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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% (DT₅₀) 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 $\mu\text{g L}^{-1}$), N = 45552
143 for AMPA (LQ = 0.02 $\mu\text{g L}^{-1}$) and N = 199833 for total phosphorus (LQ = 0.01 mg P L^{-1}).

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, $\mu\text{g P L}^{-1}$): 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 ρ ($P <$
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⁻¹; water discharge 0.03/1.25 m³ s⁻¹; dissolved organic carbon
162 6.50/12.97 mg L⁻¹; NO₃ 3.55/16.70 mg L⁻¹; soluble reactive phosphorus 0.03/0.32 mg P L⁻¹;
163 dissolved oxygen 10.02/8.15 mg L⁻¹; conductivity 297.53/654.91 μS cm⁻¹; pH 7.33/7.35;
164 glyphosate 0.00/0.25 μg L⁻¹ and AMPA 0.09/1.08 μg L⁻¹ (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 cm² 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⁻¹ of NO₃, 100 μg

180 P L⁻¹ of soluble reactive phosphorus, 8.2 mg L⁻¹ of dissolved organic carbon, 9.2 mg L⁻¹ of
181 dissolved oxygen, 244 µS cm⁻¹ 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⁻¹). 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⁻¹ 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⁻¹) by adding adequate volumes of 1 M K₂HPO₄ 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⁻¹ and HighG = 100 µg L⁻¹, nominal concentration) by adding adequate
198 volumes of 10 and 100 g L⁻¹ glyphosate aqueous solution prepared with glyphosate
199 PESTANAL[®] (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 KNO_3 (in order to maintain the initial concentration of
213 nitrate in water, i.e. 4.0 mg L^{-1}) and 1 M K_2HPO_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°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°C) to the CARSO laboratory
224 (COFRAC agreement number 1-1531, Lyon, France) and kept at 4°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⁻¹: NaCl, 8.5; Na₂HPO₄ 1.07; NaH₂PO₄, 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 (FluoroskanTM, 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 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 ExactiveTM 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 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 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_{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.

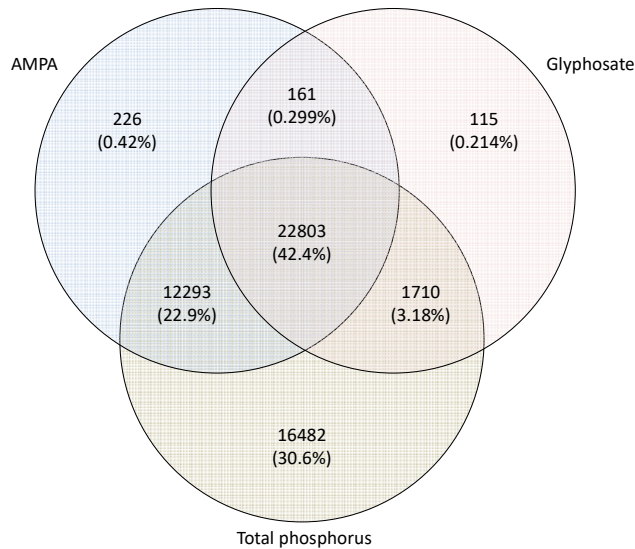
310

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 mg P L^{-1} , maximum 18 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%).

