{-1}$ ).

654 We did not observe differences in pesticide molecules accumulation and BCFs in biofilms among  
655 different hydrological treatments, indicating that droughts affected the overall pesticide dissipation  
656 but not the type of pesticide molecules, although higher accumulation and BCFs was observed for  
657 tebuconazole and terbuthylazine in long droughts (LF\_LD) compared to the other treatments (HF\_SD  
658 and IC). This results suggest that biofilms can inform us about pesticides history in stream  
659 environments, specifically for hydrophobic molecules as observed in (Mahler *et al.* 2020).  
660 Nevertheless, further research is needed to better apprehend the strong variability in BCFs observed  
661 between microcosm replicates in our study.

## 662 5. Conclusions

663 The realistic assessment of pesticides dissipation by stream biofilms in a continuous open-flow  
664 approach revealed relatively low dissipation percentages per molecule in the cocktail (2 to 35 %)   
665 which should be considered specific to the type of biofilm used and the composition and  
666 concentrations of the pesticides applied. Strikingly, the capacity of stream biofilms to dissipate  
667 pesticides performed better in a drought-stressed environment subjected to short and frequent  
668 droughts compared to an environment subjected to longer droughts or permanently immersed. The  
669 continuous open-flow exposure in our experiment suggests that mostly physicochemical interactions  
670 between pesticides and biofilms occurred rather than transformation and/or degradation processes of  
671 the pesticide molecules. Contrary to our hypotheses, neither biofilm thickness nor EPS concentration  
672 were related to an enhancement of pesticide dissipation by biofilms. Other biofilm characteristics such  
673 as sinuosity, thickness or matrix density, EPS quality, and specific functional fingerprints need to be

674 further investigated to improve understanding on the role of biofilms in controlling pesticide fluxes in  
675 contaminated streams.

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899

900 Supplementary material A

901 *Table S1. Physicochemical parameters in water from the three hydrological treatments. T= Temperature; DOperc= Dissolved oxygen percentage; DOconc=*  
 902 *Dissolved oxygen concentration; EC = Electrical conductivity; DOC = Dissolved organic carbon; TDC = Total dissolved carbon; DIC = Dissolved*  
 903 *inorganic carbon; TDN = Total dissolved nitrogen; N-NO<sub>3</sub><sup>-</sup> = nitrogen of inorganic nitrate; TDP = Total dissolved phosphorus; P-PO<sub>4</sub><sup>3-</sup>= phosphorus of*  
 904 *inorganic orthophosphate. IC: Immersed controls; HF\_SD: high frequency and short duration drought; LF\_LD: low frequency and long duration drought.*  
 905 *Values represent the mean and standard deviation values of 3 replicates, excepting for the Veyre stream water (n=6).*

Time	Treatment	T	DOperc	DOconc	EC	Light	pH	DOC	TDC	DIC	TDN	N-NO <sub>3</sub> <sup>-</sup>	TDP	P-PO <sub>4</sub> <sup>3-</sup>
		°C	%	mg L <sup>-1</sup>	µS cm <sup>-1</sup>	Lux		mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>
S1	HF_SD	18.60±0.26	94.40±0.15	8.81±0.04	312.07±1.63	1508.00±337.03	8.03±0.05	6.88±0.82	35.97±0.90	29.09±0.48	0.55±0.12	0.74±0.20	0.016±0.021	0.009±0.005
	IC	18.7±0.26	94.27±0.15	8.81±0.04	312.07±1.63	1508.00±337.03	8.03±0.05	6.88±0.82	35.97±0.90	29.09±0.48	0.55±0.12	0.74±0.20	0.016±0.021	0.009±0.005
	LF_LD	18.60±0.26	94.27±0.15	8.81±0.04	312.07±1.63	1508.00±337.03	8.03±0.05	6.88±0.82	35.97±0.90	29.09±0.48	0.55±0.12	0.74±0.20	0.016±0.021	0.009±0.005
S2	HF_SD	18.97±0.06	93.90±0.17	8.71±0.01	266.57±27.84	1592.33±220.04	8.08±0.05	3.72±0.29	23.49±2.79	19.77±3.05	0.93±0.09	0.82±0.28	0.008±0.007	0.005±0.000
	IC	19.07±0.12	94.10±0.10	8.71±0.02	230.67±31.33	1511.00±313.08	8.05±0.12	4.84±0.49	23.65±4.84	18.81±4.57	0.90±0.05	1.14±0.16	0.025±0.027	0.003±0.003
	LF_LD	19.00±0.00	93.53±0.21	8.66±0.01	235.00±7.01	1563.00±213.06	8.05±0.02	6.00±0.11	24.93±1.49	18.93±1.60	1.28±0.08	1.32±0.36	0.037±0.029	0.017±0.003
S3	HF_SD	18.93±0.35	92.33±0.06	8.57±0.06	291.07±11.93	1661.67±135.31	7.94±0.01	3.15±0.91	29.76±1.27	26.60±1.95	0.93±0.04	1.34±0.52	0.012±0.005	0.008±0.006
	IC	19.03±0.38	93.43±0.99	8.65±0.03	294.70±5.31	1597.00±381.09	7.93±0.06	3.65±0.56	31.27±0.84	27.63±0.41	0.68±0.30	0.69±0.55	0.009±0.002	0.005±0.003
	LF_LD	18.90±0.26	91.90±0.66	8.53±0.02	278.17±6.30	1623.00±288.17	7.94±0.01	3.72±0.53	29.36±0.51	25.64±0.43	0.96±0.17	0.97±0.17	0.012±0.006	0.004±0.002
S4	HF_SD	18.83±0.25	91.13±0.86	8.44±0.11	249.83±1.33	1554.67±115.61	7.74±0.29	2.06±1.19	22.26±0.62	20.20±0.88	0.88±0.10	0.54±0.03	0.020±0.013	0.010±0.007
	IC	18.90±0.35	91.93±0.35	8.52±0.02	245.53±5.39	1533.33±364.77	7.86±0.13	2.94±1.15	22.44±0.40	19.50±0.79	0.93±0.06	0.73±0.32	0.024±0.005	0.012±0.014
	LF_LD	18.83±0.21	90.10±0.80	8.36±0.07	248.57±7.69	1565.67±260.65	7.85±0.08	2.50±0.78	22.82±1.21	20.32±0.66	0.89±0.05	1.20±0.25	0.012±0.002	0.016±0.016
S5	HF_SD	18.90±0.00	94.53±0.58	8.78±0.05	321.93±10.39	1599.33±147.29	7.87±0.08	2.91±1.52	30.12±2.10	27.20±1.62	0.60±0.29	1.45±0.42	0.008±0.001	0.007±0.002
	IC	19.07±0.06	95.07±1.34	8.80±0.14	325.50±9.39	1806.67±88.51	8.01±0.05	4.27±1.21	32.40±1.16	28.12±0.78	0.71±0.47	1.07±0.14	0.006±0.001	0.015±0.006
	LF_LD	19.07±0.06	94.23±0.57	8.72±0.05	335.90±3.08	1357.00±203.16	8.07±0.02	3.14±1.22	32.26±2.09	29.13±0.99	0.89±0.22	1.10±0.10	0.010±0.001	0.011±0.008
Veyre stream water		10.30±0.00	96.37±0.14	10.79±0.01	226.60±0.00	20333.33±1366.26	7.56±0.00	0.47±0.34	20.58±0.45	20.11±0.37	1.16±0.04	0.63±0.14	0.010±0.000	0.010±0.000

