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1 **Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms**

2

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17

18 **Keywords**

19 aminomethyl phosphonic acid (AMPA); phosphorus; co-occurrence; microbial  
20 ecotoxicology; biodegradation; eutrophication.

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26        **Abstract**

27

28        One consequence of the intensive use of glyphosate is the contamination of rivers by the  
29 active substance and its metabolites aminomethyl phosphonic acid (AMPA) and sarcosine,  
30 inducing river eutrophication. Biofilms are the predominant lifestyle for microorganisms in  
31 rivers, providing pivotal roles in ecosystem functioning and pollutant removal. The  
32 persistence of glyphosate in these ecosystems is suspected to be mostly influenced by  
33 microbial biodegradation processes.

34        The present study aimed to investigate the tripartite relationship among biofilms,  
35 phosphorus and glyphosate in rivers. The first part consists of a co-occurrence analysis among  
36 glyphosate, AMPA and phosphorus using an extensive dataset of measurements (n = 56198)  
37 from French surface waters between 2013 and 2017. The second part investigated the capacity  
38 of natural river biofilms to dissipate glyphosate, depending on phosphorus availability and the  
39 exposure history of the biofilm, in a microcosm study.

40        A strong co-occurrence among glyphosate, AMPA and phosphorus was found in surface  
41 waters. More than two-thirds of samples contained phosphorous with glyphosate, AMPA or  
42 both compounds. Seasonal fluctuations in glyphosate, AMPA and phosphorus concentrations  
43 were correlated, peaking in spring/summer shortly after pesticide spreading. Laboratory  
44 experiments revealed that natural river biofilms can degrade glyphosate. However,  
45 phosphorus availability negatively influenced the biodegradation of glyphosate and induced  
46 the accumulation of AMPA in water. An increase in alkaline phosphatase activity and  
47 phosphorus uptake was observed in glyphosate-degrading biofilms, evidencing the tight link  
48 between phosphorus limitation and glyphosate degradation by biofilms.

49        The results of the present study show that phosphorus not only is a key driver of river  
50 eutrophication but also can reduce complete glyphosate degradation by biofilms and favour

51 the accumulation of AMPA in river water. The predominant role of biofilms and the trophic  
52 status of rivers must therefore be considered in order to better assess the fate and persistence  
53 of glyphosate.

54

## 55 **1. Introduction**

56 Glyphosate (N-(phosphonomethyl) glycine) is a systemic herbicide exhibiting a broad  
57 activity spectrum. This herbicide is mainly used before planting non-genetically modified  
58 crops, on glyphosate-resistant crops (Powles and Duke, 2010), in orchards (Maqueda et al.,  
59 2017), in minimum-tillage agroecosystems (Buhler, 2014) and in urban areas (Hanke et al.,  
60 2010). Since its commercialisation in 1974, glyphosate use has rapidly increased (Duke,  
61 2015). In 2012, approximately 9,000 tons of glyphosate was used in France, 127,000 tons in  
62 the USA and 700,000 tons worldwide (AGRESTE, 2018; Swanson et al., 2014; US  
63 Geological Survey, 2018). This herbicide is authorized at the European Community level  
64 (included in Annex I to Directive 91/414/EEC on 2002/07/01 by Commission Directive  
65 2001/99/EC) and at the national level in France, where a total of 192 glyphosate-based  
66 formulations are currently approved (E-Phy, 2018). Glyphosate is the most-used active  
67 substance in France, and sales remained constant over the period 2011-2015 (AGRESTE,  
68 2018). Broad contamination by glyphosate residues has recently led to awareness of its  
69 potential harmful side effects to human health and soil and aquatic ecosystems (Davoren and  
70 Schiestl, 2018; Van Bruggen et al., 2018). This global contamination has been a central  
71 question in the media and political scenes worldwide concerning the prolongation (or not) of  
72 glyphosate's use in the environment.

73 One consequence of the intensive use of glyphosate is the contamination of various  
74 environmental compartments despite the fact that this compound can be degraded by various  
75 soil and aquatic microorganisms (Sviridov et al., 2015). The primary degrading product often  
76 detected in the environment is AMPA aminomethyl phosphonic acid (AMPA) (Singh and  
77 Singh, 2016). Glyphosate can also move through soil and contaminate surface and ground  
78 waters (Van Bruggen et al., 2018). In rivers, the dissipation time to eliminate 50% (DT<sub>50</sub>) of  
79 glyphosate varied from 13.8 to 301 days, suggesting a moderate to high persistence of the

80 molecule (EFSA, 2015). In France, AMPA and glyphosate are among the most often  
81 quantified compounds in river water (63% and 43% quantification frequency, respectively).  
82 Their concentrations can reach up to 558 µg/L (AMPA) and 164 µg/L (glyphosate)  
83 (NAIADES, 2018).

84 Rivers are dynamic ecosystems that play a key role in nutrient cycling and xenobiotic  
85 mitigation (Hanna et al., 2018). Within rivers, biofilms are one of the microbial lifestyle that  
86 guarantee essential ecosystem functions and participate in biogeochemical cycles (Battin et  
87 al., 2016). The large panel of functions provided by river biofilms is due to their extreme  
88 microbial diversity. Biofilms are composed of metazoan organisms, bacteria, archaea, algae,  
89 fungi, protozoa and viruses embedded in an extracellular matrix protecting them against  
90 environmental stresses such as nutrient starvation and pollutants (Besemer, 2015). River water  
91 carries nutrients, organic matter and pollutants, which may constitute important constraints for  
92 microorganisms (Battin et al., 2016). In turn, the fact that biofilms are complex assemblages  
93 hosting a large variety of microbial species enables biofilms to degrade a large variety of  
94 xenobiotics, either by co-metabolism or by mineralization. Biofilms are also dynamic  
95 communities that can colonize different types of substrate (e.g. mud, stones, macrophytes)  
96 and adapt to xenobiotic exposure (Blanck, 2002).

97 The glyphosate molecule contains one atom of phosphorus and can therefore be used as a  
98 phosphorus source for a variety of microorganisms in biofilms (i.e., bacteria and fungi)  
99 (Sviridov et al., 2015). This compound can therefore contribute to river eutrophication (Vera  
100 et al., 2010) and reinforce the problem resulting from massive fertilizer utilization (Lasier et  
101 al., 2016) can lead to an excess of phosphorus, which interferes with the capacity of biofilms  
102 to mitigate glyphosate. The genetic and biochemical bases of glyphosate utilization as  
103 phosphorus source for microorganisms have been well described in the literature (Hove-  
104 Jensen et al., 2014). Glyphosate is cleaved through the CP-lyase pathway, which involves

105 seven or eight enzyme-catalysed reactions (e.g., phosphonate activation and C-P bound  
106 cleavage). The genes encoding the corresponding enzymes are assembled into the *phn* operon,  
107 which is widespread among bacterial species.

108 To date, the effect of phosphorus on glyphosate biodegradation has mostly been studied  
109 for isolated microbial strains (Krzysko-Lupicka et al., 2015; McMullan and Quinn, 1994)  
110 rather than for natural biofilm communities (Klátyik et al., 2017). The present study  
111 investigates the tripartite relationship among biofilms, phosphorus, and glyphosate in rivers.  
112 The first part of the study consists of the analysis of an extensive dataset of glyphosate,  
113 AMPA and total dissolved phosphorus concentrations recorded in French surface waters  
114 between 2013 and 2017 (n = 56198). The co-occurrence, seasonality and influence of trophic  
115 status on glyphosate and AMPA fate in rivers were investigated. The second part of the study  
116 was carried out in laboratory microcosms in order to investigate the capacity of natural  
117 biofilms to dissipate glyphosate. Biofilms from an upstream site (non-exposed to glyphosate)  
118 and a downstream site (chronically exposed to glyphosate) were used to test the influence of i)  
119 phosphorus availability in water and in biofilms and ii) glyphosate availability in water on  
120 glyphosate dissipation. Our main hypothesis was that glyphosate can be used as a phosphorus  
121 source for biofilm microorganisms, though eutrophic conditions might lead to the slower total  
122 degradation of glyphosate and accumulation of AMPA in rivers. Besides, biofilms chronically  
123 exposed to glyphosate would develop more efficient glyphosate degradation comparing to  
124 biofilms non-exposed.

125

## 126 **2. Material and methods**

127

128 *2.1. Meta-analysis of glyphosate, AMPA and total phosphorus concentrations in surface*  
129 *waters from France*

130 Data on glyphosate, AMPA and total phosphorus concentrations in surface waters in  
131 France were downloaded on 2018/02/16 from the NAIADES public database available at  
132 <http://www.naiades.eaufrance.fr/acces-donnees#/physicochimie> (NAIADES, 2018). This  
133 database contains the results of analyses performed by water agencies and environmental  
134 consultancies, with a total of 4733 (glyphosate), 4716 (AMPA) and 9326 (total phosphorus)  
135 sites located throughout France (excluding French overseas departments and territories).  
136 Within this huge database, the research criteria imposed for data selection in our study were  
137 as follows: time period: “2013/01/01 – 2017/12/31”; data qualification: “correct” (i.e. result of  
138 the water analysis validated by the freshwater agencies); and fraction: “raw water”. Valid data  
139 obtained separately for each parameter (glyphosate samples N = 72298, AMPA N = 72277,  
140 total phosphorus N = 215462) were then grouped together (N = 56198) and filtered again by  
141 cases in which quantification of each compound was > average of their corresponding limit of  
142 quantification (LQ). This resulted in N = 31041 for glyphosate (LQ = 0.03 µg L<sup>-1</sup>), N = 45552  
143 for AMPA (LQ = 0.02 µg L<sup>-1</sup>) and N = 199833 for total phosphorus (LQ = 0.01 mg P L<sup>-1</sup>).

144 Co-occurrence analysis was therefore performed in cases where three compounds were  
145 detected at the same station AND on the same date. The quantification of glyphosate and  
146 AMPA was also classified by trophic status (according to the concentration of total  
147 phosphorus in surface water, µg P L<sup>-1</sup>): oligotrophic (< 25, N = 1018), mesotrophic (25 – 75,  
148 N = 6988), eutrophic (75 – 100, N = 3147) and hypereutrophic (> 100, N = 11670).

149 The temporal evolution of glyphosate, AMPA and total phosphorus concentrations was  
150 assessed on a monthly basis from January 2013 to August 2017. The average concentration of  
151 each parameter was calculated for each month independent of the station (N = 56). The  
152 relationship between the AMPA/glyphosate ratio and the concentration of total phosphorus  
153 was assessed for a monthly averaged dataset using Spearman’s rank correlation test  $\rho$  ( $P <$   
154 0.05).

155

156 *2.2. Microcosm study*

157 Colonization of natural biofilms was carried out in the field at the end of spring 2017 in an  
158 upstream site (Ups, 45°43'14.4"N 3°01'16.3"E) and a downstream site (Dws, 45°47'44.8"N  
159 3°10'26.8"E) of the Artière River (Puy-de-Dôme region, France) during two weeks (Figure  
160 S1). Both sites were well distinct in terms of (mean for upstream/downstream sites): water  
161 velocity 0.21/0.74 m s<sup>-1</sup>; water discharge 0.03/1.25 m<sup>3</sup> s<sup>-1</sup>; dissolved organic carbon  
162 6.50/12.97 mg L<sup>-1</sup>; NO<sub>3</sub> 3.55/16.70 mg L<sup>-1</sup>; soluble reactive phosphorus 0.03/0.32 mg P L<sup>-1</sup>;  
163 dissolved oxygen 10.02/8.15 mg L<sup>-1</sup>; conductivity 297.53/654.91 μS cm<sup>-1</sup>; pH 7.33/7.35;  
164 glyphosate 0.00/0.25 μg L<sup>-1</sup> and AMPA 0.09/1.08 μg L<sup>-1</sup> (Artigas et al., 2017; Rossi et al.,  
165 2019). Rocks, boulders, and sand covered the streambed of the upstream site, while rocks and  
166 sand were dominant at the downstream site. Frosted glass slides with an area of 21.9 cm<sup>2</sup> were  
167 glued onto three different flagstones per site, which were randomly submerged along the  
168 stream sections to depths of 10–20 cm.