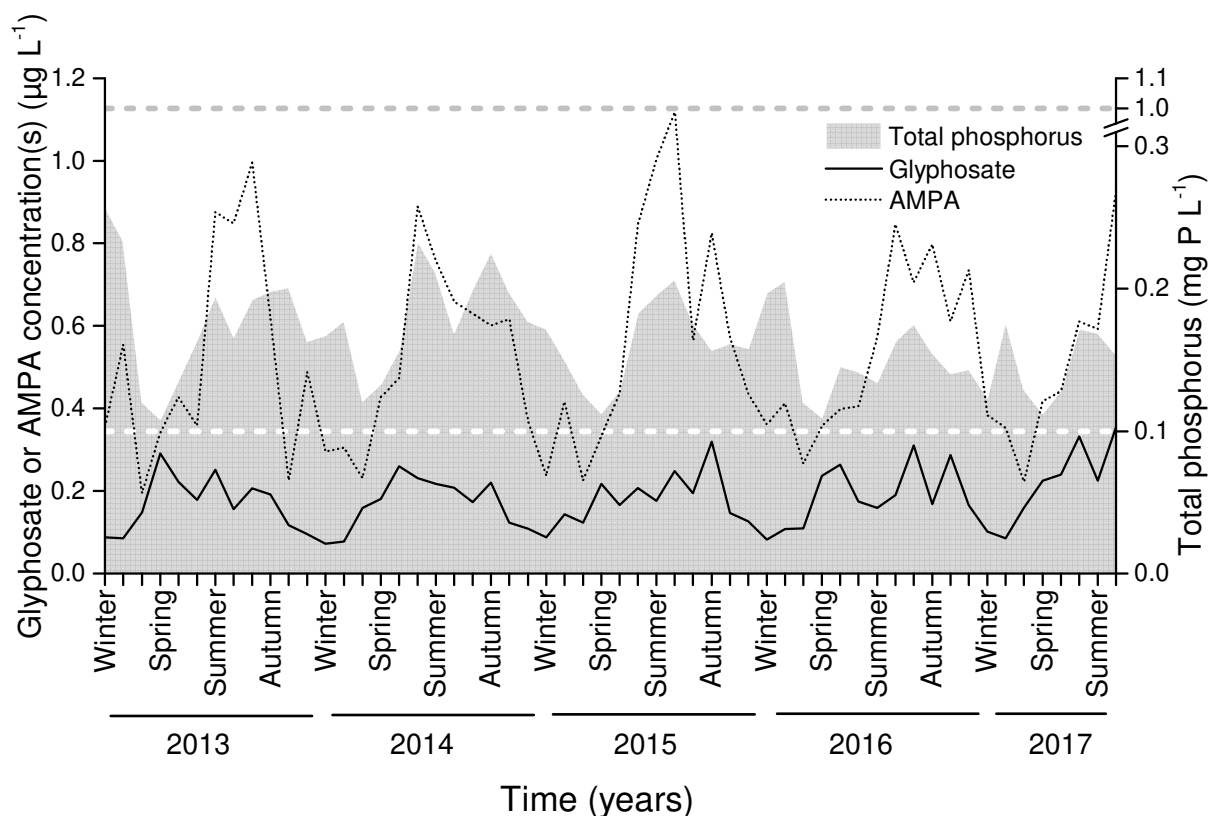


327

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).