906 *Table S2. Flow rate of each microcosm and hydrological treatments (HF\_SD = High frequency and*  
 907 *short drought exposure, IC = Immersed control, LF\_LD = Low frequency and long drought*  
 908 *exposure).*

Microcosm	Treatment	Q (L/s)
C1	HF_SD	0.00491
C2	IC	0.01110
C3	LF_LD	0.01926
C4	IC	0.0095
C5	LF_LD	0.01409
C6	HF_SD	0.00760
C7	HF_SD	0.00691
C8	LF_LD	0.01221
C9	IC	0.01418

909

910 *Table S3. Carbon substrate codes and their respective guilds for canonical variate plots.*

C substrate	C guild	C substrate code
Pyruvic Acid Methyl Ester	Carbohydrates	PAME
Tween 40	Polymers	Tw40
Tween 80	Polymers	Tw80
$\alpha$ Cyclodextrin	Polymers	$\alpha$ -Cycl
Glycogen	Polymers	Glyc
D-Cellobiose	Carbohydrates	D-Cell
$\alpha$ -D-Lactose	Carbohydrates	$\alpha$ -D-Lact
$\beta$ -Methyl-DGlucoside	Carbohydrates	$\beta$ -M-D-Gluc
D-Xylose	Carbohydrates	D-xyl
i-Erythritol	Carbohydrates	i-Er
D-Mannitol	Carbohydrates	D-Mann
N-Acetyl-DGlucosamine	Carbohydrates	N-A-D-Gluc
DGlucosaminic Acid	Carboxylic and ketonicacids	D-GlucA
Glucose-1- Phosphate	Carbohydrates	G-1-P
D,L- $\alpha$ Glycerol Phosphate	Carbohydrates	D,L- $\alpha$ -GlyPhos
D-Galactonic Acid $\gamma$ -Lactone	Carboxylic and ketonicacids	D-G-Lact
DGalacturonic Acid	Carboxylic and ketonicacids	D-GalA
2-Hydroxy Benzoic Acid	Carboxylic and ketonicacids	2-HxBA
4-Hydroxy Benzoic Acid	Carboxylic and ketonicacids	4-HxBA
$\gamma$ Hydroxybutyric Acid	Carboxylic and ketonicacids	G-HxButA
Itaconic Acid	Carboxylic and ketonicacids	ItcA
$\alpha$ -Ketobutyric Acid	Carboxylic and ketonicacids	$\alpha$ -KetA
D-Malic Acid	Carboxylic and ketonicacids	D-MalA
L-Arginine	Amino acids	L-Ar
L-Asparagine	Amino acids	L-Asp
LPhenylalanine	Amino acids	L-Phe
L-Serine	Amino acids	L-Ser
L-Threonine	Amino acids	L-Thr
Glycyl-LGlutamic Acid	Amino acids	Glyc-L-GlutA

Phenylethylamine	Amines or amides	PA
Putrescine	Amines or amides	Putr

911

912

913 *Table S4. Limits of quantification (LOQ) for different molecules of the cocktail of pesticides.*

Pesticide	LOQ ( $\mu\text{g L}^{-1}$ ) water samples	LOQ ( $\mu\text{g g}^{-1}$ ) biofilm extracts
AMPA	0.05	N/A
GLY	0.05	N/A
IMID	1	0.1
TBT	0.5	0.05
TBZ	0.5	0.05

914

915 *Table S5. Results of two-way ANOVA and  $\chi^2$  and p values of Kruskal-Wallis non-parametric tests on*  
916 *water physical and chemical characteristics. p values below 0.05 are represented in bold. T*  
917 *=Temperature; DOperc = Dissolved oxygen percentage; DOconc = Dissolved oxygen concentration;*  
918 *EC= Electrical conductivity; DOC = Dissolved organic carbon; TDN = Total Dissolved Nitrogen; N-*  
919 *NO<sub>3</sub><sup>-</sup> = Nitrogen of nitrate; TDP = Total dissolved phosphorus; P-PO<sub>4</sub><sup>3-</sup> = Phosphorus of*  
920 *orthophosphate.*