169 Twenty-four microcosms consisting of 20 L glass aquariums (rectangular parallelepiped  
170 length x width x height 39 x 19 x 23 cm) were used to determine the biodegradation potential  
171 of glyphosate in the laboratory. For the acclimation phase, glass slides were detached from  
172 flagstones and carefully placed at the bottom of the aquarium. Colonized slides were heavy  
173 enough to not to be taken by the water current supplied by the aquarium pump. The side of the  
174 slide where the biofilm was grown was placed in contact with the water column and the light  
175 source. The position of biofilms in the microcosm mimicked water depths, current velocity,  
176 and light conditions similar to those found in the field during colonization. Half of the aquaria  
177 received Ups biofilms, while the other half received Dws biofilms (11 colonized slides per  
178 aquarium). Each aquarium containing biofilms was filled with 5 L of filtered (0.5 mm) water  
179 collected from the upstream site. This water was characterized by 4.0 mg L<sup>-1</sup> of NO<sub>3</sub>, 100 μg

180 P L<sup>-1</sup> of soluble reactive phosphorus, 8.2 mg L<sup>-1</sup> of dissolved organic carbon, 9.2 mg L<sup>-1</sup> of  
181 dissolved oxygen, 244 µS cm<sup>-1</sup> of conductivity and a pH of 7.3. This water did not contain  
182 any traces of glyphosate and AMPA. Water was recirculated in each aquarium by a  
183 submerged pump (Newjet 1200, Nawa, Italy). Flow rates of aquarium pumps were adjusted in  
184 order to obtain water current velocities similar to those measured in the Artière upstream site  
185 (200 cm s<sup>-1</sup>). Temperature and photoperiod were set at 19 +/- 1°C and 13 h light: 11 h dark.  
186 The water in the aquaria was completely renewed every 3 days to avoid nutrient limitation in  
187 the biofilms during the acclimation phase. The dissolved phosphorus concentration in aquaria  
188 containing Ups biofilms was maintained at the initial P concentration at the upstream site  
189 (corresponding to the LowP condition, i.e., an average value of 100 µg P L<sup>-1</sup> during the  
190 experiment), whereas the water in Dws aquaria was adjusted to 10 x LowP (corresponding to  
191 the HighP condition, 1000 µg P L<sup>-1</sup>) by adding adequate volumes of 1 M K<sub>2</sub>HPO<sub>4</sub> salt  
192 solution.

193 After two weeks of laboratory acclimation for the biofilms, the water was removed, and  
194 each biofilm type (Upstream = colonized at a low-P site, Downstream = colonized at a high-P  
195 site) were subjected to the four experimental conditions in triplicate according to water  
196 phosphorus concentration (LowP and HighP as defined above) and glyphosate concentration  
197 (LowG = 10 µg L<sup>-1</sup> and HighG = 100 µg L<sup>-1</sup>, nominal concentration) by adding adequate  
198 volumes of 10 and 100 g L<sup>-1</sup> glyphosate aqueous solution prepared with glyphosate  
199 PESTANAL<sup>®</sup> (analytical standard, CAS Number 1071-83-6, Sigma-Aldrich, France) in 0.2  
200 µm filtered water from upstream (15 L per aquarium). These experimental conditions  
201 correspond to 1x and 10x the mean of actual concentration of glyphosate in surface waters  
202 from France. The experimental conditions in our microcosm experiment were therefore  
203 chosen to assess realistic environmental contamination conditions. Abiotic controls (0.2 µm  
204 filtered water without biofilms) were also carried out for each condition (in triplicate) in order

205 to check the absence of abiotic dissipation of glyphosate. Glyphosate and AMPA were  
206 quantified in water at the beginning and at the end of the experiment (day 27).

207 Water analyses were carried out at 0, 2, 4, 6, 9, 13, 16, 20, 23 and 27 days. One glass slide  
208 was removed from each aquarium at 0, 2, 4, 6, 13, 20 and 27 days for biofilm analysis.  
209 Scraped biofilms were suspended in 12 mL of sterile 0.8% NaCl solution. After  
210 homogenization, aliquots of the biofilm suspension were kept for subsequent analyses.

211 Nitrate and phosphorus concentrations were maintained throughout the experiment by  
212 adding adequate volumes of 1 M  $\text{KNO}_3$  (in order to maintain the initial concentration of  
213 nitrate in water, i.e.  $4.0 \text{ mg L}^{-1}$ ) and 1 M  $\text{K}_2\text{HPO}_4$  salt solutions (in order to satisfy the  
214 concentration of phosphorus of LowP and HighP described above), respectively.

215

#### 216 *2.2.1. Water analyses*

217 The concentrations of total phosphorus and soluble reactive phosphorus (SRP) in filtered  
218 water ( $0.45 \mu\text{m}$ ) were determined spectrophotometrically at 890 nm (Murphy and Riley,  
219 1962). The total phosphorus concentration was determined after an additional digestion step:  
220 10 mL of water samples was mixed with 1 mL of digestive reagent (0.185 M potassium  
221 persulfate, 0.485 M boric acid in 0.375 M NaOH) and heated at  $120^\circ\text{C}$  for 1 h 30 m.

222 Glyphosate and AMPA quantification was performed using 100 mL water samples. These  
223 samples were immediately sent in refrigerated boxes ( $4^\circ\text{C}$ ) to the CARSO laboratory  
224 (COFRAC agreement number 1-1531, Lyon, France) and kept at  $4^\circ\text{C}$  until glyphosate and  
225 AMPA measurements were performed (HPLC/FLD, internal method M\_ET143). The method  
226 consisted of the analysis of both compounds after a derivatization step (glyphosate and  
227 AMPA LQs =  $0.1 \mu\text{g L}^{-1}$ ).

228

#### 229 *2.2.2. Bacterial abundance and phosphorus content in biofilms*

230 Bacterial density was estimated for each experimental condition and sampling time using  
231 flow cytometric counts of bacterial cells. Five hundred microliters of biofilm suspension was  
232 centrifuged (13,000 *g* for 5 minutes at 4°C). The pellet was resuspended in 900 µL of sterile  
233 PBS pH 7.2 (g L<sup>-1</sup>: NaCl, 8.5; Na<sub>2</sub>HPO<sub>4</sub> 1.07; NaH<sub>2</sub>PO<sub>4</sub>, 0.39) and sonicated twice (40 W, 40  
234 kHz, 30 s) using a sonication bath (model FB 15048, Fisher Scientific, Leicestershire, U.K.).  
235 The resulting bacterial suspension was centrifuged again (800 *g* for 60 s at 4°C), fixed with  
236 formaldehyde (2% final concentration) and stored at 4°C until cytometric analysis. Ten  
237 microliters of fixed bacterial suspension was diluted 25-fold in TE buffer (10 mM Tris, 1 mM  
238 EDTA) and stained with 2.5 µL of SYBR Green I (Molecular Probes) before counting  
239 bacterial cells with a BD FACSCalibur flow cytometer (15 mW at 488 nm, Becton Dickinson,  
240 USA).

241 Biofilm phosphorus content was measured spectrophotometrically (Murphy and Riley,  
242 1962) after the digestion of 2 mL of biofilm suspension, using the same protocol described  
243 above for total phosphorus quantification in water. Biofilm P content was corrected by the  
244 biofilm dry weight. Biofilm dry weight was determined as follows: 2 mL of biofilm  
245 suspension were centrifuged (13,000 *g* for 5 minutes at 4°C), the supernatant was discarded  
246 and the pellet was dried over night at 60 °C. The weight of dry pellet was then measured  
247 using a precision balance (Sartorius CPA225D, 0.01 mg precision).

### 248 2.2.3. *Biofilm activity*

249 The potential extracellular alkaline phosphatase activity (APA, EC 3.1.3.1) in biofilms  
250 was measured for each experimental condition and sampling time using a methylumbelliferyl-  
251 phosphate (MUF-P, Sigma) substrate analogue (Chrost and Krambeck, 1986). Eight hundred  
252 microliters of biofilm suspension was mixed with the MUF-P substrate at a known saturation  
253 concentration of 0.3 mM. Samples were then incubated for 1 h at 19°C in the dark with  
254 agitation. The enzymatic activity was stopped by adding (1:1, V:V) 0.05 M glycine buffer (pH

255 10.4), and fluorescence was measured (365 nm excitation, 455 nm emission) with a  
256 microplate fluorometer (Fluoroskan<sup>TM</sup>, Thermo Scientific, France).

257 Phosphorus uptake was calculated from bi-weekly SRP concentration measurements in  
258 microcosms containing treated biofilms. Phosphorous uptake ( $\mu\text{g P g (biofilm}_{\text{dw}})^{-1} \text{ day}^{-1}$ ) was  
259 calculated as follows:  $P_{\text{uptake}} = (\text{SRP immediately after P adjustment} - \text{SRP after each}$   
260  $\text{sampling time interval}) / \text{sampling time interval (days)}$ .

#### 261 2.2.4. *Glyphosate and AMPA quantification in biofilms*

262 At the end of the experiment (day 27), the biofilms were scraped from the remaining  
263 glass slides (total surface =  $49.5 \text{ cm}^2$ ) and lyophilized before glyphosate and AMPA analysis.  
264 The dry mass of each biofilm was estimated, and samples were extracted by ultrasound-  
265 assisted solid-liquid extraction using a custom method. Briefly, ultrapure water acidified with  
266 formic acid (HFor, Sigma-Aldrich, Steinheim, Germany) at  $\text{pH} = 3.0$  was added to the  
267 extraction tubes at a ratio of 0.10 mL per 1.0  $\text{mg}_{\text{dw}}$  of collected biofilm. The tubes were  
268 placed in an ultrasonic bath for 20 minutes. Afterwards, the extracts were centrifuged (4000  
269 rpm, 10 minutes). The supernatants were vacuum-filtered through  $0.45 \mu\text{m}$  mesh nylon filters  
270 (Whatman, Maidstone, UK) and were derivatized with 9-fluorenylmethoxycarbonyl chloride  
271 (FMOC, 97%, Sigma-Aldrich) according to a previously published method (Hanke et al.,  
272 2008; Sanchís et al., 2012).

273 After 2 h of derivatization, the extracts were acidified to  $\text{pH} = 3.0$  with  $\text{HFor}_{(\text{aq})}$  and  
274 centrifuged (4000 rpm, 10 minutes). The supernatants were analysed by liquid  
275 chromatography coupled to high resolution mass spectrometry (HPLC-HRMS) with an  
276 Acquity UPLC system (Waters, Milford, MA, USA) and a Q Exactive<sup>TM</sup> mass spectrometer  
277 (Thermo Fisher Scientific, San Jose, CA, USA). Separation was achieved with a C18 column  
278 (Luna®,  $150 \times 2.0 \text{ mm}$ ; particle size,  $5 \mu\text{m}$ , Phenomenex).  $\text{HFor}_{(\text{aq})}$  (0.1%) and acetonitrile  
279 were used as mobile phases at  $0.250 \text{ mL min}^{-1}$ . Ionisation was carried out with an electrospray

280 ionisation (ESI) source in negative mode. The acquisition was carried out in full-scan mode.  
281 The deprotonated molecular ions  $[C_{18}H_{17}NO_7P]^-$  ( $m/z = 390.0748$ ) and  $[C_{16}H_{15}NO_5P]^-$  ( $m/z$   
282  $= 332.0693$ ) were used for FMOG-glyphosate and FMOG-AMPA, respectively.

283

### 284 *2.3. Modelling glyphosate dissipation and AMPA formation in microcosms*

285 The dissipation kinetics of glyphosate in water were fitted using OriginPro 2016 software  
286 (Origin Lab Corporation, USA) to a simple first-order exponential model (Exp2Mod1),  
287 characterized by the following equation:

$$288 \quad C_t = C_0 e^{-kt} \quad (\text{Equation 1})$$

289 where  $t$  is the incubation time,  $C_t$  the glyphosate concentration at time  $t$ ,  $C_0$  the initial  
290 concentration of glyphosate and  $k$  the rate constant in  $\text{day}^{-1}$ .  $DT_{50}$  is the time required for the  
291 concentration to decline to 50% of the initial value.