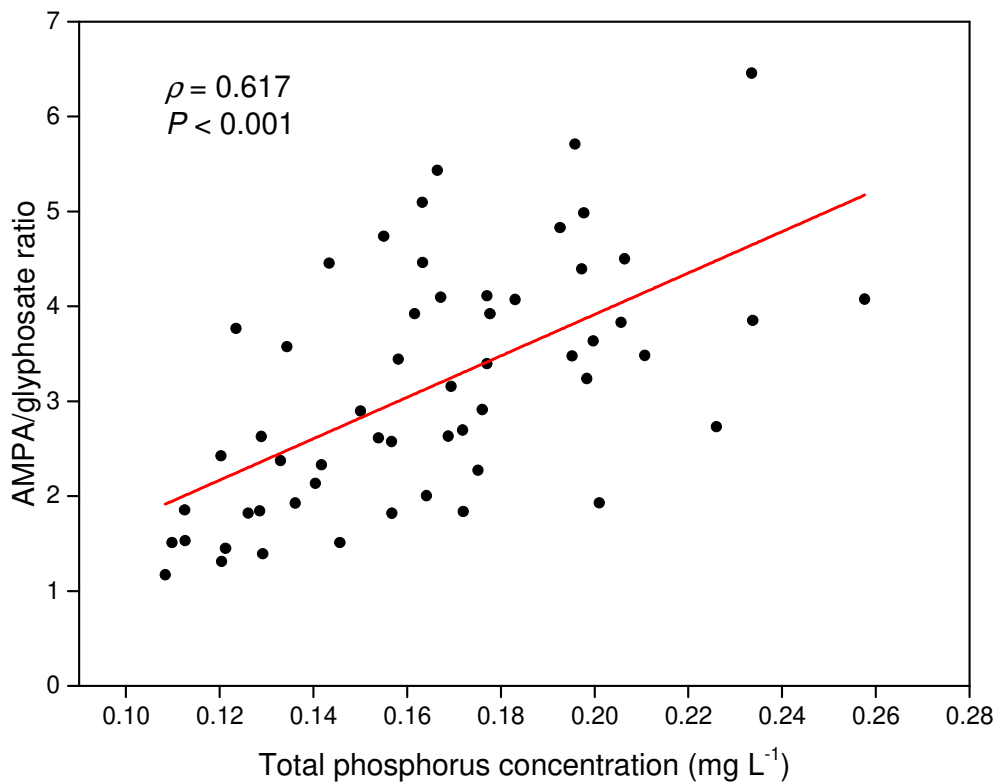
332 The temporal evolution of glyphosate, AMPA and total phosphorus concentrations
 333 followed a seasonal pattern that was consistent over the years (Figure 2). The concentrations
 334 of the three compounds increased from spring to summer (the increase in glyphosate occurred
 335 first, followed by 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 ± 0.02 and $0.13 \pm 0.01 \mu\text{g L}^{-1}$ (oligotrophic) to 0.32 ± 0.05 and 0.90 ± 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 μg
355 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 (ρ) 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 DT_{50} were inversely proportional (the higher the k value was, the lower
379 the 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 (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 DT_{50}) and AMPA (C_f) parameters. The three factors were site (Ups vs. Dws),
391 phosphorus concentration in water P_{water} (LowP vs. HighP) and glyphosate concentration in
392 water G_{water} (LowG vs. HighG), $P < 0.05$.

(A)

Glyphosate	Model parameters				R ²
	Condition	C ₀ (µg L ⁻¹)	k (day ⁻¹)	DT ₅₀ (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	
AMPA	Model parameters				R ²
Condition	C ₀ (µg L ⁻¹)	k (day ⁻¹)	h ₅₀ (days)	C _f (µg L ⁻¹)	
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	Glyphosate				AMPA	
	C ₀	DT ₅₀	C _f			
Factors/interactions	F value	P value	F value	P value	F value	P value
Site	0.1	0.763	46.9	< 0.001	4.6	< 0.05
Pwater	1.9	0.185	134.1	< 0.001	4.8	< 0.05
Gwater	1552.1	< 0.001	181.3	< 0.001	226.01	< 0.001
Site*Pwater	1.1	0.318	11.5	< 0.01	0.2	0.693
Site*Gwater	0.01	0.922	18.5	< 0.001	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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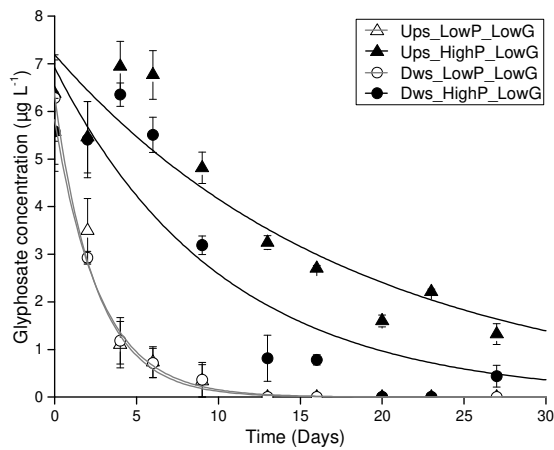
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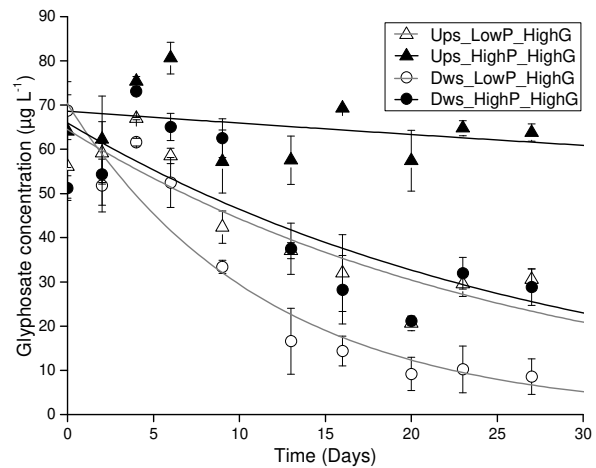
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(A)