## S2-S3-S4-S5

	TWO-WAY ANOVA repeated measures			Kruskal-Wallis		
	Treatment	Time	Treatment x Time	Treatment	Time	Treatment x Time
<i>T(°C)</i>	n.s.	n.s.	n.s.			
<i>DOperc (%)</i>	n.s.	<b>0.001 F=27.743</b>	n.s.			
<i>DOconc (mg L<sup>-1</sup>)</i>	n.s.	<b>&lt;0.0001 F=29.196</b>	n.s.			
<i>EC (<math>\mu\text{s cm}^{-1}</math>)</i>				n.s.	<b>&lt;0.0001 <math>\chi^2= 28.027</math></b>	<b>&lt;0.0001 <math>\chi^2= 31.154</math></b>
<i>pH</i>				n.s.	<b>&lt;0.0001 <math>\chi^2= 16.866</math></b>	<b>0.01 <math>\chi^2= 25.007</math></b>
<i>Light (lux)</i>				n.s.	n.s.	n.s.
<i>DOC (mg L<sup>-1</sup>)</i>	n.s.	<b>0.009 F=10.272</b>	n.s.			
<i>TDN (mg L<sup>-1</sup>)</i>				n.s.	n.s.	n.s.
<i>N-NO<sub>3</sub><sup>-</sup> (mg L<sup>-1</sup>)</i>	n.s.	n.s.	n.s.			
<i>TDP (mg L<sup>-1</sup>)</i>	n.s.	n.s.	n.s.			
<i>P-PO<sub>4</sub><sup>3-</sup> (mg L<sup>-1</sup>)</i>	n.s.	n.s.	n.s.			

921

922 *Table S6. Results of different communities of microalgal densities with F and p values of one-way*  
923 *ANOVA and  $\chi^2$  and p values of Kruskal-Wallis non-parametric tests. p values below 0.05 are*  
924 *represented in bold.*

	ONE-WAY ANOVA	Kruskal-Wallis
	Treatment	Treatment
<i>Total live microalgal densities (cells cm<sup>-2</sup>) (Ln)</i>	<b>0.002 F=19.57</b>	
<i>Live diatoms (cells cm<sup>-2</sup>) (Ln)</i>	<b>0.023 F=7.55</b>	
<i>Live green algae (cells cm<sup>-2</sup>) (Ln)</i>	<b>0.022 F=7.64</b>	

<i>Live cyanobacteria (cells cm<sup>-2</sup>)</i>	n.s.
<i>Diatoms mortality rate (%)</i>	n.s.

925

926 *Table S7. Results of all experimental approach with F and p values of two-way ANOVA. p values*  
 927 *below 0.05 are represented in bold. EPS: Extracellular polymeric substances; Resazurin: microbial*  
 928 *respiration by Resazurin method; P-UPT: Phosphorus uptake.*

<u>S2-S3-S4-S5</u>			
TWO-WAY ANOVA repeated measures			
	Treatment	Time	Treatment x Time
<i>EPS by microbial C (µg glucose µg<sup>-1</sup> microbial C) (Ln)</i>	n.s	n.s	n.s
<i>Resazurin (µg Rru µg<sup>-1</sup> cm<sup>2</sup>h<sup>-1</sup>) (Ln)</i>	<b>0.006</b> <b>F=24.209</b>	n.s.	<b>0.019</b> <b>F=4.018</b>
<i>P-UPT* (µg P-PO4 µg<sup>-1</sup> cm<sup>2</sup> min<sup>-1</sup>) (Ln)</i>	<b>0.004</b> <b>F=242.040</b>	n.s.	<b>0.023</b> <b>F=10.155</b>

\* (P-UPT only S3-S4-S5)

929

930 *Table S8. Mean and standard deviation of 3 replicates of different parameters of carbon sources*  
 931 *degradation in Biolog grouped by drought conditions (HF\_SD: high frequency and short droughts;*  
 932 *LF\_LD: low frequency and long droughts; IC: immersed control) and sampling times. AWCD:*  
 933 *Average well colour development; DT50: dissipation time at 50%.*

Treatment	Time	AWCD	DT50 of AWCD (h)	Shannon of AWCD
HF_SD	S2	0.45 ± 0.10	55.06 ± 3.32	2.59 ± 0.24
HF_SD	S3	0.51 ± 0.02	50.59 ± 7.52	2.62 ± 0.14
HF_SD	S4	0.54 ± 0.18	44.35 ± 3.74	2.67 ± 0.52
HF_SD	S5	0.49 ± 0.04	49.97 ± 5.64	3.02 ± 0.14
IC	S2	0.51 ± 0.03	60.81 ± 4.97	2.60 ± 0.17
IC	S3	0.57 ± 0.12	55.62 ± 8.78	2.83 ± 0.10
IC	S4	0.55 ± 0.08	54.35 ± 5.15	2.59 ± 0.19
IC	S5	0.53 ± 0.11	40.34 ± 4.29	3.13 ± 0.08
LF_LD	S2	0.45 ± 0.12	49.72 ± 4.11	2.47 ± 0.29
LF_LD	S3	0.49 ± 0.17	41.84 ± 9.19	2.50 ± 0.63
LF_LD	S4	0.43 ± 0.18	39.72 ± 4.62	2.55 ± 0.21
LF_LD	S5	0.60 ± 0.08	48.10 ± 4.96	3.03 ± 0.13

934

935 Table S9. 31 carbon sources of Biolog grouped by sampling times and hydrological conditions (HF\_SD: high frequency and short drought; LF\_LD: low  
 936 frequency and long drought; IC: immersed control). Table S3 for details with carbon source abbreviation. Values represented by mean  $\pm$  standard deviation.