292 The kinetics of AMPA formation in water were fitted with the sigmoid function  
293 (Boltzmann) of OriginPro 2016, characterized by the following equation:

$$294 \quad C_t = \frac{C_0 - C_f}{1 + e^{(t - h_{50})k}} + C_f \quad (\text{Equation 2})$$

295 where  $t$  is the incubation time,  $C_t$  the AMPA concentration at time  $t$ ,  $C_0$  the initial  
296 concentration of AMPA,  $C_f$  the final concentration of AMPA,  $k$  the rate constant in  $\text{day}^{-1}$  and  
297  $h_{50}$  the time required for the AMPA concentration to reach 50% of  $(C_f - C_0)$ .

298

### 299 *2.4. Statistical analyses*

300 Statistical analyses were carried out using OriginPro 2016. The differences in various  
301 parameters linked to glyphosate dissipation kinetics ( $C_0$  and  $DT_{50}$ ), AMPA formation kinetics  
302 ( $C_f$ ), glyphosate and AMPA content in biofilms, phosphorus uptake ( $P_{\text{uptake}}$ ), cell abundance,  
303 phosphorus content and phosphatase activity were assessed using three-way ANOVA

304 followed by separate post hoc comparisons (Tukey's test,  $P < 0.05$ ). The three factors tested  
305 were site (Ups vs. Dws), phosphorous in water (LowP vs. HighP) and glyphosate initial  
306 concentration in water (LowG vs. HighG). The normality and homogeneity of variance were  
307 checked prior to ANOVA analysis (Kolmogorov-Smirnov's and Levene's tests, respectively,  
308  $P < 0.05$ ) and data that were not normally distributed were transformed using logarithmic  
309 functions.

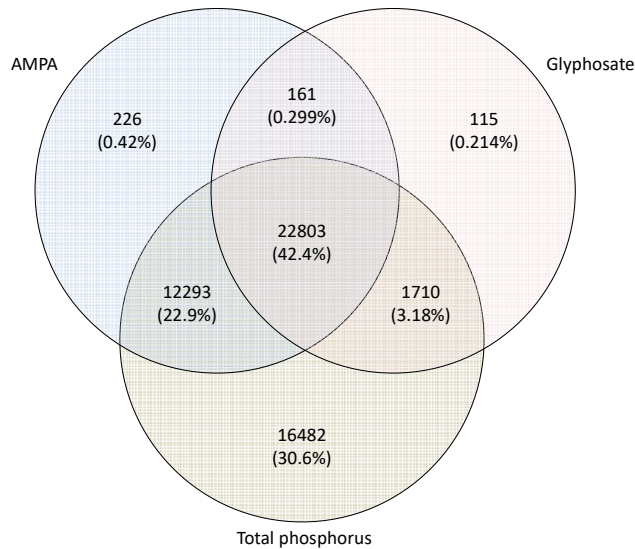
310

### 311 **3. Results**

312

#### 313 *3.1. Concentrations of glyphosate, AMPA and phosphorus in French surface waters* 314 *between 2013 and 2017*

315 The analysis performed on the public database revealed that phosphorus concentrations  
316 are relatively high (mean of  $0.1 \text{ mg P L}^{-1}$ , maximum  $18 \text{ mg P L}^{-1}$ ), corresponding to sites with  
317 high levels of eutrophication. Phosphorus was detected in all the samples analysed ( $N =$   
318  $215462$ ) and was properly quantified ( $LQ = 0.01 \text{ mg P L}^{-1}$ ) in 93% of the samples. In parallel,  
319 glyphosate and AMPA were also detected in almost all water samples (99.9% each). Our  
320 analysis was specifically focused on samples containing at least one of the analytes above its  
321 LQ. The Venn diagram showed that co-occurrence (i.e., when compounds were quantified at  
322 the same station AND on the same date) was mainly found for glyphosate – AMPA –  
323 phosphorus (42.4%), followed by AMPA – phosphorus (22.9%) (Figure 1). However, very  
324 few samples had only the combination glyphosate – phosphorus (3.2%) because glyphosate  
325 was most often quantified with its main metabolite AMPA (42.7%) compared to glyphosate  
326 without AMPA (3.4%).

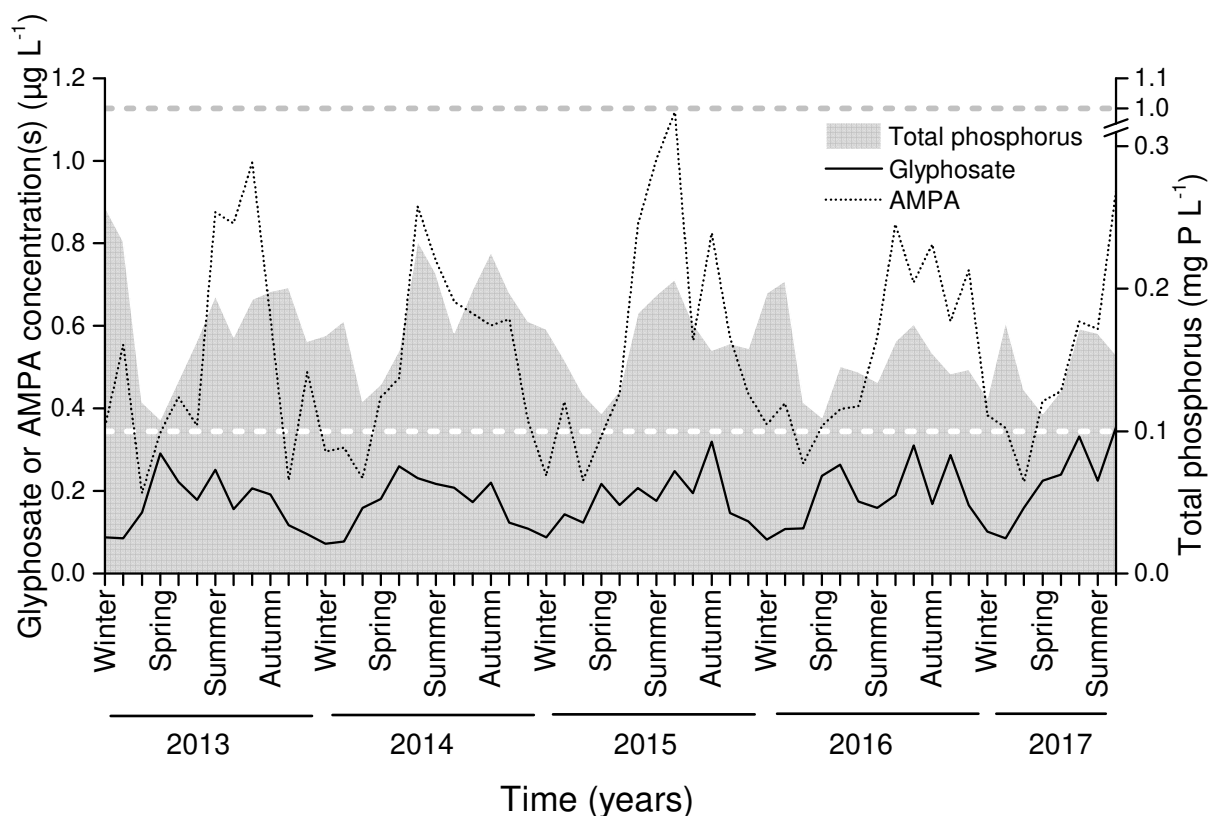


327

328 **Figure 1.** Co-occurrence of glyphosate, AMPA and phosphorus in surface water in France.

329 Values are the number of water samples in which the corresponding compound was quantified  
 330 (i.e., result of analysis > LQ). Period of time: January 2013 – August 2017 (N = 53790  
 331 measurements).

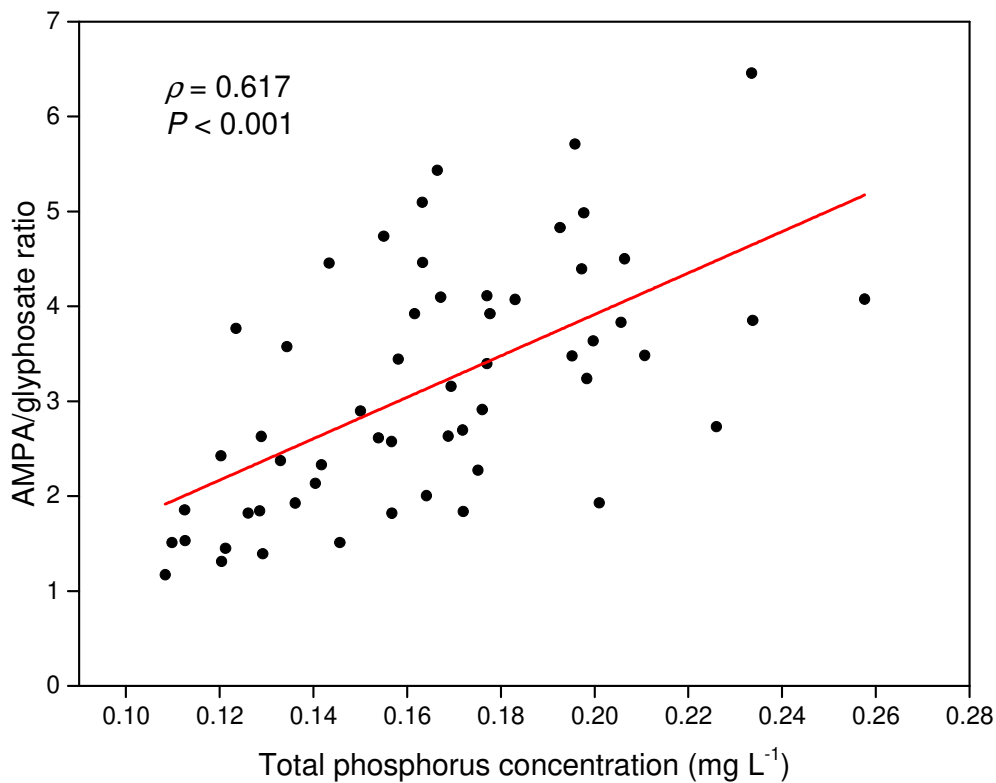
332 The temporal evolution of glyphosate, AMPA and total phosphorus concentrations  
 333 followed a seasonal pattern that was consistent over the years (Figure 2). The concentrations  
 334 of the three compounds increased from spring to summer (the increase in glyphosate occurred  
 335 first, followed by AMPA and total phosphorus) and decreased from the end of autumn to  
 336 winter. In terms of phosphorus equivalents, the results showed that the  $P_{(AMPA)}$  concentration  
 337 was always higher than the  $P_{(glyphosate)}$  concentration, by a factor of 2 to 10 depending on the  
 338 season (higher differences were obtained during spring and summer). However, the  
 339 concentration of phosphorus from both compounds was extremely low compared with the  
 340 total phosphorus concentration in water ( $P_{(AMPA)}$  and  $P_{(glyphosate)}$  represent less than 0.17% and  
 341 0.05% of total P).



342  
 343 **Figure 2.** Seasonal fluctuations in glyphosate, AMPA and phosphorus concentrations in  
 344 surface water in France between January 2013 and August 2017. A total of  $N = 22823$   
 345 measurements were monthly averaged. The average concentrations of phosphorus used in the  
 346 microcosm study are indicated by horizontal white (LowP) and grey (HighP) dashed lines.

347 Regarding P concentrations, French surface waters containing glyphosate and/or AMPA  
 348 were classified into 4 different trophic states according to the classification by Dodds *et al.*  
 349 (1998). The more eutrophic a site was, the greater were the concentrations of glyphosate and  
 350 AMPA recorded (Figure S2). The specific glyphosate and AMPA concentrations in water  
 351 varied from  $0.12 \pm 0.02$  and  $0.13 \pm 0.01 \mu\text{g L}^{-1}$  (oligotrophic) to  $0.32 \pm 0.05$  and  $0.90 \pm 0.02$   
 352  $\mu\text{g L}^{-1}$  (hypereutrophic), respectively. The results also indicate that glyphosate and AMPA  
 353 were quantified in water samples containing less than  $100 \mu\text{g P L}^{-1}$ . These compounds were

354 even quantified in oligotrophic water containing very little phosphorous (approximately 10  $\mu\text{g}$   
355  $\text{P L}^{-1}$ ).