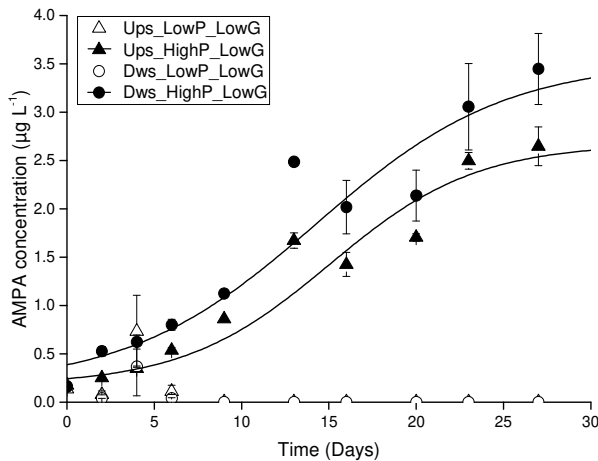
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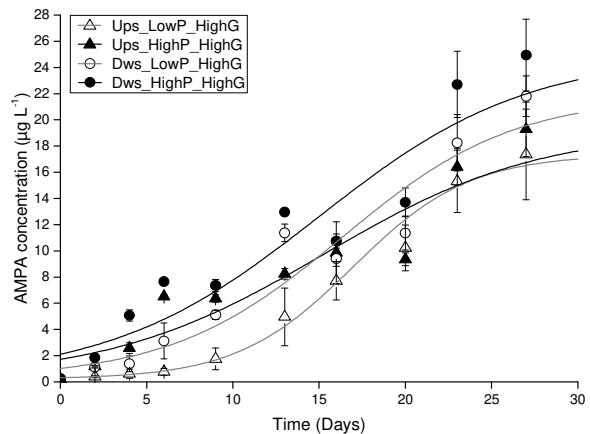
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(C)



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).

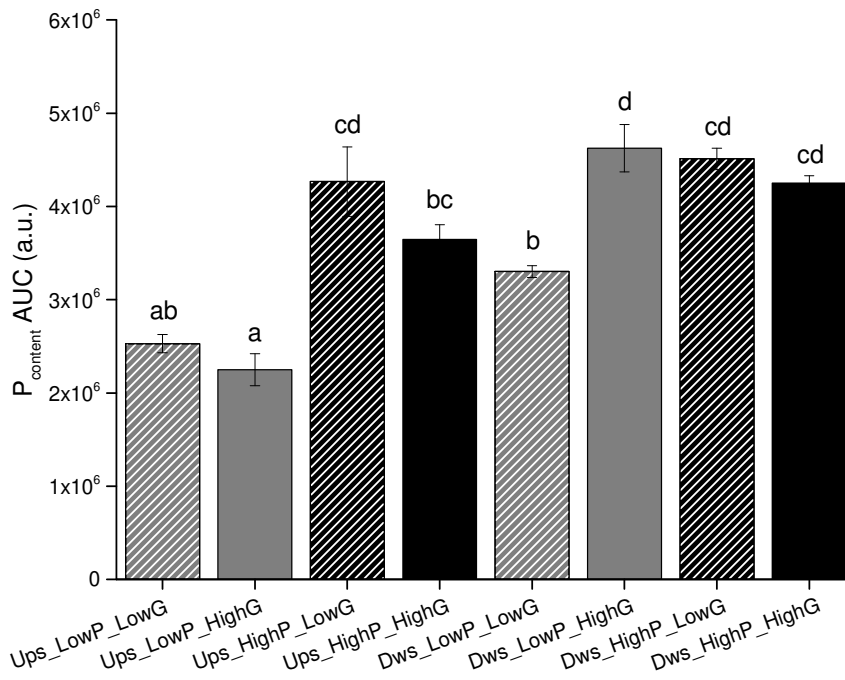
426 The formation of AMPA was also modulated by the concentration of phosphorus in water
427 ($P < 0.05$). This effect was more marked in LowG water than in HighG water (Table 1). Thus,
428 in the LowP, LowG water condition, in which glyphosate dissipation was the fastest, AMPA
429 was only a transient 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_{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 \pm standard error (SE), $n = 3$. Significant differences are indicated by
 461 lowercase letters, $a < b < c < d$ (Tukey's test, $P < 0.05$).