Time	Treatment	PAME	Tw40	Tw80	$\alpha$ -Cycl	Glyc	D-Cell	$\alpha$ -D-Lact	$\beta$ -M-D-Gluc	D-xyl	i-Er
S2	HF_SD	0.460 $\pm$	1.233 $\pm$	0.707 $\pm$	0.203 $\pm$	0.567 $\pm$	0.270 $\pm$	0.317 $\pm$	0.220 $\pm$	0.067 $\pm$	0.050 $\pm$
		0.229	0.223	0.121	0.150	0.482	0.155	0.112	0.115	0.083	0.087
		0.517 $\pm$	1.507 $\pm$	0.853 $\pm$	0.433 $\pm$	0.233 $\pm$	0.500 $\pm$	0.473 $\pm$	0.353 $\pm$	0.043 $\pm$	0.140 $\pm$
S2	IC	0.140	0.230	0.285	0.032	0.098	0.075	0.023	0.015	0.075	0.017
		0.250 $\pm$	1.030 $\pm$	0.563 $\pm$	0.507 $\pm$	0.657 $\pm$	0.747 $\pm$	0.400 $\pm$	0.250 $\pm$	0.153 $\pm$	0.000 $\pm$
S2	LF_LD	0.122	0.050	0.035	0.320	0.473	0.571	0.403	0.236	0.155	0.000
		0.383 $\pm$	1.187 $\pm$	0.718 $\pm$	0.255 $\pm$	0.386 $\pm$	0.365 $\pm$	0.394 $\pm$	0.342 $\pm$	0.198 $\pm$	0.106 $\pm$
S3	HF_SD	0.086	0.074	0.161	0.092	0.095	0.044	0.071	0.065	0.102	0.092
		0.615 $\pm$	1.303 $\pm$	0.907 $\pm$	0.367 $\pm$	0.212 $\pm$	0.456 $\pm$	0.357 $\pm$	0.319 $\pm$	0.188 $\pm$	0.162 $\pm$
S3	IC	0.063	0.047	0.299	0.038	0.055	0.025	0.075	0.057	0.199	0.120
		0.366 $\pm$	1.109 $\pm$	0.716 $\pm$	0.567 $\pm$	1.016 $\pm$	0.742 $\pm$	0.681 $\pm$	0.527 $\pm$	0.261 $\pm$	0.067 $\pm$
S3	LF_LD	0.291	0.331	0.240	0.311	0.112	0.430	0.677	0.376	0.163	0.058
		0.398 $\pm$	1.205 $\pm$	0.699 $\pm$	0.276 $\pm$	0.351 $\pm$	0.476 $\pm$	0.389 $\pm$	0.355 $\pm$	0.338 $\pm$	0.121 $\pm$
S4	HF_SD	0.295	0.266	0.145	0.124	0.206	0.211	0.088	0.133	0.154	0.121
		0.603 $\pm$	1.192 $\pm$	0.619 $\pm$	0.359 $\pm$	0.258 $\pm$	0.447 $\pm$	0.407 $\pm$	0.357 $\pm$	0.286 $\pm$	0.202 $\pm$
S4	IC	0.098	0.186	0.225	0.116	0.174	0.137	0.247	0.189	0.126	0.128
		0.260 $\pm$	0.985 $\pm$	0.488 $\pm$	0.559 $\pm$	0.947 $\pm$	0.681 $\pm$	0.603 $\pm$	0.499 $\pm$	0.370 $\pm$	0.064 $\pm$
S4	LF_LD	0.077	0.058	0.028	0.304	0.160	0.700	0.578	0.409	0.430	0.038
		0.437 $\pm$	1.280 $\pm$	0.553 $\pm$	0.453 $\pm$	0.583 $\pm$	0.737 $\pm$	0.417 $\pm$	0.323 $\pm$	0.433 $\pm$	0.153 $\pm$
S5	HF_SD	0.197	0.075	0.156	0.115	0.449	0.509	0.117	0.095	0.248	0.090
		0.580 $\pm$	1.260 $\pm$	0.833 $\pm$	0.380 $\pm$	0.533 $\pm$	0.460 $\pm$	0.457 $\pm$	0.593 $\pm$	0.377 $\pm$	0.197 $\pm$
S5	IC	0.118	0.337	0.203	0.010	0.379	0.062	0.107	0.427	0.237	0.040
		0.413 $\pm$	1.207 $\pm$	0.610 $\pm$	0.683 $\pm$	0.993 $\pm$	0.860 $\pm$	0.740 $\pm$	0.303 $\pm$	0.263 $\pm$	0.073 $\pm$
S5	LF_LD	0.193	0.298	0.164	0.136	0.119	0.740	0.436	0.031	0.127	0.029
						<b>D,L-<math>\alpha</math>-</b>					
		<b>D-Mann</b>	<b>N-A-D-Gluc</b>	<b>D-GlucA</b>	<b>G-1-P</b>	<b>GlyPhos</b>	<b>D-G-Lact</b>	<b>D-GalA</b>	<b>2-HxBA</b>	<b>4-HxBA</b>	<b>G-HxButA</b>
S2	HF_SD	0.580 $\pm$	0.293 $\pm$	0.303 $\pm$	0.137 $\pm$	0.040 $\pm$	0.393 $\pm$	1.127 $\pm$	0.080 $\pm$	0.563 $\pm$	1.120 $\pm$
		0.223	0.172	0.180	0.140	0.069	0.125	0.185	0.139	0.097	0.263
		0.627 $\pm$	0.363 $\pm$	0.300 $\pm$	0.050 $\pm$	0.117 $\pm$	0.533 $\pm$	1.240 $\pm$	0.243 $\pm$	0.663 $\pm$	0.907 $\pm$
S2	IC	0.090	0.038	0.263	0.087	0.023	0.104	0.280	0.421	0.150	0.388
		0.583 $\pm$	0.480 $\pm$	0.350 $\pm$	0.060 $\pm$	0.090 $\pm$	0.410 $\pm$	0.963 $\pm$	0.163 $\pm$	0.580 $\pm$	1.097 $\pm$
S2	LF_LD	0.081	0.401	0.161	0.044	0.131	0.121	0.127	0.257	0.374	0.531
		0.499 $\pm$	0.337 $\pm$	0.264 $\pm$	0.216 $\pm$	0.049 $\pm$	0.433 $\pm$	1.238 $\pm$	0.335 $\pm$	0.431 $\pm$	1.283 $\pm$
S3	HF_SD	0.031	0.060	0.039	0.053	0.020	0.050	0.173	0.581	0.022	0.102
		0.687 $\pm$	0.327 $\pm$	0.522 $\pm$	0.022 $\pm$	0.113 $\pm$	0.337 $\pm$	1.368 $\pm$	0.285 $\pm$	0.700 $\pm$	1.180 $\pm$
S3	IC	0.168	0.064	0.126	0.038	0.040	0.142	0.527	0.494	0.428	0.620