356  
357 **Figure 3.** Correlation between AMPA/glyphosate ratio and total phosphorus concentration in  
358 surface water in France. A total of  $N = 22823$  measurements were monthly averaged from  
359 January 2013 to August 2017. The value of Spearman's rank correlation test ( $\rho$ ) is also  
360 indicated in the graph.

361 A significant correlation was also observed between the AMPA/glyphosate ratio and total  
362 phosphorus concentration in water, as shown by the positive Spearman's rank correlation  
363 coefficient  $\rho = 0.617$  ( $P < 0.001$ ) (Figure 3). This correlation indicates that sites with high  
364 phosphorus concentrations result in low glyphosate but high AMPA accumulation, since the  
365 latter compound still contains an atom of phosphorus. In addition, a positive correlation was  
366 observed between glyphosate and AMPA ( $\rho = 0.498$ ,  $P < 0.001$ ).

367

368 3.2. *Glyphosate dissipation by river biofilms*

369 Glyphosate and AMPA concentrations in water were monitored for 27 days in  
370 microcosms. The initial concentrations of glyphosate measured in aquaria were slightly lower  
371 than the nominal concentration. However, as expected, the concentrations of glyphosate in  
372 LowG condition ( $6.5 \pm 1.1 \mu\text{g L}^{-1}$ ) were 10 times lower than those in HighG condition ( $67.0 \pm$   
373  $1.0 \mu\text{g L}^{-1}$ ) at the beginning of the experiment, as shown by  $C_0$  values (Table 1). Throughout  
374 the entire experiment, glyphosate dissipation was observed in the presence of biofilms (Figure  
375 4A and B) but not in the abiotic controls (Table S2), indicating that the dissipation of  
376 glyphosate was not explained by abiotic factors such as photolysis and/or adsorption.  
377 Glyphosate dissipation curves were well fitted to a simple first-order exponential model in  
378 which the rate ( $k$ ) and  $\text{DT}_{50}$  were inversely proportional (the higher the  $k$  value was, the lower  
379 the  $\text{DT}_{50}$ ) (Table 1).

380

381

382 **Table 1.** Kinetics of glyphosate dissipation and AMPA appearance in water. The  
383 experimental data for glyphosate and AMPA concentrations were fitted with a simple first-  
384 order model and the Boltzmann model, respectively, using OriginPro, as described in the  
385 Materials and methods section. **(A)** Values of the model parameters dissipation time 50%  
386 ( $\text{DT}_{50}$ ),  $h_{50}$  and coefficient of determination ( $R^2$ ) are reported as the mean  $\pm$  standard error  
387 (SE),  $n = 3$ . Independently for glyphosate and AMPA, significant differences between  
388 conditions for each parameter are indicated by lowercase letters,  $a < b < c < d$  (Tukey's test,  $P$   
389  $< 0.05$ ). N/A: not applicable. **(B)** Results of three-way ANOVA carried out with glyphosate  
390 ( $C_0$  and  $\text{DT}_{50}$ ) and AMPA ( $C_f$ ) parameters. The three factors were site (Ups vs. Dws),  
391 phosphorus concentration in water  $P_{\text{water}}$  (LowP vs. HighP) and glyphosate concentration in  
392 water  $G_{\text{water}}$  (LowG vs. HighG),  $P < 0.05$ .

(A)

<b>Glyphosate</b>					
Condition	Model parameters				R <sup>2</sup>
	C <sub>0</sub> (µg L <sup>-1</sup> )	k (day <sup>-1</sup> )	DT <sub>50</sub> (days)		
Ups_LowP_LowG	5.8 ± 0.7 (a)	0.363 ± 0.102 (d)	2.27 ± 0.68 (a)		0.946 ± 0.031
Ups_LowP_HighG	64.5 ± 3.8 (b)	0.038 ± 0.004 (bc)	18.99 ± 2.40 (bc)		0.696 ± 0.087
Ups_HighP_LowG	7.2 ± 0.2 (a)	0.055 ± 0.004 (bc)	12.78 ± 0.87 (bc)		0.796 ± 0.071
Ups_HighP_HighG	68.6 ± 3.3 (b)	0.004 ± 0.002 (a)	206.36 ± 81.25 (d)		0.070 ± 0.059
Dws_LowP_LowG	6.3 ± 0.9 (a)	0.396 ± 0.097 (d)	1.95 ± 0.40 (a)		0.986 ± 0.004
Dws_LowP_HighG	70.2 ± 1.1 (b)	0.087 ± 0.011 (bc)	8.23 ± 1.05 (bc)		0.888 ± 0.052
Dws_HighP_LowG	6.9 ± 0.6 (a)	0.098 ± 0.007 (c)	7.12 ± 0.54 (b)		0.811 ± 0.042
Dws_HighP_HighG	65.9 ± 2.7 (b)	0.035 ± 0.005 (b)	20.80 ± 3.57 (c)		0.577 ± 0.062
<b>AMPA</b>					
Condition	Model parameters				R <sup>2</sup>
	C <sub>0</sub> (µg L <sup>-1</sup> )	k (day <sup>-1</sup> )	h <sub>50</sub> (days)	C <sub>f</sub> (µg L <sup>-1</sup> )	
Ups_LowP_LowG	N/A	N/A	N/A	N/A	N/A
Ups_LowP_HighG	0.2 ± 0.002 (a)	0.296 ± 0.049 (b)	16.967 ± 0.753 (a)	17.4 ± 3.5 (b)	0.968 ± 0.007
Ups_HighP_LowG	0.2 ± 0.01 (a)	0.239 ± 0.026 (ab)	15.037 ± 1.384 (a)	2.7 ± 0.2 (a)	0.955 ± 0.009
Ups_HighP_HighG	0.2 ± 0.01 (a)	0.160 ± 0.005 (a)	15.256 ± 0.409 (a)	19.3 ± 2.1 (b)	0.879 ± 0.006
Dws_LowP_LowG	N/A	N/A	N/A	N/A	N/A
Dws_LowP_HighG	0.3 ± 0.01 (a)	0.201 ± 0.030 (ab)	16.328 ± 0.662 (a)	21.8 ± 1.6 (b)	0.918 ± 0.034
Dws_HighP_LowG	0.2 ± 0.01 (a)	0.185 ± 0.024 (ab)	14.268 ± 1.317 (a)	3.5 ± 0.3 (a)	0.928 ± 0.009
Dws_HighP_HighG	0.2 ± 0.03 (a)	0.166 ± 0.007 (ab)	14.949 ± 0.683 (a)	24.9 ± 2.7 (b)	0.906 ± 0.004

(B)

3-way ANOVA	<b>Glyphosate</b>				<b>AMPA</b>	
	C <sub>0</sub>		DT <sub>50</sub>		C <sub>f</sub>	
	F value	P value	F value	P value	F value	P value
Site	0.1	0.763	46.9	< <b>0.001</b>	4.6	< <b>0.05</b>
Pwater	1.9	0.185	134.1	< <b>0.001</b>	4.8	< <b>0.05</b>
Gwater	1552.1	< <b>0.001</b>	181.3	< <b>0.001</b>	226.01	< <b>0.001</b>
Site*Pwater	1.1	0.318	11.5	< <b>0.01</b>	0.2	0.693
Site*Gwater	0.01	0.922	18.5	< <b>0.001</b>	3.3	0.090
Pwater*Gwater	1.9	0.188	0.02	0.887	0.05	0.828
Site*Pwater*Gwater	0.002	0.967	2.9	0.112	0.005	0.945

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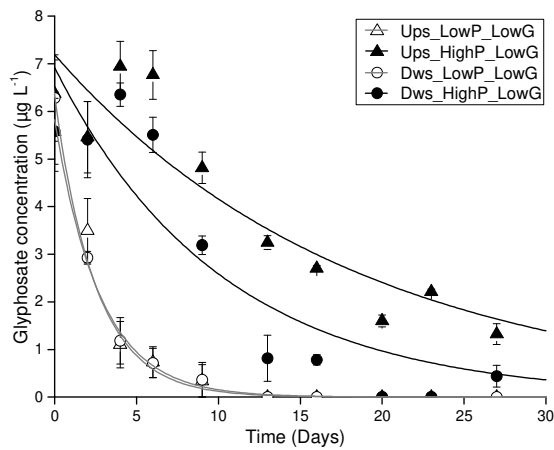
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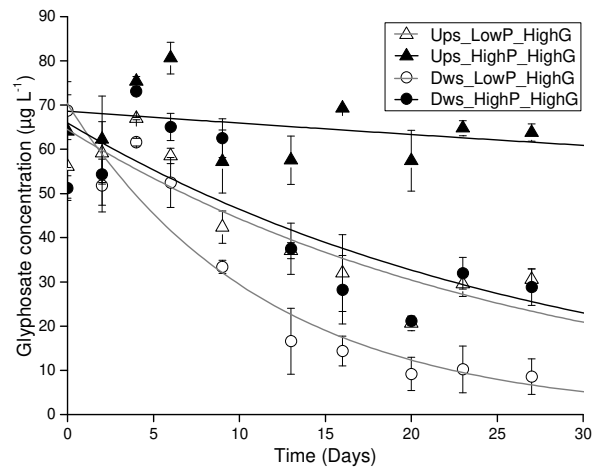
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(A)



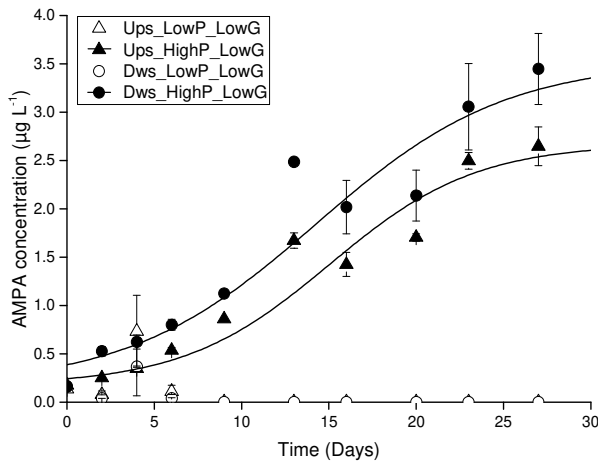
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(B)



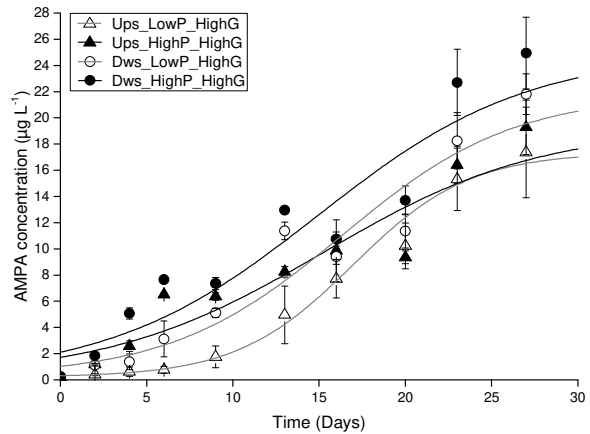
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(C)



401

(D)



402 **Figure 4.** Kinetics of glyphosate dissipation (A, B) and AMPA formation (C, D) in water.

403 Biofilms from the upstream (Ups) and downstream (Dws) sites were exposed to low (A, C)

404 and high (B, D) glyphosate concentrations and low (LowP) and high (HighP) phosphorus

405 concentrations in water. The experimental data for glyphosate and AMPA concentrations

406 were fitted with simple first-order and Boltzmann models, respectively, using OriginPro as

407 described in the Materials and Methods section. The lines correspond to the model with mean

408 parameters values for each condition. The values are means  $\pm$  standard errors ( $n = 3$ ) of the

409 experimental data.