462

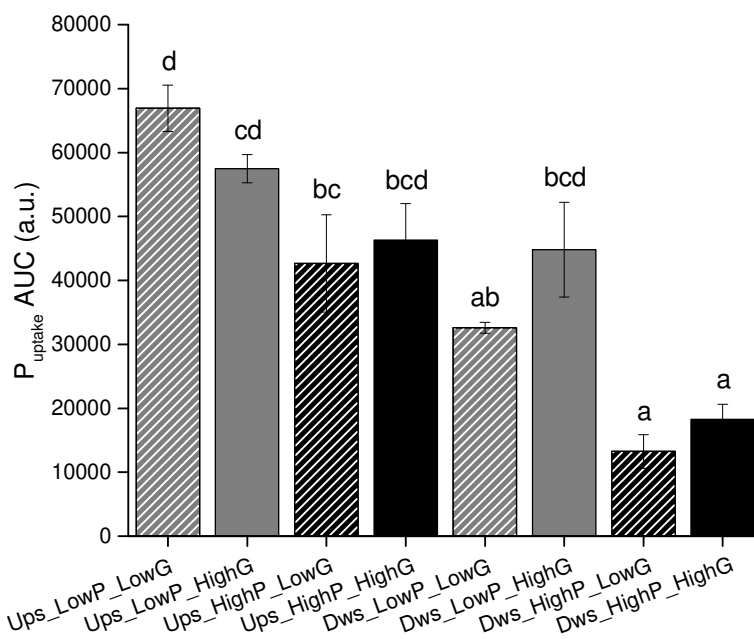
463 The phosphorus content in biofilms was significantly higher in Dws biofilms than in the
 464 Ups ones (Figure 5, $P < 0.001$). During the first 6 days of the experiment, the biofilms
 465 exhibiting the lowest P content (approximately 25 mg P g⁻¹ of biofilm dry weight) were those
 466 from the Ups 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 ($P = 0.401$).