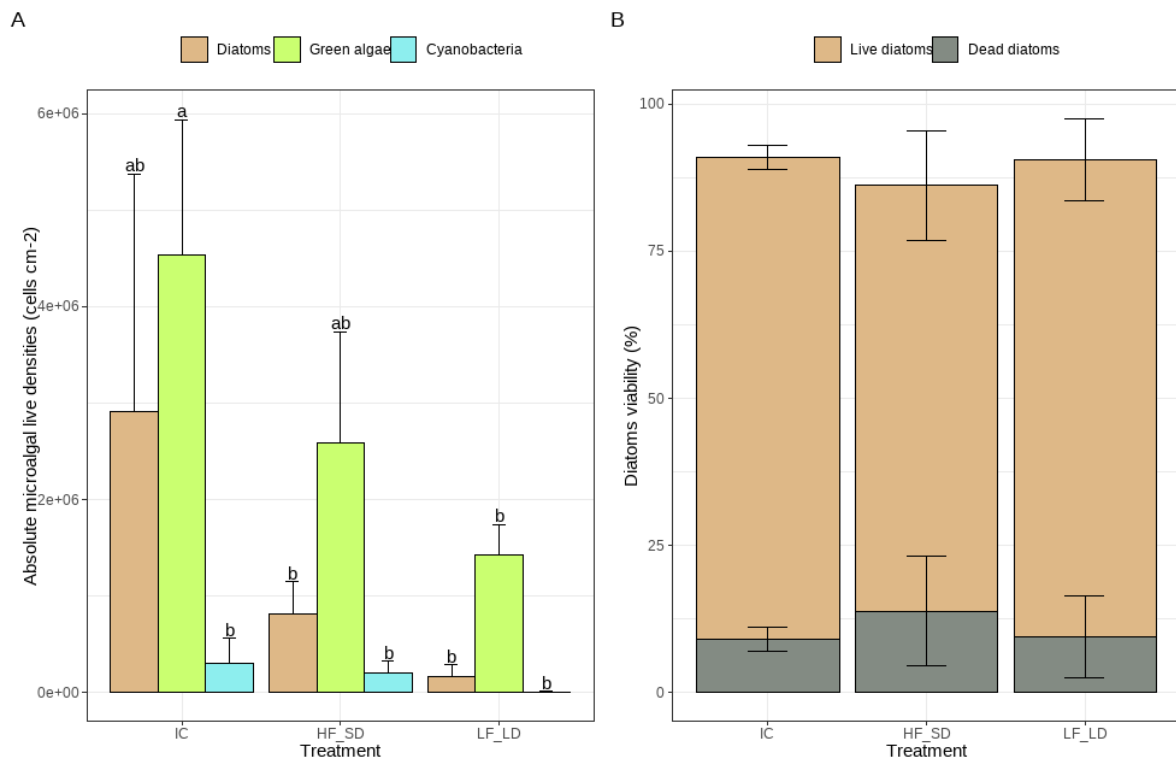
S3	LF_LD	0.611 ±	0.498 ±	0.322 ±	0.356 ±	0.115 ±	0.512 ±	0.853 ±	0.125 ±	0.313 ±	0.955 ±
		0.331	0.355	0.129	0.328	0.133	0.366	0.318	0.109	0.178	0.289
S4	HF_SD	0.520 ±	0.337 ±	0.396 ±	0.176 ±	0.108 ±	0.528 ±	1.172 ±	0.585 ±	0.353 ±	1.289 ±
		0.159	0.088	0.131	0.039	0.104	0.073	0.124	0.000	0.111	0.395
S4	IC	0.557 ±	0.670 ±	0.328 ±	0.178 ±	0.117 ±	0.494 ±	1.296 ±	0.186 ±	0.579 ±	1.003 ±
		0.136	0.595	0.191	0.124	0.074	0.113	0.195	0.179	0.233	0.131
S4	LF_LD	0.465 ±	0.710 ±	0.320 ±	0.078 ±	0.020 ±	0.326 ±	0.738 ±	0.000 ±	0.267 ±	0.920 ±
		0.199	0.742	0.062	0.134	0.017	0.109	0.342	0.000	0.150	0.564
S5	HF_SD	0.713 ±	0.397 ±	0.340 ±	0.137 ±	0.070 ±	0.797 ±	1.273 ±	0.363 ±	0.530 ±	1.387 ±
		0.196	0.038	0.017	0.031	0.061	0.367	0.210	0.629	0.062	0.283
S5	IC	0.630 ±	0.683 ±	0.557 ±	0.177 ±	0.147 ±	0.457 ±	1.447 ±	0.000 ±	0.590 ±	1.173 ±
		0.180	0.582	0.185	0.152	0.078	0.121	0.242	0.000	0.056	0.440
S5	LF_LD	0.710 ±	0.897 ±	0.317 ±	0.000 ±	0.030 ±	0.390 ±	0.527 ±	0.057 ±	0.260 ±	1.153 ±
		0.168	0.266	0.091	0.000	0.030	0.149	0.234	0.074	0.072	0.101