410

411 Glyphosate dissipation was faster for the lower concentration of glyphosate ( $DT_{50} = 4.3 \pm$   
412  $1.3$  days) than for the higher concentration ( $DT_{50} = 23.2 \pm 1.4$  days) (Table 1,  $P < 0.001$ ),  
413 though a strong effect of the concentration of phosphorus in water was also observed on  
414 dissipation coefficients (Figure 4A and B, Table 1). For instance, glyphosate was more  
415 rapidly dissipated in LowP water than in HighP water ( $P < 0.001$ ). Consequently, the highest  
416 percentages of glyphosate dissipation were obtained for biofilms in LowP and LowG water  
417 (Figure 4A). In the LowP and LowG conditions, the herbicide was completely dissipated after  
418 13 days, with  $DT_{50}$  values of 2.27 and 1.95 days for the Ups and Dws biofilms, respectively  
419 (Table 1). Concerning the origin of the biofilms, the highest  $DT_{50}$  values were obtained in Ups  
420 biofilms (Table 1,  $P < 0.001$ ). For instance, the slowest glyphosate dissipation was observed in  
421 upstream biofilms subjected to HighP and HighG (3% loss after 27 days). Irrespective of the  
422 glyphosate concentration, the influence of water phosphorus on glyphosate dissipation was  
423 more marked in upstream biofilms than in downstream biofilms. Indeed, glyphosate  $DT_{50}$  in  
424 the LowG condition was increased by a factor of 3.7 (Dws) and 5.7 (Ups) from the LowP to  
425 the HighP conditions (Table 1).

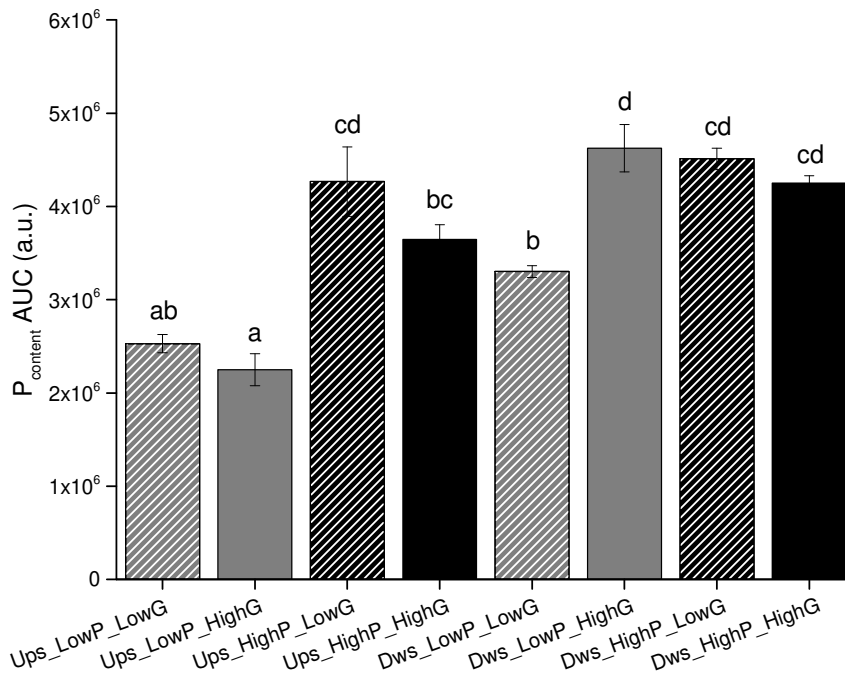
426 The formation of AMPA was also modulated by the concentration of phosphorus in water  
427 ( $P < 0.05$ ). This effect was more marked in LowG water than in HighG water (Table 1). Thus,  
428 in the LowP, LowG water condition, in which glyphosate dissipation was the fastest, AMPA  
429 was only a transient intermediate and did not accumulate (not detected after day 9, Figure  
430 4C). As expected, the final concentration of AMPA was higher in HighG than in LowG  
431 conditions (Table 1,  $P < 0.001$ ). Because the transformation of glyphosate into AMPA is  
432 equimolar, the proportion of glyphosate transformed into AMPA at the end of the experiment  
433 could be determined. Except for the LowP, LowG condition, in which AMPA did not  
434 accumulate, calculations revealed that  $82 \pm 9\%$  of glyphosate was transformed into AMPA in  
435 the LowG condition, whereas only  $55 \pm 5\%$  was transformed in the HighG condition.

436 Glyphosate and AMPA were quantified in the biofilms at the end of the experiment (day  
437 27). Very low contents of glyphosate ( $0.005 - 6.467 \mu\text{g g}_{\text{dw}}^{-1}$ ) and AMPA ( $0.112 - 0.304 \mu\text{g}$   
438  $\text{g}_{\text{dw}}^{-1}$ ) were detected in biofilms, the contents represented less than 0.1% (glyphosate) and  
439 0.07% (AMPA) of the initial amount of glyphosate added (molar equivalent) (Table S1). This  
440 trend was confirmed by the relatively low values of the partitioning coefficient  
441  $k(\text{glyphosate})_{\text{biofilm/water}}$  obtained for all conditions tested (between  $4.9 \cdot 10^{-4}$  and  $6.5 \cdot 10^{-2} \text{ L g}_{\text{dw}}^{-1}$   
442  $^{-1}$ ) (Table S1). Moreover, the phosphorus concentration in water did not influence the  
443 glyphosate content in biofilms ( $P = 0.234$ ). The AMPA content in biofilms was not influenced  
444 by any of the factors tested in this experiment (site,  $P = 0.364$ ;  $P_{\text{water}}$ ,  $P = 0.405$ ; glyphosate,  
445  $P = 0.780$ ).

446

### 447 *3.3. Bacterial abundance and phosphorus content in biofilm*

448 Bacterial growth in biofilms during glyphosate experiments was assessed by flow  
449 cytometry. The initial biofilm cell abundance was higher in biofilms from the downstream site  
450 ( $2.8 \cdot 10^7 \pm 2.6 \cdot 10^6 \text{ cell cm}^{-2}$ ) than in those from the upstream site ( $2.4 \cdot 10^6 \pm 2.5 \cdot 10^5 \text{ cell cm}^{-2}$ );  
451 among the former samples, most of the surface of the glass slide was completely colonized.  
452 However, biofilm growth during the experiment was greater for upstream (increase of  $1581.2$   
453  $\pm 197.8\%$ ) than for downstream (increase of  $66.5 \pm 25.4\%$ ) biofilms (Figure S3,  $P < 0.001$ ).  
454 More specifically, the bacterial growth in Ups biofilms was higher in LowP water (where  
455 phosphorus uptake and APA were higher, see below) than in HighP water (Figure S3,  $P <$   
456  $0.05$ ).



457

458 **Figure 5.** Biofilm phosphorus content. The area under the curve (AUC), expressed in  
 459 arbitrary units (a.u.) integrates the curves of phosphorus content over time. The results are  
 460 reported as the mean ± standard error (SE), n = 3. Significant differences are indicated by  
 461 lowercase letters, a < b < c < d (Tukey's test, P < 0.05).

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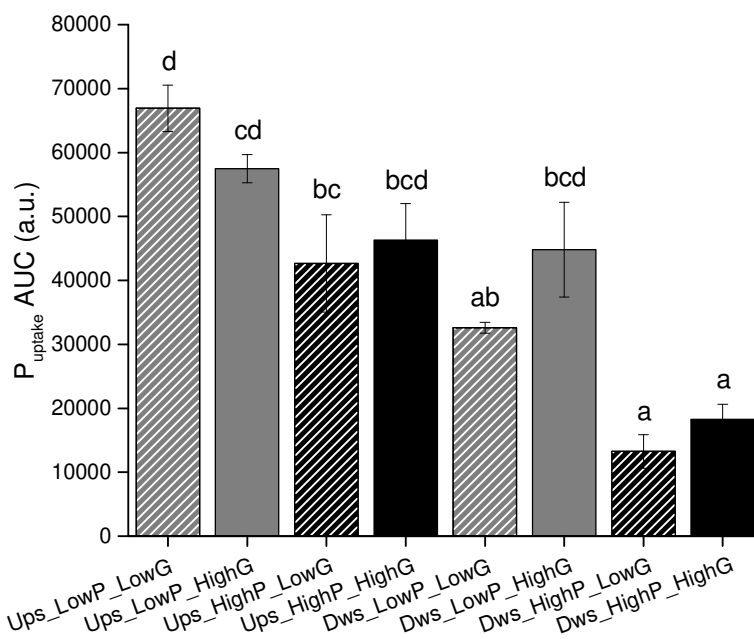
463 The phosphorus content in biofilms was significantly higher in Dws biofilms than in the  
 464 Ups ones (Figure 5, P < 0.001). During the first 6 days of the experiment, the biofilms  
 465 exhibiting the lowest P content (approximately 25 mg P g<sup>-1</sup> of biofilm dry weight) were those  
 466 from the Ups site subjected to LowP (Figure S4). The phosphorus content of biofilms  
 467 reflected the phosphorus concentration in water since biofilms placed in LowP water had a  
 468 lower P content than those placed in HighP water throughout the entire experiment (Figure 5,  
 469 P < 0.001). Although glyphosate did not significantly affect the microbial growth and P  
 470 content of biofilms, independent of the site and the phosphorus conditions in water, the high P  
 471 demand observed in Ups biofilms coincided with the fastest glyphosate dissipation without

472 AMPA accumulation, suggesting the use of glyphosate and/or AMPA as a phosphorus source  
473 for microbial growth.

474

#### 475 3.4. Phosphorus utilization by biofilms

476 Phosphorus uptake was calculated from bi-weekly SRP concentration measurements in  
477 microcosms containing treated biofilms. A significantly higher P uptake was observed for  
478 biofilms subjected to LowP water than for those subjected to HighP water (Figure 6,  $P <$   
479 0.001). Moreover, the phosphorus consumption was higher for Ups biofilms (colonized in a  
480 P-poor area) than for Dws biofilms (colonized in a P-rich area) (Figure 6,  $P < 0.001$ ).  
481 Phosphorus uptake by biofilms did not differ between glyphosate conditions ( $P = 0.401$ ).

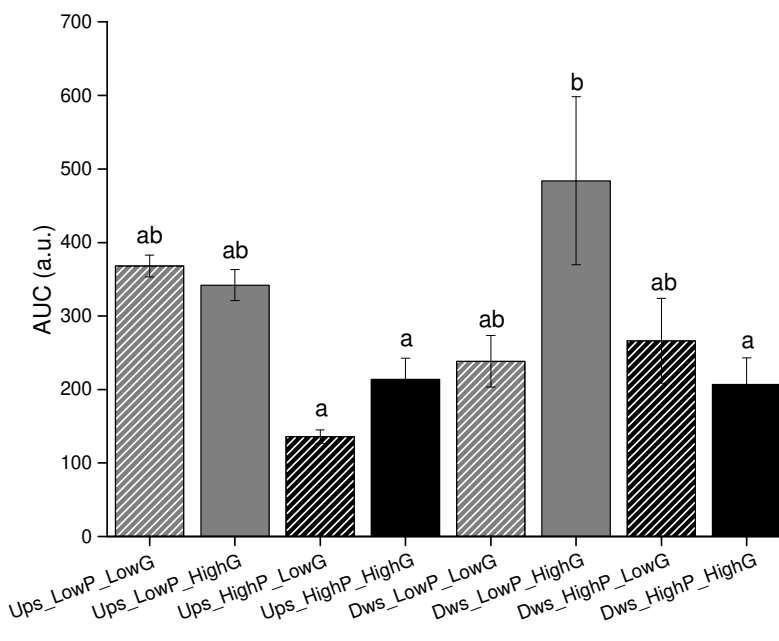


482

483 **Figure 6.** Phosphorus uptake. Phosphorus uptake curves were integrated with OriginPro 2016  
484 to obtain the area under the curve (AUC), expressed in arbitrary units (a.u.). The results are  
485 reported as the mean  $\pm$  standard error (SE),  $n = 3$ . Significant differences are indicated by  
486 lowercase letters,  $a < b < c < d$  (Tukey's test,  $P < 0.05$ ).