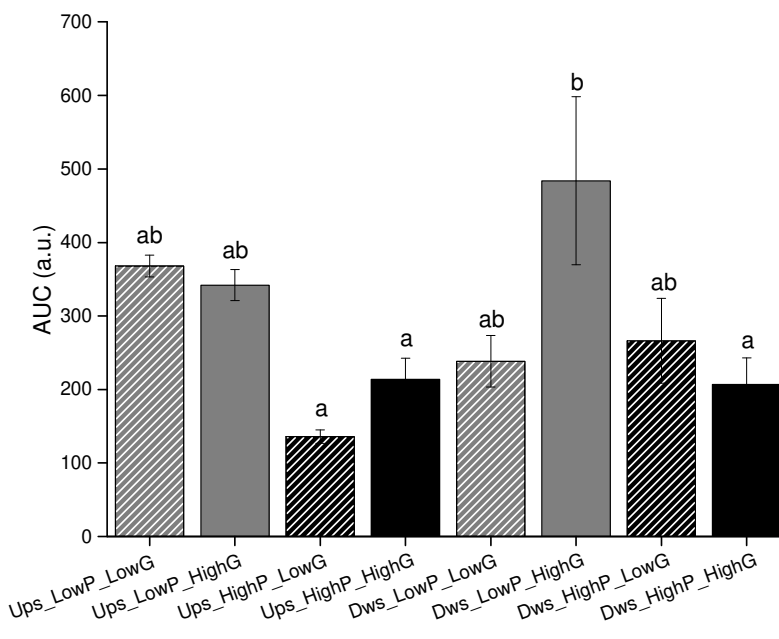


482

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 ± 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 ± 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, $P < 0.05$).
 502

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 (DT₅₀ ranging from 2.8 to 500.3 days) and high persistence in anaerobic soils
576 (DT₅₀ 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 (DT₅₀ 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.

591

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595

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599

600 **Declaration of interest:**

601 none.

602

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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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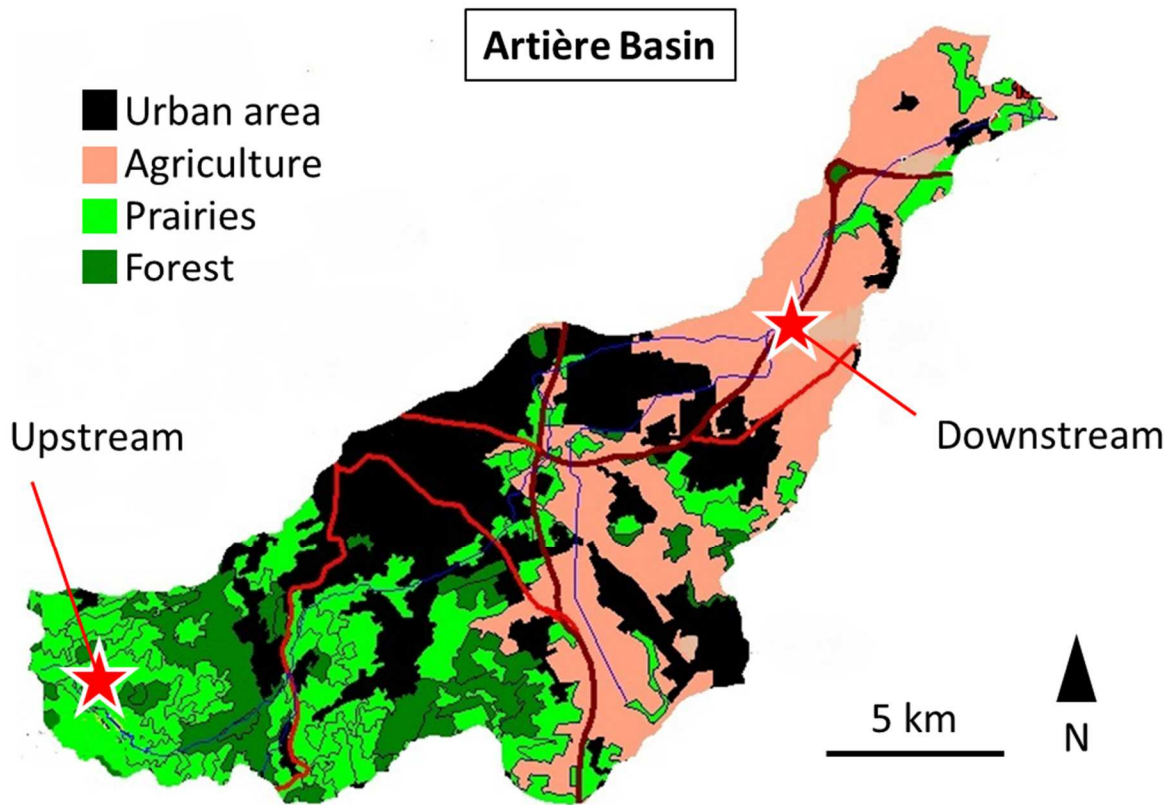
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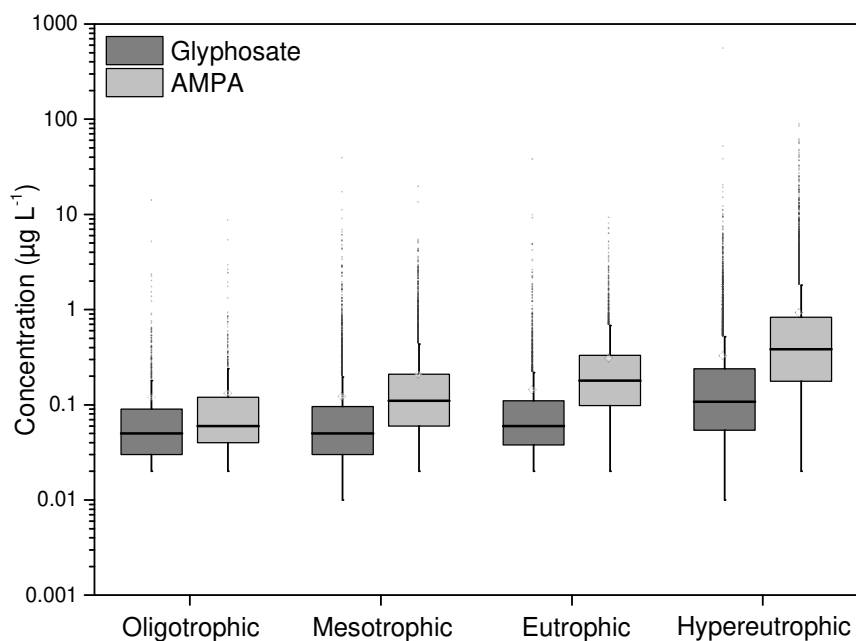