		<b>ItcA</b>	<b>α-KetA</b>	<b>D-MalA</b>	<b>L-Ar</b>	<b>L-Asp</b>	<b>L-Phe</b>	<b>L-Ser</b>	<b>L-Thr</b>	<b>Glyc-L-GlutA</b>	<b>PA</b>	<b>Putr</b>
S2	HF_SD	0.293 ±	0.137 ±	0.517 ±	0.580 ±	1.623 ±	0.137 ±	0.703 ±	0.080 ±	0.153 ±	0.467 ±	0.650 ±
		0.290	0.129	0.029	0.130	0.277	0.211	0.110	0.106	0.155	0.163	0.142
S2	IC	0.347 ±	0.290 ±	0.623 ±	0.463 ±	1.297 ±	0.320 ±	0.573 ±	0.177 ±	0.403 ±	0.673 ±	0.627 ±
		0.276	0.115	0.091	0.221	0.115	0.070	0.095	0.068	0.188	0.160	0.169
S2	LF_LD	0.143 ±	0.120 ±	0.590 ±	0.633 ±	1.630 ±	0.020 ±	0.510 ±	0.093 ±	0.177 ±	0.467 ±	0.303 ±
		0.223	0.120	0.290	0.101	0.256	0.035	0.226	0.114	0.198	0.153	0.091
S3	HF_SD	0.131 ±	0.193 ±	0.863 ±	0.656 ±	1.677 ±	0.214 ±	0.953 ±	0.183 ±	0.205 ±	0.504 ±	0.821 ±
		0.116	0.110	0.263	0.267	0.180	0.150	0.200	0.033	0.093	0.272	0.276
S3	IC	0.158 ±	0.276 ±	0.912 ±	0.816 ±	1.869 ±	0.250 ±	0.711 ±	0.212 ±	0.251 ±	0.741 ±	0.955 ±
		0.153	0.182	0.315	0.370	0.287	0.071	0.390	0.104	0.104	0.216	0.263
S3	LF_LD	0.142 ±	0.158 ±	0.478 ±	0.545 ±	1.338 ±	0.103 ±	0.653 ±	0.103 ±	0.163 ±	0.415 ±	0.407 ±
		0.166	0.089	0.382	0.273	0.264	0.038	0.169	0.093	0.075	0.114	0.183
S4	HF_SD	0.146 ±	0.141 ±	0.758 ±	0.495 ±	1.810 ±	0.239 ±	0.859 ±	0.199 ±	0.189 ±	0.567 ±	1.131 ±
		0.127	0.122	0.320	0.234	0.198	0.099	0.530	0.064	0.088	0.267	0.396
S4	IC	0.190 ±	0.254 ±	0.988 ±	0.709 ±	1.619 ±	0.211 ±	0.592 ±	0.224 ±	0.239 ±	0.775 ±	1.132 ±
		0.104	0.060	0.461	0.069	0.101	0.105	0.196	0.099	0.099	0.353	0.293
S4	LF_LD	0.089 ±	0.104 ±	0.384 ±	0.349 ±	1.464 ±	0.109 ±	0.575 ±	0.090 ±	0.125 ±	0.524 ±	0.421 ±
		0.123	0.044	0.365	0.068	0.408	0.100	0.337	0.047	0.054	0.335	0.114
S5	HF_SD	0.153 ±	0.097 ±	0.720 ±	0.713 ±	2.020 ±	0.243 ±	1.487 ±	0.190 ±	0.163 ±	0.713 ±	1.157 ±
		0.012	0.047	0.123	0.206	0.122	0.086	0.465	0.036	0.031	0.015	0.260
S5	IC	0.260 ±	0.223 ±	1.013 ±	0.857 ±	1.517 ±	0.247 ±	0.700 ±	0.207 ±	0.243 ±	0.647 ±	1.390 ±
		0.165	0.081	0.248	0.674	0.395	0.061	0.130	0.127	0.042	0.067	0.646
S5	LF_LD	0.147 ±	0.200 ±	0.310 ±	0.363 ±	1.580 ±	0.183 ±	0.803 ±	0.147 ±	0.237 ±	0.457 ±	0.347 ±
		0.090	0.082	0.197	0.150	0.262	0.057	0.067	0.006	0.085	0.106	0.142

937 *Table S10. Correlation of structural and functional variables to the three first dimensions of the PCA.*  
 938 *TBD = Total bacteria density; LC = Live cells percentage; Chl-a = Chlorophyll-a; EPS by microbial*  
 939 *carbon (C) = Extracellular polymeric substance corrected by microbial carbon; Resazurin (Rsz) by*  
 940 *microbial C = microbial respiration by Resazurin method corrected by microbial carbon; P-UPT by*  
 941 *microbial C = Phosphorus uptake corrected by microbial carbon.*