487

488 Potential APA (EC 3.1.3.1) was measured in biofilms at each sampling time, which  
 489 permitted the integration of 27 days of activity for each experimental condition. The  
 490 integrated APA did not differ between the Ups and Dws sites ( $P = 0.358$ ) or between LowG  
 491 and HighG conditions ( $P = 0.116$ , Figure 7). However, similarly to phosphorus uptake, the  
 492 phosphorus concentration in water was observed to have a significant effect on APA; the  
 493 activity was the lowest under the HighP water condition ( $P < 0.005$ ). This effect was  
 494 particularly marked for Ups biofilms in the first 4 days of the experiment (average of  $15.4 \pm 2$   
 495 and  $3.2 \pm 0.2 \mu\text{mol MUF h}^{-1} \text{g}_{\text{dw}}^{-1}$  for LowP and HighP, respectively) compared with Dws  
 496 biofilms (average of  $4.6 \pm 1.3$  and  $2.3 \pm 0.7 \mu\text{mol MUF h}^{-1} \text{g}_{\text{dw}}^{-1}$  for LowP and HighP,  
 497 respectively) (Figure S5).



498 **Figure 7.** Phosphatase activity of biofilms. Phosphatase activity curves were integrated with  
 499 OriginPro 2016 to obtain the area under the curve (AUC), expressed in arbitrary units (a.u.).  
 500 The results are reported as the mean  $\pm$  standard error (SE),  $n = 3$ . Significant differences are  
 501 indicated by lowercase letters,  $a < b$  (Tukey's test,  $P < 0.05$ ).

503

504

#### 505 4. Discussion

506 After herbicidal application, glyphosate can migrate through soil and reach aquatic  
507 compartments. This movement to surface water is limited by biodegradation by soil  
508 microorganisms (Sviridov et al., 2015) and sorption to soil particles, the latter being  
509 negatively correlated with pH and phosphorus content (Okada et al., 2016). The similarity  
510 between the chemical structures of glyphosate and phosphate molecules establishes  
511 competition between both compounds towards soil sorption sites (Kanissery et al., 2015). The  
512 observed seasonal variations in glyphosate and AMPA in French surface waters coincide with  
513 observations made in Canadian streams (Struger et al., 2015): concentrations increase from  
514 early spring to summer and decrease from the end of autumn to winter, matching perfectly  
515 with the pesticide application calendar for crops. This result suggests that glyphosate transfer  
516 between the terrestrial and aquatic ecosystems is relatively fast and that the saturation of soil  
517 sorption sites by phosphate probably accelerates this transfer. Indeed, the high level of co-  
518 occurrence among phosphorus, glyphosate, and AMPA is reflected by the strong positive  
519 correlation found between the AMPA/glyphosate ratio and phosphorus concentration in water  
520 (Figure 3), which suggests that P eutrophication is probably an important factor influencing  
521 the balance between glyphosate and AMPA in surface water.

522 AMPA derived from glyphosate transformation constitutes the main source of AMPA  
523 contamination in surface water ecosystems, the other source being AMPA derived from  
524 amino-polyphosphonate (commonly used in industrial and household applications as  
525 detergents, flame retardants, anticorrosives, etc.) degradation (Grandcoin et al., 2017). The  
526 present meta-analysis shows that glyphosate and AMPA co-occurred in water: glyphosate was  
527 quantified without AMPA in only 3.4% of 53790 water samples analysed from 2013 – 2017  
528 in France (Figure 1), and a positive correlation was observed between glyphosate and AMPA  
529 ( $\rho = 0.498$ ,  $P < 0.001$ ). These results are in accordance with a spatially broad occurrence

530 study performed in the USA, in which glyphosate was detected without AMPA in only 2.3%  
531 of 3732 water and sediment samples (Battaglin et al., 2014), and with another study that  
532 showed a co-occurrence ( $\rho = 0.76$ ) between glyphosate and AMPA in Canadian rivers  
533 (Struger et al., 2015). The co-occurrence of glyphosate and AMPA in surface waters seems  
534 therefore inevitable and suggests the incomplete mineralization of the molecule, especially at  
535 eutrophic sites.

536 The levels of surface water contamination by pesticides are influenced by a large number  
537 of drivers that may be directly influenced by anthropic activities (e.g., agricultural practices,  
538 industrial activities) or not (e.g., water flow, temperature, biodegradation by microorganisms).  
539 The present study revealed that natural river biofilms possess a strong capability for  
540 glyphosate degradation under environmentally realistic conditions (Figure 4A). Biofilms  
541 exposed to low concentrations of glyphosate ( $<10 \mu\text{g L}^{-1}$ ) can dissipate the herbicide  
542 completely after 13 days. This capability was also found in a recent study, but only in the  
543 presence of formulated glyphosate (Klátyik et al., 2017). In realistic conditions (LowG in our  
544 study), the pre-exposure history of biofilms to glyphosate and AMPA does not influence the  
545 capacity of biofilm microorganisms to degrade the two molecules (a site effect was indicated  
546 only when the HighG condition was added to analyses). These results contradict our  
547 hypothesis, and those of many other studies on pesticides (Carles et al., 2017; Mamy et al.,  
548 2005; Tuxen et al., 2002) suggesting a fast adaptation of biofilms for glyphosate degradation  
549 irrespectively of their history of exposure to the molecule.

550 The dissolved phosphorus concentration in water has been shown to modulate the  
551 dissipation of glyphosate by biofilms, and this activity is well explained by the fact that this  
552 herbicide can represent a phosphorus source for microbes (Hove-Jensen et al., 2014). For the  
553 first time, this effect has been demonstrated for natural biofilm communities, indicating that  
554 similar biochemical effects can be observed at both the population and community scales. The

555 utilization of glyphosate as phosphorus source has not yet been described for natural river  
556 biofilms; the microbial strains that are capable of using this herbicide as a sole phosphorus  
557 source have been isolated from other environments, mainly soil and activated sludge  
558 (Sviridov et al., 2015). Pure strain studies have also shown that glyphosate catabolism can be  
559 repressed by phosphorus in *Arthrobacter* sp. (Pipke et al., 1987) and *Pseudomonas* sp.  
560 PG2982 (Fitzgibbon and Braymer, 1988). Essentially, the strong effect of phosphorus  
561 availability on glyphosate dissipation, AMPA production (Figure 4), biofilm phosphorus  
562 content (Figure 5), P uptake and APA can be explained by phosphorus limitation within  
563 biofilms. Indeed, the fastest dissipation of glyphosate without AMPA production was  
564 observed in the LowP condition (Figure 4 and Table 1). Inversely, glyphosate is mostly  
565 transformed into AMPA in eutrophic water in comparison with more P-poor waters.  
566 Surprisingly, glyphosate and AMPA were still detected at oligotrophic sites, whereas the two  
567 compounds disappeared completely in the LowG/LowP condition for upstream communities  
568 in our experiment. These different responses could result from differences in i) the  
569 composition of microbial communities, ii) environmental conditions (e.g., temperature, pH),  
570 and/or iii) the chronic input of glyphosate and/or AMPA in surface waters, which was not the  
571 case in our microcosm study.

572 Although variations in environmental conditions and watershed types make it difficult to  
573 predict the environmental persistence of glyphosate, some general trends can be highlighted.  
574 The literature indicates that glyphosate has low to high persistence in soils under aerobic  
575 conditions ( $DT_{50}$  ranging from 2.8 to 500.3 days) and high persistence in anaerobic soils  
576 ( $DT_{50}$  of 135 – 1000 days) (EFSA, 2015). Some conditions could thus favour the persistence  
577 of glyphosate in soil and delay its biodegradation and/or movement to aquatic ecosystems. In  
578 water sediments, the persistence of glyphosate is moderate to high ( $DT_{50}$  range: 13.82 – 301  
579 days) (EFSA, 2015). However, the concentration of phosphorus from both glyphosate and

580 AMPA is extremely low compared with the total phosphorus concentration in surface water  
581 ( $P_{(AMPA)}$  and  $P_{(glyphosate)}$  represent less than 0.17% and 0.05% of total P). One can therefore  
582 identify two main factors influencing the levels of glyphosate contamination in surface water.  
583 The first factor is the herbicide input, which is influenced by soil biological activity  
584 (biodegradation) and retention capacity (sorption). The second factor is phosphorus  
585 availability in water, which decreases glyphosate degradation and promotes AMPA  
586 accumulation. However, it cannot be ruled out that P production derived from glyphosate  
587 degradation could display a negative feed-back on glyphosate degradation in extremely low P  
588 systems. Overall, eutrophication by phosphorus favours the incomplete degradation of  
589 glyphosate in aquatic systems. Eutrophication levels should thus be taken into account in the  
590 environmental risk assessment of glyphosate and AMPA in surface waters.

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595

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599

## 600 **Declaration of interest:**

601 none.

602

## 603 **References**

604 AGRESTE, 2018. Données de vente des produits phytosanitaires 2011 à 2015. [WWW  
605 Document]. URL [http://agreste.agriculture.gouv.fr/thematiques-872/productions-](http://agreste.agriculture.gouv.fr/thematiques-872/productions-vegetales-874/grandes-cultures-fourrages-875)  
606 [vegetales-874/grandes-cultures-fourrages-875](http://agreste.agriculture.gouv.fr/thematiques-872/productions-vegetales-874/grandes-cultures-fourrages-875) (accessed 5.22.18).

607 Artigas, J., Rossi, F., Gerphagnon, M., Mallet, C., 2017. Sensitivity of laccase activity to the  
608 fungicide tebuconazole in decomposing litter. *Sci. Total Environ.* 584–585, 1084–  
609 1092. <https://doi.org/10.1016/j.scitotenv.2017.01.167>

610 Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and its  
611 degradation product AMPA occur frequently and widely in US soils, surface water,  
612 groundwater, and precipitation. *JAWRA J. Am. Water Resour. Assoc.* 50, 275–290.

613 Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The  
614 ecology and biogeochemistry of stream biofilms. *Nat. Rev. Microbiol.* 14, 251–263.  
615 <https://doi.org/10.1038/nrmicro.2016.15>

616 Besemer, K., 2015. Biodiversity, community structure and function of biofilms in stream  
617 ecosystems. *Res. Microbiol.* 166, 774–781.  
618 <https://doi.org/10.1016/j.resmic.2015.05.006>

619 Blanck, H., 2002. A critical review of procedures and approaches used for assessing  
620 pollution-induced community tolerance (PICT) in biotic communities. *Hum. Ecol.*  
621 *Risk Assess.* 8, 1003–1034.

622 Buhler, D.D., 2014. Weed Management, in: Reference Module in Earth Systems and  
623 Environmental Sciences. Elsevier. [https://doi.org/10.1016/B978-0-12-409548-](https://doi.org/10.1016/B978-0-12-409548-9.09118-1)  
624 [9.09118-1](https://doi.org/10.1016/B978-0-12-409548-9.09118-1)

625 Carles, L., Rossi, F., Joly, M., Besse-Hoggan, P., Batisson, I., Artigas, J., 2017.  
626 Biotransformation of herbicides by aquatic microbial communities associated to  
627 submerged leaves. *Environ. Sci. Pollut. Res.* 24, 3664–3674.  
628 <https://doi.org/10.1007/s11356-016-8035-9>

629 Chrost, J., Krambeck, H.J., 1986. Fluorescence correction for measurements of enzyme  
630 activity in natural waters using methylumbelliferyl-substrates. *Arch. Hydrobiol.*  
631 *Stuttg.* 106, 79–90.

632 Davoren, M.J., Schiestl, R.H., 2018. Glyphosate Based Herbicides and Cancer Risk: A Post  
633 IARC Decision Review of Potential Mechanisms, Policy, and Avenues of Research.  
634 *Carcinogenesis.* <https://doi.org/10.1093/carcin/bgy105>

635 Dodds, W.K., Jones, J.R., Welch, E.B., 1998. Suggested classification of stream trophic state:  
636 distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus.  
637 *Water Res.* 32, 1455–1462.