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758 **Figure S1.** Map of Artière watershed. Upstream and downstream sites locations are indicated
 759 by red stars.

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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).

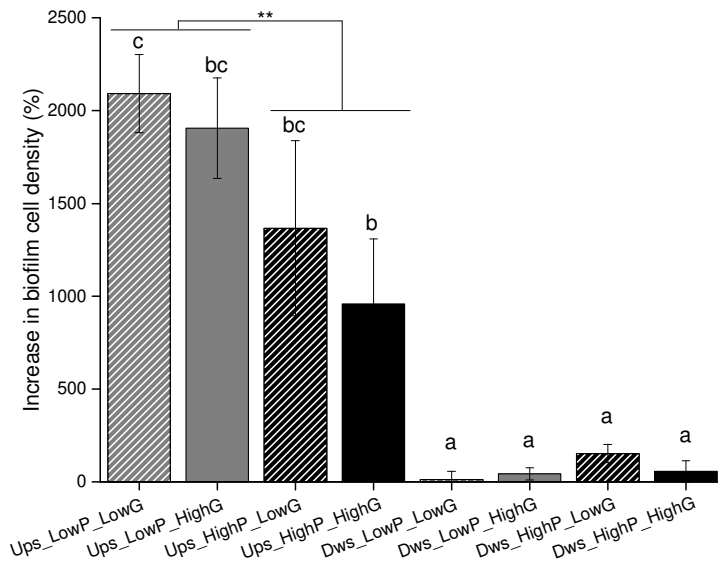
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774 **Figure S3.** Increase in biofilm cell density between day 0 and day 27. The results are reported
 775 as the mean \pm standard error (SE), n = 3. Significant differences between conditions are
 776 indicated by lowercase letters, a < b < c (Tukey's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