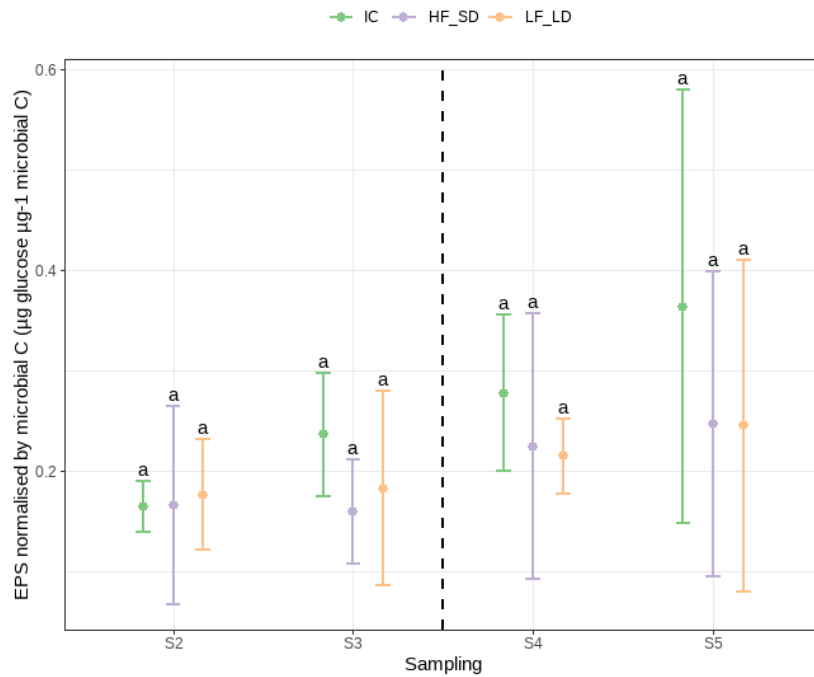
	Dim.1	Dim.2	Dim.3
TBD (cells cm <sup>-2</sup> )	-0.49	0.37	0.62
LC (%)	0.68	-0.40	-0.26
Chl-a (µg chl-a cm <sup>-2</sup> )	0.64	0.46	0.13
Total biofilm biomass (mg cm <sup>-2</sup> )	0.62	0.39	-0.13
Rsz by microbial C (µg Rsz µg microbial C <sup>-1</sup> h <sup>-1</sup> )	-0.48	-0.58	-0.24
Carbohydrates (%)	-0.58	0.65	-0.12
Polymers (%)	-0.72	-0.23	-0.17
Carboxylic and ketonic acids (%)	0.78	-0.08	0.16
Amino acids (%)	0.33	-0.79	0.18
Amines or amides (%)	0.87	0.11	0.04
PUPT by microbial C (µg P-PO <sub>4</sub> <sup>3-</sup> µg microbial C <sup>-1</sup> min <sup>-1</sup> )	-0.23	0.03	-0.54
EPS by microbial C (µg glucose µg microbial C <sup>-1</sup> )	0.23	0.35	-0.77

942



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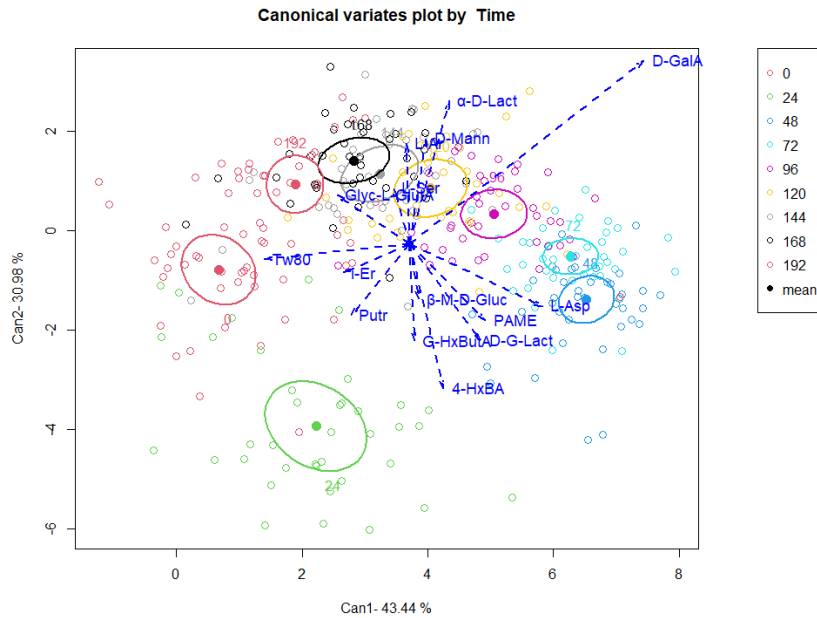
944 *Figure S1. Absolute microalgal live densities of diatoms, green algae and cyanobacteria (A) in the*  
 945 *last sampling time (S5) between different hydrological treatments (IC: immersed control; HF\_SD:*  
 946 *high frequency and short duration drought; LF\_LD = low frequency and long duration drought) and*  
 947 *diatoms viability rates (B). Letters represented the signification between treatments and different*  
 948 *microalgal communities. Values ranged by mean and standard deviation.*



949

950 *Figure S2. Extracellular polymeric substances in hydrological treatments in different sampling days.*  
 951 *Different letters indicate significant differences among treatments based on Tukey post-hoc tests (A,*  
 952 *C, D) and Wilcoxon rank-sum test (B). Dashed line represented the spike of cocktail of pesticides.*  
 953 *Values represented by means and standard deviation.*

954



955

956 *Figure S3. Canonical variates plot in function of incubation time from canonical community level*  
 957 *physiological profile (CLPP) data.*

958

## 959 **Supplementary material B**

### 960 *HPLC-MS/MS analysis for neutral pesticides from water and biofilm samples*

961 For IMID, TBZ and TBT analyses quantitation in water, 2 mL of water were filtered on Whatman®  
962 Puradisc 13 syringe filters (cellulose acetate membrane, pore size 0.45 µm). 1 mL of this filtered  
963 sample was transferred to a 1.8 mL glass vial into which 10 µL of an internal standard solution  
964 (imidacloprid d4, tebuconazole d6 and atrazine d5) at 10 ng µL<sup>-1</sup> had been initially added. The  
965 samples were analysed by HPLC-MS/MS.