638 Duke, S.O., 2015. Perspectives on transgenic, herbicide-resistant crops in the United States  
639 almost 20 years after introduction: Perspectives on transgenic, herbicide-resistant  
640 crops. *Pest Manag. Sci.* 71, 652–657. <https://doi.org/10.1002/ps.3863>

641 EFSA, 2015. Conclusion on the peer review of the pesticide risk assessment of the active  
642 substance glyphosate: Peer review of the pesticide risk assessment of the active  
643 substance glyphosate. *EFSA J.* 13, 4302. <https://doi.org/10.2903/j.efsa.2015.4302>

644 E-Phy, 2018. Le catalogue des produits phytopharmaceutiques et de leurs usages, des matières  
645 fertilisantes et des supports de culture autorisés en France.  
646 <https://ephy.anses.fr/substance/glyphosate> [WWW Document]. URL  
647 <https://ephy.anses.fr/substance/glyphosate> (accessed 7.25.18).

648 Fitzgibbon, J., Braymer, H.D., 1988. Phosphate starvation induces uptake of glyphosate by  
649 *Pseudomonas* sp. strain PG2982. *Appl. Environ. Microbiol.* 54, 1886–1888.

650 Grandcoin, A., Piel, S., Baures, E., 2017. AminoMethylPhosphonic acid (AMPA) in natural  
651 waters: Its sources, behavior and environmental fate. *Water Res.* 117, 187–197.  
652 <https://doi.org/10.1016/j.watres.2017.03.055>

- 653 Hanke, I., Singer, H., Hollender, J., 2008. Ultratrace-level determination of glyphosate,  
654 aminomethylphosphonic acid and glufosinate in natural waters by solid-phase  
655 extraction followed by liquid chromatography–tandem mass spectrometry:  
656 performance tuning of derivatization, enrichment and detection. *Anal. Bioanal. Chem.*  
657 391, 2265–2276.
- 658 Hanke, I., Wittmer, I., Bischofberger, S., Stamm, C., Singer, H., 2010. Relevance of urban  
659 glyphosate use for surface water quality. *Chemosphere* 81, 422–429.
- 660 Hanna, D.E.L., Tomscha, S.A., Ouellet Dallaire, C., Bennett, E.M., 2018. A review of  
661 riverine ecosystem service quantification: Research gaps and recommendations. *J.*  
662 *Appl. Ecol.* 55, 1299–1311. <https://doi.org/10.1111/1365-2664.13045>
- 663 Hove-Jensen, B., Zechel, D.L., Jochimsen, B., 2014. Utilization of Glyphosate as Phosphate  
664 Source: Biochemistry and Genetics of Bacterial Carbon-Phosphorus Lyase. *Microbiol.*  
665 *Mol. Biol. Rev.* 78, 176–197. <https://doi.org/10.1128/MMBR.00040-13>
- 666 Kanissery, R.G., Welsh, A., Sims, G.K., 2015. Effect of Soil Aeration and Phosphate  
667 Addition on the Microbial Bioavailability of Carbon-14-Glyphosate. *J. Environ. Qual.*  
668 44, 137. <https://doi.org/10.2134/jeq2014.08.0331>
- 669 Klátyik, S., Takács, E., Mörtl, M., Földi, A., Trábert, Z., Ács, É., Darvas, B., Székács, A.,  
670 2017. Dissipation of the herbicide active ingredient glyphosate in natural water  
671 samples in the presence of biofilms. *Int. J. Environ. Anal. Chem.* 97, 901–921.  
672 <https://doi.org/10.1080/03067319.2017.1373770>
- 673 Krzysko-Lupicka, T., Krecidlo, L., Koszalkowska, M., 2015. The Ability of Selected Bacteria  
674 to Grow in the Presence of Glyphosate. *Ecol. Chem. Eng. -Chem. Inzynieria Ekol. A*  
675 22, 185–193. [https://doi.org/10.2428/ecea.2015.22\(2\)15](https://doi.org/10.2428/ecea.2015.22(2)15)
- 676 Lasier, P.J., Urich, M.L., Hassan, S.M., Jacobs, W.N., Bringolf, R.B., Owens, K.M., 2016.  
677 Changing agricultural practices: potential consequences to aquatic organisms.  
678 *Environ. Monit. Assess.* 188, 672. <https://doi.org/10.1007/s10661-016-5691-7>
- 679 Mamy, L., Barriuso, E., Gabrielle, B., 2005. Environmental fate of herbicides trifluralin,  
680 metazachlor, metamitron and sulcotrione compared with that of glyphosate, a  
681 substitute broad spectrum herbicide for different glyphosate-resistant crops. *Pest*  
682 *Manag. Sci. Former. Pestic. Sci.* 61, 905–916.
- 683 Maqueda, C., Undabeytia, T., Villaverde, J., Morillo, E., 2017. Behaviour of glyphosate in a  
684 reservoir and the surrounding agricultural soils. *Sci. Total Environ.* 593, 787–795.
- 685 McMullan, G., Quinn, J.P., 1994. In vitro characterization of a phosphate starvation-  
686 independent carbon-phosphorus bond cleavage activity in *Pseudomonas fluorescens*  
687 23F. *J. Bacteriol.* 176, 320–324.
- 688 Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of  
689 phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.
- 690 NAIADES, 2018. Données sur la qualité des eaux de surface.  
691 <http://www.naiades.eaufrance.fr/acces-donnees#/physicochimie>. [WWW Document].  
692 URL <http://www.naiades.eaufrance.fr/acces-donnees#/physicochimie> (accessed  
693 5.23.18).
- 694 Okada, E., Costa, J.L., Bedmar, F., 2016. Adsorption and mobility of glyphosate in different  
695 soils under no-till and conventional tillage. *Geoderma* 263, 78–85.
- 696 Pipke, R., Schulz, A., Amrhein, N., 1987. Uptake of glyphosate by an *Arthrobacter* sp. *Appl.*  
697 *Environ. Microbiol.* 53, 974–978.
- 698 Powles, S.B., Duke, S.O., 2010. Glyphosate-resistant crops and weeds: now and in the future.
- 699 Rossi, F., Mallet, C., Portelli, C., Donnadieu, F., Bonnemoy, F., Artigas, J., 2019. Stimulation  
700 or inhibition: Leaf microbial decomposition in streams subjected to complex chemical

701 contamination. *Sci. Total Environ.* 648, 1371–1383.  
702 <https://doi.org/10.1016/j.scitotenv.2018.08.197>

703 Sanchís, J., Kantiani, L., Llorca, M., Rubio, F., Ginebreda, A., Fraile, J., Garrido, T., Farré,  
704 M., 2012. Determination of glyphosate in groundwater samples using an ultrasensitive  
705 immunoassay and confirmation by on-line solid-phase extraction followed by liquid  
706 chromatography coupled to tandem mass spectrometry. *Anal. Bioanal. Chem.* 402,  
707 2335–2345.

708 Singh, B., Singh, K., 2016. Microbial degradation of herbicides. *Crit. Rev. Microbiol.* 42,  
709 245–261. <https://doi.org/10.3109/1040841X.2014.929564>

710 Struger, J., Van Stempvoort, D.R., Brown, S.J., 2015. Sources of aminomethylphosphonic  
711 acid (AMPA) in urban and rural catchments in Ontario, Canada: Glyphosate or  
712 phosphonates in wastewater? *Environ. Pollut.* 204, 289–297.  
713 <https://doi.org/10.1016/j.envpol.2015.03.038>

714 Sviridov, A.V., Shushkova, T.V., Ermakova, I.T., Ivanova, E.V., Epiktetov, D.O.,  
715 Leontievsky, A.A., 2015. Microbial degradation of glyphosate herbicides (Review).  
716 *Appl. Biochem. Microbiol.* 51, 188–195.

717 Swanson, N.L., Leu, A., Abrahamson, J., Wallet, B., 2014. Genetically engineered crops,  
718 glyphosate and the deterioration of health in the United States of America. *J. Org.*  
719 *Syst.* 9, 6–37.

720 Tuxen, N., de Liphay, J.R., Albrechtsen, H.-J., Aamand, J., Bjerg, P.L., 2002. Effect of  
721 Exposure History on Microbial Herbicide Degradation in an Aerobic Aquifer Affected  
722 by a Point Source. *Environ. Sci. Technol.* 36, 2205–2212.  
723 <https://doi.org/10.1021/es0113549>

724 US Geological Survey, 2018. Pesticide Use Maps. [WWW Document]. URL  
725 [https://water.usgs.gov/nawqa/pnsp/usage/maps/show\\_map.php?year=2012&map=GL](https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2012&map=GLYPHOSATE&hilo=L&disp=Glyphosate)  
726 [YPHOSATE&hilo=L&disp=Glyphosate](https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2012&map=GLYPHOSATE&hilo=L&disp=Glyphosate) (accessed 5.22.18).

727 Van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris, J.G.,  
728 2018. Environmental and health effects of the herbicide glyphosate. *Sci. Total*  
729 *Environ.* 616, 255–268. <https://doi.org/10.1016/j.scitotenv.2017.10.309>

730 Vera, M.S., Lagomarsino, L., Sylvester, M., Perez, G.L., Rodriguez, P., Mugni, H., Sinistro,  
731 R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H., 2010. New evidences of  
732 Roundup(A (R)) (glyphosate formulation) impact on the periphyton community and  
733 the water quality of freshwater ecosystems. *Ecotoxicology* 19, 710–721.  
734 <https://doi.org/10.1007/s10646-009-0446-7>

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743 **Supplementary material**

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745 **Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms**

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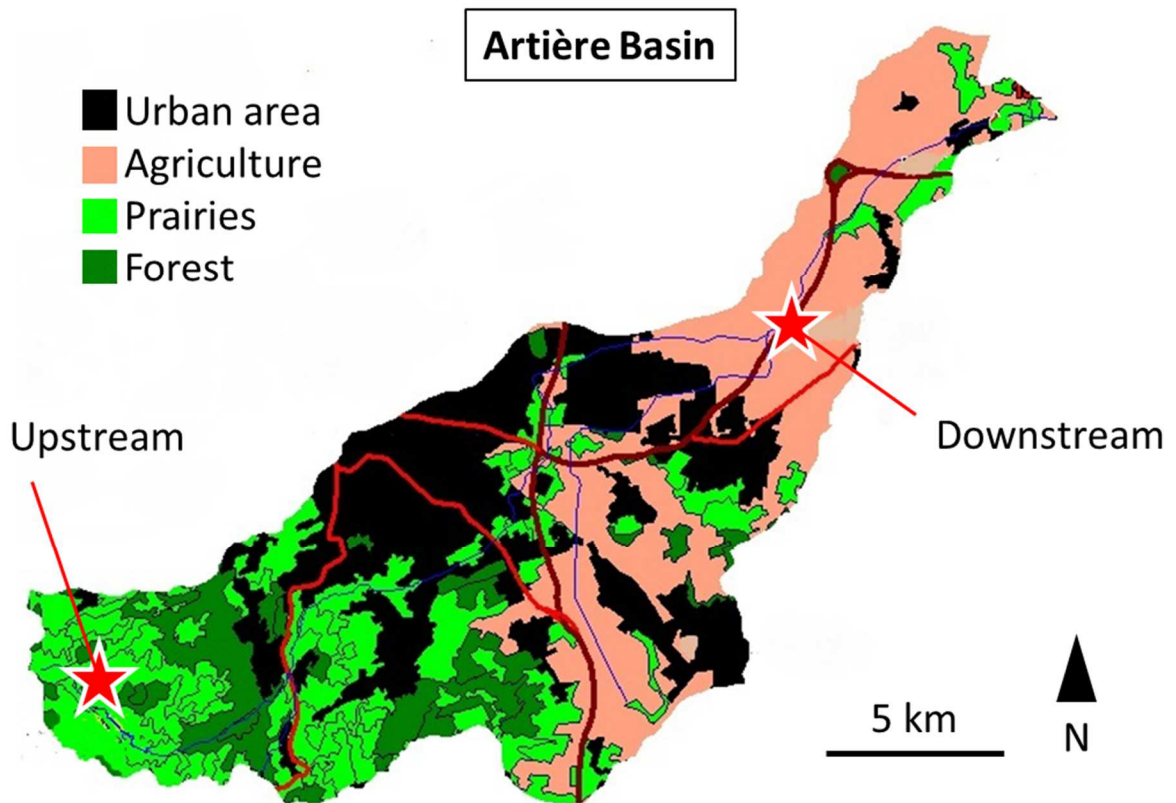
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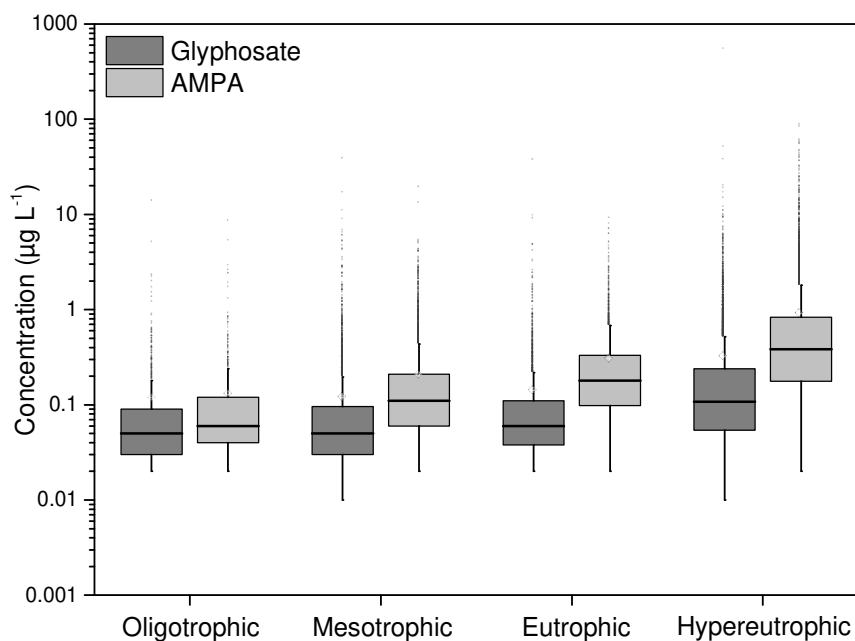