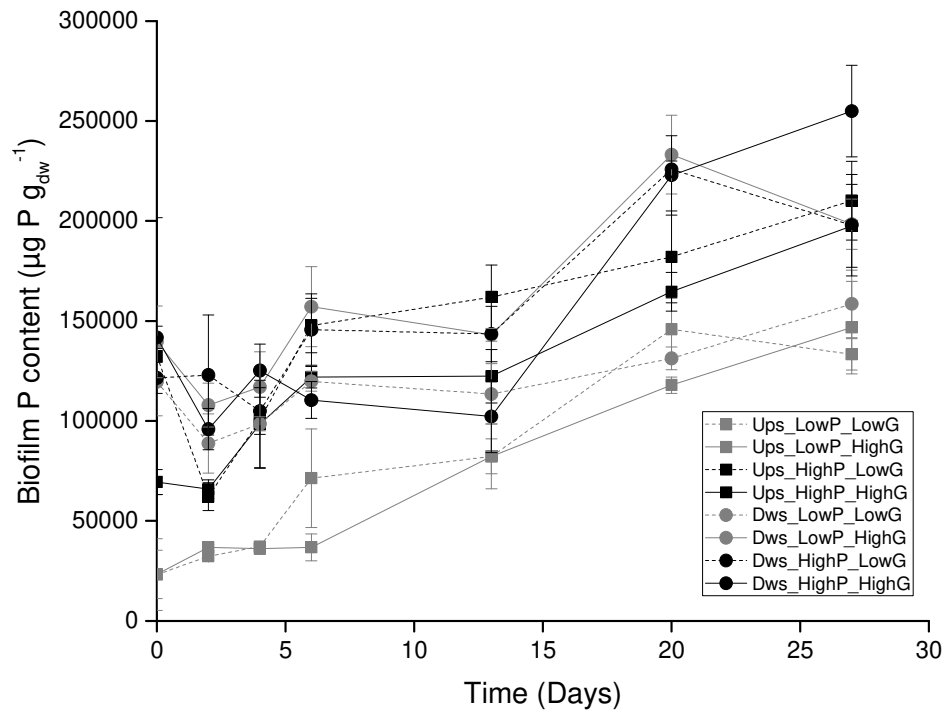
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784 **Figure S4.** Biofilm phosphorus content. The results are reported as the mean \pm standard error

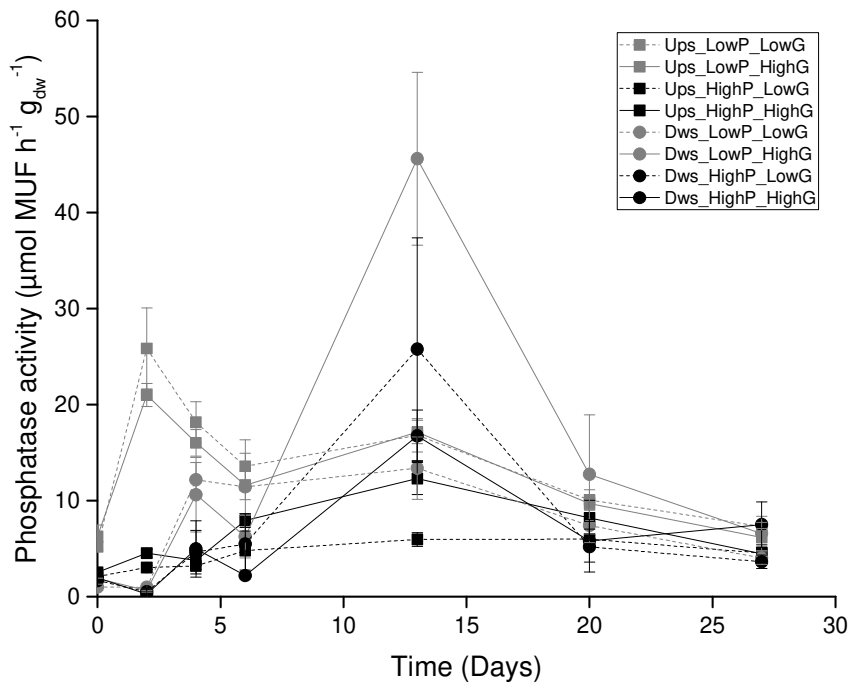
785 (SE), $n = 3$.

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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