966 For the extraction of IMID, TBZ and TBT from the biofilm matrixes, 5 mL of acetonitrile were added  
967 to a 10 mL polyethylene (PE) tube containing 10 mg (dry weight) of lyophilized biofilm sample,  
968 previously conserved at -80 °C. Before extraction, 10 µL of a surrogate solution (monuron d6,  
969 prometryn d6, simazine d5) at 1 ng µL<sup>-1</sup> was added to the tube. Firstly agitated, and then ultrasounded  
970 the suspension for 20 minutes. Then, 5 mL of the extract was transferred to a 15 mL glass tube to  
971 perform a second extraction with another 5 mL of acetonitrile for further evaporation under nitrogen.  
972 The resulting pellet was obtained and shaken in 10 mL of ultrapure water (UPW) before performing  
973 the solid phase extraction (SPE) purification step. Chromabond HR-X SPE cartridges (3 mL, 60 mg,  
974 Macherey-Nagel, France) were placed on a Visiprep (Supelco) and conditioned initially with 5 mL  
975 methanol (MeOH) and 5 mL UPW. The 10 mL of sample previously diluted in UPW was percolated  
976 under vacuum at a flow rate of 5 mL min<sup>-1</sup>. Then, samples were rinsed with 5 mL of an UPW:MeOH  
977 (80/20, v/v) mixture. Afterwards, the cartridges were dried under nitrogen for approximately 30  
978 minutes and stored at -20 °C until the elution step. This final elution step was performed by flushing  
979 the cartridge with 5 mL of acetonitrile. The purified extract was collected in a 15 mL glass tube which  
980 10 µL of a solution of internal standards (imidacloprid d4, tebuconazole d6 and atrazine d5) at 10 ng  
981 µL<sup>-1</sup> was also added. The sample was then evaporated to dryness under nitrogen, filled with 1 mL of  
982 UPW, and finally transferred to a 1.8 mL vial for analysis by HPLC-MS/MS.

983 Finally, the neutral pesticides from water samples or biofilm extracts were analysed with Dionex  
984 Ultimate 3000 HPLC (Thermo Fisher Scientific, Villebon-sur-Yvette, France). Chromatographic  
985 separation was performed with a Gemini-NX C18 3µm, 110 Å, 100 × 2 mm with a Security Guard  
986 cartridge Gemini-NX C18 4 × 2.0 mm (Phenomenex, Le Pecq, France). Detection was performed  
987 with an API 2000 tandem mass spectrometer (Sciex, Villebon-sur-Yvette, France).

### 988 *HPLC-MS/MS analysis for glyphosate and AMPA from water samples*

989 The method used in this analysis followed the recommendations of the project ISO/DIS 16308 (Water  
990 Quality – Determination of GLY and AMPA- Method using high performance liquid chromatography  
991 (HPLC) with tandem mass spectrometry detection). Briefly, 5 mL of freshwater sample were

992 transferred into 50 mL polypropylene tubes and fortified with 50  $\mu\text{L}$  of both GLY and AMPA  $^{13}\text{C}$ ,  $^{15}\text{N}$   
993 isotopes  $20 \text{ ng mL}^{-1}$  (surrogates). Then, 325  $\mu\text{L}$  of sodium borate 50 mM and 200  $\mu\text{L}$  EDTA- $\text{Na}_2$  0.1  
994 M were added. The samples were homogenised and left for 5 min. 4.5 mL of acetonitrile and 600  $\mu\text{L}$   
995 of FMOC-Cl  $50 \text{ mg mL}^{-1}$  were added, and the samples were left for 30 min in the dark at room  
996 temperature (formation of FMOC derivatives). Afterwards, acetonitrile was evaporated through an  
997 azote stream during 1h approximately (until sample volume  $< 5 \text{ mL}$ ). Liquid-liquid extractions were  
998 then performed with addition of  $3 \times 1.5 \text{ mL}$  ethyl acetate in a 15 mL graduated glass tube. The  
999 remaining ethyl acetate was removed using an azote stream for 15 min. 100  $\mu\text{L}$  of formic acid 5 %  
1000 was added, and the sample volume was adjusted to 5 mL and homogenised. The sample extract was  
1001 then loaded onto Oasis HLB cartridges (3 mL, 60 mg, 30  $\mu\text{m}$  particle size, Waters, France) after a  
1002 conditioning step (1 mL MeOH followed by 1 mL formic acid 0.1 %). After sample loading, the  
1003 cartridge was washed with 1 mL formic acid 0.1 % and 1 mL UPW, dried under azote, and eluted in a  
1004 1 mL graduated flask with 2 mL of ammonium hydroxide/UPW/MeOH 2:30:68 (v/v/v). The collected  
1005 extract was then evaporated until the volume stabilizes at 0.5 mL. Finally, the samples volume was  
1006 adjusted to 1 mL with UPW for further analysis by HPLC-MS/MS.

1007 Analyses of both GLY and AMPA were performed by HPLC-MS/MS with the same instruments  
1008 mentioned before for IMID, TBZ and TBT. Reversed phase separation was performed on a X-Bridge  
1009  $\text{C}_{18}$  3.5  $\mu\text{m}$ , 2.1 x 50 mm protected by a precolumn X-Bridge  $\text{C}_{18}$  2.1 x 10 mm (Waters, Le Pecq,  
1010 France).

1011