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758 **Figure S1.** Map of Artière watershed. Upstream and downstream sites locations are indicated  
759 by red stars.

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763 **Figure S2.** Glyphosate and AMPA concentrations in river water in France. The results are  
 764 classified by river trophic status (according to the concentration of total phosphorus in the  
 765 river water,  $\mu\text{g P L}^{-1}$ ): oligotrophic ( $< 25$ ), mesotrophic ( $25 - 75$ ), eutrophic ( $75 - 100$ ) and  
 766 hypereutrophic ( $> 100$ ) following the trophic state classification of rivers proposed by Dodds  
 767 et al. (1998). Period of time: January 2013 – August 2017 (N = 22823 measurements).

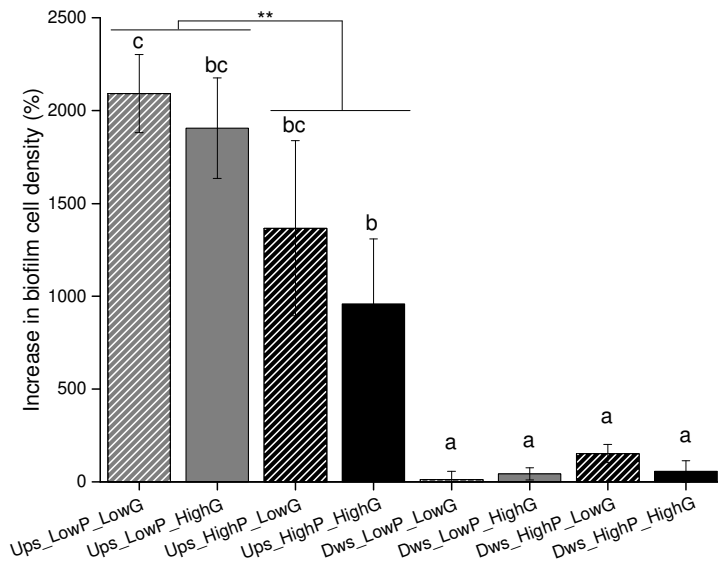
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774 **Figure S3.** Increase in biofilm cell density between day 0 and day 27. The results are reported  
 775 as the mean  $\pm$  standard error (SE), n = 3. Significant differences between conditions are  
 776 indicated by lowercase letters, a < b < c (Tukey's test, P < 0.05). \*\* P < 0.05 (three-way  
 777 ANOVA).

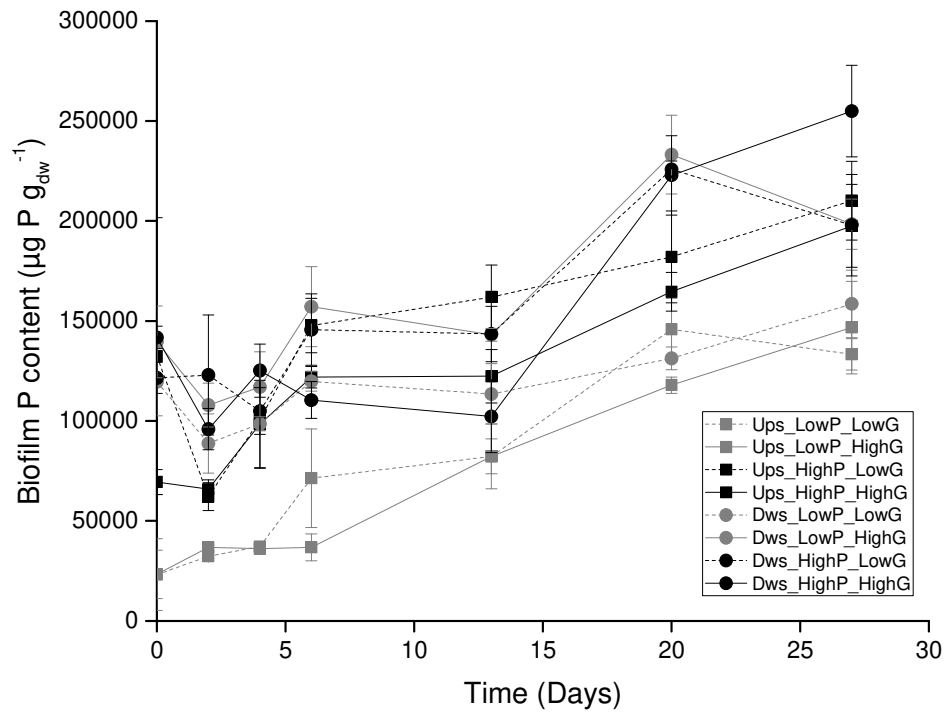
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784 **Figure S4.** Biofilm phosphorus content. The results are reported as the mean  $\pm$  standard error

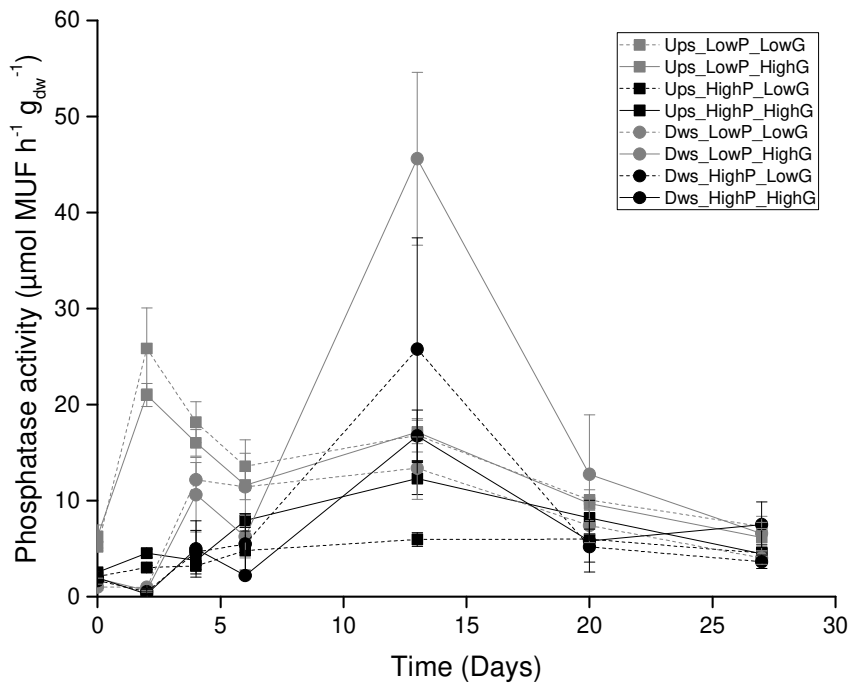
785 (SE),  $n = 3$ .

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791 **Figure S5.** Phosphatase activity of biofilms. The results are reported as the mean  $\pm$  standard

792 error (SE), n = 3.

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806 **Table S1.** Glyphosate and AMPA concentrations in biofilms at the end of the experiment (day  
807 27). Biofilms from the upstream (Ups) and downstream (Dws) sites were exposed to low  
808 (LowG) and high (HighG) glyphosate concentrations and low (LowP) and high (HighP)  
809 phosphorus concentrations in water. The glyphosate partitioning coefficient  $k$  (biofilm/water)  
810 is also given. The values are means  $\pm$  standard errors ( $n = 3$ ) of experimental data. Significant  
811 differences between conditions for glyphosate and AMPA concentrations are indicated by  
812 lowercase letters,  $a < b$  (Tukey's test,  $P < 0.05$ ).

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Condition	Glyphosate concentration ( $\mu\text{g g}_{\text{dw}}^{-1}$ )	AMPA concentration ( $\mu\text{g g}_{\text{dw}}^{-1}$ )	$k(\text{glyphosate})_{\text{biofilm/water}}$ ( $\text{L g}_{\text{dw}}^{-1}$ )
Ups_LowP_LowG	0.005 $\pm$ 0.000 (a)	0.157 $\pm$ 0.037 (a)	4.9 $10^{-4}$
Ups_LowP_HighG	3.527 $\pm$ 0.284 (b)	0.112 $\pm$ 0.056 (a)	3.5 $10^{-2}$
Ups_HighP_LowG	0.168 $\pm$ 0.032 (a)	0.304 $\pm$ 0.146 (a)	1.7 $10^{-2}$
Ups_HighP_HighG	6.467 $\pm$ 1.668 (b)	0.287 $\pm$ 0.125 (a)	6.5 $10^{-2}$
Dws_LowP_LowG	0.127 $\pm$ 0.080 (a)	0.151 $\pm$ 0.022 (a)	1.3 $10^{-2}$
Dws_LowP_HighG	0.343 $\pm$ 0.026 (a)	0.141 $\pm$ 0.046 (a)	3.4 $10^{-3}$
Dws_HighP_LowG	0.022 $\pm$ 0.010 (a)	0.129 $\pm$ 0.032 (a)	1.4 $10^{-3}$
Dws_HighP_HighG	0.542 $\pm$ 0.059 (a)	0.126 $\pm$ 0.023 (a)	5.4 $10^{-3}$

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824 **Table S2.** Glyphosate and AMPA concentrations in water (abiotic controls). The analyses  
 825 were carried out at the beginning (day 0) and at the end of the experiment (day 27). Low  
 826 (LowG) and high (HighG) glyphosate concentrations and low (LowP) and high (HighP)  
 827 phosphorus concentrations in water. The values are means  $\pm$  standard errors (n = 3) of  
 828 experimental data.

Time (days)	Phosphorus	Glyphosate	Glyphosate concentration ( $\mu\text{g L}^{-1}$ )	AMPA concentration ( $\mu\text{g L}^{-1}$ )
0	LowP	LowG	15 $\pm$ 0.1	0.25 $\pm$ 0.01
0	LowP	HighG	63 $\pm$ 1.0	2.3 $\pm$ 0.06
0	HighP	LowG	15 $\pm$ 0.1	0.22 $\pm$ 0.02
0	HighP	HighG	63 $\pm$ 0.2	1.7 $\pm$ 1.2
27	LowP	LowG	15 $\pm$ 0.4	0.07 $\pm$ 0.01
27	LowP	HighG	79 $\pm$ 0.5	0.25 $\pm$ 0.00
27	HighP	LowG	15 $\pm$ 0.5	0.02 $\pm$ 0.00
27	HighP	HighG	67 $\pm$ 2.9	0.13 $\pm$ 0.00

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# Glyphosate degradation in river

